Immunogenetic analysis of HLA Class II in premalignant disease of the cervix and correlation with HPV status

Thesis

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IMMUNOGENETIC ANALYSIS OF HLA CLASS II IN PREMALIGNANT DISEASE OF THE CERVIX AND CORRELATION WITH HPV STATUS

A thesis presented for the degree of Doctor of Philosophy, Ph.D.

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ABSTRACT

IMMUNOGENETIC ANALYSIS OF HLA CLASS II IN PREMALIGNANT DISEASE OF THE CERVIX AND CORRELATION WITH HPV STATUS

The human papilloma virus (HPV) infection has a causal association with cervical intra-epithelial neoplasia (CIN) and cervical cancer. However, pre-malignant or malignant transformation is not always observed with HPV infection. HLA molecules are important in the regulation of the immune response to foreign antigens. The role of genetic variation at the HLA class II loci (DR and DQ) in CIN was investigated in 176 British Caucasian patients and 420 controls (normal cervical cytology and negative for HPV 16, 18, 31 and 33). HLA DQB1*03 typing was performed by a novel polymerase chain reaction-restriction fragment length polymorphism method (A-RFLP). The technique uses PCR to mutate the first base of codon 40 (DQ alleles) from T to G to create an artificial restriction site for an enzyme, *MluI*, which distinguishes DQB1*03 from other alleles and is confirmed by digestion of amplified DNA with *MluI*. Further HLA DR-DQ typing was performed by PCR DNA amplification and oligonucleotide probe typing. HPV types (16, 18, 31 & 33) were detected by using type-specific oligonucleotide primers and PCR. The alleles of the DQB1*03, DRB1*04 and DRB1*11 groups were strongly associated with susceptibility to CIN. Specifically the haplotypes DRB1*0401-DQB1*0301 and DRB1*1101-DQB1*0301 were significant and indicated susceptibility. The DQB1*03 locus was more contributory to this association than the DRB1 loci. A weak protective effect was shown for the haplotype DRB1*0101-DQB1*0501. Positive correlation was also observed for HPV-positive CIN, suggesting that specific HLA alleles may be important in determining the immune response to HPV antigens and the risk for CIN after HPV infection. Immunoaffinity purification of the susceptibility and protective HLA DQ molecules was performed and the naturally processed peptides were eluted and sequenced by Edman degradation. The data obtained was used for motif prediction of HPV 16 E6, E7, L1 and L2 sequences that may be capable of binding to these HLA molecules. Motif

prediction as well as the binding affinity of predicted peptide motifs for HLA DRB1*0401 and DRB1*0101 was accomplished using the published data on the naturally bound peptide sequences bound to these HLA molecules. The results revealed significant differences in both the number and binding affinity of the HPV 16 derived peptides to the protective and susceptibility HLA molecules. These results should help in the rational design of vaccines against HPV.

DEDICATION

This work is dedicated to my wife, Ayo; and to our daughters, Tosin, Tomi and Tolu.

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CHAPTER 1: INTRODUCTION

1.1 CERVICAL CANCER

1.1.1 GENERAL INTRODUCTION

Cervical cancer constitutes a major health problem worldwide. Each year, there are approximately 465,000 new cases and in excess of 200,000 deaths from the disease[1]. The areas with the maximum incidence are in Southern and Eastern Africa and Central and tropical South America. The risk in Western European and North American countries is considered relatively low at less than 10 new cases per 100,000 women annually. The rates are 10 to 20 times higher in some parts of Northeastern Brazil where lifetime cumulative risks can approach 10%[2]. In the United Kingdom, there are approximately 3,000 new cases each year and over 2,000 deaths from the disease. Likewise, in the United States, each year there are approximately 16,000 new cases of invasive cervical cancer with 4800 deaths due to the disease[3]. For each new case of cervical cancer found by cytology screening, there are approximately 50 other cases of abnormal cervical smears that merit careful monitoring by colposcopic follow-up and eventually, biopsy. To this triage burden must be added an equal number of cases of borderline atypias (the so-called "ASCUS" smears) that are found concurrently and need to be confirmed by repeat cytology (MMWR, 1994). Although much effort has been applied to the early detection of cervical cancer by exfoliative cytology and the provision of conservative modalities of treatment for pre-invasive lesions, study of the molecular mechanisms of cervical carcinogenesis has only recently become an intense field of research.

1.1.2 PATHOGENESIS

The uterine cervix consists of the ectocervix, which is covered by glycogen rich squamous epithelium, and endocervix, lined with a single layer of columnar epithelial cells. Colposcopic and histologic examination of the cervix from perinatal to adult life discloses three epochs when columnar epithelium on the endocervix is activated by contact with the vaginal environment. These epochs, which correspond with periods of maximal estrogen

stimulation are perinatal, at the menarche, and during the first pregnancy[4]. As a result of contact with the lower pH of the vaginal environment, the columnar epithelium is induced to transform into stratified squamous epithelium. The new squamous epithelium soon becomes an undifferentiated 8 to 10 cell-thick structure composed of rounded cells. Later differentiation occurs into basal, intermediate and superficial cells. Finally, the mature epithelium comes to mimic closely the squamous epithelium of the vaginal portion of the cervix and of the vagina which it adjoins abruptly at a line running concentrically around the os. Within this line, the squamous epithelium is new and distinct from the original or native squamous epithelium outside its limits. Thus, the limits of the *transformation zone* lies between the original squamo-columnar junction (now squamo-squamous) and the new squamo-columnar junction. This site is thought to be where premalignant lesions of the cervix develop.

Cytologic examination of cervical smears is used to detect cervical abnormalities. Women with abnormal cervical (Papanicolaou) smears are subsequently examined by colposcopy. The lesions in the transformation zone that appear atypical by colposcopy are diagnosed by histological examination. Cervical lesions with the potential to progress to invasive cancer were originally histologically diagnosed as dysplasia. Cervical dysplasia is characterized by a disturbed epithelial architecture and cellular atypia. Depending on the proportion of the epithelial layer that shows dysplastic changes, lesions are classified as mild, moderate or severe dysplasia. In 1968, the concept of cervical intraepithelial neoplasia (CIN) was introduced[5]. In this scheme, CIN is a continuous spectrum of intraepithelial changes, that starts with minor atypia, progressing through increasing degrees of intra-epithelial abnormalities to invasive squamous cell carcinoma. CIN lesions are classified according to the thickness of the epithelial layer involved in neoplastic change. Involvement of the lower one third of the epithelial layer represents CIN grade 1, one third to two thirds involvement

CIN grade II, and two thirds to full thickness CIN grade III, which is equivalent to carcinoma in situ (CIS)[6].

This classification scheme led to two major problems. Firstly, the relative high inter- and intra-observer variation in interpretation by pathologists[7] and the attitude of the clinician toward treatment of CIN II lesions, with a choice between treatment and watchful expectancy. By 1988, several classifications and many modifications were in use throughout the world resulting in confusion in communications among clinicians, pathologists and researchers. The result of a workshop by the National Cancer Institute was the Bethesda System[8] to replace the various Papanicolaou designations and to standardize cytologic terminology to correlate with histologic reports. A comparison between the Bethesda System and earlier ones is summarized in Table 1.1. The term CIN was replaced by Squamous Intraepithelial Neoplasia (SIL), with CIN II and III replaced by high grade SIL (HGSIL) while CIN I was replaced by low grade SIL (LGSIL). This suggests that CIN grade I lesions constitute a different entity from CIN II or III lesions, lacking the ability to progress to cervical cancer. In reality, about 20 - 30% of cervical lesions with mild dysplasia will progress to carcinoma in situ[9-10]. In another study, 50% of patients with CIN I progressed to CIN III, while 25% either progressed to CIN II or remained at grade I for nine years[11]. In the United Kingdom, approximately 250,000 women are diagnosed annually with CIN. The much lower frequency of cervical cancer per year suggests that only a proportion of lesions diagnosed as CIN will progress to carcinoma.

The mean age for cervical cancer is 52.2 years, and the distribution is bimodal, with peaks at 35-39 years and 60-64 years[12]. The incidence of adenocarcinoma appears to be increasing relative to that of squamous cancers. Older reports indicate that 5% of all cervical cancers were adenocarcinomas[13], whereas newer reports suggest an incidence as high as

18.5-27% [14-15]. There is evidence to suggest a poorer prognosis for adenocarcinoma than for squamous cell carcinoma in every stage. Hopkins & Morley[15] performed a Cox proportional hazard analysis of 203 women with adenocarcinoma and 756 women with squamous carcinoma and reported 5-year survival rates of 90% versus 60%, 62% versus 47%, and 36% versus 8% for stages I, II, and III, respectively. Although this has been attributed to a relative resistance to radiation, it is more likely a reflection of the tendency of adenocarcinomas to grow endophytically and to be undetected until a larger volume of tumour is present. It is also unclear whether or not patients with adenosquamous carcinoma of the cervix have a poorer prognosis than those with pure adenocarcinoma or squamous carcinoma[16-17].

1.1.3 ETIOLOGY

The epidemiological pattern of cervical cancer strongly points to a sexually transmitted infectious agent as being etiologically important. As early as 1842, Rigoni Stern[18] found that compared with breast cancer, cervical cancer was eighteen times more common amongst married than unmarried women in Verona. Modern epidemiological studies have shown low cervical cancer rates among catholic nuns[19] while higher rates are found in women marrying at younger ages[20-21]. The latter is related more specifically with two aspects of sexual behaviour namely number of sexual partners and age at initiation of intercourse[22-23]. The risk associated with ten or more partners is nearly three times higher than that associated with one or fewer partners[24-25]. Women with first sexual experiences before age 16 have about twice the risk compared with women who initiate sexual intercourse after the age of 20 years and this may reflect the increased susceptibility of the "younger" cervix to carcinogenic insult[26]. Other risk factors include cigarette smoking and the use of the oral contraceptive pill.

Different candidates for a sexually transmitted agent have been proposed including syphilis, gonorrhea, Trichomonas vaginalis and Herpes simplex virus type 2 (HSV-2). In particular, HSV-2 appeared a plausible candidate, because of the high frequency of HSV-2 specific antibodies in cervical cancer patients compared with healthy controls. Although HSV was proven to be carcinogenic, in vitro and in vivo clinical studies eventually demonstrated that only a fraction of cervical carcinomas contained traces of HSV viral DNA, and epidemiological studies failed to demonstrate an association between HSV and cervical cancer[27-28].

In 1976, zur Hausen hypothesized that cervical cancer shows a similar epidemiological pattern as genital warts[29]. The formation of genital warts (condylomata acuminita) was considered to be associated with infection with the human papillomavirus (HPV). Subsequently, zur Hausen and Gissman using recombinant DNA technology were able to clone and characterize isolated HPV DNA from genital warts and papillomas[30-32]. Novel HPV types in cervical cancer were identified that differed from those associated with genital warts. Since then, there have been several compelling epidemiological, clinical, and molecular biologic data indicating that the infectious agent with a causal relationship to CIN and cancer is the human papillomavirus (discussed below).

1.2 HUMAN PAPILLOMAVIRUS (HPV)

1.2.1 GENERAL PROPERTIES

Papillomavirus belong to the family Papovaviridae. They possess a closed, circular, double stranded genome and are encapsulated in an icosahedral capsid of about 55nm in diameter, consisting only of protein. Their genome is approximately 7.9kB in size and more than 70 different types of papillomaviruses have been identified, many of which infect the ano-genital epithelium. By definition, the different types share less than 50% homology under stringent conditions[33-34]. Since the number of complete sequences of different HPV types

is rapidly increasing, and many more HPVs are being identified, a modified definition of a new HPV type has been proposed[35]. Here, a new type is defined when less than 90% sequence homology in E6, E7 and L1 region is found with any other known HPV type.

Two major groups are defined according to their epithelial affinity - types infecting the dry skin and those infecting the moist mucosal areas of the body (Table 1.2). The genital HPV types are placed into three broad categories based on the frequency of association with malignant tumors and thus the presumed oncogenic potential. The low risk groups includes types 6, 11, 42 and 44, which are common in LGSIL and less so in HGSIL and practically non existent in cancer specimens. The intermediate risk group is comprised of types 31, 33, 35, 51 and 52 whose combined frequency of association increase within the SIL spectrum but decrease in carcinomas. The high risk group includes HPV 16, 18, 45 and 56, which are strongly associated with carcinomas and exhibit diverse behaviour with respect to HGSIL[36].

HPVs infect the basal cells of the epithelium and rather than proceeding to a lytic infection in which viral replication kills the cell, viral DNA transcription and replication are maintained at very low levels (20-100 copies per cell) until more superficial epithelium is infected. At this level, viral transcription accelerates, DNA synthesis begins and virions assemble[37]. In addition, the activation of late gene expression and capsid assembly occurs concurrently with the amplification of viral DNA. In benign or pre-malignant lesions, the HPV DNA exists extra-chromosomally as a plasmid. By contrast, all cervical cancer derived cell lines that contain HPV DNA and the majority of primary tumours reveal integrated viral DNA[38].

1.2.2 HPV GENOME ORGANIZATION

The HPV genome consists of 3 regions : one regulatory non coding region, termed the long control region (LCR), and two coding regions (Fig 1.1). The ' early ' region consists of six to eight open reading frames , whereas the ' late ' region encodes two genes. By definition, early genes are expressed shortly after viral infection and prior to viral replication. The late genes code for the structural proteins forming the viral particle, and are expressed in the late phase of infection. The functions of the different reading frames are summarized in table 1.3.

E1: HPV E1 is homologous with polyoma large T domains[39] and BPV-E1[40]. The viral ring molecule is usually opened within the 3' end of the E1 or the 5' end of the E2 open reading frames and mutations in the E1 or E2 gene increases HPV 16 immortalisation efficiency in keratinocytes[41]. In monolayer cultures, both E1 and E2 gene products are required for the transient replication of viral genomes[42-43] and the origin of replication (ori) maps to a region adjacent to the E6 open reading frame (ORF), which binds the E1 protein. The E1 ORF also encodes a nuclear phosphoprotein that can bind the E2 molecule[44]. While the E1 protein may bind DNA by itself[45], the presence of E2 increases its affinity for binding[46].

E2: The E2 protein acts as a transcriptional trans-activator, via the E2 responsive elements (E2-RE), located in the LCR[47]. E2 disruption is thought to alter regulation of expression of E6 and E7 genes[48]. In high risk HPVs, the primary transcriptional activity of E2 appears to be as a repressor E6 and E7 transcription[49-50]. Two E2 sites are located 2 to 4 bp upstream from the putative TATA box and this close proximity may result in steric hinderance by bound E2 proteins.

E4: The E4 gene product is expressed as an E1-E4 fusion protein in the "late" phase of the viral life cycle. In this form, it disrupts keratin assembly in stratified suprabasal cells, allowing for viral egress[51-52].

E5: The HPV E5 encodes a membrane protein with weak transforming activity[53]. Part of its transforming function may reside in its ability to complex with epidermal growth factor (EGF) receptor, resulting in an enhanced EGF-mediated signal transduction to the nucleus, as shown by increased *c-fos* expression levels[54].

E6 and E7: Analysis of the transcription of HPV 16 in cervical cancer derived cell lines and in fresh premalignant and malignant cervical biopsies have shown that transcription of the E6 - E7 region of the HPV 16 genome is selectively retained in the neoplastic tissue[55-56]. E6 and E7 have been identified as the transforming genes of HPV16 and it appears that both are necessary for full transformation of cervical epithelial cells[57-58].

The E6 and E7 gene products of HPV 16 & 18 can each transform immortalized rodent cells such as NIH 3T3 and Rat-1 cells to an anchorage dependent state[59]. The E7 proteins can also transform primary rodent cells, but require the additional presence of an activated ras gene for full activity[60]. In the human keratinocyte, the HPV E6 and E7 act in a co-operative fashion to efficiently immortalize cells[61-62]. The E7 gene by itself can immortalize keratinocytes at low frequency, whereas the E6 protein does not exhibit any such properties. Furthermore, keratinocytes immortalized by HPV 16 and 18 E6/E7 only become tumourigenic by the addition of activated ras gene or following prolonged passage in culture[63].

L1 and L2: The L1 and L2 open reading frames encode the major and minor capsid proteins respectively.

LCR: The LCR is located between the early and late genes (also termed upstream regulatory region, URR), and contains viral gene promoter and enhancer sequences which are dependent solely on cellular factors for function[64]. These enhancers are called constitutive or "C" enhancers for HPV 11, 16 and 18 and are located in the URR 200 to 300bp upstream of the E6 ORF. C enhancers direct expression of heterologous promoters preferentially in cell lines derived from squamous cell carcinomas, as well as in primary human keratinocytes. Transcripts controlled by these enhancers initiate upstream of the E6 gene at a promoter referred to by nucleotide number p97 in HPV 16 and HPV 31 and p101 in HPV 31. These enhancers include AP-1, keratinocyte specific factor, KRF-1[65] and steroid receptors[11,66].

1.2.3 HPV DETECTION METHODS

Classical viral cultivation techniques are not applicable in HPV because the virus cannot be propagated in culture. Consequently, in order to assess the presence of HPV in clinical samples, HPV DNA detection methods using hybridization based techniques have been developed. Southern blot analysis allows highly specific HPV genotype detection with a sensitivity range between 0.1 and 0.01 HPV genome copies per cell. However, the methodology is too time-consuming and labour intensive making it unsuitable for mass screening purposes. Other methods like dot blot analysis, *in situ* hybridization and filter *in situ* hybridization, are less labour intensive, but suffer from other drawbacks such as reduced specificity and sensitivity.

The advent of the polymerase chain reaction (PCR) has considerably increased the possibility of screening a large number of samples[67-68]. The method is superior in sensitivity as compared to other HPV detection techniques, with a sensitivity of 1 copy per 10^6 cells in a clinical sample being detectable[69], and it requires low amounts of target

DNA. Furthermore, it can be applied directly to crude cell extracts without the need of DNA isolation from every clinical sample[70] and can also be applied on fixed tissue[71-72].

Many alternative sets of primers are used for detecting HPV by PCR. Two commonly used sets are the consensus primer pair MY09 and MY11[73] and the general primers GP5 and GP6[69]. In both systems, the primers used are homologous with sequences in the L1 ORF of HPVs, since this region is highly conserved. Several other sets of primers have been reported in the literature, such as consensus E1 primers, but their use is not widespread enough. In the MY 09/11 system, a PCR amplicon of about 450 nucleotides is produced and this can be subsequently typed by dot blotting of PCR products using radioactive or biotin labeled oligomers[73]. In the GP 5/6 system, an amplicon of about 140 nucleotides is produced from a region of L1 that overlaps with MY and subsequently separated by gel electrophoresis, blotted onto filters and hybridized with radioactively labeled probes. Both methods are fairly equivalent for in-vitro use, but the GP primers are better for amplification of targets from paraffin-embedded sections, because the longer MY amplicons are not synthesized as efficiently because of formalin cross-linking in the tissue.

The use of type-specific primer systems is an alternative approach for the detection of HPV. This system is of particular use when the determination of a single genotype of HPV is of interest. Additionally, these systems provide an excellent means of confirming results generated by consensus PCR or other methods of HPV DNA detection.

1.2.4 PAPILLOMAVIRUSES AS CAUSATIVE AGENTS IN CERVICAL NEOPLASIA

Genital HPV infections are highly prevalent (20 - 80%) in sexually active age groups[74-75]. The causative role of HPV in the induction of condylomata has been proven by experimental transmission from person-to-person and in animal model systems. The

etiologic role of HPV in intraepithelial neoplasias was demonstrated by observations of naturally occurring transmission between sexual partners in whom histologically similar lesions developed harbouring the same HPV type[76]. Transfection of human keratinocytes with HPV 16 induces histologic features of intraepithelial neoplasia when cells are grown in organotypic cultures allowing the formation of stratified epithelium[77]. The histology resembles CIN I at the beginning and corresponds to carcinoma in situ after several in vitro passages.

Using PCR-based and other HPV detection techniques, many cross sectional studies have been performed to study the prevalence of HPV in women with normal and abnormal cervical smears. In women with normal cervical smears, HPV prevalence rates varying from 1.5% to over 30% have been reported[74,78]. Some studies may have suffered from insensitive HPV detection and PCR contamination making comparisons of the different studies difficult. However, prospective studies of women with initially negative cytologic tests showed that the cumulative incidence of CIN II at 2 years was 28% among HPV positive women compared with 3% among HPV negative women [79]. Infection with either HPV 16 or 18 is associated with a relative risk of 11 for development of CIN.

The magnitude of the association between HPV infection and the risk of cervical neoplasia have been examined by different groups. Pooling of data based on Woolf's technique[80] leads to a combined RR for CIN from all non-PCR studies of 10.3 (95% CI, 6.9 - 15.3), whereas the RR from PCR studies was 19.8 (95% CI, 15.2 - 25.8). The difference between pooled estimates is more pronounced for studies of invasive carcinomas with RR of 3.7 (95% CI, 3.1 - 4.6) and 34.5 (95% CI, 21.5 - 55.4) for non-PCR and PCR studies, respectively. These data place HPV infection as the strongest risk factor for cervical cancer with a magnitude of association that is greater than that for the association between smoking and lung cancer and is second only to the association between the chronic

carrier state of hepatitis B infection and liver cancer, causal relations in cancer that are no longer challenged[81]. In addition, recent evidence from a large international study indicates that meticulous testing by PCR of nonfixed specimens of cervical carcinomas results in positivity rates of 95%[82]. A consensus panel of the World Health Organization's International Agency for Research on Cancer (IARC) has concluded that there is now compelling evidence both from biologic and from epidemiologic standpoints to consider that HPV infection leads to cervical cancer[83-85].

Cervical HPV infection detected by DNA hybridization techniques is found in 15 - 40% of sexually active women[74,85-86]. Most of these infections are transient, and only a small proportion of women tend to harbor the same HPV type on a persistent basis[87-89]. Prospective epidemiologic studies have indicated that the risk of subsequent cervical neoplasia seems to be proportional to the number of specimens testing positive for HPV[79]. Little is known about risk determinants for persistent HPV infection. The risk of HPV infection seems to be independently influenced by other variables such as parity, oral contraceptive use, and smoking[90].

1.2.5 MOLECULAR MECHANISMS OF HPV IN CERVICAL ONCOGENESIS

HPV E6 and E7 are small proteins that show some similarities to each other. It has been proposed that they arise following amplification and divergence of a 33 amino acid peptide. The main feature that the two share is a series of Cys-X-X-Cys motifs, which occur four times in E6 and twice in E7, and are thought to play a role in zinc binding by both proteins[91-92].

1.2.5.1 The E7 Gene

The E7 oncoprotein is a 98 amino-acid phosphoprotein localized to the cell nucleus within the nuclear matrix[93]. It possesses transforming, immortalizing and *trans* activating

properties and is phosphorylated on serine residues[94]. The amino-terminal 37 amino-acids bear significant sequence homology to conserved domains 1 and 2 (CD1 and CD2) of the Adenovirus 5 E1a oncoprotein as well as to a region of the SV40 large T oncoprotein. CD1 and CD2 have been shown to have several important biological functions such as cooperation with the ras oncogene in transformation assays, stimulation of DNA synthesis, as well as possessing binding sites for cellular proteins which may be important for E1a mediated transformation. Recent experimental evidence has shown that like E1a and SV40 large T antigen, HPV 16 and 18 E7 proteins bind to the retinoblastoma gene product, Rb[95-96]. The Rb binding domain has been localized to the region of homology with CD2 of the adenovirus E1a protein[96] and mutations in this domain eliminated Rb binding[96-98]. This region consists of a stretch of 17 amino acids[99].

The Rb gene consists of 27 exons spanning 200 kilobases of chromosomal DNA (band 13q14). The associated mRNA encodes a nuclear phosphoprotein with M.W. of 105-110Kda. It is expressed throughout the cell cycle, and it is found in the non-phosphosphorylated and phosphorylated forms that are specific for certain phases of the cell cycle[100]. In the non-phosphorylated state, it acts to restrict cell proliferation, partly by binding to the transcription factor E2F[101]. E2F is capable of transactivating several genes expressed during the S phase of the cell cycle. E2F-pRb complexes can be identified primarily in extracts of cells at the G1 phase of the cell cycle[101]. In this complex, pRb is unable to activate promoters which are important positive signals for growth such as *c-myc* and *n-myc*. In the phosphorylated state (G2/S), the control of Rb on cell growth is released[100]. Regulation of the phosphorylation is mediated in part through TGF-ß1 probably by blocking phosphorylation of Rb protein[102].

HPV16 E7 binds preferentially to the under-phosphorylated form of Rb and releases E2F from the Rb complex [98]. Furthermore, the HPV E7 gene product associates with the E2F-

cyclin A complex[103]. The complex consists of cellular proteins E2F, p107, cyclin A and cdk 2, all of which are important in the regulation of cell growth at different stages of the cell cycle. The released E2F will activate the expression of cell-cycle regulated genes such as *c-myc*, thymidine kinase, and DNA polymerase alpha, required for entry into the S-phase of the cell cycle. The functional significance of the E7-pRB interaction is underlined by the fact that the E7 proteins of low risk genital HPV types 6 and 11 bind with much lower affinity than the E7 proteins of HPV 16 and 18[96,104-105].

The E7 oncoprotein also has another biochemical function which it shares with an area of structural homology to E1a in the carboxy terminal region of CD2. Aminoacids 31 to 37 represent a substrate for casein kinase II (CKII), which phosphorylates serine 31 and 32[97]. Replacement of the 2 serines by non-phosphorylatable amino acids lead to a reduction in transforming activity and abolished phosphorylation, but not Rb binding. CKII has been implicated in the regulation of RNA and protein synthesis as well as DNA metabolism by phosphorylating the enzymes and proteins mediating these processes. It also mediates the phosphorylation of c-myc encoded proteins suggesting that it may be involved in cell cycle regulation.

1.2.5.2 The E6 Gene

The E6 protein of HPV has been shown to possess various transforming and immortalizing activities, the most important of which seems to be the ability to co-operate with HPV 16 E7 in the efficient immortalization of primary human epithelial cells[58,106]. The protein consists of approximately 150 amino acids which are believed to form 2 zinc binding fingers. The base of each finger contains four cysteines (Cys) in two pairs of the motif Cys-X-X-Cys, where X varies among the viruses. These E6 fingers comprise 29-30 amino acids and have been shown to specifically bind zinc in an in vitro binding assay[107].

The oncogenic activity of the E6 proteins of the high risk HPVs has recently been correlated with their ability to interact and inactivate the cellular p53 protein[108-109]. The documented effects of wild type p53 on cell proliferation include regulation of the transition from G1 to S phase of the cell cycle[110-112] and a role in determining cell death through apoptosis. p53 also appears to function normally as a G1-S checkpoint control for DNA damage[113-114]. Thus normal p53 may function as a 'molecular policeman' monitoring the integrity of the genome[114]. Removal of policing activities of p53 allows for continuous cycling of cells and the more rapid appearance of chromosomal abnormalities.

E6 binding of p53 leads to an increased rate of p53 degradation by a ubiquitin-directed system[109]. The enhancement of p53 degradation has been shown to be mediated only by E6 proteins of 'high risk' HPV types. Crook et al[115] have shown that a C-terminal region of E6 is involved in the binding of p53 while a region in the N-terminus is involved in degradation. It would appear that all genital HPV E6 proteins bind p53 but only high risk viruses have the ability to bind with high affinity. The E6 protein targets all quaternary forms of wild-type p53, while mutant p53 proteins are variably resistant to E6 mediated regulation and this correlates with PAb 1620 reactivity[116]. It appears that the PAb 1620+ conformation is important for recognition of p53 by E6 but is not the actual target for degradation. The function of p53 seems to be dependent on a conformationally flexible domain encompassing about 150 residues in the central portion of the protein.

The enzymatic reactions involved in the ubiquitination of proteins are well characterized. Ubiquitin is a 76 amino acid protein which is found in all eukaryotic organisms. The E1 ubiquitin-activating enzyme stimulates the ATP-dependent formation of a high energy thioester between the carboxyl group of the last amino acid of ubiquitin and a thiol group of a cysteine residue of the E1 protein. The E1 protein then transfers the activated ubiquitin to a cysteine of an E2 ubiquitin conjugating enzyme, with retention of a high energy thioester

bond. The E2 proteins usually require an E3 ubiquitin ligase protein to specify proteins that are to be multiubiquinated. In the case of HPV 16 and 18 E6 interaction with p53, this ligase is a 100KDa cellular protein, called E6 associated protein (E6-AP). Neither E6 nor E6-AP alone can stably associate with p53. The 3 functional domains of E6-AP which are important for the association has been characterized to an 18 amino acid region from amino acid 391 to 408 for binding, a 502 amino acid region from 280 to 781 for the E6 dependent association of E6-AP with p53, and the C terminal 84 amino acids for the E6 and E6-AP dependent ubiquitination of p53[117].

Several studies of p53 sequences in tumours and tumour cell lines have shown that while HPV- negative tumours express mutant p53 sequences, only wild type p53 is detected in HPV- positive cancers[115-118]. However, Kessis et al[119] provided recent evidence that HPV infection and p53 mutations are not mutually exclusive and that some HPV negative carcinomas may arise from a pathway independent of p53 inactivation. Indeed, an overview of data from several studies suggested that overall, the rate of p53 mutations in HPV positive carcinoma is only 3%; whereas in HPV negative tumours , it is 15%[120].

1.2.5.3 The E5 and E2 Genes

Both HPV 6 and HPV 16-E5 proteins form complexes with the p16 component of the vacoular ATPase, which serves the acidification of intracellular compartments[121]. E5 of HPV 6 also associates with the receptors for platelet-derived growth factor (PDGFR) and epidermal growth factor (EGFR)[122]. The HPV 16 E5 stimulates the transforming activity of EGFR by enhancing growth factor mediated signal transduction to the nucleus[54,123].

The lack of immortalizing activity of a HPV 16 variant from normal human cervical keratinocytes with a mutation in the E2 gene may point to a fourth viral oncogene[124]. It is unlikely that E2 and E5 have a major role in the maintenance of the malignant phenotype of

cancer cells because they are frequently destroyed by integration of the viral genome into cellular DNA.

1.2.5.4 Physical State of Viral DNA

The integration of HPV 16 or 18 DNA into the genome of cancer cells appear to be a potentially important step in tumour progression. Opening of the viral genome at the time of integration frequently disrupts the regulator genes E1 and E2 and engineered mutants in these genes revealed increased transformation efficiency in vitro[41]. HPV 18 DNA is integrated in most cancers. However, a substantial proportion of HPV 16-positive tumours and one cancer derived cell line revealed only episomal viral DNA[122,125]. This demonstrates that integration is not a necessary prerequisite for tumour progression.

A specific mechanism for upregulation of E6/E7 expression has recently been shown to operate in carcinomas containing only episomal HPV 16 DNA. The promoter of E6/E7 of the wild type HPV 16 genome is downregulated by a silencer element in the viral control region, which depends on interactions with cellular transcriptional regulator yin-yang 1 (YY1) with four binding sites[126]. Analysis of six cancers carrying exclusively extrachromosomal HPV 16 DNA revealed deletions affecting one to four YY1-binding sites[126-127]. All of these mutations resulted in a four to six fold increased activity of the E6/E7 promoter suggesting that deletion or mutation in the target sequences for the cellular repressor represents a repeatedly used strategy of HPV 16 to escape from cellular control. A deletion of 38bp from integrated HPV 16 DNA in the cervical carcinoma cell line SiHa[128] removes one YY-1 binding site indicating that inactivation of YY1 target sequences is not restricted to episomal HPV DNA in cancers.

1.2.6 Summary of evidence for the role of HPV in cervical oncogenesis

1. The incidence of HPV-16 DNA in CIN lesions increases proportionately with their severity.

2. PCR-detectable HPV-16 DNA occurs in more than 50-90% of cervical cancer biopsies.

3. HPV-16 DNA in cervical cancers is often integrated into host DNA.

4. HPV-16 DNA is retained in continuous cell lines from cervical cancers.

5. HPV-16 DNA can transform and immortalize human keratinocytes in vitro, whereas non-cancer associated HPVs do not.

6. HPV-16 E6 and E7 proteins inactivate endogenous tumour suppresor proteins p53 and pRb, respectively.

1.3 THE HUMAN IMMUNE SYSTEM

The human immune system is equipped with several different functional cell types which are involved in the identification and subsequent destruction of infectious agents. A division can be made between the specific and the non-specific immune response. The latter is represented by natural killer cells (NKs) and macrophages, which do not require specific priming for lytic functions and lack immunological memory. Macrophages can be enhanced in their lytic activity by cytokines such as g-IFN, IL-2 and M-CSF. Activated macrophages can kill target cells by production of cytotoxic products, including TNF-α, or by mediating antibody-dependent cell-mediated cytotoxicity (ADCC), using their Fc-receptor. NK cells may recognize and kill target cells which lack MHC class I molecules on their cell surface, according to the hypothesis of 'missing self' recognition[129]. In addition, NK cells can also engage via ADCC using their Fc receptor.

The two main categories of specific immune response are the humoral immune response, making use of antibodies, and the cellular immune response, mediated directly by T cells.

1.3.1 ACTIVATION OF THE IMMUNE RESPONSE

Upon invasion of a host, a pathogen may reside either in the extracellular space or within a cell's interior. Cell associated receptors can readily detect extracellular material but cannot directly recognize ligand separated from the receptor by a lipid bilayer. The detection task is further complicated by the existence of two distinct subcompartments for intracellular pathogen residence within a cell: the cytoplasm and membrane-bound endocytic organelles.

A central role is played by the 'professional' antigen presenting cell (APC) in the onset of both the cellular and humoral immune response (Fig 1.2). These cells, including the Langerhans' cells and interdigitating cells[130], retain the ability to take up antigenic proteins, degrade these in the endocytic route and present small protein fragments or peptides at their cell surface to lymphocytes. Also B cells and monocytes/macrophages can process and present antigens in a similar fashion. The peptides are presented at the cell surface by major histocompatibility complex class II (HLA class II) molecules, after which the specific HLA/peptide interaction can be recognized by the T cell receptor (TcR) of CD4+ helper cells (Th). Recognition of the antigen as non-self leads to the activation of the specific Th cell, which then proliferates and starts producing different lymphokines, that stimulate various other immune cells. Activated Th cells have occasionally been found to exert cytolytic functions as well[131].

Two subsets of CD4+ cells, designated Th1 and Th2 have been recognized based on the cytokines they express as well as functional properties[132-133]. Th1 clones produce IL-2, γ-IFN and TNF-ß, thereby providing help to cell mediated effector responses. Th2 clones secrete IL-3, IL-4, IL-5, IL-6 and IL-10, which stimulates B cells to produce antibodies[133]. The conditions that dictate which Th clone develops after antigenic stimulation are not fully understood and may be determined, at least in part by the invading virus. The cytokines produced by one Th clone can inhibit cytokine production by the other

Th clone[134]. Also transforming growth factor-ß (TGF-ß) inhibits IL-4 and -5 production by Th, while g-IFN and IL-2 production remain unaffected, suggesting that in particular, Th1 stimulation is promoted[135]. Furthermore, co-stimulatory signals from the APC may dictate which Th clone develops, since the requirements of costimulatory signals is different for Th1 and Th2 cell[136].

1.3.2 HUMORAL IMMUNE RESPONSE

Generally, B-cell responses require help from T cells. A B-cell recognizes a determinant on a native antigen via its membrane bound immunoglobulin (ig). The antigen is internalized, processed and the resulting peptides are presented on the cell surface by HLA class II. The subsequent recognition by the TcR of a CD4+ Th cell leads to activation of the B cell, either as a result of direct contact with the TcR (cognate interaction) or by lymphokine production by the activated Th cell. Activation of B cells leads to clonal expansion and differentiation into antibody producing plasma cells[137]. Antibodies recognize intact protein structures , which allows them to bind and recognize free viral particles. In addition, by binding to structures present at the cell surface of host cells, antibodies can effect complement fixation ('classical' complement fixation pathway), promoting phagocytosis and damage to plasma membranes via the membrane attack complex (MAC). Furthermore, antibodies can enhance the effector functions of the non-specific cellular response via antibody-dependent cell-mediated cytotoxicity (ADCC).

1.3.3 CELLULAR IMMUNE RESPONSE

Cellular immune response involves two types of reactions mediated by different T cell subsets: delayed type hypersensitivity (DTH), initiated by CD4+ T cells and T cell mediated cytotoxicity mediated by CD8+ T cells (cytotoxic T-lymphocytes, CTL). Both require antigen specific priming and retain immunological memory.

In DTH, sensitized Th cells are activated by antigen, presented by APC and the resulting cytokine production recruits and activates lymphocytes and macrophages capable of inflicting local tissue damage. In T cell mediated cytotoxicity, CTLs constitute the main effector targeted towards endogenous antigens such as viral proteins. Also the CTL response is dependent on cytokine production by the Th cells, mainly IL-2 produced by Th1. Since the present work concerns immune response to the human papillomavirus, the CTL-mediated cytolytic pathway is discussed in more depth below.

1.3.3.1 T cell mediated cytotoxicity

CTLs are CD8+ T cells that recognize antigenic peptides presented by HLA class I[138]. Interaction of the TcR with MHC-I/peptide leads to the activation of CTL and expression of its IL-2 receptor. Subsequent clonal expansion is mostly dependent on IL-2 production by the activated Th1 [139-140]. This results in an increased number of antigen specific CTL that express cytolytic agents, packaged in cytosolic granules. The contents of such granules include serine proteases or granzymes[141], the pore-forming macromolecular complex perforin or cytolysin[142] and the calcium-binding protein cal-reticulin[143].

When the activated granzyme expressing CTL engages with its target cell, the TcR is trigerred by the appropriate MHC-I/peptide combination. This activates protein kinase-C (PKC), resulting in phosphorylation of lymphocyte function-associated antigen-1 (LFA-1)[144]. As a consequence, the affinity of LFA-1 for its ligand intercellular adhesion molecule-1 (ICAM-1), present on the target cell is increased[145]. Furthermore, phosphorylated LFA-1 associates with the cytoskeletal protein talin[146], which stabilizes LFA-1 expression at the cell surface. The enhanced LFA-1/ICAM-1 interaction further consolidates the CTL-target cell contact.

In addition, engagement of the TcR results in a rapid reorientation of granules in the CTL towards the target cell[147]. This probably involves reorganization of the microtubuli organization centre (MTOC) and the golgi apparatus[146], which promotes the intracellular flow of vesicles towards the CTL-target cell contact site. Furthermore, the association of LFA-1 with talin stabilizes talin clusters under the CTL membrane in the proximity of the CTL-target cell contact area, which promotes fusion of secretory vesicles with the CTL membrane. The combined action of the MTOC/Golgi reorientation, and the talin/LFA-1 association results in exocytosis of the cytolytic granules into the luminal cleft between the CTL and the target cell[147]. Either through the pore-forming function of perforin[148], or by specific adherence and subsequent endocytosis of the granule[149], the cytolytic components are delivered to the target cell. A number of other cytotoxicity pathways involving CTLs have been described. These include secretion of lymphokines such as TNF-α and TNF-ß by CTL which are cytotoxic to some cells[150].

1.4 THE HLA COMPLEX

The HLA gene complex is found on the short arm of chromosome 6 in the 6p21.31 to 6p.33 region where it encompasses approximately 3,500 to 4,000 Kilobases of DNA (Fig 1.3). The HLA class I region spans approximately 1600 to 2000 Kb and contains genes encoding the classic class I antigens: HLA-A, HLA-B, and HLA-C as well as the three non-HLA-A, B, C class I genes: HLA-E, HLA-F, and HLA-G. The HLA D region contains the genes for HLA-DR, DQ, DP, DN, and DO and spans 1000 to 1200 Kb of DNA. Between the class I and class II regions lies the class III region, which contains at least 35 genes including complement factors (C2, C4 and Bf), steroid 21-hydroxylase (CYP21), heat shock protein 70, opposite strand gene (OSG), and tumor necrosis factor alpha and beta. Recently, genes encoding molecules involved in antigen processing and assembly of class I molecules as subunits of a large multifunctional protease (LMP) and as

a membrane transporter associated with antigen processing (TAP) were mapped to the class II region[151].

The HLA complex covers a relatively small segment of the chromosome corresponding to approximately 2 centimorgans. This means that genetic recombination occurs very infrequently and the complex can be considered as a single genetic unit. The genetic unit composed of HLA alleles present on the HLA-A, HLA- B, HLA-C, and HLA-D loci on each of the two homologous chromosome 6 is called an HLA *haplotype*. The two haplotypes present in each individual constitute the HLA *genotype*. The gene products of each of the class I and class II loci are co-dominantly expressed as cell surface antigens. This means that each individual expresses two HLA-A antigens, two HLA-B antigens, two HLA-C antigens, and two sets of HLA-D gene products. These HLA antigens constitute an individual's *phenotype*. An important characteristic of the HLA gene complex is the existence of *linkage disequilibrium* between the alleles of the loci. In a random mating population at Hardy-Weinberg equilibrium, the joint frequency of 2 alleles from 2 different loci will be the product of their individual gene frequencies. If the observed value of the joint frequency is significantly different from the expected frequency, the 2 alleles are said to be in linkage disequilibrium.

Most expressed HLA genes exhibit a remarkable degree of allelic polymorphism. This is the occurrence in the population of two or more genetically determined forms in such frequencies that the rarest forms could not be maintained by mutation alone. The molecular genetic basis for polymorphisms of HLA class I and class II alleles is due to differences in nucleotide sequences within the coding regions of the individual HLA genes. HLA polymorphism has several unique features: most have many alleles, no allele dominates in frequency, and alleles differ by many amino acid substitutions. Although the reasons for this extensive genetic polymorphism are currently unknown, there are two dominant

theories, namely, retention of ancestral polymorphisms and hypermutational diversification[152-153]. It is evident that most major MHC allelic types diverged prior to the origin of the species in which they are found based on sequence data from rodent and primate MHC genes[152,154-155]. The rate of amino acid altering substitutions exceeds that of silent substitutions in codons of contact amino acids in the antigen binding site of MHC class I and Class II molecules, indicating that selection operates directly on the antigen binding site[156]. The high degree of polymorphism, long persistence of alleles, low frequency of homozygotes, and high rate of replacement substitutions is probably best explained by overdominant selection.

1.4.1 CLASS I AND CLASS II HLA MOLECULES

The main biological function of HLA molecules is to bind peptide fragments of processed protein antigens and present them to T cells. Class I molecules consist of two common subunits; a polymorphic 45-kDa heavy chain glycoprotein that is non-covalently associated with a conserved 12 k-Da $ß_2$-microglobulin ($ß_2M$) light chain[157-158]. Class I molecules are expressed on virtually all nucleated cells[159]. The class I heterodimer is expressed as a transmembrane complex at the cell surface with three N-terminal heavy chain domains called α-1, α-2, and α-3, extending outward from the membrane. The heavy chain-ß2M complex bound with its antigenic peptide is anchored by a single transmembrane segment on the heavy chain that is followed by a short intracytoplasmic sequence of variable length. The membrane proximal external domain, α-3, folds in a manner similar to that of an immunoglobulin domain and has several extensive contact with the ß2M light chain. The structure of several class I molecules as determined by X-ray crystallography (Fig 1.4) reveal that the two most N-terminal domains fold as a unit to form a prominent groove on the top face of the molecule[138,160-161]. Two parallel α helices and eight antiparallel ß sheets comprise the walls and base of the groove, respectively. The groove was found to be of dimensions appropriate to accommodate short peptides (8 - 10 residues).

Class II MHC proteins consist of a 33 KDa alpha chain that is noncovalently associated with a 28 KDa beta chain[162-163]. Both chains are glycosylated transmembrane proteins, and each consists of two extracellular domains (α1 and α2 ; and ß1 and ß2), a hydrophobic domain, and a short cytoplasmic segment. Class II molecules are found on B cells, activated T cells, macrophages, monocytes, dendritic cells and endothelium, except under the influence of the cytokine gamma interferon, which induces class II expression on diverse cell types. As determined by X-ray crystallography, the N-terminal α-1 and ß-1 domains of the class II subunits fold in a manner analogous to that observed on class I (Fig 1.4) and form a groove similar in overall structure to that observed on class I, with the notable exception that unlike class I, the ends of the class II groove are open[164].

One of the most important features of HLA molecules is their ability to form stable complexes with several different peptide sequences. This enormous binding capacity arises from hydrogen bond interaction between conserved HLA residues and the peptide main chain, thus providing sequence-independent affinity for peptide ligands[161,165-166]. HLA-peptide interaction also involves polymorphic residues in the HLA molecule and specific side chains of the peptide. Some of the peptide side chains contact residues within the HLA cleft and increase the overall binding affinity and specificity of the associated peptides (anchor residues)[167-170]; others interfere with residues of the HLA cleft and reduce binding (inhibitory residues)[171-173]. These sequence-dependent interactions are due to "pockets" which stud the grooves of both HLA classes[164] and the side chains of polymorphic residues contribute to the walls and floors of these pockets. Thus, the distinct chemical and size characteristics of these pockets in different MHC molecules result in strong preferences for interacting with certain amino acids side chains. For example, a negatively charged side chain in one HLA molecule may preferentially interact with positively charged peptide residues, whereas a positively charged side chain in another HLA molecule may only bind to negatively charged peptide residues. The residues that fit optimally (anchor residues) into

these pockets occur with high frequency in specific positions in peptides associating tightly with particular HLA class I or class II molecules [169-170] and most HLA molecules require two to three anchors in a peptide for optimal binding. For any given HLA allele, the anchor positions are at fixed distances from one another and involve only a few specific amino acids. They can therefore be described by simple motifs, which is proving to be a useful way of predicting which segments of a protein may be efficiently presented by a given HLA allele. Once they have been produced and transported to the plasma membrane, peptide-MHC molecule complexes function by interacting with clonally distributed receptors of T lymphocytes.

In contrast to peptides associated with HLA class I, those associated with HLA class II are commonly presented as nested sets and are typically 10-34 residues in length[174-175]. The term 'nested' set refers to a family of peptides sharing a common core sequence with extensions/truncations at either the N- or C- terminal ends. The ability of peptides to vary considerably in length is consistent with the open ended structure of the HLA class II binding groove[164]. Most peptides bound to HLA class II have either an aliphatic or aromatic residue near the N-terminus, which presumably fits into the 'hydrophobic' pocket formed by α22, α26, α31, α54, ß85 and ß86 residues of the HLA class II molecule. This pocket seems to be capable of accommodating many different hydrophobic residues (e.g Ile, Leu, Met, Val, Phe, Tyr or Trp) [170,172]. The fact that many different hydrophobic residues are accepted in this position, combined with the occurrence of substantial variations in peptide length, has hindered the identification of other anchor positions by simple sequence alignment.

1.4.1.1 Viral Antigen Processing and Presentation: Class I Pathway

Both infectious and noninfectious forms of viral antigen can enter the endocytic pathway of a professional APC to be processed and presented, in the context of HLA class II

molecules, to CD4+ cells. However, infection with live virus is a requirement for induction of class I-restricted CD8+ T cell responses[176,177]. This difference results from the distinct intracellular location of processing activities for HLA class I and class II antigen presentation. Viral epitopes presented by class I molecules are derived from viral proteins synthesized *de novo* in an infected cell, whereas viral antigens that enter the endosomal/lysosomal compartments have been specifically routed to these compartments, usually after capture from an extracellular location.

The cytosolic proteolytic processing enzymes required for the generation of class I-presented viral peptide fragment have not been positively identified. However, proteasomes and a larger ubiquitin-dependent complex are attractive candidates for generating peptides from larger proteins because of their broad specificity, ability to cleave on the carboxyl side of hydrophobic, basic, or acidic residues, and the demonstration that proteasomes can process proteins into oligomers within an appropriate length range for class I binding without further degradation to single amino acids[178]. The finding that two proteasome subunits, LMP-2 and LMP-7 (for low molecular mass polypeptide), are encoded in the HLA class II region further implicated the involvement of proteasomes in antigenic processing[179]. Recently, it was demonstrated that the MHC encoded LMP gene products specifically alter the peptidase activity of the proteasome to favor cleavages that result in peptides possessing basic residues on their C-termini [180-181] which is necessary to anchor peptide binding in class I grooves.

The intracellular association of appropriate octamer or nonamer peptides with class I heavy chains is essential for stable assembly and transport of peptide-loaded class I complexes to the cell surface[182-183]. Peptides bind newly synthesized and translocated class I molecules in the endoplasmic reticulum (ER)[184-185]. The empty class I molecule may temporarily be stabilized in ER by complexing with p88 (also termed calnexin), a chaperone-like

molecule[186-187]. Binding of peptide results in the release of p88[187]. The genes responsible for peptide translocation over the ER membrane have been identified[188-189], and are now designated the transporter for antigen presentation (TAP) 1 and 2 genes, previously known as peptide supply factor (PSF) or Really Interesting New Gene (RING-4 and -11). They belong to the superfamily of ABC transporters, displaying properties like an ATP-binding cassette.

1.4.1.2 Viral Antigen Processing and Presentation: Class II Pathway

HLA class II molecules present peptide fragments derived from exogenous protein antigens, including structural components of virus particles or secreted viral proteins, to CD4+ cells. Exogenous viral antigens are taken up into endosomal compartments, cleaved into short peptides that associate with class II molecules targeted to this compartment, and then the peptide-MHC II complexes are routed to the cell surface for T cell recognition[137,190-191].

On translocation to the ER, HLA class II α and ß chains rapidly associate with one another together with a third, nonpolymorphic or invariant (Ii) chain[192]. The Ii chain is a type II transmembrane protein, with the amino terminus extending into the cytosol and the C terminus residing in the lumen of the ER[193]. The Ii chain has been demonstrated to prevent exogenous peptides from binding the associated HLA class II[194-195]. The α/ß-Ii trimeric complex is transported through the Golgi to the trans-Golgi reticulum (TG), where the cytoplasmic domain of the Ii chain targets the class II molecule into the endocytic compartments[196]. Subsequently, the Ii chain is degraded by proteases in the acidic environment of the endosomes, which renders the class II molecule free for peptide binding[197].

Proteolytic cleavage of the Ii chain yields large fragments termed LIP and SLIP[198-199] as well as a set of nested invariant chain fragments termed CLIP[200]. The CLIP epitope resides in the class II binding groove potentially to prevent peptide loading in early biosynthetic compartments. Release of these invariant chain fragments allows antigenic peptides to bind to class II proteins. Although the spontaneous dissociation of CLIP from class II molecules is observed at low pH[201-202], a novel MHC heterodimer DM has been identified that enzymatically catalyzes rapid CLIP dissociation[203-204]. The ability of HLA-DM to release invariant chain fragments has led to the proposal that DM functions as a peptide editor and triggers the dissociation of unstable peptides from class II proteins[205]. In this way, DM may catalyze the release of suboptimal peptides from class II proteins and directly influence epitope selection. However, alternate mechanisms within APC may also control peptide loading, including antigen trafficking, sites of processing, and the protease content of the APC.

Where in the endocytic route the class II molecule picks up peptide is not clear, and may differ per cell type and per antigen. Endocytosis of soluble antigens can be accomplished by internalization of surface Ig plus bound antigen (B cells), by absorption of soluble antibody-antigen complexes by Fc-receptors (NK cells and macrophages), or by fluid phase endocytosis. The endocytosed antigen proceeds through the early and late endosomes to lysosomes. The proteolytic enzymes involved in antigen breakdown, such as cathepsin D and E[206-207], are probably of sufficent concentration in the late endosomal/lysosomal stage[197]. Here these peptides may be protected from further breakdown into single amino acids by binding to class II, which makes the late endosomal/lysosomal compartments the most likely site where the class II molecule meets its antigenic peptide. Similar to class I, the stable cell surface expression of HLA class II is enhanced by binding of peptide.

1.4.2 HLA DNA TYPING STRATEGIES

Until recently, HLA typing was dominated by serological and cellular techniques. Within the last 10 years, more powerful DNA-based typing methods have evolved, and these have proven considerably more accurate and reproducible than conventional serological or cellular typing.

Prior to the advent of the polymerase chain reaction, the most widely used DNA-based HLA class II typing method was restriction fragment length polymorphism (RFLP) analysis[208]. RFLP entails the restriction endonuclease digestion of genomic DNA followed by electrophoretic resolution of the endonucleotic fragments which are denatured in situ and hybridized to a nylon membrane. The membrane is then probed with a homologous labeled cDNA or genomic probes which yield hybridization signals characteristic of various HLA alleles. Although RFLP is considerably more accurate than DR and DQ serotyping, it has certain disadvantages. The technique is technically demanding, takes around 7 days to complete, relies heavily upon linkage disequilibrium between DR and DQ loci for identification of certain alleles, and it does not define allelic variation at the level of the second exon of the gene.

The development of the polymerase chain reaction (PCR)[209] allowed the evolution of improved molecular HLA-typing techniques. PCR is used to generate specific amplified stretches of DNA sequences in vitro through repeated cycles of DNA denaturation, annealing of specific primer to a single strand, and nucleotide extension from primer pairs using a DNA polymerase. Currently, most HLA class II typing methods rely on the amplification of the second exon of the polymorphic DRB, DQA, DQB and DPB genes followed by a simplified analysis of allele-specific nucleotide sequences within the hypervariable regions of the exon. The techniques for analyzing polymorphisms in

amplified DNA can be divided into two basic groups: probe hybridization and direct amplicon analysis.

Probe hybridization techniques rely on amplification of a target DNA sequence which is generally immobilized onto a support membrane (known as dot blotting). The initial amplification is normally generic but may be a mosaic of amplifications which when used together amplify all possible alleles of a given locus. The polymorphisms in the immobilized DNA are subsequently detected by using specific single-stranded DNA probes in combination with highly stringent washes to remove non-specifically bound probe[210]. In addition to the use of radioactive isotopes, hybridized probes can be detected by a variety of non-radioactive methods, such as horseradish peroxidase[211] and digoxigenin labelling[212]. The technique became known as PCR-SSOP (PCR followed by sequence specific oligonucleotide probing, also discussed in chapter 2). PCR-SSOP was first applied to histocompatibility testing in HLA DQA1 by Saiki et al (1986). Subsequently, the method was applied to HLA DRB1[213-214], HLA DQB1[215] and a combination of DR, DQB1, DPA1, and DPB1[216].

The method is useful for analyzing a large number of samples at once. However, it is a time consuming and expensive method to use to define HLA types in a small number of samples. This led to the development of an alternative strategy, the reverse PCR-SSOP method[217], where a panel of SSO probes are immobilized on a single membrane by means of poly-T tails, leaving the detection end of the probe free to interact with target DNA[211]. Biotin-labeled PCR-amplified target DNA is hybridized with the membrane bound SSO probes. Following stringent washing, the specificity of hybridization is revealed using streptavidin-horse radish peroxidase as the conjugate. This converts a chromogenic substrate into a coloured precipitate[218]. Another modification of PCR-SSOP is PCR-HPA (hybridization protection assay), and is based on nucleotide hybridization utilizing

acridium ester labeled SSO in the liquid phase. As in the case of the PCR-SSO method, the design of the SSO is critical for the accuracy of this technique.

Current PCR-SSOP approaches require lengthy post-PCR steps. This has led to the development of direct amplicon analysis techniques, These methods, while not so efficient for large numbers of samples, are more suitable for rapid limited sample number throughput. These include PCR-RFLP, PCR-SSP, nested PCR-SSP, heteroduplex analysis, and other conformational assays.

The first of these methods, the PCR-RFLP depends on sequence recognition by restriction enzymes. The main advantages of this technique are that sequence variations at different positions can be recognized at once if there are several restriction sites in the region analyzed, it may be performed in less than 5 hours, and it eliminates the requirements for radioisotopes, probes or reporter molecules. However, some of the currently known alleles cannot be easily distinguished because of the unavailability of restriction enzymes recognizing their sequence variations. Furthermore, the identification of some allelic combinations in heterozygous individuals is not possible[219] or is complicated by incomplete digestion of PCR products.

Another method, allele specific amplification of an allele or group of alleles is based on the fact that PCR cannot be accomplished if the 3' end of primer has a mismatch(es) with a given allele. Newton et al[220] described the detection of a single point mutation using one generic sense primer and two antisense primers: one antisense primer was specific for the "normal" form and was refractory to PCR on "mutant" DNA, and the other antisense primer for the "mutant" was refractory to PCR on "normal" DNA. This was termed the amplification refractory mutation system (ARMS). The technique works because *Taq* polymerase lacks 3' to 5' exonucleotic proof-reading activity. For efficient ARMS

amplification without false priming, the conditions need to be highly stringent. The first comprehensive ARMS HLA typing system was described in 1992 by Olerup and Zetterquist[221] for low resolution HLA DRB1 typing, including group specific detection of DRB3 and DRB4 by ARMS using 19 PCR reactions. Olerup and Zetterquist[221] renamed the assay PCR-SSP (PCR using sequence-specific primers). Modern PCR-SSP features multiple PCR reactions where each reaction is specific for an allele, or group of alleles. The method requires a large number of primers to detect a specific allele and is therefore used more often to detect groups of alleles. For example, PCR primers, complimentary to conserved flanking sequences of second exons from a group of DRB1 loci, will generate a mixture of PCR products, depending on the DR haplotype. This is useful since it permits dot-blot, reverse dot-blot PCR-SSO and PCR-RFLP typing of the individual alleles simultaneously.

A modification of the PCR-SSP method is the nested PCR-SSP technique first described by Bein et al[222]. In this method, the region of interest is amplified in the first step and this amplicon is used instead of genomic DNA for the second sequence-specific amplifications using primers which are internal to the first set of amplification primers. The results obtained by this method are similar to one-step PCR-SSP. The advantages of the nested PCR-SSP over conventional PCR-SSP include the very small amount of DNA required for the former, and the possibility of subtyping highly polymorphic alleles.

PCR-heteroduplex formation or 'DNA crossmatching' is another direct amplicon analysis method. At the end of any PCR cycle, the individual strands may re-anneal with each other to form homoduplexes, or they may re-anneal with an unrelated DNA strand to form a heteroduplex, or they may remain as single stranded structures[223]. These different forms of PCR products have unique conformational structures which may be differentiated by their electrophoretic mobilities in a temperature or denaturing gradient gel. PCR heteroduplex

analysis has never gained popularity for identifying HLA polymorphisms due to the complexity of the gel analysis and the technically challenging conditions. However, it has been used to match individuals for HLA DR and HLA DP by "DNA crossmatching"[224].

The single-stranded conformation polymorphism (SSCP) analysis depends on the fact that single-stranded DNA molecules of differing sequences exhibit conformational changes as a result of intra-strand complementary base pairing[225]. The single-stranded products exhibit different mobilities in nondenaturing polyacrylamide gel electrophoresis that can be used to ascertain the genotype of an individual. So far SSCP has been successfully applied to HLA A, DRB1, DQB1, DQA1, DPA1 and DPB1 typing, and HLA-DR4 subtyping. However, like heteroduplex analysis, the complexity of both the technique and the interpretation has prevented the widespread application of this technique.

Finally, HLA typing by direct amplicon analysis can be accomplished by directly sequencing the PCR products (sequence-based typing, SBT). The principles of SBT are that the polymorphic regions of any given allele are amplified by flanking PCR primers. The resulting PCR products are sequenced by one of a variety of methods and are analyzed by computer to ascertain the type. Computer analysis is required because the sequenced product from a heterozygous individual will contain two superimposed sequences that need to be aligned with all previously known sequences in order to be identified and separated. SBT was initially described for HLA DRB1, DQB1 and DQA1 by Santamaria et al[226] and for HLA DPB1 by Rozemuller et al[227]. The main drawbacks of SBT are the equipment costs and the time required to fully sequence one individual. Offset against this is the tremendous advantage of having high resolution typing. However, sequencing is not infalliable and some sequenced alleles have had to be retracted due to errors, most commonly GC inversions.

1.5 IMMUNE RESPONSE TO HUMAN PAPILLOMAVIRUS

Advances in the understanding of the nature of immune responses to HPV infection has been hampered by three major technological problems. Firstly, there exists no useful animal model of the disease (with the exception of the analogous bovine papillomavirus that is associated with malignancies of the gastrointestinal tract). Secondly, until recently[228], there was no permissive system for propagating the virus in culture in vitro and thirdly, it is difficult to isolate intact genital HPVs from lesions or tumours. Nevertheless, there is now a substantial body of evidence which indicate that humans can mount immunological responses to the genital HPVs. However, the different steps of onset and the efficacy of the immune response is little understood. It is noteworthy that there is no viremia associated with viral replication in lesions and the infected cells are relatively inaccessible to the elements of the immune system that are not associated with the skin. Thus, the principal mediators of the immune reactions to papillomavirus infection are the keratinocytes, intraepithelial lymphocytes, and the dendritic Langerhans cells[229-231].

A significant higher prevalence of sera with antibodies to HPV 16-E6 and E-7 have been observed in cervical cancer patients (33% and 23% respectively) compared with healthy controls[232]. Antibodies to recombinant proteins and to synthetic peptides corresponding to HPV 16 early and late proteins have been detected in sera from patients with CIN, but the concentrations of these antibodies are generally low[233]. In the case of the rabbit papillomavirus model, carcinogenic progression seems to be accompanied by variable levels of antibody response to the viral proteins, but the antibodies have little or no ability to induce regression[234]. Overall, whilst antibodies to early proteins may represent predictive markers of disease progression[235], they are unlikely to confer any protection against subsequent HPV 16 infections. However, neutralizing antibodies to HPV 16 in cervical secretions may prevent reinfections and effective cell mediated immune responses probably explain why the majority of untreated CIN lesions do not progress to malignancy.

There are several lines of evidence indicating that cell mediated immune response is important in the control of papillomavirus infection. There is increased frequency of HPV infection in therapeutically immunosuppressed patients or in immunodeficiencies specifically involving cell mediated immunity[236-237] as well as in patients with HIV infection[238]. HPV infections are also found up to 9 times more often in renal transplant recipients compared to the general population[239] and these patients have an increased incidence of CIN lesions[240]. The presence of HPV 16 or 18 in CIN correlates with a decreased number of Langerhans cells[241] and decreased numbers of CD4+ cells are observed in CIN lesions[242]. Furthermore, patients with HPV positive CIN or cancer exhibit decreased natural killer cell activity[243]. Regression of warts shows many characteristics of a cell mediated immune response. Histological examination reveals intense mononuclear cell infiltrate in the dermis, and the majority of the infiltrating cells in regressing warts and CIN are CD4+ T cells[244]. Finally, patients with common variable immunodeficiency (characterized by failure to produce antibodies) appear not to be unduly susceptible to the development of HPV lesions[245].

Although antibodies may play a direct role in the clearance of some viruses[246], cellular mechanisms are probably the most important tools for the defense against HPV infection. Thus, the mechanisms underlying the impaired production of IgG antibodies may be important in increasing the risk for persistent HPV infection and cancer. Because a class switch from IgM to IgG antibody production in response to a certain antigen is induced by CD4+ regulatory lymphocytes, HLA class II antigens are likely to be involved in the recognition of foreign peptides by these lymphocytes[247]. CD4+ T lymphocytes are also involved in the function of class I restricted cytotoxic T cells, which are thought to be responsible for lysis of virally infected cells and malignant transformed cells.

The ability to respond to HPV antigens therefore revolves around the capacity of infected cells to effectively present viral epitopes to T cells, and the host immunogenetic background such as the HLA class I or II type is an important parameter in the overall cellular immune response.

1.6 HLA ASSOCIATIONS WITH HUMAN PAPILLOMAVIRUS AND CERVICAL CANCER

The early population studies on HLA association with cervical cancer were from the United States. In the largest of these studies[248] in which 253 patients were HLA typed, HLA A-A11 was significantly decreased in the patients. However, in another report[249], deviations in the frequencies of A1, A9 and B12 were noted. The frequency of HLA B5 was increased in the data of Tarpley et al[250] on 67 patients while the frequency of HLA B8 was increased in 33 patients studied by Twomey et al[251]. In the combined analysis of data on Caucasian patients from these early studies (391 from the United states and 64 from Germany), HLA B8 was not significantly increased. There are two early reports from South Africa on Indian[252] and Black[253] populations. There was no indication of any HLA associations in these studies. Koenig et al[254] examined the sera of 89 German patients for antibodies against Herpes Simplex type 1 and type 2 viruses. The titre for type 2 virus was significantly higher in patients positive for HLA B12. Furthermore, in their study of 120 patients, a small but non-significant association between HLA B12 and increased risk of cervical carcinoma was found.

More recently, there have been a renewed interest in HLA association with cervical cancer (Table 1.4). The results have not been consistent probably because of differences in the size and type of population examined and techniques used for the HLA and HPV typing. Wank & Thomssen[255] reported on the frequencies of the HLA class II phenotypes in a German population of 66 patients with squamous cell carcinoma of the cervix and

compared with two control groups. The first control group was a local panel of 109 individuals, and the other control group was a caucasian panel of 2,019 individuals from the Ninth International Histocompatibility Workshop. Using serological typing, the frequency of HLA DQW3 antigen in the local panel was 50.4% (Ninth workshop panel, 41.2%), whereas the frequency in the patient group was 87.8%, suggesting that a caucasian female with the HLA DQW3 antigen has a 7.1 times greater chance of developing squamous cell carcinoma compared with females without this antigen ($p = 0.0009$). In addition, there was a weaker association with HLA DR5 which is in linkage disequilibrium with HLA DQW3, but a 12.7 fold decreased relative risk with HLA DR6.

The limitations of this initial report was that serological typing methods were used, the local control panel were not very well defined, and there was no information on the HPV status of patients or controls. Two subsequent reports by the authors[256-257] using sequence-specific oligonucleotides to define DQ alleles in the same group of patients showed a preferential increase in the frequency of DQB1*0301 (40 of 57 patients; relative risk 8.71, $p = 0.0001$) and *0303 alleles (9 of 57 patients, relative risk 4.5, $p = 0.0012$) in patients with squamous cell carcinoma of the cervix. However, these findings were not correlated with the HPV status of the patients or controls. In addition, these authors also found that 11 of 22 patients with SCC from Tanzania had the HLA DQB1*0602 allele[257]. The latter study utilized data from South African donors as controls (22.7% frequency of DQB1*0602) and suggested that this antigen may be important ($p = 0.0041$). A full discussion of the limitations of studies by other groups is found in chapter 6.

1.7 SIGNIFICANCE OF HLA ASSOCIATIONS WITH DISEASE: REVERSE IMMUNOGENETICS

The discoveries of HLA associations with certain diseases represent a significant break through in the understanding of the genetics of these diseases. The primary data showing

the associations are increased frequencies of certain HLA antigens in groups of patients as compared with a sample of normal individuals, and usually none of the observed associations are absolute.

The association of HLA with some autoimmune and infectious diseases are well established. These include rheumatoid arthritis, ankylosing spondylithis, Behcet's disease, insulin dependent diabetes mellitus, malaria, schistosomiasis, tuberculosis and hepatitis B. In addition, previous studies have revealed several associations between the HLA system and malignant disease. For instance, Hodgkin's disease-associated with Epstein-Barr virus (EBV)[258], thyroid carcinomas[259], non-melanoma skin carcinomas associated with HPV[260], cutaneous melanoma[261]and nasopharyngeal carcinoma[262].

The process of identifying an HLA association with an infectious disease and then using this information to identify candidate antigens involved in immunity has been termed "reverse immunogenetics". Classical immunogenetics for infectious disease uses an approach in which antigens are identified (often by relatively unrelated criteria such as reactivity with murine monoclonal antibodies) and then immune responses to these are studied. It is then possible to analyze the MHC restriction of the response to these antigens and to map T-cell epitopes. The difficulty with this approach is that there will be immune responses to many antigens of a pathogen, and only some of these may mediate protection. Therefore, it is necessary to assess whether responsiveness to a particular antigen correlates with protection, which is often a difficult task in clinical practice.

"Reverse immunogenetics" has the advantage that its starting point is an observed resistance or susceptibility to a disease in a subset of a population bearing a particular HLA type. The mechanisms of this resistance or susceptibility can then be analyzed by the identification of antigens derived from the pathogen and recognized in the context of the

significant HLA molecules. This approach has been applied to the investigation of HLA B53 mediated resistance to severe malaria and has been used in this thesis.

1.8 APPROACHES TO DEFINING HLA CLASS II BINDING MOTIFS

The characterization of naturally processed peptides bound to HLA class II molecules associated with susceptibility and protection to HPV infection provides an approach towards understanding both antigen processing and peptide binding events in *vivo*. Crystallographic analysis of HLA class II /peptide complexes have shown that class II molecules bind peptides by forming hydrogen bonds to the peptide backbone and by the sequestration of the side chains of the peptide anchor amino acids inside the pockets of the groove of the class II molecule[165]. Different class II molecules have different pockets and bind different sets of peptides[263-264]. The positions and type of residues which anchor a peptide to a particular class II groove determine the peptide binding "motif" for that class II molecule[264].

The binding specificity of HLA class II molecules has been analyzed by a variety of methods. Direct binding to class II molecules has been measured using synthetic variants of high affinity binding peptides[172,265-266]. More recently, in vitro binding studies have been employed using libraries of random peptides encoded in the coat protein of M13 bacteriophage[170], or by studying binding of peptide libraries[173]. Another approach is the sequencing of individual peptides and pools of peptides eluted from affinity-purified class II molecules[267].

1.8.1 The Use of Large Peptide Repertoires to Identify General HLA Class II motifs

Of all class II isotypes, HLA-DR is the best characterized structurally and functionally. Thus, for class II HLA-DR molecules, motifs have been identified by the analysis of large

peptide pools selected from M13 bacteriophage peptide display libraries [170,173,268]. This technique is based on the ability of filamentous bacteriophage to display peptides on their outside surface and involves the screening and enrichment of bacteriophage-displaying peptides that bind to a particular protein. By inserting oligonucleotide-encoding peptides known to bind to HLA-DRB1*0101 into the protein-III encoding gene of bacteriophage M13, Hammer et al[268] demonstrated that the bacteriophage displaying the appropriate class II ligand can bind specifically to the DR groove. Based on this observation, a large DRB1*0101 binding peptide repertoire was selected from a M13 peptide display library consisting of millions of random peptides. Sequence analysis of the DNA encoding the DRB1*0101-selected peptides led to the identification of peptide positions in which amino acids with similar side chains occurred with increased frequency (anchor residues), thus resulting in a DRB1*0101 peptide-binding motif [268]. The motif consists of four anchors at relative positions 1, 4, 6 and 9 that are fixed at distances from one another, thus reflecting the architecture of the DRB1*0101 groove in that both the spacing and chemical characteristics of anchor residues correspond to the major pockets 1, 4, 6, and 9 of the HLA-DR cleft.

The screening of bacteriophage libraries has also been applied to other HLA-DR alleles such as DRB1*0401 and DRB1*1101[170]. The results show the presence of conserved anchor residues, i.e., anchors found in each of the HLA-DR selected peptide pools, as well as allele specific anchor residues. For example, most of the HLA DRB1*0101, DRB1*0401, and DRB1*1101 selected peptide pools were found to have aromatic and aliphatic amino acids at positions 1 and 4 respectively, whereas strong allele-specific amino acid preferences were identified at position 6: Ala and Gly for DRB1*0101, Ser and Thr for DRB1*0401, and Arg and Lys for DRB1*1101. These results provided the molecular basis for both the promiscuity and specificity of peptide recognition by HLA-DR

molecules. Further, by varying the conditions used to elute bacteriophage from the class II cleft, it is possible to identify secondary anchors at positions 2, 3, and 7[173].

General HLA class II motifs can also be identified by the characterizing large endogenous bound peptide pools. The technique was originally developed for the definition of class I motifs[169]. In this approach, endogenous class II-bound peptide pools are eluted and subsequently analyzed by Edman sequencing[267,269]. Because the class II-binding cleft is open at both ends and endogenous peptides are not aligned due to the variable length of class II ligands, pool sequencing approaches with class II-eluted peptides failed to reveal patterns as clear as those of class I ligands. However, pool sequencing combined with the alignment of natural ligands and the consideration of predicted pocket structure resulted in class II motifs similar to the ones obtained by the bacteriophage technology[270].

1.8.2 The Use of Single-Substitution Experiments on Naturally Processed Peptides to Identify Specific HLA Class II-Binding Motifs

The effects of single residue substitutions in naturally HLA-bound peptides have been studied to identify residues critical to the interaction of these peptides with HLA class II molecules. For example, in the case of HLA DRB1*0101, the importance of an aromatic residue at relative position 1 was initially found by Ala substitutions of the influenza hemagglutinin (HA) epitope 307-319[168]. More extensive truncation and single-residue substitution studies on HA 307-319 or tetanus toxoid 830-843 revealed specific class II binding motifs for DRB1*0401, DRB1*1101, and DRB1*0701[172,265,271]. Substitution experiments on myelin basic protein peptide 84-102 also revealed differential binding for DRB1*1501 and DRB5*0101[272-273].

Comparison of the results from single-substitution studies with other methods, such as M13-displayed peptide repertoires have confirmed the generality of motifs derived from

single-substitution experiments. Furthermore, only this approach is able to reveal the presence of side chains that interfere with peptide binding. These inhibitory residues are of similar importance for binding with class II molecules as the presence of anchor residues[171-173].

1.8.3 The Use of Quantitative Matrices to Identify HLA Class II motifs

Data from X-ray crystallographic studies, large peptide repertoires and single substitution experiments indicate that peptide side chain effects (anchor, inhibitory, or neutral) seem to depend on the position within a particular peptide frame rather than on neighbouring amino acids. These observations led to the approximation that each amino acid in a peptide sequence contributes to the affinity of the peptide independently of the neighbouring amino acids[173,274-275]. The determination of the effects of each amino acid at all peptide positions resulted in matrices that define quantitatively HLA class II ligand specificity. DRB1*0401 and DRB1*0101 matrices have been determined using 9- and 13-residue-long[275] designer peptides (see also chapter 5). More recently, this approach has been extended to the use of "pocket-specificity" profiles to generate quantitative matrices for many HLA class II alleles[276].

1.8 AIMS OF THIS THESIS

Despite the compelling evidence implicating HPV in cervical oncogenesis, the majority of women infected with 'high risk' HPV do not develop cervical intra-epithelial neoplasia (CIN) or cancer. It is clear that competent cell-mediated immune response is required to control HPV infection and prevent the development of CIN or cancer, and this in turn is dependent on proper HLA-mediated antigen presentation. The aims of this thesis are:

1. To examine in detail, the association between HLA-DQ and -DR alleles, the human papillomavirus and premalignant disease of the uterine cervix.

2. To identify susceptibility and protective HLA DQ-DR haplotypes in relation to human papillomavirus and premalignant disease of the cervix.

3. To identify naturally processed peptide sequences bound to susceptibility and protective HLA molecules and use this for motif prediction of HPV 16 L1, L2, E6 and E7 sequences that will bind with high affinity to these HLA molecules. This should lay the basis for future work in evaluating HLA DQ and DR restricted immune responses to HPV infection as well as peptide based vaccine approaches for the prevention and treatment of CIN and cervical cancer.

Bethesda System	Equivalent Terminology
ASCUS	Squamous atypia, Pap class II
LSIL	Mild dysplasia, CIN1, Koilocytotic atypia, condylomatous atypia, HPV related changes
HSIL	Moderate dysplasia, CIN 2, severe dysplasia, carcinoma in-situ, CIN 3

Table 1.1: The Bethesda system of classification of squamous abnormalities compared with other nomenclature

HPV Genotype	Lesion
Cutaneous 1,2,3,4,5,7,8,9,10,12,14,15,17,19,20,21, 22,23,24,25,26,27,28,29,36,37,38,41,46, 47,48,49,50,60,63,65	Cutaneous warts, flat warts, plantar warts, butcher's warts. Cutaneous plaques and papillomas in patients with Epidermo-dysplasia Verruciformis (EV). Skin carcinomas in renal allograft patients and EV patients.
Mucosal 6,11,13,16,18,30,31,32,33,34,35,39,40, 42,43,44,45,51,52,53,54,55,56,57,58,59, 61,62,64,66,67,68	Laryngeal papillomas, condylomata acuminatum, CIN; vulvar, penile and perianal intraepithelial neoplasia; cervical cancer; vulva, penile, perianal and anal cancer, verrucous carcinoma of vulva and penis, Buschke -Lowenstein tumor.

Table 1.2: HPV genotypes from cutaneous and mucosal lesions (Walboomers et al., 1994).

Reading Frame	Function
E1	DNA replication
E2	Transcription, DNA replication
E4	Cytoskeletal disintegration
E5	Transformation
E6	Transformation
E7	Transformation
L1	Viral capsid
L2	Viral capsid

Table 1.3: Function of HPV gene products

Table 1.4: Summary of Studies on HLA Associations with HPV, CIN and Cervical Cancer

Reference	Patients	Controls	Population	HLA Typing Method	HPV Detection	Main Results
Vandenvelde et al. (1993)	71 (24 CIN I, 21 CINII, 26 CIN III)	323 (CIN0, not typed for HPV)	Belgian	PCR-ASO	Done	1. DQB1*03: RR = 2.647 for HPV associated CIN
David et al. (1993)	50 (5 CIN I, 15 CIN II, 30 CIN III)	99 (CIN0, blood donors)	British	PCR-SSO	Not done	1. DQB1*03 : RR = 2.5 for CIN III
Mehal et al. (1994)	66 (27 CIN I, 15 CIN II, 24 CIN III)	60	British	PCR-RFLP	PCR	Increased risk of CIN for DQB1*03
Apple et al. (1995)	128 (55 slight /moderate dysplasia, 73 severe dysplasia / CIS)	220 (CIN0)	Hispanic	PCR-SSO	PCR	1. DRB1*0407-DQB1*0301: OR=2.22 2. DRB1*1501-DQB1*0602: OR=3.03 3. DRB1*1501: OR=4.75 4. DRB1*1102-DQA1*0501: OR=0.19 for HPV positive severe dysplasia/CIS.
Sanjeevi et al. (1996)	74 (10 CIN I, 41 CIN II-III, 23 CIN ND)	164	Swedish	PCR-SSO	Serology (HPV 6,16)	1. DQB1*0602: OR=5.67 for HPV 16 seropositive cases/controls. 2. DQB1*0303: OR=2.98 for all cases/control. 3. DQA1*0102-DQB1*0602: OR=6.00 for all cases/controls. 4.DQA1*0501-DQB1*0301: OR=3.00 for seronegative cases/controls. 5. DR15-DQA1*0102-DQB1*0602: OR=6.8

Reference	Patients	Controls	Population	HLA Typing Method	HPV Detection	Main Results
Helland et al. (1998)	92 (10 CIN II, 82 CIN III)	225 (CIN 0)	Norweigian	PCR-SSO	PCR	1. DQB1*0602: OR=3.2 for HPV positive cases. 2. DQB1*0604: OR=0.1 3. DQA1*0102-DQB1*0601: OR=3.2 for HPV positive cases.
Wank & Thomssen (1992)	66 (SCC)	109 (local panel)	German	PCR-SSO	Not done	Increased risk of SCC for DQB1*0301 & 0302
Helland et al. (1992)	213 (SCC)	181	Norwegian	PCR-SSO	Not done	1. DQW3: RR=2.0
Amar et al (1993)	30 (SCC)	400 (local panel)	Jewish	PCR-SSO	Not done	No HLA associations
Glew et al. (1993)	65 (SCC)	857 (organ donors)	British	PCR-SSO & Serology	PCR	No HLA associations
Nawa et al. (1994)	23 (SCC)	Int. workshop	Japanese	PCR-RFLP	PCR	Increased risk of SCC for DQB1*03: p=0.0003
Apple et al. (1994)	98 (SCC)	220 (CIN0)	Hispanic	PCR-SSO	PCR	1.DRB1*1501-DQB1*0602: OR=2.87 OR=4.78 for HPV 16 +ve cases 2.DRB1*0407-DQB1*0302: OR=2.19 3.DR13: OR = 0.29 (-ve) 4.DQB1*03: No association.
Gregoire et al. (1994)	66 (SCC)	214	African-American	PCR-SSO	PCR	1. DQB1*03; RR=2.3 2. DQB1*0303 ; RR=5.2 3. DQB1*0604; RR=5.2

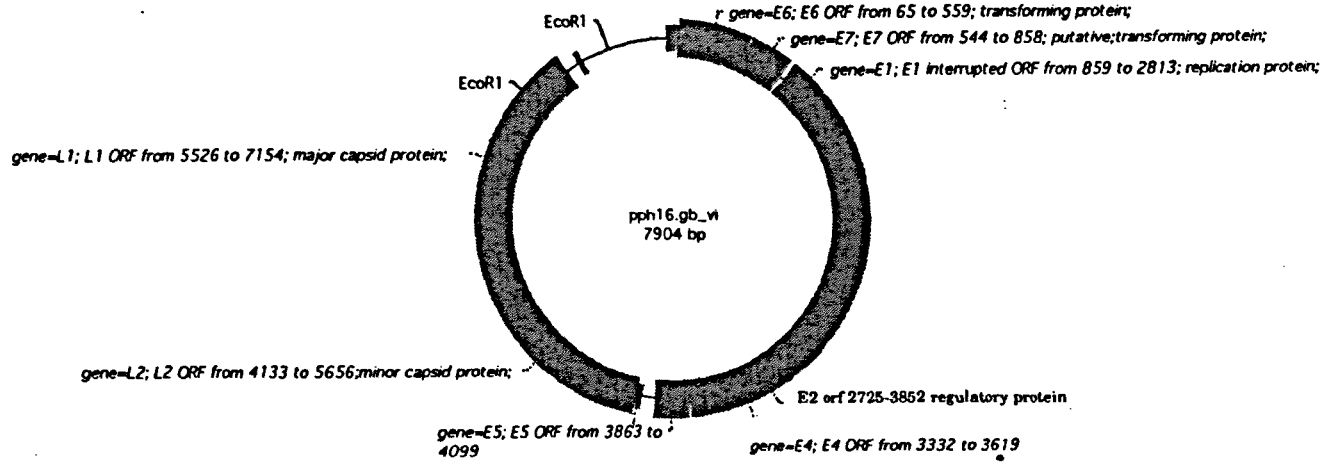

Fig 1.1: Schematic presentation of the HPV 16 genome. The numbers indicate the first and last nucleotide of the different open reading frames (ORF). E = early ORF, L = late ORF.

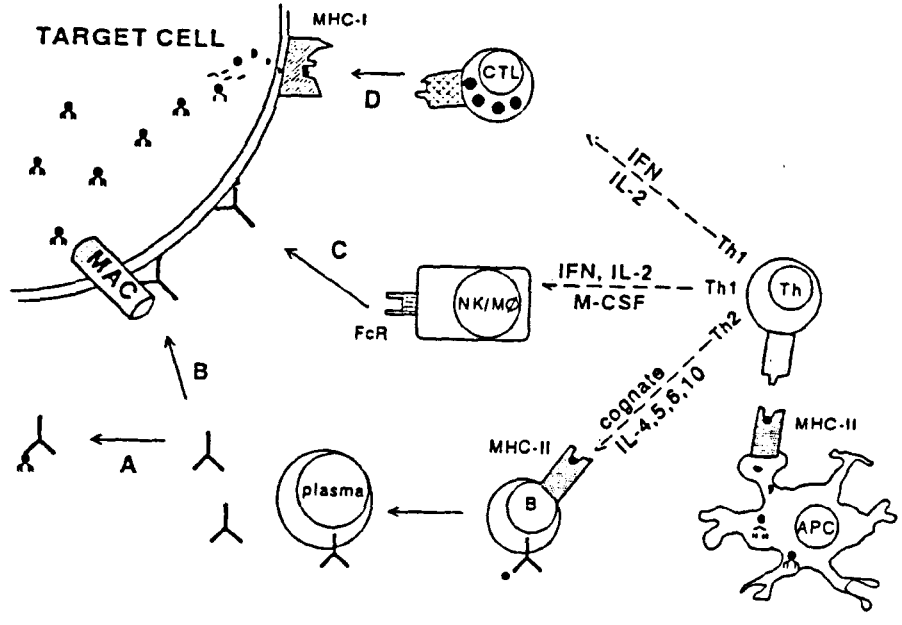

Fig 1.2: Antigen presentation by HLA class II molecules on professional APCs leads to T-helper activation. Subsequently different Th subsets stimulate different effector function: Th1 cells stimulate NK cells and macrophage activity by lymphokine production (g-IFN, IL-2 and M-CSF). In addition, Th1 stimulate clonal expansion of primed CTLs by IL-2 and g-IFN production. Th2 cells stimulate primed B-cells to proliferate and produce antibodies by the secretion of IL-4,5,6 and 10. Alternatively, the interaction between the T-cell receptor of Th2 and HLA class II on the activated B-cell leads to antibody production (cognate interaction pathway). Arrows indicated A-D represent different effector pathways: A, viral particle neutralization by antibodies; B, antibody-directed complement fixation,resulting in membrane damage by MAC; C, antibody dependent cell-mediated cytotoxicity (ADCC), in which NKs and macrophages bind via their Fc-receptor to antibodies adhered to the target cell; D, MHC-1 restricted CTL mediated killing.

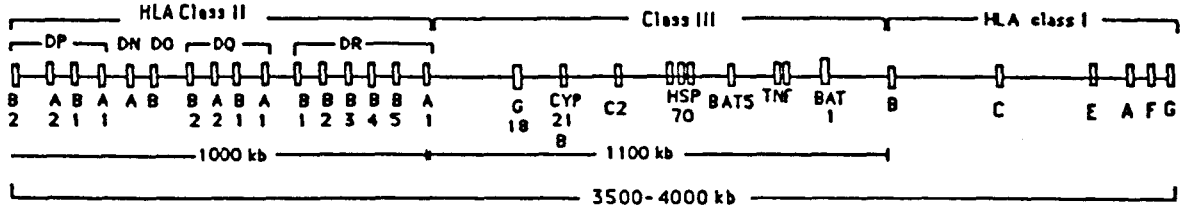

Fig 1.3: The HLA gene complex showing the 3500-4000kb of DNA with the locations and distances of Class II, Class I, complement (C2,C4, Bf, hydoxylase genes 21B and 21A), the heat shock protein genes 70 (HSP70), tumour necrosis factor, and HLA-B-associated transcripts (BATS).

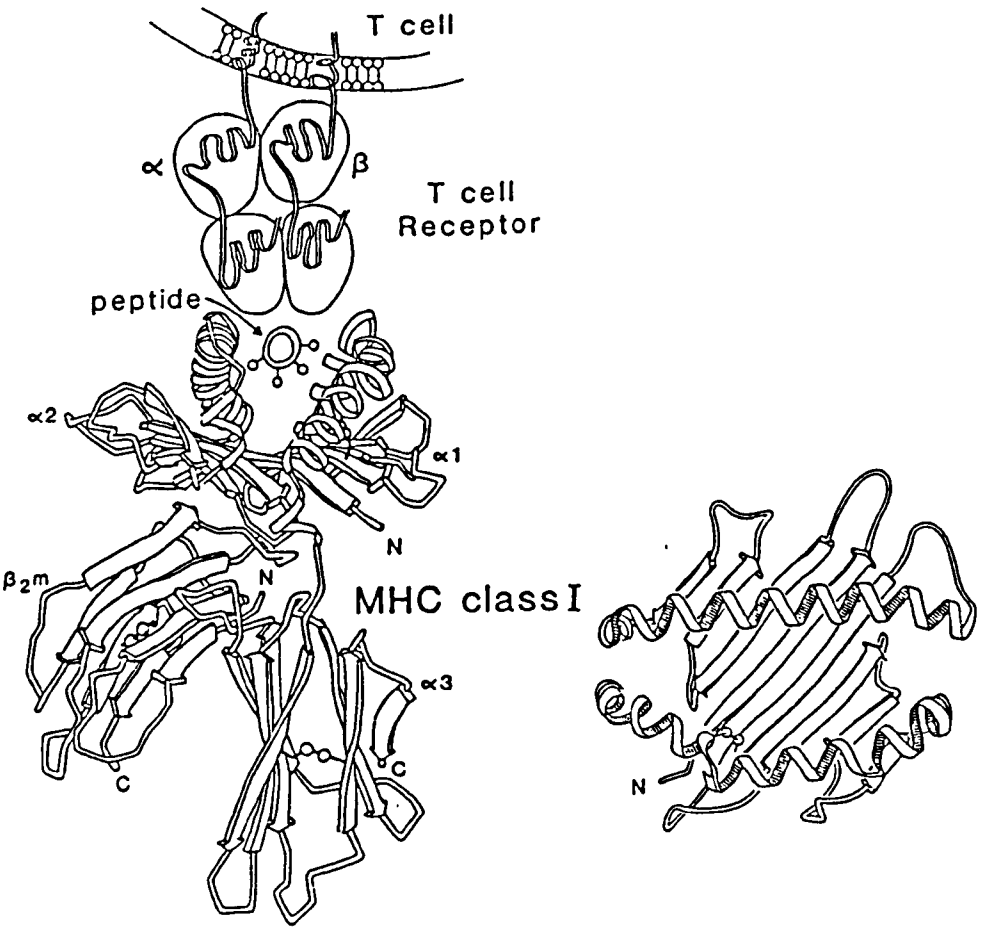

Fig 1.4: A representation of the trimolecular relationship between the MHC molecule, peptide, and T cell receptor. The class I molecule is shown. Class II molecules have a similar structure but different domain organization. On the right side, a top view of the peptide-binding site which consists of a ß-pleated sheet formed by eight anti-parallel ß strands, and the sides are formed by two alpha helical segments. Polymorphic residues in both Class I and Class II proteins are clustered in this peptide-binding region and are responsible for the different peptide specificities observed for different HLA proteins.

CHAPTER 2: METHODS AND MATERIALS

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2.15 CHEMICAL AND MATERIAL SUPPLIERS

2.1 SAMPLE COLLECTION AND GENOMIC DNA EXTRACTION

2.1.1 SAMPLE COLLECTION

Cervical smears were taken from healthy women and those with CIN attending the outpatient clinics at City Hospital, Nottingham; Whittington Hospital, London, and the Margaret Pyke Center, London. In most cases, the referral for colposcopy was based on current British guidelines, i.e. a single moderate or severely dyskaryotic smear or a persistent mild abnormality. At the time of colposcopy, another smear was taken with an Ayre spatula and sent for routine cytological examination. The same spatula was used to collect additional cells, which were then agitated in phosphate buffered saline, and stored at -20^{0}C. Any areas of abnormal epithelium found on colposcopy were biopsied (punch biopsy, loop diathermy, or laser cone as appropriate), and sent for routine histological assessment. Women with no visible colposcopic abnormality were not biopsied and were assumed to be histologically normal. Patients with colposcopic and histologic diagnosis of CIN formed the test population. Patients with normal cervical cytology who tested negative for HPV infection formed the control population. Histological classification into normal, CIN1, and CIN III were carried out according to established criteria[6,277].

2.1.2 GENOMIC DNA PREPARATION

After thawing, exfoliated cells were pelleted and washed twice in PBS. Cell pellets were digested with SDS (0.5%) and proteinase K (500µg/ml) for 6 hours or overnight at 37^{0}C. An equal volume of equilibrated phenol was added and the solution was mixed with gentle rocking for 30 min at room temperature. The aqueous layer was removed by suction using a wide-bore pipette and re-extracted with phenol two to three times until the interface was clear. The aqueous layer was extracted once with an equal volume of phenol/chloroform and once more with chloroform. DNA was precipitated from the aqueous phase by the addition of two volumes of absolute alcohol, washed once with 70% ethanol and resuspended in 10mM Tris (pH 8) and 1mM EDTA (TE) and digested with 100µg/ml of RNAse for 1 hour at 37^{0}C. After re-extraction (once with phenol, once with phenol/chloroform and once with chloroform), the DNA was precipitated, washed with 70%

ethanol and dissolved in 50µl of TE. The amount of DNA recovered from each specimen was determined by spotting 1µl of serial dilutions on a commercially available dipstick (Invitrogen).

2.2 HPV TYPING

2.2.1 POLYMERASE CHAIN REACTION WITH TYPE SPECIFIC PRIMERS

Separate PCR reaction were run for each of the HPV types 16, 18, 31, 33 using the primers shown in table 2.1. The PCR primers were chosen from the literature to be type specific. This was confirmed using cloned HPV plasmids and by the results obtained on some clinical specimens using alternative type specific primer pairs which gave entirely consistent results.

2.2.1.1 PCR AMPLIFICATION CONDITIONS

PCR amplifications were performed in either a Techne PHC-3 or Perkin Elmer Cetus machine. The reactions were performed in 50µl containing 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, 0.01% gelatin and 50pmol of each primer. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. 1.25 units of Ampli Taq polymerase (Perkin Elmer Cetus) was added at 70^0C after the initial denaturation.

2.1.1.2 PCR TEMPERATURE CONDITIONS

HPV 16: Sense and antisense primers for HPV 16 were used with the conditions described by Seedorf et al[278]. Initial denaturation was for 8 minutes followed by 35 cycles of 94^0C for 15 seconds, 54^0C for 15 seconds and 72°C for 30 seconds, with a final extension at 72^0C for 8 minutes.

HPV 18: The primers for HPV 18 were used with the conditions described by Coles and Danos[279]. Initial denaturation was for 8 minutes followed by 35 cycles of 94^0C for 15 seconds, 70^0C for 15 seconds and 72°C for 30 seconds, with a final extension at 72^0C for 8 minutes.

HPV 31: Sense and antisense primers for HPV 31 were used with the conditions described by Goldsborough et al[280]. Initial denaturation was for 8 minutes followed by 35 cycles of 94^{0}C for 15 seconds, 54^{0}C for 15 seconds and 72^{o}C for 30 seconds, with a final extension at 72^{0}C for 8 minutes.

HPV 33: Sense and antisense primers for HPV 33 were used with the conditions described by Cole and Streeck[281]. Initial denaturation was for 8 minutes followed by 35 cycles of 94^{0}C for 15 seconds, 65^{0}C for 15 seconds and 72^{o}C for 30 seconds, with a final extension at 72^{0}C for 8 minutes.

2.2.1.3 AGAROSE GEL ELECTROPHORESIS

Agarose (Sigma) gels of 2% concentration (wt/vol) were made with and run in 1X Tris-acetate-EDTA (TAE) buffer pH 8.0[282]. The PCR product were mixed with 2µl of loading dye[282] and loaded into the wells of the gel. The gels were run at room temperature at a constant voltage of 70v for approximately three hours. Variations of both voltage and run times were used for convenience and for better resolution of the DNA fragments. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm. The gels were photographed. As an aid to fragment size identification, 123 bp marker from EcoRI/Bam HI fragments of adenovirus type 2 was included.

2.3 HLA DQB1*03 TYPING: ARTIFICIAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (A-RFLP)

2.3.1 PRINCIPLES OF A-RFLP

Restriction analysis of PCR products is one of the earliest techniques used for analyzing amplified products[209]. This approach is applicable for distinguishing alleles in which the polymorphic residue results in the creation or removal of a restriction enzyme site. Unfortunately, many

polymorphisms are not associated with restriction enzyme site change and thus are not amenable to this analysis. However, by using site directed mutagenesis using primers with mismatches near the 3' ends, it is possible to create an artificial RFLP (A-RFLP) for almost all naturally occurring DNA polymorphisms[283]. Fig 2.1 illustrates the principles of this approach.

2.3.2 DESIGN OF PRIMERS FOR A-RFLP

An A-RFLP primer can be designed using a semi-automatic approach by using a computer programme which will search for restriction enzyme sites for a given sequence, e.g DNA Strider. The process is illustrated in Fig 2.2. If it is assumed that the polymorphic residue is P and restriction enzymes with recognition sites of up to 6 bases are needed. The five bases on either side of P are entered into the computer programme from -5 to +5 and the programme is used to search for a restriction enzyme site encompassing P. If a restriction enzyme site is found which is only present in one allele but not in the other one, then no further searching is required. If no restriction site polymorphism is found, then the nucleotides from -2 to -5 and +2 to +5 are changed one at a time with a computer search being carried out after each alteration. For each position, the nucleotide A, T, C and G is substituted in turn. The -1 or +1 position is avoided as this may reduce amplification efficiency and is used as the last base of the PCR primer. All possibilities are investigated as more than one solution may be possible for a given polymorphism and some restriction enzymes work better than others.

For HLA DQB1*03 primer design, all DQB1*03 alleles possess an A at the last base of codon 38 followed by CGC (Codon 39) and TTC (codon 40). Thus if the first base of codon 40 can be mutated from "T" to "G", then a *Mlu* I site (ACGCGT) will be created for the DQB1*03 alleles. The non DQB1*03 alleles, on the other hand, possess a "G" in the last base of codon 38. No *Mlu* I site will therefore be created by mutating the first base of codon 40 (Fig. 2.3). Following endonuclease restriction, the PCR product from the allele with the restriction site will have the portion containing the ARFLP primer cleaved off, thus resulting in smaller size fragment on gel

electrophoresis. The forward primer "A" is used in conjunction with the reverse mutagenesis primer "B".

A: 5' AGG GAT CCC CGC AGA GGA TTT CGT GTACC 3' (forward)

B: 5' CCG GTA CAC CCC CAC GTC GCT GTC GAC GCG 3' (reverse)

(The mutating base is underlined)

2.3.3 PCR AMPLIFICATION CONDITIONS

PCR amplifications were performed in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. The initial denaturation was at 94^0C for 8 minutes followed by 30 cycles each at 94^0C for 1 minute, 60^0C for 1 minute and 72^0C for 1 minute. There was a final extension step at 72^0C for 15 minutes. All PCR reactions were performed with both negative and positive controls.

2.3.4 RESTRICTION ANALYSIS

Following amplification 10µl of the PCR product was restricted with 50 units of *Mlu* I (Boehringer Mannheim) in a volume of 20µl at 37^0C overnight using manufacturer's buffer. The products were analyzed by electrophoresis on 4% agarose gels (Metaphor, Flowgen). The agarose gels were made with and run in 1X TAE buffer, pH 8.0. The genomic digests were mixed with 2µl of loading dye and loaded into wells of the gel. For fragment size identification, 123 bp marker from EcoRI/Bam HI fragments of adenovirus type 2 was included. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm and the gels photographed.

2.4 POLYMERASE CHAIN REACTION WITH SEQUENCE SPECIFIC PRIMERS (PCR-SSP) FOR HLA DQB1*03 SUBTYPING

2.4.1 PRINCIPLES OF PCR-SSP

PCR amplification of the HLA DQ locus with sequence specific primers is a powerful method for detecting genetic variability, including single base pair mismatches. The technique is based on the principle that a completely matched primer will be more efficiently utilized in the PCR reaction than a primer with one or several mismatches in the 3' end. The resolution of the method is high, especially in heterozygotes, as each primer pair identifies two sequence motifs located on the same chromosome, i.e. in *cis*. The post amplification processing of samples consists of determining whether amplification has occurred or not, since the discrimination between alleles takes place during the enzymatic *in vitro* DNA amplification. The PCR-SSP technique for HLA DQ typing was introduced by Olerup et al[284] with good reproducibility, and the results were 100% concordant with allelic assignment by *Taq* I DRB-DQA-DQB haplotype analysis.

2.4.2 PRIMERS FOR AMPLIFICATION OF DQB1*03 ALLELES

Eight primer pairs (Table 2.2) were used to identify the DQB1*03 alleles. The primers were defined by Olerup et al[284] based on the nucleotide sequences of the first 92 amino acids of the DQB1 alleles.

2.4.3 PCR AMPLIFICATION CONDITIONS

PCR amplifications were performed in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 1.5mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. The initial denaturation was at 94^{0}C for 8 minutes followed by 30 cycles each at 94^{0}C for 1 minute, 60^{0}C for 1 minute and 72^{0}C for 1 minute. There was a final extension step at 72^{0}C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology

(BISHI). Agarose gel electrophoresis was performed as described above. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm. The gels were photographed. 123 bp marker from EcoRI/Bam HI fragments of adenovirus type 2 was included.

2.5 POLYMERASE CHAIN REACTION - DIGOXIGENIN LABELED OLIGONUCLEOTIDE HYBRIDIZATION FOR HLA DQ-DR TYPING

2.5.1 INTRODUCTION AND PRINCIPLES

The amplification of specific DNA sequences by polymerase chain reaction followed by hybridization with sequence-specific oligonucleotide probes (SSO) has become a powerful technique for detailed analysis of genetic variations[209,285]. Each probe is constructed so as to be exactly complementary to an allele specific motif within one of the hypervariable regions of the exon. When hybridized under the appropriate conditions, these synthetic DNA probes (usually 15-20 bases in length) will anneal to their complementary target sequences in the sample DNA only if they are completely matched. The PCR product is denatured, spotted onto a charged nylon membrane, hybridized with a labeled SSO probe, washed at a stringent temperature and examined by an autoradiographic, colourimetric or chemiluminescence assay. In the case of an absolute nucleotide sequence match between the SSO probe and the membrane bound target DNA, washing at a stringent temperature fails to denature the probe-target hybrid and this is shown by a positive signal from the probe. A mismatch of one or more nucleotides results in denaturation of the probe-target hybrid and elution of the labeled probe, and consequently, no signal is generated. With an appropriate selection of oligonucleotide probes, the relevant genetic content of a DNA sample can be completely described.

The temperature and salt concentration at which the membrane is washed are influenced by the nucleotide composition and length of individual SSO probes, and therefore a variety of probe specific washing temperatures are used. The allele specificity of the target DNA may thus be determined using a series of SSO probes. This requires the preparation of replicate membranes, one for each probe to be tested. Alternatively, a single or small number of membranes may be used, necessitating the removal of each probe after signal development, before reprobing with another SSO.

Traditionally, the PCR-SSO technique has relied on 5'- end labeling (usually ^{32}P-labeled) of the SSO. Radioactive labeling is associated with several disadvantages and a number of non-radioactive alternatives have become available. In these systems (see section 1.4.1), probe target hybridization is revealed by the use of reporter molecules such as streptavidin-enzyme or specific antibody-enzyme conjugates, in colourimetric or chemiluminescence assays. The complexity of the dot-blot PCR-SSO typing system is proportional to the number of SSO probes required to discriminate between each allele at a given locus.

The 11th Histocompatibility Protocol for PCR-SSO[216] with some modifications have been used in this work. A complete listing of probes and reagents are at the end of this chapter.

2.5.2 DIGOXIGENIN LABELING OF SSO PROBES

Digoxigenin is a steroid hapten (Fig 2.4). DNA probes may be labeled with DIG-11-dUTP via random primed labeling, nick translation, cDNA synthesis or Taq DNA polymerase. Oligonucleotide probes can be 3'-end labeled with DIG-11-ddUTP, tailed with DIG-11-dUTP by terminal transferase. In this study, probes were labeled at the 5' end with Digoxigenin-NHS Ester (Digoxigenin-3-O-methylcarbonyl-E aminocaproic acid-N-hydroxysuccinimide Ester).

The synthesis and labeling of these probes was done by Dr. Ian Goldsmith at the Clare Hall Laboratories of the Imperial Cancer Research Fund. The oligonucleotide was synthesized and deprotected according to standard protocol by treatment with 25% aqueous ammonia which was subsequently removed by lyophilization. Ethanol precipitation was performed by dissolving the oligomer in a mixture of 300µl of distilled water and 30µl of sodium acetate buffer, 3mol/l; pH 8.5, and transferred to a microfuge tube. 9ml of ice cold ethanol was added, mixed and kept at -20^{0}C for 2 hours. The solution was centrifuged for 15 minutes at 10,000g and the supernatant decanted. The pellet was washed with 100µl of ice-cold ethanol, centrifuged for 5 min and the supernatant was removed. The pellet was dissolved in 200µl of sodium borate buffer, 0.1mol/l; pH 8.5. 1mg of Digoxigenin-NHS Ester was dissolved in 600µl of ethanol, and 200µl of this solution was added to the solution of oligonucleotide and kept overnight at ambient temperature in a shaker. Separation of labeled oligonucleotide from the unlabeled compound was achieved by using reversed phase HPLC.

2.5.3 HLA DQB GENERIC AMPLIFICATION

Generic HLA DQB1 amplification was performed using primers:

DQBAMP-A 5'CATGTGCTACTTCACCAACGG-3' and

DQBAMP-B 5'CTGGTAGTTGTGTCTGCACAC-3'

PCR amplifications were performed in 96 well microtitre plates in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 2.0mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. Each final reaction mixture was overlaid with several drops (about 30µl) of mineral oil. The initial denaturation was at 95^{0}C for 5 minutes followed by 35 cycles each at 95^{0}C for 45 seconds, 60^{0}C for 1 minute and 72^{0}C for 1 minute. There was a final extension step at 72^{0}C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology (BISHI).

2.5.4 HLA DRB GENERIC AMPLIFICATION

Generic HLA DRB amplification was performed using primers:

DRBAMP-A 5'CCCCACAGCACGTTTCTTG-3' and

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

PCR amplifications were performed in 96 well microtitre plates in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 2.0mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. Each final reaction mixture was overlaid with several drops (about 30µl) of mineral oil. The initial denaturation was at 95^0C for 5 minutes followed by 35 cycles each at 95^0C for 45 seconds, 60^0C for 1 minute and 72^0C for 1 minute. There was a final extension step at 72^0C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology (BISHI).

After completion of thermal cycles, an aliquot (3µl) of each reaction sample was subject to agarose gel electrophoresis. Once the DNA had run for sufficient size fractionation, the gel was removed and placed in 0.5µg/ml of ethidium bromide (Sigma). After 10 minutes, the DNA fragments were visualized on a UV light transilluminator, wavelength 254nm. The gels were photographed. 123 bp marker from EcoRI/Bam HI fragments of adenovirus type 2 was included. Generic DQB1 amplification generated a 214bp fragment while DRB1 generated a 274bp fragment.

2.5.5 PREPARATION OF DOT BLOTS

Hybond-N positively charged nylon membranes (Amersham International plc, Aylesbury, Bucks, UK) were used. The membranes were cut to appropriate size allowing $1cm^2$ per dot, corresponding to the size of a 96 well microtitre plate. The membranes were not prewetted.

PCR products were heated to 95 degrees for 10 minutes and placed on ice. Spotting was performed using the Biomek 1000 Laboratory Automation Workstation. The equipment was programmed to perform multi-tip pipetting of 2µl PCR products from microtitre plates and dotted on the nylon membranes. Using its 8 channel pipetting tool, the Biomek spots samples on to the membrane held on a purpose made vacuum blotter. Spots are placed in an 8x12 array for compatibility with a standard 96-well microtitre plate. The membranes were allowed to air dry for at least 10 minutes before being placed in a UV cross linker. DNA cross linking was performed using the auto power setting. This provides 254nm UV lamp of $0.12J/cm^2$. The membranes are stored at 4^0C until required.

2.5.6 PREHYBRIDIZATION/HYBRIDIZATION AND TMACl WASHES

The baked membranes were placed in 50ml Falcon tube with no overlap. The membrane was blocked in 5ml blocking solution (Boehringer Mannheim) at room temperature on rotisserie for at least 30 mins. The blocking solution was poured off and to the tube was added 5ml prehybridization solution {4X SSPE, 0.1% laurylsarcosine, 1% blocking reagent}, 50µl (10mg/ml) sonicated/boiled salmon sperm DNA, which has been preheated to appropriate temperature (52^0C for DQB and 54^0C for DRB). Prehybridization was performed for 1hour. The solution was poured off and 2pM SSO (listed below) per ml of hybridization solution was added to the tube and incubated at appropriate temperature for 1 hour 30 minutes (52^0C for DQB and 54^0C for DRB).

The hybridized membranes were removed from the tubes and washed twice in 1L 2X SSPE/0.1% SDS for 10 minutes at room temperature, in trays on an orbital shaker. The membranes were then washed twice in 50mM Tris (pH 8), 0.1% SDS, 2mM EDTA (pH8), 3M TMACl (Tetramethylammonium chloride, Sigma) solution at 58^0C. This allows A-T rich probe to remain annealed at 10^0C higher than the predicted temperature of dissociation. Also, as a means of standardizing posthybridization washing temperatures, TMACl was used in the washing solutions.

It allows a common washing temperature for each probe used, provided that they contain the same number of nucleotides. The membranes were gently blotted and stored moist in polythene at 5^0C. Once washed, the membranes were stored for up to 24 hours before the detection procedure.

2.5.7 CHEMILUMINESCENT DETECTION OF DIGOXIGENIN LABELED PROBES WITH CSPD

Disodium3-(4-methoxyspiro{1,2-dioxetane3,2'(5'chloro)tricyclo [3.3.1.13,7]decan}-4-yl) phenyl phosphate (CSPD, Boerhinger Mannheim), is a chemiluminescent substrate for alkaline phosphatase that enables sensitive and fast detection of biomolecules by producing visible light which is recorded on film. Enzymatic dephosphorylation of CSPD by alkaline phosphatase leads to the metastable phenolate anion which decomposes and emits light at a wavelength of 477nm.

All steps were carried out at room temperature. The membranes were washed in 1L of buffer 1 in a tray on an orbital shaker for at least 5 minutes. The membranes were blotted dry and placed in clean plastic tubes dot side up. 5 ml of buffer 2 was added to each tube and placed on rotisserie for at least 30 minutes. 1µl of Anti-digoxigenin-Alkaline Phosphatase, Fab fragments (Boehringer Mannheim) was added to the solution (1:10,000 dilution). The tubes were placed on rotisserie for 40 minutes . The membranes were then washed thrice in buffer 1 to remove any excess Anti-DIG fragments. The membranes were blotted dry, placed in plastic tubes and equilibrated in buffer 3 for 5 minutes. For membranes that required to be reprobed, buffer 3 was used without magnesium.

CSPPD was prepared by diluting the 10mg/ml solution in buffer 3, 1:100 and placed in a container with a large surface area to volume ratio. The membranes were placed face down in solution, for 5 minutes, ensuring there were no air bubbles at the interface. The membranes were removed and gently blotted dry. For the briefest exposure to X-ray film, the alkaline phosphatase chemiluminescent reaction must be at a steady state. This was brought about by a 15 minute

incubation at +37^{0}C. DRB probes were exposed at 45 minutes and DQB at 1 hour 30 minutes. Because not all of the SSO may be labeled to the same extent, long exposure (12 hours) was also performed.

2.5.8 STRIPPING OF MEMBRANES

Membranes were stripped to enable reprobing. The membranes were incubated twice for 10 minutes in 0.2N NaOH, 0.1% SDS solution at 37^{0}C. This incubation removed the DIG-labeled probe. The membranes were then rinsed thoroughly in 2X SSPE for 15minutes. They were either stored moist at 4^{0}C or reprobing was commenced with the prehybridization step of the desired hybridization procedure.

2.5.9 HLA DRB GROUP SPECIFIC AMPLIFICATION

From the hybridization patterns in response to the SSO, individuals were assigned as belonging to one or more of the following groups.

A. DR1 group

B. DR2 group

C. DR4 group

D. DR52 associated group (DR3, DR5, DR6, DR8).

E. DR 52 group

The DRB1 genes that can be typed directly by the generic amplification procedure are DRB1*07 (corresponding to DRB1*0701 or DRB1*0702), DRB1*0901, and DRB1*1001. The DRB3*0101 and DRB4*0101 can also be assigned for the DRB3 and DRB4 genes respectively. Further subtyping utilizes group specific amplification followed by SSO hybridization.

For group specific amplification, samples from the different groups were amplified as follows.

A. DR1 group with DR1-DRB1 specific primer pair

DRBAMP-1 5'TTCTTGTGGCAGCTTAAGTT-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

B. DR2 group with DR2-DRB1 specific primer pair

DRBAMP-2 5'TTCCTGTGGCAGCCTAAGAGG-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

C. DR4 group with DR4-DRB1 specific primer pair

DRBAMP-4 5'GTTTCTTGGAGCAGGTTAAAC-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

D. DR52-associated group with DR52-associated group-DRB1 specific primer

DRBAMP-3 5' CACGTTTCTTGGAGTACTCTAC-3'

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

E. DR52 group with DR52-DRB3 specific primer

DRBAMP-52 5' CCCAGCACGTTTCTTGGAGCT

DRBAMP-B 5'CCGCTGCACTGTGAAGCTCT-3'

PCR amplifications were performed in 96 well microtitre plates in 50µl volume containing 10pmol of each primer, 100ng of specimen DNA, 10mM Tris-HCl pH 8.3, 50mM potassium chloride, 2.0mM magnesium chloride, and 1U of Taq DNA polymerase. The nucleotides (dATP, dCTP, dGTP, dTTP) were each at a final concentration of 100µM. Each final reaction mixture was overlaid with several drops (about 30µl) of mineral oil. The initial denaturation was at 95^0C for 5 minutes followed by 35 cycles each at 95^0C for 45 seconds, 60^0C for 1 minute and 72^0C for 1 minute. There was a final extension step at 72^0C for 15 minutes. All PCR reactions were performed with both negative and positive controls obtained from the British Society for Histocompatibility and Immunology (BISHI).

Each reaction sample was subject to agarose gel electrophoresis. The product sizes were 261, 261, 263, 266 and 271 for DR1-DRB1, DR2-DRB1, DR4-DRB1, DR52 associated-DRB1, and DR52-

DRB3 respectively. Oligonucleotide hybridization of the amplified products were performed as described above using the SSO probes in section 2.5.12.

2.5.10 SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DQB TYPING

DQB2301	GAC CGA GCT CGT GCG GGG
DQB2302N	AAC GGG ACC GAG CGC GTG
DQB2601	CGG GGT GTG ACC AGA CAC
DQB2602	CGT TAT GTG ACC AGA TAC
DQB2603	CGT CTT GTG ACC AGA TAC
DQB2604	CGT CTT GTA ACC AGA CAC
DQB2605	CGT CTT GTG AGC AGA AGC
DQB2606	CGT CTT GTA ACC AGA TAC
DQB3701	AGG AGT ACG TGC GCT TCG
DQB3702N	AGG AGG ACG TGC GCT TCG
DQB3703	TAA CCG AGA AGA GTA CGT
DQB4501N	GAC GTG GAG GTG TAC CGG
DQB4901	GGT GTA CCG GGC AGT GAC
DQB4902N	GGT GTA TCG GGC GGT GAC
DQB5701	GCG GCC TGT TGC CGA GTA
DQB5702	GCG GCC TAG CGC CGA GTA
DQB5703	GGC GGC CTG ACG CCG AGT
DQB5704	GCG GCC TGA TGC CGA GTA
DQB5705	GGC TGC CTG CCG CCG AGT
DQB5706	GGC CGC CTG ACG CCG AGT
DQB5707	GGC CGC CTG CCG CCG AGT
DQB5708	GCG GCT TGA CGC CGA GTA
DQB7001	GAC CCG AGC GGA GTT GGA

DQB7003 GAG GGG ACC CGG GCG GAG

DQB7005 GAA ACG GGC GGC GGT GGA

2.5.10.1 HLA DQB PROBE SPECIFICITY

Probe Name	HLADQ Specificity
1. DQB2301	0401
2. DQB2302	03031,0402
3. DQB2601	0501, 0502, 05031, 05032
4. DQB2604	0603, 0604
5. DQB2606	0605
6. DQB3702N	0601
7. DQB4901	0501
8. DQB5701	0501, 0604, 0605
9. DQB15702	0502, 0504
10. DQB5703	05031, 0601
11. DQB5704	05032, 0602, 0603
12. DQB5705	0201

2.5.11 SEQUENCE SPECIFIC OLIGONUCLEOTIDES FOR HLA DRB

DRB1005 AGA AAT AAC ACT ACA CCG

DRB1006 TGG CAG GGT AAG TAT AAG

DRB1007 GAA GCA GGA TAA GTT TGA

DRB1008 GAG GAG GTT AAG TTT GAG

DRB2802 GGT TAC TGG AGA GAC ACT

DRB2807 GCG GTA CCT GGA CAG ATA

DRB 2810 GCG AGT GTG GAA CCT GAT

DRB3701	CCA AGA GGA GTC CGT GCG
DRB3707	AAC CAA GAG GAG AAC GTG
DRB3712	CAG GAG GAG TTC GTG CGC
DRB3713	GCG CAC GTA CTC CTC TTG
DRB5701	GCC TGA TGC CGA GTA CTG
DRB5702	GCC TAG CGC CGA GTA CTG
DRB5703	GCC TGA TGA GGA GTA CTG
DRB5704	GCC TGC TGC GGA GCA CTG
DRB5705	GCC TGT CGC CGA GTC CTG
DRB5708	GCC TGA TGC TGA GTA CTG
DRB7001	TCC TGG AGC AGA GGC GGG
DRB7002	GAC TTC CTG GAA GAC AGG
DRB7003	GAC CTC CTG GAA GAC AGG
DRB7004	GGC CGG GTG GAC AAC TAC
DRB7005	ACC GCG GCC CGC TTC TGC
DRB7006	GCA GAG GCG GGC CGA GGT
DRB7007	ACA TCC TGG AAG ACG AGC
DRB7008	ACT TCC TGG AAG ACG AGC
DRB7009	AGC GGA GGC GGG CCG AGG
DRB7011	GAC ATC CTG GAG CAG GCG
DRB8601	AAC TAC GGG GTT GGT GAG
DRB8602	AAC TAC GGG GCT GTG GAG
DRB8603	AAC TAC GGG GTT GTG GAG

2.5.11.1 HLA DRB PROBE SPECIFICITY

A. Generic

Probe name	HLA DR specificity
1. DRB1001	DR1
2. DRB1009	DRB5

3. DRB1004	DR4
4. DRB1010N	DRB3*0101
5. DRB1006	DR7
6. DRB1007	DR9
7. DRB1008	DR10
8. DRB2802	DR12
9. DRB3709	DRB5*0101
10. DRB5703	DR11
11. DRB2810	DR53
12. DRB1003	DR52 Associated group (DR3, DR11, DR13,DR14)
13. DRB1005	DR52 Associated group (DR12, DR8, DR14)
14. DRB 1011	DRB3*0201+ DRB3*0202
15. DRB1002	DRB3*0301

B. GROUP SPECIFIC

(i) HLA DRB1*04 PROBES

	Probe 3701	Probe 5701	Probe 5702	Probe 7001	Probe 7005	Probe 7006	Probe 7007	Probe 8601
DRB1*0401	-	+	-	-	+	-	-	+
DRB1*0402	-	+	-	-	-	-	+	-
DRB1*0403	-	+	-	+	-	+	-	-
DRB1*0404	-	+	-	+	-	-	-	-
DRB1*0405	-	-	+	+	-	-	-	+
DRB1*0406	+	+	-	+	-	+	-	-
DRB1*0407	-	+	-	+	-	+	-	+
DRB1*0408	-	+	-	+	-	-	-	+
DRB1*0409	-	-	+	-	+	-	-	+
DRB1*0410	-	-	+	+	-	-	-	-
DRB1*0411	-	-	+	+	-	+	-	-

(ii) HLA DR2/DRB5 PROBES

	Probe 2813	Probe 3707	Probe 7002	Probe 7003	Probe 8601	Probe 8603
DRB1*1501	+	-	-	-	-	+
DRB1*1502	+	-	-	-	+	-
DRB1*1601	+	-	+	-	+	-
DRB1*1602	+	-	-	+	+	-
DRB1*1503	-	-	-	-	-	+
DRB5*0101	-	-	-	-	+	-
DRB5*0102	-	+	-	-	+	-
DRB5*0201	-	+	-	-	-	-
DRB5*0202	-	+	-	-	-	-

(iii) HLA DR1 PROBES

	Probe 7001	Probe 7007	Probe 8602
DRB1*0101	+	-	-
DRB1*0102	+	-	+
DRB1*0103	-	+	-

(iv) DR52-ASSOCIATED DRB1 PROBES

	Probe 1003	1005	1013	2802	2807	2809	2813	3707	3712	3713	5701	5702	5703	5704	5705	5708	7001	7002	7003	7004	7007	7008	7009	7010	8601	8602	8603
DRB1*0301	+	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
DRB1*0302	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-
DRB1*1101	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-
DRB1*1102	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	c	-	-	+
DRB1*1103	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+
DRB1*1104	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+
DRB1*1201	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
DRB1*1202	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-
DRB1*1301	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	c	-	-	+
DRB1*1302	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	c	+	-	-
DRB1*1303	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	c	+	-	-
DRB1*1304	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	-	c	-	-	+
DRB1*1305	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
DRB1*1401	+	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+
DRB1*1402	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
DRB1*1403	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
DRB1*1404	-	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+
DRB1*1405	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	+
DRB1*1406	+	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
DRB1*1407	+	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-
DRB1*1408	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
DRB1*0801	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
DRB1*0802	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
DRB1*0803	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
DRB1*08031	-	+	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
DRB1*08042	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+

c = Cross hybridized.

2.6 HAPLOTYPIC ASSIGNMENT

HLA DRB1, DRB3, DRB4, DRB5 and DQB1 haplotypes were inferred based on known patterns of linkage disequilibrium in Caucasians for these loci[286-287]. If a sample contained DRB1*0401, DRB1*0101 at the DRB1 locus and DQB1*0301, DQB1*0501 at the DQB1 locus, then the correct inferred haplotypes would be DRB1*0401-DQB1*0301 and DRB1*0101-DQB1*0501 which is known to occur naturally. For supertypic antigens HLA DRB3, DRB4 and DRB5, the 3-locus haplotypes were inferred[287].

2.7 STATISTICAL ANALYSIS

Odds ratios and their approximate 95% confidence intervals were calculated for all variables by the χ^2 test for 2 x 2 tables without a continuity correction[288]. For small samples, exact 'p' values were calculated. For 2 x k tables, the χ^2 test for trend was calculated[289]. The unit of sampling was the allele in all analysis except when studying the effect of homozygosity versus heterozygosity. For other analysis, each allele or haplotype was taken as an independent observation so that the sample size was twice as large for these comparisons. No formal adjustments of 'p' values for multiple comparisons were made.

2.8 CELL LINES AND CULTURE CONDITIONS

In the present study, the haplotype HLADRB1*0401-DQB1*0301 was shown to correlate with susceptibility to HPV and CIN while DRB1*0101-DQB1*0501 indicated protection (Details of results in chapters 3 and 4). Although the binding motifs of several HLA-DR molecules have been defined, studies on the binding motifs of HLA-DQ molecules are few[269,290-297].Therefore, the peptide pools eluted from HLA {DQA1*0301/DQB1*0301} and {DQA1*0101/DQB1*0501} were sequenced. Amino acid preferences based on peptide sequence alignment with HPV 16 and polymorphic residue substitutions in the binding cleft of HLA DQ are discussed in chapters 5 and 6.

2.8.1 JHF CELL LINE

The JHF cell line, Xth International Histocompatibility Workshop No. 9030, is a B lymphoblastoid cell line obtained from the ECACC. The cell line is homozygous for the following HLA alleles:

HLA-A*31011; HLA-C*15; HLA-DRB1*0407; HLA-DRB4*0101; HLA-DQA1*0301; **HLA-DQB1*0301**; HLA-DPA1*01; HLA-DPB1*0301.

2.8.2 JESTHOM CELL LINE

The Jesthom cell line, Xth International Histocompatibility Workshop No. 9004, is a B lymphoblastoid cell line from the ECACC. The cell line is homozygous for the following HLA alleles:

HLA-C*01; HLA-DRA*0101; HLA-DRB1*0101; HLA-DRB6*0101; HLA-DQA1*0101; **HLA-DQB1*0501**; HLA-DPA1*01; HLA-DPB1*0401.

2.8.3 CELL CULTURE CONDITIONS

The cells were grown in RPMI 1640 supplemented with 5% fetal calf serum, 5% CO2, 2% bicarbonate, 2mM glutamine, 50U/ml penicillin G, and 50μg/ml streptomycin in roller bottles at 37^0C. Cultures were split every 3-7 days to two- to fivefold volume, depending on the expansion rate. When the required number of cells was reached (10^{11}), they were spun down at 1,000 r.p.m. The supernatant was removed and the pellet washed with PBSA. The wash was repeated twice and the final pellet was frozen at -80 degrees until used.

2.9 IMMUNOAFFINITY PURIFICATION

Immunoaffinity purification is a powerful technique for the isolation of proteins. Under proper conditions, purifications of 1,000 to 10,000-fold can be achieved in a single step. The factors that affect the success of the technique include the starting purity of the antigen,

the affinity of the antibody for the antigen, and the ease with which the antigen-antibody bond can be broken. The affinity of the antibody for the antigen determines the total amount of antigen that can be removed. For example, antibodies of high affinity ($>10^8$ / mol), quantitative removal can be achieved in less than 1 hour. Even at high antibody concentrations, low-affinity antibodies (10^6 /mol) will not bind all of the antigen in solution. The ideal antibody for immunoaffinity purification is one that has a high affinity for the antigen and whose binding can be reversed by a simple but gentle change in pH.

Immunoaffinity purification was performed in three steps: preparation of the antibody column, the binding of antigen to the antibody-bead matrix, and the elution of the antigen from the column.

2.9.1 ANTIBODY FOR IMMUNOAFFINITY PURIFICATION

The anti-HLA-DQ IA3 (Winchester et al.) is a pan HLA-DQ monoclonal antibody and was kindly provided by Dr Robert Winchester (Columbia University, New York, NY).

2.9.2 TECHNIQUE OF AFFINITY CHROMATOGRAPHY

Crude membrane fractions of the cell lines were prepared by hypotonic lysis and differential centrifugation. After washing in ice-cold phosphate buffered saline (PBS) , 10g of cell pellet were lysed in PBS with 3% nonidet-P40 (NP40), 1μg/ml leupeptin, 1μg/ml pepstatin and 5mM ethylenediaminetetraacetic acid (EDTA). Cell lysates were cleared for nuclei and debris by centrifugation at 100,000 x g for 90 minutes at 4^0C.

Immunoaffinity chromatography columns of anti-DQ IA3-Cyanogen Bromide-activated sepharose (Pharmacia) were prepared[293]. The mAb IA-3 was mixed with the CNBr-activated sepharose beads and incubated at room temperature with gentle rocking overnight. The beads were washed twice with 0.5M sodium phosphate (pH 7.5) and once with 1M

Nacl, 0.05M sodium phosphate (pH 7.5). 10 volumes of 100mM ethanolamine (pH 7.5) was added and incubated overnight with gentle mixing. The beads were further washed twice with PBS, 0.01% merthiolate was added and they were stored at 4^0C until used.

The detergent soluble membrane fractions from the cell lines were passed over a pre-column of Sepharose CL 4B (pharmacia) followed by passage over the affinity column with cyanogen bromide-activated Sepharose beads linked to the anti-DQ IA-3. After the lysates have been passed over the columns, the columns were washed extensively and then eluted with 0.05M diethlamine (pH = 11.5). The DQ molecules were immediately neutralized with 1M Tris (pH 6.8) and concentrated by ultrafiltration (Centripep; Amicon, Beverley, MA). 50µl aliquots of eluates were analyzed by 12% SDS-PAGE and silver staining to confirm protein purity (Fig 5.1).

2.10 PEPTIDE ELUTION

The HLA DQ eluates obtained after immunoaffinity purification were concentrated on a CENTRICON-10 Microconcentrator (Amicon, Beverly, MA). The centricon tube was washed in 0.1% tri-fluoroacetic acid (TFA) for 1 hour. The tube was filled with 1 ml of TFA, the eluate was added and centrifuged at 5000 x g for 1 hour to obtain an ultrafiltrate. 1ml of TFA was added and centrifugation performed for 1 hour to obtain another ultrafiltrate stored in a different tube. This step was repeated to obtain more ultrafiltrate. The ultrafiltrates containing HLA-DQ bound peptides were stored at -70^0C until characterization.

2.11 SEPARATION OF PEPTIDES: REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (r-HPLC)

The reversed-phase (RP) HPLC separation of any peptide or protein mixture is dependent upon the strength of the hydrophobic interactions of each component in the mixture with

the hydrophobic surface of the column matrix and the elution strength of the organic solvent in the mobile phase. When peptides or protein mixtures are applied to a RP column, the adsorbed peptides or proteins are eluted in order of least to most strongly bound molecules by increasing the organic solvent concentration in the elution buffer, collected as individual chromatographic fractions, and analyzed separately.

Organic solvent (acetonitrile) was removed from the RP column with degassed, HPLC-grade water using a gradient from 100% organic solvent to 100% water over 15 minutes at 1ml/min. The RP column was then equilibrated by pumping 100% trifluoroacetic acid (TFA)/acetonitrile buffer at 1ml/min. This was gradually switched to 100% TFA buffer with a 10 to 15 min linear gradient and equilibrated at 100% TFA buffer for an additional 20minutes. Equilibration was achieved when the pressure and detector absorbance were constant. A blank run without any injection for the equilibrated column was made. This was by pumping at 1ml/min, a linear gradient from 0 to 100%TFA/acetonitrile buffer over 45 min at isocratic conditions, at 100% TFA/acetonitrile buffer for 5 min, returned to 100% TFA buffer for 15 min (total run time from gradient start to completion of requilibration was 80min). Detection settings was 0.1 absorption units full scale (AUFS) at ~210 to 220nm for 50 to 200pmol peptide.

The DQB1*0301 and DQB1*0501 eluted peptides and the RP peptide standard (transferrin), were centrifuged at 5000xg for 5 min. An aliquot of each solution was withdrawn into an HPLC syringe that was rinsed with TFA buffer through a needle compatible with the HPLC injector. The injection loop was loaded with 10µl of the peptide. The HLA DQB1*0301 and DQB1*0501 peptides were separated by HPLC on a Vydac microbore C18 reversed phase chromatography (RPC) column (250X2.1mm; 300Å; 5µm). Chromatographic analysis was monitored at multiple UV wavelengths simultaneously.

2.12 PEPTIDE SEQUENCING

Peptide sequencing was performed at the HHMI Biopolymer and W. M. Keck Foundation Biotechnology Resource Laboratoty at the Yale University School of Medicine, New Haven, Connecticut, USA.

2.12.1 EDMAN DEGRADATION

The chemical process employed by automated protein/peptide sequencers is derived from the technique originated by Edman in the 1950s for the sequential degradation of peptide chains[298-299]. The first step in this degradation is the selective coupling of a peptide's amino-terminal amino acid with the Edman reagent, phenylisothiocyanate (PITC), a reaction catalyzed by an organic base delievered with the coupling reagent. The second step is cleavage of this derived amino acid from the remainder of the peptide, a reaction accomplished by treating the peptide with a strong organic acid. Each repeated coupling/cleavage cycle occurs at the newly formed amino-terminal amino acid left by the previous cycle. These repetitive cycles provide sequential separation of the amino acids which form the primary structure of the peptide.

The identity of the amino acid removed by Edman degradation is determined by converting the cleaved amino acid derivative (anilinothiazolinone, ATZ) to the more stable derivative (penylthiohydantoin, PTH) (Fig 2.5). In modern sequencers, this conversion is accomplished automatically, using an aqueous solution of a strong organic acid, in a reaction vessel separate from that in which the Edman degradation occurs. The PTHs produced from each degradation cycle are then transferred directly and automatically from the sequencer conversion vessel to an on-line analysis system.

For pool sequencing of peptides bound to HLA DQB1*0301 and DQB1*0501, dominant peaks identified by HPLC were first removed, then the remaining fractions were pooled,

speedvaced to dryness and redissolved in 0.05% TFA and 50% acetonitrile. The sample was then subjected to 17 cycles of Edman degradation using an Applied Biosystems (Foster City, CA) 477A pulsed liquid protein sequencer equipped with on-line HPLC. Cysteine was not quantitated. The pool sequence data are shown in tables 5.1 and 5.2. From the analysis of the pool sequence, a motif for peptide binding to HLA DQB1*0301 and HLA DQB1*0501 were derived. The source proteins of peptides were identified by searching the Protein Identification Resource (PIR), Genpept and Swiss Protein Databases.

2.13 SOLUTIONS AND REAGENTS

Blocking Solution: 500ml

4X SSPE	100ml 20X stock
0.1% Lauroylsarcosine	5ml 10% stock
1.0% Blocking Reagent	5g
dH_2O	390ml

Blocking reagent from Boerhinger Cat No 1096 176

Lauroylsarcosine Sigma L-5125

2X SSPE/0.1% SDS: 500ml

2X SSPE	50ml	20X Stock
0.1% SDS	2.5ml	20X Stock

TMACl Wash Solution: 500ml

3M TMACl	300ml	5M Stock
50mM Tris	25ml	1M Tris/HCl (PH 8)

0.1% SDS	2.5ml	20% Stock
2mM EDTA	2ml	0.5M (PH 8)

Buffer 1 : 10 litres of 10X Stock

1M Tris	1211g
1.5MNaCl	876.6g

pH solution to 7.5 with conc HCl

Dilute 1/10 before use.

Buffer 2: 500ml

0.1M Tris	50ml	1M Stock (pH 7.5)
0.15M Nacl	75ml	1M Stock
1% Blocking reagent	5g	
dH_2O	370ml	

Buffer 3: 500ml

0.1M Tris	50ml	1M Tris/HCl (pH 9.5)
0.1M Nacl	50ml	1M Stock
50mM $MgCl_2$	25ml	1M Stock

CSPPD (Lumigen) Solution : 100ml

1ml of CSPPD is added 100ml of filtered buffer 3. The container is wrapped in tin foil and stored at 4^0C.

Boerhinger

Anti-digoxigenin-AP, Fab fragments:

Boerhinger

2.14 DNA CONTROL KIT

DRB1 and DQB DNA control kits of the British Society for Haematology and Immunology (BISHI) were obtained from the United Kingdom Transplant Support Service Authority, Bristol.

2.15 CHEMICAL AND MATERIAL SUPPLIERS

AMERSHAM INTERNATIONAL PLC
Amersham place, Little Chalfont, Amersham, Buckinghamshire
HP7 9NA.

BDH
Merck Ltd., Merck House, Poole, Dorset, BH15 1TD.

BIO-RAD LABORATORIES LTD.
Bio-Rad House, Maylands Avenue, Hemel Hempstead,
Hertfordshire, HP2 7TD.

BOEHRINGER MANNHEIM UK (DIAGNOSTICS AND BIOCHEMICALS) LTD.
Bell Lane, Lewes, East Sussex, BNG ILG.

DIFCO LABORATORIES LTD.
P. O. Box 14B, Central Avenue, East Molesey, Surrey, KT8 0SE.

DUPONT (UK) LTD.
Diagnostics and Biotechnology Systems, Wedgwood Way,
Stevenage, Hertfordshire, SG1 4QN.

GIBCO BRL
Life Technologies Limited, Unit 4, Cowley Mill Trading Estate,
Longbridge Way, Uxbridge, UB8 2YG.

FLUKA CHEMIKA-BIOCHEMIKA
Fluka Chemicals Ltd., The Old Brickyard, New Road, Gillingham,

Dorset, SP8 4JL.

FMC BIOPRODUCTS
Flowgen Instruments Ltd., Broad Oak Enterprise Village, Broad Oak Road,
Sittingbourne, Kent, ME9 8AQ.

IBI
International Biotechnologies Inc., 36 Clifton Road,
Cambridge, CB1 4ZR.

ICN BIOCHEMICALS
Division of ICN Biomedicals Inc., Cleveland, OH 44128.

NBS BIOLOGICALS
New Brunswick Scientific (UK) Ltd., Edison House,
163 Dixons Hill Road, North Mymms, Hatfield, AL9 7JE.

NEW ENGLAND BIOLABS
CP Laboratories, P. O. Box 22, Bishop's Stortford,
Herts, CM23 3DX.

PHARMACIA LKB
Pharmacia Biosystems Limited, Biotechnology Division, Davy Avenue,
Knowlhill, Milton Keynes, MK5 8PH.

STRATAGENE CLONING SYSTEMS
Stratagene Ltd., 140 Cambridge Innovation Centre, Cambridge Science Park/Milton Road,
Cambridge, CB4 4GF.

SIGMA CHEMICAL COMPANY
Fancy Road, Poole, Dorset, BH17 7NH.

UNITED STATES BIOCHEMICAL CORPORATION
Cambridge Bioscience, 25 Signet Court, Newmarket Road,
Cambridge, CB5 8LA.

Type	Primer	Location (nt) and product size
HPV 16	Sense: 5'-AAGGCCAACTAAATGTCAC-3' Antisense: 5'-(GCGGATCC)TGTCTGCTTTTATACTAA-3' (Seedorf et al, 1985)[278]	7763-7781 78-61 (+5'*Bam*HI site) 228bp
HPV 18	Sense: 5'-CACGGCGACCCTACAAGCTACCTG-3' Antisense: 5'-TGCAGCACGAATGGCACTGGCCTC-3' (Coles & Danos, 1987)[279]	127-150 531-508 405bp
HPV 31	Sense: 5'-AGAAAGACCTCGGAAATTG-3' Antisense: 5'-TACCTCTGTTTCTGTTAAC-3' (Goldsborough et al., 1989)[280]	125-143 233-215 109bp
HPV 33	Sense: 5'-CTACAGTGCGTGGAATGCAAAAAACC-3' Antisense: 5'-CGGGACCTCCAACACGCCGCAC-3' (Cole &Streeck, 1986)[281]	190-215 536-515 347bp

Table 2.1: Type Specific Primers used for HPV amplification and annealing temperatures

HLA Allele	Primer sequences FAMP/RAMP	Size/ PCR product.
DQB1*0201	5' GTGCGTCTTGTGAGCAGAAG 3'	205bp
	5' GCAAGG TCGTGCCGAGCT 3'	
DQB1*0201/ 0302	5' GACGGAGCGCGTGCGTCT 3'	129bp
	5' CTGTTCCAGTACTCGGCGG 3'	
DQB1*0301/ 0304	5' GACGGAGCGCGTGCGTTA 3'	122bp
	5' AGTACTCGGCGTCAGGCG 3'	
DQB1*0302/ 0303	5' GACGGAGCGCGTGCGTTA 3'	122bp
	5' AGTACTCGGCGTCAGGCG 3'	
DQB1*0303	5' GACGGAGCGCGTGCGTTA 3'	129bp
	5' CTGTTCCAGTACTCGGCGT 3'	
DQB1*0601	5' GCCATGTGCTACTTCACCAAT 3'	198bp
	5' CACCGTGTCCAACTCCGCT 3'	
DQB1*0601/ 0301	5' GACGGAGCGCGTGCGTTA 3'	129bp
	5' CTGTTCCAGTACTCGGCGT 3'	
DQB1*0304	5' GACGGAGCGCGTGCGTTA 3'	129bp
	5' CTGTTCCAGTACTCGGCGG 3'	

Table 2.2: Sequence specific primer pairs for typing the HLA DQB1*03 locus.

FAMP Forward amplification primer; RAMP reverse amplification primer

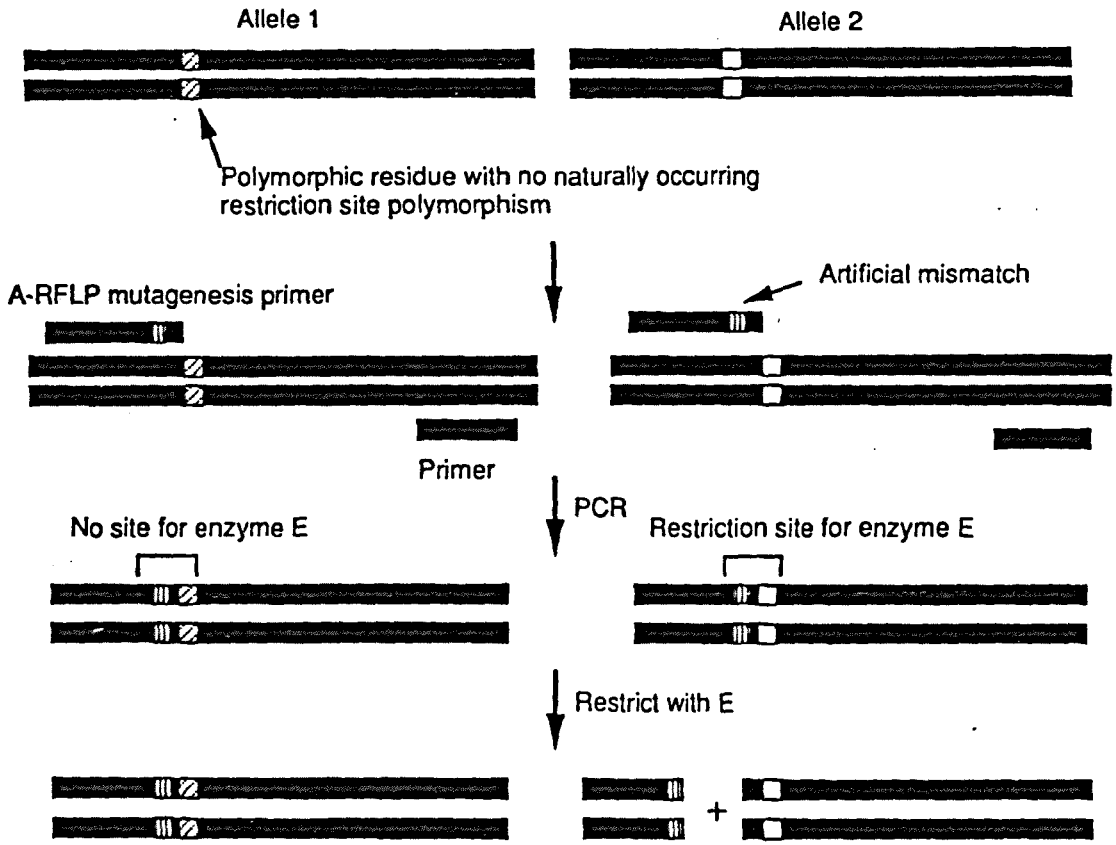

Fig 2.1: Principles of A-RFLP

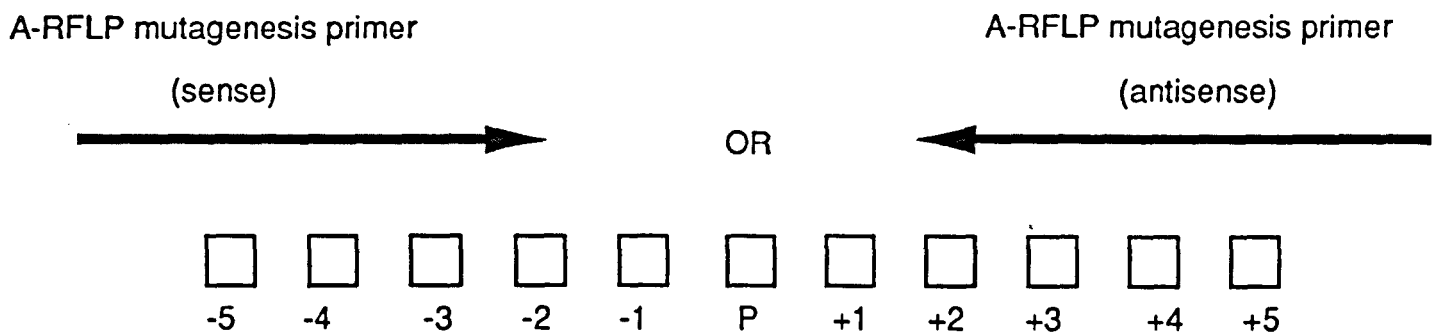

Fig 2.2: The design of A-RFLP Primers

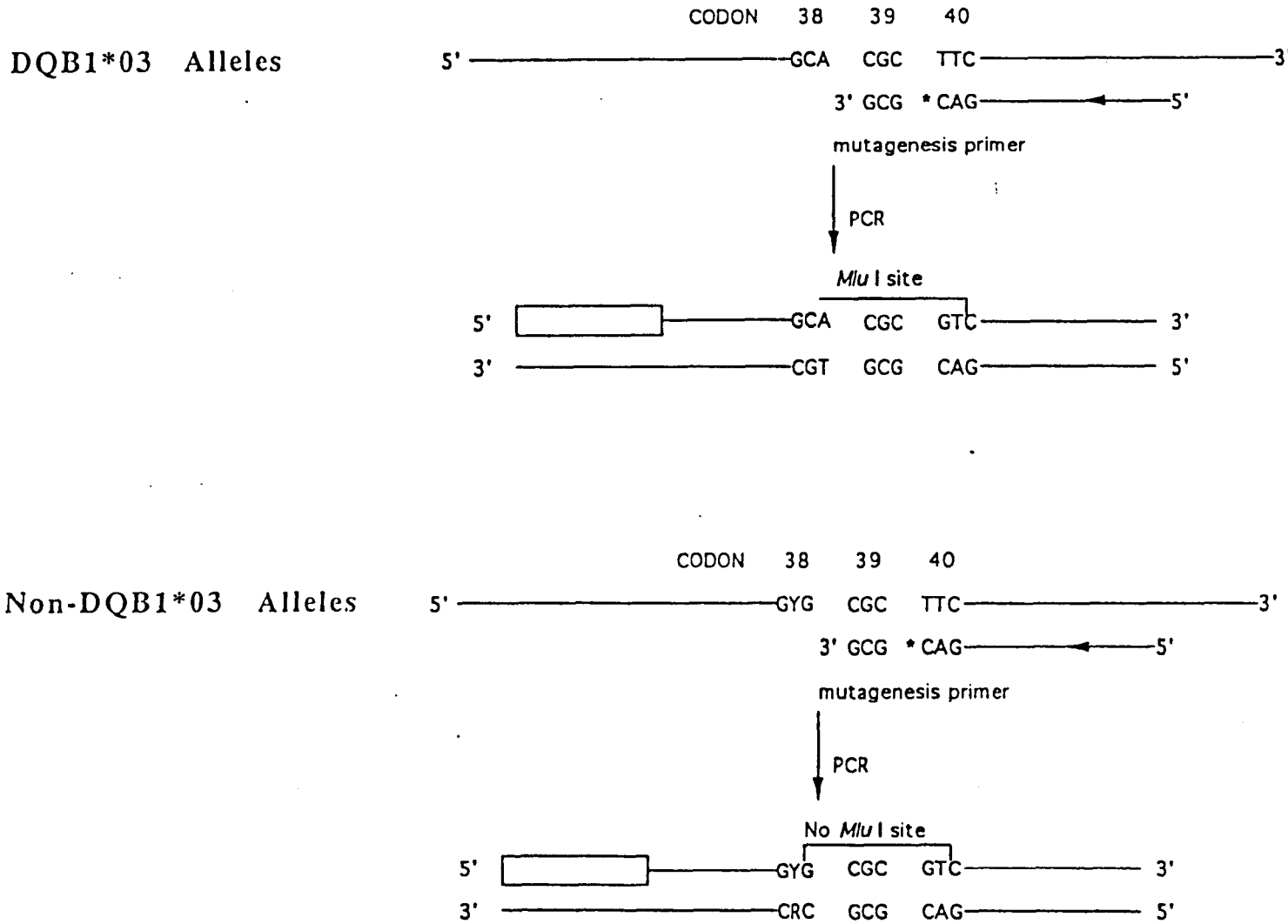

Fig 2.3: A-RFLP for HLA DQB1*03

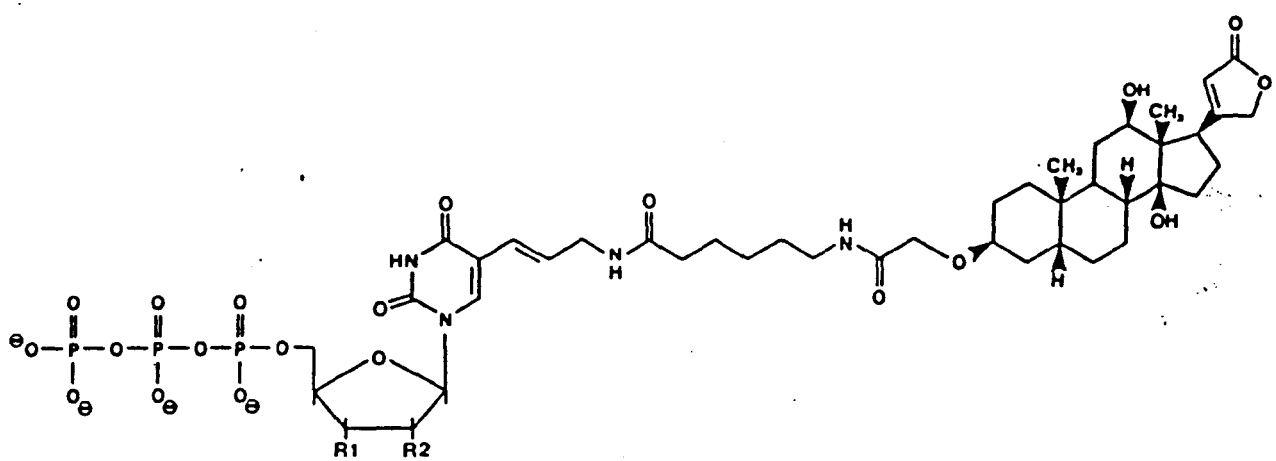

Fig 2.4: Digoxigenin-UTP (R1 = OH, R2 = OH)/Digoxigenin-dUTP (R1 = OH, R2 = H)/Digoxigenin-ddUTP (R1 = H, R2 = H)

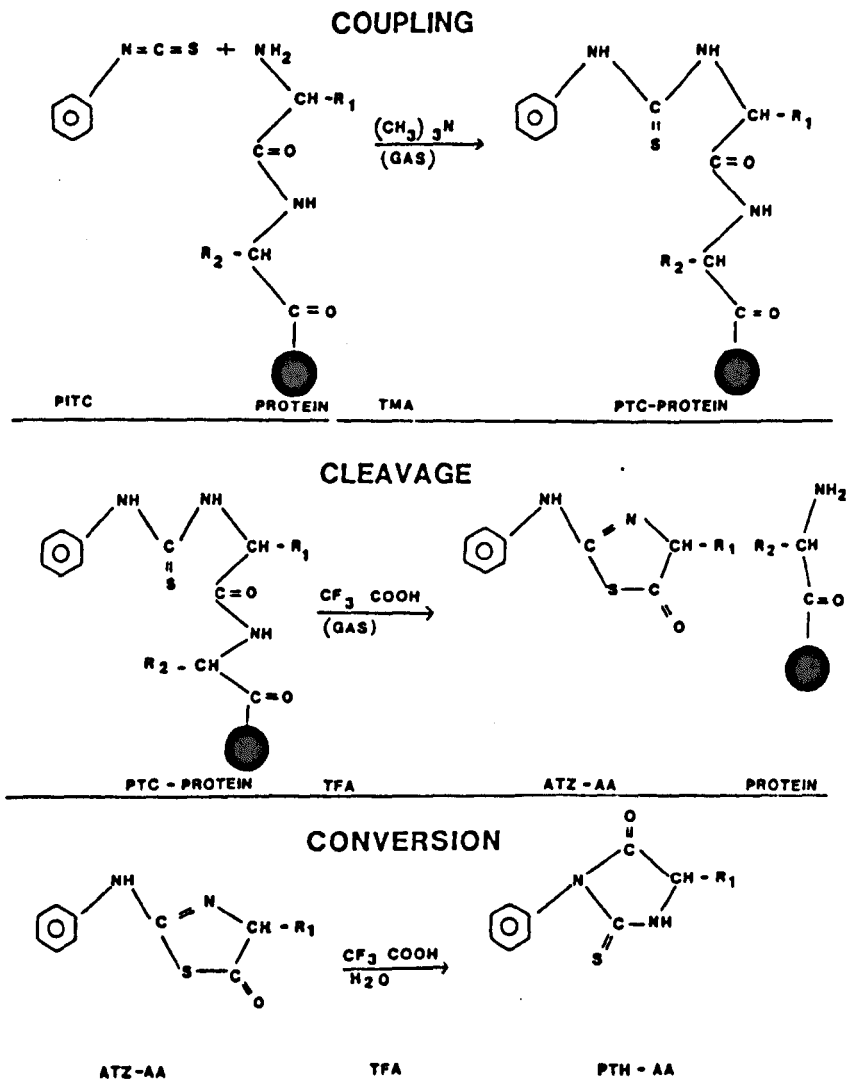

Fig 2.5: Schematic illustration of the principles of Edman chemistry showing the coupling, cleavage and conversion steps.

CHAPTER 3: ASSOCIATION BETWEEN HLA DQB1*03 AND CERVICAL INTRA-EPITHELIAL NEOPLASIA

3.1 INTRODUCTION

Recently, Wank & Thomssen[255] showed a significant association between HLA DQB1*03 and cervical cancer. Subsequent reports have not consistently confirmed this observation (reviewed in chapter 1 and Odunsi & Ganesan[300]). Evidently there is heterogeneity within results depending on the size and type of population examined and techniques used for the HLA and HPV typing. This chapter reports the results of HPV and HLA DQB1*03 typing conducted in a Caucasian population.

Allelic products of the polymorphic DQA1 and DQB1 genes encode functional DQ molecules through *cis*- and *trans*-complementations. Cis-dimers comprise α and ß chains encoded by DQA1 and DQB1 genes of the same chromosome, and trans-dimers are encoded by genes on homologous chromosomes. Although it is clear that HLA-DP, -DQ and -DR products can all present antigen to human CD4+ T cells, HLA-DR restriction overwhelmingly predominates. The apparent inefficiency of HLA-DQ as an antigen restriction molecule presents a perplexing paradox: HLA-DQ restricted T cell clones are rare, reflecting the low expression of the dimer on antigen presenting cells, yet disease association studies relatively frequently implicate HLA-DQ, rather than -DR alleles in predispostion to autoimmune and some infectious diseases. Human T-cell clones so far characterized show a marked bias against HLA-DQ restriction, reflecting the low level of expression on APCs in the periphery. Although this low frequency of DQ-restricted clones may reflect a truly marginal role in the immune response, the stimulation requirements or effector functions of DQ-restricted clones may differ from those in conventional studies.

3.2 RESULTS

The ARFLP-PCR technique on DNA from cervical smears, following *Mlu* I digestion, can lead to three possible results: negative for DQB1*03, heterozygous or homozygous for DQB1*03 (Fig 3.1).

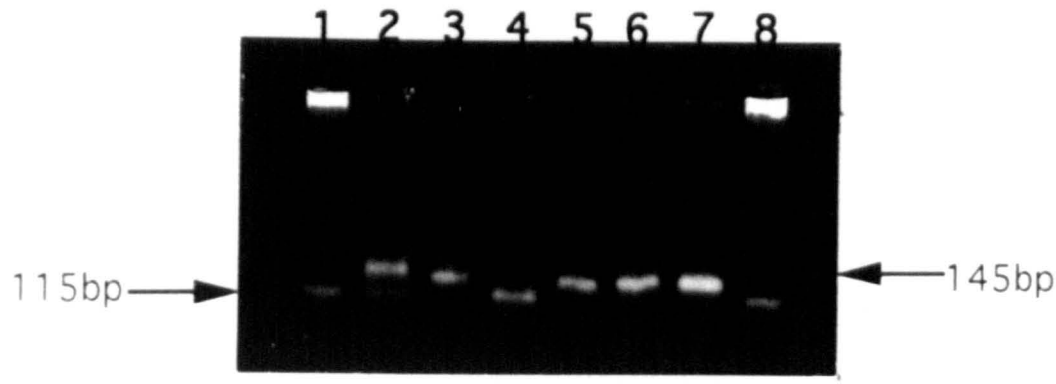

Fig 3.1: A 4% metaphor agarose gel showing amplified DNA after PCR with primers A and B with and without digestion by *MluI*

The size of amplified DNA is 145bp and on digestion with *MluI*, a 115 and 30bp product is produced in DQB1*03 homozygotes. DNA for all controls were from the British Society for Histocompatibility and Immunogenetics. Arrows show the 145 and 115bp products. Lanes 2 and 3 show heterozygous DQB1*03 control with and without digestion by *MluI*. Lanes 4 and 5 show homozygous DQB1*03 control with and without digestion with *MluI*. Lanes 6 and 7 show non-DQB1*03 control with and without digestion with *MluI*. Lanes 1 and 8 are 123 bp markers.

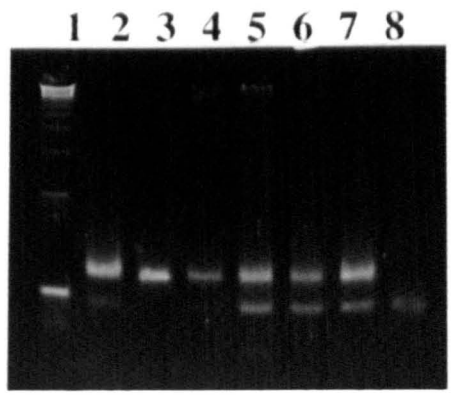

Fig 3.2: Example of A-RFLP on study samples.Lane 1: 123bp ladder DNA; Lane 2: JRA 28, DQB1*03 heterozygous cell line after digestion; Lane 3: JRA 28, DQB1*03 heterozygous cell line before digestion; Lane 4: Amai, Non DQB1*03 cell line after digestion; Lanes 5-8: samples from patients with CIN- 5: heterozygous DQB1*03; 6: Heterozygous DQB1*03; 7: Heterozygous DQB1*03; 8: Homozygous DQB1*03.

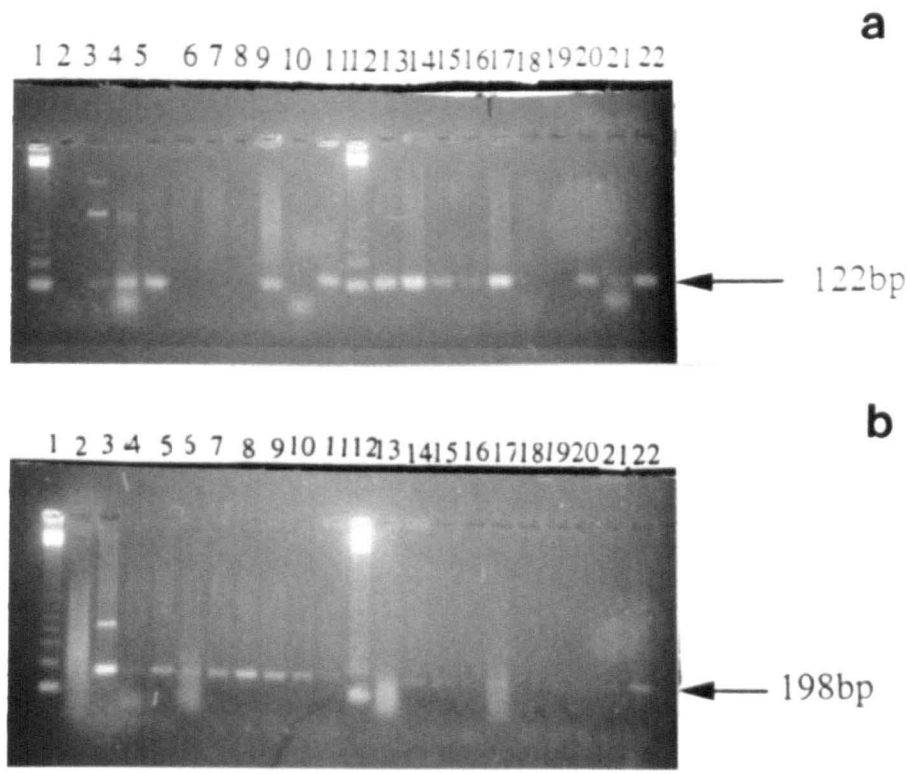

Fig 3.3: PCR products obtained by HLA DQB1*03 sequence specific primers. (a) 122 bp product obtained with primer pair PB5'09 and PB3'09 to identify DQB1*0301/0304. Lanes 1 and 12: 123 bp marker; Lanes 2 and 18: negative control; Lane 3: positive control DNA. An internal amplification control primer pair PC'5 and PC'3 (amplifies the third intron of DRB1 genes) was included in this reaction to give a 796 bp fragment; the rest represent study samples. (b) 198 bp product obtained on the same set of samples with primer pair PB5'03 and PB3'04 to identify DQB1*0601. Lanes 1 and 12: 123bp marker; Lanes 2 and 18 are negative controls; Lane 3: positive control DNA and the remaining lanes are study samples. Fig. for DQB1*0304 not shown since all were negative for this set of primers. Allelic assignment was by comparing and integrating positive results.

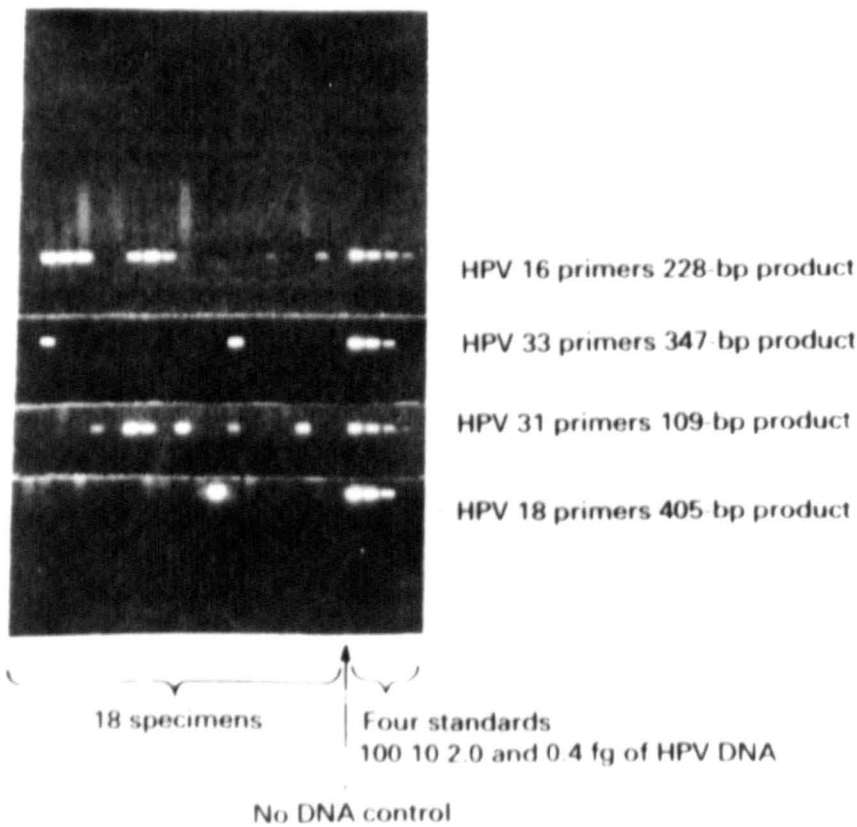

Fig 3.4: Type specific amplification of HPV.

3.3 OVERALL RESULTS

HLA DQB1*03 typing was performed on DNA from cervical smears of 178 women with CIN (CIN I = 66; CIN III = 112) and 420 healthy women who had a normal smear. All samples were successfully amplified for the locus. HPV typing was performed for types 16, 18, 31 and 33 on all the test and control samples. The HLA DQB1*03 and HPV results on individual samples are included in the tables showing the complete HLA DQ-DR typing results (Refer to Appendix 1). As shown in appendix 1, the women are either homozygous for the HLA DQB1*03 alleles (0301, 0302, 0303 and 0304) or heterozygous (DQB1*03 allele in combination with any other DQB allele). The analysis of HLA DQB1*03 is presented first.

Table 3.1 is a summary of the distribution of HLA DQB1*03. Of CIN cases, 61% were positive (56% of CIN 1, 64% of CIN III) for the HLA DQB1*03 type, compared to 34% of controls. The association was significant (χ^2 trend = 37.3, $p<0.001$), and the odds ratio for CIN overall was 3.03 (95% CI 2.11-4.35). The association was significant for both CIN III (odds ratio 3.45 vs 2.45) and CIN I, stronger for CINIII, but not significantly different from CINI.

One hundred and thirty-one patients with CIN (73.5%) were positive for one or more HPV types 16, 18, 31, 33 . Of HPV-positive CIN, 64% were of the type DQB1*03. There was a significant association between DQB1*03 and HPV (χ^2 trend= 38.6, $p<0.001$) with a odds ratio of 3.43 (95% CI 2.28-5.15).

3.4 ASSOCIATION BETWEEN HLA DQB1*03 AND CIN (Tables 3.2 and 3.3)

Of women with CIN, 38% were negative for DQB1*03, while 37% were heterozygous and 23% homozygous for the DQB1*03 locus (χ^2 trend= 39.01, $p<0.001$). Compared

with controls, the odds ratio was greater for homozygosity (4.0, 95% CI 2.43-6.6) than for heterozygosity (2.63, 95% CI 1.75-3.94). Further typing of the DQB1*03 locus in positive samples by PCR-SSP showed that the 0301 allele that was present in 40% of CIN as opposed to 9% of controls (odds ratio 2.53, 95% CI 1.79-3.57; χ^2 trend =28.6, p<0.001). DQB1*0302 was present in 32% and 10% of CIN and controls respectively (odds ratio 1.84, 95% CI 1.29-2.62). The association between HLA DQB1*03 and CIN is shown in table 3.2 while association between DQB1*03 alleles and CIN is shown in table 3.3.

3.5 ASSOCIATION BETWEEN HLA DQB1*03 AND HPV (Tables 3.4, 3.5 and 3.6)

HPV typing was performed for the major oncogenic types, HPV 16,18, 31 and 33. Of CIN cases, 57% were positive for HPV 16, 7% for HPV 18, 12% for HPV 31 and 7% for HPV 33 and 16% were positive for multiple types. All types correlated strongly with DQB1*03 but there was insufficient data to find a difference between the types. The highest odd ratio was found for women with HPV 18 or multiple types. There was a significant correlation with "gene dosage" at the DQB1*03 locus, with 39% of HPV positive CIN being heterozygous and 24% homozygous for DQB1*03 (χ^2 trend =37.9, p<0.001). Homozygosity was significantly associated with HPV positive CIN (odds ratio 4.47, 95% CI 2.58-7.77). Further typing of the HLA DQB1*03 locus in positive samples showed that the 0301 allele was most strongly associated with HPV infection (odds ratio 2.69, 95% CI 1.88-3.94; χ^2 trend=32.9, p<0.001). Table 3.4 shows the association between HLA DQB1*03 and HPV type while table 3.5 shows the effect of zygosity at the DQB1*03 locus. Table 3.6 shows the association between individual DQB1*03 alleles and HPV.

SUMMARY AND DISCUSSION

Cervical cancer and CIN have been shown to be strongly associated with the oncogenic types of the human papillomaviruses (16,18,31,33,35,39,45,51,52,56 and 58) in several cross-sectional studies[68,85]. However, additional factors must operate to determine the progression from normal epithelium to CIN and cervical cancer after HPV infection. One host factor is possibly immunological, as in other virus induced cancers, such as nasopharyngeal carcinoma due to Epstein-Barr virus[301]. In cervical disease this is supported by the fact that spontaneous regression of low grade CIN is frequently observed. Thus immunological mechanisms, in particular the cellular immune response, may play a significant role in the development of CIN and cervical cancer after HPV infection.

To address these issues this large study, of sufficient test samples and controls evaluates the significance of DQB1*03 association with cervical intra-epithelial neoplasia. This study was performed in CIN, as it is the precursor lesion of cervical cancer, and the results of HLA typing may be relevant particularly when correlated with the HPV status. Further it is quite important to evaluate the association between CIN, HPV and DQB1*03 using controls that are negative for HPV and have a normal cytology. The interpretation and reporting of negative HPV results must be interpreted in the context of the detection system used. In this study, HPV 16, 18, 31 and 33 were tested for and a negative result simply means that the specimen does not contain any of these HPV types.

The use of consensus primers in PCR for HPV can result in competition between non-specifically primed human DNA with HPV DNA and between different types of HPV DNA in individual clinical specimens, and the apparent level of any particular type may be distorted after amplification. For this reason, type specific primers were used in this study.

Consensus primers are reserved for qualitative demonstration of the presence of HPV types other those specific types tested for.

The typing for HLA DQB1*03 was performed with a rapid technique which was concordant with data based on sequencing[302]. The advantage of this method lies in the need for a single mutagenic primer, which is used in a single step PCR amplification. This technique is also informative in assessing whether the individual sample is heterozygous or homozygous for the DQB1*03 locus. Likewise, the PCR-SSP technique is an accurate and rapid technique for detecting genetic variability with a high degree of resolution. Each primer pair identifies two cis-located sequence motifs, which allows the separation of all homozygous and heterozygous combinations of DQB1*03. For instance, a DQB1*0301/DQB1*0302 cannot be distinguished by PCR-SSO typing. However, the two alleles can be unequivocally assigned by the PCR-SSP technique. Since the method is ideal for analyzing a small number of samples, it was not used for typing the remaining DQB alleles.

The results show a significant association between CIN and DQB1*03 that is only slightly stronger for CIN III than CIN I. The association between CIN and DQB1*03 that was found (odds ratio 3.03) was less strong than that reported by Wank and Thomssen, but a slightly stronger association in HPV positive CIN (odds ratio 3.43) was observed than that reported by Van den velde et al[303]. Homozygosity at DQB1*03, was significantly associated (odds ratio 4.0) with CIN and was more strongly related than heterozygosity, a result not reported so far in any previous studies. The 0301 allele was the most strongly associated with CIN (odds ratio 2.53, χ^2= 28.6, $p<0.001$) but 0302 was also positively related. This agrees with Wank and Thomssen's DNA typing data for 0301 on their original sample of cervical cancer patients[257].

A significant association with HPV positive CIN and DQB1*03 was found for all HPV types tested (16,18,31,33). Again homozygosity at the DQB1*03 was strongly associated with HPV positive CIN (odds ratio 4.47) with intermediate risk found for heterozygotes. Typing for HPV has not been uniformly performed in all the previous studies, but in general HPV positive CIN was significantly associated with the DQB1*03 phenotype. In this study type specific primers for the major oncogenic types of HPV were used and it is possible that some of the HPV negative CIN are positive for other types. Detailed typing for other HPV types is only likely to increase the strength of the association. The results also show that the association between DQB1*03 and HPV positive CIN is intermediate in risk for CINI and greater for CINIII, in agreement with the natural history of the disease. These results suggest that probably the DQB1*03 locus may be an important determinant in allowing the HPV infection to be tolerated and permit the progression to CIN or cancer.

Another disease due to HPV infection, recurrent respiratory papillomatosis, has been shown to be associated with the DQB1*03 phenotype[304]. In an analysis of 16 patients, 75% were positive for DQB1*03. Analysis of HLA class I and II using restriction fragment length polymorphisms, in New Zealand rabbits infected with Shope cotton-tail rabbit papillomavirus, showed a strong linkage between wart regressions and DR locus, and an increased risk of malignant transformation with the DQ locus[305]. Thus based on this study and others, the DQB1*03 locus seems to be important for HPV associated disease. The results of the analysis of HLA DR and DQ in squamous cell carcinoma reported by Apple et al[306] in a Hispanic population showed no significant association with the DQB1*03 locus, although the haplotype DRB1*0407-DQB1*0302 was associated with increased risk of cervical carcinoma.

In summary, it is possible that women who are positive for the DQB1*03 phenotype may be unable to mount an effective cytotoxic T cell response against HPV infection. This is

particularly important as it has been shown that HPV16 E_7 is a target for cytotoxic T cells and to mediate tumour rejection[307].

Table 3.1: Summary of Distribution of HLA DQB1*03

Patients (No.)	HLA DQB1*03 (Positive) %	Odds Ratio (95% CI)
CIN (178)	109 (61)	3.03 (2.11-4.35)
CIN 1 (66)	37 (56)	2.45 (1.45-4.12)
CIN 3 (112)	72 (64)	3.45 (2.23-5.33)
HPV negative CIN (47)	25 (53)	2.18 (1.19-3.97)
HPV positive CIN (131)	84 (64)	3.43 (2.28-5.15)
Controls (420) (HPV negative)	144 (34)	1*

χ^2 (trend) for (controls, CIN 1 and CIN 3) =37.3, $p< 0.001$.

χ^2 (trend) for controls, HPV negative and HPV positive) = 38.6, $p<0.001$.

* reference category

Table 3.2: Association between HLA DQB1*03 and CIN

HLA	Controls (%)	CIN (%)	Odds Ratio (95% CI)
non DQB1*03	276 (65)	69 (38)	1*
Heterozygous for DQB1*03	102 (24)	67 (37)	2.63 (1.75-3.94)
Homozygous for DQB1*03	42 (10)	42 (23)	4.0 (2.43-6.60)
Total	420	178	

* reference category

χ^2 (trend) = 39.01, $p<0.001$.

Table 3.3: Association between HLA DQB1*03 allele and CIN

HLA DQB1*03 allele	CIN 3 (2n=224) (%)	CIN1 (2n=132) (%)	Controls (2n=840) (%)	Odds Ratio (95% CI)	χ^2 (trend)
0301	49 (21)	25 (19)	79 (9)	2.53 (1.79-3.57)	28.6 (p<0.001)
0302	45 (20)	16 (12)	85 (10)	1.84 (1.29-2.62)	15.5 (p<0.001)
0303	5 (2)	7 (5)	21 (2.5)	1.36 (0.67-2.76)	0.05 p=0.82
0304	0	0	1 (0.1)	0	
non DQB1*03	125	84	654	1*	
Total	224	132	840		

* reference category

Table 3.4: Association between HLA DQB1*03 and HPV type

HPV Type present	Number of Patients	DQB1 *03 (positive) (%)	Odds Ratio (95% CI)
16	75	45 (60)	2.88 (1.74-4.74)
18	9	7 (77)	6.71 (1.56-∞)
31	16	11 (68)	4.22 (1.5-11.84)
33	10	6 (60)	2.88 (0-.86-9.64)
Multiple types	21	15 (71)	4.79 (1.88-12.2)
Controls (HPV negative)	420	144 (34)	1*

* reference category

Table 3.5: Association between HLA DQB1*03 and HPV

HLA	Controls (%)	HPV positive CIN (%)	Odds Ratio (95% CI)
non DQB1*03	276 (65%)	47 (35%)	1*
Heterozygous for DQB1*03	102 (24%)	52 (39%)	2.99 (1.90-4.71)
Homozygous for DQB1*03	42 (10%)	32 (24%)	4.47 (2.58-7.77)
Total	420	131	

* reference category

χ^2 (trend) =37.9, p<0.001.

Table 3.6: Association between HLA DQB1*03 allele and HPV

HLA DQB1*03 allele	CIN HPV (positive) (2n=262) (%)	CIN HPV (negative) (2n=94) (%)	Controls HPV (negative) (2n= 840) (%)	Odds Ratio (95% CI)	χ^2 (trend)
0301	60 (22)	14 (15)	79 (9)	2.69 (1.88-3.94)	32.9 ($p<0.001$)
0302	45 (17)	16 (17)	85 (10)	1.71 (1.17-2.50)	10.6 ($p<0.001$)
0303	9 (3)	3 (3)	21 (2.5)	1.35 (0.63-2.89)	0.71 ($p<0.4$)
0304	0	0	1 (0.1)	0	
non DQB1*03	148 (56)	61 (65)	654 (78)	1*	
Total	262	94	840		

* reference category

CHAPTER 4: ANALYSIS OF HLA DR-DQ ASSOCIATIONS WITH HPV AND CERVICAL INTRA-EPITHELIAL NEOPLASIA

4.1: INTRODUCTION

In the preceding chapter, it was shown that there is an increased risk for HPV positive CIN in women with HLA DQB1*03, specifically DQB1*0301 (O.R. 2.53) and DQB1*0302 (O.R. 1.84) alleles. The next phase of the thesis was to perform a detailed analysis of the HLA DR and DQ alleles in patients with CIN and healthy controls in a British Caucasian population and identify haplotypes which confer both susceptibility and protection in the development of CIN after HPV infection. By defining susceptibility and protective alleles and haplotypes, these studies may help to provide a framework for understanding peptide binding and T cell recognition events in the immunological response to HPV infection. This chapter reports the detailed analysis of HLA DR-DQ in HPV associated cervical intra-epithelial neoplasia.

4.2: RESULTS

HLA DR and DQ typing was performed on DNA from cervical smears of 176 women with CIN (CIN I=63; CIN III=113) and 416 healthy women who had a normal cervical smear. All cervical samples from patients with CIN and controls were typed for DRB1, DRB3, DRB4, DRB5 and DQB1 using the PCR/SSO technique except for DQB1*03 alleles which were individually detected by allele specific primers and PCR[308]. The DR/DQ haplotypes were inferred based on known patterns of linkage disequilibrium for these loci[286-287]. HPV typing was performed on all the test and control samples and 131 of 176 (75%) cases of CIN were positive for one or more of the HPV types which were examined, and all the controls were selected to be negative for HPV. The overall results on individual samples are shown in Appendix 1. Statistical analysis was performed to evaluate for correlation between HLA type and CIN, CIN I, CIN III and HPV positive CIN.

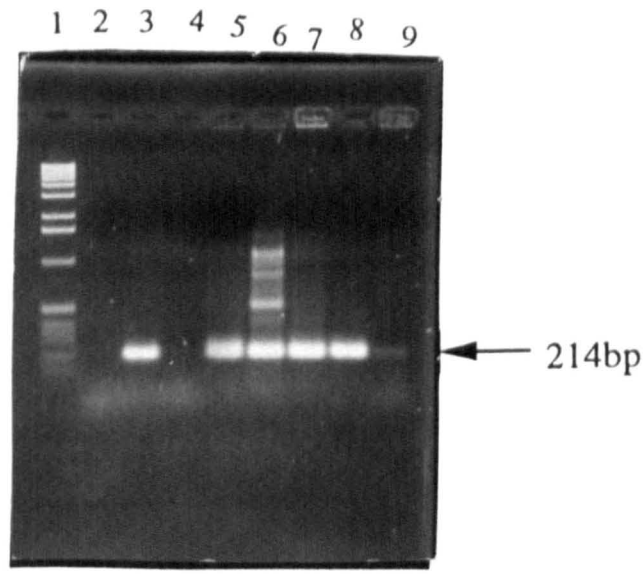

Fig. 4.1: Generic DQB1 amplification using the primer pair DQBAMP and DQRAMP to obtain a 214bp product. Lane 1: 123bp marker; Lane 2: Negative control; Lane 3: positive control DNA from cell line BVR (DQB1*0501); Lane 4: Negative control; Lanes 5 to 9: samples from patients with CIN.

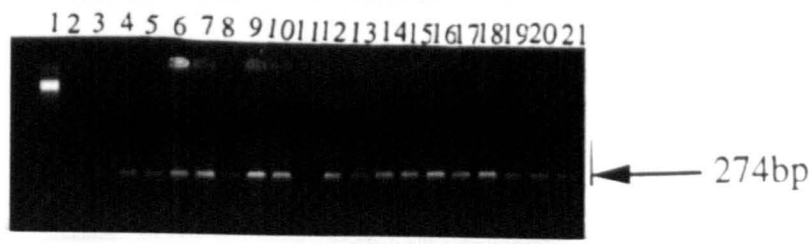

Fig. 4.2: Generic DRB1 amplification using the primer pair DRBAMP and DRRAMP to obtain a 274bp product. Lane 1: 123bp marker; Lanes 2 and 11: Negative controls; Lanes 3 and 4: positive control DNA from cell line PREISS (DRB1*0401); Lanes 5 to 10 and 12 to 21: samples from patients with CIN.

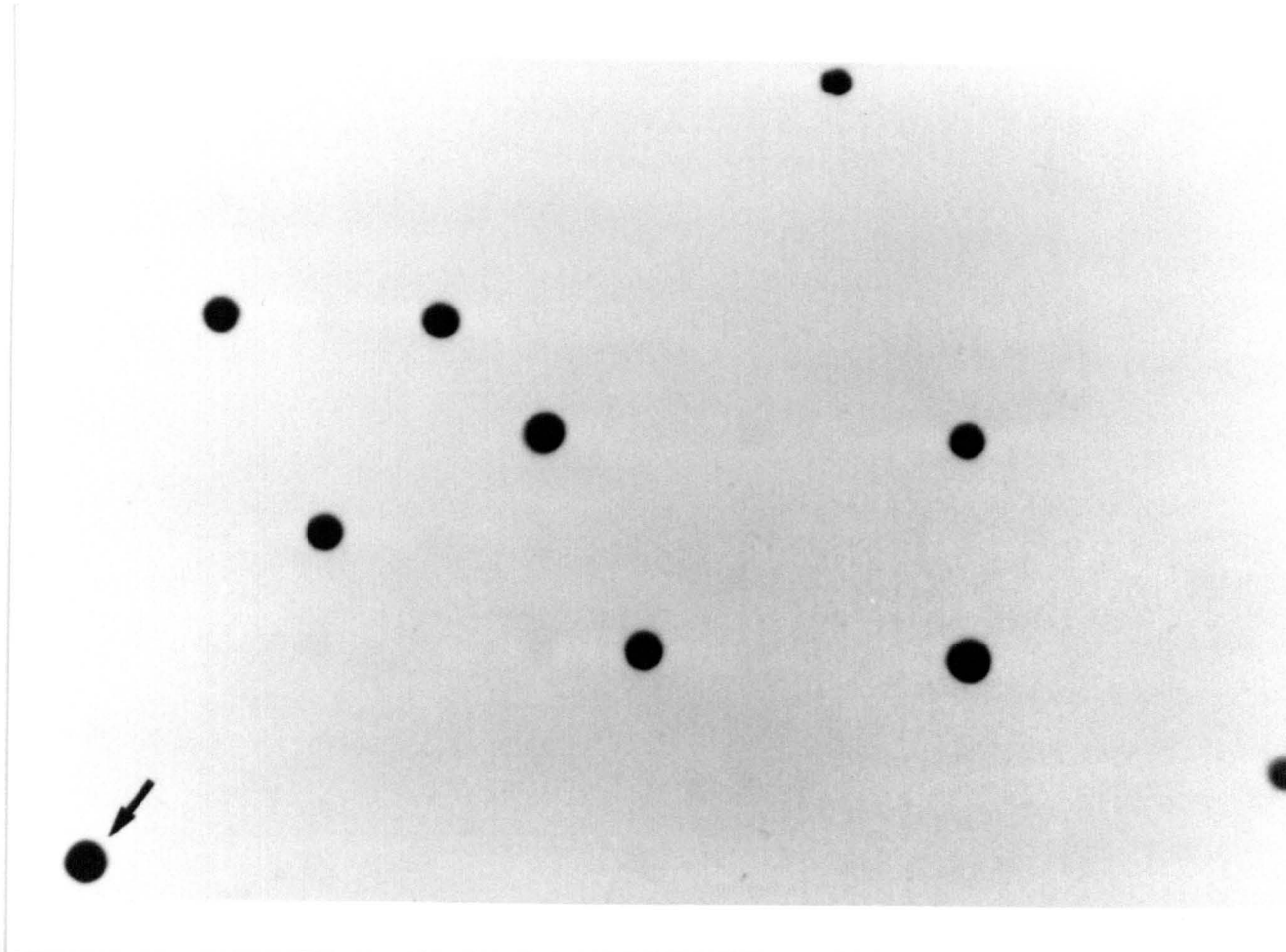

Fig 4.3: Digoxigenin labeled oligonucleotide hybridization after generic HLA DQ amplification. In this example, the probe DQB4901 identifies the HLA DQB1*0501 allele in a set of control samples. The arrow shows a positive signal from the HLA DQB1*0501 control cell line BVR, obtained from BISHI. Further confirmation of HLA DQB1*0501 in these individuals was by demonstrating positive signals from probes DQB2601 (0501, 0502, 05031, 05032) and DQB5701 (0501, 0604 and 0605).

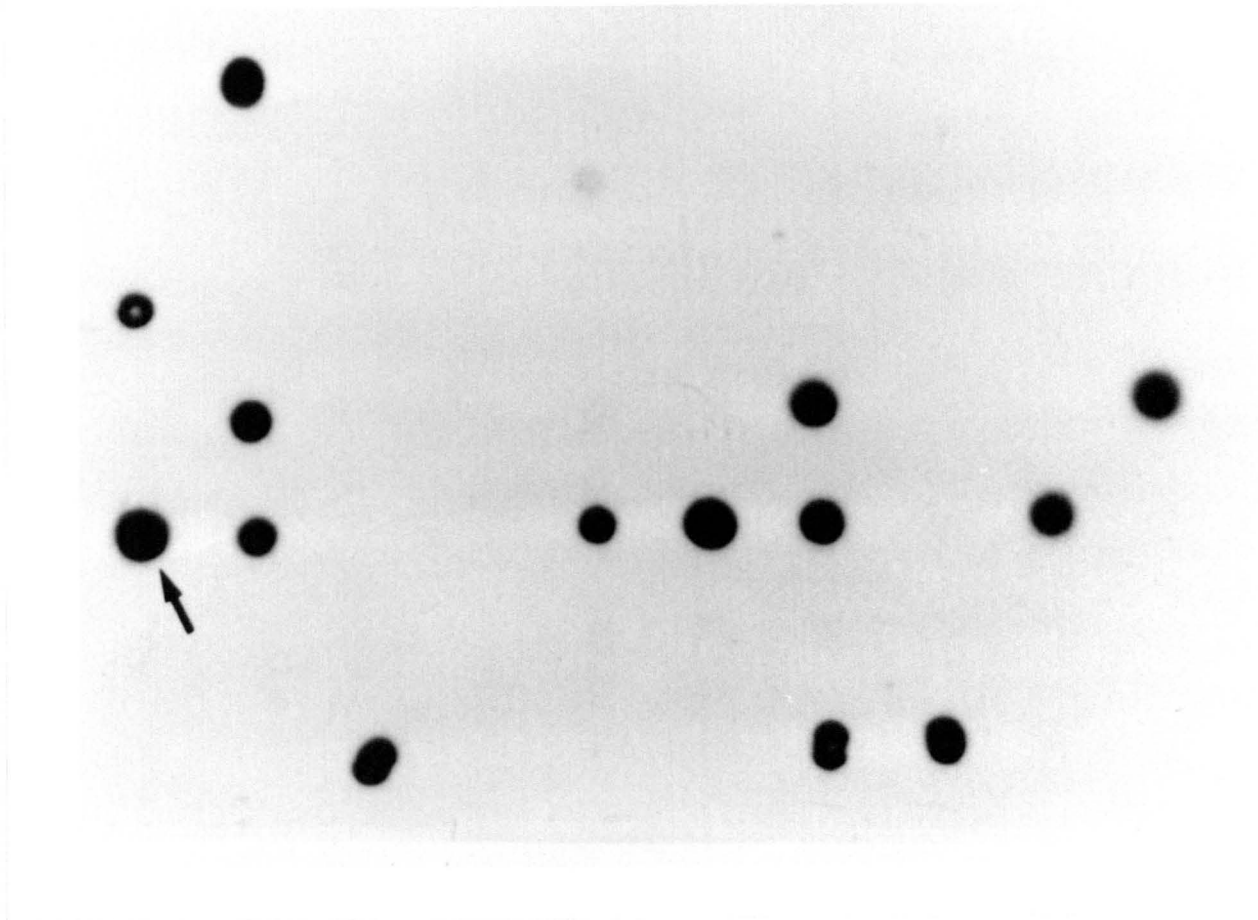

Fig 4.4: Digoxigenin labeled oligonucleotide hybridization after generic HLA DR amplification. In this example, the probe DRB1004 identifies the HLA DR4 group of alleles in a set of samples from patients with CIN. The arrow shows a positive signal from the HLA DRB1*0401 control cell line PREISS, obtained from BISHI. Further subtyping was by group specific amplification followed by DIG-labeled hybridization with the appropriate probes (section 2.5.11).

4.3 CORRELATION BETWEEN INDIVIDUAL ALLELES OF HLA DRB1, DRB3, DRB4 AND DRB5 WITH CIN (Table 4.1)

The occurrence of different DR allele groups is clearly related to CIN when analysed for heterogeneity (χ^2=28.76, d.f.=12, p=0.004). The DR4 group correlated significantly with CIN (O.R. 1.76 {1.28-2.40}; χ^2=12, p=0.001).Within the DR4 group there was also evidence for heterogeneity (χ^2=22.5, d.f=8, p=0.004). The DR4 alleles principally DRB1*0401 (O.R. 1.99, p=0.002); DRB1*0403 (O.R. 3.61, p=0.02); DRB1*0406 (O.R. 3.74- , p=0.0007) correlated significantly with CIN. In addition, DRB1*1101 also correlated with increased susceptibility for CIN (O.R. 2.35, p=0.004).

There were several DR alleles which suggested a protective effect for CIN and HPV positive CIN. In particular, DRB1*0101 (O.R. 0.48, p=0.01); DRB1*0701/0702 (O.R. 0.58, p=0.02) and DRB5*0101 (O.R. 0.45, p=0.03) indicated a protective effect. HLA DRB1*1301 showed a protective effect for CIN III only (O.R. 0.32, p=0.004).

4.4 CORRELATION BETWEEN INDIVIDUAL HLA DQB1 ALLELES AND CIN (Table 4.2)

Different DQB1 alleles showed a relationship with CIN when analysed for heterogeneity (χ^{22}=49.39, d.f=4, p<0.0001). HLA DQB1*03was the most significant, and there was no evidence of heterogeneity within it (χ^2=2.74, p=n.s.). The DQB1*0301 demonstrated the stronger association (O.R. 2.49; p<0.0001), but DQB1*0302 (O.R. 1.82, p=0.001) was also significantly more common. Further analysis showed that the positive association with DQB1*0301 was also significantly more common in CIN I (O.R. 2.02, P=0.01).

Similarly, the frequency of the DQB1 alleles, DQB1*0501 (O.R. 0.48, p=0.004); DQB1*0402 (O.R. 0.49, p=0.06); DQB1*0603 (O.R. 0.47, p=0.03); and DQB1*0604 (O.R. 0.6, p=0.06) showed a protective effect with either CIN. However when the data

was re-analysed after excluding the positively associated DQ and DR alleles, none of these protective associations were significant.

The significant individual DRB1 and DQB1 alleles were analysed to assess whether homozygosity conferred an additional risk for CIN. Only at the DQB1*0301 locus could homozygosity be shown to increase risk (O.R., 4.39{1.84-10.50}; p=0.002). There were insufficient homozygotes of other alleles to yield clear conclusions.

4.5 CORRELATION BETWEEN SIGNIFICANT INDIVIDUAL HLA DQB1 AND HLA DRB ALLELES AND HPV (Table 4.3)

The same alleles that were found to significantly correlate with CIN were found to correlate with HPV positive CIN. The susceptibility alleles were HLA DQB1*0301 (O.R. 2.77, P=0.00001); DQB1*0302 (O.R. 1.85, P=0.003); DRB1*0401 (O.R. 2.34, P=0.0004); DRB1*0403 (O.R. 3.23, P=0.04); DRB1*0406 (O.R. 5.05-∞, P=0.0002); and DRB1*1101 (O.R. 2.19, P=0.02). The alleles that showed protection to HPV positive CIN were DQB1*0501 (O.R. 0.54, P=0.04); DQB1*0603 (O.R. 0.44, P=0.04); DQB1*0604 (O.R. 0.55, P=0.06); DRB1*0101 (O.R. 0.56, P=0.06); DRB1*1301 (O.R. 0.52, P=0.05) and DRB5*0101 (O.R. 0.40, P=0.03).

4.6 CORRELATION BETWEEN HLA DR/DQ HAPLOTYPES AND CIN (Table 4.4)

The analysis for specific haplotypes was performed for all the possible DR-DQ combinations. The most common naturally occurring haplotypes (where n 10) in British and Caucasian populations[286-287] and ones where there was a significant correlation are displayed in Table 4.4. The two locus haplotypes DRB1*0401-DQB1*0301 (O.R. 2.22, p=0.02), and DRB1*1101-DQB1*0301 (O.R. 3.95, p=0.003) showed significantly strong associations with CIN and in particular with CIN III. Other haplotypes also demonstrated

nominally significant positive associations, but these were difficult to assess because of small numbers and multiple testing. They included haplotypes principally from the DRB1*04 group, i.e., DRB1*0401-DQB1*0302 (O.R. 1.90, $p<0.05$), DRB1*0403-DQB1*0302 (O.R. 4.34, $p=0.007$) and DRB1*0406-DQB1*0302 (O.R. 2.48-∞, $p=0.008$).

The only haplotype to confer a significant protective effect for CIN was DRB1*0101-DQB1*0501 (O.R. 0.48, $p=0.01$). The haplotype was also protective for CIN III (O.R. 0.37, $p=0.01$). None of the three locus haplotypes correlated positively or negatively with CIN. There were insufficient cases with homozygous DR-DQ haplotypes to analyse for correlation with risk for CIN.

4.7 CORRELATION BETWEEN THE SIGNIFICANT HLA HAPLOTYPES AND HPV (Table 4.5)

The haplotypes that correlated with CIN showed similar results for HPV positive CIN (Table 4.5). In addition, two rare haplotypes DRB1*0701-DQB1*0302 (O.R. 3.24, $p=0.03$) and DRB1*0801-DQB1*0301 (O.R. 9.63, $p=0.05$) also correlated significantly with HPV positive CIN.

4.8 CORRELATION BETWEEN SIGNIFICANT HLA DR/DQ HAPLOTYPES AND HPV TYPE (Table 4.6)

The significant susceptible and protective haplotypes were analysed to examine for correlation with individual HPV types. 131 cases of CIN were positive for one of the HPV types either alone or in combination. HPV 16 was present in 75 (57%) cases, HPV 18 in 9 (7%), HPV 31 in 17 (13%), HPV 33 in 9 (7%) and there were multiple HPV types detected in 21(16%) cases. The relation between the most significant haplotypes and specific HPV types are shown in Table 4.6. No clear association with HPV type is

apparent, but because HPV16 positive CIN was the most common it is not possible to comment on associations with other HPV types.

4.9 DISCUSSION

In this study the two DR-DQ haplotypes most clearly associated with CIN particularly CIN III were DRB1*0401-DQB1*0301 and DRB1*1101-DQB1*0301. Two other DR4 associated haplotypes DRB1*0401-DQB1*0302 and DRB1*0403-DQB1*0302 also had a significant correlation with CIN. The major susceptibility haplotypes are different to those reported by Apple et al[309] and this may be partly explained by ethnic differences as they examined a Hispanic population. Nevertheless DR4 associated haplotypes have been identified in both studies as significant although the individual alleles are different. In this study the haplotypes DRB1*0401-DQB1*0301 (n=22), DRB1*0401-DQB1*0302 (n=23) and DRB1*0404-DQB1*0302 (n=21) were the most frequent in controls, and representative of a Caucasian population[286-287]. In contrast, the haplotypes DRB1*0407-DQB1*0302 (n=18) and DRB1*0404-DQB1*0302 (n=14) were the most common in the Hispanic population[309]. It is probable that both studies together suggest that the DR4 associated haplotypes confer increased risk in the development of CIN. The other major susceptibility haplotype DRB1*1101-DQB1*0301 in this study, was not identified as significant in the Hispanic study[309]. Instead DRB1*1501-DQB1*0602 was reported as a positively associated haplotype, despite both being observed at comparable frequencies in the control population. This is likely to be a genuine difference between the studies, particularly as a protective effect was observed with DRB1*1101-DQB1*0301 in their study[309]. Two further haplotypes, less common in Caucasians, i.e. DRB1*0701-DQB1*0302 and DRB1*0801-DQB1*0301 were also found to be significant in this study though the observations were few. The haplotypes were all significant when found at the heterozygous level, but additional risk for homozygosity was not observed. On further analysis, the Hardy-Weinberg law was not maintained in the controls with an excess of

homozygotes typed for DQB1. This is probably due to false negative scoring of some controls as homozygotes on PCR-SSO. However, further analyses using the individual as the unit and combining heterozygotes and homozygotes for each allele gave similar results. The linkage disequilibrium between the individual alleles of the significant haplotypes is too strong to determine the individual allele contributing to the overall risk[286]. However, in this study positively associated haplotypes all contained DQB1*03 alleles and the simplest explanation of the data is that the relevant factor is most closely linked to the DQB1*03 locus and the resulting association with DRB1*0401 or DRB1*1101 is due to linkage disequilibrium.

The haplotype DRB1*0101-DQB1*0501 was the only one found in this study to be negatively associated with CIN (0.R. 0.48, p=0.01). Other individual DQB1 (0402,0603,0604) and DR (DRB1*1301, DRB1*0701, DRB5*0101) alleles that were more weakly negatively associated, did not reach statistical significance at haplotype level. The protective haplotypes identified for CIN in the Hispanic study[309] are completely different and may be partly due to genetic differences between the populations. However, significant results for protective haplotypes have to be interpreted cautiously, because of the number of comparisons and both our observations and that found in the Hispanic study may still be due to chance. This is suggested by the absence of a significant protective effect for any of the DR or DQ alleles when the data are reanalysed excluding the positively associated DRB1*04 and DQB1*03 alleles.

All the significant haplotypes correlated with HPV positive CIN. Firm conclusions could not be drawn, with respect to type specific correlation due to insufficient number. The presence of multiple HPV types (16%) in CIN, unlike cervical cancer, also dilutes the ability to delineate the contribution of an individual HPV type in calculating risks[310].

TABLE 4.1 - CORRELATION BETWEEN INDIVIDUAL DRB1, DRB3, DRB4, DRB5 ALLELES AND CIN.

DR	Controls	CIN I	CIN III	Total	Odds ratio (95% C.I.)				Trend 'p'
DRB1					CIN	p	CIN III	p	
0101	71	7	8	86	0.48 (0.27-0.84)	0.01	0.39 (0.19-0.82)	0.01	0.01
0102	8	1	0	9	0.29				
0103	15	3	8	26	1.76		2.00		
0301	132	24	38	194	1.13		1.07		
0302	12	1	1	14	0.39		0.30		
0401	53	12	30	95	1.99 (1.30-3.04)	0.002	2.25 (1.40-3.60)	0.0012	0.0007
0402	13	4	1	18	0.91		0.28		
0403	6	3	6	15	3.61 (1.33-9.82)	0.019	3.75(1.26-11.16)	0.03	0.02
0404	30	3	9	42	0.94		1.11		
0405	1	0	0	1	-	-	-	-	
0406	0	1	5	6	(3.74-∞)	0.0007	(4.87-∞)	0.0004	0.01
0407	4	1	3	8	2.38		2.78		
0408	5	0	0	5	-		-		
0410	4	0	0	4	-		-		
0801	22	5	5	32	1.08		0.83		
0802	3	1	3	7	3.18		3.72		
0803	7	1	1	9	0.67		0.52		
08031	1	0	1	2	2.37		3.69		
08042	1	3	0	4	7.14		-		
1101*	24	8	15	47	2.35 (1.32-4.20)	0.005	2.39 (1.25-4.60)	0.015	0.006
1102	1	0	0	1	-		-		
1103	2	0	0	2	-		-		
1104	13	2	2	17	0.72		0.56		
1201	7	0	3	10	1.01		1.59		
1202	1	0	1	2	2.37		3.69		
1301	65	13	6	84	0.67 (0.40-1.13)		0.32 (0.14-0.74)	0.004	0.03
1302	28	5	7	40	1.01		0.92		
1303	2	0	2	4	2.37		3.71		
1304	2	0	2	4	2.37		3.71		

* CINI vs controls O.R. 2.28, p=0.06

DRB1	Controls	CIN I	CIN III	Total	Odds ratio (95% C.I.)				Trend 'p'
					CIN	p	CINIII	p	
1305	5	0	0	5	-		-		
1401	27	4	8	39	1.05		1.09		
1402	2	2	0	4	2.37		-		
1403	1	0	0	1	-		-		
1404	4	0	0	4	-		-		
1406	3	0	0	6	2.38		3.72		
1407	3	0	0	3	-		-		
1501	75	9	17	101	0.8		0.82		
1502	17	3	7	27	1.40		1.53		
1601	12	0	4	16	0.79		1.23		
1602	3	0	3	6	2.38		3.72		
0701/0702	90	7	16	113	0.58 (0.36-0.92)	0.02	0.63 (0.36-1.09)	0.1	0.05
0901	37	3	8	48	0.69		0.79		
1001	20	0	3	23	0.35		0.55		
Total	n=832	n=126	n=226	n=1184					
DRB3									
0101	139	25	30	194	0.92		0.76		
0201	16	2	3	21	0.73		0.69		
0202	96	16	33	145	1.24		1.31		
0301	27	5	5	37	0.87		0.67		
Null	554	78	151						
DRB4									
DR53	200	27	57	284	0.99		1.07		
Null	632	99	169						
DRB5									
0101	46	4	5	55	0.45 (0.22-0.91)	0.03	0.39(0.16-0.96)	0.04	0.03
0102	15	2	7	24	1.43		1.74		
0201/2	35	5	18	58	1.59		1.97(1.10-3.53)	0.03	0.03
Null	736	115	196						
Total	n=832	n=126	n=226	n=1184					

TABLE 4.2 - CORRELATION BETWEEN INDIVIDUAL DQ ALLELES AND CIN

DQ	Controls	CINI	CINIII	Total	Odds Ratio (95% C.I.)				Trend 'p'
					CIN	p	CINIII	p	
0201	180	27	46	253	0.95		0.93		
0301*	79	22	51	152	2.49 (1.77-3.52)	0.0001	2.78 (1.89-4.09)	0.0001	0.0001
0302	86	16	45	147	1.82 (1.28-2.59)	0.001	2.16 (1.45-3.20)	0.0002	0.0002
0303	20	7	5	32	1.43		0.92		
0304	1	0	0	1	-	-	-	-	
0401	17	1	4	22	0.69		0.86		
0402	42	7	2	51	0.49 (0.24-1.01)	0.06	0.17 (0-0.63)	0.004	0.02
0501	89	9	10	108	0.48 (0.29-0.79)	0.004	0.39 (0.20-0.75)	0.003	0.003
0502	31	3	8	42	0.83		0.95		
0503	1	0	0	1	-	-	-	-	
05031	20	1	6	27	0.82		1.11		
05032	11	2	3	16	1.08		1.00		
0504	17	1	2	20	0.41		0.43		
0601	69	12	17	98	0.99		0.90		
0602	31	2	9	42	0.83		1.07		
0603	49	6	4	59	0.47 (0.24-0.92)	0.03	0.29 (0.11-0.78)	0.009	0.02
0604	72	9	10	91	0.6 (0.36-1.01)	0.06	0.49 (0.25-0.95)	0.04	0.04
0605	17	1	3	21	0.55		0.64		
0606	0	0	1	1	-	-	-	-	
Total	n=832	n=126	n=226	n=1184					

* CIN I vs controls 0.R. 2.02, $p<0.01$.

TABLE 4.3 - CORRELATION BETWEEN SIGNIFICANT INDIVIDUAL DQB1 AND DRB1 ALLELES AND HPV

DQB1	Controls	HPV- CIN+	HPV+ CIN+	Total	Odds ratio (95% C.I.) HPV+CIN vs Controls	'p'
0301	79	14	59	152	2.77 (1.91-4.01)	0.00001
0302	86	15	46	147	1.85 (1.25-2.72)	0.003
0402	42	5	4	51	0.29 (0.11-0.79)	0.01
0501	89	3	16	108	0.54 (0.31-0.94)	0.03
0603	49	3	7	59	0.44 (0.20-0.96)	0.04
0604	72	6	13	91	0.55 (0.30-1.00)	0.06
DRB1						
0101	71	2	13	86	0.56 (0.31-1.02)	0.06
0401	53	6	36	95	2.34 (1.50-3.66)	0.0004
0403	6	3	6	15	3.23 (1.09-9.58)	0.04
0406	0	0	6	6	(5.05-∞)	0.0002
1101	24	7	16	47	2.19 (1.16-4.15)	0.02
1301	65	8	11	84	0.52 (0.27-0.99)	0.05
DRB5						
0101	46	3	6	55	0.40 (0.17-0.93)	0.03
Total	n=832	n=90	n=262	n=1184		

TABLE 4.4 - CORRELATION BETWEEN DR/DQ HAPLOTYPES AND CIN

DR/DQ haplotype*	Controls	CIN I	CIN III	Total	Odds ratio (95% C.I.)				Trend 'p'
					CIN vsControls.	'p'	CIN 3 vs Controls	'p'	
0101/0501	66	7	7	80	0.48 (0.27-0.86)	0.02	0.37 (0.17-0.81)	0.01	0.01
0103/0301	13	2	6	21	1.47		1.72		
0301/0201	127	22	38	187	1.14		1.12		
0401/0301	22	5	15	42	2.22 (1.20-4.08)	0.02	2.62 (1.35-5.08)	0.007	0.005
0401/0302	23	5	13	41	1.90 (1.02-3.53)	0.05	2.15 (1.08-4.26)	0.04	0.03
0402/0302	10	2	1	13	0.71		0.37		
0403/0302	5	3	6	14	4.34 (1.51-12.43)	0.007	4.51 (1.45-14.05)	0.02	0.01
0404/0302	21	3	6	30	1.01		1.05		
0406/0302	0	1	3	4	∝ (2.48-∝)	0.008	∝ (2.90-∝)	0.009	0.03
0406/0301	0	0	2	2	∝ (1.23-∝)	0.09	∝ (1.93-∝)	0.05	
0701/0201	36	3	5	44	0.51 (0.24-1.10)	0.09	0.50 (0.20-1.25)	0.18	
0701/0302†	8	0	8	16	2.40 (0.92-6.21)	0.1	3.78 (1.45-9.84)	0.009	0.01
0801/0402	18	3	1	22	0.52 (0.18-1.48)	0.3	0.20 (0-1.19)	0.09	
1101/0301‡	8	4	9	21	3.95 (1.66-9.37)	0.003	4.27 (1.68-10.85)	0.004	0.002
1101/0603	12	3	2	17	0.98		0.61		
1301/0303§	9	5	1	15	1.59		0.41		
1301/0603	19	3	1	23	0.49 (0.17-1.39)	0.25	0.19 (0-1.12)	0.1	
1301/0604	30	3	3	36	0.46		0.36		
1302/0604	23	5	5	33	1.03		0.80		
1401/05031	14	1	4	19	0.84		1.05		
1501/0601	42	7	10	59	0.95		0.87		
1501/0602	18	0	6	24	0.78		1.23		
1502/0601	13	2	5	20	1.28		1.43		
1601/0502	11	0	4	15	0.86		1.34		
Total	n=832	n=126	n=226	n=1184					

* DR/DQ haplotypes where there were 10 or more total alleles or for which a †significant association was found.
‡ CIN I vs controls O.R. 3.38, p=0.06; § CIN I vs controls O.R. 3.78, p=0.03.

TABLE 4.5 - CORRELATION BETWEEN SIGNIFICANT DR/DQ HAPLOTYPES AND HPV

DR/DQ haplotype	Controls	HPV-CIN+	HPV+CIN+	Total	Odds ratio (95% C.I.) HPV+CIN vs Controls	'p'
0101/0501	66	2	12	80	0.56 (0.30-1.04)	0.07
0401/0301	22	1	19	42	2.88 (1.55-5.36)	0.001
0401/0302	23	5	13	41	1.84 (0.93-3.64)	0.1
0403/0302	5	3	6	14	3.88 (1.25-12.06)	0.03
0406/0302	0	0	4	4	(3.34-∞)	0.003
0406/0301	0	0	2	2	(1.66-∞)	0.06
0701/0302	8	0	8	16	3.24 (1.25-8.43)	0.03
0801/0301	1	0	3	4	9.63 (1.37-∞)	0.05
1101/0301	8	3	10	21	4.09 (1.64-10.16)	0.004
Total	n=832	n=90	n=262	n=1184		

TABLE 4.6 - CORRELATION BETWEEN SIGNIFICANT DRB1/DQB1 HAPLOTYPES AND HPV TYPE

		DRB1/DQB1 haplotypes														
		0401/0301			0401/0302			1101/0301			0701/0302			0101/0501		
HPV type	No	N	O. R. (95%CI)	'p'	N	O.R.	'p'	N	O.R. (95% CI)	'p'	N	O.R.	'p'	N	O.R.	'p'
16	150	10	2.63 (1.2-5.6)	0.02	8	1.98	0.09	8	5.80 (2.2-15)	0.001	5	3.55 (1.22-10.05)	0.04	8	0.65	0.4
18	18	1	2.17	0.4	1	2.07	0.4	0	0	-	0	0	-	1	0.7	1
31	34	3	3.56	0.07	1	1.07	0.6	2	6.44 (1.3-32)	0.05	0	0	-	0	0	0.1
33	18	0	0	-	1	2.07	0.4	0	0	-	1	6.06	0.2	1	0.7	1
Multiple	42	5	4.98 (1.9-13)	0.007	2	1.76	0.3	0	0	-	2	5.15	0.08	2	0.58	0.8
Controls (HPV-ve)	832	22	1*		23	1*		8	1*		8	1*		66	1*	

* reference category

O.R. odds ratio, (95% CI)

CHAPTER 5: POOL SEQUENCING OF NATURALLY PROCESSED PEPTIDES BOUND TO HLA-DQB1*0301 AND DQB1*0501; PREDICTION OF PEPTIDE MOTIFS FROM HUMAN PAPILLOMAVIRUS TYPE 16

5.1 INTRODUCTION

The pioneering work of Buus et al[311] was the first that detailed the acid extraction of naturally processed self peptides bound to MHC molecules. The application and refinement of this technique has produced substantial information on HLA-associated peptides. Thus, it has been shown that the majority of peptides that associate with HLA class I are 8-10 residues long, with allotype specific binding motifs containing up to three anchor positions[169,312]. This is consistent with the multiple pockets and the close-ended structure of the HLA class I peptide binding groove. However, only a few peptide side-chains are actively involved, as the majority of the binding energy is obtained through conserved binding sites at the terminal ends of the peptide and extensive hydrogen-bonding networks along the peptide backbone[313-314].

To further the understanding of the mechanisms of HLA associated susceptibility to HPV induced cervical carcinogenesis, it is reasonable to suppose that susceptibility to HPV infection reflects the presence or absence of immunodominant peptide binding motifs. Therefore, the identification of the type of peptides bound by the susceptibility and protective HLA molecules may contribute to the understanding of HPV induced cervical carcinogenesis. Furthermore, knowledge of the motif requirements of peptide binding to these molecules may allow the modification of the immune response to the human papillomavirus.

In this study, the haplotype HLADRB1*0401-DQB1*0301 was shown to correlate with susceptibility to HPV and CIN while DRB1*0101-DQB1*0501 indicated protection. The extended 3-locus haplotypes, DQA1*0301-DQB1*0301-DRB1*0401 and DQA1*0301-DQB1*0302-DRB1*0401 have been found at a frequency of 23.1% in British caucasoids[287]. Similarly, DQA1*0101-DQB1*0501-DRB1*0101 occurs at a frequency of 19.8% in British caucasoids[287]. Therefore, in the present analysis, the peptide pools eluted

from HLA {DQA1*0301/DQB1*0301} and {DQA1*0101/DQB1*0501} were sequenced (Since these significant haplotypes cover more than 20% of the British population). Amino acid preferences based on peptide sequence alignment with HPV 16 L1, L2, E6 and E7 are discussed.

5.2 ELUTED POOL SEQUENCE DATA FROM PEPTIDES ELUTED FROM HLA DQB1*0501 AND DQB1*0301

A representative SDS-PAGE analysis of HLA DQA1*0101-DQB1*0501 from the JESTHOM cell line is shown in fig 5.1. The rpHPLC absorption profile for (210nm) for eluted peptides from HLA DQA1*0101-DQB1*0501 and HLA DQA1*0301-DQB1*0301 are shown in figures 5.2 and 5.3 respectively. The profiles illustrate the heterogeneity of the eluted protein material. Pooled fractions from the eluted HLA DQA1*0101-DQB1*0501 and HLA DQA1*0301-HLA DQB1*0301 were used for further analysis (Edman sequencing). Only 50% of the HPLC fractions were used for this pool.

Tables 5.1 and 5.2 show the pool sequencing data for HLA DQA1*0101-DQB1*0501 and HLA DQA1*0301-DQB1*0301. Norvaline (nv) was used as solvent to dissolve PTH amino acid and to verify the injection. Norleucine was used as standard. For interpretation of the data, the amount of increase of yield of PTH-amino acid at each cycle was considered more significant than the actual number itself. Two levels of arbitrary significance were employed. Values at least 50% higher as compared with either of the three previous cycles are considered highly significant (similar to the evaluation in Falk et al[169]). Signals with high absolute values and either a small increase as compared to the previous cycle, or a decrease lower than the expected lag for the residue are considered likely to be presented in a proportion of peptides in the mixture. These significant residues are underlined. In this way, pool sequence data for DQB1*0501 and DQB1*0301 ligands were obtained.

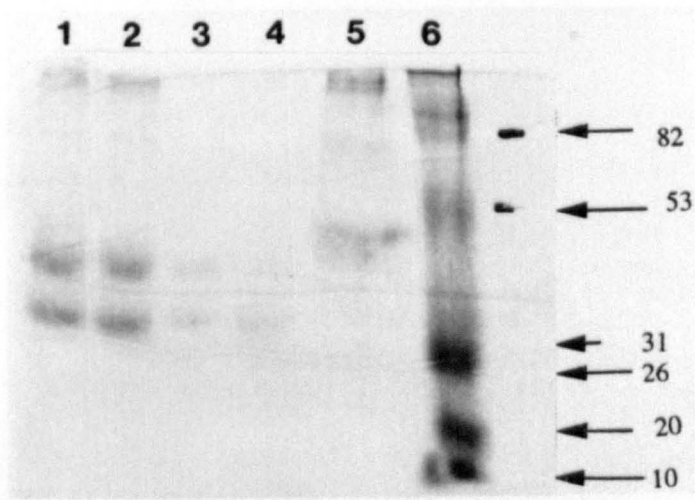

Fig 5.1: 12% SDS-PAGE Analysis of HLA DQA1*0101-DQB1*0501 obtained from the JESTHOM cell line. Lane 1: DR-1 (control); Lane 2: DQB1*0501 from JESTHOM; Lane 3: DR1; Lane 4: DQB1*0501; Lane 5: Flow through column; Lane 6: Marker.

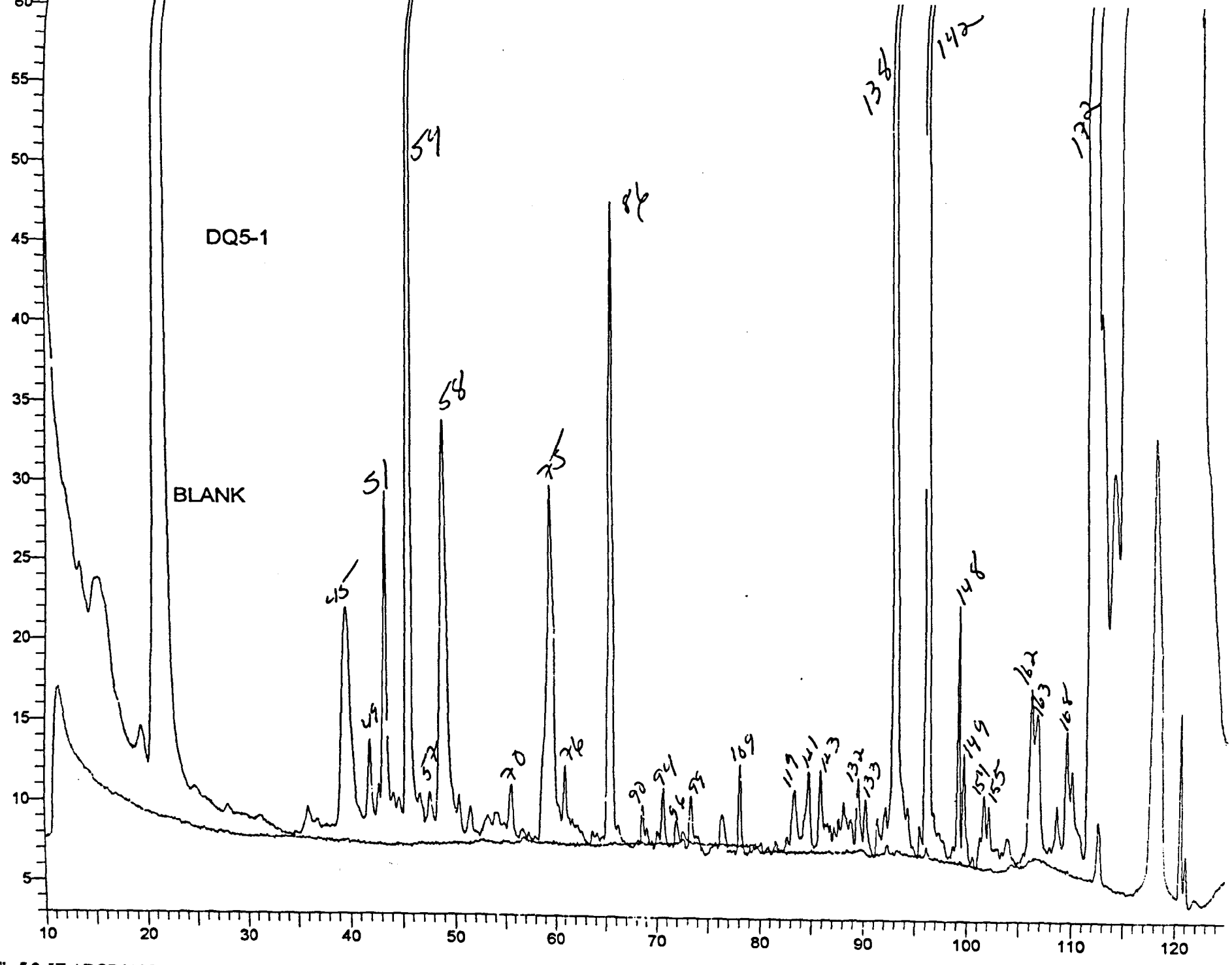

Fig 5.2: HLADQB1*0501 peptide pools were separated by r-HPLC. Each HPLC chromatogram represents the peptide repertoire as detected by UV absorbance at 210nm

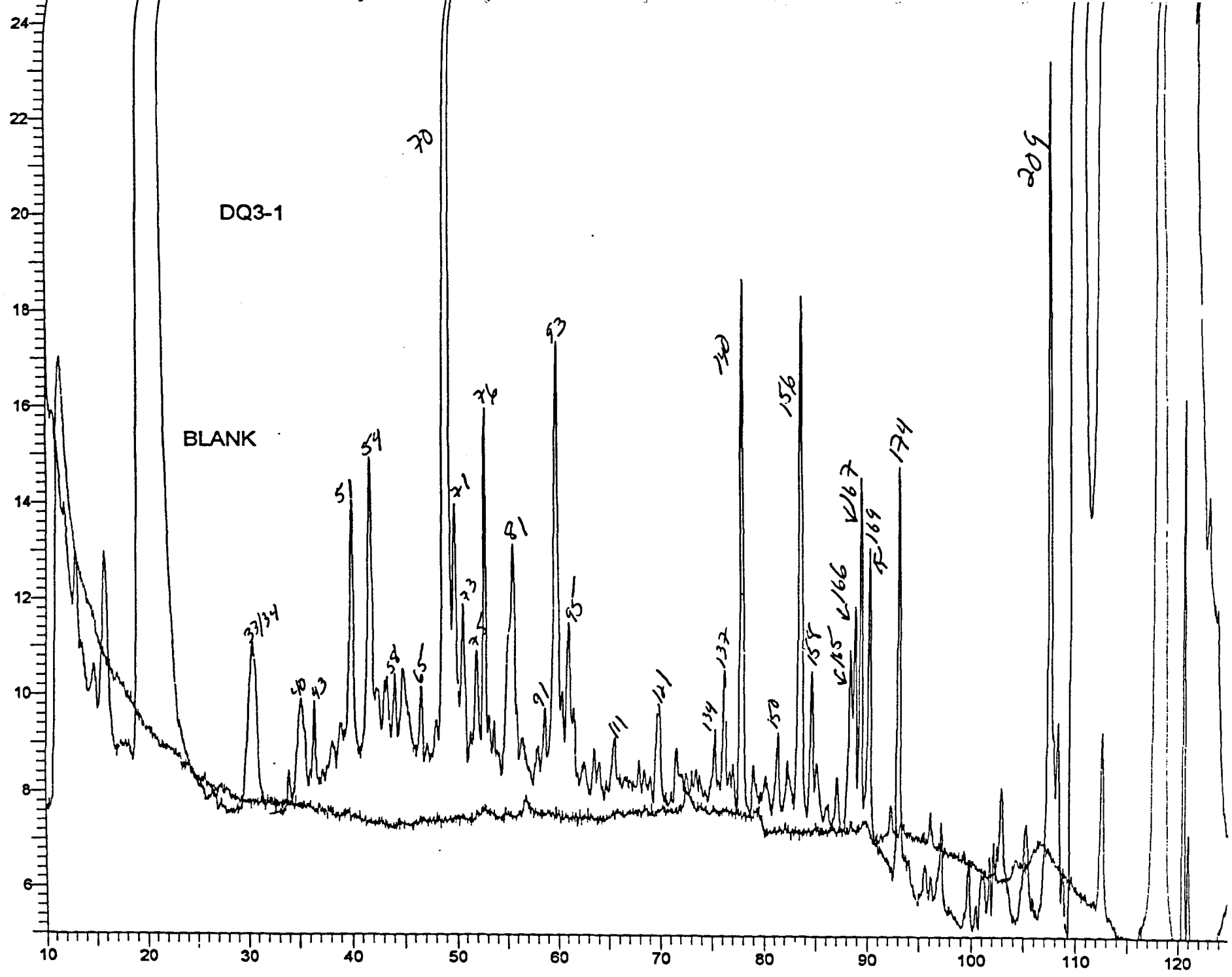

Fig 5.3: HLADQB1*0301 peptide pools were separated by r-HPLC. Each HPLC chromatogram represents the peptide repertoire as detected by UV absorbance at 210nm

Amino acid residues (in pmol)

cycle	A	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	smc	T	V	W	Y	nl	nv
1	22.18	14.27	6.78	17.36	15.09	2.62	0.00	11.76	6.78	1.94	3.24	1.90	0.00	0.32	26.00	0.02	11.00	6.28	0.00	3.77	4.55	2.53
2	5.01	6.96	4.14	2.20	10.05	1.69	2.84	4.54	4.59	0.54	1.75	2.17	1.68	4.40	9.25	0.01	3.50	3.03	0.00	2.12	0.22	1.65
3	2.30	5.63	3.39	1.18	6.70	1.29	2.00	2.63	2.94	0.48	1.63	2.01	1.14	4.10	3.92	0.02	2.00	1.84	0.00	2.02	0.03	1.52
4	1.67	3.89	3.09	1.02	4.07	0.94	1.57	1.36	2.56	0.27	1.18	1.36	1.60	4.20	1.62	0.03	1.00	1.32	0.00	1.03	0.06	1.40
5	1.74	3.05	2.21	0.80	2.77	0.68	1.48	0.66	2.22	0.27	1.38	0.98	1.37	5.49	1.40	0.01	1.16	1.17	0.00	0.84	0.06	1.35
6	1.86	2.92	2.03	0.98	2.42	0.61	1.93	0.67	2.53	0.33	1.06	1.07	1.20	4.25	1.20	0.03	1.03	1.07	0.00	0.97	4.08	1.34
7	1.69	1.92	1.36	1.00	1.65	0.44	0.00	2.14	1.34	0.28	1.01	0.99	0.79	3.15	0.99	0.01	0.76	0.85	0.00	0.90	0.32	1.23
8	2.27	1.83	1.19	0.93	1.81	0.41	0.00	1.79	1.43	0.25	0.75	0.79	0.78	3.51	1.04	0.01	0.86	0.98	0.00	0.84	0.04	1.30
9	1.36	2.06	1.04	0.61	1.34	0.37	0.00	2.29	1.86	0.20	0.70	0.73	0.74	3.10	0.85	0.02	0.60	0.82	0.00	0.52	0.03	1.29
10	1.17	1.43	0.80	0.44	1.02	0.29	0.00	2.43	1.03	0.14	0.57	0.55	0.80	2.27	0.62	0.01	0.41	0.60	0.00	0.35	0.05	1.21
11	1.55	1.21	0.71	0.38	1.11	0.23	0.00	0.00	0.69	0.10	0.50	0.49	0.59	2.08	0.67	0.01	0.41	0.52	0.00	0.32	2.89	1.21
12	1.29	1.19	0.88	0.47	1.44	0.29	0.00	1.86	0.78	0.14	0.50	0.47	0.66	2.37	0.77	0.01	0.46	0.86	0.00	0.37	0.49	1.31
13	0.81	0.89	0.84	0.28	1.05	0.21	0.00	1.66	0.79	0.12	0.45	0.37	0.55	2.37	0.57	0.01	0.37	0.53	0.00	0.32	0.20	1.23
14	0.65	0.84	0.58	0.25	0.78	0.16	0.54	0.06	0.50	0.07	0.43	0.33	0.42	3.15	0.42	0.01	0.24	0.45	0.00	0.25	0.37	1.19
15	0.64	0.70	0.47	0.27	0.77	0.20	0.00	1.05	0.58	0.04	0.36	0.33	0.43	3.36	0.43	0.01	0.32	0.56	0.00	0.42	0.00	1.25
16	0.69	0.62	0.41	0.26	0.78	0.17	0.00	1.06	0.46	0.11	0.28	0.32	0.44	2.42	0.41	0.01	0.26	0.41	0.00	0.77	1.78	1.28
17	0.58	0.56	0.39	0.29	0.80	0.23	0.48	0.11	0.46	0.11	0.27	0.27	0.38	1.97	0.40	0.02	0.35	0.39	0.00	0.45	0.54	1.27
18	0.59	0.46	0.33	0.25	0.73	0.16	0.00	0.96	0.45	0.09	0.27	0.25	0.36	1.67	0.38	0.01	0.53	0.36	0.00	0.30	0.00	1.27
19	0.54	0.47	0.28	0.22	0.62	0.12	0.56	0.05	0.41	0.07	0.25	0.23	0.33	1.50	0.35	0.01	0.14	0.35	0.00	0.43	0.00	1.26

Table 5.1: Sequencing of DQB1*0501 ligands. The pools were sequenced by Edman degradation. The numbers indicate pmols of individual amino acids residues detected at each cycle. SMC = S-methyl cysteine; nl = norleucine (standard); nv = norvaline.

Amino acid residues (in pmol)

cycle	A	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	smc	T	V	W	Y	nl	nv
1	24.48	14.63	8.55	6.78	18.71	2.97	8.92	18.00	6.40	1.55	4.59	2.25	3.93	10.77	30.89	0.37	14.10	7.02	0.00	3.93	8.47	3.39
2	6.23	7.93	4.75	2.56	10.39	2.48	4.03	6.99	2.82	0.81	2.89	6.31	2.41	5.60	10.90	0.17	4.39	3.34	0.00	1.76	0.08	1.79
3	5.32	6.78	4.46	1.91	6.42	1.93	2.02	4.91	1.85	1.09	0.00	2.82	1.65	4.32	4.95	0.07	1.76	1.99	0.00	1.22	0.00	1.52
4	5.70	7.36	5.44	0.67	4.86	1.40	1.06	3.51	1.39	0.92	0.00	1.70	1.61	4.62	2.06	0.05	1.40	1.59	0.00	0.84	0.04	1.38
5	5.68	4.04	4.88	0.87	4.13	1.28	0.77	3.26	1.17	0.55	0.00	1.33	1.35	5.40	1.63	0.08	2.51	1.42	0.00	0.73	0.04	1.35
6	8.06	2.18	3.94	0.55	3.79	0.84	0.70	2.01	0.98	0.44	0.97	1.37	1.46	4.62	1.45	0.04	1.02	1.86	0.00	0.55	6.41	1.34
7	8.47	1.38	2.17	0.82	4.20	0.69	0.95	1.88	0.97	0.30	0.80	1.09	1.47	4.19	1.43	0.03	0.84	1.89	0.00	1.57	0.26	1.37
8	7.43	1.00	1.25	0.46	3.23	0.00	0.49	1.27	1.13	0.26	0.79	1.37	1.43	4.52	1.30	0.02	0.93	1.79	0.00	0.52	0.05	1.31
9	7.46	0.82	0.93	0.30	1.78	0.00	1.00	1.38	1.14	0.20	0.78	1.50	1.02	4.66	1.55	0.02	0.72	2.52	0.00	0.40	0.00	1.30
10	5.65	0.70	0.87	0.29	1.70	0.00	0.54	0.99	0.89	0.24	0.77	1.48	0.99	4.15	1.22	0.05	1.27	2.76	0.00	0.28	0.05	1.30
11	5.14	0.58	0.70	0.24	1.83	0.50	0.95	0.23	0.55	0.23	0.67	1.45	0.90	3.15	1.04	0.02	0.61	1.47	0.00	0.26	5.38	1.30
12	3.31	0.27	0.59	0.15	1.67	0.00	0.47	0.27	0.59	0.18	0.33	1.54	0.81	3.01	0.98	0.01	0.44	1.01	0.00	0.23	0.34	1.25
13	2.41	0.61	0.61	0.17	1.77	0.00	0.29	0.42	0.59	0.13	0.56	0.93	0.79	2.47	0.70	0.01	0.45	0.63	0.00	0.16	0.08	1.29
14	1.40	0.54	0.52	0.19	1.16	0.79	0.26	0.46	0.51	0.06	0.49	0.95	0.52	2.11	0.66	0.01	0.23	0.42	0.00	0.13	0.05	1.29
15	0.95	0.21	0.39	0.17	0.88	0.00	0.00	0.00	0.52	0.08	0.27	0.68	0.58	1.54	0.58	0.03	0.27	0.36	0.00	0.10	0.04	1.28
16	0.65	0.27	0.32	0.15	0.83	0.00	0.12	0.00	0.30	0.07	0.41	0.51	0.47	1.27	0.47	0.01	0.25	0.26	0.00	0.12	2.42	1.26
17	0.50	0.20	0.27	0.08	0.68	0.00	0.00	0.00	0.29	0.15	0.29	0.40	0.42	0.93	0.37	0.01	0.22	0.24	0.00	0.13	0.21	1.30

Table 5.2: Sequencing of DQB1*0301 ligands. The pools were sequenced by Edman degradation. The numbers indicate pmols of individual amino acids residues detected at each cycle. SMC = S-methyl cysteine; nl = norleucine (standard); nv = norvaline.

Based on the above pool sequence data, the peptide ligand for HLA DQA1*0101/**DQB1*0501** is as follows:

								(Leu)			
								(Val)			
						(Leu)		(Thr)	(Ile)		
					(Asn)	(Ile)		(Gly)	(Asp)		
Sequence:	X	- (Pro)	- X	- (Gln)	- (Arg)	- (Phe)	- X	- Ala	-Leu	-	X
CYCLE # :	1	2	3	4	5	6	7	8	9		10

		(Glu)						(Thr)			
		(Gly)				Val		(His)			
Sequence:	Ala	- Val	- X	- Arg	- Tyr	- Tyr	-	-	-		Thr
CYCLE#	11	12	13	14	15	16		17			

This sequence shows that proline gives an outstanding signal at position 2. This most likely reflects the consequence of processing and not of MHC-binding requirements. In support of this notion is the absence of the influence of Pro residues in peptide binding studies[268,315]. This Pro residue might protect the epitope from degradation by exopeptidases some of which, like aminopeptidase N, are hindered by prolines.

Based on the crystal structure of HLA-DR1, the peptide binding groove usually anchors a stretch of peptides which in most cases is nine amino acids long (P1 to P9). The number of amino acids between the amino terminus and P1 differs considerably between different ligands (0-10). Sequencing studies of pools of class II ligands that have had those peptides with a high copy number removed have indicated that the distance from the amino terminus to P1 is 3+/- 1 amino acids for the majority of peptides[267]. There have been only one previously reported motif for HLA-DQ1 (DQA1*0101/DQB1*0501)[293] and one for HLA DQ7 (DQA1*0501/DQB1*0301)[267]. The latter report suggested that proline gives an

outstanding signal at position 2. The DQ7 motif was shown to have three anchors focused at positions 5, 9, and 11. The first and the last are dominated by aromatic residues, whereas the middle one is mainly aliphatic and only four intermittent polar clusters were found.

The DQB1*0501 motif is characterized by Asn/Arg (amidic amino acid and amino acid with basic or positively charged side chain) at P1 (absolute position 5), suggesting that a small or polar amino acid is preferred at this position. The central amino-acids are mainly aliphatic/aromatic. P5 (absolute position 9) is the focus of a hydrophobic cluster with a small contribution by small polar residues. There is another cluster of aromatic residues towards the C- terminus.

Based on the above pool sequence data, the peptide ligand for HLA-DQA1*0301/**DQB1*0301** is as follows:

			(Gly)			(Gln)	(Gly)	(Leu)	(Ser) (Pro)
			(Ser)		(Arg)	(Val)	Ala	(Pro)	(Ile) (Val)
Sequence:	X -	Pro -	X -	Glu -	Thr -	Ala -	Tyr -	X -	X
CYCLE # :	1	2	3	4	5	6	7	8	9

	(Val)	(Ile)	(Pro)			
Sequence:	Thr -	X -	X -	X -	HIS	
CYCLE#	10	11	12	13	14	

This sequence also shows that proline gives an outstanding signal at position 2. This is similar to the sequence of HLA DQB1*0501 and most likely reflects the consequence of processing and not of MHC-binding requirements.

In this study, the pool sequencing data beyond Pro at position 2 shows that the DQB1*0301 motif is characterized by Thr/Arg (small polar amino acid and amino acid with positively charged side chain) at P1 (absolute position 5), suggesting that a small or polar amino acid is preferred at this position. The central amino-acids are mainly aliphatic/aromatic. P5 (absolute position 9) is the focus of an aliphatic cluster with a small contribution by small polar residues. Proline gives a signal again at absolute position 12 which is towards the C-terminus. This suggests trimming of the peptide also by carboxypeptidases which are also hindered by proline.

5.3 PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM E6, E7, L1 AND L2 of HPV 16 to HLA DQB1*0301, AND DQB1*0501

On the basis of the peptide sequence data above, the E6, E7, L1 and L2 proteins of HPV 16 were examined to identify sequences which are likely to bind to HLA DQB1*0301 and DQB1*0501. The examination was performed systematically in an overlapping fashion for 13-mer peptides.

The motif analysis of the HPV 16- E_7 protein (98 amino acids) shows that a total of 26 peptides possess at least one preferred residue at P1 or P5 for binding to HLA DQB1*0301. However, since most HLA molecules require two to three anchors in a peptide for optimal binding, further analysis revealed that two of these peptides (7.7%) possess the preferred amino acid residues at both P1 and P5. These are **$E_7$68-80: CVQSTHVDIRTLE**; and **$E_7$82-94: LLMGTLGIVCPIC** (Table 5.3). By contrast, a total of 28 peptides derived from HPV 16-E_7 posses at least one preferred residue at P1 or P5 for binding to HLA DQB1*0501 and one of these (3.6%), **$E_7$73-85: HVDIRTLEDLLMG**, possess the preferred residues at P1 and P5 (Table 5.4).

In the analysis of HPV 16-E_6 (158 amino acids), a total of 43 peptides possess at least one preferred residue at P1 or P5 for binding to HLA DQB1*0301. Of these, four peptides (9.3%) possess the preferred residues at both P1 and P5. These are **$E_6$25-37: ELQTTIHDIILEC; $E_6$51-63:DFAERDLCIVYRD; $E_6$58-70: CIVYRDGNPYAVC;** and **$E_6$127-140: DKKQRFHNIRGRW** (Table 5.5). In the case of HLA DQB1*0501, a total of 46 peptides from HPV 16 E6 are probably capable of binding and four of these (8.7%) possess the preferred residues at P1 and P5 (Table 5.6).

In the analysis of the L1 protein of HPV 16 (531 amino acids), a total of 166 peptides posses at least one preferred residue at P1 or P5 for binding to HLA DQB1*0301. Of these, 14 peptides (8.4%) possess the preferred residues at both P1 and P5 (Table 5.7). The comparable predicted motifs for HLA DQB1*0501 show that a total of 128 peptides that are probably capable of binding to HPV 16 L1 derived peptides with 11 peptides (8.6%) possessing the preferred residues at P1 and P5. These peptides are listed in table 5.8.

Motif analysis of the HPV 16 L2 protein (473 amino acids) for binding to HLA DQB1*0301 revealed that a total of 216 peptides posses at least one preferred residue at P1 or P5. Of these, 22 peptides (10.2%) possess the preferred residues at both P1 and P4. These peptides are listed in table 5.9. Comparative analysis of binding to HLA DQB1*0501 reveal a total of 119 peptides with at least one preferred residue, and 7 of these (6.0%) possess the preferred residues at P1 and P5 (Table 5.10).

The finding that there are no significant differences in the number of HPV 16 derived peptides that are probably capable of binding the HLA DQB1*0501 molecule compared with HLA DQB1*0301 supports the notion that the protective effect of the former on HPV associated disease relates more to peptide binding affinity. In this way, the HLA

DQB1*0501-HPV peptide complex may lead to the generation of a more effective immune response.

Table 5.3: The predicted peptide motifs from HPV 16-E7 to HLADQB1*0301.

HPV 16 E7	Peptide Sequence (DQB1*0301)
68-80	CVQSTHVDIRTLE
82-94	LLMGTLGIVCPIC

Table 5.4: The predicted peptide motifs from HPV 16-E7 to HLADQB1*0501.

HPV 16 E7	Peptide Sequence (DQB1*0501)
73-85	HVDIRTLEDLLMG

Table 5.5: The predicted peptide motifs from HPV 16-E6 to HLADQB1*0301.

HPV 16 E6	Peptide Sequence (DQB1*0301)
25-37	ELQTTIHDIILEC
51-63	DFAERDLCIVYRD
58-70	CIVYRDGNPYAVC
127-139	DKKQRFHNIRGRW

Table 5.6: The predicted peptide motifs from HPV 16-E6 to HLADQB1*0501.

HPV 16 E6	Peptide Sequence (DQB1*0501)
11-23	DPQERPRKLPQLC
43-55	QLLRREVYDFAFR
51-63	DFAFRDLCIVYRD
127-139	DKKQRFHNIRGRW

Table 5.7: The predicted peptide motifs from HPV 16-L1 to HLADQB1*0301.

HPV 16 L1	Peptide Sequence (DQB1*0301)
32-44	PSEATVYLPPVPV
46-58	KVVSTDEYVARTN
63-75	AGTSRLLAVGHPY
93-105	GLQYRVFRIHLPD
117-129	YNPDTQRLVWACV
151-163	VGISGHPLLNKLD
166-178	GVDNRECISMDYK
198-210	GSPCTNVAVNPGD
285-297	HLFNRAGTVGNV
316-328	NYFPTPSGSMVTS
439-451	TLEDTYRFVTQAI
453-465	CQKHTPPAPKEDD
467-479	LKKYTFWEVNLKE
507-519	TLGKRKATPTTSS

Table 5.8: The predicted peptide motifs from HPV 16-L1 to HLADQB1*0501

HPV 16 L1	Peptide Sequence (DQB1*0501)
15-27	ENDVNVYHIFFQM
78-90	LKKPNNNKILVPK
79-91	KKPNNNKILVPKV
93-105	GLQYRVFRIHLPD
131-143	VEVGRGQPLGVGI
146-158	HPLLNKLDDTENA
160-172	AYAANAGVDNREC
165-177	AGVDNRECISMDY
292-304	TVGENVPDDLYIK
417-429	ITLTADVMTYIHS
488-500	FPLGRKFLLQAGL

Table 5.9: The predicted peptide motifs from HPV 16-L2 to HLADQB1*0301.

HPV 16 L2	Peptide Sequence (DQB1*0301)
6-18	SAKRTKRASATQL
65-77	GTGGRTGYIPLGT
66-78	TGGRTGYIPLGTR
81-93	TATDTLAPVRPPL
90-102	RPPLTVDPVGPSD
117-129	AGAPYSVPSIPPD
135-147	ITTSTDTTPAILD
137-149	TSTDTTPAILDIN
178-190	GGHFTLSSSTIST
196-208	IPMDTFIVSTNPN
205-217	TNPNTVTSSTPIP
227-239	PNTVTSSTPIPGS
247-259	TKLITYDNPAYEG
261-273	DVDNTLYFSSNDN
293-305	ALTSRRTGIRYSR
298-310	RTGIRYSRIGNKQ
339-351	IELQTITPSTYTT
373-385	FITDTSTTPVPSV
375-387	TDTSTTPVPSVPS
376-388	DTSTTPVPSVPST
417-429	PINITDQAPSLIP
455-467	LRKRRKRLPYFFS

Table 5.10: The predicted peptide motifs from HPV 16-L2 to HLADQB1*0501

HPV 16 L2	Peptide Sequence (DQB1*0501)
188-200	ISTHNYEEIPMDT
267-279	YFSSNDNSINIAP
269-281	SSNDNSINIAPDP
272-284	DNSINIAPDPDFL
298-310	RTGIRYSRIGNKQ
359-371	TSINNGLYDIYAD
454-466	MLRKRRKRLPYFF

5.4 PREDICTION OF PEPTIDE MOTIFS FOR BINDING FROM L1, L2, E6 and E7 of HPV 16 to HLA DRB1*0101 AND DRB1*0401

The requirements for peptide binding to several HLA DR alleles have been defined by the analysis of peptide analogs and have been shown to be remarkably simple[168,170,268 315-318]. Other than the peptide backbone, only a single hydrophobic amino acid side chain appears to be critical. This was initially demonstrated in an analysis of monosubstituted analogues of an influenza hemaglutinin peptide to bind DRB1*0101[168,317]. Of the twelve residues examined, only substitutions for a tyrosine near the amino terminus dramatically reduced binding. The importance of this single side chain was established unequivocally by demonstrating that a peptide of equal length as the natural hemaglutinin sequence, but with all amino acids other than the tyrosine and a single lysine replaced with alanine, bound both DRB1*0101 and DRB1*0401 better than the natural peptide. The ability of simplified analogues to bind as well, if not better than, the parent T cell determinants suggest that the other amino acids in the peptide ligand either made minor contributions, were neutral, or were deleterious to binding.

More extensive quantitative studies using simplified polyalanine peptides have demonstrated for binding to both DRB1*0101 and DRB1*0401 that:

i The optimal position for the hydrophobic amino acid in the context of a thirteen amino-acid peptide was the third position (P3).

ii The structural requirements at P3 were quite tolerant, with aromatic being superior to aliphatic side chains for binding to DRB1*0101 and DRB1*0401

iii An analog with tyrosine at P3 bound with an IC_{50} value three orders of magnitude lower than a peptide with alanine at this position, and more than five orders of magnitude lower than peptides containing polar amino acids at this position[317].

The minimal requirements for binding shared by eight DR alleles suggested that the important hydrophobic side chain interacted with a subsite composed principally of conserved residues[318]. An appropriate site was the relatively deep hydrophobic pocket in the region of residues 24α, 26α, 54α of the α-chain and 86ß of the ß-chain. The only polymorphic amino acid present in this subsite was 86ß which is either a glycine or valine in the DR proteins. The side chains in the peptides that appeared to be responsible for allele specificity were determined by correlating their common structural features with complementary polymorphic residues in the binding site. The importance of the peptide side chains was tested by incorporating them into a polyalanine backbone and was confirmed by the ability of these residues to transfer allele specificity to these simplified analogues. Although polymorphic contacts affect peptide affinity, the majority of the free energy of binding in all cases arose from interactions with the peptide backbone and the single hydrophobic amino acid at the third position. These constraints appear to orient all peptides in a similar location, forcing them to adopt a closely related conformation in the binding site. The corresponding side chain in each peptide contacted the same pocket in the binding site, regardless of the allele. This apparent similarity allows for the analysis of any DR allele by extrapolation from the DRB1*0101 crystal structure.

The data from the analysis of monosubstituted polyalanine peptides binding to a set of DR alleles indicated that the free energy of binding can be viewed as a simple sum of the interactions of the peptide side chains and backbone with distinct regions of the binding site[318-319]. Interactions of more than a single peptide side chain with a particular subsite of the binding cleft is unlikely because of the extended conformation the peptide adopts in the binding site. Thus, the free energy of binding can be represented as a simple polynomial with separate terms for backbone interactions and the side chains. Therefore, the apparent affinity of any sequence of common length should be predictable based on the rules of binding and data on the relative effects of the natural amino acids at each position.

A suitable database for this prediction has been constructed (Rothbard, J; Stanford University, USA, personal communication) and was used in this thesis to predict HPV peptide motifs for DRB1*0101 and DRB1*0401. The program is simple, written using Microsoft Excel and is able to parse any open reading frame into 13 amino acid peptides and calculate their affinity for DRB1*0401 and DRB1*0101.

In designing the database, the contributions of the individual side chains of a peptide from influenza hemaglutinin and tetanus toxin were measured by assaying analogs of AAYAAAKAAAAAA containing the corresponding amino acid at each position. This polyalanine peptide was chosen because alanine can be viewed as having a neutral side chain due to lack of size and charge. Consequently, any substitution could be viewed as advantageous, neutral or deleterious depending on its effect as compared to the parent peptide. Assuming that the tyrosine and the peptide backbone oriented all peptides equivalently, any differences in the IC_{50} value was due to the effect of the added amino acid. Multiplying each of the ratios for each analogue together resulted in a composite ratio, that when multiplied by the IC_{50} value of the parent peptide (14.7nM) resulted in a predicted IC_{50} value that was very close to experimentally determined value for both peptides. A thorough study of prediction has been done using the ratios derived from all possible monosubstituted analogues at the central eleven positions of a simplified peptide[320]. Prediction data from myelin basic protein, human serum albumin show that the predicted affinity in all cases, was within a factor of four of the experimentally derived value[320] Rothbard J, personal communication). This degree of error is comparable to that observed for the binding assays.

The peptide motifs of HPV 16 and their IC_{50} based on the approach described above are attached as appendix II. The motifs with IC_{50} less than 20nM are listed as follows: Tables 5.11 and 5.12 show the motifs derived from L1; Tables 5.13 and 5.14 show the motifs

derived from L2; Tables 5.15 and 5.16 the motifs derived from E7; and Tables 5.17 and 5.18 the motifs derived from E6.

Table 5.11: The predicted binding affinity of peptides derived from HPV 16-L1 to DRB1*0101. The first nine peptides are shown.

HPV 16 -L1	Peptide Sequence	IC50 DRB1*0101 (nM)
58-70	NIYYHAGTSRLLA	1.50
3-15	VTFIYILVITCYE	1.85
398-410	LQFIFQLCKITLT	2.85
414-426	MTYIHSMNSTILE	3.25
442-454	DTYRFVTQAIACQ	4.42
300-312	DLYIKGSGSTANL	5.05
317-327	SNYFPTPSGSMVT	6.40
59-71	IYYHAGTSRLLAV	7.25
159-171	SAYAANAGVDNRE	22.50

Table 5.12: The predicted binding affinity of peptides derived from HPV 16-L1 to DRB1*0401. The first five peptides are shown.

HPV 16 -L1	Peptide Sequence	IC50 DRB1*0401 (nM)
442-454	DTYRFVTQAIACQ	1.15
492-504	RKFLLQAGLKAKP	3.00
94-106	LQYRVFRIHLPDP	4.30
3-15	VTFIYILVITCYE	10.50
124-136	LVWACVGVEVGRG	21.00

Table 5.13: The predicted binding affinity of peptides derived from HPV 16-L2 to DRB1*0101. The first nine peptides are shown.

HPV 16 -L2	Peptide Sequence	IC50 DRB1*0101 (nM)
240-252	PAFVTTPTKLITY	2.70
444-456	GDFYLHPSYYMLR	3.35
52-64	GVFFGGLGIGTGS	7.20
301-313	IRYSRIGNKQTLR	11.50
200-212	TFIVSTNPNTVTS	13.00
445-457	DFYLHPSYYMLRK	13.00
70-82	TGYIPLGTRPPTA	14.00
418-430	INITDQAPSLIPI	19.00
161-173	PTFTDPSVLQPPT	32.00

Table 5.14: The predicted binding affinity of peptides derived from HPV 16-L2 to DRB1*0401. The first four peptides are shown.

HPV 16 -L2	Peptide Sequence	IC50 DRB1*0401 (nM)
46-58	LQYGSMGVFFGGL	9.80
266-278	LYFSSNDNSINIA	11.00
391-403	SGYIPANTTIPFG	12.00
444-456	GDFYLHPSYYMLR	20.20

Table 5.15: The predicted binding affinity of peptides derived from HPV 16-E7 to DRB1*0101. The first four peptides are shown.

HPV 16 -E7	Peptide Sequence	IC50 DRB1*0101 (nM)
81-93	DLLMGTLGIVCPI	45.20
80-92	EDLLMGTLGIVCP	230.00
52-64	YNIVTFCCKCDST	300.00
55-67	VTFCCKCDSTLRL	1120.00

Table 5.16: The predicted binding affinity of peptides derived from HPV 16-E7 to DRB1*0401. The first four peptides are shown.

HPV 16 -E7	Peptide Sequence	IC50 DRB1*0401 (nM)
21-33	DLYCYEQLNDSSE	105.00
9-21	HEYMLDLQPETTD	210.00
84-96	MGTLGIVCPICSQ	230.00
63-75	STLRLCVQSTHVD	452.00

Table 5.17: The predicted binding affinity of peptides derived from HPV 16-E6 to DRB1*0101. The first five peptides are shown.

HPV 16 -E6	Peptide Sequence	IC50 DRB1*0101 (nM)
59-71	IVYRDGNPYAVCD	4.00
33-45	IILECVYCKQQLL	12.00
142-154	RCMSCCRSSRTRR	12.50
75-87	KFYSKISEYRHYC	18.20
84-96	RHYCYSLYGTTLE	34.50

Table 5.18: The predicted binding affinity of peptides derived from HPV 16-E6 to DRB1*0401. The first four peptides are shown.

HPV 16 -E6	Peptide Sequence	IC50 DRB1*0401 (nM)
59-71	IVYRDGNPYAVCD	24.00
52-64	FAFRDLCIVYRDG	35.00
75-87	KFYSKISEYRHYC	83.00
86-98	YCYSLYGTTLEQQ	120.00

5.5 SUMMARY AND DISCUSSION

In this chapter, data obtained from immunoaffinity purification and sequencing of peptides bound to the susceptibility and protective HLA DQ alleles were used to predict peptide motifs from HPV 16. There are two limitations of this data. Firstly, sequencing of the individual peptide peaks obtained after rpHPLC would have provided additional information on both sequence match and protein source of the peptides. These peptides could then be aligned with the motif derived from the pool sequence to derive anchor residues with certainty. Indeed, an attempt was made to obtain this data using electrosplay ionization mass spectrometry (LC-ESI-MS/MS) but proper identification could not be made using the parameters described by Dongre et al (1997) because of insufficient samples (Data not shown). Secondly, in order to confirm the motif derived from pool sequencing, binding studies on synthetic variants of the eluted peptides could be performed. Nevertheless, the results suggest that many more peptide motifs are capable of binding to HLA DQB1*0501 than DQB1*0301. This may partly explain the mechanisms of the association of these alleles to HPV related cervical carcinogenesis (see also chapter 6).

For HLA DQ molecules, information on ligand specificity has only recently been available. Single-substitution experiments defined a simple motif for DQA1*0301/DQB1*0301 that was quite different from the motifs recognized by DR molecules[290]. Its prominent feature is the requirement of two small and/or hydrophobic residues spaced at relative positions i+2 and i+4. However, because these features can basically be found in almost every natural peptide frame, this motif is not suitable for predicting HLA-DQ ligands. Simple motifs have also been described for the autoimmune disease-linked HLA DQA1*0501/DQB1*0201[291,296,321] and DQA1*0301/DQB1*0302[295,322]. As for DQA1*0301/DQB1*0302, these motifs were different from the ones recognized by DR molecules. For example, no prominent position 1 anchors were found, as indicated by Ala-

substitution experiments. Both motifs consisted mainly of inhibitory residues, with the exception of a negatively charged anchor at residue at position 9.

The tendency of HLA DQ ligands to be less dependent on the interaction of peptide side chains with the class II cleft than HLA DR ligands has recently been confirmed by the determination of a quantitative matrix-based motif for DQA1*0501/DQB1*0301[323]. This motif revealed the ability of DQA1*0501/DQB1*0301 molecules to bind peptide structures without the involvement of large peptide side chains. Based on this finding, it was possible to modify DR-selected peptide repertoires such that they loose the binding capacity for HLA DR molecules and bind exclusively to the DQA1*0501/DQB1*0301, thus demonstrating, at least in part, a complementary function of HLA DR and DQ isotypes in antigen presentation[323]. These differential binding capabilities between HLA DR and HLA DQ may maximize the diversity of peptide repertoires available for T cell recognition. This may result in an additive positive or negative effect on the immune response depending on an individual's HLA class II haplotype.

Published data for the HLA DRB1*0101 and 0401 alleles were also used for motif prediction from HPV 16. Although a threshold of 20nM was arbitrarily chosen in this study to define peptides that bind with high affinity, it has been determined that an affinity threshold of 500nM determines the capacity of a peptide to elicit a CTL response in a series of HLA-A2 motif peptides evaluated in transgenic mice and in vitro recall responses in patients with acute hepatitis infection[324]. There is currently no available information on the affinity threshold for HLA class II. Nevertheless, in most cases, ranking of peptides according to binding affinity for a particular HLA allele seems to correlate with their immunogenic potential.

The results include information on the binding affinity of the peptides (IC_{50}) and show a number of interesting features. For the HPV 16 L1 protein, DRB1*0101 is capable of binding twice as many peptides with high affinity ($IC_{50}<20$) than DRB1*0401 (Tables 5.11 and 5.12). Even when these HLA molecules bind to the same set of peptides, the binding affinity to DRB1*0101 is several orders of magnitude higher than for DRB1*0401. For example, HPV16 **$L_1$58-70: NIYYHAGTSRLLA** binds DRB1*0101 with an IC_{50} of 1.5. The corresponding IC_{50} for DRB1*0401 is 105 (almost 100-fold difference).

HLA DRB1*0101 is also capable of binding twice as many L2 peptides with high affinity ($IC_{50}<20$) than DRB1*0401 (Tables 5.13 and 5.14). The peptide **$L_2$240-252: PAFVTTPTKLITY** that binds with the highest affinity to DRB1*0101 ($IC_{50}=2.70$), binds to DRB1*0401 with an IC_{50} of 4900, a difference of over 4,000-fold.

None of the HPV16 E7 derived peptides is capable of binding to either HLA DRB1*0101 or HLA DRB1*0401 with high affinity (i.e $IC_{50}<20$) (Tables 5.15 and 5.16). Nevertheless, the peptide that binds best to DRB1*0101, **$E_7$81-93: DLLMGTLGIVCPI** ($IC_{50}=45.20$) does so at two orders of magnitude better than the peptide with the highest binding affinity to DRB1*0401 (**$E_7$21-33: DLYCYEQLNDSSE; $IC_{50}=105.00$**). In addition, the IC_{50} for $E_7$81-93 with respect to DRB1*0401 is 710, an almost 20-fold difference.

Four HPV 16 E6 derived peptides are capable of binding with high affinity ($IC_{50}<20$) to HLA DRB1*0101 compared with none for HLA DRB1*0401. Peptide **$E_6$59-71: IVYRDGNPYAVCD** binds to DRB1*0101 with an IC_{50} of 4.00. The corresponding IC_{50} using the same peptide for DRB1*0401 is 24.00, a six fold difference in binding affinity.

In conclusion, there are significant differences in both the number and binding affinity of HPV derived peptides to the susceptibility and protective HLA DQ and DR alleles. Since peptide binding to HLA molecules is an important step in the generation of effective immune response, these differences would likely account for the observations of HLA associations with HPV induced cervical carcinogenesis. In addition, HLA molecules influence the choice between Th1 and Th2 response from CD4+ cells. A Th1 response is required to provide an adequate response to intracellular pathogens such as viruses. If a particular HPV-derived peptide is presented in the context of a susceptibility allele, it could induce a Th2 response, and disease could progress or persist. Since CD4+ T lymphocytes play a central role in the complex immune network that leads to antigen-specific reactivity, future studies (discussed in chapter 6) should be directed at identifying HLA class II specific immunodominant epitopes from HPV that may be useful for the prevention and treatment of CIN and cervical cancer.

CHAPTER 6: DISCUSSION

6.1 : SUMMARY

The primary objective of this project was to examine the association between HLA-DQ and -DR alleles, the human papillomavirus and premalignant disease of the uterine cervix. This was accomplished by HPV and HLA DNA typing. The latter consisted of three phases. The first phase involved the development of a novel polymerase chain reaction-restriction fragment length polymorphism (artificial restriction fragment length polymorphism) for HLA DQB1*03 typing[308]. The results show a significant association between CIN and DQB1*03 that is only slightly stronger for CIN III than CIN I. Homozygosity at the DQB1*03 locus, was significantly associated with CIN and was more strongly related than heterozygosity, a result not reported so far in any previous studies. A significant association with HPV positive CIN and DQB1*03 was found for all HPV types tested (16,18,31,33) and homozygosity at the DQB1*03 locus was strongly associated with HPV positive CIN.

The second phase consisted of polymerase chain reaction with sequence specific primers for HLA DQB1*03. The DQB1*0301 allele was shown to be most strongly associated with CIN and HPV, but 0302 was also positively related[308].

The third phase of HLA DNA typing involved polymerase chain reaction followed by sequence specific oligonucleotide hybridization with digoxigenin labeled probes using the 11th Histocompatibility Protocol with some modifications. This enabled the identification of susceptibility and protective HLA DQ-DR haplotypes in relation to human papillomavirus and premalignant disease of the cervix. The haplotype HLADRB1*0401-DQB1*0301 was shown to correlate with susceptibility to HPV and CIN while DRB1*0101-DQB1*0501 indicated protection[325].

In an attempt to further understand HPV antigen processing events, the final phase of the project consisted of immunoaffinity purification of the susceptibility and protective HLA DQ molecules and sequencing of the naturally processed peptide sequences bound to these HLA molecules. The data obtained was used for motif prediction of HPV 16 E6, E7, L1 and L2 sequences that are probably capable of binding to these HLA molecules. Motif prediction as well as the binding affinity of predicted peptide motifs for HLA DRB1*0401 and DRB1*0101, the DR alleles associated with susceptibility and protection respectively, was accomplished using published data on the naturally processed peptide sequences bound to these molecules. The data revealed significant differences in both the number and binding affinity of the HPV 16 derived peptides to the protective and susceptibility HLA molecules.

6.2: DISCUSSION

During the course of this project, a number of studies were published on the association between HLA class II and premalignant and malignant disease of the cervix. The purpose of this section is to discuss these studies including their merits and limitations. The results from these studies have been inconsistent, probably for the following reasons:

i. Differences in sample size.

ii. Difficulties in obtaining representative control groups.

iii. Methodological differences in HLA typing (obviated by PCR-based methods in more recent studies).

iv. Lack of information on HPV status of patients and controls in many studies.

In a report from Norway, Helland and co-workers[326-327] using polymerase chain reaction and a DNA hybridization technique, found that 67% of 213 patients with squamous cell carcinoma of the cervix carried the DQB1 gene encoding HLA DQB1*03 compared with 51% of 118 controls (RR=2.0, $p<0.002$). However, the report provided no information

on the HPV status of the patients and controls. Another report[328] on a population of 66 African American women with cervical cancer using a PCR based technique showed an increased risk with HLA DQB1*03 compared with 214 controls (RR 2.3, p= 0.004) and the risk was highest for HLA DQB1*0303 (RR 2.7, p= 0.017). Apple et al. [306] examined a Hispanic population of 98 women with cervical cancer and 220 controls. Although no association between cervical carcinoma and HLA DQB1*03 alone was found, an increased risk of cervical cancer was found with the DRB1*0407-DQB1*0302 haplotype (OR 2.19, p=0.030). The highest risk in the study was with the HLA DRB1*1501-DQB1*0602 haplotype (OR 2.87, p=0.005) and this increase was greater for HPV 16 positive cases relative to controls (OR 4.78, 95%CI 1.90-11.83; p=0.00007). The authors suggested that based on the co-occurrence of HPV16 and DRB1*1501-DQB1*0602, the combined relative risk was 75. In addition, protective haplotypes were identified, all in the DR 13 group. These were DRB1*1301-DQB1*0603, DRB1*1302-DQB1*0604 and DRB1*1303-DQB1*0301. The DRB1*1302-DQB1*0604 among HPV 16 positive cases was found to be strong enough to be significant independently (p=0.048). Although the control population in the study had normal cervical smears, the study suffers from the drawback that the HPV status of the control group was not examined. It is well recognized that between 5 -50% of women with normal cervical cytology may have HPV infection, and of these up to 50% may harbour high risk or oncogenic HPV infection[85].

Nawa et al[329] examined the HLA DQB1 frequency in 23 Japanese patients (age 23-35 years) with invasive squamous cell carcinoma of the cervix using a PCR-RFLP technique. Twenty patients (87%) carried a DQB1 gene encoding the HLA-DQB1*03 alleles, compared with 49.4% Japanese control subjects in the International Histocompatibility Workshop panel (p=0.0003). However, the correlation between DQB1*03 alleles and HPV infection was not statistically significant. The limitations of the study include the

small sample size, typing only for HPV 16 and 18, and the use of a control group that may not necessarily be comparable. By contrast, Amar et al[330] investigated HLA class II in a population of 30 Jewish patients with invasive squamous cell carcinoma of the cervix and compared with 400 local healthy controls. The results showed no significant association of any of the HLA DQ alleles with cancer.

A total of three other reports were published in 1996. Sastre-Garau et al[331] performed PCR-SSO reverse dot blot for HLA DRB1 typing and PCR-SSO for HLA DQ typing in a population of 126 French women with invasive squamous carcinoma of the cervix. Controls were 165 randomly selected individuals previously typed for HLA DR and DQ. The results showed a decreased frequency of the DRB1*1301/02 alleles in patients (11%) compared with controls (29%) (p=0.0004, OR=0.33) and the decrease was limited to HPV positive tumors. The haplotype DRB1*1301/02-DQA1*0103-DQB1*0601 was also lower in patients (2%) than in controls (9%) (p=0.001, OR=0.25) and the decrease was again limited to the HPV positive tumours. Although the study used PCR-based strategies for HLA and HPV typing, the major drawback is the limited information on the control group, as they may not necessarily be comparable.

In a Swedish study by Allen et al[332], 150 patients with invasive squamous cell carcinoma of the cervix were examined using PCR-based HLA and HPV typing. The results were compared with data from a general Swedish population and showed the DRB1*0401-DQB1*0302 haplotype to be positively associated with disease (p=0.05).

In contrast to the results of the above studies, Glew et al[333] reported no significant differences in HLA class II antigen frequencies in a group of 58 patients with squamous cell carcinoma of the cervix from Northwestern England. Further, the study showed no significant differences in the HLA antigen frequencies of patients with HPV 16 positive or

negative tumours. There were also no differences in antigen frequencies in relation to stage of disease. The control population in the study were 857 organ donors (347 males and 510 females) from the same geographical area. The relatively small patient group in the study may reflect a type -1 statistical error rather than the true biologic pattern of disease. Furthermore, the patient and control population are not necessarily comparable as the HPV status of the control group was not known. In a more recent report from Northwestern England[334] an HPV 16 oncogene variant leading to an amino acid change from arginine to glycine at position 10 from the E6 consensus start codon was identified in 32% (7 out of 22) of HLA B7 - positive patients. The altered sequence was not found in HLA B7 - negative individuals. Although the substitution could have a profound effect on the interaction of the epitope to HLA B7, binding studies showed that the variant peptide binds to HLA B7 in a similar manner to its wild type equivalent. However, computer modeling suggests that the alteration may affect the amino acid residues which are exposed for interaction with the T cell receptor. This raises the possibility that both the HLA type and/or presence of mutations in specific T cell epitopes of HPV oncoproteins act in concert to determine the risk of developing cervical carcinoma or progressing from low grade SIL to cancer.

As cervical intraepithelial neoplasia occurs at a stage prior to the development of cancer and is about 50 times as common, any HLA association identified will be important in establishing the role of immunological factors in the progression to invasive cervical cancer. Indeed it has been estimated that 20 - 30 % of CIN III progress to SCC in 5 - 10 years[11]. To date, five studies have specifically addressed this question. In a Belgian population, Vandenvelde et al[303] using an HLA DQB1*03 allele-specific oligonucleotide (ASO) primed fast PCR technique, found a significant difference between normal women (174/323=0.539) and CIN I (18/24=0.750, p=0.045) and CIN II patients (16/21=0.762, p=0.046) but not CIN III (15/26=0.577, p=0.707). The data from the study suggest a

greater risk of high risk HPV associated dysplastic transformation of the normal cervix in DQB1*03 positive women (RR=2.647, p=0.022), but not a higher risk of malignant transformation (RR=1.168, p=0.707). This conclusion is slightly different from the results of another study by David et al[335] from northwestern England which showed a higher risk of both dysplastic change as well as malignant transformation in HLA DQB1*03 positive women. Using the PCR-SSO technique, the DQB1*03 frequency in 50 patients with CIN compared with 49 age-matched controls without abnormal cervical cytology were 40% in CIN III, 22% in CIN I/II, 26% in cytology negative controls, and 21% in a local panel of blood donors (RR=2.5 for CIN III, p= 0.017). In another report by Apple et al[309], the frequency of distribution of HLA DR-DQ haplotypes among 128 Hispanic women with HPV 16 positive severe dysplasia was significantly different from a control population of 220 women, whereas severe dysplasia containing HPV types other than HPV 16 did not reveal any significant differences. The study also showed that the DR-DQ haplotypes previously found[306] to be associated with HPV 16 positive cervical carcinomas were also associated with HPV 16 positive severe dysplasia/CIS.

In a Swedish study, Sanjeevi et al[336] examined a population of 74 women with CIN and 164 controls using PCR-SSO for HLA DQ typing, low resolution PCR-SSP for HLA DR typing, and serological typing for HPV 16 and 6. The results showed increased risk of CIN in patients with DQB1*0602 compared with controls (OR 2.23, p<0.01), and the association was stronger for HPV 16 seropositive patients (OR 3.37, P<0.05). In addition, DRB1*15 was associated with disease (OR 2.20; p<0.01), stronger for HPV 16 seropositive patients (OR 5.82, p<0.05). The DQA1*0501-DQB1*0301 was also found to be increased among HPV seronegative patients. The study suffers significant drawbacks because cervical cytology (not biopsy) was used to diagnose CIN in the majority of cases and only 2 HPV types were tested serologically. Furthermore, serum antibodies against HPV 16 are absent in a significant proportion of patients with CIN and cancer[232].

Finally, Helland et al[337], in a Norwegian population-based case-control study examined 91 patients with histologically verified CIN grade II-III and 213 control subjects. The control population were randomly selected through the Central Population Register, and were without CIN at study entry. HPV typing was performed using PCR with general nested primers, followed by type specific primers for HPV 6, 11, 16, 18, 31, 33 and X. HLA DQA1 and DQB1 typing was performed using PCR-SSO. There were no differences in frequencies of the individual alleles when the cases were compared with controls. However, the haplotype DQA1*0102-DQB1*0602 was increased in HPV positive cases (OR 3.2; p = 0.02) and this association was stronger for HPV 16 positive cases (OR10.1, p = 0.01). It is unlikely that the discordance between the Norwegian and Swedish studies is due to genetic heterogeneity in HLA frequencies, and may reflect differences in HPV detection methodologies.

In the present study, the patients and control groups have been well characterized. The diagnosis of CIN was made by histological examination of material from women with abnormal pap smears. The control population were drawn from women attending the same clinics and had negative cytology. Furthermore, the latter population were further characterized to be negative for HPV 16, 18, 31 and 33. Thus the study comprises important subsets for analysis namely:

(i) HPV negative and CIN negative (controls).

(ii) HPV negative, CIN positive

(iii) HPV positive, CIN positive.

Analysis was performed for trend as well as for direct correlation. HLA DQB1*0301 and 0302 correlated significantly with increased risk for both CIN and HPV positive CIN. DQB1*0301 had the most significant association (O.R. 2.49; p<0.00001). The DR alleles that correlated significantly with increased risk of CIN were the DR4 group (O.R. 1.76,

p<0.001) principally DRB1*0401 (O.R. 1.99, p=0.002); DRB1*0403 (O.R. 3.61; P=0.02) and DRB1*0406 (O.R. 3.74-@; p=0.0007). In addition, DRB1*1101 also correlated with increased susceptibility to CIN (O.R. 2.31, p=0.004). These alleles were also found to correlate significantly with CIN3 and HPV positive CIN.

DRB1*0101 and DRB1*1301 were significantly associated with protection (O.R. 0.48 and 0.67 respectively) for both HPV infection and CIN. The most significant DQB1 allele associated with protection from HPV and CIN was DQB1*0501 (O.R. 0.48, P<0.005). Homozygosity at only the DQB1*0301 locus conferred an increased risk (O.R. 4.39, p<0.002).

In addition, the two locus haplotypes DRB1*0401-DQB1*0301 (O.R.2.22,p<0.01), DRB1*0401-DQB1*0302 (O.R. 1.90, p<0.05), DRB1*0403-DQB1*0302 (O.R. 4.34, p<0.01) and DRB1*1101-DQB1*0301 (O.R. 3.95, p<0.003) were significantly associated with HPV and CIN and indicated susceptibility. The haplotype which significantly correlated with protection from HPV positive CIN was DRB1*0101-DQB1*0501 (O.R. 0.48, p<0.01). The significant protective and susceptibility alleles were analysed to examine for associations with individual HPV types. HPV 16 was present in 75 (57%) of cases, HPV 18 in 9 (7%), HPV31 in 17 (13%), HPV 33 in 9 (7%), and multiple HPV types were detected in 21(16%). The best correlation was with HPV 16 and the susceptible haplotypes were DRB1*0401-DQB1*0301 (O.R. 2.63, p<0.02) and DRB1*1101-DQB1*0301 (O.R 5.80, p<0.001). There was a weak positive correlation with DRB1*1101-DQB1*0301 and HPV 31 (O.R. 6.44, p<0.05). The haplotype which conferred a protective effect did not show any significant correlation with any of the HPV types.

Of interest, the studies in which HLA class I frequencies were determined by serological typing in cervical cancer and controls showed an increase in HLA B12 and HLA B7[333,255] while there was a negative association with HLA B35. HLA B12 is known to be in linkage disequilibrium with DRB1*0401-DQB1*0301[286] suggesting that the observed increase in HLA B12 may be due to linkage disequilibrium rather than an independent effect. The negative association with HLA B35 may likewise be due linkage disequilibrium with DRB1*0101-DQB1*0501[286], which was found to be important in the present study.

The natural history of CIN has shown that the majority of low grade lesions regress spontaneously. The plurality of HPV types associated with low grade lesions is greater than that observed in high grade lesions or in invasive cancer, suggesting the existence of HPV-type specific regression mechanisms. Taken together, the evidence from this and other studies indicate that genetic factors are involved in the control of HPV-induced tumors. Although a familial trend has not been reported in cervical cancer, a familial aggregation has been reported in about 10% of cases of epidermodysplasia verruciformis, a disease characterized by a high susceptibility to cutaneous HPV[338]. The study of immunogenetic mechanisms controlling the regression or the development of genital neoplasia should shed light on mechanisms involved in the progression or regression of HPV-associated tumors.

6.3 MECHANISMS OF HLA CLASS II ASSOCIATION WITH HPV AND CIN

The significance of HLA association with cervical cancer is supported by data on the analysis of HLA class I and II using restriction fragment length polymorphisms in New Zealand rabbits infected with Shope cotton-tail rabbit papillomavirus, which showed a strong linkage between wart regressions and DR locus, and an increased risk of malignant transformation with the DQ locus[305]. A number of diseases have been associated with the

DQB1*03 group of alleles. These include autoimmune disorders as well as malignancies. The former group include the lupus-anticoagulant response[339], vitiligo[340], ocular cicatricial phemphigoid[341], and the herpes-associated form of erythema multiforme[342]. Malignant diseases associated with HLA DQB1*0301 include malignant melanoma[261], adult T-cell leukemia, T-cell lymphoma, human T-cell leukemia virus type 1 carrier state[343] and gastric adenocarcinoma[344].

There are at least three possible ways by which the association between DQB1*03 and HPV positive disease may be explained. Firstly, these women may present peptide antigen to CD4+ T cells ineffectively; secondly there may be clonal deletion of antigen specific T cell during thymic maturation may occur; or thirdly there may be active suppression of immune response to HPV in DQB1*03 positive women. Indeed, there is a high level of expression of HLA DQ in the thymic cortex[345] and a role for negative selection for HPV specific T cell clones would fit predisposition to HPV positive CIN by the DQB1*03 alleles.

The other possible mechanism is based on observations of HLA associated immunological low responsiveness to antigens such as streptococcal cell wall[346], schistosoma[347], mycobaterium leprae[348], tetanus toxoid[349] and hepatitis surface antigen[350] either after natural exposure or after vaccination. Despite the controversy regarding the function of suppressor T cells, there is evidence to suggest that HLA DQ maybe the preferred restriction element for immunological suppression mediated by CD8+ T suppressor cells[351-352]. It is possible that women who are positive for the DQB1*03 phenotype maybe unable to mount an effective cytotoxic T cell response against HPV infection. This is particularly important as it has been shown that HPV16 E7 is a target for cytotoxic T cells and to mediate tumour rejection[307].

There are several lines of evidence that cloned T suppressor (Ts) cells express conventional α and ß genes[353-354]. These clones respond to peptides presented in the context of MHC class I or II molecules. There are a number of ways by which this class of T cells can suppress immune responses in an antigen-specific manner. The first mechanism involves soluble antigen specific factors. These factors are comprised, at least in part, of some form of TCR α- and/or ß chains[355]. A second mechanism of suppression is cytotoxicity, which requires specific recognition of antigen-MHC complexes and is achieved by specific (cell mediated) or non-specific (cytokine-mediated) means. For example, it has been shown that CD8+ T cells can kill a CD4+ T cell line that mediates experimental autoimmune encephalomyelitis and neutralize their ability to mediate disease in vivo[356], and a Ts clone that kills T helper (Th) cells has been described[357]. Specific suppression could be achieved by killing T cells bearing clonally distributed TCRs (via recognition of TCR-peptide-MHC complexes).

In the case of HLA DR associations with HPV infection this may be due to polymorphisms in the second exon of the molecule. For example , position 86 of the β chain is dimorphic and the amino acids glycine (Gly) and valine (Val) are found at this position. The functional significance of the Gly/Val dimorphism at this position has been explained by the resolution of the tertiary structure of HLA DRB1*0101. β86 contributes to the formation of the 'hydrophobic' pocket and its substitution by valine restricts the size of the peptide side chain which can bind to HLA DRB1*0101 and therefore the peptide which can bind to the T cell receptor. A possible explanation for the protective effect of HLA DRB1*0101 in this study is that an immunodominant HPV epitope might contain a large hydrophobic side chain (Trp, Tyr, Phe, Leu, Ileu) as a major anchor in the β86 Gly pocket and this epitope binds with high affinity to DRB1*0101.

6.4 POLYMORPHIC STRUCTURAL FEATURES OF HLA-DQ MOLECULES ASSOCIATED WITH SUSCEPTIBILITY OR RESISTANCE TO HPV ASSOCIATED CIN

An attractive hypothesis for the molecular basis of the association between HLA DQ and HPV associated CIN may be based on the results of motif analysis in chapter 5: HPV epitopes bind with higher affinity to the resistant than to susceptible DQ molecules, leading to a more effective elimination of the virus. Although this hypothesis is not contrary to current immunological paradigms, very little is known about the function of DQ molecules. They are only constitutively present on a subfraction of antigen-presenting cells, and at much lower density than DR molecules[358]. However, their expression on APCs can be induced by gamma interferon[359], and infectious agents such as Epstein Barr Virus[360] and Human Papillomavirus[361]. It has been postulated that DQ molecules exercise epistatic action over DR molecules[362] and that they are the mediators of immunosuppression[351].

In order to examine this hypothesis of differential binding to susceptibility and protective alleles, a model of the structure of HLA DQ molecules based on the HLA DR1 structure can be inferred[363]. This model suggests that they possess the mould of a class II histocompatibility molecule with an antigen-binding groove in the $\alpha 1\beta 1$ domain that is bounded by a "floor" of eight β-sheets and two "walls" of antiparallel α-helices. There is an $\alpha 2\beta 2$ domain that contains the homodimerisation region, the CD4-binding area and the Arg-Gly-Asp loop. This domain and the $\alpha 1\beta 1$ domain dimerise in some alleles with their counterparts in an identical DQ molecule forming a homodimer of $\alpha\beta$ heterodimers.

The polar residues lining the antigen binding groove of DR1 and participating in hydrogen bonding with antigenic peptide are located in exactly the same positions in modeled molecules[164-165], with nearly identical orientations[348]. Within the binding groove are five pockets which can trap specific residues of antigenic peptides. The first pocket of DQ

molecules (formed by α10, 27, 34, 35, 46 and β85, 86, 89 and 90) is either amphiphilic or hydrophilic, as judged from the amino acids that line-up this formation. The hydrophilic variant of this pocket appears shallower, because of the presence of bulky residues in β85, 86 and 89 (Leu, Glu and Thr instead of Val, Ala, and Gly respectively for the amphiphilic variant). The character of the first pocket is also modified by residue α34 (Glu or Gln). Thus, the HLA DQB1*0501, which is protective for HPV associated CIN in this study has valine, alanine and glycine in the β85, 86 and 89 positions respectively and is therefore amphiphilic. By contrast, the susceptibility alleles HLA DQB1*0301, 0302 and 0303 have leucine, glutamate and threonine in the same positions and are therefore hydrophilic.

The second HLA DQ pocket appears to be the most prominent or anchoring pocket. This is probably due to the presence of small residues in position α9 and β13 of DQ molecules in contrast to the bulky glutamine or phenylalanine respectively in DR1. There is extensive polymorphism in the four residues from the β1 helix of this pocket (70, 71, 74 and 78). In the case of HLA DQB1*0501, which correlates with resistance to HPV associated CIN, these residues consist of glycine, alanine, serine and valine respectively. All the DQB1*03 susceptibility alleles have arginine, threonine, glutamate and valine in these positions and the size of these residues may restrict peptide binding to an immunodominant HPV epitope.

The presence of aspartate in β57 which is part of the fifth pocket is a major determinant of peptide affinity[318]. This residue consists of valine in HLA DQB1*0501. HLA-DQB1*0302 and DQB1*0303 differ only in Ala to Asp polymorphism at codon 57, whereas DQB1*0301 encodes Asp 57 and three additional polymorphisms at positions 13, 26, and 45. The aspartic acid residue on DQB1*0301 and 0303 likely interacts with an arginine at residue 79 of the DQA1 chain to form a salt bridge by analogy with a similar structure of HLA-DR1[165]. Although an influence of this potential salt bridge has been

suggested[322,364], it does not appear to significantly influence the HLA-DQ3 association with HPV infection and CIN since all three alleles result in increased risk of disease.

There are important differences in the β49-56 dimerisation patch[164] of all the HPV associated susceptible DQ molecules when compared with the patch of the DQB1*0501 resistant molecule. In the DR molecule, there is a monomorphic dimerisation patch, with dimerisation probably promoted after T cell receptor binding[164], by symmetrical salt bridges between β52Glu of one heterodimer and β55Arg of the opposite heterodimer. By contrast DQ alleles are polymorphic in this region leading to β49-56 sequences that may be very hydrophobic, amphiphilic or hydrophilic. The alleles DQB1*0301, 0302 and 0303 have a hydrophilic patch. On the other hand, the protective DQB1*0501 allele is amphiphilic. It contains an arginine at position 55, and glutamine in position 53 and proline at position 56, right opposite each other at the first turn of the β1 helix. The relative ease of homodimerisation by the protective DQB1*0501 molecule means that in case any cognate T-cell clones exist in the periphery, their activation upon recognition of this protective DQ molecule complexed with an HPV peptide would be easy. By contrast, susceptible DQ molecules will form homodimers with more difficulty leading to less effective activation of cognate T-cell clones. In the case of the α2β2 homodimerisation domain, it involves a large surface area of the DQ molecule and the dimerisation is stabilized by multiple interactions involving charged and hydrophobic residues[164,363]. There appears to be no difference between susceptible and protective DQ molecules in this domain.

The CD4 binding area is formed by the homodimerisation of DQ molecules (β2 of one DQ heterodimer to α2 of another DQ heterodimer), and has been shown in DR to be composed of the sequence β134-148 and several residues on the alpha chain apposed to this sequence. Of the residues shown to be critical for CD4 binding to HLA DRβ, by site directed mutagenesis (β137Glu, 142Val, 143Val)[365], all remain invariant in the DQB

alleles suggesting that this region of the DQ molecule is unlikely to be important in determining susceptibility or resistance to HPV.

The Arg-Gly-Asp loop on ß167-169, present in the protective allele, HLA DQB1*0501, is absent in HLA DQB1*0301 where β167 is histidine. The exact function of this RGD loop in DQ molecules is unknown but probably functions in cell adhesion as in other integral membrane proteins and proteins of the extracellular matrix involved in such function[366-367], and may be important in the DQ restricted T-cell clone activation.

A scheme where the three structural features of the DQ molecules segregate in the two phenotypes of susceptible and protective HLA-DQB alleles that confer susceptibility or protection to HPV-associated CIN is shown in table 6.1. The difference in the physicochemical properties of the antigen-binding groove of susceptibility and protective DQ alleles would translate into different affinities for an "immunodominant" epitope in HPV. Such differences would certainly play a role both in the ontogenesis of the immune system and in the mounting of a specific DQ restricted immune response in the mature organism. Indeed, the human embryonic thymus is very rich in DQ molecules that probably function as restriction elements[368]. Therefore, the CD4+ T-cell clones recognizing the combination of susceptible DQ molecules with its bound peptide epitope would be eliminated. In the periphery, the susceptible DQ molecules expressed under proper stimulation on antigen presenting cells could bind to HPV derived peptide(s) and present such complexes to cognate CD4+ T cells. The ensuing immune reaction may be insufficient for viral clearance.

The dominant effect of the protective DQ molecule in the periphery could be exercised in the same manner. The differences in the physicochemical character of the antigen-binding groove assures preferential peptide binding. The difficulty of dimerization by the

susceptibility molecules ensures that even though the peptide has been trapped, the activation of cognate T-cell clones that might have escaped elimination in the thymus would be very difficult.

It is to be noted that in offering an explanation for the involvement of HLA DQ molecules in the susceptibility to HPV-associated CIN based on HLA structural features, account is not taken of the polymorphisms of DQ molecules in the intracellular amino acid sequences that participate in signal transduction[369], or the possible differences in the level of expression of various DQ alleles. Also, a number of regulatory sequences have significant effects on DQB genes[370] and their possible role in the HPV induced cervical carcinogenesis is unknown.

Domain	Features	Character	DQB1*03 (Susceptible)	DQB1*0501 (Protective)
α1β1	Antigen binding groove	Polymorphic		
	First residue binding pocket	Dimorphic, hydrophilic, amphiphilic	Hydrophilic	Amphiphilic
	Residue at β57	Polymorphic	0301 Asp, 0302 Ala, 0303 Asp	Valine
	β49-55 dimerisation patch	Polymorphic, very hydrophilic, amphiphilic, hydrophilic		Amphiphilic
α2β2	CD4 binding region		No discernible differences	
	β167-169 RGD loop	Probably involved in cell adhesion	Absent in 0301	Present

Table 6.1: Summary of the structural features of HLA DQB1*03 and HLA DQB1*0501.

6.5 CLINICAL IMPLICATIONS : HLA TYPING AND VACCINE DEVELOPMENT

This study raises the question as to whether women infected with HPV will benefit from HLA typing to predict disease susceptibility and/or severity. At the present, there is no data to suggest that information on HLA type will alter current clinical practice. However, the information would be useful in a research setting in screening programmes, evaluation of treatment outcome (surgery, radiation therapy, and chemotherapy), on-going vaccination trials as well in the design of novel immunomodulatory strategies for the prevention and treatment of HPV associated cervical cancer.

Screening with the Papanicolaou smear remains the best available method of reducing the incidence and mortality of invasive cervical cancer. There are large numbers of women with Papanicolaou smears showing squamous intraepithelial lesions (SIL) each year. Only a minority of these women will progress to invasive cancer, and it would be advantageous to develop predictive markers to identify those women. The need for predictive markers is even more important in the category of patients with atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells of undetermined significance (AGUS) and low-grade SIL (LGSIL), lesions that are usually managed expectantly. However, Cox et al[371], Wright et al[372] and Kinney et al[373] reported that 6.9%, 6.1% and 7.3% of women with ASCUS cytology, respectively harboured histologic high-grade SIL (HGSIL). Attempts to improve the triage of these women with HPV typing assays have yielded conflicting results. The addition of HLA class II typing may allow "low-risk" women (with ASCUS, AGUS, LGSIL or HGSIL) to avoid costly and potentially morbid diagnostic and therapeutic procedures. Further, women in the different categories could be followed longitudinally over several years to determine the effects of HLA type on the natural history of disease.

Another major clinical impact of this study is likely to be in the area of vaccine design for HPV associated CIN and cervical cancer. The rationale for the use of HPV epitopic determinants as prophylactic and therapeutic cancer vaccines is supported by the following:

i. In a recent study utilizing the sorting signal of the lysosomal-associated membrane protein-1 (LAMP-1) to reroute HPV 16-E7 into the MHC class II processing pathway, there was enhanced presentation to CD4+ cells, greater E7 specific lymphoproliferative activity, antibody titres, and CTL activity[374].

ii. Using the LAMP-1 / HPV E-7 chimera expressed in a recombinant vaccinia virus, Lin et al[375] showed that 80% of vaccinated mice remained tumour free 3 months after injection compared to progressive tumour growth in all wild type E7 injected mice. Further, vaccination cured mice with small established tumours, whereas the wild type E7 vaccinia showed no effect on established tumour.

iii. It has been recognized that in the case of other tumours, especially human melanoma, systemic administration of melanoma-associated antigens (MAA) derived peptides can elicit anti-tumor CTL activity in-vivo[376-377].

As an increasing number of HPV 16 and 18 epitopes are reported, the practical question to be raised is which, given limited resources, should be given priority for clinical trials. The identification within the context of a specific HLA restriction element of the *immunorelevant* antigen among the repertoire of several possible HPV peptide molecules may allow a more focused selection of the most appropriate target antigen for vaccination. The HPV peptide epitopes identified in this study (chapter 5) as probably being capable of binding with high affinity to both susceptibility and protective alleles may be utilized in in-vitro and in-vivo vaccine design experiments using the LAMP-1 sorting signal to route the peptides into the HLA class II pathway.

The processing of endogenous HPV proteins for class II restricted presentation is of considerable practical interest because it allows direct recognition of HPV infected cells by CD4+ cells. Although CD4+CD8- T cells are often referred to as belonging to the helper/inducer subset, this population is heterogeneous in terms of their effector functions such as lymphokine production and secretion or cytolysis. In humans, CD4+ cytotoxic T cells have been described as an effector population in a variety of viral infections including EBV[378], hepatitis B [379] and herpes simplex[380]. Furthermore, CD4+ T cells have been shown to be critical in generating immune responses against several solid malignancies in murine[381-382] and human systems [383-384]. Given that an appropriate peptide epitope in association with HLA molecules might be expressed on the surface of infected cells, lysis of these cells by CD4+ cytotoxic T cells is likely to be an important mechanism for the protection against persistent HPV infection.

The advantages of peptide vaccines include stability, ease of preparation, transportation, and injection; and they do not pose the biological risks that may occur with the use of intact proteins. The potential disadvantages include the requirement for knowledge of the epitopic determinant for each HLA allele, the potential limitation of the targeting of only one restriction element among several expressed by a given tumour, and dependence for immunogenicity on the stability of the peptide/HLA complex[385]. Although the use of peptide mixtures that will bind to several class I alleles may overcome some of these problems, the immunogenicity of these mixtures will need to be determined. Since MHC class II peptide binding exhibit allele specificity as well as promiscuity, knowledge from studies of class II association with HPV infection should lay the framework for the development of "promiscuous" immunogenic peptides that would be presented via the class II pathway.

Vaccination trials in mice and rats have clearly demonstrated the feasibility of inducing immunity that can protect against the growth of HPV 16 containing tumours. A number of immunization approaches have been used with varying degrees of success. It was shown that immunization of C3H/HeN mice with syngeneic fibroblasts transfected with HPV 16 E7 gene conferred protection against E7 transfected syngeneic tumour cells[307]. Similarly, immunization of mice with HPV 16 gene transfected fibroblasts induced regression of transplanted tumours expressing E6[386]. Populations of CTL isolated from the spleens of mice which rejected the tumour challenge were shown to specifically lyse E6 expressing target cells in vitro. Meneguzzi et al[387] used recombinant vaccinia virus expressing E6 or E7 to immunize rats which were then challenged with cells co-transformed with HPV 16 and Ras. The study showed tumour development to be delayed or prevented in immunized rats. Vaccines based on recombinant live virus have the advantage of physiologic antigen delivery and is not HLA haplotype dependent since different HLA alleles will select different peptides from the naturally processed peptide pool. The major disadvantage is safety concern but this may be obviated by designing vaccines with non transforming mutant variants without compromising the immunogenicity of the parent protein.

In another approach, synthetic peptides corresponding to residues 49-57 of the E7 protein were used to immunize C57BL/6 mice and they showed complete or partial protection against tumour formation by transformed cells containing HPV 16 and Ras[388]. The immunogenicity of HPV 16 E6 and E7 proteins were analyzed extensively by Kast et al[389]. A set of 240 nonamer peptides derived from E6 and E7 were synthesized and tested for binding to several of the most common HLA-A alleles. From these studies, a number of high affinity binding peptides were determined and the immunogenicity of these peptides was tested *in vivo* by immunization of HLA-A2.1$^+$ transgenic mice and *in vitro* by stimulation of CTLs from normal HLA-A2.1+ human peripheral blood lymphocytes[390]. Four high-affinity binding peptides were immunogenic in the transgenic mice and three of

these peptides were also immunogenic to CTLs from normal donors. Human HLA-2.1-restricted CTL clones specific for these peptides were able to recognize and lyse peptide-pulsed targets as well as HLA-A2.1+ cervical carcinoma cell line CaSki that expresses the HPV-16 E6 and E7 genes. These results suggest that these peptides are naturally processed T cell epitopes of HPV-16 and may act as cervical carcinoma tumour antigens.

There are at least six human papillomavirus vaccine trials that have been initiated worldwide in the past 12 months. Although the growing number of HPV vaccine trials has raised hopes for the future of vaccine therapy in cervical cancer, differences among the trials make the details of that future still far from clear. All of the trials are small phase I or phase I/II studies, and are all testing vaccines against HPV 16 and 18. There is however an array of different vaccine formulations and a variety of patients, as seen in the following summary of the studies:

1. National Cancer Institute (NCI) phase I trial of HPV 16 E7 lipopeptide vaccine for recurrent or refractory cervical cancer. In this study, a vaccine consisting of a lipidated HPV E7 peptide epitope (Cytel Corporation, San Diego) linked to a nonspecific helper peptide (PADRE) is used in HLA-2 and HPV 16-positive patients with recurrent or refractory cancer.

2. NCI phase II pilot study of HPV 16 E6 and E7 peptide vaccines for advanced or recurrent cervical cancer. The trial involves the use of antigen-presenting cells pulsed with synthetic peptide corresponding to the tumour's HPV 16 E6 or E7.

3. Multicenter European trial using a vaccine as an adjunct to surgery and radiation therapy in women with early stage invasive disease.

4. Two HPV vaccine trials, one at the University of Wales in Cardiff and the other at the Norris Cancer Center of the University of Southern California, Los Angeles - will give HPV vaccines to women with high grade preinvasive lesions (CIN2/3).

In the future, the development of a prophylactic or therapeutic vaccine for cervical cancer may offer an attractive and cost-effective immunologic approach to reduce the need for expensive screening and surveillance prevention programs and substantially decrease the worldwide morbidity from this disease.

6.6 CONCLUSIONS

Genetic variation at the HLA loci accounts for differences in immune recognition between individuals and similarly underlies differences in disease susceptibility to HPV associated CIN. One of the important functional consequences of this genetic variation is the generation of distinct patterns of peptide recognition and antigen presentation. Understanding the structural basis for these functional properties of specific HLA molecules is helping to unravel the peptide-binding properties that are inherent to each distinct allele. In studying HLA class II genes with HPV associated CIN, these peptide-specific interactions presumably form the basis for genetically regulated events in immune activation and disease.

There is compelling evidence to suggest that the HLA class II type is important in determining the risk of HPV infection and progression to CIN and cancer. Taken together, the most consistent finding in several studies is the increased risk of HPV infection, CIN and cancer in individuals with HLA DQB1*03. A number of other HLA class II alleles have been shown to correlate with susceptibility or protection in different populations. It is possible that differences in results are either due to variations in methodologies employed in the different studies or different patterns of linkage disequilibria with the disease susceptibility gene in different populations. Difficulties of single mechanisms to explain HLA association with HPV and cervical cancer is to be expected, since the development of cancer is a complex process influenced by many factors, environmental and genetic. Nevertheless, all these studies should add a new insight into the development of

immunomodulatory strategies for the prevention and treatment of cervical cancer. Several vaccination approaches against HPV infection are currently being evaluated and it is expected that further refinements in vaccine design and delivery will be made based on rapidly emerging information on the role of HLA class II in HPV infection.

6.7 FUTURE STUDIES

6.7.1 HLA ASSOCIATION STUDIES

While ethnic variations in HLA haplotype frequencies may explain the differences between the Mexican-American and African-American patient cohorts on the one hand, and the European patient cohorts on the other, the heterogeneity on HLA frequencies among the North European populations is hardly sufficient to explain the observed differences in association reported for English, German, Norwegian and Swedish patients. The differences may really be due to statistical error or heterogeneity in an as yet undetermined genetic or environmental fashion, including the HPV genome. The former possibility can be addressed by replicating the analysis in an unrelated set of patients.

The possible susceptibility and protective haplotypes identified in this study need to be confirmed in a larger sample size especially if relations to specific HPV types are to be determined. Additional studies on patients with invasive cervical cancer are also needed to determine the contribution of HLA class II alleles in progression to invasive cancer. A complete HLA class I typing of cases with CIN will allow the identification of the complete haplotype and also determine the contribution of individual alleles towards susceptibility and protection.

6.7.2 ASSOCIATION WITH HLA RELATED GENES AND P53

Differences in the distribution of HLA class II genes observed after a comparison of patients and controls may suggest that the immune response to HPV may be determined, at

least in part, by specific class II alleles. However, these differences could be related to a linkage disequilibrium with other MHC-related genes such as TAP-1, the TNFα gene promoter, or antigen processing regulator genes. Furthermore, recently, it was shown that patients with HPV associated tumors have an overrepresentation of homozygous arginine-72p53 compared with the normal population[391]. This finding will need to be confirmed in larger populations and in different geographic regions to determine the combined roles of HLA and p53 polymorphisms on HPV associated cervical carcinogenesis.

6.7.3 IMMUNODOMINANCE AND ANTIGEN PRESENTATION

The ability of the immune system to direct T-cell responses against a select number of peptides is termed immunodominance[392]. Epitopes that trigger potent T-cell activation and proliferation are classified as immunodominant. By contrast, epitopes that are poor activators of cellular immune response are termed subdominant, whereas those peptides that fail to elicit any response are cryptic. These terms indicate that there may be a discrepancy between the number of peptides within an antigenic protein that could be predicted to potentially bind to a particular HLA molecule and the number of epitopes actually recognized in a CTL response to that protein. The molecular events that control immunodominance appear to be complex with both APC and T cells regulating the process. Since the binding affinity of naturally processed peptides for class II proteins plays a significant role in influencing the heirachy of epitopes displayed to T cells[392], in-vitro studies of the binding affinity of predicted motifs of L1, L2, E6 and E7 may be used to select a library of peptides for evaluation of CTLs from normal human peripheral blood lymphocytes. In addition, the peptides could be tested *in vivo* by immunization of HLA DQ and DR transgenic mice.

6.7.4 VACCINATION TRIALS

The expression of HPV E6 and E7 genes is constitutive in cervical tumors and required for the maintenance of the transformed state. Because of their continued expression in tumor cells, the E6 and E7 proteins are promising targets for immune intervention. Immunodominant epitopes from E6 and E7 identified as above (6.7.3) could be used in clinical trials for the treatment of HPV associated cervical cancer. On the other hand, immunodominant epitopes from L1 and L2 could be used in the clinical trials for prevention and treatment of HPV associated CIN. In this way, HPV vaccine design will be based on a firm knowledge of the HPV epitopes involved in antigen processing and presentation to T lymphocytes.

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APPENDIX I: FULL HLA DR-DQ RESULTS ON PATIENTS WITH CIN

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Sample No	CIN	HPV	DQB1	DRB1	DRB3	DRB4	DRB5	Inferred Haplo.1	Inferred Haplo.2	CephDQ-DRB1.1	CephDQ-DRB1.2	DQ-DRB1-DRB345.1	DQ-DRB1-DRB345.2
2	MP1001	CIN1	33I	0303/0601	0301/1301	0301/0202	0	0	0	0	0303/1301	0601/0301*	0301/1301/B3*0301	0601/0301/B3*0202
3	MP108	CIN1	31H	0201/0302	0301/0404	*0101	DR53	0	0	0	0201/0301	0302/0404	0201/0301/B3*0101	0302/0604/B4*0101(2)
4	MP1122	CIN3	18H	0303/05031	1301/1401	*0101	0	0	0	0	0303/1301	05031/1401	0303/1301/B3*0101	05031/1301/B3*0101
5	MP1139	CIN1	Negative	0201/0603	0301/1301	*0101	0	0	0	0	0201/0301	0603/1301	0201/0301/B3*0101	0603/1301/B3*0101
6	MP1152	CIN1	Negative	0201/0601	0301/1501	*0201	0	*0101	0	0	0201/0301	0601/1501	0201/0301/B3*0201	0601/1501/B5*0101
7	MP1171	CIN1	33H	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
8	MP1221	CIN1	16I	0401/0602	0401/1502	0	DR53	*0101	0	0	0401/0401**	0602/1502**	0401/0401/B4*0101(2)	0602/1502/B5*0101
9	MP1230	CIN3	16H	0301/0601	1501/1201	*0202	0	*0201/2	0	0	0601/1501**	0301/1201	0601/1501/B5*0201(2)	0301/1201/B3*0202
10	MP1236	CIN3	16H	0201/0501	0301/DR10	*0101	0	0	0	0	0201/0301	0501/1001	0201/0301/B3*0101	0501/1001/0
11	MP1237	CIN1	16L 31H 33H	0301/0605	0401/DR7	0	DR53	0	0	0	0301/0401	0605/0701(2)*	0301/0401/B4*0101(2)	0605/0701(2)/B4*0101(2)
12	MP137	CIN3	16H	0301/0301	0801/0801	0	0	0	0301/0801	0301/0801	0301/0801**	0301/0801**	0301/0801/0	0301/0801/0
13	MP1425	CIN3	16H	0302/0601	1501/0401	0	DR53	0201/2	0	0	0601/1501**	0302/0401	0601/1501/B5*0201(2)	0302/0401/B4*0101(2)
14	MP143	CIN3	16H 18H	0201/0302	0301/0404	*0202	DR53	0	0	0	0201/0301	0302/0404	0201/0301/B3*0202	0302/0404/D4*0101(2)
15	MP1446	CIN3	18H	0201/0602	0301/1502	*0202	0	*0102	0	0	0201/0301	0602/1502**	0201/0301/B3*0202	0602/1502/B5*0102
16	MP145	CIN1	18H	0301/0603	1101/0401	*0202	DR53	0	0	0	0603/1101**	0301/0401	0603/1101/B3*0202	0301/0401/B4*0101(2)
17	MP1460	CIN1	18I	0201/0604	0301/1302	*0101	0	0	0	0	0201/0301	0604/1302	0201/0301/B3*0101	0604/1302/B3*0101*
18	MP1490	CIN3	31H	0201/0601	0301/1501	*0101	0	0201/2	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0201(2)*
19	MP1505	CIN1	Negative	0201/0402	0801/0301	*0101	0	0	0	0	0402/0801**	0201/0301	0402/0801/0	0201/0301/B3*0101
20	MP1629	CIN3	Negative	0201/0601	0301/1501	*0101	0	*0201/2	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0201(2)*
21	MP196	CIN3	16H	0301/0303	0103/DR9	0	0	0	0	0	0301/0103**	0303/0901**	0301/0103/0	0303/0901/0*
22	MP197	CIN3	16H 31H 33H	0201/0302	0301/0402	*0202	DR53	0	0	0	0201/0301	0302/0402	0201/0301/B3*0202	0302/0402/B4*0101(2)
23	MP200	CIN3	18H 31H	0301/0302	0401/0404	0	DR53	0	0	0	0301/0401	0302/0404	0301/0401/B4*0101(2)	0302/0404/B4*0101(2)
24	MP201	CIN1	31H	0201/0302	0404/DR7	0	DR53	0	0	0	0302/0404	0201/0701(2)	0302/0404/B4*0101(2)	0202/0701(2)/B4*0101(2)
25	MP230	CIN1	16H	0301/0601	0407/DR7	0	DR53	0	0	0	0301/0407*	0601/0701(2)*	0301/0407/B4*0101(2)	0601/0701(2)/B4*0101(2)
26	MP247	CIN3	16H	0302/05031	0401/1401	*0101	0	0	0	0	0302/0401	05031/1401	0302/0401/0*	05031/1401/B3*0101
27	MP253	CIN1	Negative	0301/0301	1101/1101	*0202	0	0	1101/0301	1101/0301	1101/0301	1101/0301	1101/0301/B3*0202	1101/0301/B3*0202
28	MP255	CIN3	Negative	0302/0301	0403/1303	*0202	DR53	0	0	0	0302/0403	0301/1303	0302/0403/B4*0101(2)	0301/1303/B3*0202
29	MP262	CIN1	Negative	0303/0604	1301/1301	*0101/0301	DR53	0	0303/1301	0604/1301	0303/1301	0604/1301**	0303/1301/B3*0301	0604/1301/B3*0101
30	MP306	CIN1	33H	0201/0501	DR7/0101	0	DR53	0	0	0	0201/0701(2)	0501/0101	0201/0701(2)/B4*0101(2)	0501/0101/0
31	MP320	CIN3	16I	0201/0301	0301/0401	*0101	DR53	0	0	0	0201/0301	0301/0401	0201/0301/B3*0101	0301/0401/B4*0101(2)
32	MP352	CIN1	Negative	0301/0201	0301/0402	*0101	DR53	0	0	0	0201/0301	0301/0402**	0201/0301/B3*0101	0301/0402/B4*0101(2)
33	MP401	CIN1	31H	0301/0402	0801/0801	0	0	0	0301/0801	0402/0801	0301/0801**	0402/0801**	0301/0801/0	0402/0801/0
34	MP446	CIN3	31H	0201/0601	0301/1502	*0101	0	*0102	0	0	0201/0301	0601/1502	0201/0301/B3*0101	0601/1502/B5*0102
35	MP449	CIN3	31H	0301/0501	0401/0802	*0202	DR53	0	0	0	0301/0401	0501/0802*	0301/0401/B4*0101(2)	0501/0802/B3*0202*
36	MP53	CIN1	Negative	0501/0601	1501/0103	0	0	*0201/2	0	0	0601/1501**	0501/0103	0601/1501/B5*0201(2)	0501/0103/0
37	MP567	CIN1	Negative	0301/0601	1501/0401	0	DR53	*0201/2	0	0	0601/1501**	0301/0401	0601/1501/B5*0201(2)	0301/0401/B4*0101
38	MP569	CIN3	16H	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
39	MP584	CIN1	16H	0302/0201	0301/0403	*0101	DR53	0	0	0	0201/0301	0302/0403	0201/0301/B3*0101	0302/0403/B4*0101(2)
40	MP621	CIN3	16H	0201/0301	0406/0301	0	DR53	0	0	0	0301/0406	0201/0301	0301/0406/B4*0101(2)	0201/0301/0*
41	MP63	CIN3	16H 18H	0301/0302	DR9/0103	0	DR53	0	0	0	0302/0901*	0301/0103**	0302/0901/B4*0101(2)	0301/0103/0
42	MP633	CIN3	16H	0301/0601	1301/08031	*0202	0	0	0	0	0301/1301**	0601/08031*	0301/1301/B3*0202	0601/08031/0
43	MP652	CIN1	16H	0303/0303	DR7/DR9	0	DR53	0	0303/0701(2)	0303/0901	0303/0701(2)	0303/0901**	0303/0701(2)/B4*0101(2)	0303/0901/B4*0101(2)
44	MP686	CIN3	16H	0301/0303	0103/DR9	0	DR53	0	0	0	0301/0103**	0303/0901**	0301/0103/0	0303/0901/B4*0101(2)
45	MP706	CIN1	33H	0201/0302	0301/0406	*0101	DR53	0	0	0	0201/0301	0302/0406*	0201/0301/B3*0101	0302/0406/B4*0101(2)
46	MP742	CIN1	18H	0301/0501	0101/0402	0	DR53	0	0	0	0501/0101	0301/0402**	0501/0101/0	0301/0402/B4*0101(2)
47	MP743	CIN3	Negative	0201/0302	0404/0301	*0101	DR53	0	0	0	0302/0404	0201/0301	0302/0404/B4*0101(2)	0201/0301/B3*0101
48	MP752	CIN3	Negative	0302/0602	0404/0801	*0201/0202	DR53	0	0	0	0302/0404	0602/0801*	0302/0404/B4*0101(2)	0602/0801/B3*0201*
49	MP765	CIN1	Negative	0502/0604	1501/1302	*0301	0	*0201/2	0	0	0502/1501	0604/1302	0502/1501/B5*0201(2)	0604/1302/B3*0301
50	MP810	CIN1	Negative	05032/0501	1401/0101	*0101	0	0	0	0	05032/1401	0501/0101	05032/1401/B3*0101	0501/0101/0
51	MP899	CIN1	33H	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
52	MP904	CIN1	31H	0301/0301	1101/1104	*0202	0	0	0301/1101	0301/1104	0301/1101	0301/1104	0301/1101/B3*0202	0301/1104/B3*0202
53	MP922	CIN3	18L 33I	0302/0602	1501/DR7	0	DR53	*0201/2	0	0	0602/1501	0302/0701(2)**	0602/1501/B5*0201(2)	0302/0701(2)/B4*0101(2)
54	MP945	CIN1	Negative	0601/0603	1501/1301	*0202	0	*0101	0	0	0601/1501**	0603/1301	0601/1501/B5*0101	0603/1301/B3*0202
55	MP969	CIN1	Negative	0201/0301	0301/1104	*0202	0	0	0	0	0201/0301	0301/1104	0201/0301/B3*0202	0301/1104/B3*0202
56	MP995	CIN3	16H	0201/0302	0301/0404	*0101	DR53	0	0	0	0201/0301	0302/0404	0201/0301/B3*0101	0302/0404/B4*0101(2)
57	NT106	CIN3	16H	0301/0601	0401/1502	0	DR53	*0102	0	0	0301/0401	0601/1502	0301/0401/B4*0101(2)	0601/1502/B5*0102
58	NT111	CIN3	31L	0201/0602	0301/1501	*0101	0	*0201/2	0	0	0201/0301	0602/1501	0201/0301/B3*0101	0602/1501/B5*0201(2)
59	NT112	CIN1	31L 33L	0301/0402	0401/1301	*0101	DR53	0	0	0	0301/0401	0402/1301*	0301/0401/B4*0101(2)	0402/1301/B3*0101
60	NT115	CIN3	Negative	0302/0201	0401/0301	*0101	DR53	0	0	0	0302/0401	0201/0301	0302/0401/B4*0101(2)	0201/0301/B3*0101
61	NT116	CIN3	16H 18L 33H	0302/0301	0401/0406	0	DR53	0	0	0	0301/0401	0302/0406*	0301/0401/B4*0101(2)	0302/0406/B4*0101(2)
62	NT117	CIN3	31H	0201/0301	0301/1304	*0202	0	0	0	0	0201/0301	0301/1304**	0201/0301/B3*0202	0301/1304/B3*0202
63	NT126	CIN1	16L	0201/0201	0401/0301	*0202	DR53	0	0201/0401	0201/0301	0201/0401*	0201/0301	0201/0401/B4*0101(2)	0201/0301/B3*0202
64	NT128	CIN3	18H	0301/0602	1501/0802	*0202	0	*0201/2	0	0	0602/1501	0301/0802**	0602/1501/B5*0201(2)	0301/0802/B3*0202*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
65	NT129	CIN3	31H	0301/0302	0401/0406	0	DR53	0	0	0	0301/0401	0302/0406*	0301/0401/B4*0101(2)	0302/0406/B4*0101(2)
66	NT131	CIN3	16h18l31h33H	0201/0301	0404/DR7	0	DR53	0	0	0	0301/0404*	0201/0701(2)	0301/0404/B4*0101(2)	0201/0701(2)/B4*0101(2)
67	NT133	CIN3	16H 33H	0302/0302	0401/DR7	0	DR53	0	0302/0401	0302/0701(2)	0302/0401	0302/0701(2)**	0302/0401/B4*0101(2)	0302/0701(2)/B4*0101(2)
68	NT137	CIN3	16H	0301/0201	0301/0401	0	DR53	0	0	0	0201/0301	0301/0401	0201/0301/0*	0301/0401/B4*0101(2)
69	NT143	CIN3	16H 31l	0301/05032	1401/0103	*0202	0	0	0	0	05032/1401	0301/0103**	05032/1401/B3*0202	0301/0103/0
70	NT144	CIN3	Negative	0201/0301	1101/0301	*0202	0	0	0	0	0301/1101	0201/0301	0301/1101/B3*0202	0201/0301/B3*0202
71	NT160	CIN3	Negative	0201/0302	0401/0301	*0101	DR53	0	0	0	0301/0401	0201/0301	0302/0401/B4*0101(2)	0201/0301/B3*0101
72	NT161	CIN3	18H	0302/0402	1303/0803	*0202	0	0	0	0	0302/1303*	0402/0803*	0302/1303/B3*0202	0402/0803/0
73	NT28	CIN3	18L 33H	0501/0502	0101/1501	0	0	*0201/2	0	0	0501/0101	0502/1501	0501/0101/0	0502/1501/B5*0201(2)
74	NT29	CIN3	16l31L33H	0402/0502	1601/0801	0	0	*0201/2	0	0	0502/1601	0402/0801**	0502/1601/B5*0201(2)	0402/0801/0
75	NT30	CIN3	16l	0201/0602	0301/1501	*0101	0	*0201/2	0	0	0201/0301	0602/1501	0201/0301/B3*0101	0602/1501/B5*0201(2)
76	NT33	CIN3	16H	0301/0301	0401/1101	*0202	DR53	0	0301/0401	0301/1101	0301/0401	0301/1101	0301/0401/B4*0101(2)	0301/1101/B3*0202
77	NT36	CIN3	16L 18L	0201/0603	1301/0301	*0101	0	0	0	0	0603/1301	0201/0301	0603/1301/B3*0101	0201/0301/B3*0101
78	NT41	CIN3	16H	0604/0605	1302/0101	*0301	0	0	0	0	0604/1302	0605/0101*	0604/1302/B3*0301	0605/0101/0
79	NT42	CIN3	16H	0302/0302	1406/1406	*0101	0	0	0302/1406	0302/1406	0302/1406*	0302/1406*	0302/1406/B3*0101	0302/1406/B3*0101
80	NT46	CIN3	16H 31H	0301/0501	0101/1201	*0202	0	0	0	0	0501/0101	0301/1201	0501/0101/0	0301/1201/0202
81	NT47	CIN3	16H 31L	0302/0302	0407/0407	0	DR53	0	0302/0407	0302/0407	0302/0407	0302/0407	0302/0407/B4*0101(2)	0302/0407/B4*0101(2)
82	NT53	CIN3	18L 31H	0401/0601	0401/1501	0	DR53	0201/2	0	0	0401/0401**	0601/1501**	0401/0401/B4*0101(2)	0601/1501/B5*0201(2)
83	NT56	CIN3	18L 31H	0301/0301	0401/0406	0	DR53	0	0301/0406	0301/0401	0301/0406*	0301/0401	0301/0406/B4*0101(2)	0301/0401/B4*0101(2)
84	NT58	CIN1	Negative	0603/0604	1101/1302	*0202/0301	0	0	0	0	0603/1101**	0604/1302	0603/1101/B3*0202	0604/1302/B3*0301
85	NT62	CIN3	16H	0301/0601	0407/1501	0	DR53	*0101	0	0	0301/0407	0601/1501**	0301/0407/B4*0101(2)	0601/1501/B5*0101
86	NT64	CIN3	16H 18H	0601/0601	1501/1502	0	0	*0201/2	0601/1501	0601/1502	0601/1501**	0601/1502	0601/1501/B5*0201(2)	0601/1502/B5*0201(2)
87	NT67	CIN3	16H	0604/0504	1401/1302	*0301/0101	0	0	0	0	0504/1401	0604/1302	0504/1401/B3*0101	0604/1302/B3*0301
88	NT71	CIN3	31H	0201/0601	0301/1101	*0202	0	0	0	0	0201/0301	0601/1101*	0201/0301/B3*0202	0601/1101/B3*0202
89	NT72	CIN3	16L 31H	0302/0601	0401/1502	0	DR53	*0102	0	0	0302/0401	0601/1502	0302/0401/B4*0101(2)	0601/1502/B5*0102
90	NT73	CIN3	16H	0401/0502	1602/0404	0	DR53	0201/2	0	0	0502/1602**	0401/0404*	0502/1602/B5*0201(2)	0401/0404/B4*0101(2)
91	NT74	CIN3	16H	0302/0201	DR7/DR7	0	DR53	0	0302/0701(2)	0201/0701(2)	0302/0701(2)**	0201/0701	0302/0701(2)/B4*0101(2)	0201/0701(2)/B4*0101(2)
92	NT79	CIN3	16H	0302/0302	0403/DR7	0	DR53	0	0302/0403	0302/0701(2)	0302/0403	0302/0701(2)**	0302/0403/B4*0101(2)	0302/0701(2)/B4*0101(2)
93	NT80	CIN3	16H	0302/0601	1501/0401	0	DR53	*0101	0	0	0601/1501	0302/0401	0601/1501/B5*0101	0302/0401/B4*0101(2)
94	NT83	CIN3	31H	0302/0602	1502/0403	0	DR53	*0102	0	0	0602/1502	0302/0403	0602/1502/B5*0102	0302/0403/B4*0101
95	NT86	CIN3	16l	0201/0602	0301/1501	*0101	0	*0101	0	0	0201/0301	0602/1501	0201/0301/B3*0101	0602/1501/B5*0101
96	NT90	CIN3	31H	0301/0301	1201/0401	*0101	DR53	0	0301/1201	0301/0401	0301/1201	0301/0401	0301/1201/B3*0101	0301/0401/B4*0101(2)
97	NT96	CIN3	16L	0401/0604	0103/1301	*0301	0	0	0	0	0401/0103*	0604/1301	0401/0103/0	0604/1301/B3*0301
98	W10	CIN1	Negative	0201/0301	0301/08042	*0101	0	0	0	0	0201/0301	0301/08042	0201/0301/B3*0101	0301/08042/0
99	W100	CIN3	16H	05031/0604	DR7/DR9	0	DR53	0	0	0	0604/0701(2)*	05031/0901*	0604/0701(2)/B4*0101(2)	05031/0901/B4*0101(2)
100	W102	CIN3	31L	05032/0604	1602/1301	*0101	0	*0102	0	0	05032/1602*	0604/1301**	05032/1602/B5*0102	0604/1301/B3*0101
101	W104	CIN1	Negative	0201/0602	0302/DR9	*0101	DR53	0	0	0	0201/0302*	0602/0901*	0201/0302/B3*0101	0602/0901/B4*0101(2)
102	W11	CIN1	31H	0301/0302	0103/0402	0	DR53	0	0	0	0301/0103**	0302/0402	0301/0103/0	0302/0402/B4*0101(2)
103	W111	CIN3	16L	0401/0501	0401/0101	0	DR53	0	0	0	0401/0401**	0501/0101	0401/0401/B4*0101(2)	0501/0101/0
104	W112	CIN3	16H	0301/0301	1101/1202	*0202	0	0	0301/1101	0301/1202	0301/1101	0301/1202**	0301/1101/B3*0202	0301/1202/B3*0202
105	W12	CIN1	16L	0301/0504	0401/1401	*0202	DR53	0	0	0	0301/0401	0504/1401**	0301/0401/B4*0101(2)	0504/1401/B3*0202
106	W120	CIN3	16H	0301/0301	0401/0404	0	DR53	0	0301/0401	0301/0404	0301/0401	0301/0404*	0301/0401/B4*0101(2)	0301/0404/B4*0101(2)
107	W121	CIN3	Negative	0502/05031	1401/1601	*0202	0	0201/2	0	0	05031/1401	0502/1601	05031/1401/B3*0202	0502/1601/B5*0201(2)
108	W125	CIN3	Negative	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
109	W128	CIN3	Negative	0302/0302	1101/1101	*0101	0	0	0302/1101	0302/1101	0302/1101	0302/1101	0302/1101/B3*0101	0302/1101/B3*0101
110	W132	CIN1	Negative	0303/0601	1501/1301	*0101	0	*0201/2	0	0	0601/1501**	0303/1301	0601/1501/B5*0201(2)	0303/1301/B3*0101
111	W134	CIN3	16H	0201/0301	0401/DR7	0	DR53	0	0	0	0301/0401	0201/0701(2)	0301/0401/B4*0101(2)	0201/0701(2)/B4*0101(2)
112	W135	CIN3	16H	0201/0301	0401/DR7	0	DR53	0	0	0	0301/0401	0201/0701(2)	0301/0401/B4*0101(2)	0201/0701(2)/B4*0101(2)
113	W137	CIN3	16H	0301/0301	0103/0401	0	DR53	0	0301/0103	0301/0401	0301/0103**	0301/0401	0301/0103/0	0301/0401/B4*0101(2)
114	W143	CIN3	33H	0302/0502	0403/1601	0	DR53	0201/2	0	0	0302/0403	0502/1601	0302/0403/B4*0101(2)	0502/1601/B5*0201(2)
115	W147	CIN3	16H	0501/0604	0101/1302	*0101	0	0	0	0	0501/0101	0604/1302	0501/0101/0	0604/1302/B3*0101
116	W148	CIN1	Negative	0301/0601	1502/1101	*0202	0	*0102	0	0	0601/1502	0301/1101	0601/1502/B5*0102	0301/1101/B3*0202
117	W15	CIN3	16H	0302/0601	1501/0401	0	DR53	*0101	0	0	0601/1501**	0302/0401	0601/1501/B5*0101	0302/0401/B4*0101
118	W154	CIN3	16H	0201/0301	0301/1101	*0101	0	0	0	0	0201/0301	0301/1101	0201/0301/B3*0101	0301/1101/B3*0101
119	W158	CIN1	Negative	0302/0303	1301/0401	*0101	DR53	0	0	0	0303/1301	0302/0401	0303/1301/B3*0101	0302/0401/B4*0101
120	W164	CIN1	Negative	0502/0604	1301/1301	*0101	0	0	0502/1301	0604/1301	0502/1301*	0604/1301	0502/1301/B3*0101	0604/1301/B3*0101
121	W169	CIN1	16H	0201/0603	0301/1301	*0202	0	0	0	0	0201/0301	0603/1301	0201/0301/B3*0202	0603/1301/B3*0202
122	W165	CIN3	16H	0501/0606	0101/DR9	0	DR53	0	0	0	0501/0101	0606/0901*	0501/0101/0	0601/0901/B4*0101(2)
123	W166	CIN3	31H	0301/0301	1101/1104	*0201/0202	0	0	0301/1101	0301/1104	0301/1101	0301/1104	0301/1101/B3*0201	0301/1104/B3*0202
124	W18	CIN3	16l	0201/0302	0301/0401	*0101	DR53	0	0	0	0201/0301	0302/0401	0201/0301/B3*0101	0302/0401/B4*0101(2)
125	W180	CIN3	16H	0301/0601	1501/1304	*0202	0	*0201/2	0	0	0601/1501**	0301/1304**	0601/1501/B5*0201(2)	0301/1304/B3*0202
126	W182	CIN3	16H	0302/0603	1101/0404	*0202	DR53	0	0	0	0603/1101**	0302/0404	0603/1101/B3*0202	0302/0404/B4*0101(2)
127	W186	CIN3	16H	0302/0302	0403/0401	0	DR53	0201/2	0302/0403	0302/0401	0302/0403	0302/0401	0302/0403/B4*0101(2)	0302/0401/B4*0101(2)
128	W191	CIN1	Negative	0301/0601	DR7/1502	0	DR53	*0102	0	0	0301/0701(2)*	0601/1502	0301/0701(2)/B4*0101(2)	0601/1502/B5*0102

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
129	W198	CIN3	16H	0303/0604	DR10/1301	*0202	0	0	0	0	0604/1301**	0303/1001*	0604/1301/B3*0202	0303/1001/0
130	MP198	CIN3	16H	0301/0201	0301/DR7	*0202	DR53	0	0	0	0201/0301	0301/0701(2)*	0201/0301/B3*0202	0301/0701(2)/B4*0101(2)
131	W199	CIN3	16L	0201/0201	0301/0301	*0101/0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0202
132	W2	CIN3	33H	0302/0303	DR7/DR7	0	DR53	0	0302/0701(2)	0303/0701(2)	0302/0701(2)**	0303/0701(2)	0302/0701(2)/B4*0101(2)	0303/0701(2)/B4*0101(2)
133	W200	CIN3	16H	05032/0604	1302/1302	*0301	0	0	05032/1302	0604/1302	05032/1302*	0604/1302	05032/1302/B3*0301	0604/1302/B3*0301
134	W21	CIN1	Negative	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
135	W22	CIN1	Negative	0302/0302	0801/0802	0	0	0	0302/0801	0302/0802	0302/0801*	0302/0802*	0302/0801/0	0302/0802/0
136	W24	CIN1	16I	0302/0302	0401/0404	0	DR53	0	0302/0401	0302/0404	0302/0401	0302/0404	0302/0401/B4*0101	0302/0404/B4*0101(2)
137	W26	CIN1	Negative	05031/0604	1302/1401	*0301/0101	0	0	0	0	0604/1302	05031/1401	0604/1302/B3*0301	05031/1401/B3*0101
138	W27	CIN1	Negative	0201/0302	0301/0402	*0101	DR53	0	0	0	0201/0301	0302/0402	0201/0301/B3*0101	0302/0402/B4*0101(2)
139	W3	CIN1	16L	0301/0303	1301/1101	*0202	0	0	0	0	0301/1101	0303/1301	0301/1101/B3*0202	0303/1301/B3*0202
140	W30	CIN1	Negative	0501/0201	0301/0101	*0202	0	0	0	0	0201/0301	0501/0101	0201/0301/B3*0202	0501/0101/0
141	W32	CIN3	16H	0201/0603	0301/1104	*0202	0	0	0	0	0201/0301	0603/1104**	0201/0301/B3*0202	0603/1104/B3*0202
142	W33	CIN3	16H	0302/0302	DR7/0406	0	DR53	0	0	0	0302/0701(2)**	0302/0406*	0302/0701(2)/B4*0101	0302/0406/B4*0101
143	W34	CIN3	16H	0602/0201	1501/DR7	0	DR53	*0101	0	0	0602/1501	0201/0701(2)	0602/1501/B5*0101	0201/0701(2)/B4*0101(2)
144	W37	CIN1	Negative	0402/0402	1401/0801	*0101	0	0	0402/1401	0402/0801	0402/1401*	0402/0801**	0402/1401/B3*0101	0402/0801/0
145	W39	CIN3	16I	0301/0301	1101/1101	*0101	0	0	0301/1101	0301/1101	0301/1101	0301/1101	0301/1101/B3*0101	0301/1101/B3*0101
146	W4	CIN3	16I	0201/0201	0301/DR9	*0202	DR53	0	0201/0301	0201/0901	0201/0301	0201/0901**	0201/0301/B3*0202	0201/0901/B4*0101(2)
147	W41	CIN3	16I	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
148	W43	CIN1	Negative	05032/0601	1501/1501	0	0	0201/2	05032/1501	0601/1501	05032/1501*	0601/1501**	05032/1501/B5*0202(2)	0601/1501/B5*0201(2)
149	W45	CIN3	16H	0504/0605	1401/1101	*0201	0	0	0	0	0504/1401**	0605/1101*	0504/1401/B3*0201	0605/1101/B3*0201
150	W5	CIN3	16H	0301/0302	0401/1101	*0202	DR53	0	0	0	0301/1101	0302/0401	0301/1101B3*0202	0302/0401/B3*0202
151	W51	CIN1	16L	0501/0501	0101/0102	0	0	0	0501/0101	0501/0102	0501/0101	0501/0102	0501/0101/0	0501/0102/0
152	W52	CIN3	16H	0301/0301	0302/0103	*0101	0	0	0301/0103	0301/0302	0301/0103**	0301/0302*	0301/0103/0	0301/0302/B3*0101
153	W58	CIN1	16L	0603/0604	1101/1301	*0101	0	0	0	0	0603/1101**	0604/1301**	0603/1101/B3*0101	0604/1301/B3*0101
154	W6	CIN1	Negative	0201/0601	1501/0301	*0101	0	*0101	0	0	0601/1501**	0201/0301	0601/1501/B5*0101	0201/0301/B3*0101
155	W60	CIN1	16L	0501/0604	0101/1402	*0202	0	0	0	0	0501/0101	0604/1402*	0501/0101/0	0604/1402/B3*0202
156	W64	CIN3	16I	0501/0501	0101/0103	0	0	0	0501/0101	0501/0103	0501/0101	0501/0103	0501/0101/0	0501/0103/0
157	W66	CIN3	Negative	0302/0605	0403/1302	*0101	DR53	0	0	0	0302/0403	0605/1302	0302/0403/B4*0101	0605/1302/B3*0101
158	W7	CIN3	16I	0301/0501	0101/1101	*0101	0	0	0	0	0501/0101	0301/1101	0501/0101/0	0301/1101/B3*0101
159	W70	CIN1	Negative	0302/0604	1302/0403	*0202	DR53	0	0	0	0604/1302	0302/0403	0604/1302/B3*0202	0302/0403/B4*0101(2)
160	W72	CIN3	33L	0201/0502	DR10/0301	*0202	0	0	0	0	0502/1001*	0201/0301	0502/1001/0	0201/0301/B3*0202
161	W74	CIN1	Negative	0301/0301	08042/08042	*0202	0	0	0301/08042	0301/08042	0301/08042**	0301/08042**	0301/08042/B3*0202*	0301/08042/0
162	W78	CIN3	Negative	0301/0302	0802/0401	0	DR53	0	0	0	0301/08042**	0302/0401	0301/0802/0	0302/0401/B4*0101(2)
163	W8	CIN3	Negative	0201/0201	0301/1406	*0202	0	0	0201/0301	0201/1406	0201/0301	0201/1406*	0201/0301/B3*0202	0201/1406/B3*0202
164	W80	CIN3	16H	0201/0301	0801/DR9	*0201	0	0	0	0	0201/0801*	0301/0901	0201/0801/B3*0201*	0301/0901/0*
165	W81	CIN1	33H	0302/0302	0401/0403	0	DR53	0	0302/0401	0302/0403	0302/0401	0302/0403	0302/0401/B4*0101(2)	0302/0403/B4*0101(2)
166	W82	CIN3	16H	0201/0604	0301/1302	*0301/0202	0	0	0	0	0201/0301	0604/1302	0201/0301/B3*0202	0604/1302/B3*0301
167	W84	CIN1	Negative	0402/0402	0301/0803	*0201	0	0	0402/0301	0402/0803	0402/0301*	0402/0803	0402/0301/B3*0201	0402/0803/0
168	W86	CIN3	16H	0502/05031	1601/1602	0	0	0201/2	0	0	0502/1601	05031/1602*	0502/1601/B5*0201(2)	05031/1602/B5*0201(2)
169	W87	CIN1	16H	0201/0601	DR7/DR9	0	DR53	0	0	0	0201/0701(2)	0601/0901*	0201/0701(2)/B4*0101(2)	0601/0901/B4*0101(2)
170	W88	CIN1	18L	0201/0302	0301/0401	*0101	DR53	0	0	0	0201/0301	0302/0401	0201/0301/B3*0101	0302/0401/B4*0101(2)
171	W9	CIN3	16H	0302/0302	DR7/DR7	0	DR53	0	0302/0701(2)	0302/0701(2)	0302/0701(2)**	0302/0701(2)**	0302/0701(2)/B4*0101(2)	0302/0701(2)/B4*0101(2)
172	W90	CIN3	16I	0301/0604	0401/DR9	0	DR53	0	0	0	0301/0401	0604/0901*	0301/0401/B4*0101(2)	0604/0901/B4*0101(2)
173	W91	CIN3	31L	0302/0603	0401/1101	*0202	DR53	0	0	0	0302/0401	0603/1101**	0302/0401/B4*0101(2)	0603/1101/B3*0202
174	W92	CIN1	16I	0501/0502	0101/1402	*0101	0	0	0	0	0501/0101	0502/1402	0501/0101/0	0502/1402/B3*0101
175	W96	CIN3	16I 18I	0201/0601	0301/1502	*0202	0	*0102	0	0	0201/0301	0601/1502	0201/0301/B3*0202	0601/1502/B5*0102
176	W97	CIN1	Negative	0302/0301	0103/0401	0	DR53	0	0	0	0301/0103**	0302/0401	0301/0103/0	0302/0401/B4*0101(2)
177	W98	CIN3	16H	05031/0502	1401/1401	*0202	0	0	0	0	05031/1401	0502/1401*	05031/1401/B3*0202	0502/1401/B3*0202
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APPENDIX II: FULL HLA DR-DQ RESULTS ON CONTROL POPULATION

	1	2	3	4	5	6	7	8	9	10	11	12
1	Sample No	DQB1	DRB1	DRB3(DR52)	DRB4(DR53)	DRB5	Inferred Haplo.1	Inferred Haplo.2	CephDQ-DRB1.1	CephDQ-DRB1.2	DQ-DRB1-DRB345.1	DQ-DRB1-DRB345.2
2	MP508	0301/0301	1104/1305	*0202	0	0	0301/1104	0301/1305	0301/1104	0301/1305	0301/1104/B3*0202	0301/1305/B3*0202
3	MP1	0201/0301	0301/1101	*0202	0	0	0	0	0201/0301	0301/1101	0201/0301/B3*0202	0301/1101/B3*0202
4	MP10	0301/0501	1101/0101	*0202	0	0	0	0	0301/1101	0501/0101	0301/1101/B3*0202	0501/0101/0
5	MP100	0301/0601	1501/0401	0	DR53	*0201/2	0	0	0601/1501**	0301/0401	0601/1501/B5*0201(2)	0301/0401/B4*0101(2)
6	MP1002	0401/0501	0101/0404	0	DR53	0	0	0	0501/0101	0401/0404*	0501/0101/0	0401/0404/B4*0101(2)
7	MP1003	0201/0602	DR9/1501	0	DR53	*0101	0	0	0602/1501	0201/0901*	0602/1501/B5*0101	0201/0901/B4*0101(2)
8	MP1004	0501/0604	0101/1301	*0202	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0202
9	MP1005	0501/0502	0101/DR7	0	DR53	0	0	0	0501/0101	0502/0701(2)*	0501/0101/0	0502/0701(2)/B4*0101(2)
10	MP1007	0201/0602	0301/1406	*0202	DR53	0	0	0	0201/0301	0602/1406*	0201/0301/B3*0202	0602/1406/B3*0202
11	MP1008	0501/0604	0102/1302	*0301	0	0	0	0	0501/0102	0604/1302	0501/0101/0	0604/1302/B3*0301
12	MP1014	0201/05031	0301/1401	*0101	0	0	0	0	0201/0301	05031/1401	0201/0301/B3*0101	05031/1401/B3*0101
13	MP1015	0201/0604	0301/1301	*0101	0	0	0	0	0201/0301	0604/1301**	0201/0301/B3*0101	0604/1301/B3*0101
14	MP102	0201/0501	0410/0101	0	DR53	0	0	0	0201/0410*	0501/0101	0201/0401/B4*0101(2)	0501/0101/0
15	MP103	05031/0402	0801/DR9	0	DR53	0	0	0	0402/0801**	05031/0901*	0402/0801/0	05031/0901/B4*0101(2)
16	MP105	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
17	MP106	0301/0601	0401/1501	0	DR53	*0101	0	0	0301/0401	0601/1501	0301/0401/B4*0101(2)	0601/1501/B5*0101
18	MP107	0401/05032	1401/DR9	*0202	DR53	0	0	0	0401/0901*	0503/1401	0401/0901/B4*0101(2)	0503/1401/B3*0202
19	MP11	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
20	MP111	0201/0605	1501/0301	0	0	0201/2	0	0	0201/0301	0605/1501*	0201/0301/0*	0605/1501/B5*0201(2)
21	MP112	0201/0601	1502/0301	*0101	0	*0101	0	0	0201/0301	0601/1502	0201/0301/B3*0101	0601/1502/B5*0101
22	MP113	0501/0604	1301/0101	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
23	MP114	0302/0602	1501/0404	0	DR53	*0101	0	0	0302/0404	0602/1501	0302/0404/B4*0101	0602/1501/B5*0101
24	MP115	0302/0201	0404/DR7	0	DR53	0	0	0	0201/0701(2)	0302/0404	0201/0701(2)/B4*0101(2)	0302/0404/B4*0101
25	MP117	0201/0601	0301/DR9	*0202	DR53	0	0	0	0201/0301	0601/0901*	0201/0301/B3*0202	0601/0901/B4*0101
26	MP1185	0302/0402	0407/DR10	0	DR53	0	0	0	0302/0407	0402/1001*	0302/0407/B4*0101	0402/1001/0
27	MP119	0501/0603	DR10/1104	*0202	0	0	0	0	0501/1001	0603/1104**	0501/1001/0	0603/1104/B3*0202
28	MP1193	0302/0302	0401/0404	0	DR53	0	0302/0401	0302/0404	0302/0401	0302/0404	0302/0401/B4*0101(2)	0302/0404/B4*0101(2)
29	MP120	05032/0601	DR10/1502	0	0	*0102	0	0	0601/1502	05032/1001*	0602/1502/B5*0102	05032/1001/0
30	MP122	0502/05031	1401/DR7	0	DR53	0	0	0	05031/1401	0502/0701(2)*	05031/1401/0*	0502/0701(2)/B4*0101(2)
31	MP123	0501/0501	0101/0103	0	0	0	0501/0101	0501/0103	0501/0101	0501/0103	0501/0101/0	0501/0103/0
32	MP124	0301/0301	1104/DR9	*0101	DR53	0	0301/1104	0301/0901	0301/1104	0301/0901*	0301/1104/B3*0101	0301/0901/B4*0101(2)
33	MP125	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301	0501/0101/0	0604/1301/B3*0101
34	MP1257	0402/0501	0101/0803	0	0	0	0	0	0501/0101	0402/0803**	0501/0101/0	0402/0803/0
35	MP1259	0301/0302	DR9/0401	0	DR53	0	0	0	0301/0401	0302/0901*	0301/0401/B4*0101(2)	0302/0901/B4*0101(2)
36	MP126	0301/0201	1501/0103	0	0	*0101	0	0	0301/0103**	0201/1501*	0301/0103/0	0201/1501/B5*0101
37	MP1260	0303/0201	1301/0301	*0101	0	0	0	0	0303/1301	0201/0301	0303/1301/B3*0101	0201/0301/B3*0101
38	MP1262	0302/0302	0401/0403	0	DR53	0	0302/0401	0302/0403	0302/0401	0302/0403	0302/0401/B4*0101(2)	0302/0403/B4*0101(2)
39	MP127	0601/0601	1501/1502	0	0	*0101/2	0601/1501	0601/1502	0601/1501**	0601/1502	0601/1501/B5*0101	0601/1502/B5*0102
40	MP1401	0201/0601	0	0	0	0	0	0	0	0	0	0
41	MP1420	0301/0301	0401/0103	0	DR53	0	0301/0401	0301/0103	0301/0401	0301/0103**	0301/0401/B4*0101(2)	0301/0103/0
42	MP1421	0201/0402	0301/0803	*0101	0	0	0	0	0201/0301	0402/0803**	0201/0301/B3*0101	0402/0803/0
43	MP1422	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
44	MP1424	0402/0604	1302/0801	*0301	0	0	0	0	0402/0801**	0604/1302	0402/0801/0	0604/1302/B3*0301
45	MP148	0201/0302	0301/0403	*0101	DR53	0	0	0	0201/0301	0302/0403	0201/0301/B3*0101	0302/0403/B4*0101
46	MP1461	0201/0603	1301/DR7	*0101	DR53	0	0	0	0201/0701(2)	0603/1301	0201/0701(2)/B4*0101(2)	0603/1301/B3*0101
47	MP1462	0303/0501	0101/1301	*0101	0	0	0	0	0501/0101	0303/1301	0501/0101/0	0303/1301/B3*0101
48	MP1465	0302/0603	1301/0401	*0202	DR53	0	0	0	0302/0401	0603/1301	0302/0401/B4*0101(2)	0603/1301/B3*0202
49	MP1466	0301/05031	1401/1201	*0202	0	0	0	0	0301/1201	05031/1401	0301/1201/B3*0202	05031/1401/B3*0202
50	MP1467	0201/0303	DR7/DR7	0	DR53	0	0201/0701(2)	0303/0701(2)	0201/0701(2)	0303/0701(2)	0201/0701(2)/B4*0101(2)	0303/0701(2)/B4*0101(2)
51	MP1468	0302/0301	0404/0404	0	DR53	0	0302/0404	0301/0404	0302/0404	0301/0404*	0302/0404/B4*0101(2)	0301/0404/B4*0101(2)
52	MP1469	0501/0501	0101/DR7	0	DR53	0	0501/0101	0501/0701(2)	0501/0101	0501/0701(2)*	0501/0101/0	0501/0701(2)/B4*0101(2)
53	MP147	0301/0501	0101/0103	0	0	0	0	0	0301/0103**	0501/0101	0301/0103/0	0501/0101/0
54	MP1470	0201/0201	0301/DR7	*0101	DR53	0	0201/0301	0201/0701(2)	0201/0301	0201/0701(2)	0201/0301/B3*0101	0201/0701(2)/B4*0101(2)
55	MP1471	0301/0301	0103/0401	0	DR53	0	0301/0103	0301/0401	0301/0103**	0301/0401	0301/0103/0	0301/0401/B4*0101(2)
56	MP1472	0201/0603	0301/1301	*0202	0	0	0	0	0201/0301	0603/1301	0201/0301/B3*0202	0603/1301/B3*0202
57	MP1473	0402/0604	1301/0801	*0202	0	0	0	0	0402/0801**	0604/1301**	0402/0801/0	0604/1301/B3*0202
58	MP1474	0501/0605	0101/1302	*0201	0	0	0	0	0501/0101	0605/1302*	0501/0101/0	0605/1302/B3*0201

	1	2	3	4	5	6	7	8	9	10	11	12
59	MP1475	0402/0501	0801/DR7	0	DR53	0	0	0	0402/0801**	0501/0701(2)*	0402/0801/0	0501/0701(2)/B4*0101(2)
60	MP1476	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
61	MP148	0302/0302	1101/DR7	*0202	DR53	0	0302/1101	0302/0701(2)	0302/1101	0302/0701(2)	0302/1101/B3*0202	0302/0701(2)/B4*0101(2)
62	MP149	0201/0301	0301/0401	*0101	DR53	0	0	0	0201/0301	0301/0401	0201/0301/B3*0101	0301/0401/B4*0101
63	MP1496	0402/05031	0803/1401	*0202	0	0	0	0	0402/0803**	05031/1401	0402/0803/0	05031/1401/B3*0202
64	MP1497	0402/0604	1301/0801	*0101	0	0	0	0	0402/0801	0604/1301	0402/0801/0	0604/1301/B3*0101
65	MP1498	0201/0601	1501/0301	*0101	0	*0101	0	0	0201/0301	0601/1501	0201/0301/B3*0101	0601/1501/B5*0101
66	MP1499	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
67	MP15	0301/0501	0101/0401	0	DR53	0	0	0	0301/0401	0501/0101	0301/0401/B4*0101(2)	0501/0101
68	MP150	0201/0603	1103/0301	*0101/0202	0	0	0	0	0201/0301	0601/1103*	0201/0301/B3*0101	0601/1103/B3*0202
69	MP1500	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
70	MP1503	0401/0501	0101/0401	0	DR53	0	0	0	0501/0101	0401/0401**	0501/0101/0	0401/0401/B4*0101(2)
71	MP1504	0201/0601	0301/1501	*0101	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0101
72	MP1506	0301/0302	0404/0404	0	DR53	0	0301/0404	0302/0404	0301/0404**	0302/0404	0301/0404/B4*0101	0302/0404/B4*0101
73	MP1507	0501/0604	0101/1302	*0301	0	0	0	0	0501/0101	0604/1302	0501/0101/0	0604/1302/B3*0301
74	MP1509	0301/0303	1201/1201	*0201	0	0	0301/1201	0303/1201	0301/1201	0303/1201*	0301/1201/B3*0201	0303/1201/B3*0201
75	MP151	0501/0601	0101/1502	0	0	*0102	0	0	0501/0101	0601/1502	0501/0101/0	0601/1502/B5*0102
76	MP152	0302/0201	0401/0301	*0101	DR53	0	0	0	0201/0301	0302/0401	0201/0301/B3*0101	0302/0401/B4*0101
77	MP153	0501/0604	0101/1302	*0301	0	0	0	0	0501/0101	0604/1302	0501/0101/0	0604/1302/B3*0301
78	MP154	0501/0604	0101/0302	*0101	0	0	0	0	0501/0101	0604/0302*	0501/0101/0	0604/0302/B3*0101
79	MP155	0201/0601	0301/1301	*0301	0	0	0	0	0201/0301	0601/1301*	0201/0301/B3*0301	0601/1301/B3*0301
80	MP156	0303/0502	1501/DR7	0	DR53	*0101	0	0	0502/1501	0303/0701(2)	0502/1501/B5*0101	0303/0701(2)/B4*0101
81	MP157	0201/0301	0301/1202	*0101	0	0	0	0	0201/0301	0301/1202**	0201/0301/B3*0101	0301/1202/B3*0101
82	MP158	0302/0504	0404/1404	*0101	DR53	0	0	0	0302/0404	0504/1404**	0302/0404/B4*0101	0504/1404/B3*0101
83	MP159	0501/0604	0101/1302	*0101	0	0	0	0	0501/0101	0604/1302	0501/0101/0	0604/1302/B3*0101
84	MP1591	0302/0201	DR9/DR10	0	DR53	0	0	0	0302/0901*	0201/1001*	0302/0901/B4*0101	0201/1001/0
85	MP1592	0201/0302	0404/0301	*0101	DR53	0	0	0	0201/0301	0302/0404	0201/0301/B3*0101	0302/0404/B4*0101
86	MP1593	0302/0302	0404/0802	0	DR53	0	0302/0404	0302/0802	0302/0404	0302/0802	0302/0404/B4*0101	0302/0802/0
87	MP1596	0301/0301	0401/0404	0	DR53	0	0301/0401	0301/0404	0301/0401	0301/0404**	0301/0401/B4*0101	0301/0404/B4*0101
88	MP1594	0201/0301	0301/1201	*0101	0	0	0	0	0201/0301	0301/1201	0201/0301/B3*0101	0301/1201/B3*0101
89	MP1598	0201/0601	0	0	0	0	0	0	0	0	0	0
90	MP1599	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
91	MP16	0301/0402	1201/0801	*0101	0	0	0	0	0301/1201	0402/0801**	0301/1201/B3*0101	0402/0801/0
92	MP160	0601/0603	DR7/DR10	0	DR53	0	0	0	0601/0701(2)	0603/1001*	0601/0701(2)/B4*0101	0603/1001/0
93	MP1600	0501/0604	0101/0404	0	DR53	0	0	0	0501/0101	0604/0404*	0501/0101/0	0604/0404/B4*0101(2)
94	MP1601	0604/0604	1301/DR7	*0301	DR53	0	0604/1301	0604/0701(2)	0604/1301**	0604/0701(2)*	0604/1301/B3*0301	0604/0701(2)/B4*0101(2)
95	MP1602	0201/0601	0301/DR7	*0101	DR53	0	0	0	0201/0701(2)	0601/0301*	0201/0701(2)/B4*0101(2)	0601/0301/B3*0101
96	MP1604	0401/0402	0401/0801	0	DR53	0	0	0	0402/0801**	0401/0401**	0402/0801/0	0401/0401/B4*0101(2)
97	MP1606	0201/0201	0301/DR7	*0101	DR53	0	0201/0301	0201/0701(2)	0201/0301	0201/0701(2)	0201/0301/B3*0101	0201/0701(2)/B4*0101(2)
98	MP1607	0501/0604	0101/1302	*0201	0	0	0	0	0501/0101	0604/1302	0501/0101/0	0604/1302/B3*0201
99	MP1608	0603/0605	1301/DR7	*0202	0	0	0	0	0603/1301	0605/0701(2)*	0603/1301/B3*0202	0605/0701(2)/B4*0101(2)
100	MP161	0601/0601	1501/1501	0	0	*0101	0601/1501	0601/1501	0601/1501**	0601/1501**	0601/1501/B5*0101	0601/1501/B5*0101
101	MP163	0501/0601	1501/0101	0	0	0201/2	0	0	0501/0101	0601/1501**	0501/0101/0	0601/1501/B5*0201(2)
102	MP1633	0201/0201	0301/DR10	*0101	0	0	0201/0301	0201/1001	0201/0301	0201/1001*	0201/0301/B3*0101	0201/1001/0
103	MP1634	0502/05031	1601/1401	*0101	0	0201/2	0	0	0502/1601	05031/1401	0502/1601/B5*0201(2)	05031/1401/B3*0101
104	MP1635	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
105	MP1636	0302/0601	0404/1501	0	DR53	*0101	0	0	0302/0404	0601/1501**	0302/0404/B4*0101	0601/1501/B5*0101
106	MP1637	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
107	MP164	0201/0303	DR7/DR7	0	DR53	0	0201/0701(2)	0201/0701(2)	0201/0701(2)	0201/0701(2)	0201/0701(2)/B4*0101(2)	0201/0701(2)/B4*0101(2)
108	MP165	0601/0603	1501/1301	*0202	0	*0101	0	0	0601/1501**	0603/1301	0601/1501/B5*0101	0603/1301/B3*0202
109	MP166	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
110	MP167	0601/0601	1501/1501	0	0	*0101	0601/1501	0601/1501	0601/1501**	0601/1501**	0601/1501/B5*0101	0601/1501/B5*0101
111	MP168	0502/05032	1501/1401	*0202	0	*0101	0	0	0502/1501	5032/1401	0502/1501/B5*0101	05032/1401/B3*0202
112	MP169	0501/0504	0101/1401	*0202	0	0	0	0	0501/0101	0504/1401**	0501/0101/0	0504/1401/B3*0202
113	MP17	0201/0303	0301/DR7	*0101	DR53	0	0	0	0201/0301	0303/0701(2)	0201/0301/B3*0101	0303/0701(2)/B4*0101(2)
114	MP170	0201/0603	0301/1301	*0101	0	0	0	0	0201/0301	0603/1301	0201/0301/B3*0101	0603/1301/B3*0101
115	MP171	0301/0601	1104/1305	*0202	0	0	0	0	0301/1104	0601/1305*	0301/1104/B3*0202	0601/1305/B3*0202
116	MP172	0201/0601	0301/1501	*0101	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0101

	1	2	3	4	5	6	7	8	9	10	11	12
117	MP173	0201/0504	DR9/DR7	0	DR53	0	0	0	0201/0701(2)	0504/0901*	0201/0701(2)/B4*0101	0504/0901/B4*0101(2)
118	MP174	0301/0504	1401/0401	*0101	DR53	0	0	0	0301/0401	0504/1401**	0301/0401/B4*0101(2)	0504/1401/B3*0101
119	MP175	0201/0601	1501/0302	*0101	0	0201/2	0	0	0201/0302*	0601/1501**	0201/0302/B3*0101	0601/1501/B5*0201(2)
120	MP176	0501/0604	0101/0801	0	0	0	0	0	0501/0101	0604/0801	0501/0101/0	0604/0801/0
121	MP177	0301/0402	DR7/DR10	0	DR53	0	0	0	0301/0701(2)**	0402/1001*	0301/0701(2)/B4*0101(2)	0402/1001/0
122	MP178	0201/0601	0301/1501	*0101	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0101
123	MP179	0504/0601	1501/DR10	0	0	0201/2	0	0	0601/1501	0504/1001*	0601/1501/B5*0201(2)	0504/1001/0
124	MP18	0302/0201	0401/DR7	0	DR53	0	0	0	0201/0701(2)	0302/0401	0201/0701(2)/B4*0101(2)	0302/0401/B4*0101(2)
125	MP180	0201/0604	0301/1302	*0101	0	0	0	0	0201/0301	0604/1302	0201/0301/B3*0101	0604/1302/B3*0101
126	MP181	0302/0501	0101/DR7	0	DR53	0	0	0	0501/0101	0302/0701(2)**	0501/0101/0	0302/0701(2)/B4*0101(2)
127	MP182	0201/0601	1502/0301	*0202	0	*0101	0	0	0201/0301	0601/1502	0201/0301/B3*0202	0601/1502/B5*0101
128	MP183	0302/0601	0407/1501	0	DR53	*0101	0	0	0302/0407**	0601/1501**	0302/0407/B4*0101(2)	0601/1501/B5*0101
129	MP184	0301/0201	0301/1101	*0202	0	0	0	0	0301/1101	0201/0301	0301/1101/B3*0202	0201/0301/B3*0202
130	MP185	0201/0601	0302/1502	*0101	0	*0102	0	0	0201/0302*	0601/1502	0201/0302/B3*0101	0601/1502/B5*0102
131	MP186	0302/0302	0401/0402	0	DR53	0	0302/0401	0302/0402	0302/0401	0302/0402	0302/0401/B4*0101(2)	0302/0402/B4*0101(2)
132	MP187	0201/0601	0301/1501	*0101	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0101
133	MP188	0402/0603	1501/1501	0	0	0201/2	0402/1501	0603/1501	0402/1501*	0603/1501*	0402/1501/B5*0202(2)	0603/1501/B5*0201(2)
134	MP189	05031/0504	0404/DR10	0	DR53	0	0	0	0504/1001*	05031/0404*	0504/1001/0	05031/0404/B4*0101(2)
135	MP19	0302/0201	0301/0401	*0101	DR53	0	0	0	0201/0301	0302/0401	0201/0301/B3*0101	0302/0401/B4*0101(2)
136	MP190	0601/0603	1501/1301	*0101	0	*0101	0	0	0601/1501**	0603/1301	0601/1501/B5*0101	0603/1301/B3*0101
137	MP2	0301/0603	0103/1101	*0202	0	0	0	0	0301/0103	0603/1101	0301/0103/0	0603/1101/B3*0202
138	MP21	05032/0602	1401/1501	*0101	0	*0101	0	0	0602/1501	05032/1401	0602/1501/B5*0101	05032/1401/B3*0101
139	MP202	0601/0604	1501/1301	*0101	0	*0101	0	0	0601/1501**	0604/1301**	0601/1501/B5*0101	0604/1301/B3*0101
140	MP203	0301/0302	1101/0401	*0201	DR53	0	0	0	0302/0401	0301/1101	0302/0401/B4*0101(2)	0301/1101/B3*0201
141	MP207	0303/0601	DR9/1501	0	DR53	*0101	0	0	0303/0901**	0601/1501**	0303/0901/B4*0101(2)	0601/1501/B5*0101
142	MP208	0401/0604	0101/DR7	0	DR53	0	0	0	0401/0701(2)*	0604/0101*	0401/0701/B4*0101(2)	0604/0101/0
143	MP209	0301/0504	1401/0103	*0202	0	0	0	0	0301/0103**	0504/1401**	0301/0103/0	0504/1401/B3*0202
144	MP210	0302/0601	1501/0404	0	DR53	*0101	0	0	0302/0404	0601/1501**	0302/0404/B4*0101(2)	0302/0404/B4*0101(2)
145	MP211	0601/0601	1501/1502	0	0	*0101/0102	0601/1501	0601/1502	0601/1501**	0601/1502	0601/1501/B5*0101	0601/1502/B5*0102
146	MP212	0603/0604	0302/1407	*0101	0	0	0	0	0603/0302*	0604/1407*	0603/0302/B3*0101	0604/1407/B3*0101
147	MP22	0501/0501	0101/0101	0	0	0	0501/0101	0501/0101	0501/0101	0501/0101	0501/0101/0	0501/0101/0
148	MP23	0201/0302	0301/0401	*0101	DR53	0	0	0	0201/0301	0302/0401	0201/0301/B3*0101	0302/0401/B4*0101(2)
149	MP234	0301/0402	1304/1304	*0202	0	0	0301/1304	0402/1304	0301/1304**	0402/1304**	0301/1304/B3*0202	0402/1304/B3*0202
150	MP236	0201/0302	0301/0402	*0202	DR53	0	0	0	0201/0301	0302/0402	0201/0301/B3*0202	0302/0402/B4*0101(2)
151	MP237	0603/0604	1301/1302	*0202/0301	0	0	0	0	0603/1301	0604/1302	0603/1301/B3*0202	0604/1302/B3*0301
152	MP238	0402/0501	0801/0802	*0101	0	0	0	0	0402/0801	0501/0801*	0402/0801/B3*0101	0501/0801/0
153	MP239	0201/0601	0302/0302	*0101	0	0	0201/0302	0601/0302	0201/0302*	0601/0302*	0201/0302/B3*0101	0601/0302/B3*0101
154	MP24	0201/0602	0301/1406	*0202	0	0	0	0	0201/0301	0602/1406*	0201/0301/B3*0202	0602/1406/B3*0202
155	MP240	0601/0601	1501/1502	0	0	0201/2	0601/1501	0601/1502	0601/1501	0601/1502	0601/1501/B5*0201(2)	0601/1502/B5*0201(2)
156	MP241	0302/0603	0404/1301	*0101	DR53	0	0	0	0302/0404	0603/1301	0302/0404/B4*0101(2)	0603/1301/B3*0101
157	MP242	0201/0603	0301/DR10	*0101	0	0	0	0	0201/0301	0603/1001*	0201/0301/B3*0101	0603/1001/0
158	MP243	0302/0504	0401/1401	*0101	DR53	0	0	0	0302/0401	0504/1401**	0302/0401/B4*0101(2)	0504/1401/B3*0101
159	MP244	0201/0601	1501/DR9	0	DR53	*0101	0	0	0601/1501	0201/0901*	0601/1501/B5*0101	0201/0901/B4*0101(2)
160	MP246	0302/0201	0301/0404	*0101	DR53	0	0	0	0302/0404	0201/0301	0302/0404/B4*0101(2)	0201/0301/B3*0101
161	MP249	0201/0302	DR7/1101	*0202	DR53	0	0	0	0201/0701(2)	0302/1101	0201/0701(2)/B4*0101(2)	0302/1101/B3*0202
162	MP250	0502/0605	1404/0802	*0202	0	0	0	0	0502/1404*	0605/0802*	0502/1404/B3*0202	0605/0802/0
163	MP251	0301/0302	1104/0403	*0101	DR53	0	0	0	0301/1104	0302/0403	0301/1104/B3*0101	0302/0403/B4*0101
164	MP252	0501/0603	0101/1301	*0101	0	0	0	0	0501/0101	0603/1301	0501/0101/0	0603/1301/B3*0101
165	MP254	0302/0401	0404/0404	0	DR53	0	0302/0404	0401/0404	0302/0404	0401/0404*	0302/0404/B4*0101(2)	0401/0404/B4*0101(2)
166	MP256	0201/0301	0301/0401	*0202	DR53	0	0	0	0201/0301	0301/0401	0201/0301/B3*0202	0301/0401/B4*0101(2)
167	MP257	0302/0504	0402/1401	*0101	DR53	0	0	0	0302/0402	0504/1401**	0302/0402/B4*0101(2)	0504/1401/B3*0101
168	MP258	0301/0604	1101/1305	*0202	0	0	0	0	0301/1101	0604/1305*	0301/1101/B3*0202	0604/1305/B3*0202
169	MP259	0401/05032	DR9/1501	0	DR53	0	0	0	0401/0901*	05032/1501*	0401/0901/B4*0101(2)	05032/1501/B5*?
170	MP26	0201/0302	0408/0301	*0101	DR53	0	0	0	0201/0301	0302/0408**	0201/0301/B3*0101	0302/0408/B4*0101(2)
171	MP261	0501/0604	0101/1302	*0101	0	0	0	0	0501/0101	0604/1302	0501/0101/0	0604/1302/B3*0101
172	MP267	0302/0201	DR7/0301	*0201	DR53	0	0	0	0201/0301	0302/0701(2)**	0201/0301/B3*0201	0302/0701(2)/B4*0101(2)
173	MP268	0302/0601	1601/0403	0	DR53	*0102	0	0	0302/0403	0601/1601*	0302/0403/B4*0101(2)	0601/1601/B5*0101
174	MP269	0301/0501	0101/0401	0	DR53	0	0	0	0501/0101	0301/0401	0501/0101/0	0301/0401/B4*0101(2)

	1	2	3	4	5	6	7	8	9	10	11	12
175	MP270	0501/0604	0103/1302	*0301	DR53	0	0	0	0501/0103	0604/1302	0501/0103/0	0604/1302/B3*0301
176	MP271	0402/05031	DR7/1401	*0101	DR53	0	0	0	05031/1401	0402/0701(2)*	0503:/1401/B3*0101	0402/0701(2)/B4*0101(2)
177	MP272	0201/0302	DR7/0404	0	DR53	0	0	0	0201/0701(2)	0302/0404	0201/0701(2)/B4*0101(2)	0302/0404/B4*0101(2)
178	MP273	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
179	MP274	0302/0501	0101/0405	0	DR53	0	0	0	0501/0101	0302/0405	0501/0101/0	0302/0405/B3*0101
180	MP275	0502/0603	0101/1302	*0301	0	0	0	0	0502/0101*	0602/1302*	0502/0101/0	0603/1302/B3*0301
181	MP278	0201/0302	DR7/0407	0	DR53	0	0	0	0201/0701(2)	0302/0407	0201/0701(2)/B4*0101(2)	0302/0407/B4*0101(2)
182	MP279	0201/0603	0301/1301	*0202	0	0	0	0	0201/0301	0603/1301	0201/0301/B3*0202	0603/1301/B3*0202
183	MP28	0302/0603	DR7/1101	*0202	DR53	0	0	0	0302/0701(2)	0603/1101**	0302/0701(2)/B4*0101(2)	0603/1101/B3*0202
184	MP280	0603/0604	1301/1301	*0101	0	0	0603/1301	0604/1301	0603/1301	0604/1301**	0603/1301/B3*0101	0604/1301/B3*0101
185	MP281	0601/0601	1501/1501	0	0	0201/2	0601/1501	0601/1501	0601/1501**	0601/1501**	0601/1501/B5*0201(2)	0601/1501/B5*0201(2)
186	MP288	0201/0602	1501/0301	*0101	0	*0101	0	0	0201/0301	0602/1501	0201/0301/B3*0101	0602/1501/B5*0101
187	MP289	0402/0402	0801/1104	*0202	0	0	0402/0801	0402/1104	0402/0801**	0402/1104*	0402/0801/0	0402/1104/B3*0202
188	MP29	0301/0501	0101/0103	0	0	0	0	0	0301/0103**	0501/0101	0301/0103/0	0501/0101/0
189	MP290	0301/0402	0402/1301	*0202	DR53	0	0	0	0301/1301	0402/0402*	0301/1301/B3*0202	0402/0402/B4*0101(2)
190	MP293	0201/0603	0301/1101	*0201	0	0	0	0	0201/0301	0603/1101**	0201/0301/B3*0201	0603/1101/B3*0201
191	MP294	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
192	MP296	05031/0604	0101/1302	*0301	0	0	0	0	0604/1302	05031/0101*	0604/1302/B3*0301	05031/0101/0
193	MP297	0201/0402	DR10/0801	*0202	0	0	0	0	0201/1001*	0402/0801	0201/1001/0	0402/0801/
194	MP299	0201/0402	DR7/0803	0	DR53	0	0	0	0201/0701(2)	0402/0803**	0201/0701(2)/B4*0101	0402/0803/0
195	MP3	0201/0502	1501/0301	*0101	0	0201/2	0	0	0201/0301	0502/1501	0201/0301/B3*0101	0502/1501/B5*0101(2)
196	MP30	0402/0605	0801/1401	*0101	0	0	0	0	0402/0801**	0605/1401*	0402/0801/0	0605/1401/B3*0101
197	MP300	0402/0201	DR7/1201	*0202	DR53	0	0	0	0201/0701(2)	0402/1201*	0201/0701(2)/B4*0101(2)	0402/1201/B3*0202
198	MP32	0301/0601	0404/1501	0	DR53	*0101	0	0	0301/0404**	0601/1501**	0301/0404/B4*0101	0601/1501/B5*0101
199	MP34	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
200	MP340	05032/0504	DR7/1404	*0201	DR53	0	0	0	05032/1404**	0504/0701(2)*	05032/1404/B3*0201	0504/0701(2)/B4*0101(2)
201	MP341	0201/0301	0301/1101	*0101	0	0	0	0	0201/0301	0301/1101	0201/0301/B3*0101	0301/1101/B3*0101
202	MP342	0301/0303	1303/DR7	*0101	DR53	0	0	0	0301/1303	0303/0701(2)	0301/1303/B3*0101	0303/0701(2)/B4*0101(2)
203	MP343	0302/0303	0402/DR9	0	DR53	0	0	0	0302/0402	0303/0901**	0302/0402/B4*0101	0303/0901/B4*0101(2)
204	MP344	0502/0601	1501/0301	*0202	0	0201/2	0	0	0601/1501**	0502/0301*	0601/1501/B5*0201(2)	0502/0301/B3*0202
205	MP345	0201/0301	1103/0301	*0101	0	0	0	0	0201/0301	0301/1103	0201/0301/B3*0101	0301/1103/B3*0101
206	MP346	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
207	MP347	0201/0302	0404/0301	*0201	DR53	0	0	0	0201/0301	0302/0404	0201/0301/B3*0201	0302/0404/B4*0101
208	MP35	0603/0604	1301/1302	*0201	0	0	0	0	0603/1301	0604/1302	0603/1301/B3*0201	0604/1302/B3*0201
209	MP350	0201/0201	DR10/0301	*0101	0	0	0	0	0201/1001*	0201/0301	0201/1001/0	0201/0301/B3*0101
210	MP355	0301/0601	1102/1502	*0301	0	*0102	0	0	0301/1102	0601/1502	0301/1102/B4*0301	0601/1502/B5*0102
211	MP356	0201/0201	DR7/0301	*0101	DR53	0	0201/0701(2)	0201/0301	0201/0701(2)	0201/0301	0201/0701(2)/B4*0101	0201/0301/B3*0101
212	MP357	0301/0301	0301/0801	*0101	0	0	0301/0801	0301/0301	0301/0801**	0301/0301*	0301/0801/0	0301/0301/B3*0101
213	MP358	0602/0401	0401/1302	*0301	DR53	0	0	0	0401/0401**	0602/1302*	0401/0401/B4*0101	0602/1302/B3*0301
214	MP359	0301/0602	0401/1501	0	DR53	*0101	0	0	0301/0401	0602/1501	0301/0401/B4*0101	0602/1501/B5*0101
215	MP36	0501/0502	0101/1101	*0101	0	0	0	0	0501/0101	0502/1101*	0501/0101/0	0502/1101/B3*0101
216	MP360	0201/0201	DR7/0301	*0101	DR53	0	0201/0701(2)	0201/0301	0201/0701(2)	0201/0301	0201/0701(2)/B4*0101	0201/0301/B4*0101
217	MP361	0402/0601	DR7/0302	*0202	0	0	0	0	0402/0701(2)*	0601/0302*	0402/0701(2)/B4*0101	0601/0302/B3*0202
218	MP362	0201/0502	0301/1601	*0101	0	*0201/2	0	0	0201/0301	0502/1601	0201/0301/B3*0101	0502/1601/B5*0201(2)
219	MP363	0501/0504	0101/1401	*0201	0	0	0	0	0501/0101	0504/1401**	0501/0101/0	0504/1401/B3*0201
220	MP364	0201/0301	0401/0301	*0101	DR53	0	0	0	0201/0301	0301/0401	0201/0301/B3*0101	0301/0401/B4*0101
221	MP366	0504/0602	DR7/DR10	0	DR53	0	0	0	0504/0701*	0602/1001*	0504/0701/B4*0101	0602/1001/0
222	MP37	0201/0603	DR9/1501	0	DR53	0201/2	0	0	0201/1501*	0603/0901*	0201/1501/B5*0201(2)	0603/0901/B4*0101
223	MP372	0604/0605	1302/0401	*0301	DR53	0	0	0	0604/1302	0605/0401*	0604/1302/B3*0301	0605/0401/B4*0101
224	MP373	0601/0604	1501/1302	*0301	0	0201/2	0	0	0601/1501**	0604/1302	0601/1501/B5*0201(2)	0604/1302/B3*0301
225	MP374	0301/0302	0401/0404	0	DR53	0	0	0	0301/0401	0302/0404	0301/0401/B4*0101	0302/0404/B4*0101
226	MP375	0504/0602	1501/0302	*0101	0	0201/2	0	0	0602/1501	0504/0302*	0602/1501/B5*0201(2)	0504/0302/B3*0101
227	MP376	0201/0602	0301/1501	*0202	0	*0101	0	0	0201/0301	0602/1501	0201/0301/B3*0202	0602/1501/B5*0101
228	MP378	0402/0501	0101/0401	0	DR53	0	0	0	0501/0101	0402/0401*	0501/0101/0	0402/0401/B4*0101(2)
229	MP379	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
230	MP38	0402/0503	0801/1401	*0101	0	0	0	0	0402/0801**	0503/1401	0402/0801/0	0503/1401/B3*0101
231	MP380	0602/0603	1301/1501	*0202	0	*0101	0	0	0602/1501	0603/1301	0602/1501/B5*0101	0603/1301/B3*0202
232	MP381	0201/0201	0301/0301	*0101	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101

	1	2	3	4	5	6	7	8	9	10	11	12
233	MP382	0201/0501	DR9/0301	*0101	DR53	0	0	0	0201/0301	0501/0901*	0201/0301/B3*0101	0501/0901/B4*0101
234	MP383	05031/0604	1501/1302	*0301	0	0201/2	0	0	05031/1501*	0604/1302	05031/1501/0201(2)	0604/1302/B3*0301
235	MP384	0501/0605	DR9/0101	0	DR53	0	0	0	0501/0101	0605/0901*	0501/0101/0	0605/0901/B4*0101
236	MP386	0301/0604	DR7/0407	0	DR53	0	0	0	0301/0407	0604/0701(2)*	0301/0407/B4*0101	0604/0701/B4*0101(2)
237	MP387	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
238	MP389	0201/0501	DR9/0102	0	DR53	0	0	0	0501/0102	0201/0901*	0501/0102/0	0201/0901/B4*0101
239	MP39	0201/0601	1501/0301	*0202	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0202	0601/1501/B5*0101
240	MP391	0602/0603	1501/1301	*0101	0	*0101	0	0	0602/1501	0603/1301	0602/1501/B5*0101	0603/1301/B3*0101
241	MP392	0201/0602	1501/0301	*0101	0	0201/2	0	0	0201/0301	0602/1501	0201/0301/B3*0101	0602/1501/B5*0201(2)
242	MP393	0302/0303	0402/0402	0	DR53	0	0302/0402	0303/0402	0302/0402	0303/0402*	0302/0402/B4*0101(2)	0303/0402/B4*0101(2)
243	MP394	0301/0302	0103/0410	0	DR53	0201/2	0	0	0301/0103**	0302/0410**	0301/0103/0	0302/0410/B4*0101(2)
244	MP395	0201/0602	DR7/1501	0	DR53	*0101	0	0	0201/0701(2)	0602/1501	0201/0701(2)/B4*0101	0602/1501/B5*0101
245	MP41	0302/0302	0401/0401	0	DR53	0	0302/0401	0302/0401	0302/0401	0302/0401	0302/0401/B4*0101(2)	0302/0401/B4*0101(2)
246	MP426	0602/0602	1501/1502	0	0	*0201/2	0602/1501	0602/1502	0602/1501	0602/1501**	0602/1501/B5*0201(2)	0602/1502/B5*0201(2)
247	MP427	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
248	MP428	0401/0502	DR10/1501	0	0	0201/2	0	0	0502/1501	0401/1001*	0502/1501/B5*0201(2)	0401/1001/0
249	MP429	0201/0601	1501/0301	*0101	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0101
250	MP43	0301/0601	1201/1501	*0202	0	*0101	0	0	0301/1201	0601/1501**	0301/1201/B3*0202	0601/1501/B5*0101
251	MP430	0501/0501	0101/0101	0	0	0	0501/0101	0501/0101	0501/0101	0501/0101	0501/0101/0	0501/0101/0
252	MP431	0201/0402	0801/0301	*0202	0	0	0	0	0201/0301	0402/0801**	0201/0301/B3*0202	0402/0801/0
253	MP432	05032/0603	1401/1104	*0202	0	0	0	0	05032/1401	0603/1104**	05032/1401/B3*0202	0603/1104/B3*0202
254	MP433	0602/0603	1101/DR7	*0202	DR53	0	0	0	0603/1101**	0602/0701(2)*	0603/1101/B3*0202	0602/0701(2)/B4*0101
255	MP434	0502/0602	1501/1601	0	0	*0201/2	0	0	0502/1601	0602/1501	0502/1601/B5*0201(2)	0602/1501/B5*0201(2)
256	MP435	0201/0601	DR7/1406	*0301	DR53	0	0	0	0201/0701(2)	0601/1406*	0201/0701(2)/B4*0101	0601/1406/B3*0301
257	MP438	0501/0501	0101/DR7	0	DR53	0	0501/0101	0501/0701(2)	0501/0101	0501/0701(2)*	0501/0101/0	0501/0701(2)/B4*0101(2)
258	MP44	0201/0402	DR9/0301	*0202	0	0	0	0	0201/0301	0402/0901*	0201/0301/B3*0202	0402/0901/B4*0101(2)
259	MP440	0201/0304	0402/0301	*0101	DR53	0	0	0	0201/0301	0304/0402*	0201/0301/B3*0101	0304/0402/B4*0101(2)
260	MP441	05031/0604	1302/1401	*0101/*0301	0	0	0	0	05031/1401	0604/1302	05031/1401/B3*0101	0604/1302/B3*0301
261	MP442	0501/0604	0101/1301	*0202	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0202
262	MP444	0302/0302	0402/0410	0	DR53	0	0302/0402	0302/0410	0302/0402	0302/0410**	0302/0402/B4*0101(2)	0302/0410/B4*0101(2)
263	MP445	0501/0501	DR7/0102	0	DR53	0	0501/0102	0501/0701(2)	0501/0102	0501/0701(2)*	0501/0102/0	0501/0701(2)/B4*0101(2)
264	MP45	0601/0601	DR7/1501	0	DR53	0201/2	0601/1501	0601/0701(2)	0601/1501**	0601/0701(2)*	0601/1501/B5*0201(2)	0601/0701(2)/B4*0101(2)
265	MP46	0301/0603	0401/1302	*0301	DR53	0	0	0	0301/0401	0603/1302*	0301/0401/B4*0101	0603/1302/B3*0301
266	MP47	0401/0502	0101/1602	0	0	*0201/2	0	0	0502/1602	0401/0101*	0502/1602/B5*0201(2)	0401/0101/0
267	MP475	0201/0302	DR7/0401	0	DR53	0	0	0	0201/0701(2)	0302/0401	0201/0701/B4*0101(2)	0302/0401/B4*0101(2)
268	MP476	05032/0601	1501/DR7	0	DR53	*0101	0	0	0601/1501**	05032/1701(2)*	0601/1501/B5*0101	05032/0701/B4*0101
269	MP477	0501/0604	0101/1402	*0301	0	0	0	0	0501/0101	0604/1402*	0501/0101/0	0604/1402/B3*0301
270	MP478	0302/0402	0801/08042	*0101	0	0	0	0	0302/08041**	0402/0801*	0302/08042/0	0402/0801/0
271	MP48	0301/0301	0103/DR10	0	0	0	0301/0103	0301/1001	0301/0103**	0301/1001*	0301/0103/0	0301/1001/0
272	MP480	0603/0604	1101/1302	*0301/0202	0	0	0	0	0603/1101**	0604/1302	0603/1101/B3*0202	0604/1302/B3*0301
273	MP481	0301/0302	0401/0404	0	DR53	0	0	0	0301/0401	0302/0404	0301/0401/B4*0101(2)	0302/0404/B4*0101(2)
274	MP482	0502/0604	1601/1301	*0101	0	*0201/2	0	0	0502/1601	0604/1301**	0502/1601/B5*0201(2)	0604/1301/B3*0101
275	MP483	0201/0303	1301/0301	*0202	0	0	0	0	0201/0301	0303/1301	0201/0301/B3*0202	0303/1301/B3*0202
276	MP484	0201/0601	1501/0301	*0202	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0202	0601/1501/B5*0101
277	MP486	0602/0603	DR7/DR9	0	DR53	0	0	0	0602/0701(2)*	0603/0901*	0602/0701(2)/B4*0101(2)	0603/0901/B4*0101
278	MP487	0501/0605	0101/0301	*0202	0	0	0	0	0501/0101	0605/0301*	0501/0101/0	0605/0301/B3*0202
279	MP488	05031/0602	1401/DR7	*0101	DR53	0	0	0	05031/1401	0602/0701(2)	05031/1401/B3*0101	0602/0701(2)/B4*0101(2)
280	MP489	0301/0601	0401/1502	0	DR53	*0102	0	0	0601/1502	0301/0401	0601/1502/B5*0102	0301/0401/B4*0101
281	MP49	0201/0601	0301/DR9	*0202	DR53	0	0	0	0201/0301	0601/0901*	0201/0301/B3*0202	0601/0901/B4*0101
282	MP491	0402/05032	DR7/0803	0	DR53	0	0	0	0402/0803**	05032/0701(2)*	0402/0803/0	05032/0701(2)/B4*0101(2)
283	MP492	0201/0201	DR7/DR7	0	DR53	0	0201/0701(2)	0201/0701(2)	0201/0701(2)	0201/0701(2)	0201/0701(2)/B4*0101(2)	0201/0701(2)/B4*0101(2)
284	MP493	0501/0504	DR9/0101	0	DR53	0	0	0	0501/0101	0504/0901*	0501/0101/0	0504/0901/B4*0101
285	MP494	0302/0302	DR7/0401	0	DR53	0	0302/0401	0302/0701(2)	0302/0401	0302/0701(2)	0302/0701(2)/B4*0101	0302/0401/B4*0101(2)
286	MP495	0402/0605	0801/1301	*0202	0	0	0	0	0402/0801**	0605/1301*	0402/0801/0	0605/1301/B3*0202
287	MP496	0302/0302	0404/1101	*0202	DR53	0	0302/0404	0302/1101	0302/0404	0302/1101	0302/0404/B4*0101	0302/1101/B3*0202
288	MP497	0605/0605	1301/0404	*0101	DR53	0	0605/1301	0605/0404	0605/1301*	0605/0404*	0605/1301/B3*0101	0605/0404/B4*0101(2)
289	MP498	0302/0303	0404/1301	*0101	DR53	0	0	0	0302/0404	0303/1301	0302/0404/B4*0101(2)	0303/1301/B3*0101
290	MP499	0504/0601	1104/1502	*0202	0	*0102	0	0	0601/1502	0504/1104*	0601/1502/B5*0102	0504/1104/B3*0202

	1	2	3	4	5	6	7	8	9	10	11	12
291	MP50	0302/0302	0401/0402	0	DR53	0	0302/0401	0302/0402	0302/0401	0302/0402	0302/0401/B4*0101(2)	0302/0402/B4*0101(2)
292	MP500	0401/0402	0401/0803	*0101	DR53	0	0	0	0401/0401**	0402/0803**	0401/0401/B4*0101(2)	0402/0803/0
293	MP501	0502/0604	1601/1301	*0101	0	*0201/2	0	0	0502/1601	0604/1301**	0502/1601/B5*0201(2)	0604/1301/B3*0101
294	MP507	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
295	MP502	0303/0303	DR9/DR9	0	DR53	0	0303/0901	0303/0901	0303/0901**	0303/0901**	0303/0901/B4*0101(2)	0303/0901/B4*0101(2)
296	MP503	0201/0604	DR7/1301	*0201	0	0	0	0	0201/0701(2)	0604/1301**	0201/0701(2)/B4*0101(2)	0604/1301/B3*0201
297	MP506	0402/0501	DR7/0101	0	DR53	0	0	0	0501/0101	0402/0701(2)*	0501/0101/0	0402/0701(2)/B4*0101(2)
298	MP52	0201/0604	DR9/1301	*0202	DR53	0	0	0	0604/1301**	0201/0901*	0604/1301/B3*0202	0201/0901/B4*0101(2)
299	MP54	0603/0604	1104/1301	*0202	0	0	0	0	0603/1104**	0604/1301**	0603/1104/B3*0202	0604/1301/B3*0202
300	MP55	0302/0603	0402/1101	*0202	DR53	0	0	0	0603/1101**	0302/0402	0603/1101/B3*0202	0302/1402/B4*0101(2)
301	MP56	0501/0604	DR9/1301	*0202	DR53	0	0	0	0604/1301**	0501/0901*	0604/1301/B3*0202	0501/0901/B4*0101(2)
302	MP57	0301/0504	0103/1404	*0202	0	0	0	0	0301/0103**	0504/1104**	0301/0103/0	0504/1404/B3*0202
303	MP58	0201/0201	DR7/0301	*0202	DR53	0	0201/0301	0201/0701(2)	0201/0301	0201/0701(2)	0201/0301/B3*0202	0201/0701(2)/B4*0101(2)
304	MP59	0302/05031	0401/1401	*0101	DR53	0	0	0	0302/0401	05032/1401	0302/0401/B4*0101(2)	05032/1401/B3*0101
305	MP6	0201/0601	1407/0301	*0202	0	0	0	0	0201/0301	0601/1407*	0201/0301/B3*0202	0601/1407/B3*0202
306	MP60	0301/0502	1301/1601	*0202	0	0201/2	0	0	0301/1301	0502/1601	0301/1301/B3*0202	0502/1601/B5*0201(2)
307	MP61	0201/0301	0301/1101	*0101	0	0	0	0	0201/0301	0301/1101	0201/0301/B3*0101	0301/1101/B3*0101
308	MP62	0502/0604	1601/1301	*0101	0	*0102	0	0	0502/1601	0604/1301**	0502/1601/B5*0102	0604/1301/B3*0101
309	MP65	0201/0201	DR9/0301	*0101	DR53	0	0201/0301	0201/0901	0201/0301	0201/0901*	0201/0301/B3*0101	0201/0901/B4*0101(2)
310	MP66	0301/0402	1301/0801	*0101	0	0	0	0	0301/1301	0402/0801**	0301/1301/B3*0101	0402/0801/0
311	MP67	0201/0501	0101/0301	*0202	0	0	0	0	0201/0301	0501/0101	0201/0301/B3*0202	0501/0101/0
312	MP687	0501/0502	0101/1501	0	0	0201/2	0	0	0501/0101	0502/1501	0501/0101/0	0502/1501/B5*0201(2)
313	MP689	0501/0603	DR7/0102	0	DR53	0	0	0	0501/0102	0603/0701(2)*	0501/0102/0	0603/0701(2)/B4*0101(2)
314	MP69	0302/0602	0401/08031	0	DR53	0	0	0	0302/0401	0602/08031*	0302/0401/B4*0101(2)	0602/08031/0
315	MP691	0201/0201	DR7/DR7	0	DR53	0	0201/0701(2)	0201/0701(2)	0201/0701(2)	0201/0701(2)	0201/0701(2)/B4*0101(2)	0201/0701(2)/B4*0101(2)
316	MP692	0604/0605	DR7/1301	*0101	DR53	0	0	0	0604/1301**	0605/0701(2)*	0604/1301/B3*0101	0605/0701(2)/B4*0101(2)
317	MP693	0201/0502	1601/0301	*0202	0	*0201/2	0	0	0201/0301	0502/1601	0201/0301/B3*0202	0502/1601/B5*0201(2)
318	MP695	0201/0604	0301/1301	*0202	0	0	0	0	0201/0301	0604/1301**	0201/0301/B3*0202	0604/1301/B3*0202
319	MP697	0201/0602	0301/1501	*0202	0	*0101	0	0	0201/0301	0602/1501	0201/0301/B3*0202	0602/1501/B5*0101
320	MP698	0302/0603	0401/1101	*0202	DR53	0	0	0	0302/0401	0603/1101**	0302/0401/B4*0101	0603/1101/B3*0202
321	MP699	0501/0604	0101/1301	*0101	0	0	0	0	0501/0101	0604/1301**	0501/0101/0	0604/1301/B3*0101
322	MP7	0502/0603	0302/1303	*0202	0	0	0	0	0502/0302*	0603/1303*	0502/0302/B3*0202	0603/1303/B3*0202
323	MP70	0201/0201	0408/0301	*0101	DR53	0	0201/0408	0201/0301	0201/0408*	0201/0301	0201/0408/B4*0101(2)	0201/0301/B3*0101
324	MP701	0301/0301	1104/1305	*0101	0	0	0301/1104	0301/1305	0301/1104	0301/1305	0301/1104/B3*0101	0301/1305/B3*0101
325	MP702	0501/0501	DR7/0101	0	DR53	0	0501/0101	0501/0701(2)	0501/0101	0501/0701(2)*	0501/0101/0	0501/0701(2)/B4*0101(2)
326	MP703	0302/0302	DR7/0801	0	DR53	0	0302/0701(2)	0302/0801	0302/0701(2)**	0302/0801*	0302/0701(2)/B4*0101(2)	0302/0801/0
327	MP704	0201/0605	DR9/0301	*0202	DR53	0	0	0	0201/0301	0605/0901*	0201/0301/B3*0202	0605/0901/B4*0101(2)
328	MP753	0201/0601	0301/1502	*0101	0	*0102	0	0	0201/0301	0601/1502	0201/0301/B3*0101	0601/1502/B5*0102
329	MP754	0602/0604	DR9/1501	0	DR53	*0101	0	0	0602/1501	0604/0901*	0602/1501/B5*0101	0604/0901/B4*0101(2)
330	MP756	0401/0502	DR7/1501	0	DR53	0201/2	0	0	0401/1501*	0502/1701(2)*	0401/1501/B5*0201(2)	0502/0701(2)/B4*0101(2)
331	MP758	0201/0302	0301/0401	*0101	DR53	0	0	0	0201/0301	0302/0401	0201/0301/B3*0101	0302/0401/B4*0101(2)
332	MP760	0501/0605	0101/1301	*0201	0	0	0	0	0501/0101	0605/1301	0501/1010/0	0605/1301/B3*0201
333	MP762	0603/0604	DR9/1301	*0101	DR53	0	0	0	0603/1301	0604/0901*	0603/1301/B3*0101	0604/0901/B4*0101
334	MP763	0201/0601	0301/1501	*0101	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0101
335	MP766	0201/0602	DR9/0301	*0101	DR53	0	0	0	0201/0301	0602/0901*	0201/0301/B3*0101	0602/0901/B4*0101
336	MP767	0302/0201	0402/0301	*0101	DR53	0	0	0	0302/0402	0201/0301	0302/0402/B4*0101	0201/0301/B3*0101
337	MP768	0402/05031	DR7/0302	*0202	DR53	0	0	0	0402/0701(2)*	05031/0302*	0402/0701(2)/B4*0101(2)	05031/0302/B3*0202
338	MP770	0301/0301	0401/0103	0	DR53	0	0301/0401	0301/0103	0301/0401	0301/0103**	0301/0401/B4*0101(2)	0301/0103/0
339	MP771	0201/0502	0301/1602	*0101	0	*0201/2	0	0	0201/0301	0502/1602**	0201/0301/B3*0101	0502/1602/B5*0201(2)
340	MP772	0302/0401	DR7/0401	0	DR53	0	0	0	0302/0401	0401/0401**	0302/0401/B4*0101(2)	0401/0401/B4*0101(2)
341	MP773	0201/0604	DR9/0301	*0101	DR53	0	0	0	0201/0301	0604/0901*	0201/0301/B3*0101	0604/0901/B4*0101
342	MP774	0201/0302	DR7/0301	*0202	DR53	0	0	0	0201/0301	0302/0701(2)**	0201/0301/B3*0202	0302/0701(2)/B4*0101
343	MP775	05031/0601	1501/1401	*0101	0	*0101	0	0	0601/1501**	05031/1401	0601/1501/B5*0101	05031/1401/B3*0101
344	MP777	0501/0501	DR9/0101	0	DR53	0	0501/0101	0501/0901	0501/0101	0501/0901*	0501/0101/0	0501/0901/B4*0101(2)
345	MP778	0201/0301	0301/0401	*0101	DR53	0	0	0	0201/0301	0301/0401	0201/0301/B3*0101	0301/0401/B4*0101
346	MP780	0402/0501	0	0	0	0	0	0	0	0	0	0
347	MP781	0201/0302	DR7/0408	0	DR53	0	0	0	0201/0701(2)	0302/0408**	0201/0701/B4*0101	0302/0408/B4*0101(2)
348	MP782	0303/0303	1301/1301	*0101	0	0	0303/1301	0303/1301	0303/1301	0303/1301	0303/1301/B3*0101	0303/1301/B3*0101

	1	2	3	4	5	6	7	8	9	10	11	12
349	MP79	0201/0301	0401/0302	*0202	DR53	0	0	0	0201/0302*	0301/0401	0201/0302/B3*0202	0301/0401/B4*0101
350	MP799	0201/0604	0301/1407	*0101	0	0	0	0	0201/0301	0604/1407*	0201/0301/B3*0101	0604/1407/B3*0101
351	MP8	0502/0603	DR10/1101	*0202	0	0	0	0	0502/1001*	0603/1101**	0502/1001/0	0603/1101/B3*0202
352	MP80	0201/0601	0301/1501	*0101	0	0	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/*
353	MP800	0401/0402	DR7/0401	0	DR53	0	0	0	0401/0401	0402/0701(2)*	0401/0401/B4*0101(2)	0402/0701(2)/B4*0101(2)
354	MP801	0201/0602	DR7/1502	0	DR53	*0102	0	0	0201/0701	0602/1502**	0201/0701/B4*0101(2)	0602/1502/B5*0102
355	MP802	0401/0501	DR7/0101	0	DR53	0	0	0	0501/0101	0401/0701(2)*	0501/0101/0	0401/0701(2)/B4*0101(2)
356	MP803	0302/0302	DR10/0404	0	DR53	0	0302/0404	0302/1001	0302/0404	0302/1001*	0302/0404/B4*0101(2)	0302/1001/0
357	MP804	0502/05031	1601/1401	*0101	0	0	0	0	0502/1601	05031/1401	0502/1601/*	05031/1401/B3*0101
358	MP805	0201/0301	DR7/0103	0	DR53	0	0	0	0201/0701	0301/0103**	0201/0701(2)/B4*0101(2)	0301/0103/0
359	MP806	0501/0604	DR7/0101	*0101	0	0	0	0	0501/0101	0604/0701(2)*	0501/0101/0	0604/0701(2)/0
360	MP807	0201/0301	DR7/0408	0	DR53	0	0	0	0201/0701(2)	0301/0408	0201/0701(2)/B4*0101(2)	0301/0408/B4*0101(2)
361	MP808	0201/0601	0301/1501	*0101	0	*0101	0	0	0201/0301	0601/1501**	0201/0301/B3*0101	0601/1501/B5*0101
362	MP809	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
363	MP81	0504/0603	1104/1305	0301/0201	0	0	0	0	0603/1104**	0504/1305*	0603/1104/B3*0201	0504/1305/B3*0301
364	MP811	0401/0603	1101/0401	*0202	DR53	0	0	0	0401/1401**	0603/1101**	0401/0401/B4*0101	0603/1101/B3*0202
365	MP812	0201/0302	DR7/0401	0	DR53	0	0	0	0201/0701(2)	0302/0401	0201/0701(2)/B4*0101(2)	0302/0401/B4*0101(2)
366	MP813	0302/0302	0402/0403	0	DR53	0	0302/0402	0302/0403	0302/0402	0302/0403	0302/0402/B4*0101	0302/0403/B4*0101(2)
367	MP814	0501/0604	0102/1302	*0301	0	0	0	0	0501/0102	0604/1302	0501/0102/0	0604/1302/B3*0301
368	MP815	0201/0603	1301/0301	*0202	0	0	0	0	0201/0301	0603/1301	0201/0301/B3*0202	0603/1301/B3*0202
369	MP816	0402/0501	0101/0801	0	0	0	0	0	0501/0101	0402/0801**	0501/0101/0	0402/0801/0
370	MP817	0201/0604	0301/1301	*0101	0	0	0	0	0201/0301	0604/1301**	0201/0301/B3*0101	0604/1301/B3*0101
371	MP818	0201/0604	DR7/0301	*0201	0	0	0	0	0201/0301	0604/0701(2)*	0201/0301/B3*0201	0604/0701(2)/B4*0101(2)
372	MP819	0201/0604	0301/1302	*0101/0301	0	0	0	0	0201/0301	0604/1302	0201/0301/B3*0101	0604/1302/B3*0301
373	MP82	05032/0601	0102/1501	0	0	0201/2	0	0	0601/1501**	05032/0102*	0601/1501/B5*0201(2)	05032/0102/0
374	MP820	0501/05031	DR10/1403	*0301	0	0	0	0	05031/1403**	0501/1001	05031/1403/B3*0301	0501/1001/0
375	MP821	0302/0303	0401/1301	0	DR53	0	0	0	0302/0401	0303/1301	0302/0401/B4*0101(2)	0303/1301/B3*
376	MP822	0301/0201	DR9/0301	*0101	DR53	0	0	0	0301/0901*	0201/0301	0301/0901/B4*0101(2)	0201/0301/B3*0101
377	MP828	0303/0501	0	0	0	0	0	0	0	0	0	0
378	MP823	0201/0604	DR7/DR7	0	DR53	0	0201/0701(2)	0604/0701(2)	0201/0701(2)	0604/0701(2)*	0201/0701(2)/B4*0101(2)	0604/0701/B4*0101(2)
379	MP824	0603/0604	DR7/1301	*0101	DR53	0	0	0	0603/1301	0604/0701(2)*	0603/1301/B3*0101	0604/0701(2)/B4*0101(2)
380	MP825	0301/0301	0103/0401	0	DR53	0	0301/0103	0301/0401	0301/0103**	0301/0401	0301/0103/0	0301/0401/B4*0101(2)
381	MP826	0501/0502	DR7/1601	0	DR53	*0201/2	0	0	0502/1601	0501/0701(2)*	0502/1601/B5*0201(2)	0501/0701/B4*0101(2)
382	MP827	0301/0301	DR9/1104	*0101	DR53	0	0301/0901	0301/1104	0301/0901*	0301/1104	0301/0901/B4*0101(2)	0301/1104/B3*0101
383	MP83	0301/0603	1101/0403	*0202	DR53	0	0	0	0301/0403**	0603/1101**	0301/0403/B4*0101(2)	0603/1101/B3*0202
384	MP84	0201/0303	DR7/1301	*0101	DR53	0	0	0	0201/0701	0303/1301	0201/0701/B4*0101(2)	0303/1301/B3*0101
385	MP85	0601/0605	1501/1501	0	0	0201/2	0601/1501	0605/1501	0601/1501**	0605/1501*	0601/1501/B5*0201(2)	0605/1501/B5*0201(2)
386	MP86	0201/0201	0301/0301	*0202	0	0	0201/0301	0201/0301	0201/0301	0201/0301	0201/0301/B3*0202	0201/0301/B3*0202
387	MP87	0502/0603	1602/1104	*0202	0	*0201/2	0	0	0502/1602**	0603/1104**	0502/1602/B5*0201(2)	0603/1104/B3*0202
388	MP88	0602/0604	1501/1401	*0101	0	0	0	0	0602/1501	0604/1401*	0602/1501/B5*	0604/1401/B3*0101
389	MP89	0201/0501	DR10/0101	0	0	0	0	0	0501/0101	0201/1001*	0501/0101/0	0201/1001/0
390	MP9	0604/0604	1301/1302	*0201/0202	0	0	0604/1301	0604/1302	0604/1301**	0604/1302	0604/1301/B3*0201	0604/1302/B3*0202
391	MP90	0301/0302	DR7/1101	*0202	DR53	0	0	0	0301/1101	0302/0701(2)**	0301/1101/B3*0202	0302/0701(2)/B4*0101(2)
392	MP91	0603/0604	0302/1302	*0101	0	0	0	0	0603/0302*	0604/1302	0603/0302/B3*0101	0604/1302/B3*0101
393	MP92	0502/0602	DR10/1501	0	0	*0101	0	0	0502/1501	0602/1001*	0502/1501/B5*0101	0602/1001/0
394	MP93	0201/0301	DR7/0401	0	DR53	0	0	0	0201/0701(2)	0301/0401	0201/0701(2)/B4*0101(2)	0301/0401/B4*0101(2)
395	MP934	0501/0501	DR7/0102	0	DR53	0	0501/0102	0501/0701(2)*	0501/0102	0501/0701(2)*	0501/0102/0	0501/0701(2)/B4*0101(2)
396	MP935	0302/0302	0401/0801	0	DR53	0	0302/0401	0302/0801	0302/0401	0302/0801*	0302/0401/B4*0101	0302/0801/0
397	MP936	05031/0603	DR7/1401	*0202	DR53	0	0	0	05031/1401	0603/0701(2)*	05031/1401/B3*0202	0603/0701(2)/B4*0101(2)
398	MP937	0501/0604	0102/1302	*0301	0	0	0	0	0501/0102	0604/1302	0501/0102/0	0604/1302/B3*0301
399	MP938	0402/0402	0801/1302	*0301	0	0	0402/0801	0402/1302	0402/0801**	0402/1302*	0402/0801/0	0402/1302/B3*0301
400	MP939	0201/0601	0408/0301	*0101	DR53	0	0	0	0201/0301	0601/0408*	0201/0301/B3*0101	0601/0408/B4*0101(2)
401	MP94	0302/0303	0404/1301	*0101	DR53	0	0	0	0302/0404	0303/1301	0302/0404/B4*0101	0303/1301/B3*0101
402	MP940	0501/0501	0101/0101	0	0	0	0501/0101	0501/0101	0501/0101	0501/0101	0501/0101/0	0501/0101/0
403	MP941	0603/0604	1101/1301	*0101	0	0	0	0	0603/1101**	0604/1301**	0603/1101/B3*0101	0604/1301/B3*0101
404	MP942	0605/0605	DR7/0301	*0202	DR53	0	0605/0701(2)	0605/0301	0605/0701(2)*	0605/0301*	0605/0701/B4*0101(2)	0605/0301/B3*0202
405	MP943	0401/0502	DR9/0410	0	DR53	0	0	0	0401/0410*	0502/0901*	0401/0410/B4*0101(2)	0502/0901/B4*0101(2)
406	MP944	0201/0301	0101/0301	*0101	0	0	0	0	0201/0301	0301/0101*	0201/0301/B3*0101	0301/0101/0

	1	2	3	4	5	6	7	8	9	10	11	12
407	MP946	0402/05031	0803/1401	*0101	0	0	0	0	0402/0803**	05031/1401	0402/0803/0	05031/1401/B3*0101
408	MP947	0501/0502	DR9/0101	0	DR53	0	0	0	0501/0101	0502/0901*	0501/0101/0	0502/0901/B4*0101(2)
409	MP948	0201/0601	0301/1502	*0202	0	*0102	0	0	0201/0301	0601/1502	0201/0301/B3*0202	0601/1502/B5*0102
410	MP949	0201/0201	0301/0301	*0101	0	0	0	0	0201/0301	0201/0301	0201/0301/B3*0101	0201/0301/B3*0101
411	MP95	0302/05032	1501/1402	*0101	0	*0101	0	0	0302/1501*	05032/1402	0302/1501/B5*0101	05032/1402/B3*0101
412	MP950	0402/0602	DR7/1502	0	DR53	*0102	0	0	0602/1502**	0402/0701*	0602/1502/B5*0102	0402/0701/B4*0101
413	MP951	0602/0201	1501/DR7	0	DR53	*0101	0	0	0201/0701(2)	0602/1501	0201/0701(2)/B4*0101(2)	0602/1501/B5*0101
414	MP952	0501/0604	DR7/DR7	0	DR53	0	0501/0701	0604/0701	0501/0701(2)*	0604/0701(2)*	0501/0701/B4*0101(2)	0604/0701(2)/B4*0101(2)
415	MP953	0502/0603	1301/1601	*0101	0	*0201/2	0	0	0502/1601	0603/1301	0502/1601/B5*0201(2)	0603/1301/B3*0101
416	MP954	0201/0602	DR7/1502	0	DR53	*0102	0	0	0201/0701(2)	0602/1502**	0201/0701(2)/B4*0101(2)	0602/1502/B5*0102
417	MP955	0402/05031	DR9/1401	*0201	DR53	0	0	0	05031/1401	0402/0901*	0503/1401/B3*0201	0402/0901/B4*0101(2)
418	MP956	0201/0604	0301/1302	*0301/0202	0	0	0	0	0201/0301	0604/1302	0201/0301/B3*0202	0604/1302/B3*0301
419	MP96	0603/0604	1101/1301	*0202	0	0	0	0	0603/1101**	0604/1301**	0603/1101/B3*0202	0604/1301/B3*0202
420	MP97	0501/0601	DR9/0101	0	DR53	0	0	0	0501/0101	0601/0901*	0501/0101/0	0601/0901/B4*0101
421	MP98	0201/0501	0301/0101	*0202	0	0	0	0	0201/0301	0501/0101	0201/0301/B3*0202	0501/0101/0
422												

APPENDIX III: FULL MOTIF PREDICTION FROM HPV 16 E6, E7, L1 AND L2 FOR BINDING TO HLA DRB1*0101 AND DRB1*0401

															IC50 DRB1*0101(nm)
59	71	I	V	Y	R	D	G	N	P	Y	A	V	C	D	4
33	45	I	I	L	E	C	V	Y	C	K	Q	Q	L	L	12
142	154	R	C	M	S	C	C	R	S	S	R	T	R	R	12.5
75	87	K	F	Y	S	K	I	S	E	Y	R	H	Y	C	18.2
84	96	R	H	Y	C	Y	S	L	Y	G	T	T	L	E	34.5
130	142	Q	R	F	H	N	I	R	G	R	W	T	G	R	56.2
106	118	L	L	I	R	C	I	N	C	Q	K	P	L	C	170
101	113	K	P	L	C	D	L	L	I	R	C	I	N	C	185
93	105	T	T	L	E	Q	Q	Y	N	K	P	L	C	D	265
133	145	H	N	I	R	G	R	W	T	G	R	C	M	S	362
109	121	R	C	I	N	C	Q	K	P	L	C	P	E	E	365
36	48	E	C	V	Y	C	K	Q	Q	L	L	R	R	E	415
74	86	L	K	F	Y	S	K	I	S	E	Y	R	H	Y	440
50	62	Y	D	F	A	F	R	D	L	C	I	V	Y	R	520
37	49	C	V	Y	C	K	Q	Q	L	L	R	R	E	V	622
105	117	D	L	L	I	R	C	I	N	C	Q	K	P	L	770
52	64	F	A	F	R	D	L	C	I	V	Y	R	D	G	825
81	93	S	E	Y	R	H	Y	C	Y	S	L	Y	G	T	880
88	100	Y	S	L	Y	G	T	T	L	E	Q	Q	Y	N	1300
31	43	H	D	I	I	L	E	C	V	Y	C	K	Q	Q	1900
58	70	C	I	V	Y	R	D	G	N	P	Y	A	V	C	2600
28	40	T	T	I	H	D	I	I	L	E	C	V	Y	C	3200
48	60	E	V	Y	D	F	A	F	R	D	L	C	I	V	3350
57	69	L	C	I	V	Y	R	D	G	N	P	Y	A	V	4000
24	36	T	E	L	Q	T	T	I	H	D	I	I	L	E	4600
80	92	I	S	E	Y	R	H	Y	C	Y	S	L	Y	G	5900
47	59	R	E	V	Y	D	F	A	F	R	D	L	C	I	6120
97	109	Q	Q	Y	N	K	P	L	C	D	L	L	I	R	6500
32	44	D	I	I	L	E	C	V	Y	C	K	Q	Q	L	7000
127	139	D	K	K	Q	R	F	H	N	I	R	G	R	W	7350
65	77	N	P	Y	A	V	C	D	K	C	L	K	F	Y	10200
72	84	K	C	L	K	F	Y	S	K	I	S	E	Y	R	11000
78	90	S	K	I	S	E	Y	R	H	Y	C	Y	S	L	11000
137	149	G	R	W	T	G	R	C	M	S	C	C	R	S	12000
43	55	Q	L	L	R	R	E	V	Y	D	F	A	F	R	29200
67	79	Y	A	V	C	D	K	C	L	K	F	Y	S	K	35000
71	83	D	K	C	L	K	F	Y	S	K	I	S	E	Y	37000
6	18	T	A	M	F	Q	D	P	Q	E	R	P	R	K	39500
140	152	T	G	R	C	M	S	C	C	R	S	S	R	T	45000
104	116	C	D	L	L	I	R	C	I	N	C	Q	K	P	60000
139	151	W	T	G	R	C	M	S	C	C	R	S	S	R	65200
86	98	Y	C	Y	S	L	Y	G	T	T	L	E	Q	Q	74500
7	19	A	M	F	Q	D	P	Q	E	R	P	R	K	L	84200
128	140	K	K	Q	R	F	H	N	I	R	G	R	W	T	110000
89	101	S	L	Y	G	T	T	L	E	Q	Q	Y	N	K	155000
55	67	R	D	L	C	I	V	Y	R	D	G	N	P	Y	162000
115	127	K	P	L	C	P	E	E	K	Q	R	H	L	D	170000
103	115	L	C	D	L	L	I	R	C	I	N	C	Q	K	180000
136	148	R	G	R	W	T	G	R	C	M	S	C	C	R	182000
42	54	Q	Q	L	L	R	R	E	V	Y	D	F	A	F	200000
77	89	Y	S	K	I	S	E	Y	R	H	Y	C	Y	S	230000
145	157	S	C	C	R	S	S	R	T	R	R	E	T	Q	245000
83	95	Y	R	H	Y	C	Y	S	L	Y	G	T	T	L	265000
124	136	R	H	L	D	K	K	Q	R	F	H	N	I	R	272000
87	99	C	Y	S	L	Y	G	T	T	L	E	Q	Q	Y	305000
17	29	R	K	L	P	Q	L	C	T	E	L	Q	T	T	390000
49	61	V	Y	D	F	A	F	R	D	L	C	I	V	Y	415000

107	119	L	I	R	C	I	N	C	Q	K	P	L	C	P	422000
35	47	L	E	C	V	Y	C	K	Q	Q	L	L	R	R	430000
53	65	A	F	R	D	L	C	I	V	Y	R	D	G	N	475000
39	51	Y	C	K	Q	Q	L	L	R	R	E	V	Y	D	480000
98	110	Q	Y	N	K	P	L	C	D	L	L	I	R	C	530000
125	137	H	L	D	K	K	Q	R	F	H	N	I	R	G	580000
30	42	I	H	D	I	I	L	E	C	V	Y	C	K	Q	612000
82	94	E	Y	R	H	Y	C	Y	S	L	Y	G	T	T	650000
143	155	C	M	S	C	C	R	S	S	R	T	R	R	E	770000
56	68	D	L	C	I	V	Y	R	D	G	N	P	Y	A	805000
1	13	M	H	Q	K	R	T	A	M	F	Q	D	P	Q	1000000
2	14	H	Q	K	R	T	A	M	F	Q	D	P	Q	E	1000000
3	15	Q	K	R	T	A	M	F	Q	D	P	Q	E	R	1000000
4	16	K	R	T	A	M	F	Q	D	P	Q	E	R	P	1000000
5	17	R	T	A	M	F	Q	D	P	Q	E	R	P	R	1000000
8	20	M	F	Q	D	P	Q	E	R	P	R	K	L	P	1000000
9	21	F	Q	D	P	Q	E	R	P	R	K	L	P	Q	1000000
10	22	Q	D	P	Q	E	R	P	R	K	L	P	Q	L	1000000
11	23	D	P	Q	E	R	P	R	K	L	P	Q	L	C	1000000
12	24	P	Q	E	R	P	R	K	L	P	Q	L	C	T	1000000
13	25	Q	E	R	P	R	K	L	P	Q	L	C	T	E	1000000
14	26	E	R	P	R	K	L	P	Q	L	C	T	E	L	1000000
15	27	R	P	R	K	L	P	Q	L	C	T	E	L	Q	1000000
16	28	P	R	K	L	P	Q	L	C	T	E	L	Q	T	1000000
18	30	K	L	P	Q	L	C	T	E	L	Q	T	T	I	1000000
19	31	L	P	Q	L	C	T	E	L	Q	T	T	I	H	1000000
20	32	P	Q	L	C	T	E	L	Q	T	T	I	H	D	1000000
21	33	Q	L	C	T	E	L	Q	T	T	I	H	D	I	1000000
22	34	L	C	T	E	L	Q	T	T	I	H	D	I	I	1000000
23	35	C	T	E	L	Q	T	T	I	H	D	I	I	L	1000000
25	37	E	L	Q	T	T	I	H	D	I	I	L	E	C	1000000
26	38	L	Q	T	T	I	H	D	I	I	L	E	C	V	1000000
27	39	Q	T	T	I	H	D	I	I	L	E	C	V	Y	1000000
29	41	T	I	H	D	I	I	L	E	C	V	Y	C	K	1000000
34	46	I	L	E	C	V	Y	C	K	Q	Q	L	L	R	1000000
38	50	V	Y	C	K	Q	Q	L	L	R	R	E	V	Y	1000000
40	52	C	K	Q	Q	L	L	R	R	E	V	Y	D	F	1000000
41	53	K	Q	Q	L	L	R	R	E	V	Y	D	F	A	1000000
44	56	L	L	R	R	E	V	Y	D	F	A	F	R	D	1000000
45	57	L	R	R	E	V	Y	D	F	A	F	R	D	L	1000000
46	58	R	R	E	V	Y	D	F	A	F	R	D	L	C	1000000
51	63	D	F	A	F	R	D	L	C	I	V	Y	R	D	1000000
54	66	F	R	D	L	C	I	V	Y	R	D	G	N	P	1000000
60	72	V	Y	R	D	G	N	P	Y	A	V	C	D	K	1000000
61	73	Y	R	D	G	N	P	Y	A	V	C	D	K	C	1000000
62	74	R	D	G	N	P	Y	A	V	C	D	K	C	L	1000000
63	75	D	G	N	P	Y	A	V	C	D	K	C	L	K	1000000
64	76	G	N	P	Y	A	V	C	D	K	C	L	K	F	1000000
66	78	P	Y	A	V	C	D	K	C	L	K	F	Y	S	1000000
68	80	A	V	C	D	K	C	L	K	F	Y	S	K	I	1000000
69	81	V	C	D	K	C	L	K	F	Y	S	K	I	S	1000000
70	82	C	D	K	C	L	K	F	Y	S	K	I	S	E	1000000
73	85	C	L	K	F	Y	S	K	I	S	E	Y	R	H	1000000
76	88	F	Y	S	K	I	S	E	Y	R	H	Y	C	Y	1000000
79	91	K	I	S	E	Y	R	H	Y	C	Y	S	L	Y	1000000
85	97	H	Y	C	Y	S	L	Y	G	T	T	L	E	Q	1000000
90	102	L	Y	G	T	T	L	E	Q	Q	Y	N	K	P	1000000
91	103	Y	G	T	T	L	E	Q	Q	Y	N	K	P	L	1000000

92	104	G	T	T	L	E	Q	Q	Y	N	K	P	L	C	1000000
94	106	T	L	E	Q	Q	Y	N	K	P	L	C	D	L	1000000
95	107	L	E	Q	Q	Y	N	K	P	L	C	D	L	L	1000000
96	108	E	Q	Q	Y	N	K	P	L	C	D	L	L	I	1000000
99	111	Y	N	K	P	L	C	D	L	L	I	R	C	I	1000000
100	112	N	K	P	L	C	D	L	L	I	R	C	I	N	1000000
102	114	P	L	C	D	L	L	I	R	C	I	N	C	Q	1000000
108	120	I	R	C	I	N	C	Q	K	P	L	C	P	E	1000000
110	122	C	I	N	C	Q	K	P	L	C	P	E	E	K	1000000
111	123	I	N	C	Q	K	P	L	C	P	E	E	K	Q	1000000
112	124	N	C	Q	K	P	L	C	P	E	E	K	Q	R	1000000
113	125	C	Q	K	P	L	C	P	E	E	K	Q	R	H	1000000
114	126	Q	K	P	L	C	P	E	E	K	Q	R	H	L	1000000
116	128	P	L	C	P	E	E	K	Q	R	H	L	D	K	1000000
117	129	L	C	P	E	E	K	Q	R	H	L	D	K	K	1000000
118	130	C	P	E	E	K	Q	R	H	L	D	K	K	Q	1000000
119	131	P	E	E	K	Q	R	H	L	D	K	K	Q	R	1000000
120	132	E	E	K	Q	R	H	L	D	K	K	Q	R	F	1000000
121	133	E	K	Q	R	H	L	D	K	K	Q	R	F	H	1000000
122	134	K	Q	R	H	L	D	K	K	Q	R	F	H	N	1000000
123	135	Q	R	H	L	D	K	K	Q	R	F	H	N	I	1000000
126	138	L	D	K	K	Q	R	F	H	N	I	R	G	R	1000000
129	141	K	Q	R	F	H	N	I	R	G	R	W	T	G	1000000
131	143	R	F	H	N	I	R	G	R	W	T	G	R	C	1000000
132	144	F	H	N	I	R	G	R	W	T	G	R	C	M	1000000
134	146	N	I	R	G	R	W	T	G	R	C	M	S	C	1000000
135	147	I	R	G	R	W	T	G	R	C	M	S	C	C	1000000
138	150	R	W	T	G	R	C	M	S	C	C	R	S	S	1000000
141	153	G	R	C	M	S	C	C	R	S	S	R	T	R	1000000
144	156	M	S	C	C	R	S	S	R	T	R	R	E	T	1000000
146	158	C	C	R	S	S	R	T	R	R	E	T	Q	L	1000000

															IC50 DRB1*0401 (nM)
59	71	I	V	Y	R	D	G	N	P	Y	A	V	C	D	24
52	64	F	A	F	R	D	L	C	I	V	Y	R	D	G	35
75	87	K	F	Y	S	K	I	S	E	Y	R	H	Y	C	83
86	98	Y	C	Y	S	L	Y	G	T	T	L	E	Q	Q	120
31	43	H	D	I	I	L	E	C	V	Y	C	K	Q	Q	135
58	70	C	I	V	Y	R	D	G	N	P	Y	A	V	C	250
81	93	S	E	Y	R	H	Y	C	Y	S	L	Y	G	T	265
106	118	L	L	I	R	C	I	N	C	Q	K	P	L	C	290
43	55	Q	L	L	R	R	E	V	Y	D	F	A	F	R	360
142	154	R	C	M	S	C	C	R	S	S	R	T	R	R	370
133	145	H	N	I	R	G	R	W	T	G	R	C	M	S	425
109	121	R	C	I	N	C	Q	K	P	L	C	P	E	E	532
139	151	W	T	G	R	C	M	S	C	C	R	S	S	R	620
32	44	D	I	I	L	E	C	V	Y	C	K	Q	Q	L	660
28	40	T	T	I	H	D	I	I	L	E	C	V	Y	C	732
88	100	Y	S	L	Y	G	T	T	L	E	Q	Q	Y	N	862
105	117	D	L	L	I	R	C	I	N	C	Q	K	P	L	930
137	149	G	R	W	T	G	R	C	M	S	C	C	R	S	1550
37	49	C	V	Y	C	K	Q	Q	L	L	R	R	E	V	1600
78	90	S	K	I	S	E	Y	R	H	Y	C	Y	S	L	2200
74	86	L	K	F	Y	S	K	I	S	E	Y	R	H	Y	2500
50	62	Y	D	F	A	F	R	D	L	C	I	V	Y	R	2720
27	39	Q	T	T	I	H	D	I	I	L	E	C	V	Y	3400
48	60	E	V	Y	D	F	A	F	R	D	L	C	I	V	5300
82	94	E	Y	R	H	Y	C	Y	S	L	Y	G	T	T	6700
77	89	Y	S	K	I	S	E	Y	R	H	Y	C	Y	S	7300
130	142	Q	R	F	H	N	I	R	G	R	W	T	G	R	8620
65	77	N	P	Y	A	V	C	D	K	C	L	K	F	Y	10500
84	96	R	H	Y	C	Y	S	L	Y	G	T	T	L	E	10500
91	103	Y	G	T	T	L	E	Q	Q	Y	N	K	P	L	11000
103	115	L	C	D	L	L	I	R	C	I	N	C	Q	K	12000
24	36	T	E	L	Q	T	T	I	H	D	I	I	L	E	17500
104	116	C	D	L	L	I	R	C	I	N	C	Q	K	P	20000
57	69	L	C	I	V	Y	R	D	G	N	P	Y	A	V	20200
30	42	I	H	D	I	I	L	E	C	V	Y	C	K	Q	27000
33	45	I	I	L	E	C	V	Y	C	K	Q	Q	L	L	28000
47	59	R	E	V	Y	D	F	A	F	R	D	L	C	I	45200
72	84	K	C	L	K	F	Y	S	K	I	S	E	Y	R	48000
100	112	N	K	P	L	C	D	L	L	I	R	C	I	N	51500
20	32	P	Q	L	C	T	E	L	Q	T	T	I	H	D	54000
92	104	G	T	T	L	E	Q	Q	Y	N	K	P	L	C	60000
83	95	Y	R	H	Y	C	Y	S	L	Y	G	T	T	L	64500
6	18	T	A	M	F	Q	D	P	Q	E	R	P	R	K	67000
42	54	Q	Q	L	L	R	R	E	V	Y	D	F	A	F	68500
26	38	L	Q	T	T	I	H	D	I	I	L	E	C	V	75000
97	109	Q	Q	Y	N	K	P	L	C	D	L	L	I	R	79000
93	105	T	T	L	E	Q	Q	Y	N	K	P	L	C	D	81200
36	48	E	C	V	Y	C	K	Q	Q	L	L	R	R	E	82000
136	148	R	G	R	W	T	G	R	C	M	S	C	C	R	95200
87	99	C	Y	S	L	Y	G	T	T	L	E	Q	Q	Y	98000

138	150	R	W	T	G	R	C	M	S	C	C	R	S	S	110000
80	92	I	S	E	Y	R	H	Y	C	Y	S	L	Y	G	115000
89	101	S	L	Y	G	T	T	L	E	Q	Q	Y	N	K	120000
127	139	D	K	K	Q	R	F	H	N	I	R	G	R	W	130000
4	16	K	R	T	A	M	F	Q	D	P	Q	E	R	P	175000
66	78	P	Y	A	V	C	D	K	C	L	K	F	Y	S	180000
135	147	I	R	G	R	W	T	G	R	C	M	S	C	C	225000
18	30	K	L	P	Q	L	C	T	E	L	Q	T	T	I	320000
16	28	P	R	K	L	P	Q	L	C	T	E	L	Q	T	390000
55	67	R	D	L	C	I	V	Y	R	D	G	N	P	Y	402000
85	97	H	Y	C	Y	S	L	Y	G	T	T	L	E	Q	472000
71	83	D	K	C	L	K	F	Y	S	K	I	S	E	Y	550000
90	102	L	Y	G	T	T	L	E	Q	Q	Y	N	K	P	615000
95	107	L	E	Q	Q	Y	N	K	P	L	C	D	L	L	760000
101	113	K	P	L	C	D	L	L	I	R	C	I	N	C	780000
108	120	I	R	C	I	N	C	Q	K	P	L	C	P	E	785000
54	66	F	R	D	L	C	I	V	Y	R	D	G	N	P	915000
1	13	M	H	Q	K	R	T	A	M	F	Q	D	P	Q	1000000
2	14	H	Q	K	R	T	A	M	F	Q	D	P	Q	E	1000000
3	15	Q	K	R	T	A	M	F	Q	D	P	Q	E	R	1000000
5	17	R	T	A	M	F	Q	D	P	Q	E	R	P	R	1000000
7	19	A	M	F	Q	D	P	Q	E	R	P	R	K	L	1000000
8	20	M	F	Q	D	P	Q	E	R	P	R	K	L	P	1000000
9	21	F	Q	D	P	Q	E	R	P	R	K	L	P	Q	1000000
10	22	Q	D	P	Q	E	R	P	R	K	L	P	Q	L	1000000
11	23	D	P	Q	E	R	P	R	K	L	P	Q	L	C	1000000
12	24	P	Q	E	R	P	R	K	L	P	Q	L	C	T	1000000
13	25	Q	E	R	P	R	K	L	P	Q	L	C	T	E	1000000
14	26	E	R	P	R	K	L	P	Q	L	C	T	E	L	1000000
15	27	R	P	R	K	L	P	Q	L	C	T	E	L	Q	1000000
17	29	R	K	L	P	Q	L	C	T	E	L	Q	T	T	1000000
19	31	L	P	Q	L	C	T	E	L	Q	T	T	I	H	1000000
21	33	Q	L	C	T	E	L	Q	T	T	I	H	D	I	1000000
22	34	L	C	T	E	L	Q	T	T	I	H	D	I	I	1000000
23	35	C	T	E	L	Q	T	T	I	H	D	I	I	L	1000000
25	37	E	L	Q	T	T	I	H	D	I	I	L	E	C	1000000
29	41	T	I	H	D	I	I	L	E	C	V	Y	C	K	1000000
34	46	I	L	E	C	V	Y	C	K	Q	Q	L	L	R	1000000
35	47	L	E	C	V	Y	C	K	Q	Q	L	L	R	R	1000000
38	50	V	Y	C	K	Q	Q	L	L	R	R	E	V	Y	1000000
39	51	Y	C	K	Q	Q	L	L	R	R	E	V	Y	D	1000000
40	52	C	K	Q	Q	L	L	R	R	E	V	Y	D	F	1000000
41	53	K	Q	Q	L	L	R	R	E	V	Y	D	F	A	1000000
44	56	L	L	R	R	E	V	Y	D	F	A	F	R	D	1000000
45	57	L	R	R	E	V	Y	D	F	A	F	R	D	L	1000000
46	58	R	R	E	V	Y	D	F	A	F	R	D	L	C	1000000
49	61	V	Y	D	F	A	F	R	D	L	C	I	V	Y	1000000
51	63	D	F	A	F	R	D	L	C	I	V	Y	R	D	1000000
53	65	A	F	R	D	L	C	I	V	Y	R	D	G	N	1000000
56	68	D	L	C	I	V	Y	R	D	G	N	P	Y	A	1000000
60	72	V	Y	R	D	G	N	P	Y	A	V	C	D	K	1000000

61	73	Y	R	D	G	N	P	Y	A	V	C	D	K	C	1000000
62	74	R	D	G	N	P	Y	A	V	C	D	K	C	L	1000000
63	75	D	G	N	P	Y	A	V	C	D	K	C	L	K	1000000
64	76	G	N	P	Y	A	V	C	D	K	C	L	K	F	1000000
67	79	Y	A	V	C	D	K	C	L	K	F	Y	S	K	1000000
68	80	A	V	C	D	K	C	L	K	F	Y	S	K	I	1000000
69	81	V	C	D	K	C	L	K	F	Y	S	K	I	S	1000000
70	82	C	D	K	C	L	K	F	Y	S	K	I	S	E	1000000
73	85	C	L	K	F	Y	S	K	I	S	E	Y	R	H	1000000
76	88	F	Y	S	K	I	S	E	Y	R	H	Y	C	Y	1000000
79	91	K	I	S	E	Y	R	H	Y	C	Y	S	L	Y	1000000
94	106	T	L	E	Q	Q	Y	N	K	P	L	C	D	L	1000000
96	108	E	Q	Q	Y	N	K	P	L	C	D	L	L	I	1000000
98	110	Q	Y	N	K	P	L	C	D	L	L	I	R	C	1000000
99	111	Y	N	K	P	L	C	D	L	L	I	R	C	I	1000000
102	114	P	L	C	D	L	L	I	R	C	I	N	C	Q	1000000
107	119	L	I	R	C	I	N	C	Q	K	P	L	C	P	1000000
110	122	C	I	N	C	Q	K	P	L	C	P	E	E	K	1000000
111	123	I	N	C	Q	K	P	L	C	P	E	E	K	Q	1000000
112	124	N	C	Q	K	P	L	C	P	E	E	K	Q	R	1000000
113	125	C	Q	K	P	L	C	P	E	E	K	Q	R	H	1000000
114	126	Q	K	P	L	C	P	E	E	K	Q	R	H	L	1000000
115	127	K	P	L	C	P	E	E	K	Q	R	H	L	D	1000000
116	128	P	L	C	P	E	E	K	Q	R	H	L	D	K	1000000
117	129	L	C	P	E	E	K	Q	R	H	L	D	K	K	1000000
118	130	C	P	E	E	K	Q	R	H	L	D	K	K	Q	1000000
119	131	P	E	E	K	Q	R	H	L	D	K	K	Q	R	1000000
120	132	E	E	K	Q	R	H	L	D	K	K	Q	R	F	1000000
121	133	E	K	Q	R	H	L	D	K	K	Q	R	F	H	1000000
122	134	K	Q	R	H	L	D	K	K	Q	R	F	H	N	1000000
123	135	Q	R	H	L	D	K	K	Q	R	F	H	N	I	1000000
124	136	R	H	L	D	K	K	Q	R	F	H	N	I	R	1000000
125	137	H	L	D	K	K	Q	R	F	H	N	I	R	G	1000000
126	138	L	D	K	K	Q	R	F	H	N	I	R	G	R	1000000
128	140	K	K	Q	R	F	H	N	I	R	G	R	W	T	1000000
129	141	K	Q	R	F	H	N	I	R	G	R	W	T	G	1000000
131	143	R	F	H	N	I	R	G	R	W	T	G	R	C	1000000
132	144	F	H	N	I	R	G	R	W	T	G	R	C	M	1000000
134	146	N	I	R	G	R	W	T	G	R	C	M	S	C	1000000
140	152	T	G	R	C	M	S	C	C	R	S	S	R	T	1000000
141	153	G	R	C	M	S	C	C	R	S	S	R	T	R	1000000
143	155	C	M	S	C	C	R	S	S	R	T	R	R	E	1000000
144	156	M	S	C	C	R	S	S	R	T	R	R	E	T	1000000
145	157	S	C	C	R	S	S	R	T	R	R	E	T	Q	1000000
146	158	C	C	R	S	S	R	T	R	R	E	T	Q	L	1000000

															IC50 DRB1*0101(nM)
81	93	D	L	L	M	G	T	L	G	I	V	C	P	I	45.2
80	92	E	D	L	L	M	G	T	L	G	I	V	C	P	230
52	64	Y	N	I	V	T	F	C	C	K	C	D	S	T	300
55	67	V	T	F	C	C	K	C	D	S	T	L	R	L	1120
10	22	E	Y	M	L	D	L	Q	P	E	T	T	D	L	1600
77	89	R	T	L	E	D	L	L	M	G	T	L	G	I	1800
74	86	V	D	I	R	T	L	E	D	L	L	M	G	T	2200
21	33	D	L	Y	C	Y	E	Q	L	N	D	S	S	E	4550
63	75	S	T	L	R	L	C	V	Q	S	T	H	V	D	5600
50	62	A	H	Y	N	I	V	T	F	C	C	K	C	D	8020
67	79	L	C	V	Q	S	T	H	V	D	I	R	T	L	8950
36	48	D	E	I	D	G	P	A	G	Q	A	E	P	D	9420
65	77	L	R	L	C	V	Q	S	T	H	V	D	I	R	11000
20	32	T	D	L	Y	C	Y	E	Q	L	N	D	S	S	13000
79	91	L	E	D	L	L	M	G	T	L	G	I	V	C	18500
9	21	H	E	Y	M	L	D	L	Q	P	E	T	T	D	25000
85	97	G	T	L	G	I	V	C	P	I	C	S	Q	K	28200
23	35	Y	C	Y	E	Q	L	N	D	S	S	E	E	E	28500
46	58	E	P	D	R	A	H	Y	N	I	V	T	F	C	33500
35	47	E	D	E	I	D	G	P	A	G	Q	A	E	P	47500
83	95	L	M	G	T	L	G	I	V	C	P	I	C	S	51000
82	94	L	L	M	G	T	L	G	I	V	C	P	I	C	56500
53	65	N	I	V	T	F	C	C	K	C	D	S	T	L	60000
17	29	P	E	T	T	D	L	Y	C	Y	E	Q	L	N	64500
62	74	D	S	T	L	R	L	C	V	Q	S	T	H	V	67500
57	69	F	C	C	K	C	D	S	T	L	R	L	C	V	97500
13	25	L	D	L	Q	P	E	T	T	D	L	Y	C	Y	140000
51	63	H	Y	N	I	V	T	F	C	C	K	C	D	S	315000
59	71	C	K	C	D	S	T	L	R	L	C	V	Q	S	405000
84	96	M	G	T	L	G	I	V	C	P	I	C	S	Q	410000
6	18	P	T	L	H	E	Y	M	L	D	L	Q	P	E	435000
58	70	C	C	K	C	D	S	T	L	R	L	C	V	Q	572000
72	84	T	H	V	D	I	R	T	L	E	D	L	L	M	670000
69	81	V	Q	S	T	H	V	D	I	R	T	L	E	D	720000
60	72	K	C	D	S	T	L	R	L	C	V	Q	S	T	815000
61	73	C	D	S	T	L	R	L	C	V	Q	S	T	H	850000
1	13	M	H	G	D	T	P	T	L	H	E	Y	M	L	1000000
2	14	H	G	D	T	P	T	L	H	E	Y	M	L	D	1000000
3	15	G	D	T	P	T	L	H	E	Y	M	L	D	L	1000000
4	16	D	T	P	T	L	H	E	Y	M	L	D	L	Q	1000000
5	17	T	P	T	L	H	E	Y	M	L	D	L	Q	P	1000000
7	19	T	L	H	E	Y	M	L	D	L	Q	P	E	T	1000000
8	20	L	H	E	Y	M	L	D	L	Q	P	E	T	T	1000000
11	23	Y	M	L	D	L	Q	P	E	T	T	D	L	Y	1000000
12	24	M	L	D	L	Q	P	E	T	T	D	L	Y	C	1000000
14	26	D	L	Q	P	E	T	T	D	L	Y	C	Y	E	1000000
15	27	L	Q	P	E	T	T	D	L	Y	C	Y	E	Q	1000000
16	28	Q	P	E	T	T	D	L	Y	C	Y	E	Q	L	1000000
18	30	E	T	T	D	L	Y	C	Y	E	Q	L	N	D	1000000
19	31	T	T	D	L	Y	C	Y	E	Q	L	N	D	S	1000000

22	34	L	Y	C	Y	E	Q	L	N	D	S	S	E	E	1000000
24	36	C	Y	E	Q	L	N	D	S	S	E	E	E	D	1000000
25	37	Y	E	Q	L	N	D	S	S	E	E	E	D	E	1000000
26	38	E	Q	L	N	D	S	S	E	E	E	D	E	I	1000000
27	39	Q	L	N	D	S	S	E	E	E	D	E	I	D	1000000
28	40	L	N	D	S	S	E	E	E	D	E	I	D	G	1000000
29	41	N	D	S	S	E	E	E	D	E	I	D	G	P	1000000
30	42	D	S	S	E	E	E	D	E	I	D	G	P	A	1000000
31	43	S	S	E	E	E	D	E	I	D	G	P	A	G	1000000
32	44	S	E	E	E	D	E	I	D	G	P	A	G	Q	1000000
33	45	E	E	E	D	E	I	D	G	P	A	G	Q	A	1000000
34	46	E	E	D	E	I	D	G	P	A	G	Q	A	E	1000000
37	49	E	I	D	G	P	A	G	Q	A	E	P	D	R	1000000
38	50	I	D	G	P	A	G	Q	A	E	P	D	R	A	1000000
39	51	D	G	P	A	G	Q	A	E	P	D	R	A	H	1000000
40	52	G	P	A	G	Q	A	E	P	D	R	A	H	Y	1000000
41	53	P	A	G	Q	A	E	P	D	R	A	H	Y	N	1000000
42	54	A	G	Q	A	E	P	D	R	A	H	Y	N	I	1000000
43	55	G	Q	A	E	P	D	R	A	H	Y	N	I	V	1000000
44	56	Q	A	E	P	D	R	A	H	Y	N	I	V	T	1000000
45	57	A	E	P	D	R	A	H	Y	N	I	V	T	F	1000000
47	59	P	D	R	A	H	Y	N	I	V	T	F	C	C	1000000
48	60	D	R	A	H	Y	N	I	V	T	F	C	C	K	1000000
49	61	R	A	H	Y	N	I	V	T	F	C	C	K	C	1000000
54	66	I	V	T	F	C	C	K	C	D	S	T	L	R	1000000
56	68	T	F	C	C	K	C	D	S	T	L	R	L	C	1000000
64	76	T	L	R	L	C	V	Q	S	T	H	V	D	I	1000000
66	78	R	L	C	V	Q	S	T	H	V	D	I	R	T	1000000
68	80	C	V	Q	S	T	H	V	D	I	R	T	L	E	1000000
70	82	Q	S	T	H	V	D	I	R	T	L	E	D	L	1000000
71	83	S	T	H	V	D	I	R	T	L	E	D	L	L	1000000
73	85	H	V	D	I	R	T	L	E	D	L	L	M	G	1000000
75	87	D	I	R	T	L	E	D	L	L	M	G	T	L	1000000
76	88	I	R	T	L	E	D	L	L	M	G	T	L	G	1000000
78	90	T	L	E	D	L	L	M	G	T	L	G	I	V	1000000
86	98	T	L	G	I	V	C	P	I	C	S	Q	K	P	1000000

															IC50 DRB1*0401 (nM)
21	33	D	L	Y	C	Y	E	Q	L	N	D	S	S	E	105
9	21	H	E	Y	M	L	D	L	Q	P	E	T	T	D	210
84	96	M	G	T	L	G	I	V	C	P	I	C	S	Q	230
63	75	S	T	L	R	L	C	V	Q	S	T	H	V	D	452
81	93	D	L	L	M	G	T	L	G	I	V	C	P	I	710
85	97	G	T	L	G	I	V	C	P	I	C	S	Q	K	920
53	65	N	I	V	T	F	C	C	K	C	D	S	T	L	1700
80	92	E	D	L	L	M	G	T	L	G	I	V	C	P	1800
50	62	A	H	Y	N	I	V	T	F	C	C	K	C	D	2600
74	86	V	D	I	R	T	L	E	D	L	L	M	G	T	2650
67	79	L	C	V	Q	S	T	H	V	D	I	R	T	L	3700
17	29	P	E	T	T	D	L	Y	C	Y	E	Q	L	N	4750
82	94	L	L	M	G	T	L	G	I	V	C	P	I	C	5120
65	77	L	R	L	C	V	Q	S	T	H	V	D	I	R	5600
20	32	T	D	L	Y	C	Y	E	Q	L	N	D	S	S	6720
48	60	D	R	A	H	Y	N	I	V	T	F	C	C	K	7120
13	25	L	D	L	Q	P	E	T	T	D	L	Y	C	Y	7300
10	22	E	Y	M	L	D	L	Q	P	E	T	T	D	L	9320
6	18	P	T	L	H	E	Y	M	L	D	L	Q	P	E	9700
54	66	I	V	T	F	C	C	K	C	D	S	T	L	R	11500
52	64	Y	N	I	V	T	F	C	C	K	C	D	S	T	16500
51	63	H	Y	N	I	V	T	F	C	C	K	C	D	S	30000
83	95	L	M	G	T	L	G	I	V	C	P	I	C	S	53000
57	69	F	C	C	K	C	D	S	T	L	R	L	C	V	60000
55	67	V	T	F	C	C	K	C	D	S	T	L	R	L	62500
75	87	D	I	R	T	L	E	D	L	L	M	G	T	L	68000
49	61	R	A	H	Y	N	I	V	T	F	C	C	K	C	76200
5	17	T	P	T	L	H	E	Y	M	L	D	L	Q	P	96500
79	91	L	E	D	L	L	M	G	T	L	G	I	V	C	110000
46	58	E	P	D	R	A	H	Y	N	I	V	T	F	C	122000
64	76	T	L	R	L	C	V	Q	S	T	H	V	D	I	132000
76	88	I	R	T	L	E	D	L	L	M	G	T	L	G	145000
86	98	T	L	G	I	V	C	P	I	C	S	Q	K	P	202000
77	89	R	T	L	E	D	L	L	M	G	T	L	G	I	260000
18	30	E	T	T	D	L	Y	C	Y	E	Q	L	N	D	535000
62	74	D	S	T	L	R	L	C	V	Q	S	T	H	V	580000
26	38	E	Q	L	N	D	S	S	E	E	E	D	E	I	590000
60	72	K	C	D	S	T	L	R	L	C	V	Q	S	T	695000
71	83	S	T	H	V	D	I	R	T	L	E	D	L	L	870000
24	36	C	Y	E	Q	L	N	D	S	S	E	E	E	D	952000
1	13	M	H	G	D	T	P	T	L	H	E	Y	M	L	1000000
2	14	H	G	D	T	P	T	L	H	E	Y	M	L	D	1000000
3	15	G	D	T	P	T	L	H	E	Y	M	L	D	L	1000000
4	16	D	T	P	T	L	H	E	Y	M	L	D	L	Q	1000000
7	19	T	L	H	E	Y	M	L	D	L	Q	P	E	T	1000000
8	20	L	H	E	Y	M	L	D	L	Q	P	E	T	T	1000000
11	23	Y	M	L	D	L	Q	P	E	T	T	D	L	Y	1000000
12	24	M	L	D	L	Q	P	E	T	T	D	L	Y	C	1000000
14	26	D	L	Q	P	E	T	T	D	L	Y	C	Y	E	1000000
15	27	L	Q	P	E	T	T	D	L	Y	C	Y	E	Q	1000000

16	28	Q	P	E	T	T	D	L	Y	C	Y	E	Q	L	1000000
19	31	T	T	D	L	Y	C	Y	E	Q	L	N	D	S	1000000
22	34	L	Y	C	Y	E	Q	L	N	D	S	S	E	E	1000000
23	35	Y	C	Y	E	Q	L	N	D	S	S	E	E	E	1000000
25	37	Y	E	Q	L	N	D	S	S	E	E	E	D	E	1000000
27	39	Q	L	N	D	S	S	E	E	E	D	E	I	D	1000000
28	40	L	N	D	S	S	E	E	E	D	E	I	D	G	1000000
29	41	N	D	S	S	E	E	E	D	E	I	D	G	P	1000000
30	42	D	S	S	E	E	E	D	E	I	D	G	P	A	1000000
31	43	S	S	E	E	E	D	E	I	D	G	P	A	G	1000000
32	44	S	E	E	E	D	E	I	D	G	P	A	G	Q	1000000
33	45	E	E	E	D	E	I	D	G	P	A	G	Q	A	1000000
34	46	E	E	D	E	I	D	G	P	A	G	Q	A	E	1000000
35	47	E	D	E	I	D	G	P	A	G	Q	A	E	P	1000000
36	48	D	E	I	D	G	P	A	G	Q	A	E	P	D	1000000
37	49	E	I	D	G	P	A	G	Q	A	E	P	D	R	1000000
38	50	I	D	G	P	A	G	Q	A	E	P	D	R	A	1000000
39	51	D	G	P	A	G	Q	A	E	P	D	R	A	H	1000000
40	52	G	P	A	G	Q	A	E	P	D	R	A	H	Y	1000000
41	53	P	A	G	Q	A	E	P	D	R	A	H	Y	N	1000000
42	54	A	G	Q	A	E	P	D	R	A	H	Y	N	I	1000000
43	55	G	Q	A	E	P	D	R	A	H	Y	N	I	V	1000000
44	56	Q	A	E	P	D	R	A	H	Y	N	I	V	T	1000000
45	57	A	E	P	D	R	A	H	Y	N	I	V	T	F	1000000
47	59	P	D	R	A	H	Y	N	I	V	T	F	C	C	1000000
56	68	T	F	C	C	K	C	D	S	T	L	R	L	C	1000000
58	70	C	C	K	C	D	S	T	L	R	L	C	V	Q	1000000
59	71	C	K	C	D	S	T	L	R	L	C	V	Q	S	1000000
61	73	C	D	S	T	L	R	L	C	V	Q	S	T	H	1000000
66	78	R	L	C	V	Q	S	T	H	V	D	I	R	T	1000000
68	80	C	V	Q	S	T	H	V	D	I	R	T	L	E	1000000
69	81	V	Q	S	T	H	V	D	I	R	T	L	E	D	1000000
70	82	Q	S	T	H	V	D	I	R	T	L	E	D	L	1000000
72	84	T	H	V	D	I	R	T	L	E	D	L	L	M	1000000
73	85	H	V	D	I	R	T	L	E	D	L	L	M	G	1000000
78	90	T	L	E	D	L	L	M	G	T	L	G	I	V	1000000

															IC50 DRB1*0101 (nM)
58	70	N	I	Y	Y	H	A	G	T	S	R	L	L	A	1.5
3	15	V	T	F	I	Y	I	L	V	I	T	C	Y	E	1.85
398	410	L	Q	F	I	F	Q	L	C	K	I	T	L	T	2.85
414	426	M	T	Y	I	H	S	M	N	S	T	I	L	E	3.25
442	454	D	T	Y	R	F	V	T	Q	A	I	A	C	Q	4.42
300	312	D	L	Y	I	K	G	S	G	S	T	A	N	L	5.05
315	327	S	N	Y	F	P	T	P	S	G	S	M	V	T	6.4
59	71	I	Y	Y	H	A	G	T	S	R	L	L	A	V	7.25
159	171	S	A	Y	A	A	N	A	G	V	D	N	R	E	22.5
21	33	H	H	I	F	F	Q	M	S	L	W	L	P	S	28.5
124	136	L	V	W	A	C	V	G	V	E	V	G	R	G	28.5
234	246	M	D	F	T	T	L	Q	A	N	K	S	E	V	33
5	17	F	I	Y	I	L	V	I	T	C	Y	E	N	D	36
468	480	K	K	Y	T	F	W	E	V	N	L	K	E	K	36
273	285	F	F	Y	L	R	R	E	Q	M	F	V	R	H	54
280	292	Q	M	F	V	R	H	L	F	N	R	A	G	T	57
67	79	R	L	L	A	V	G	H	P	Y	F	P	I	K	65.5
1	13	M	Q	V	T	F	I	Y	I	L	V	I	T	C	67.5
281	293	M	F	V	R	H	L	F	N	R	A	G	T	V	84
94	106	L	Q	Y	R	V	F	R	I	H	L	P	D	P	86
175	187	M	D	Y	K	Q	T	Q	L	C	L	I	G	C	89.5
22	34	H	I	F	F	Q	M	S	L	W	L	P	S	E	90.2
45	57	S	K	V	V	S	T	D	E	Y	V	A	R	T	92
366	378	T	N	M	S	L	C	A	A	I	S	T	S	E	93
114	126	T	S	F	Y	N	P	D	T	Q	R	L	V	W	96.5
212	224	P	P	L	E	L	I	N	T	V	I	Q	D	G	110
337	349	Y	W	L	Q	R	A	Q	G	H	N	N	G	I	115
504	516	P	K	F	T	L	G	K	R	K	A	T	P	T	115
123	135	R	L	V	W	A	C	V	G	V	E	V	G	R	120
52	64	E	Y	V	A	R	T	N	I	Y	Y	H	A	G	122
335	347	K	P	Y	W	L	Q	R	A	Q	G	H	N	N	130
84	96	N	K	I	L	V	P	K	V	S	G	L	Q	Y	150
492	504	R	K	F	L	L	Q	A	G	L	K	A	K	P	150
76	88	F	P	I	K	K	P	N	N	N	K	I	L	V	152
405	417	C	K	I	T	L	T	A	D	V	M	T	Y	I	152
6	18	I	Y	I	L	V	I	T	C	Y	E	N	D	V	160
85	97	K	I	L	V	P	K	V	S	G	L	Q	Y	R	165
150	162	N	K	L	D	D	T	E	N	A	S	A	Y	A	190
285	297	H	L	F	N	R	A	G	T	V	G	E	N	V	195
23	35	I	F	F	Q	M	S	L	W	L	P	S	E	A	200
115	127	S	F	Y	N	P	D	T	Q	R	L	V	W	A	205
412	424	D	V	M	T	Y	I	H	S	M	N	S	T	I	240
51	63	D	E	Y	V	A	R	T	N	I	Y	Y	H	A	260
129	141	V	G	V	E	V	G	R	G	Q	P	L	G	V	265
12	24	T	C	Y	E	N	D	V	N	V	H	H	I	F	432
9	21	L	V	I	T	C	Y	E	N	D	V	N	V	H	505
248	260	L	D	I	C	T	S	I	C	K	Y	P	D	Y	540
394	406	E	E	Y	D	L	Q	F	I	F	Q	L	C	K	540
271	283	S	L	F	F	Y	L	R	R	E	Q	M	F	V	550
445	457	R	F	V	T	Q	A	I	A	C	Q	K	H	T	570

270	282	D	S	L	F	F	Y	L	R	R	E	Q	M	F	582
299	311	D	D	L	Y	I	K	G	S	G	S	T	A	N	630
36	48	T	V	Y	L	P	P	V	P	V	S	K	V	V	680
347	359	N	G	I	C	W	G	N	Q	L	F	V	T	V	700
182	194	L	C	L	I	G	C	K	P	P	I	G	E	H	835
411	423	A	D	V	M	T	Y	I	H	S	M	N	S	T	845
27	39	M	S	L	W	L	P	S	E	A	T	V	Y	L	860
430	442	F	G	L	Q	P	P	P	G	G	T	L	E	D	860
173	185	I	S	M	D	Y	K	Q	T	Q	L	C	L	I	912
224	236	G	D	M	V	H	T	G	F	G	A	M	D	F	1720
89	101	P	K	V	S	G	L	Q	Y	R	V	F	R	I	1900
258	270	P	D	Y	L	K	M	V	S	E	P	Y	G	D	1950
423	435	T	I	L	E	D	W	N	F	G	L	Q	P	P	2250
237	249	T	T	L	Q	A	N	K	S	E	V	P	L	D	2500
336	348	P	Y	W	L	Q	R	A	Q	G	H	N	N	G	2620
225	237	D	M	V	H	T	G	F	G	A	M	D	F	T	2650
399	411	Q	F	I	F	Q	L	C	K	I	T	L	T	A	2800
353	365	N	Q	L	F	V	T	V	V	D	T	T	R	S	2950
331	343	Q	I	F	N	K	P	Y	W	L	Q	R	A	Q	3050
193	205	E	H	W	G	K	G	S	P	C	T	N	V	A	3250
310	322	A	N	L	A	S	S	N	Y	F	P	T	P	S	3300
180	192	T	Q	L	C	L	I	G	C	K	P	P	I	G	3600
444	456	Y	R	F	V	T	Q	A	I	A	C	Q	K	H	3700
330	342	A	Q	I	F	N	K	P	Y	W	L	Q	R	A	3720
357	369	V	T	V	V	D	T	T	R	S	T	N	M	S	3750
18	30	V	N	V	H	H	I	F	F	Q	M	S	L	W	3800
246	258	V	P	L	D	I	C	T	S	I	C	K	Y	P	4150
323	335	G	S	M	V	T	S	D	A	Q	I	F	N	K	4200
426	438	E	D	W	N	F	G	L	Q	P	P	P	G	G	4350
219	231	T	V	I	Q	D	G	D	M	V	H	T	G	F	4550
202	214	T	N	V	A	V	N	P	G	D	C	P	P	L	4750
141	153	V	G	I	S	G	H	P	L	L	N	K	L	D	5020
379	391	T	T	Y	K	N	T	N	F	K	E	Y	L	R	5050
122	134	Q	R	L	V	W	A	C	V	G	V	E	V	G	5320
8	20	I	L	V	I	T	C	Y	E	N	D	V	N	V	5400
25	37	F	Q	M	S	L	W	L	P	S	E	A	T	V	5450
88	100	V	P	K	V	S	G	L	Q	Y	R	V	F	R	5500
231	243	F	G	A	M	D	F	T	T	L	Q	A	N	K	5750
396	408	Y	D	L	Q	F	I	F	Q	L	C	K	I	T	5900
329	341	D	A	Q	I	F	N	K	P	Y	W	L	Q	R	5950
252	264	T	S	I	C	K	Y	P	D	Y	L	K	M	V	6250
16	28	N	D	V	N	V	H	H	I	F	F	Q	M	S	6600
131	143	V	E	V	G	R	G	Q	P	L	G	V	G	I	7220
261	273	L	K	M	V	S	E	P	Y	G	D	S	L	F	7220
364	376	R	S	T	N	M	S	L	C	A	A	I	S	T	7600
92	104	S	G	L	Q	Y	R	V	F	R	I	H	L	P	7920
470	482	Y	T	F	W	E	V	N	L	K	E	K	F	S	8150
165	177	A	G	V	D	N	R	E	C	I	S	M	D	Y	8320
483	495	A	D	L	D	Q	F	P	L	G	R	K	F	L	8600
138	150	P	L	G	V	G	I	S	G	H	P	L	L	N	9950
365	377	S	T	N	M	S	L	C	A	A	I	S	T	S	11000

97	109	R	V	F	R	I	H	L	P	D	P	N	K	F	12500
349	361	I	C	W	G	N	Q	L	F	V	T	V	V	D	12500
303	315	I	K	G	S	G	S	T	A	N	L	A	S	S	13000
384	396	T	N	F	K	E	Y	L	R	H	G	E	E	Y	13000
479	491	E	K	F	S	A	D	L	D	Q	F	P	L	G	13000
493	505	K	F	L	L	Q	A	G	L	K	A	K	P	K	13000
137	149	Q	P	L	G	V	G	I	S	G	H	P	L	L	14500
400	412	F	I	F	Q	L	C	K	I	T	L	T	A	D	15200
465	477	D	P	L	K	K	Y	T	F	W	E	V	N	L	15200
441	453	E	D	T	Y	R	F	V	T	Q	A	I	A	C	16000
73	85	H	P	Y	F	P	I	K	K	P	N	N	N	K	17000
127	139	A	C	V	G	V	E	V	G	R	G	Q	P	L	17000
155	167	T	E	N	A	S	A	Y	A	A	N	A	G	V	17200
388	400	E	Y	L	R	H	G	E	E	Y	D	L	Q	F	19200
34	46	E	A	T	V	Y	L	P	P	V	P	V	S	K	19500
387	399	K	E	Y	L	R	H	G	E	E	Y	D	L	Q	21000
438	450	G	T	L	E	D	T	Y	R	F	V	T	Q	A	21000
156	168	E	N	A	S	A	Y	A	A	N	A	G	V	D	21500
171	183	E	C	I	S	M	D	Y	K	Q	T	Q	L	C	21500
153	165	D	D	T	E	N	A	S	A	Y	A	A	N	A	23200
232	244	G	A	M	D	F	T	T	L	Q	A	N	K	S	23500
13	25	C	Y	E	N	D	V	N	V	H	H	I	F	F	24000
278	290	R	E	Q	M	F	V	R	H	L	F	N	R	A	24000
306	318	S	G	S	T	A	N	L	A	S	S	N	Y	F	25200
410	422	T	A	D	V	M	T	Y	I	H	S	M	N	S	25200
513	525	A	T	P	T	T	S	S	T	S	T	T	A	K	25500
56	68	R	T	N	I	Y	Y	H	A	G	T	S	R	L	26500
272	284	L	F	F	Y	L	R	R	E	Q	M	F	V	R	26500
408	420	T	L	T	A	D	V	M	T	Y	I	H	S	M	27500
126	138	W	A	C	V	G	V	E	V	G	R	G	Q	P	31500
259	271	D	Y	L	K	M	V	S	E	P	Y	G	D	S	32000
317	329	Y	F	P	T	P	S	G	S	M	V	T	S	D	33500
373	385	A	I	S	T	S	E	T	T	Y	K	N	T	N	36000
497	509	Q	A	G	L	K	A	K	P	K	F	T	L	G	39000
47	59	V	V	S	T	D	E	Y	V	A	R	T	N	I	39500
415	427	T	Y	I	H	S	M	N	S	T	I	L	E	D	40200
77	89	P	I	K	K	P	N	N	N	K	I	L	V	P	41200
86	98	I	L	V	P	K	V	S	G	L	Q	Y	R	V	45000
214	226	L	E	L	I	N	T	V	I	Q	D	G	D	M	45000
324	336	S	M	V	T	S	D	A	Q	I	F	N	K	P	46000
2	14	Q	V	T	F	I	Y	I	L	V	I	T	C	Y	47000
316	328	N	Y	F	P	T	P	S	G	S	M	V	T	S	47200
475	487	V	N	L	K	E	K	F	S	A	D	L	D	Q	48200
309	321	T	A	N	L	A	S	S	N	Y	F	P	T	P	51000
176	188	D	Y	K	Q	T	Q	L	C	L	I	G	C	K	54500
401	413	I	F	Q	L	C	K	I	T	L	T	A	D	V	54500
443	455	T	Y	R	F	V	T	Q	A	I	A	C	Q	K	55500
50	62	T	D	E	Y	V	A	R	T	N	I	Y	Y	H	56000
301	313	L	Y	I	K	G	S	G	S	T	A	N	L	A	58000
63	75	A	G	T	S	R	L	L	A	V	G	H	P	Y	58500
283	295	V	R	H	L	F	N	R	A	G	T	V	G	E	65000

37	49	V	Y	L	P	P	V	P	V	S	K	V	V	S	66000
204	216	V	A	V	N	P	G	D	C	P	P	L	E	L	66000
276	288	L	R	R	E	Q	M	F	V	R	H	L	F	N	66000
29	41	L	W	L	P	S	E	A	T	V	Y	L	P	P	67200
502	514	A	K	P	K	F	T	L	G	K	R	K	A	T	70500
352	364	G	N	Q	L	F	V	T	V	V	D	T	T	R	71000
107	119	N	K	F	G	F	P	D	T	S	F	Y	N	P	72000
179	191	Q	T	Q	L	C	L	I	G	C	K	P	P	I	74000
327	339	T	S	D	A	Q	I	F	N	K	P	Y	W	L	74000
488	500	F	P	L	G	R	K	F	L	L	Q	A	G	L	74500
222	234	Q	D	G	D	M	V	H	T	G	F	G	A	M	76000
196	208	G	K	G	S	P	C	T	N	V	A	V	N	P	79000
494	506	F	L	L	Q	A	G	L	K	A	K	P	K	F	82000
91	103	V	S	G	L	Q	Y	R	V	F	R	I	H	L	86000
322	334	S	G	S	M	V	T	S	D	A	Q	I	F	N	89200
35	47	A	T	V	Y	L	P	P	V	P	V	S	K	V	92500
40	52	P	P	V	P	V	S	K	V	V	S	T	D	E	93500
82	94	N	N	N	K	I	L	V	P	K	V	S	G	L	99000
422	434	S	T	I	L	E	D	W	N	F	G	L	Q	P	100000
19	31	N	V	H	H	I	F	F	Q	M	S	L	W	L	102000
355	367	L	F	V	T	V	V	D	T	T	R	S	T	N	102000
471	483	T	F	W	E	V	N	L	K	E	K	F	S	A	102000
360	372	V	D	T	T	R	S	T	N	M	S	L	C	A	110000
218	230	N	T	V	I	Q	D	G	D	M	V	H	T	G	112000
340	352	Q	R	A	Q	G	H	N	N	G	I	C	W	G	112000
266	278	E	P	Y	G	D	S	L	F	F	Y	L	R	R	115000
223	235	D	G	D	M	V	H	T	G	F	G	A	M	D	120000
7	19	Y	I	L	V	I	T	C	Y	E	N	D	V	N	125000
199	211	S	P	C	T	N	V	A	V	N	P	G	D	C	130000
358	370	T	V	V	D	T	T	R	S	T	N	M	S	L	130000
362	374	T	T	R	S	T	N	M	S	L	C	A	A	I	130000
428	440	W	N	F	G	L	Q	P	P	P	G	G	T	L	130000
215	227	E	L	I	N	T	V	I	Q	D	G	D	M	V	132000
57	69	T	N	I	Y	Y	H	A	G	T	S	R	L	L	140000
198	210	G	S	P	C	T	N	V	A	V	N	P	G	D	142000
183	195	C	L	I	G	C	K	P	P	I	G	E	H	W	145000
60	72	Y	Y	H	A	G	T	S	R	L	L	A	V	G	150000
262	274	K	M	V	S	E	P	Y	G	D	S	L	F	F	152000
429	441	N	F	G	L	Q	P	P	P	G	G	T	L	E	155000
157	169	N	A	S	A	Y	A	A	N	A	G	V	D	N	162000
64	76	G	T	S	R	L	L	A	V	G	H	P	Y	F	165000
498	510	A	G	L	K	A	K	P	K	F	T	L	G	K	165000
140	152	G	V	G	I	S	G	H	P	L	L	N	K	L	170000
152	164	L	D	D	T	E	N	A	S	A	Y	A	A	N	170000
370	382	L	C	A	A	I	S	T	S	E	T	T	Y	K	172000
28	40	S	L	W	L	P	S	E	A	T	V	Y	L	P	180000
343	355	Q	G	H	N	N	G	I	C	W	G	N	Q	L	182000
118	130	N	P	D	T	Q	R	L	V	W	A	C	V	G	200000
314	326	S	S	N	Y	F	P	T	P	S	G	S	M	V	200000
381	393	Y	K	N	T	N	F	K	E	Y	L	R	H	G	202000
369	381	S	L	C	A	A	I	S	T	S	E	T	T	Y	205000

65	77	T	S	R	L	L	A	V	G	H	P	Y	F	P	210000
189	201	P	P	I	G	E	H	W	G	K	G	S	P	C	210000
93	105	G	L	Q	Y	R	V	F	R	I	H	L	P	D	220000
121	133	T	Q	R	L	V	W	A	C	V	G	V	E	V	220000
359	371	V	V	D	T	T	R	S	T	N	M	S	L	C	225000
346	358	N	N	G	I	C	W	G	N	Q	L	F	V	T	232000
33	45	S	E	A	T	V	Y	L	P	P	V	P	V	S	235000
53	65	Y	V	A	R	T	N	I	Y	Y	H	A	G	T	240000
170	182	R	E	C	I	S	M	D	Y	K	Q	T	Q	L	240000
397	409	D	L	Q	F	I	F	Q	L	C	K	I	T	L	240000
447	459	V	T	Q	A	I	A	C	Q	K	H	T	P	P	242000
286	298	L	F	N	R	A	G	T	V	G	E	N	V	P	245000
46	58	K	V	V	S	T	D	E	Y	V	A	R	T	N	250000
255	267	C	K	Y	P	D	Y	L	K	M	V	S	E	P	250000
120	132	D	T	Q	R	L	V	W	A	C	V	G	V	E	252000
30	42	W	L	P	S	E	A	T	V	Y	L	P	P	V	260000
117	129	Y	N	P	D	T	Q	R	L	V	W	A	C	V	270000
424	436	I	L	E	D	W	N	F	G	L	Q	P	P	P	270000
403	415	Q	L	C	K	I	T	L	T	A	D	V	M	T	275000
404	416	L	C	K	I	T	L	T	A	D	V	M	T	Y	335000
48	60	V	S	T	D	E	Y	V	A	R	T	N	I	Y	340000
274	286	F	Y	L	R	R	E	Q	M	F	V	R	H	L	380000
495	507	L	L	Q	A	G	L	K	A	K	P	K	F	T	380000
260	272	Y	L	K	M	V	S	E	P	Y	G	D	S	L	382000
339	351	L	Q	R	A	Q	G	H	N	N	G	I	C	W	385000
511	523	R	K	A	T	P	T	T	S	S	T	S	T	T	390000
287	299	F	N	R	A	G	T	V	G	E	N	V	P	D	392000
356	368	F	V	T	V	V	D	T	T	R	S	T	N	M	400000
158	170	A	S	A	Y	A	A	N	A	G	V	D	N	R	402000
54	66	V	A	R	T	N	I	Y	Y	H	A	G	T	S	415000
269	281	G	D	S	L	F	F	Y	L	R	R	E	Q	M	430000
240	252	Q	A	N	K	S	E	V	P	L	D	I	C	T	440000
20	32	V	H	H	I	F	F	Q	M	S	L	W	L	P	442000
267	279	P	Y	G	D	S	L	F	F	Y	L	R	R	E	455000
99	111	F	R	I	H	L	P	D	P	N	K	F	G	F	462000
81	93	P	N	N	N	K	I	L	V	P	K	V	S	G	485000
62	74	H	A	G	T	S	R	L	L	A	V	G	H	P	500000
393	405	G	E	E	Y	D	L	Q	F	I	F	Q	L	C	512000
109	121	F	G	F	P	D	T	S	F	Y	N	P	D	T	555000
307	319	G	S	T	A	N	L	A	S	S	N	Y	F	P	555000
154	166	D	T	E	N	A	S	A	Y	A	A	N	A	G	562000
416	428	Y	I	H	S	M	N	S	T	I	L	E	D	W	572000
66	78	S	R	L	L	A	V	G	H	P	Y	F	P	I	575000
134	146	G	R	G	Q	P	L	G	V	G	I	S	G	H	575000
418	430	H	S	M	N	S	T	I	L	E	D	W	N	F	580000
334	346	N	K	P	Y	W	L	Q	R	A	Q	G	H	N	605000
484	496	D	L	D	Q	F	P	L	G	R	K	F	L	L	605000
100	112	R	I	H	L	P	D	P	N	K	F	G	F	P	625000
446	458	F	V	T	Q	A	I	A	C	Q	K	H	T	P	635000
247	259	P	L	D	I	C	T	S	I	C	K	Y	P	D	660000
201	213	C	T	N	V	A	V	N	P	G	D	C	P	P	670000

449	461	Q	A	I	A	C	Q	K	H	T	P	P	A	P	710000
344	356	G	H	N	N	G	I	C	W	G	N	Q	L	F	752000
31	43	L	P	S	E	A	T	V	Y	L	P	P	V	P	770000
111	123	F	P	D	T	S	F	Y	N	P	D	T	Q	R	770000
348	360	G	I	C	W	G	N	Q	L	F	V	T	V	V	770000
105	117	D	P	N	K	F	G	F	P	D	T	S	F	Y	775000
112	124	P	D	T	S	F	Y	N	P	D	T	Q	R	L	782000
367	379	N	M	S	L	C	A	A	I	S	T	S	E	T	782000
83	95	N	N	K	I	L	V	P	K	V	S	G	L	Q	802000
220	232	V	I	Q	D	G	D	M	V	H	T	G	F	G	810000
321	333	P	S	G	S	M	V	T	S	D	A	Q	I	F	810000
439	451	T	L	E	D	T	Y	R	F	V	T	Q	A	I	842000
489	501	P	L	G	R	K	F	L	L	Q	A	G	L	K	905000
282	294	F	V	R	H	L	F	N	R	A	G	T	V	G	915000
354	366	Q	L	F	V	T	V	V	D	T	T	R	S	T	980000
26	38	Q	M	S	L	W	L	P	S	E	A	T	V	Y	995000
4	16	T	F	I	Y	I	L	V	I	T	C	Y	E	N	1000000
10	22	V	I	T	C	Y	E	N	D	V	N	V	H	H	1000000
11	23	I	T	C	Y	E	N	D	V	N	V	H	H	I	1000000
14	26	Y	E	N	D	V	N	V	H	H	I	F	F	Q	1000000
15	27	E	N	D	V	N	V	H	H	I	F	F	Q	M	1000000
17	29	D	V	N	V	H	H	I	F	F	Q	M	S	L	1000000
24	36	F	F	Q	M	S	L	W	L	P	S	E	A	T	1000000
32	44	P	S	E	A	T	V	Y	L	P	P	V	P	V	1000000
38	50	Y	L	P	P	V	P	V	S	K	V	V	S	T	1000000
39	51	L	P	P	V	P	V	S	K	V	V	S	T	D	1000000
41	53	P	V	P	V	S	K	V	V	S	T	D	E	Y	1000000
42	54	V	P	V	S	K	V	V	S	T	D	E	Y	V	1000000
43	55	P	V	S	K	V	V	S	T	D	E	Y	V	A	1000000
44	56	V	S	K	V	V	S	T	D	E	Y	V	A	R	1000000
49	61	S	T	D	E	Y	V	A	R	T	N	I	Y	Y	1000000
55	67	A	R	T	N	I	Y	Y	H	A	G	T	S	R	1000000
61	73	Y	H	A	G	T	S	R	L	L	A	V	G	H	1000000
68	80	L	L	A	V	G	H	P	Y	F	P	I	K	K	1000000
69	81	L	A	V	G	H	P	Y	F	P	I	K	K	P	1000000
70	82	A	V	G	H	P	Y	F	P	I	K	K	P	N	1000000
71	83	V	G	H	P	Y	F	P	I	K	K	P	N	N	1000000
72	84	G	H	P	Y	F	P	I	K	K	P	N	N	N	1000000
74	86	P	Y	F	P	I	K	K	P	N	N	N	K	I	1000000
75	87	Y	F	P	I	K	K	P	N	N	N	K	I	L	1000000
78	90	I	K	K	P	N	N	N	K	I	L	V	P	K	1000000
79	91	K	K	P	N	N	N	K	I	L	V	P	K	V	1000000
80	92	K	P	N	N	N	K	I	L	V	P	K	V	S	1000000
87	99	L	V	P	K	V	S	G	L	Q	Y	R	V	F	1000000
90	102	K	V	S	G	L	Q	Y	R	V	F	R	I	H	1000000
95	107	Q	Y	R	V	F	R	I	H	L	P	D	P	N	1000000
96	108	Y	R	V	F	R	I	H	L	P	D	P	N	K	1000000
98	110	V	F	R	I	H	L	P	D	P	N	K	F	G	1000000
101	113	I	H	L	P	D	P	N	K	F	G	F	P	D	1000000
102	114	H	L	P	D	P	N	K	F	G	F	P	D	T	1000000
103	115	L	P	D	P	N	K	F	G	F	P	D	T	S	1000000

104	116	P	D	P	N	K	F	G	F	P	D	T	S	F	1000000
106	118	P	N	K	F	G	F	P	D	T	S	F	Y	N	1000000
108	120	K	F	G	F	P	D	T	S	F	Y	N	P	D	1000000
110	122	G	F	P	D	T	S	F	Y	N	P	D	T	Q	1000000
113	125	D	T	S	F	Y	N	P	D	T	Q	R	L	V	1000000
116	128	F	Y	N	P	D	T	Q	R	L	V	W	A	C	1000000
119	131	P	D	T	Q	R	L	V	W	A	C	V	G	V	1000000
125	137	V	W	A	C	V	G	V	E	V	G	R	G	Q	1000000
128	140	C	V	G	V	E	V	G	R	G	Q	P	L	G	1000000
130	142	G	V	E	V	G	R	G	Q	P	L	G	V	G	1000000
132	144	E	V	G	R	G	Q	P	L	G	V	G	I	S	1000000
133	145	V	G	R	G	Q	P	L	G	V	G	I	S	G	1000000
135	147	R	G	Q	P	L	G	V	G	I	S	G	H	P	1000000
136	148	G	Q	P	L	G	V	G	I	S	G	H	P	L	1000000
139	151	L	G	V	G	I	S	G	H	P	L	L	N	K	1000000
142	154	G	I	S	G	H	P	L	L	N	K	L	D	D	1000000
143	155	I	S	G	H	P	L	L	N	K	L	D	D	T	1000000
144	156	S	G	H	P	L	L	N	K	L	D	D	T	E	1000000
145	157	G	H	P	L	L	N	K	L	D	D	T	E	N	1000000
146	158	H	P	L	L	N	K	L	D	D	T	E	N	A	1000000
147	159	P	L	L	N	K	L	D	D	T	E	N	A	S	1000000
148	160	L	L	N	K	L	D	D	T	E	N	A	S	A	1000000
149	161	L	N	K	L	D	D	T	E	N	A	S	A	Y	1000000
151	163	K	L	D	D	T	E	N	A	S	A	Y	A	A	1000000
160	172	A	Y	A	A	N	A	G	V	D	N	R	E	C	1000000
161	173	Y	A	A	N	A	G	V	D	N	R	E	C	I	1000000
162	174	A	A	N	A	G	V	D	N	R	E	C	I	S	1000000
163	175	A	N	A	G	V	D	N	R	E	C	I	S	M	1000000
164	176	N	A	G	V	D	N	R	E	C	I	S	M	D	1000000
166	178	G	V	D	N	R	E	C	I	S	M	D	Y	K	1000000
167	179	V	D	N	R	E	C	I	S	M	D	Y	K	Q	1000000
168	180	D	N	R	E	C	I	S	M	D	Y	K	Q	T	1000000
169	181	N	R	E	C	I	S	M	D	Y	K	Q	T	Q	1000000
172	184	C	I	S	M	D	Y	K	Q	T	Q	L	C	L	1000000
174	186	S	M	D	Y	K	Q	T	Q	L	C	L	I	G	1000000
177	189	Y	K	Q	T	Q	L	C	L	I	G	C	K	P	1000000
178	190	K	Q	T	Q	L	C	L	I	G	C	K	P	P	1000000
181	193	Q	L	C	L	I	G	C	K	P	P	I	G	E	1000000
184	196	L	I	G	C	K	P	P	I	G	E	H	W	G	1000000
185	197	I	G	C	K	P	P	I	G	E	H	W	G	K	1000000
186	198	G	C	K	P	P	I	G	E	H	W	G	K	G	1000000
187	199	C	K	P	P	I	G	E	H	W	G	K	G	S	1000000
188	200	K	P	P	I	G	E	H	W	G	K	G	S	P	1000000
190	202	P	I	G	E	H	W	G	K	G	S	P	C	T	1000000
191	203	I	G	E	H	W	G	K	G	S	P	C	T	N	1000000
192	204	G	E	H	W	G	K	G	S	P	C	T	N	V	1000000
194	206	H	W	G	K	G	S	P	C	T	N	V	A	V	1000000
195	207	W	G	K	G	S	P	C	T	N	V	A	V	N	1000000
197	209	K	G	S	P	C	T	N	V	A	V	N	P	G	1000000
200	212	P	C	T	N	V	A	V	N	P	G	D	C	P	1000000
203	215	N	V	A	V	N	P	G	D	C	P	P	L	E	1000000

205	217	A	V	N	P	G	D	C	P	P	L	E	L	I	1000000
206	218	V	N	P	G	D	C	P	P	L	E	L	I	N	1000000
207	219	N	P	G	D	C	P	P	L	E	L	I	N	T	1000000
208	220	P	G	D	C	P	P	L	E	L	I	N	T	V	1000000
209	221	G	D	C	P	P	L	E	L	I	N	T	V	I	1000000
210	222	D	C	P	P	L	E	L	I	N	T	V	I	Q	1000000
211	223	C	P	P	L	E	L	I	N	T	V	I	Q	D	1000000
213	225	P	L	E	L	I	N	T	V	I	Q	D	G	D	1000000
216	228	L	I	N	T	V	I	Q	D	G	D	M	V	H	1000000
217	229	I	N	T	V	I	Q	D	G	D	M	V	H	T	1000000
221	233	I	Q	D	G	D	M	V	H	T	G	F	G	A	1000000
226	238	M	V	H	T	G	F	G	A	M	D	F	T	T	1000000
227	239	V	H	T	G	F	G	A	M	D	F	T	T	L	1000000
228	240	H	T	G	F	G	A	M	D	F	T	T	L	Q	1000000
229	241	T	G	F	G	A	M	D	F	T	T	L	Q	A	1000000
230	242	G	F	G	A	M	D	F	T	T	L	Q	A	N	1000000
233	245	A	M	D	F	T	T	L	Q	A	N	K	S	E	1000000
235	247	D	F	T	T	L	Q	A	N	K	S	E	V	P	1000000
236	248	F	T	T	L	Q	A	N	K	S	E	V	P	L	1000000
238	250	T	L	Q	A	N	K	S	E	V	P	L	D	I	1000000
239	251	L	Q	A	N	K	S	E	V	P	L	D	I	C	1000000
241	253	A	N	K	S	E	V	P	L	D	I	C	T	S	1000000
242	254	N	K	S	E	V	P	L	D	I	C	T	S	I	1000000
243	255	K	S	E	V	P	L	D	I	C	T	S	I	C	1000000
244	256	S	E	V	P	L	D	I	C	T	S	I	C	K	1000000
245	257	E	V	P	L	D	I	C	T	S	I	C	K	Y	1000000
249	261	D	I	C	T	S	I	C	K	Y	P	D	Y	L	1000000
250	262	I	C	T	S	I	C	K	Y	P	D	Y	L	K	1000000
251	263	C	T	S	I	C	K	Y	P	D	Y	L	K	M	1000000
253	265	S	I	C	K	Y	P	D	Y	L	K	M	V	S	1000000
254	266	I	C	K	Y	P	D	Y	L	K	M	V	S	E	1000000
256	268	K	Y	P	D	Y	L	K	M	V	S	E	P	Y	1000000
257	269	Y	P	D	Y	L	K	M	V	S	E	P	Y	G	1000000
263	275	M	V	S	E	P	Y	G	D	S	L	F	F	Y	1000000
264	276	V	S	E	P	Y	G	D	S	L	F	F	Y	L	1000000
265	277	S	E	P	Y	G	D	S	L	F	F	Y	L	R	1000000
268	280	Y	G	D	S	L	F	F	Y	L	R	R	E	Q	1000000
275	287	Y	L	R	R	E	Q	M	F	V	R	H	L	F	1000000
277	289	R	R	E	Q	M	F	V	R	H	L	F	N	R	1000000
279	291	E	Q	M	F	V	R	H	L	F	N	R	A	G	1000000
284	296	R	H	L	F	N	R	A	G	T	V	G	E	N	1000000
288	300	N	R	A	G	T	V	G	E	N	V	P	D	D	1000000
289	301	R	A	G	T	V	G	E	N	V	P	D	D	L	1000000
290	302	A	G	T	V	G	E	N	V	P	D	D	L	Y	1000000
291	303	G	T	V	G	E	N	V	P	D	D	L	Y	I	1000000
292	304	T	V	G	E	N	V	P	D	D	L	Y	I	K	1000000
293	305	V	G	E	N	V	P	D	D	L	Y	I	K	G	1000000
294	306	G	E	N	V	P	D	D	L	Y	I	K	G	S	1000000
295	307	E	N	V	P	D	D	L	Y	I	K	G	S	G	1000000
296	308	N	V	P	D	D	L	Y	I	K	G	S	G	S	1000000
297	309	V	P	D	D	L	Y	I	K	G	S	G	S	T	1000000

298	310	P	D	D	L	Y	I	K	G	S	G	S	T	A	1000000
302	314	Y	I	K	G	S	G	S	T	A	N	L	A	S	1000000
304	316	K	G	S	G	S	T	A	N	L	A	S	S	N	1000000
305	317	G	S	G	S	T	A	N	L	A	S	S	N	Y	1000000
308	320	S	T	A	N	L	A	S	S	N	Y	F	P	T	1000000
311	323	N	L	A	S	S	N	Y	F	P	T	P	S	G	1000000
312	324	L	A	S	S	N	Y	F	P	T	P	S	G	S	1000000
313	325	A	S	S	N	Y	F	P	T	P	S	G	S	M	1000000
318	330	F	P	T	P	S	G	S	M	V	T	S	D	A	1000000
319	331	P	T	P	S	G	S	M	V	T	S	D	A	Q	1000000
320	332	T	P	S	G	S	M	V	T	S	D	A	Q	I	1000000
325	337	M	V	T	S	D	A	Q	I	F	N	K	P	Y	1000000
326	338	V	T	S	D	A	Q	I	F	N	K	P	Y	W	1000000
328	340	S	D	A	Q	I	F	N	K	P	Y	W	L	Q	1000000
332	344	I	F	N	K	P	Y	W	L	Q	R	A	Q	G	1000000
333	345	F	N	K	P	Y	W	L	Q	R	A	Q	G	H	1000000
338	350	W	L	Q	R	A	Q	G	H	N	N	G	I	C	1000000
341	353	R	A	Q	G	H	N	N	G	I	C	W	G	N	1000000
342	354	A	Q	G	H	N	N	G	I	C	W	G	N	Q	1000000
345	357	H	N	N	G	I	C	W	G	N	Q	L	F	V	1000000
350	362	C	W	G	N	Q	L	F	V	T	V	V	D	T	1000000
351	363	W	G	N	Q	L	F	V	T	V	V	D	T	T	1000000
361	373	D	T	T	R	S	T	N	M	S	L	C	A	A	1000000
363	375	T	R	S	T	N	M	S	L	C	A	A	I	S	1000000
368	380	M	S	L	C	A	A	I	S	T	S	E	T	T	1000000
371	383	C	A	A	I	S	T	S	E	T	T	Y	K	N	1000000
372	384	A	A	I	S	T	S	E	T	T	Y	K	N	T	1000000
374	386	I	S	T	S	E	T	T	Y	K	N	T	N	F	1000000
375	387	S	T	S	E	T	T	Y	K	N	T	N	F	K	1000000
376	388	T	S	E	T	T	Y	K	N	T	N	F	K	E	1000000
377	389	S	E	T	T	Y	K	N	T	N	F	K	E	Y	1000000
378	390	E	T	T	Y	K	N	T	N	F	K	E	Y	L	1000000
380	392	T	Y	K	N	T	N	F	K	E	Y	L	R	H	1000000
382	394	K	N	T	N	F	K	E	Y	L	R	H	G	E	1000000
383	395	N	T	N	F	K	E	Y	L	R	H	G	E	E	1000000
385	397	N	F	K	E	Y	L	R	H	G	E	E	Y	D	1000000
386	398	F	K	E	Y	L	R	H	G	E	E	Y	D	L	1000000
389	401	Y	L	R	H	G	E	E	Y	D	L	Q	F	I	1000000
390	402	L	R	H	G	E	E	Y	D	L	Q	F	I	F	1000000
391	403	R	H	G	E	E	Y	D	L	Q	F	I	F	Q	1000000
392	404	H	G	E	E	Y	D	L	Q	F	I	F	Q	L	1000000
395	407	E	Y	D	L	Q	F	I	F	Q	L	C	K	I	1000000
402	414	F	Q	L	C	K	I	T	L	T	A	D	V	M	1000000
406	418	K	I	T	L	T	A	D	V	M	T	Y	I	H	1000000
407	419	I	T	L	T	A	D	V	M	T	Y	I	H	S	1000000
409	421	L	T	A	D	V	M	T	Y	I	H	S	M	N	1000000
413	425	V	M	T	Y	I	H	S	M	N	S	T	I	L	1000000
417	429	I	H	S	M	N	S	T	I	L	E	D	W	N	1000000
419	431	S	M	N	S	T	I	L	E	D	W	N	F	G	1000000
420	432	M	N	S	T	I	L	E	D	W	N	F	G	L	1000000
421	433	N	S	T	I	L	E	D	W	N	F	G	L	Q	1000000

425	437	L	E	D	W	N	F	G	L	Q	P	P	P	G	1000000
427	439	D	W	N	F	G	L	Q	P	P	P	G	G	T	1000000
431	443	G	L	Q	P	P	P	G	G	T	L	E	D	T	1000000
432	444	L	Q	P	P	P	G	G	T	L	E	D	T	Y	1000000
433	445	Q	P	P	P	G	G	T	L	E	D	T	Y	R	1000000
434	446	P	P	P	G	G	T	L	E	D	T	Y	R	F	1000000
435	447	P	P	G	G	T	L	E	D	T	Y	R	F	V	1000000
436	448	P	G	G	T	L	E	D	T	Y	R	F	V	T	1000000
437	449	G	G	T	L	E	D	T	Y	R	F	V	T	Q	1000000
440	452	L	E	D	T	Y	R	F	V	T	Q	A	I	A	1000000
448	460	T	Q	A	I	A	C	Q	K	H	T	P	P	A	1000000
450	462	A	I	A	C	Q	K	H	T	P	P	A	P	K	1000000
451	463	I	A	C	Q	K	H	T	P	P	A	P	K	E	1000000
452	464	A	C	Q	K	H	T	P	P	A	P	K	E	D	1000000
453	465	C	Q	K	H	T	P	P	A	P	K	E	D	D	1000000
454	466	Q	K	H	T	P	P	A	P	K	E	D	D	P	1000000
455	467	K	H	T	P	P	A	P	K	E	D	D	P	L	1000000
456	468	H	T	P	P	A	P	K	E	D	D	P	L	K	1000000
457	469	T	P	P	A	P	K	E	D	D	P	L	K	K	1000000
458	470	P	P	A	P	K	E	D	D	P	L	K	K	Y	1000000
459	471	P	A	P	K	E	D	D	P	L	K	K	Y	T	1000000
460	472	A	P	K	E	D	D	P	L	K	K	Y	T	F	1000000
461	473	P	K	E	D	D	P	L	K	K	Y	T	F	W	1000000
462	474	K	E	D	D	P	L	K	K	Y	T	F	W	E	1000000
463	475	E	D	D	P	L	K	K	Y	T	F	W	E	V	1000000
464	476	D	D	P	L	K	K	Y	T	F	W	E	V	N	1000000
466	478	P	L	K	K	Y	T	F	W	E	V	N	L	K	1000000
467	479	L	K	K	Y	T	F	W	E	V	N	L	K	E	1000000
469	481	K	Y	T	F	W	E	V	N	L	K	E	K	F	1000000
472	484	F	W	E	V	N	L	K	E	K	F	S	A	D	1000000
473	485	W	E	V	N	L	K	E	K	F	S	A	D	L	1000000
474	486	E	V	N	L	K	E	K	F	S	A	D	L	D	1000000
476	488	N	L	K	E	K	F	S	A	D	L	D	Q	F	1000000
477	489	L	K	E	K	F	S	A	D	L	D	Q	F	P	1000000
478	490	K	E	K	F	S	A	D	L	D	Q	F	P	L	1000000
480	492	K	F	S	A	D	L	D	Q	F	P	L	G	R	1000000
481	493	F	S	A	D	L	D	Q	F	P	L	G	R	K	1000000
482	494	S	A	D	L	D	Q	F	P	L	G	R	K	F	1000000
485	497	L	D	Q	F	P	L	G	R	K	F	L	L	Q	1000000
486	498	D	Q	F	P	L	G	R	K	F	L	L	Q	A	1000000
487	499	Q	F	P	L	G	R	K	F	L	L	Q	A	G	1000000
490	502	L	G	R	K	F	L	L	Q	A	G	L	K	A	1000000
491	503	G	R	K	F	L	L	Q	A	G	L	K	A	K	1000000
496	508	L	Q	A	G	L	K	A	K	P	K	F	T	L	1000000
499	511	G	L	K	A	K	P	K	F	T	L	G	K	R	1000000
500	512	L	K	A	K	P	K	F	T	L	G	K	R	K	1000000
501	513	K	A	K	P	K	F	T	L	G	K	R	K	A	1000000
503	515	K	P	K	F	T	L	G	K	R	K	A	T	P	1000000
505	517	K	F	T	L	G	K	R	K	A	T	P	T	T	1000000
506	518	F	T	L	G	K	R	K	A	T	P	T	T	S	1000000
507	519	T	L	G	K	R	K	A	T	P	T	T	S	S	1000000

508	520	L·	G	K	R	K	A	T	P	T	T	S	S	T	1000000
509	521	G	K	R	K	A	T	P	T	T	S	S	T	S	1000000
510	522	K	R	K	A	T	P	T	T	S	S	T	S	T	1000000
512	524	K	A	T	P	T	T	S	S	T	S	T	T	A	1000000
514	526	T	P	T	T	S	S	T	S	T	T	A	K	R	1000000
515	527	P	T	T	S	S	T	S	T	T	A	K	R	K	1000000
516	528	T	T	S	S	T	S	T	T	A	K	R	K	K	1000000
517	529	T	S	S	T	S	T	T	A	K	R	K	K	R	1000000
518	530	S	S	T	S	T	T	A	K	R	K	K	R	K	1000000
519	531	S	T	S	T	T	A	K	R	K	K	R	K	L	1000000

															IC50 DRB1*0401 (nM)
442	454	D	T	Y	R	F	V	T	Q	A	I	A	C	Q	1.15
492	504	R	K	F	L	L	Q	A	G	L	K	A	K	P	3
94	106	L	Q	Y	R	V	F	R	I	H	L	P	D	P	4.3
3	15	V	T	F	I	Y	I	L	V	I	T	C	Y	E	10.5
124	136	L	V	W	A	C	V	G	V	E	V	G	R	G	21
412	424	D	V	M	T	Y	I	H	S	M	N	S	T	I	27.5
5	17	F	I	Y	I	L	V	I	T	C	Y	E	N	D	28.2
159	171	S	A	Y	A	A	N	A	G	V	D	N	R	E	31.5
444	456	Y	R	F	V	T	Q	A	I	A	C	Q	K	H	40
6	18	I	Y	I	L	V	I	T	C	Y	E	N	D	V	71.5
23	35	I	F	F	Q	M	S	L	W	L	P	S	E	A	88.2
394	406	E	E	Y	D	L	Q	F	I	F	Q	L	C	K	92
400	412	F	I	F	Q	L	C	K	I	T	L	T	A	D	96.5
28	40	S	L	W	L	P	S	E	A	T	V	Y	L	P	102
58	70	N	I	Y	Y	H	A	G	T	S	R	L	L	A	105
97	109	R	V	F	R	I	H	L	P	D	P	N	K	F	105
9	21	L	V	I	T	C	Y	E	N	D	V	N	V	H	110
414	426	M	T	Y	I	H	S	M	N	S	T	I	L	E	110
415	427	T	Y	I	H	S	M	N	S	T	I	L	E	D	120
301	313	L	Y	I	K	G	S	G	S	T	A	N	L	A	135
45	57	S	K	V	V	S	T	D	E	Y	V	A	R	T	145
1	13	M	Q	V	T	F	I	Y	I	L	V	I	T	C	162
21	33	H	H	I	F	F	Q	M	S	L	W	L	P	S	165
25	37	F	Q	M	S	L	W	L	P	S	E	A	T	V	200
422	434	S	T	I	L	E	D	W	N	F	G	L	Q	P	242
281	293	M	F	V	R	H	L	F	N	R	A	G	T	V	245
182	194	L	C	L	I	G	C	K	P	P	I	G	E	H	265
428	440	W	N	F	G	L	Q	P	P	P	G	G	T	L	280
51	63	D	E	Y	V	A	R	T	N	I	Y	Y	H	A	310
234	246	M	D	F	T	T	L	Q	A	N	K	S	E	V	310
237	249	T	T	L	Q	A	N	K	S	E	V	P	L	D	310
259	271	D	Y	L	K	M	V	S	E	P	Y	G	D	S	322
398	410	L	Q	F	I	F	Q	L	C	K	I	T	L	T	322
355	367	L	F	V	T	V	V	D	T	T	R	S	T	N	330
258	270	P	D	Y	L	K	M	V	S	E	P	Y	G	D	342
449	461	Q	A	I	A	C	Q	K	H	T	P	P	A	P	352
300	312	D	L	Y	I	K	G	S	G	S	T	A	N	L	425
4	16	T	F	I	Y	I	L	V	I	T	C	Y	E	N	430
261	273	L	K	M	V	S	E	P	Y	G	D	S	L	F	445
324	336	S	M	V	T	S	D	A	Q	I	F	N	K	P	505
22	34	H	I	F	F	Q	M	S	L	W	L	P	S	E	540
52	64	E	Y	V	A	R	T	N	I	Y	Y	H	A	G	550
426	438	E	D	W	N	F	G	L	Q	P	P	P	G	G	612
123	135	R	L	V	W	A	C	V	G	V	E	V	G	R	650
366	378	T	N	M	S	L	C	A	A	I	S	T	S	E	710
280	292	Q	M	F	V	R	H	L	F	N	R	A	G	T	732
468	480	K	K	Y	T	F	W	E	V	N	L	K	E	K	745
372	384	A	A	I	S	T	S	E	T	T	Y	K	N	T	815
115	127	S	F	Y	N	P	D	T	Q	R	L	V	W	A	835
405	417	C	K	I	T	L	T	A	D	V	M	T	Y	I	880

59	71	I	Y	Y	H	A	G	T	S	R	L	L	A	V	922
271	283	S	L	F	F	Y	L	R	R	E	Q	M	F	V	1050
273	285	F	F	Y	L	R	R	E	Q	M	F	V	R	H	1420
337	349	Y	W	L	Q	R	A	Q	G	H	N	N	G	I	1420
16	28	N	D	V	N	V	H	H	I	F	F	Q	M	S	1450
141	153	V	G	I	S	G	H	P	L	L	N	K	L	D	1500
335	347	K	P	Y	W	L	Q	R	A	Q	G	H	N	N	1500
175	187	M	D	Y	K	Q	T	Q	L	C	L	I	G	C	1700
421	433	N	S	T	I	L	E	D	W	N	F	G	L	Q	1850
388	400	E	Y	L	R	H	G	E	E	Y	D	L	Q	F	1900
150	162	N	K	L	D	D	T	E	N	A	S	A	Y	A	2300
57	69	T	N	I	Y	Y	H	A	G	T	S	R	L	L	2500
53	65	Y	V	A	R	T	N	I	Y	Y	H	A	G	T	2650
347	359	N	G	I	C	W	G	N	Q	L	F	V	T	V	2700
67	79	R	L	L	A	V	G	H	P	Y	F	P	I	K	2720
315	327	S	N	Y	F	P	T	P	S	G	S	M	V	T	2800
361	373	D	T	T	R	S	T	N	M	S	L	C	A	A	3200
407	419	I	T	L	T	A	D	V	M	T	Y	I	H	S	3320
204	216	V	A	V	N	P	G	D	C	P	P	L	E	L	3400
218	230	N	T	V	I	Q	D	G	D	M	V	H	T	G	3520
445	457	R	F	V	T	Q	A	I	A	C	Q	K	H	T	3600
12	24	T	C	Y	E	N	D	V	N	V	H	H	I	F	3700
96	108	Y	R	V	F	R	I	H	L	P	D	P	N	K	3850
46	58	K	V	V	S	T	D	E	Y	V	A	R	T	N	4020
285	297	H	L	F	N	R	A	G	T	V	G	E	N	V	4020
202	214	T	N	V	A	V	N	P	G	D	C	P	P	L	4100
8	20	I	L	V	I	T	C	Y	E	N	D	V	N	V	4250
418	430	H	S	M	N	S	T	I	L	E	D	W	N	F	4300
2	14	Q	V	T	F	I	Y	I	L	V	I	T	C	Y	4350
229	241	T	G	F	G	A	M	D	F	T	T	L	Q	A	4350
66	78	S	R	L	L	A	V	G	H	P	Y	F	P	I	4500
362	374	T	T	R	S	T	N	M	S	L	C	A	A	I	4700
291	303	G	T	V	G	E	N	V	P	D	D	L	Y	I	4720
219	231	T	V	I	Q	D	G	D	M	V	H	T	G	F	4820
274	286	F	Y	L	R	R	E	Q	M	F	V	R	H	L	4820
139	151	L	G	V	G	I	S	G	H	P	L	L	N	K	5000
479	491	E	K	F	S	A	D	L	D	Q	F	P	L	G	5150
387	399	K	E	Y	L	R	H	G	E	E	Y	D	L	Q	5900
310	322	A	N	L	A	S	S	N	Y	F	P	T	P	S	6020
408	420	T	L	T	A	D	V	M	T	Y	I	H	S	M	6200
120	132	D	T	Q	R	L	V	W	A	C	V	G	V	E	7220
225	237	D	M	V	H	T	G	F	G	A	M	D	F	T	7300
35	47	A	T	V	Y	L	P	P	V	P	V	S	K	V	7450
214	226	L	E	L	I	N	T	V	I	Q	D	G	D	M	7900
73	85	H	P	Y	F	P	I	K	K	P	N	N	N	K	7950
164	176	N	A	G	V	D	N	R	E	C	I	S	M	D	8120
354	366	Q	L	F	V	T	V	V	D	T	T	R	S	T	8700
329	341	D	A	Q	I	F	N	K	P	Y	W	L	Q	R	8800
231	243	F	G	A	M	D	F	T	T	L	Q	A	N	K	9200
309	321	T	A	N	L	A	S	S	N	Y	F	P	T	P	9550
353	365	N	Q	L	F	V	T	V	V	D	T	T	R	S	9950

342	354	A	Q	G	H	N	N	G	I	C	W	G	N	Q	11000
138	150	P	L	G	V	G	I	S	G	H	P	L	L	N	11200
336	348	P	Y	W	L	Q	R	A	Q	G	H	N	N	G	11200
303	315	I	K	G	S	G	S	T	A	N	L	A	S	S	12000
378	390	E	T	T	Y	K	N	T	N	F	K	E	Y	L	12500
411	423	A	D	V	M	T	Y	I	H	S	M	N	S	T	13200
180	192	T	Q	L	C	L	I	G	C	K	P	P	I	G	13500
399	411	Q	F	I	F	Q	L	C	K	I	T	L	T	A	13500
245	257	E	V	P	L	D	I	C	T	S	I	C	K	Y	14000
358	370	T	V	V	D	T	T	R	S	T	N	M	S	L	14500
155	167	T	E	N	A	S	A	Y	A	A	N	A	G	V	15500
365	377	S	T	N	M	S	L	C	A	A	I	S	T	S	16200
122	134	Q	R	L	V	W	A	C	V	G	V	E	V	G	16500
64	76	G	T	S	R	L	L	A	V	G	H	P	Y	F	17000
179	191	Q	T	Q	L	C	L	I	G	C	K	P	P	I	17000
427	439	D	W	N	F	G	L	Q	P	P	P	G	G	T	18000
396	408	Y	D	L	Q	F	I	F	Q	L	C	K	I	T	18200
84	96	N	K	I	L	V	P	K	V	S	G	L	Q	Y	19000
470	482	Y	T	F	W	E	V	N	L	K	E	K	F	S	19500
357	369	V	T	V	V	D	T	T	R	S	T	N	M	S	20000
65	77	T	S	R	L	L	A	V	G	H	P	Y	F	P	22200
290	302	A	G	T	V	G	E	N	V	P	D	D	L	Y	22200
373	385	A	I	S	T	S	E	T	T	Y	K	N	T	N	23500
323	335	G	S	M	V	T	S	D	A	Q	I	F	N	K	24000
279	291	E	Q	M	F	V	R	H	L	F	N	R	A	G	24500
85	97	K	I	L	V	P	K	V	S	G	L	Q	Y	R	26000
368	380	M	S	L	C	A	A	I	S	T	S	E	T	T	26500
108	120	K	F	G	F	P	D	T	S	F	Y	N	P	D	28000
34	46	E	A	T	V	Y	L	P	P	V	P	V	S	K	29200
248	260	L	D	I	C	T	S	I	C	K	Y	P	D	Y	30000
132	144	E	V	G	R	G	Q	P	L	G	V	G	I	S	31000
246	258	V	P	L	D	I	C	T	S	I	C	K	Y	P	31000
232	244	G	A	M	D	F	T	T	L	Q	A	N	K	S	33200
215	227	E	L	I	N	T	V	I	Q	D	G	D	M	V	34000
514	526	T	P	T	T	S	S	T	S	T	T	A	K	R	34500
230	242	G	F	G	A	M	D	F	T	T	L	Q	A	N	36000
340	352	Q	R	A	Q	G	H	N	N	G	I	C	W	G	37000
18	30	V	N	V	H	H	I	F	F	Q	M	S	L	W	39500
29	41	L	W	L	P	S	E	A	T	V	Y	L	P	P	40500
212	224	P	P	L	E	L	I	N	T	V	I	Q	D	G	40500
351	363	W	G	N	Q	L	F	V	T	V	V	D	T	T	40500
364	376	R	S	T	N	M	S	L	C	A	A	I	S	T	40500
178	190	K	Q	T	Q	L	C	L	I	G	C	K	P	P	42200
193	205	E	H	W	G	K	G	S	P	C	T	N	V	A	43000
443	455	T	Y	R	F	V	T	Q	A	I	A	C	Q	K	43000
307	319	G	S	T	A	N	L	A	S	S	N	Y	F	P	44000
515	527	P	T	T	S	S	T	S	T	T	A	K	R	K	45500
140	152	G	V	G	I	S	G	H	P	L	L	N	K	L	49500
379	391	T	T	Y	K	N	T	N	F	K	E	Y	L	R	53200
107	119	N	K	F	G	F	P	D	T	S	F	Y	N	P	53500
196	208	G	K	G	S	P	C	T	N	V	A	V	N	P	55200

469	481	K	Y	T	F	W	E	V	N	L	K	E	K	F	57500
55	67	A	R	T	N	I	Y	Y	H	A	G	T	S	R	58000
152	164	L	D	D	T	E	N	A	S	A	Y	A	A	N	58200
126	138	W	A	C	V	G	V	E	V	G	R	G	Q	P	59200
252	264	T	S	I	C	K	Y	P	D	Y	L	K	M	V	59500
171	183	E	C	I	S	M	D	Y	K	Q	T	Q	L	C	60500
63	75	A	G	T	S	R	L	L	A	V	G	H	P	Y	61200
217	229	I	N	T	V	I	Q	D	G	D	M	V	H	T	62000
147	159	P	L	L	N	K	L	D	D	T	E	N	A	S	62200
266	278	E	P	Y	G	D	S	L	F	F	Y	L	R	R	63500
224	236	G	D	M	V	H	T	G	F	G	A	M	D	F	64000
331	343	Q	I	F	N	K	P	Y	W	L	Q	R	A	Q	65000
284	296	R	H	L	F	N	R	A	G	T	V	G	E	N	66000
134	146	G	R	G	Q	P	L	G	V	G	I	S	G	H	66500
137	149	Q	P	L	G	V	G	I	S	G	H	P	L	L	67500
446	458	F	V	T	Q	A	I	A	C	Q	K	H	T	P	68200
504	516	P	K	F	T	L	G	K	R	K	A	T	P	T	69000
436	448	P	G	G	T	L	E	D	T	Y	R	F	V	T	71000
148	160	L	L	N	K	L	D	D	T	E	N	A	S	A	73000
42	54	V	P	V	S	K	V	V	S	T	D	E	Y	V	74200
36	48	T	V	Y	L	P	P	V	P	V	S	K	V	V	74500
176	188	D	Y	K	Q	T	Q	L	C	L	I	G	C	K	76500
384	396	T	N	F	K	E	Y	L	R	H	G	E	E	Y	78200
410	422	T	A	D	V	M	T	Y	I	H	S	M	N	S	83500
402	414	F	Q	L	C	K	I	T	L	T	A	D	V	M	91000
240	252	Q	A	N	K	S	E	V	P	L	D	I	C	T	93200
270	282	D	S	L	F	F	Y	L	R	R	E	Q	M	F	97500
131	143	V	E	V	G	R	G	Q	P	L	G	V	G	I	98000
360	372	V	D	T	T	R	S	T	N	M	S	L	C	A	98000
223	235	D	G	D	M	V	H	T	G	F	G	A	M	D	100000
494	506	F	L	L	Q	A	G	L	K	A	K	P	K	F	102000
89	101	P	K	V	S	G	L	Q	Y	R	V	F	R	I	105000
129	141	V	G	V	E	V	G	R	G	Q	P	L	G	V	105000
173	185	I	S	M	D	Y	K	Q	T	Q	L	C	L	I	112000
201	213	C	T	N	V	A	V	N	P	G	D	C	P	P	112000
313	325	A	S	S	N	Y	F	P	T	P	S	G	S	M	112000
213	225	P	L	E	L	I	N	T	V	I	Q	D	G	D	115000
306	318	S	G	S	T	A	N	L	A	S	S	N	Y	F	115000
332	344	I	F	N	K	P	Y	W	L	Q	R	A	Q	G	125000
56	68	R	T	N	I	Y	Y	H	A	G	T	S	R	L	130000
111	123	F	P	D	T	S	F	Y	N	P	D	T	Q	R	130000
367	379	N	M	S	L	C	A	A	I	S	T	S	E	T	132000
250	262	I	C	T	S	I	C	K	Y	P	D	Y	L	K	135000
11	23	I	T	C	Y	E	N	D	V	N	V	H	H	I	140000
79	91	K	K	P	N	N	N	K	I	L	V	P	K	V	140000
325	337	M	V	T	S	D	A	Q	I	F	N	K	P	Y	140000
475	487	V	N	L	K	E	K	F	S	A	D	L	D	Q	140000
127	139	A	C	V	G	V	E	V	G	R	G	Q	P	L	145000
19	31	N	V	H	H	I	F	F	Q	M	S	L	W	L	150000
54	66	V	A	R	T	N	I	Y	Y	H	A	G	T	S	150000
304	316	K	G	S	G	S	T	A	N	L	A	S	S	N	150000

438	450	G	T	L	E	D	T	Y	R	F	V	T	Q	A	150000
471	483	T	F	W	E	V	N	L	K	E	K	F	S	A	150000
194	206	H	W	G	K	G	S	P	C	T	N	V	A	V	160000
345	357	H	N	N	G	I	C	W	G	N	Q	L	F	V	160000
247	259	P	L	D	I	C	T	S	I	C	K	Y	P	D	170000
489	501	P	L	G	R	K	F	L	L	Q	A	G	L	K	170000
321	333	P	S	G	S	M	V	T	S	D	A	Q	I	F	172000
493	505	K	F	L	L	Q	A	G	L	K	A	K	P	K	175000
283	295	V	R	H	L	F	N	R	A	G	T	V	G	E	180000
27	39	M	S	L	W	L	P	S	E	A	T	V	Y	L	190000
7	19	Y	I	L	V	I	T	C	Y	E	N	D	V	N	192000
200	212	P	C	T	N	V	A	V	N	P	G	D	C	P	195000
356	368	F	V	T	V	V	D	T	T	R	S	T	N	M	195000
136	148	G	Q	P	L	G	V	G	I	S	G	H	P	L	200000
198	210	G	S	P	C	T	N	V	A	V	N	P	G	D	200000
338	350	W	L	Q	R	A	Q	G	H	N	N	G	I	C	205000
363	375	T	R	S	T	N	M	S	L	C	A	A	I	S	205000
397	409	D	L	Q	F	I	F	Q	L	C	K	I	T	L	220000
490	502	L	G	R	K	F	L	L	Q	A	G	L	K	A	225000
114	126	T	S	F	Y	N	P	D	T	Q	R	L	V	W	230000
317	329	Y	F	P	T	P	S	G	S	M	V	T	S	D	230000
191	203	I	G	E	H	W	G	K	G	S	P	C	T	N	235000
352	364	G	N	Q	L	F	V	T	V	V	D	T	T	R	235000
465	477	D	P	L	K	K	Y	T	F	W	E	V	N	L	250000
13	25	C	Y	E	N	D	V	N	V	H	H	I	F	F	252000
312	324	L	A	S	S	N	Y	F	P	T	P	S	G	S	255000
272	284	L	F	F	Y	L	R	R	E	Q	M	F	V	R	260000
156	168	E	N	A	S	A	Y	A	A	N	A	G	V	D	262000
305	317	G	S	G	S	T	A	N	L	A	S	S	N	Y	262000
349	361	I	C	W	G	N	Q	L	F	V	T	V	V	D	265000
516	528	T	T	S	S	T	S	T	T	A	K	R	K	K	272000
473	485	W	E	V	N	L	K	E	K	F	S	A	D	L	275000
47	59	V	V	S	T	D	E	Y	V	A	R	T	N	I	282000
348	360	G	I	C	W	G	N	Q	L	F	V	T	V	V	282000
406	418	K	I	T	L	T	A	D	V	M	T	Y	I	H	290000
437	449	G	G	T	L	E	D	T	Y	R	F	V	T	Q	290000
413	425	V	M	T	Y	I	H	S	M	N	S	T	I	L	305000
255	267	C	K	Y	P	D	Y	L	K	M	V	S	E	P	315000
62	74	H	A	G	T	S	R	L	L	A	V	G	H	P	330000
320	332	T	P	S	G	S	M	V	T	S	D	A	Q	I	330000
506	518	F	T	L	G	K	R	K	A	T	P	T	T	S	340000
298	310	P	D	D	L	Y	I	K	G	S	G	S	T	A	342000
401	413	I	F	Q	L	C	K	I	T	L	T	A	D	V	352000
119	131	P	D	T	Q	R	L	V	W	A	C	V	G	V	355000
299	311	D	D	L	Y	I	K	G	S	G	S	T	A	N	360000
286	298	L	F	N	R	A	G	T	V	G	E	N	V	P	365000
99	111	F	R	I	H	L	P	D	P	N	K	F	G	F	380000
268	280	Y	G	D	S	L	F	F	Y	L	R	R	E	Q	380000
308	320	S	T	A	N	L	A	S	S	N	Y	F	P	T	380000
153	165	D	D	T	E	N	A	S	A	Y	A	A	N	A	382000
330	342	A	Q	I	F	N	K	P	Y	W	L	Q	R	A	390000

243	255	K	S	E	V	P	L	D	I	C	T	S	I	C	392000
350	362	C	W	G	N	Q	L	F	V	T	V	V	D	T	395000
222	234	Q	D	G	D	M	V	H	T	G	F	G	A	M	400000
508	520	L	G	K	R	K	A	T	P	T	T	S	S	T	415000
262	274	K	M	V	S	E	P	Y	G	D	S	L	F	F	430000
416	428	Y	I	H	S	M	N	S	T	I	L	E	D	W	440000
404	416	L	C	K	I	T	L	T	A	D	V	M	T	Y	450000
189	201	P	P	I	G	E	H	W	G	K	G	S	P	C	455000
92	104	S	G	L	Q	Y	R	V	F	R	I	H	L	P	475000
226	238	M	V	H	T	G	F	G	A	M	D	F	T	T	480000
32	44	P	S	E	A	T	V	Y	L	P	P	V	P	V	490000
109	121	F	G	F	P	D	T	S	F	Y	N	P	D	T	492000
441	453	E	D	T	Y	R	F	V	T	Q	A	I	A	C	492000
488	500	F	P	L	G	R	K	F	L	L	Q	A	G	L	510000
403	415	Q	L	C	K	I	T	L	T	A	D	V	M	T	515000
467	479	L	K	K	Y	T	F	W	E	V	N	L	K	E	515000
482	494	S	A	D	L	D	Q	F	P	L	G	R	K	F	525000
177	189	Y	K	Q	T	Q	L	C	L	I	G	C	K	P	560000
511	523	R	K	A	T	P	T	T	S	S	T	S	T	T	560000
50	62	T	D	E	Y	V	A	R	T	N	I	Y	Y	H	572000
10	22	V	I	T	C	Y	E	N	D	V	N	V	H	H	575000
509	521	G	K	R	K	A	T	P	T	T	S	S	T	S	610000
167	179	V	D	N	R	E	C	I	S	M	D	Y	K	Q	630000
294	306	G	E	N	V	P	D	D	L	Y	I	K	G	S	642000
149	161	L	N	K	L	D	D	T	E	N	A	S	A	Y	650000
409	421	L	T	A	D	V	M	T	Y	I	H	S	M	N	650000
477	489	L	K	E	K	F	S	A	D	L	D	Q	F	P	665000
76	88	F	P	I	K	K	P	N	N	N	K	I	L	V	700000
486	498	D	Q	F	P	L	G	R	K	F	L	L	Q	A	710000
82	94	N	N	N	K	I	L	V	P	K	V	S	G	L	720000
451	463	I	A	C	Q	K	H	T	P	P	A	P	K	E	722000
112	124	P	D	T	S	F	Y	N	P	D	T	Q	R	L	730000
157	169	N	A	S	A	Y	A	A	N	A	G	V	D	N	730000
311	323	N	L	A	S	S	N	Y	F	P	T	P	S	G	730000
70	82	A	V	G	H	P	Y	F	P	I	K	K	P	N	735000
91	103	V	S	G	L	Q	Y	R	V	F	R	I	H	L	745000
220	232	V	I	Q	D	G	D	M	V	H	T	G	F	G	765000
265	277	S	E	P	Y	G	D	S	L	F	F	Y	L	R	765000
30	42	W	L	P	S	E	A	T	V	Y	L	P	P	V	785000
20	32	V	H	H	I	F	F	Q	M	S	L	W	L	P	795000
154	166	D	T	E	N	A	S	A	Y	A	A	N	A	G	795000
369	381	S	L	C	A	A	I	S	T	S	E	T	T	Y	840000
383	395	N	T	N	F	K	E	Y	L	R	H	G	E	E	882000
480	492	K	F	S	A	D	L	D	Q	F	P	L	G	R	895000
343	355	Q	G	H	N	N	G	I	C	W	G	N	Q	L	900000
113	125	D	T	S	F	Y	N	P	D	T	Q	R	L	V	920000
121	133	T	Q	R	L	V	W	A	C	V	G	V	E	V	922000
341	353	R	A	Q	G	H	N	N	G	I	C	W	G	N	925000
174	186	S	M	D	Y	K	Q	T	Q	L	C	L	I	G	945000
491	503	G	R	K	F	L	L	Q	A	G	L	K	A	K	960000
17	29	D	V	N	V	H	H	I	F	F	Q	M	S	L	985000

33	45	S	E	A	T	V	Y	L	P	P	V	P	V	S	985000
165	177	A	G	V	D	N	R	E	C	I	S	M	D	Y	985000
14	26	Y	E	N	D	V	N	V	H	H	I	F	F	Q	1000000
15	27	E	N	D	V	N	V	H	H	I	F	F	Q	M	1000000
24	36	F	F	Q	M	S	L	W	L	P	S	E	A	T	1000000
26	38	Q	M	S	L	W	L	P	S	E	A	T	V	Y	1000000
31	43	L	P	S	E	A	T	V	Y	L	P	P	V	P	1000000
37	49	V	Y	L	P	P	V	P	V	S	K	V	V	S	1000000
38	50	Y	L	P	P	V	P	V	S	K	V	V	S	T	1000000
39	51	L	P	P	V	P	V	S	K	V	V	S	T	D	1000000
40	52	P	P	V	P	V	S	K	V	V	S	T	D	E	1000000
41	53	P	V	P	V	S	K	V	V	S	T	D	E	Y	1000000
43	55	P	V	S	K	V	V	S	T	D	E	Y	V	A	1000000
44	56	V	S	K	V	V	S	T	D	E	Y	V	A	R	1000000
48	60	V	S	T	D	E	Y	V	A	R	T	N	I	Y	1000000
49	61	S	T	D	E	Y	V	A	R	T	N	I	Y	Y	1000000
60	72	Y	Y	H	A	G	T	S	R	L	L	A	V	G	1000000
61	73	Y	H	A	G	T	S	R	L	L	A	V	G	H	1000000
68	80	L	L	A	V	G	H	P	Y	F	P	I	K	K	1000000
69	81	L	A	V	G	H	P	Y	F	P	I	K	K	P	1000000
71	83	V	G	H	P	Y	F	P	I	K	K	P	N	N	1000000
72	84	G	H	P	Y	F	P	I	K	K	P	N	N	N	1000000
74	86	P	Y	F	P	I	K	K	P	N	N	N	K	I	1000000
75	87	Y	F	P	I	K	K	P	N	N	N	K	I	L	1000000
77	89	P	I	K	K	P	N	N	N	K	I	L	V	P	1000000
78	90	I	K	K	P	N	N	N	K	I	L	V	P	K	1000000
80	92	K	P	N	N	N	K	I	L	V	P	K	V	S	1000000
81	93	P	N	N	N	K	I	L	V	P	K	V	S	G	1000000
83	95	N	N	K	I	L	V	P	K	V	S	G	L	Q	1000000
86	98	I	L	V	P	K	V	S	G	L	Q	Y	R	V	1000000
87	99	L	V	P	K	V	S	G	L	Q	Y	R	V	F	1000000
88	100	V	P	K	V	S	G	L	Q	Y	R	V	F	R	1000000
90	102	K	V	S	G	L	Q	Y	R	V	F	R	I	H	1000000
93	105	G	L	Q	Y	R	V	F	R	I	H	L	P	D	1000000
95	107	Q	Y	R	V	F	R	I	H	L	P	D	P	N	1000000
98	110	V	F	R	I	H	L	P	D	P	N	K	F	G	1000000
100	112	R	I	H	L	P	D	P	N	K	F	G	F	P	1000000
101	113	I	H	L	P	D	P	N	K	F	G	F	P	D	1000000
102	114	H	L	P	D	P	N	K	F	G	F	P	D	T	1000000
103	115	L	P	D	P	N	K	F	G	F	P	D	T	S	1000000
104	116	P	D	P	N	K	F	G	F	P	D	T	S	F	1000000
105	117	D	P	N	K	F	G	F	P	D	T	S	F	Y	1000000
106	118	P	N	K	F	G	F	P	D	T	S	F	Y	N	1000000
110	122	G	F	P	D	T	S	F	Y	N	P	D	T	Q	1000000
116	128	F	Y	N	P	D	T	Q	R	L	V	W	A	C	1000000
117	129	Y	N	P	D	T	Q	R	L	V	W	A	C	V	1000000
118	130	N	P	D	T	Q	R	L	V	W	A	C	V	G	1000000
125	137	V	W	A	C	V	G	V	E	V	G	R	G	Q	1000000
128	140	C	V	G	V	E	V	G	R	G	Q	P	L	G	1000000
130	142	G	V	E	V	G	R	G	Q	P	L	G	V	G	1000000
133	145	V	G	R	G	Q	P	L	G	V	G	I	S	G	1000000

135	147	R	G	Q	P	L	G	V	G	I	S	G	H	P	1000000
142	154	G	I	S	G	H	P	L	L	N	K	L	D	D	1000000
143	155	I	S	G	H	P	L	L	N	K	L	D	D	T	1000000
144	156	S	G	H	P	L	L	N	K	L	D	D	T	E	1000000
145	157	G	H	P	L	L	N	K	L	D	D	T	E	N	1000000
146	158	H	P	L	L	N	K	L	D	D	T	E	N	A	1000000
151	163	K	L	D	D	T	E	N	A	S	A	Y	A	A	1000000
158	170	A	S	A	Y	A	A	N	A	G	V	D	N	R	1000000
160	172	A	Y	A	A	N	A	G	V	D	N	R	E	C	1000000
161	173	Y	A	A	N	A	G	V	D	N	R	E	C	I	1000000
162	174	A	A	N	A	G	V	D	N	R	E	C	I	S	1000000
163	175	A	N	A	G	V	D	N	R	E	C	I	S	M	1000000
166	178	G	V	D	N	R	E	C	I	S	M	D	Y	K	1000000
168	180	D	N	R	E	C	I	S	M	D	Y	K	Q	T	1000000
169	181	N	R	E	C	I	S	M	D	Y	K	Q	T	Q	1000000
170	182	R	E	C	I	S	M	D	Y	K	Q	T	Q	L	1000000
172	184	C	I	S	M	D	Y	K	Q	T	Q	L	C	L	1000000
181	193	Q	L	C	L	I	G	C	K	P	P	I	G	E	1000000
183	195	C	L	I	G	C	K	P	P	I	G	E	H	W	1000000
184	196	L	I	G	C	K	P	P	I	G	E	H	W	G	1000000
185	197	I	G	C	K	P	P	I	G	E	H	W	G	K	1000000
186	198	G	C	K	P	P	I	G	E	H	W	G	K	G	1000000
187	199	C	K	P	P	I	G	E	H	W	G	K	G	S	1000000
188	200	K	P	P	I	G	E	H	W	G	K	G	S	P	1000000
190	202	P	I	G	E	H	W	G	K	G	S	P	C	T	1000000
192	204	G	E	H	W	G	K	G	S	P	C	T	N	V	1000000
195	207	W	G	K	G	S	P	C	T	N	V	A	V	N	1000000
197	209	K	G	S	P	C	T	N	V	A	V	N	P	G	1000000
199	211	S	P	C	T	N	V	A	V	N	P	G	D	C	1000000
203	215	N	V	A	V	N	P	G	D	C	P	P	L	E	1000000
205	217	A	V	N	P	G	D	C	P	P	L	E	L	I	1000000
206	218	V	N	P	G	D	C	P	P	L	E	L	I	N	1000000
207	219	N	P	G	D	C	P	P	L	E	L	I	N	T	1000000
208	220	P	G	D	C	P	P	L	E	L	I	N	T	V	1000000
209	221	G	D	C	P	P	L	E	L	I	N	T	V	I	1000000
210	222	D	C	P	P	L	E	L	I	N	T	V	I	Q	1000000
211	223	C	P	P	L	E	L	I	N	T	V	I	Q	D	1000000
216	228	L	I	N	T	V	I	Q	D	G	D	M	V	H	1000000
221	233	I	Q	D	G	D	M	V	H	T	G	F	G	A	1000000
227	239	V	H	T	G	F	G	A	M	D	F	T	T	L	1000000
228	240	H	T	G	F	G	A	M	D	F	T	T	L	Q	1000000
233	245	A	M	D	F	T	T	L	Q	A	N	K	S	E	1000000
235	247	D	F	T	T	L	Q	A	N	K	S	E	V	P	1000000
236	248	F	T	T	L	Q	A	N	K	S	E	V	P	L	1000000
238	250	T	L	Q	A	N	K	S	E	V	P	L	D	I	1000000
239	251	L	Q	A	N	K	S	E	V	P	L	D	I	C	1000000
241	253	A	N	K	S	E	V	P	L	D	I	C	T	S	1000000
242	254	N	K	S	E	V	P	L	D	I	C	T	S	I	1000000
244	256	S	E	V	P	L	D	I	C	T	S	I	C	K	1000000
249	261	D	I	C	T	S	I	C	K	Y	P	D	Y	L	1000000
251	263	C	T	S	I	C	K	Y	P	D	Y	L	K	M	1000000

253	265	S	I	C	K	Y	P	D	Y	L	K	M	V	S	1000000
254	266	I	C	K	Y	P	D	Y	L	K	M	V	S	E	1000000
256	268	K	Y	P	D	Y	L	K	M	V	S	E	P	Y	1000000
257	269	Y	P	D	Y	L	K	M	V	S	E	P	Y	G	1000000
260	272	Y	L	K	M	V	S	E	P	Y	G	D	S	L	1000000
263	275	M	V	S	E	P	Y	G	D	S	L	F	F	Y	1000000
264	276	V	S	E	P	Y	G	D	S	L	F	F	Y	L	1000000
267	279	P	Y	G	D	S	L	F	F	Y	L	R	R	E	1000000
269	281	G	D	S	L	F	F	Y	L	R	R	E	Q	M	1000000
275	287	Y	L	R	R	E	Q	M	F	V	R	H	L	F	1000000
276	288	L	R	R	E	Q	M	F	V	R	H	L	F	N	1000000
277	289	R	R	E	Q	M	F	V	R	H	L	F	N	R	1000000
278	290	R	E	Q	M	F	V	R	H	L	F	N	R	A	1000000
282	294	F	V	R	H	L	F	N	R	A	G	T	V	G	1000000
287	299	F	N	R	A	G	T	V	G	E	N	V	P	D	1000000
288	300	N	R	A	G	T	V	G	E	N	V	P	D	D	1000000
289	301	R	A	G	T	V	G	E	N	V	P	D	D	L	1000000
292	304	T	V	G	E	N	V	P	D	D	L	Y	I	K	1000000
293	305	V	G	E	N	V	P	D	D	L	Y	I	K	G	1000000
295	307	E	N	V	P	D	D	L	Y	I	K	G	S	G	1000000
296	308	N	V	P	D	D	L	Y	I	K	G	S	G	S	1000000
297	309	V	P	D	D	L	Y	I	K	G	S	G	S	T	1000000
302	314	Y	I	K	G	S	G	S	T	A	N	L	A	S	1000000
314	326	S	S	N	Y	F	P	T	P	S	G	S	M	V	1000000
316	328	N	Y	F	P	T	P	S	G	S	M	V	T	S	1000000
318	330	F	P	T	P	S	G	S	M	V	T	S	D	A	1000000
319	331	P	T	P	S	G	S	M	V	T	S	D	A	Q	1000000
322	334	S	G	S	M	V	T	S	D	A	Q	I	F	N	1000000
326	338	V	T	S	D	A	Q	I	F	N	K	P	Y	W	1000000
327	339	T	S	D	A	Q	I	F	N	K	P	Y	W	L	1000000
328	340	S	D	A	Q	I	F	N	K	P	Y	W	L	Q	1000000
333	345	F	N	K	P	Y	W	L	Q	R	A	Q	G	H	1000000
334	346	N	K	P	Y	W	L	Q	R	A	Q	G	H	N	1000000
339	351	L	Q	R	A	Q	G	H	N	N	G	I	C	W	1000000
344	356	G	H	N	N	G	I	C	W	G	N	Q	L	F	1000000
346	358	N	N	G	I	C	W	G	N	Q	L	F	V	T	1000000
359	371	V	V	D	T	T	R	S	T	N	M	S	L	C	1000000
370	382	L	C	A	A	I	S	T	S	E	T	T	Y	K	1000000
371	383	C	A	A	I	S	T	S	E	T	T	Y	K	N	1000000
374	386	I	S	T	S	E	T	T	Y	K	N	T	N	F	1000000
375	387	S	T	S	E	T	T	Y	K	N	T	N	F	K	1000000
376	388	T	S	E	T	T	Y	K	N	T	N	F	K	E	1000000
377	389	S	E	T	T	Y	K	N	T	N	F	K	E	Y	1000000
380	392	T	Y	K	N	T	N	F	K	E	Y	L	R	H	1000000
381	393	Y	K	N	T	N	F	K	E	Y	L	R	H	G	1000000
382	394	K	N	T	N	F	K	E	Y	L	R	H	G	E	1000000
385	397	N	F	K	E	Y	L	R	H	G	E	E	Y	D	1000000
386	398	F	K	E	Y	L	R	H	G	E	E	Y	D	L	1000000
389	401	Y	L	R	H	G	E	E	Y	D	L	Q	F	I	1000000
390	402	L	R	H	G	E	E	Y	D	L	Q	F	I	F	1000000
391	403	R	H	G	E	E	Y	D	L	Q	F	I	F	Q	1000000

392	404	H	G	E	E	Y	D	L	Q	F	I	F	Q	L	1000000
393	405	G	E	E	Y	D	L	Q	F	I	F	Q	L	C	1000000
395	407	E	Y	D	L	Q	F	I	F	Q	L	C	K	I	1000000
417	429	I	H	S	M	N	S	T	I	L	E	D	W	N	1000000
419	431	S	M	N	S	T	I	L	E	D	W	N	F	G	1000000
420	432	M	N	S	T	I	L	E	D	W	N	F	G	L	1000000
423	435	T	I	L	E	D	W	N	F	G	L	Q	P	P	1000000
424	436	I	L	E	D	W	N	F	G	L	Q	P	P	P	1000000
425	437	L	E	D	W	N	F	G	L	Q	P	P	P	G	1000000
429	441	N	F	G	L	Q	P	P	P	G	G	T	L	E	1000000
430	442	F	G	L	Q	P	P	P	G	G	T	L	E	D	1000000
431	443	G	L	Q	P	P	P	G	G	T	L	E	D	T	1000000
432	444	L	Q	P	P	P	G	G	T	L	E	D	T	Y	1000000
433	445	Q	P	P	P	G	G	T	L	E	D	T	Y	R	1000000
434	446	P	P	P	G	G	T	L	E	D	T	Y	R	F	1000000
435	447	P	P	G	G	T	L	E	D	T	Y	R	F	V	1000000
439	451	T	L	E	D	T	Y	R	F	V	T	Q	A	I	1000000
440	452	L	E	D	T	Y	R	F	V	T	Q	A	I	A	1000000
447	459	V	T	Q	A	I	A	C	Q	K	H	T	P	P	1000000
448	460	T	Q	A	I	A	C	Q	K	H	T	P	P	A	1000000
450	462	A	I	A	C	Q	K	H	T	P	P	A	P	K	1000000
452	464	A	C	Q	K	H	T	P	P	A	P	K	E	D	1000000
453	465	C	Q	K	H	T	P	P	A	P	K	E	D	D	1000000
454	466	Q	K	H	T	P	P	A	P	K	E	D	D	P	1000000
455	467	K	H	T	P	P	A	P	K	E	D	D	P	L	1000000
456	468	H	T	P	P	A	P	K	E	D	D	P	L	K	1000000
457	469	T	P	P	A	P	K	E	D	D	P	L	K	K	1000000
458	470	P	P	A	P	K	E	D	D	P	L	K	K	Y	1000000
459	471	P	A	P	K	E	D	D	P	L	K	K	Y	T	1000000
460	472	A	P	K	E	D	D	P	L	K	K	Y	T	F	1000000
461	473	P	K	E	D	D	P	L	K	K	Y	T	F	W	1000000
462	474	K	E	D	D	P	L	K	K	Y	T	F	W	E	1000000
463	475	E	D	D	P	L	K	K	Y	T	F	W	E	V	1000000
464	476	D	D	P	L	K	K	Y	T	F	W	E	V	N	1000000
466	478	P	L	K	K	Y	T	F	W	E	V	N	L	K	1000000
472	484	F	W	E	V	N	L	K	E	K	F	S	A	D	1000000
474	486	E	V	N	L	K	E	K	F	S	A	D	L	D	1000000
476	488	N	L	K	E	K	F	S	A	D	L	D	Q	F	1000000
478	490	K	E	K	F	S	A	D	L	D	Q	F	P	L	1000000
481	493	F	S	A	D	L	D	Q	F	P	L	G	R	K	1000000
483	495	A	D	L	D	Q	F	P	L	G	R	K	F	L	1000000
484	496	D	L	D	Q	F	P	L	G	R	K	F	L	L	1000000
485	497	L	D	Q	F	P	L	G	R	K	F	L	L	Q	1000000
487	499	Q	F	P	L	G	R	K	F	L	L	Q	A	G	1000000
495	507	L	L	Q	A	G	L	K	A	K	P	K	F	T	1000000
496	508	L	Q	A	G	L	K	A	K	P	K	F	T	L	1000000
497	509	Q	A	G	L	K	A	K	P	K	F	T	L	G	1000000
498	510	A	G	L	K	A	K	P	K	F	T	L	G	K	1000000
499	511	G	L	K	A	K	P	K	F	T	L	G	K	R	1000000
500	512	L	K	A	K	P	K	F	T	L	G	K	R	K	1000000
501	513	K	A	K	P	K	F	T	L	G	K	R	K	A	1000000

502	514	A	K	P	K	F	T	L	G	K	R	K	A	T	1000000
503	515	K	P	K	F	T	L	G	K	R	K	A	T	P	1000000
505	517	K	F	T	L	G	K	R	K	A	T	P	T	T	1000000
507	519	T	L	G	K	R	K	A	T	P	T	T	S	S	1000000
510	522	K	R	K	A	T	P	T	T	S	S	T	S	T	1000000
512	524	K	A	T	P	T	T	S	S	T	S	T	T	A	1000000
513	525	A	T	P	T	T	S	S	T	S	T	T	A	K	1000000
517	529	T	S	S	T	S	T	T	A	K	R	K	K	R	1000000
518	530	S	S	T	S	T	T	A	K	R	K	K	R	K	1000000
519	531	S	T	S	T	T	A	K	R	K	K	R	K	L	1000000

															IC50 DRB1*0101(nM)
240	252	P	A	F	V	T	T	P	T	K	L	I	T	Y	2.7
444	456	G	D	F	Y	L	H	P	S	Y	Y	M	L	R	3.35
52	64	G	V	F	F	G	G	L	G	I	G	T	G	S	7.2
301	313	I	R	Y	S	R	I	G	N	K	Q	T	L	R	11.5
200	212	T	F	I	V	S	T	N	P	N	T	V	T	S	13
445	457	D	F	Y	L	H	P	S	Y	Y	M	L	R	K	13
70	82	T	G	Y	I	P	L	G	T	R	P	P	T	A	14
418	430	I	N	I	T	D	Q	A	P	S	L	I	P	I	19
161	173	P	T	F	T	D	P	S	V	L	Q	P	P	T	32
144	156	A	I	L	D	I	N	N	T	V	T	T	V	T	34
150	162	N	T	V	T	T	V	T	T	H	N	N	P	T	40
285	297	D	I	V	A	L	H	R	P	A	L	T	S	R	40
199	211	D	T	F	I	V	S	T	N	P	N	T	V	T	44
196	208	I	P	M	D	T	F	I	V	S	T	N	P	N	61.5
208	220	N	T	V	T	S	S	T	P	I	P	G	S	R	63
17	29	Q	L	Y	K	T	C	K	Q	A	G	T	C	P	68
388	400	T	S	L	S	G	Y	I	P	A	N	T	T	I	69
112	124	T	S	F	I	D	A	G	A	P	T	S	V	P	70.5
347	359	S	T	Y	T	T	T	S	H	A	A	S	P	T	79.5
450	462	P	S	Y	Y	M	L	R	K	R	R	K	R	L	102
43	55	E	Q	I	L	Q	Y	G	S	M	G	V	F	F	135
325	337	H	Y	Y	Y	D	L	S	T	I	D	P	A	E	135
281	293	P	D	F	L	D	I	V	A	L	H	R	P	A	140
265	277	T	L	Y	F	S	S	N	D	N	S	I	N	I	142
266	278	L	Y	F	S	S	N	D	N	S	I	N	I	A	160
428	440	I	P	I	V	P	G	S	P	Q	Y	T	I	I	190
292	304	P	A	L	T	S	R	R	T	G	I	R	Y	S	210
425	437	P	S	L	I	P	I	V	P	G	S	P	Q	Y	222
282	294	D	F	L	D	I	V	A	L	H	R	P	A	L	275
84	96	D	T	L	A	P	V	R	P	P	L	T	V	D	305
404	416	G	A	Y	N	I	P	L	V	S	G	P	D	I	432
255	267	P	A	Y	E	G	I	D	V	D	N	T	L	Y	462
51	63	M	G	V	F	F	G	G	L	G	I	G	T	G	550
310	322	Q	T	L	R	T	R	S	G	K	S	I	G	A	600
241	253	A	F	V	T	T	P	T	K	L	I	T	Y	D	650
408	420	I	P	L	V	S	G	P	D	I	P	I	N	I	725
233	245	Q	Q	V	K	V	V	D	P	A	F	V	T	T	955
181	193	F	T	L	S	S	S	T	I	S	T	H	N	Y	1100
400	412	I	P	F	G	G	A	Y	N	I	P	L	V	S	1100
49	61	G	S	M	G	V	F	F	G	G	L	G	I	G	1150
398	410	T	T	I	P	F	G	G	A	Y	N	I	P	L	1400
103	115	P	S	I	V	S	L	V	E	E	T	S	F	I	1700
235	247	V	K	V	V	D	P	A	F	V	T	T	P	T	1900
226	238	G	L	Y	S	R	T	T	Q	Q	V	K	V	V	2050
167	179	S	V	L	Q	P	P	T	P	A	E	T	G	G	2250
391	403	S	G	Y	I	P	A	N	T	T	I	P	F	G	2300
452	464	Y	Y	M	L	R	K	R	R	K	R	L	P	Y	2500
339	351	I	E	L	Q	T	I	T	P	S	T	Y	T	T	2620
385	397	V	P	S	T	S	L	S	G	Y	I	P	A	N	2800
67	79	G	G	R	T	G	Y	I	P	L	G	T	R	P	3100

91	103	P	P	L	T	V	D	P	V	G	P	S	D	P	3300
220	232	R	P	V	A	R	L	G	L	Y	S	R	T	T	3600
34	46	P	K	V	E	G	K	T	I	A	E	Q	I	L	3650
53	65	V	F	F	G	G	L	G	I	G	T	G	S	G	3720
248	260	K	L	I	T	Y	D	N	P	A	Y	E	G	I	4050
225	237	L	G	L	Y	S	R	T	T	Q	Q	V	K	V	4100
264	276	N	T	L	Y	F	S	S	N	D	N	S	I	N	4200
50	62	S	M	G	V	F	F	G	G	L	G	I	G	T	4550
31	43	D	I	I	P	K	V	E	G	K	T	I	A	E	4950
416	428	I	P	I	N	I	T	D	Q	A	P	S	L	I	5000
451	463	S	Y	Y	M	L	R	K	R	R	K	R	L	P	5600
326	338	Y	Y	Y	D	L	S	T	I	D	P	A	E	E	6300
39	51	K	T	I	A	E	Q	I	L	Q	Y	G	S	M	6700
87	99	A	P	V	R	P	P	L	T	V	D	P	V	G	6850
364	376	G	L	Y	D	I	Y	A	D	D	F	I	T	D	7300
127	139	P	P	D	V	S	G	F	S	I	T	T	S	T	7400
258	270	E	G	I	D	V	D	N	T	L	Y	F	S	S	7500
299	311	T	G	I	R	Y	S	R	I	G	N	K	Q	T	7650
435	447	P	Q	Y	T	I	I	A	D	A	G	D	F	Y	7750
322	334	A	K	V	H	Y	Y	Y	D	L	S	T	I	D	7800
30	42	P	D	I	I	P	K	V	E	G	K	T	I	A	8000
426	438	S	L	I	P	I	V	P	G	S	P	Q	Y	T	8900
63	75	G	S	G	T	G	G	R	T	G	Y	I	P	L	9450
315	327	R	S	G	K	S	I	G	A	K	V	H	Y	Y	10200
346	358	P	S	T	Y	T	T	T	S	H	A	A	S	P	10200
93	105	L	T	V	D	P	V	G	P	S	D	P	S	I	11000
250	262	I	T	Y	D	N	P	A	Y	E	G	I	D	V	11000
304	316	S	R	I	G	N	K	Q	T	L	R	T	R	S	11000
331	343	S	T	I	D	P	A	E	E	I	E	L	Q	T	13500
186	198	S	T	I	S	T	H	N	Y	E	E	I	P	M	14000
179	191	G	H	F	T	L	S	S	S	T	I	S	T	H	14200
222	234	V	A	R	L	G	L	Y	S	R	T	T	Q	Q	15500
438	450	T	I	I	A	D	A	G	D	F	Y	L	H	P	15500
349	361	Y	T	T	T	S	H	A	A	S	P	T	S	I	16000
44	56	Q	I	L	Q	Y	G	S	M	G	V	F	F	G	18000
147	159	D	I	N	N	T	V	T	T	V	T	T	H	N	18000
247	259	T	K	L	I	T	Y	D	N	P	A	Y	E	G	18200
284	296	L	D	I	V	A	L	H	R	P	A	L	T	S	19000
180	192	H	F	T	L	S	S	S	T	I	S	T	H	N	21000
374	386	I	T	D	T	S	T	T	P	V	P	S	V	P	21000
437	449	Y	T	I	I	A	D	A	G	D	F	Y	L	H	21200
153	165	T	T	V	T	T	H	N	N	P	T	F	T	D	22200
46	58	L	Q	Y	G	S	M	G	V	F	F	G	G	L	23000
372	384	D	F	I	T	D	T	S	T	T	P	V	P	S	24000
397	409	N	T	T	I	P	F	G	G	A	Y	N	I	P	25000
317	329	G	K	S	I	G	A	K	V	H	Y	Y	Y	D	25200
429	441	P	I	V	P	G	S	P	Q	Y	T	I	I	A	25500
61	73	G	T	G	S	G	T	G	G	R	T	G	Y	I	26200
56	68	G	G	L	G	I	G	T	G	S	G	T	G	G	28000
211	223	T	S	S	T	P	I	P	G	S	R	P	V	A	29500
228	240	Y	S	R	T	T	Q	Q	V	K	V	V	D	P	32000

453	465	Y	M	L	R	K	R	R	K	R	L	P	Y	F	33000
352	364	T	S	H	A	A	S	P	T	S	I	N	N	G	37200
283	295	F	L	D	I	V	A	L	H	R	P	A	L	T	38500
152	164	V	T	T	V	T	T	H	N	N	P	T	F	T	39000
178	190	G	G	H	F	T	L	S	S	S	T	I	S	T	39000
195	207	E	I	P	M	D	T	F	I	V	S	T	N	P	40000
143	155	P	A	I	L	D	I	N	N	T	V	T	T	V	42000
409	421	P	L	V	S	G	P	D	I	P	I	N	I	T	43000
57	69	G	L	G	I	G	T	G	S	G	T	G	G	R	44500
384	396	S	V	P	S	T	S	L	S	G	Y	I	P	A	45000
82	94	A	T	D	T	L	A	P	V	R	P	P	L	T	46000
3	15	H	K	R	S	A	K	R	T	K	R	A	S	A	46500
359	371	T	S	I	N	N	G	L	Y	D	I	Y	A	D	47200
345	357	T	P	S	T	Y	T	T	T	S	H	A	A	S	50500
214	226	T	P	I	P	G	S	R	P	V	A	R	L	G	51500
166	178	P	S	V	L	Q	P	P	T	P	A	E	T	G	53200
328	340	Y	D	L	S	T	I	D	P	A	E	E	I	E	58000
118	130	G	A	P	T	S	V	P	S	I	P	P	D	V	65200
201	213	F	I	V	S	T	N	P	N	T	V	T	S	S	65500
402	414	F	G	G	A	Y	N	I	P	L	V	S	G	P	67000
159	171	N	N	P	T	F	T	D	P	S	V	L	Q	P	69500
172	184	P	T	P	A	E	T	G	G	H	F	T	L	S	71000
37	49	E	G	K	T	I	A	E	Q	I	L	Q	Y	G	73200
136	148	T	T	S	T	D	T	T	P	A	I	L	D	I	74000
114	126	F	I	D	A	G	A	P	T	S	V	P	S	I	75000
113	125	S	F	I	D	A	G	A	P	T	S	V	P	S	76000
377	389	T	S	T	T	P	V	P	S	V	P	S	T	S	76200
286	298	I	V	A	L	H	R	P	A	L	T	S	R	R	78500
81	93	T	A	T	D	T	L	A	P	V	R	P	P	L	80000
78	90	R	P	P	T	A	T	D	T	L	A	P	V	R	86000
216	228	I	P	G	S	R	P	V	A	R	L	G	L	Y	88500
96	108	D	P	V	G	P	S	D	P	S	I	V	S	L	95000
300	312	G	I	R	Y	S	R	I	G	N	K	Q	T	L	96000
249	261	L	I	T	Y	D	N	P	A	Y	E	G	I	D	96200
107	119	S	L	V	E	E	T	S	F	I	D	A	G	A	102000
356	368	A	S	P	T	S	I	N	N	G	L	Y	D	I	110000
7	19	A	K	R	T	K	R	A	S	A	T	Q	L	Y	112000
294	306	L	T	S	R	R	T	G	I	R	Y	S	R	I	112000
106	118	V	S	L	V	E	E	T	S	F	I	D	A	G	115000
268	280	F	S	S	N	D	N	S	I	N	I	A	P	D	122000
382	394	V	P	S	V	P	S	T	S	L	S	G	Y	I	122000
419	431	N	I	T	D	Q	A	P	S	L	I	P	I	V	122000
16	28	T	Q	L	Y	K	T	C	K	Q	A	G	T	C	125000
244	256	T	T	P	T	K	L	I	T	Y	D	N	P	A	125000
18	30	L	Y	K	T	C	K	Q	A	G	T	C	P	P	130000
353	365	S	H	A	A	S	P	T	S	I	N	N	G	L	130000
109	121	V	E	E	T	S	F	I	D	A	G	A	P	T	140000
395	407	P	A	N	T	T	I	P	F	G	G	A	Y	N	140000
341	353	L	Q	T	I	T	P	S	T	Y	T	T	T	S	142000
42	54	A	E	Q	I	L	Q	Y	G	S	M	G	V	F	145000
202	214	I	V	S	T	N	P	N	T	V	T	S	S	T	145000

461	473	R	L	P	Y	F	F	S	D	V	S	L	A	A	145000
55	67	F	G	G	L	G	I	G	T	G	S	G	T	G	150000
15	27	A	T	Q	L	Y	K	T	C	K	Q	A	G	T	155000
40	52	T	I	A	E	Q	I	L	Q	Y	G	S	M	G	160000
177	189	T	G	G	H	F	T	L	S	S	S	T	I	S	162000
198	210	M	D	T	F	I	V	S	T	N	P	N	T	V	165000
336	348	A	E	E	I	E	L	Q	T	I	T	P	S	T	180000
48	60	Y	G	S	M	G	V	F	F	G	G	L	G	I	200000
371	383	D	D	F	I	T	D	T	S	T	T	P	V	P	202000
170	182	Q	P	P	T	P	A	E	T	G	G	H	F	T	205000
431	443	V	P	G	S	P	Q	Y	T	I	I	A	D	A	205000
99	111	G	P	S	D	P	S	I	V	S	L	V	E	E	210000
263	275	D	N	T	L	Y	F	S	S	N	D	N	S	I	210000
260	272	I	D	V	D	N	T	L	Y	F	S	S	N	D	215000
38	50	G	K	T	I	A	E	Q	I	L	Q	Y	G	S	220000
338	350	E	I	E	L	Q	T	I	T	P	S	T	Y	T	222000
90	102	R	P	P	L	T	V	D	P	V	G	P	S	D	235000
396	408	A	N	T	T	I	P	F	G	G	A	Y	N	I	235000
146	158	L	D	I	N	N	T	V	T	T	V	T	T	H	240000
227	239	L	Y	S	R	T	T	Q	Q	V	K	V	V	D	250000
358	370	P	T	S	I	N	N	G	L	Y	D	I	Y	A	260000
191	203	H	N	Y	E	E	I	P	M	D	T	F	I	V	270000
342	354	Q	T	I	T	P	S	T	Y	T	T	T	S	H	270000
141	153	T	T	P	A	I	L	D	I	N	N	T	V	T	272000
14	26	S	A	T	Q	L	Y	K	T	C	K	Q	A	G	280000
399	411	T	I	P	F	G	G	A	Y	N	I	P	L	V	282000
423	435	Q	A	P	S	L	I	P	I	V	P	G	S	P	290000
259	271	G	I	D	V	D	N	T	L	Y	F	S	S	N	292000
105	117	I	V	S	L	V	E	E	T	S	F	I	D	A	305000
102	114	D	P	S	I	V	S	L	V	E	E	T	S	F	310000
205	217	T	N	P	N	T	V	T	S	S	T	P	I	P	315000
131	143	S	G	F	S	I	T	T	S	T	D	T	T	P	320000
379	391	T	T	P	V	P	S	V	P	S	T	S	L	S	325000
59	71	G	I	G	T	G	S	G	T	G	G	R	T	G	330000
219	231	S	R	P	V	A	R	L	G	L	Y	S	R	T	330000
296	308	S	R	R	T	G	I	R	Y	S	R	I	G	N	330000
279	291	P	D	P	D	F	L	D	I	V	A	L	H	R	345000
420	432	I	T	D	Q	A	P	S	L	I	P	I	V	P	345000
348	360	T	Y	T	T	T	S	H	A	A	S	P	T	S	355000
110	122	E	E	T	S	F	I	D	A	G	A	P	T	S	370000
41	53	I	A	E	Q	I	L	Q	Y	G	S	M	G	V	375000
207	219	P	N	T	V	T	S	S	T	P	I	P	G	S	375000
72	84	Y	I	P	L	G	T	R	P	P	T	A	T	D	390000
414	426	P	D	I	P	I	N	I	T	D	Q	A	P	S	390000
376	388	D	T	S	T	T	P	V	P	S	V	P	S	T	392000
446	458	F	Y	L	H	P	S	Y	Y	M	L	R	K	R	392000
280	292	D	P	D	F	L	D	I	V	A	L	H	R	P	395000
160	172	N	P	T	F	T	D	P	S	V	L	Q	P	P	410000
185	197	S	S	T	I	S	T	H	N	Y	E	E	I	P	410000
324	336	V	H	Y	Y	Y	D	L	S	T	I	D	P	A	412000
363	375	N	G	L	Y	D	I	Y	A	D	D	F	I	T	432000

6	18	S	A	K	R	T	K	R	A	S	A	T	Q	L	455000
269	281	S	S	N	D	N	S	I	N	I	A	P	D	P	465000
132	144	G	F	S	I	T	T	S	T	D	T	T	P	A	475000
142	154	T	P	A	I	L	D	I	N	N	T	V	T	T	475000
236	248	K	V	V	D	P	A	F	V	T	T	P	T	K	480000
403	415	G	G	A	Y	N	I	P	L	V	S	G	P	D	480000
183	195	L	S	S	S	T	I	S	T	H	N	Y	E	E	485000
381	393	P	V	P	S	V	P	S	T	S	L	S	G	Y	492000
111	123	E	T	S	F	I	D	A	G	A	P	T	S	V	510000
45	57	I	L	Q	Y	G	S	M	G	V	F	F	G	G	542000
433	445	G	S	P	Q	Y	T	I	I	A	D	A	G	D	545000
246	258	P	T	K	L	I	T	Y	D	N	P	A	Y	E	562000
11	23	K	R	A	S	A	T	Q	L	Y	K	T	C	K	570000
128	140	P	D	V	S	G	F	S	I	T	T	S	T	D	585000
217	229	P	G	S	R	P	V	A	R	L	G	L	Y	S	635000
267	279	Y	F	S	S	N	D	N	S	I	N	I	A	P	642000
121	133	T	S	V	P	S	I	P	P	D	V	S	G	F	655000
98	110	V	G	P	S	D	P	S	I	V	S	L	V	E	670000
193	205	Y	E	E	I	P	M	D	T	F	I	V	S	T	710000
230	242	R	T	T	Q	Q	V	K	V	V	D	P	A	F	710000
354	366	H	A	A	S	P	T	S	I	N	N	G	L	Y	820000
370	382	A	D	D	F	I	T	D	T	S	T	T	P	V	842000
406	418	Y	N	I	P	L	V	S	G	P	D	I	P	I	855000
392	404	G	Y	I	P	A	N	T	T	I	P	F	G	G	870000
367	379	D	I	Y	A	D	D	F	I	T	D	T	S	T	910000
138	150	S	T	D	T	T	P	A	I	L	D	I	N	N	930000
307	319	G	N	K	Q	T	L	R	T	R	S	G	K	S	965000
190	202	T	H	N	Y	E	E	I	P	M	D	T	F	I	985000
1	13	M	R	H	K	R	S	A	K	R	T	K	R	A	1000000
2	14	R	H	K	R	S	A	K	R	T	K	R	A	S	1000000
4	16	K	R	S	A	K	R	T	K	R	A	S	A	T	1000000
5	17	R	S	A	K	R	T	K	R	A	S	A	T	Q	1000000
8	20	K	R	T	K	R	A	S	A	T	Q	L	Y	K	1000000
9	21	R	T	K	R	A	S	A	T	Q	L	Y	K	T	1000000
10	22	T	K	R	A	S	A	T	Q	L	Y	K	T	C	1000000
12	24	R	A	S	A	T	Q	L	Y	K	T	C	K	Q	1000000
13	25	A	S	A	T	Q	L	Y	K	T	C	K	Q	A	1000000
19	31	Y	K	T	C	K	Q	A	G	T	C	P	P	D	1000000
20	32	K	T	C	K	Q	A	G	T	C	P	P	D	I	1000000
21	33	T	C	K	Q	A	G	T	C	P	P	D	I	I	1000000
22	34	C	K	Q	A	G	T	C	P	P	D	I	I	P	1000000
23	35	K	Q	A	G	T	C	P	P	D	I	I	P	K	1000000
24	36	Q	A	G	T	C	P	P	D	I	I	P	K	V	1000000
25	37	A	G	T	C	P	P	D	I	I	P	K	V	E	1000000
26	38	G	T	C	P	P	D	I	I	P	K	V	E	G	1000000
27	39	T	C	P	P	D	I	I	P	K	V	E	G	K	1000000
28	40	C	P	P	D	I	I	P	K	V	E	G	K	T	1000000
29	41	P	P	D	I	I	P	K	V	E	G	K	T	I	1000000
32	44	I	I	P	K	V	E	G	K	T	I	A	E	Q	1000000
33	45	I	P	K	V	E	G	K	T	I	A	E	Q	I	1000000
35	47	K	V	E	G	K	T	I	A	E	Q	I	L	Q	1000000

36	48	V	E	G	K	T	I	A	E	Q	I	L	Q	Y	1000000
47	59	Q	Y	G	S	M	G	V	F	F	G	G	L	G	1000000
54	66	F	F	G	G	L	G	I	G	T	G	S	G	T	1000000
58	70	L	G	I	G	T	G	S	G	T	G	G	R	T	1000000
60	72	I	G	T	G	S	G	T	G	G	R	T	G	Y	1000000
62	74	T	G	S	G	T	G	G	R	T	G	Y	I	P	1000000
64	76	S	G	T	G	G	R	T	G	Y	I	P	L	G	1000000
65	77	G	T	G	G	R	T	G	Y	I	P	L	G	T	1000000
66	78	T	G	G	R	T	G	Y	I	P	L	G	T	R	1000000
68	80	G	R	T	G	Y	I	P	L	G	T	R	P	P	1000000
69	81	R	T	G	Y	I	P	L	G	T	R	P	P	T	1000000
71	83	G	Y	I	P	L	G	T	R	P	P	T	A	T	1000000
73	85	I	P	L	G	T	R	P	P	T	A	T	D	T	1000000
74	86	P	L	G	T	R	P	P	T	A	T	D	T	L	1000000
75	87	L	G	T	R	P	P	T	A	T	D	T	L	A	1000000
76	88	G	T	R	P	P	T	A	T	D	T	L	A	P	1000000
77	89	T	R	P	P	T	A	T	D	T	L	A	P	V	1000000
79	91	P	P	T	A	T	D	T	L	A	P	V	R	P	1000000
80	92	P	T	A	T	D	T	L	A	P	V	R	P	P	1000000
83	95	T	D	T	L	A	P	V	R	P	P	L	T	V	1000000
85	97	T	L	A	P	V	R	P	P	L	T	V	D	P	1000000
86	98	L	A	P	V	R	P	P	L	T	V	D	P	V	1000000
88	100	P	V	R	P	P	L	T	V	D	P	V	G	P	1000000
89	101	V	R	P	P	L	T	V	D	P	V	G	P	S	1000000
92	104	P	L	T	V	D	P	V	G	P	S	D	P	S	1000000
94	106	T	V	D	P	V	G	P	S	D	P	S	I	V	1000000
95	107	V	D	P	V	G	P	S	D	P	S	I	V	S	1000000
97	109	P	V	G	P	S	D	P	S	I	V	S	L	V	1000000
100	112	P	S	D	P	S	I	V	S	L	V	E	E	T	1000000
101	113	S	D	P	S	I	V	S	L	V	E	E	T	S	1000000
104	116	S	I	V	S	L	V	E	E	T	S	F	I	D	1000000
108	120	L	V	E	E	T	S	F	I	D	A	G	A	P	1000000
115	127	I	D	A	G	A	P	T	S	V	P	S	I	P	1000000
116	128	D	A	G	A	P	T	S	V	P	S	I	P	P	1000000
117	129	A	G	A	P	T	S	V	P	S	I	P	P	D	1000000
119	131	A	P	T	S	V	P	S	I	P	P	D	V	S	1000000
120	132	P	T	S	V	P	S	I	P	P	D	V	S	G	1000000
122	134	S	V	P	S	I	P	P	D	V	S	G	F	S	1000000
123	135	V	P	S	I	P	P	D	V	S	G	F	S	I	1000000
124	136	P	S	I	P	P	D	V	S	G	F	S	I	T	1000000
125	137	S	I	P	P	D	V	S	G	F	S	I	T	T	1000000
126	138	I	P	P	D	V	S	G	F	S	I	T	T	S	1000000
129	141	D	V	S	G	F	S	I	T	T	S	T	D	T	1000000
130	142	V	S	G	F	S	I	T	T	S	T	D	T	T	1000000
133	145	F	S	I	T	T	S	T	D	T	T	P	A	I	1000000
134	146	S	I	T	T	S	T	D	T	T	P	A	I	L	1000000
135	147	I	T	T	S	T	D	T	T	P	A	I	L	D	1000000
137	149	T	S	T	D	T	T	P	A	I	L	D	I	N	1000000
139	151	T	D	T	T	P	A	I	L	D	I	N	N	T	1000000
140	152	D	T	T	P	A	I	L	D	I	N	N	T	V	1000000
145	157	I	L	D	I	N	N	T	V	T	T	V	T	T	1000000

148	160	I	N	N	T	V	T	T	V	T	T	H	N	N	1000000
149	161	N	N	T	V	T	T	V	T	T	H	N	N	P	1000000
151	163	T	V	T	T	V	T	T	H	N	N	P	T	F	1000000
154	166	T	V	T	T	H	N	N	P	T	F	T	D	P	1000000
155	167	V	T	T	H	N	N	P	T	F	T	D	P	S	1000000
156	168	T	T	H	N	N	P	T	F	T	D	P	S	V	1000000
157	169	T	H	N	N	P	T	F	T	D	P	S	V	L	1000000
158	170	H	N	N	P	T	F	T	D	P	S	V	L	Q	1000000
162	174	T	F	T	D	P	S	V	L	Q	P	P	T	P	1000000
163	175	F	T	D	P	S	V	L	Q	P	P	T	P	A	1000000
164	176	T	D	P	S	V	L	Q	P	P	T	P	A	E	1000000
165	177	D	P	S	V	L	Q	P	P	T	P	A	E	T	1000000
168	180	V	L	Q	P	P	T	P	A	E	T	G	G	H	1000000
169	181	L	Q	P	P	T	P	A	E	T	G	G	H	F	1000000
171	183	P	P	T	P	A	E	T	G	G	H	F	T	L	1000000
173	185	T	P	A	E	T	G	G	H	F	T	L	S	S	1000000
174	186	P	A	E	T	G	G	H	F	T	L	S	S	S	1000000
175	187	A	E	T	G	G	H	F	T	L	S	S	S	T	1000000
176	188	E	T	G	G	H	F	T	L	S	S	S	T	I	1000000
182	194	T	L	S	S	S	T	I	S	T	H	N	Y	E	1000000
184	196	S	S	S	T	I	S	T	H	N	Y	E	E	I	1000000
187	199	T	I	S	T	H	N	Y	E	E	I	P	M	D	1000000
188	200	I	S	T	H	N	Y	E	E	I	P	M	D	T	1000000
189	201	S	T	H	N	Y	E	E	I	P	M	D	T	F	1000000
192	204	N	Y	E	E	I	P	M	D	T	F	I	V	S	1000000
194	206	E	E	I	P	M	D	T	F	I	V	S	T	N	1000000
197	209	P	M	D	T	F	I	V	S	T	N	P	N	T	1000000
203	215	V	S	T	N	P	N	T	V	T	S	S	T	P	1000000
204	216	S	T	N	P	N	T	V	T	S	S	T	P	I	1000000
206	218	N	P	N	T	V	T	S	S	T	P	I	P	G	1000000
209	221	T	V	T	S	S	T	P	I	P	G	S	R	P	1000000
210	222	V	T	S	S	T	P	I	P	G	S	R	P	V	1000000
212	224	S	S	T	P	I	P	G	S	R	P	V	A	R	1000000
213	225	S	T	P	I	P	G	S	R	P	V	A	R	L	1000000
215	227	P	I	P	G	S	R	P	V	A	R	L	G	L	1000000
218	230	G	S	R	P	V	A	R	L	G	L	Y	S	R	1000000
221	233	P	V	A	R	L	G	L	Y	S	R	T	T	Q	1000000
223	235	A	R	L	G	L	Y	S	R	T	T	Q	Q	V	1000000
224	236	R	L	G	L	Y	S	R	T	T	Q	Q	V	K	1000000
229	241	S	R	T	T	Q	Q	V	K	V	V	D	P	A	1000000
231	243	T	T	Q	Q	V	K	V	V	D	P	A	F	V	1000000
232	244	T	Q	Q	V	K	V	V	D	P	A	F	V	T	1000000
234	246	Q	V	K	V	V	D	P	A	F	V	T	T	P	1000000
237	249	V	V	D	P	A	F	V	T	T	P	T	K	L	1000000
238	250	V	D	P	A	F	V	T	T	P	T	K	L	I	1000000
239	251	D	P	A	F	V	T	T	P	T	K	L	I	T	1000000
242	254	F	V	T	T	P	T	K	L	I	T	Y	D	N	1000000
243	255	V	T	T	P	T	K	L	I	T	Y	D	N	P	1000000
245	257	T	P	T	K	L	I	T	Y	D	N	P	A	Y	1000000
251	263	T	Y	D	N	P	A	Y	E	G	I	D	V	D	1000000
252	264	Y	D	N	P	A	Y	E	G	I	D	V	D	N	1000000

253	265	D	N	P	A	Y	E	G	I	D	V	D	N	T	1000000
254	266	N	P	A	Y	E	G	I	D	V	D	N	T	L	1000000
256	268	A	Y	E	G	I	D	V	D	N	T	L	Y	F	1000000
257	269	Y	E	G	I	D	V	D	N	T	L	Y	F	S	1000000
261	273	D	V	D	N	T	L	Y	F	S	S	N	D	N	1000000
262	274	V	D	N	T	L	Y	F	S	S	N	D	N	S	1000000
270	282	S	N	D	N	S	I	N	I	A	P	D	P	D	1000000
271	283	N	D	N	S	I	N	I	A	P	D	P	D	F	1000000
272	284	D	N	S	I	N	I	A	P	D	P	D	F	L	1000000
273	285	N	S	I	N	I	A	P	D	P	D	F	L	D	1000000
274	286	S	I	N	I	A	P	D	P	D	F	L	D	I	1000000
275	287	I	N	I	A	P	D	P	D	F	L	D	I	V	1000000
276	288	N	I	A	P	D	P	D	F	L	D	I	V	A	1000000
277	289	I	A	P	D	P	D	F	L	D	I	V	A	L	1000000
278	290	A	P	D	P	D	F	L	D	I	V	A	L	H	1000000
287	299	V	A	L	H	R	P	A	L	T	S	R	R	T	1000000
288	300	A	L	H	R	P	A	L	T	S	R	R	T	G	1000000
289	301	L	H	R	P	A	L	T	S	R	R	T	G	I	1000000
290	302	H	R	P	A	L	T	S	R	R	T	G	I	R	1000000
291	303	R	P	A	L	T	S	R	R	T	G	I	R	Y	1000000
293	305	A	L	T	S	R	R	T	G	I	R	Y	S	R	1000000
295	307	T	S	R	R	T	G	I	R	Y	S	R	I	G	1000000
297	309	R	R	T	G	I	R	Y	S	R	I	G	N	K	1000000
298	310	R	T	G	I	R	Y	S	R	I	G	N	K	Q	1000000
302	314	R	Y	S	R	I	G	N	K	Q	T	L	R	T	1000000
303	315	Y	S	R	I	G	N	K	Q	T	L	R	T	R	1000000
305	317	R	I	G	N	K	Q	T	L	R	T	R	S	G	1000000
306	318	I	G	N	K	Q	T	L	R	T	R	S	G	K	1000000
308	320	N	K	Q	T	L	R	T	R	S	G	K	S	I	1000000
309	321	K	Q	T	L	R	T	R	S	G	K	S	I	G	1000000
311	323	T	L	R	T	R	S	G	K	S	I	G	A	K	1000000
312	324	L	R	T	R	S	G	K	S	I	G	A	K	V	1000000
313	325	R	T	R	S	G	K	S	I	G	A	K	V	H	1000000
314	326	T	R	S	G	K	S	I	G	A	K	V	H	Y	1000000
316	328	S	G	K	S	I	G	A	K	V	H	Y	Y	Y	1000000
318	330	K	S	I	G	A	K	V	H	Y	Y	Y	D	L	1000000
319	331	S	I	G	A	K	V	H	Y	Y	Y	D	L	S	1000000
320	332	I	G	A	K	V	H	Y	Y	Y	D	L	S	T	1000000
321	333	G	A	K	V	H	Y	Y	Y	D	L	S	T	I	1000000
323	335	K	V	H	Y	Y	Y	D	L	S	T	I	D	P	1000000
327	339	Y	Y	D	L	S	T	I	D	P	A	E	E	I	1000000
329	341	D	L	S	T	I	D	P	A	E	E	I	E	L	1000000
330	342	L	S	T	I	D	P	A	E	E	I	E	L	Q	1000000
332	344	T	I	D	P	A	E	E	I	E	L	Q	T	I	1000000
333	345	I	D	P	A	E	E	I	E	L	Q	T	I	T	1000000
334	346	D	P	A	E	E	I	E	L	Q	T	I	T	P	1000000
335	347	P	A	E	E	I	E	L	Q	T	I	T	P	S	1000000
337	349	E	E	I	E	L	Q	T	I	T	P	S	T	Y	1000000
340	352	E	L	Q	T	I	T	P	S	T	Y	T	T	T	1000000
343	355	T	I	T	P	S	T	Y	T	T	T	S	H	A	1000000
344	356	I	T	P	S	T	Y	T	T	T	S	H	A	A	1000000

350	362	T	T	T	S	H	A	A	S	P	T	S	I	N	1000000
351	363	T	T	S	H	A	A	S	P	T	S	I	N	N	1000000
355	367	A	A	S	P	T	S	I	N	N	G	L	Y	D	1000000
357	369	S	P	T	S	I	N	N	G	L	Y	D	I	Y	1000000
360	372	S	I	N	N	G	L	Y	D	I	Y	A	D	D	1000000
361	373	I	N	N	G	L	Y	D	I	Y	A	D	D	F	1000000
362	374	N	N	G	L	Y	D	I	Y	A	D	D	F	I	1000000
365	377	L	Y	D	I	Y	A	D	D	F	I	T	D	T	1000000
366	378	Y	D	I	Y	A	D	D	F	I	T	D	T	S	1000000
368	380	I	Y	A	D	D	F	I	T	D	T	S	T	T	1000000
369	381	Y	A	D	D	F	I	T	D	T	S	T	T	P	1000000
373	385	F	I	T	D	T	S	T	T	P	V	P	S	V	1000000
375	387	T	D	T	S	T	T	P	V	P	S	V	P	S	1000000
378	390	S	T	T	P	V	P	S	V	P	S	T	S	L	1000000
380	392	T	P	V	P	S	V	P	S	T	S	L	S	G	1000000
383	395	P	S	V	P	S	T	S	L	S	G	Y	I	P	1000000
386	398	P	S	T	S	L	S	G	Y	I	P	A	N	T	1000000
387	399	S	T	S	L	S	G	Y	I	P	A	N	T	T	1000000
389	401	S	L	S	G	Y	I	P	A	N	T	T	I	P	1000000
390	402	L	S	G	Y	I	P	A	N	T	T	I	P	F	1000000
393	405	Y	I	P	A	N	T	T	I	P	F	G	G	A	1000000
394	406	I	P	A	N	T	T	I	P	F	G	G	A	Y	1000000
401	413	P	F	G	G	A	Y	N	I	P	L	V	S	G	1000000
405	417	A	Y	N	I	P	L	V	S	G	P	D	I	P	1000000
407	419	N	I	P	L	V	S	G	P	D	I	P	I	N	1000000
410	422	L	V	S	G	P	D	I	P	I	N	I	T	D	1000000
411	423	V	S	G	P	D	I	P	I	N	I	T	D	Q	1000000
412	424	S	G	P	D	I	P	I	N	I	T	D	Q	A	1000000
413	425	G	P	D	I	P	I	N	I	T	D	Q	A	P	1000000
415	427	D	I	P	I	N	I	T	D	Q	A	P	S	L	1000000
417	429	P	I	N	I	T	D	Q	A	P	S	L	I	P	1000000
421	433	T	D	Q	A	P	S	L	I	P	I	V	P	G	1000000
422	434	D	Q	A	P	S	L	I	P	I	V	P	G	S	1000000
424	436	A	P	S	L	I	P	I	V	P	G	S	P	Q	1000000
427	439	L	I	P	I	V	P	G	S	P	Q	Y	T	I	1000000
430	442	I	V	P	G	S	P	Q	Y	T	I	I	A	D	1000000
432	444	P	G	S	P	Q	Y	T	I	I	A	D	A	G	1000000
434	446	S	P	Q	Y	T	I	I	A	D	A	G	D	F	1000000
436	448	Q	Y	T	I	I	A	D	A	G	D	F	Y	L	1000000
439	451	I	I	A	D	A	G	D	F	Y	L	H	P	S	1000000
440	452	I	A	D	A	G	D	F	Y	L	H	P	S	Y	1000000
441	453	A	D	A	G	D	F	Y	L	H	P	S	Y	Y	1000000
442	454	D	A	G	D	F	Y	L	H	P	S	Y	Y	M	1000000
443	455	A	G	D	F	Y	L	H	P	S	Y	Y	M	L	1000000
447	459	Y	L	H	P	S	Y	Y	M	L	R	K	R	R	1000000
448	460	L	H	P	S	Y	Y	M	L	R	K	R	R	K	1000000
449	461	H	P	S	Y	Y	M	L	R	K	R	R	K	R	1000000
454	466	M	L	R	K	R	R	K	R	L	P	Y	F	F	1000000
455	467	L	R	K	R	R	K	R	L	P	Y	F	F	S	1000000
456	468	R	K	R	R	K	R	L	P	Y	F	F	S	D	1000000
457	469	K	R	R	K	R	L	P	Y	F	F	S	D	V	1000000

458	470	R	R	K	R	L	P	Y	F	F	S	D	V	S	1000000
459	471	R	K	R	L	P	Y	F	F	S	D	V	S	L	1000000
460	472	K	R	L	P	Y	F	F	S	D	V	S	L	A	1000000

															IC50 DRB1*0401 nM)
46	58	L	Q	Y	G	S	M	G	V	F	F	G	G	L	9.8
266	278	L	Y	F	S	S	N	D	N	S	I	N	I	A	11
391	403	S	G	Y	I	P	A	N	T	T	I	P	F	G	12
444	456	G	D	F	Y	L	H	P	S	Y	Y	M	L	R	20.2
179	191	G	H	F	T	L	S	S	S	T	I	S	T	H	23.2
199	211	D	T	F	I	V	S	T	N	P	N	T	V	T	26
325	337	H	Y	Y	Y	D	L	S	T	I	D	P	A	E	44.5
208	220	N	T	V	T	S	S	T	P	I	P	G	S	R	62
131	143	S	G	F	S	I	T	T	S	T	D	T	T	P	66.5
347	359	S	T	Y	T	T	T	S	H	A	A	S	P	T	86
70	82	T	G	Y	I	P	L	G	T	R	P	P	T	A	91
17	29	Q	L	Y	K	T	C	K	Q	A	G	T	C	P	112
112	124	T	S	F	I	D	A	G	A	P	T	S	V	P	120
143	155	P	A	I	L	D	I	N	N	T	V	T	T	V	122
43	55	E	Q	I	L	Q	Y	G	S	M	G	V	F	F	150
326	338	Y	Y	Y	D	L	S	T	I	D	P	A	E	E	150
51	63	M	G	V	F	F	G	G	L	G	I	G	T	G	170
150	162	N	T	V	T	T	V	T	T	H	N	N	P	T	175
299	311	T	G	I	R	Y	S	R	I	G	N	K	Q	T	192
281	293	P	D	F	L	D	I	V	A	L	H	R	P	A	250
53	65	V	F	F	G	G	L	G	I	G	T	G	S	G	285
264	276	N	T	L	Y	F	S	S	N	D	N	S	I	N	305
337	349	E	E	I	E	L	Q	T	I	T	P	S	T	Y	335
52	64	G	V	F	F	G	G	L	G	I	G	T	G	S	340
200	212	T	F	I	V	S	T	N	P	N	T	V	T	S	385
372	384	D	F	I	T	D	T	S	T	T	P	V	P	S	420
388	400	T	S	L	S	G	Y	I	P	A	N	T	T	I	445
367	379	D	I	Y	A	D	D	F	I	T	D	T	S	T	450
437	449	Y	T	I	I	A	D	A	G	D	F	Y	L	H	530
181	193	F	T	L	S	S	S	T	I	S	T	H	N	Y	580
233	245	Q	Q	V	K	V	V	D	P	A	F	V	T	T	585
103	115	P	S	I	V	S	L	V	E	E	T	S	F	I	602
128	140	P	D	V	S	G	F	S	I	T	T	S	T	D	655
201	213	F	I	V	S	T	N	P	N	T	V	T	S	S	912
49	61	G	S	M	G	V	F	F	G	G	L	G	I	G	940
39	51	K	T	I	A	E	Q	I	L	Q	Y	G	S	M	1100
371	383	D	D	F	I	T	D	T	S	T	T	P	V	P	1100
301	313	I	R	Y	S	R	I	G	N	K	Q	T	L	R	1200
247	259	T	K	L	I	T	Y	D	N	P	A	Y	E	G	1300
435	447	P	Q	Y	T	I	I	A	D	A	G	D	F	Y	1400
324	336	V	H	Y	Y	Y	D	L	S	T	I	D	P	A	1500
346	358	P	S	T	Y	T	T	T	S	H	A	A	S	P	1650
400	412	I	P	F	G	G	A	Y	N	I	P	L	V	S	1700
285	297	D	I	V	A	L	H	R	P	A	L	T	S	R	1800
265	277	T	L	Y	F	S	S	N	D	N	S	I	N	I	1900
416	428	I	P	I	N	I	T	D	Q	A	P	S	L	I	1900
402	414	F	G	G	A	Y	N	I	P	L	V	S	G	P	1950
248	260	K	L	I	T	Y	D	N	P	A	Y	E	G	I	2100
146	158	L	D	I	N	N	T	V	T	T	V	T	T	H	2220
445	457	D	F	Y	L	H	P	S	Y	Y	M	L	R	K	2300

255	267	P	A	Y	E	G	I	D	V	D	N	T	L	Y	2320
312	324	L	R	T	R	S	G	K	S	I	G	A	K	V	2850
425	437	P	S	L	I	P	I	V	P	G	S	P	Q	Y	3000
58	70	L	G	I	G	T	G	S	G	T	G	G	R	T	3350
284	296	L	D	I	V	A	L	H	R	P	A	L	T	S	3400
152	164	V	T	T	V	T	T	H	N	N	P	T	F	T	3620
14	26	S	A	T	Q	L	Y	K	T	C	K	Q	A	G	3700
66	78	T	G	G	R	T	G	Y	I	P	L	G	T	R	3700
397	409	N	T	T	I	P	F	G	G	A	Y	N	I	P	4000
418	430	I	N	I	T	D	Q	A	P	S	L	I	P	I	4200
203	215	V	S	T	N	P	N	T	V	T	S	S	T	P	4800
240	252	P	A	F	V	T	T	P	T	K	L	I	T	Y	4900
180	192	H	F	T	L	S	S	S	T	I	S	T	H	N	5000
161	173	P	T	F	T	D	P	S	V	L	Q	P	P	T	5220
292	304	P	A	L	T	S	R	R	T	G	I	R	Y	S	5220
106	118	V	S	L	V	E	E	T	S	F	I	D	A	G	5400
386	398	P	S	T	S	L	S	G	Y	I	P	A	N	T	5500
446	458	F	Y	L	H	P	S	Y	Y	M	L	R	K	R	5700
249	261	L	I	T	Y	D	N	P	A	Y	E	G	I	D	5850
263	275	D	N	T	L	Y	F	S	S	N	D	N	S	I	5920
153	165	T	T	V	T	T	H	N	N	P	T	F	T	D	6000
84	96	D	T	L	A	P	V	R	P	P	L	T	V	D	6200
38	50	G	K	T	I	A	E	Q	I	L	Q	Y	G	S	6400
207	219	P	N	T	V	T	S	S	T	P	I	P	G	S	6450
258	270	E	G	I	D	V	D	N	T	L	Y	F	S	S	6620
282	294	D	F	L	D	I	V	A	L	H	R	P	A	L	6800
225	237	L	G	L	Y	S	R	T	T	Q	Q	V	K	V	7000
438	450	T	I	I	A	D	A	G	D	F	Y	L	H	P	7000
259	271	G	I	D	V	D	N	T	L	Y	F	S	S	N	7150
342	354	Q	T	I	T	P	S	T	Y	T	T	T	S	H	7420
134	146	S	I	T	T	S	T	D	T	T	P	A	I	L	8000
55	67	F	G	G	L	G	I	G	T	G	S	G	T	G	8920
209	221	T	V	T	S	S	T	P	I	P	G	S	R	P	9250
135	147	I	T	T	S	T	D	T	T	P	A	I	L	D	9300
349	361	Y	T	T	T	S	H	A	A	S	P	T	S	I	9850
339	351	I	E	L	Q	T	I	T	P	S	T	Y	T	T	9950
56	68	G	G	L	G	I	G	T	G	S	G	T	G	G	11000
133	145	F	S	I	T	T	S	T	D	T	T	P	A	I	13000
268	280	F	S	S	N	D	N	S	I	N	I	A	P	D	14000
322	334	A	K	V	H	Y	Y	Y	D	L	S	T	I	D	15200
363	375	N	G	L	Y	D	I	Y	A	D	D	F	I	T	15200
8	20	K	R	T	K	R	A	S	A	T	Q	L	Y	K	17200
144	156	A	I	L	D	I	N	N	T	V	T	T	V	T	17500
154	166	T	V	T	T	H	N	N	P	T	F	T	D	P	17500
186	198	S	T	I	S	T	H	N	Y	E	E	I	P	M	17500
226	238	G	L	Y	S	R	T	T	Q	Q	V	K	V	V	17500
364	376	G	L	Y	D	I	Y	A	D	D	F	I	T	D	18000
198	210	M	D	T	F	I	V	S	T	N	P	N	T	V	18500
185	197	S	S	T	I	S	T	H	N	Y	E	E	I	P	21500
91	103	P	P	L	T	V	D	P	V	G	P	S	D	P	22000
404	416	G	A	Y	N	I	P	L	V	S	G	P	D	I	23200

377	389	T	S	T	T	P	V	P	S	V	P	S	T	S	23500
414	426	P	D	I	P	I	N	I	T	D	Q	A	P	S	24000
350	362	T	T	T	S	H	A	A	S	P	T	S	I	N	25500
149	161	N	N	T	V	T	T	V	T	T	H	N	N	P	26500
42	54	A	E	Q	I	L	Q	Y	G	S	M	G	V	F	27000
450	462	P	S	Y	Y	M	L	R	K	R	R	K	R	L	28200
387	399	S	T	S	L	S	G	Y	I	P	A	N	T	T	29000
331	343	S	T	I	D	P	A	E	E	I	E	L	Q	T	29500
110	122	E	E	T	S	F	I	D	A	G	A	P	T	S	30000
309	321	K	Q	T	L	R	T	R	S	G	K	S	I	G	30200
50	62	S	M	G	V	F	F	G	G	L	G	I	G	T	31000
151	163	T	V	T	T	V	T	T	H	N	N	P	T	F	39000
246	258	P	T	K	L	I	T	Y	D	N	P	A	Y	E	41000
317	329	G	K	S	I	G	A	K	V	H	Y	Y	Y	D	41000
175	187	A	E	T	G	G	H	F	T	L	S	S	S	T	41500
44	56	Q	I	L	Q	Y	G	S	M	G	V	F	F	G	43000
236	248	K	V	V	D	P	A	F	V	T	T	P	T	K	43000
224	236	R	L	G	L	Y	S	R	T	T	Q	Q	V	K	46000
220	232	R	P	V	A	R	L	G	L	Y	S	R	T	T	47000
16	28	T	Q	L	Y	K	T	C	K	Q	A	G	T	C	48200
348	360	T	Y	T	T	T	S	H	A	A	S	P	T	S	51500
273	285	N	S	I	N	I	A	P	D	P	D	F	L	D	53500
358	370	P	T	S	I	N	N	G	L	Y	D	I	Y	A	59000
113	125	S	F	I	D	A	G	A	P	T	S	V	P	S	61200
359	371	T	S	I	N	N	G	L	Y	D	I	Y	A	D	61500
431	443	V	P	G	S	P	Q	Y	T	I	I	A	D	A	65000
460	472	K	R	L	P	Y	F	F	S	D	V	S	L	A	65000
93	105	L	T	V	D	P	V	G	P	S	D	P	S	I	66000
182	194	T	L	S	S	S	T	I	S	T	H	N	Y	E	72500
423	435	Q	A	P	S	L	I	P	I	V	P	G	S	P	73000
196	208	I	P	M	D	T	F	I	V	S	T	N	P	N	74500
409	421	P	L	V	S	G	P	D	I	P	I	N	I	T	77000
433	445	G	S	P	Q	Y	T	I	I	A	D	A	G	D	78000
315	327	R	S	G	K	S	I	G	A	K	V	H	Y	Y	79000
57	69	G	L	G	I	G	T	G	S	G	T	G	G	R	87000
374	386	I	T	D	T	S	T	T	P	V	P	S	V	P	87200
428	440	I	P	I	V	P	G	S	P	Q	Y	T	I	I	88000
223	235	A	R	L	G	L	Y	S	R	T	T	Q	Q	V	88500
318	330	K	S	I	G	A	K	V	H	Y	Y	Y	D	L	94000
443	455	A	G	D	F	Y	L	H	P	S	Y	Y	M	L	97500
328	340	Y	D	L	S	T	I	D	P	A	E	E	I	E	102000
392	404	G	Y	I	P	A	N	T	T	I	P	F	G	G	102000
320	332	I	G	A	K	V	H	Y	Y	Y	D	L	S	T	110000
441	453	A	D	A	G	D	F	Y	L	H	P	S	Y	Y	110000
373	385	F	I	T	D	T	S	T	T	P	V	P	S	V	120000
109	121	V	E	E	T	S	F	I	D	A	G	A	P	T	125000
257	269	Y	E	G	I	D	V	D	N	T	L	Y	F	S	130000
354	366	H	A	A	S	P	T	S	I	N	N	G	L	Y	130000
60	72	I	G	T	G	S	G	T	G	G	R	T	G	Y	140000
105	117	I	V	S	L	V	E	E	T	S	F	I	D	A	150000
360	372	S	I	N	N	G	L	Y	D	I	Y	A	D	D	150000

436	448	Q	Y	T	I	I	A	D	A	G	D	F	Y	L	150000
64	76	S	G	T	G	G	R	T	G	Y	I	P	L	G	160000
244	256	T	T	P	T	K	L	I	T	Y	D	N	P	A	160000
271	283	N	D	N	S	I	N	I	A	P	D	P	D	F	162000
167	179	S	V	L	Q	P	P	T	P	A	E	T	G	G	165000
54	66	F	F	G	G	L	G	I	G	T	G	S	G	T	175000
19	31	Y	K	T	C	K	Q	A	G	T	C	P	P	D	180000
176	188	E	T	G	G	H	F	T	L	S	S	S	T	I	180000
72	84	Y	I	P	L	G	T	R	P	P	T	A	T	D	190000
241	253	A	F	V	T	T	P	T	K	L	I	T	Y	D	190000
96	108	D	P	V	G	P	S	D	P	S	I	V	S	L	195000
393	405	Y	I	P	A	N	T	T	I	P	F	G	G	A	200000
230	242	R	T	T	Q	Q	V	K	V	V	D	P	A	F	205000
111	123	E	T	S	F	I	D	A	G	A	P	T	S	V	210000
124	136	P	S	I	P	P	D	V	S	G	F	S	I	T	210000
104	116	S	I	V	S	L	V	E	E	T	S	F	I	D	220000
357	369	S	P	T	S	I	N	N	G	L	Y	D	I	Y	220000
37	49	E	G	K	T	I	A	E	Q	I	L	Q	Y	G	222000
408	420	I	P	L	V	S	G	P	D	I	P	I	N	I	235000
130	142	V	S	G	F	S	I	T	T	S	T	D	T	T	240000
303	315	Y	S	R	I	G	N	K	Q	T	L	R	T	R	242000
398	410	T	T	I	P	F	G	G	A	Y	N	I	P	L	245000
401	413	P	F	G	G	A	Y	N	I	P	L	V	S	G	245000
193	205	Y	E	E	I	P	M	D	T	F	I	V	S	T	260000
245	257	T	P	T	K	L	I	T	Y	D	N	P	A	Y	265000
63	75	G	S	G	T	G	G	R	T	G	Y	I	P	L	270000
197	209	P	M	D	T	F	I	V	S	T	N	P	N	T	270000
194	206	E	E	I	P	M	D	T	F	I	V	S	T	N	280000
344	356	I	T	P	S	T	Y	T	T	T	S	H	A	A	292000
61	73	G	T	G	S	G	T	G	G	R	T	G	Y	I	295000
321	333	G	A	K	V	H	Y	Y	Y	D	L	S	T	I	312000
379	391	T	T	P	V	P	S	V	P	S	T	S	L	S	320000
304	316	S	R	I	G	N	K	Q	T	L	R	T	R	S	325000
385	397	V	P	S	T	S	L	S	G	Y	I	P	A	N	330000
59	71	G	I	G	T	G	S	G	T	G	G	R	T	G	340000
345	357	T	P	S	T	Y	T	T	T	S	H	A	A	S	350000
67	79	G	G	R	T	G	Y	I	P	L	G	T	R	P	360000
382	394	V	P	S	V	P	S	T	S	L	S	G	Y	I	365000
188	200	I	S	T	H	N	Y	E	E	I	P	M	D	T	372000
79	91	P	P	T	A	T	D	T	L	A	P	V	R	P	375000
11	23	K	R	A	S	A	T	Q	L	Y	K	T	C	K	382000
120	132	P	T	S	V	P	S	I	P	P	D	V	S	G	385000
107	119	S	L	V	E	E	T	S	F	I	D	A	G	A	400000
366	378	Y	D	I	Y	A	D	D	F	I	T	D	T	S	415000
189	201	S	T	H	N	Y	E	E	I	P	M	D	T	F	420000
145	157	I	L	D	I	N	N	T	V	T	T	V	T	T	440000
20	32	K	T	C	K	Q	A	G	T	C	P	P	D	I	442000
183	195	L	S	S	S	T	I	S	T	H	N	Y	E	E	450000
451	463	S	Y	Y	M	L	R	K	R	R	K	R	L	P	462000
10	22	T	K	R	A	S	A	T	Q	L	Y	K	T	C	470000
34	46	P	K	V	E	G	K	T	I	A	E	Q	I	L	490000

136	148	T	T	S	T	D	T	T	P	A	I	L	D	I	490000
139	151	T	D	T	T	P	A	I	L	D	I	N	N	T	510000
118	130	G	A	P	T	S	V	P	S	I	P	P	D	V	545000
160	172	N	P	T	F	T	D	P	S	V	L	Q	P	P	545000
159	171	N	N	P	T	F	T	D	P	S	V	L	Q	P	575000
234	246	Q	V	K	V	V	D	P	A	F	V	T	T	P	585000
81	93	T	A	T	D	T	L	A	P	V	R	P	P	L	590000
177	189	T	G	G	H	F	T	L	S	S	S	T	I	S	590000
172	184	P	T	P	A	E	T	G	G	H	F	T	L	S	630000
426	438	S	L	I	P	I	V	P	G	S	P	Q	Y	T	630000
47	59	Q	Y	G	S	M	G	V	F	F	G	G	L	G	635000
362	374	N	N	G	L	Y	D	I	Y	A	D	D	F	I	640000
121	133	T	S	V	P	S	I	P	P	D	V	S	G	F	650000
341	353	L	Q	T	I	T	P	S	T	Y	T	T	T	S	665000
141	153	T	T	P	A	I	L	D	I	N	N	T	V	T	685000
310	322	Q	T	L	R	T	R	S	G	K	S	I	G	A	710000
406	418	Y	N	I	P	L	V	S	G	P	D	I	P	I	730000
338	350	E	I	E	L	Q	T	I	T	P	S	T	Y	T	732000
280	292	D	P	D	F	L	D	I	V	A	L	H	R	P	745000
294	306	L	T	S	R	R	T	G	I	R	Y	S	R	I	752000
166	178	P	S	V	L	Q	P	P	T	P	A	E	T	G	770000
242	254	F	V	T	T	P	T	K	L	I	T	Y	D	N	780000
384	396	S	V	P	S	T	S	L	S	G	Y	I	P	A	795000
356	368	A	S	P	T	S	I	N	N	G	L	Y	D	I	802000
155	167	V	T	T	H	N	N	P	T	F	T	D	P	S	810000
222	234	V	A	R	L	G	L	Y	S	R	T	T	Q	Q	860000
75	87	L	G	T	R	P	P	T	A	T	D	T	L	A	880000
440	452	I	A	D	A	G	D	F	Y	L	H	P	S	Y	910000
375	387	T	D	T	S	T	T	P	V	P	S	V	P	S	922000
9	21	R	T	K	R	A	S	A	T	Q	L	Y	K	T	945000
417	429	P	I	N	I	T	D	Q	A	P	S	L	I	P	952000
190	202	T	H	N	Y	E	E	I	P	M	D	T	F	I	982000
1	13	M	R	H	K	R	S	A	K	R	T	K	R	A	1000000
2	14	R	H	K	R	S	A	K	R	T	K	R	A	S	1000000
3	15	H	K	R	S	A	K	R	T	K	R	A	S	A	1000000
4	16	K	R	S	A	K	R	T	K	R	A	S	A	T	1000000
5	17	R	S	A	K	R	T	K	R	A	S	A	T	Q	1000000
6	18	S	A	K	R	T	K	R	A	S	A	T	Q	L	1000000
7	19	A	K	R	T	K	R	A	S	A	T	Q	L	Y	1000000
12	24	R	A	S	A	T	Q	L	Y	K	T	C	K	Q	1000000
13	25	A	S	A	T	Q	L	Y	K	T	C	K	Q	A	1000000
15	27	A	T	Q	L	Y	K	T	C	K	Q	A	G	T	1000000
18	30	L	Y	K	T	C	K	Q	A	G	T	C	P	P	1000000
21	33	T	C	K	Q	A	G	T	C	P	P	D	I	I	1000000
22	34	C	K	Q	A	G	T	C	P	P	D	I	I	P	1000000
23	35	K	Q	A	G	T	C	P	P	D	I	I	P	K	1000000
24	36	Q	A	G	T	C	P	P	D	I	I	P	K	V	1000000
25	37	A	G	T	C	P	P	D	I	I	P	K	V	E	1000000
26	38	G	T	C	P	P	D	I	I	P	K	V	E	G	1000000
27	39	T	C	P	P	D	I	I	P	K	V	E	G	K	1000000
28	40	C	P	P	D	I	I	P	K	V	E	G	K	T	1000000

29	41	P	P	D	I	I	P	K	V	E	G	K	T	I	1000000
30	42	P	D	I	I	P	K	V	E	G	K	T	I	A	1000000
31	43	D	I	I	P	K	V	E	G	K	T	I	A	E	1000000
32	44	I	I	P	K	V	E	G	K	T	I	A	E	Q	1000000
33	45	I	P	K	V	E	G	K	T	I	A	E	Q	I	1000000
35	47	K	V	E	G	K	T	I	A	E	Q	I	L	Q	1000000
36	48	V	E	G	K	T	I	A	E	Q	I	L	Q	Y	1000000
40	52	T	I	A	E	Q	I	L	Q	Y	G	S	M	G	1000000
41	53	I	A	E	Q	I	L	Q	Y	G	S	M	G	V	1000000
45	57	I	L	Q	Y	G	S	M	G	V	F	F	G	G	1000000
48	60	Y	G	S	M	G	V	F	F	G	G	L	G	I	1000000
62	74	T	G	S	G	T	G	G	R	T	G	Y	I	P	1000000
65	77	G	T	G	G	R	T	G	Y	I	P	L	G	T	1000000
68	80	G	R	T	G	Y	I	P	L	G	T	R	P	P	1000000
69	81	R	T	G	Y	I	P	L	G	T	R	P	P	T	1000000
71	83	G	Y	I	P	L	G	T	R	P	P	T	A	T	1000000
73	85	I	P	L	G	T	R	P	P	T	A	T	D	T	1000000
74	86	P	L	G	T	R	P	P	T	A	T	D	T	L	1000000
76	88	G	T	R	P	P	T	A	T	D	T	L	A	P	1000000
77	89	T	R	P	P	T	A	T	D	T	L	A	P	V	1000000
78	90	R	P	P	T	A	T	D	T	L	A	P	V	R	1000000
80	92	P	T	A	T	D	T	L	A	P	V	R	P	P	1000000
82	94	A	T	D	T	L	A	P	V	R	P	P	L	T	1000000
83	95	T	D	T	L	A	P	V	R	P	P	L	T	V	1000000
85	97	T	L	A	P	V	R	P	P	L	T	V	D	P	1000000
86	98	L	A	P	V	R	P	P	L	T	V	D	P	V	1000000
87	99	A	P	V	R	P	P	L	T	V	D	P	V	G	1000000
88	100	P	V	R	P	P	L	T	V	D	P	V	G	P	1000000
89	101	V	R	P	P	L	T	V	D	P	V	G	P	S	1000000
90	102	R	P	P	L	T	V	D	P	V	G	P	S	D	1000000
92	104	P	L	T	V	D	P	V	G	P	S	D	P	S	1000000
94	106	T	V	D	P	V	G	P	S	D	P	S	I	V	1000000
95	107	V	D	P	V	G	P	S	D	P	S	I	V	S	1000000
97	109	P	V	G	P	S	D	P	S	I	V	S	L	V	1000000
98	110	V	G	P	S	D	P	S	I	V	S	L	V	E	1000000
99	111	G	P	S	D	P	S	I	V	S	L	V	E	E	1000000
100	112	P	S	D	P	S	I	V	S	L	V	E	E	T	1000000
101	113	S	D	P	S	I	V	S	L	V	E	E	T	S	1000000
102	114	D	P	S	I	V	S	L	V	E	E	T	S	F	1000000
108	120	L	V	E	E	T	S	F	I	D	A	G	A	P	1000000
114	126	F	I	D	A	G	A	P	T	S	V	P	S	I	1000000
115	127	I	D	A	G	A	P	T	S	V	P	S	I	P	1000000
116	128	D	A	G	A	P	T	S	V	P	S	I	P	P	1000000
117	129	A	G	A	P	T	S	V	P	S	I	P	P	D	1000000
119	131	A	P	T	S	V	P	S	I	P	P	D	V	S	1000000
122	134	S	V	P	S	I	P	P	D	V	S	G	F	S	1000000
123	135	V	P	S	I	P	P	D	V	S	G	F	S	I	1000000
125	137	S	I	P	P	D	V	S	G	F	S	I	T	T	1000000
126	138	I	P	P	D	V	S	G	F	S	I	T	T	S	1000000
127	139	P	P	D	V	S	G	F	S	I	T	T	S	T	1000000
129	141	D	V	S	G	F	S	I	T	T	S	T	D	T	1000000

132	144	G	F	S	I	T	T	S	T	D	T	T	P	A	1000000
137	149	T	S	T	D	T	T	P	A	I	L	D	I	N	1000000
138	150	S	T	D	T	T	P	A	I	L	D	I	N	N	1000000
140	152	D	T	T	P	A	I	L	D	I	N	N	T	V	1000000
142	154	T	P	A	I	L	D	I	N	N	T	V	T	T	1000000
147	159	D	I	N	N	T	V	T	T	V	T	T	H	N	1000000
148	160	I	N	N	T	V	T	T	V	T	T	H	N	N	1000000
156	168	T	T	H	N	N	P	T	F	T	D	P	S	V	1000000
157	169	T	H	N	N	P	T	F	T	D	P	S	V	L	1000000
158	170	H	N	N	P	T	F	T	D	P	S	V	L	Q	1000000
162	174	T	F	T	D	P	S	V	L	Q	P	P	T	P	1000000
163	175	F	T	D	P	S	V	L	Q	P	P	T	P	A	1000000
164	176	T	D	P	S	V	L	Q	P	P	T	P	A	E	1000000
165	177	D	P	S	V	L	Q	P	P	T	P	A	E	T	1000000
168	180	V	L	Q	P	P	T	P	A	E	T	G	G	H	1000000
169	181	L	Q	P	P	T	P	A	E	T	G	G	H	F	1000000
170	182	Q	P	P	T	P	A	E	T	G	G	H	F	T	1000000
171	183	P	P	T	P	A	E	T	G	G	H	F	T	L	1000000
173	185	T	P	A	E	T	G	G	H	F	T	L	S	S	1000000
174	186	P	A	E	T	G	G	H	F	T	L	S	S	S	1000000
178	190	G	G	H	F	T	L	S	S	S	T	I	S	T	1000000
184	196	S	S	S	T	I	S	T	H	N	Y	E	E	I	1000000
187	199	T	I	S	T	H	N	Y	E	E	I	P	M	D	1000000
191	203	H	N	Y	E	E	I	P	M	D	T	F	I	V	1000000
192	204	N	Y	E	E	I	P	M	D	T	F	I	V	S	1000000
195	207	E	I	P	M	D	T	F	I	V	S	T	N	P	1000000
202	214	I	V	S	T	N	P	N	T	V	T	S	S	T	1000000
204	216	S	T	N	P	N	T	V	T	S	S	T	P	I	1000000
205	217	T	N	P	N	T	V	T	S	S	T	P	I	P	1000000
206	218	N	P	N	T	V	T	S	S	T	P	I	P	G	1000000
210	222	V	T	S	S	T	P	I	P	G	S	R	P	V	1000000
211	223	T	S	S	T	P	I	P	G	S	R	P	V	A	1000000
212	224	S	S	T	P	I	P	G	S	R	P	V	A	R	1000000
213	225	S	T	P	I	P	G	S	R	P	V	A	R	L	1000000
214	226	T	P	I	P	G	S	R	P	V	A	R	L	G	1000000
215	227	P	I	P	G	S	R	P	V	A	R	L	G	L	1000000
216	228	I	P	G	S	R	P	V	A	R	L	G	L	Y	1000000
217	229	P	G	S	R	P	V	A	R	L	G	L	Y	S	1000000
218	230	G	S	R	P	V	A	R	L	G	L	Y	S	R	1000000
219	231	S	R	P	V	A	R	L	G	L	Y	S	R	T	1000000
221	233	P	V	A	R	L	G	L	Y	S	R	T	T	Q	1000000
227	239	L	Y	S	R	T	T	Q	Q	V	K	V	V	D	1000000
228	240	Y	S	R	T	T	Q	Q	V	K	V	V	D	P	1000000
229	241	S	R	T	T	Q	Q	V	K	V	V	D	P	A	1000000
231	243	T	T	Q	Q	V	K	V	V	D	P	A	F	V	1000000
232	244	T	Q	Q	V	K	V	V	D	P	A	F	V	T	1000000
235	247	V	K	V	V	D	P	A	F	V	T	T	P	T	1000000
237	249	V	V	D	P	A	F	V	T	T	P	T	K	L	1000000
238	250	V	D	P	A	F	V	T	T	P	T	K	L	I	1000000
239	251	D	P	A	F	V	T	T	P	T	K	L	I	T	1000000
243	255	V	T	T	P	T	K	L	I	T	Y	D	N	P	1000000

250	262	I	T	Y	D	N	P	A	Y	E	G	I	D	V	1000000
251	263	T	Y	D	N	P	A	Y	E	G	I	D	V	D	1000000
252	264	Y	D	N	P	A	Y	E	G	I	D	V	D	N	1000000
253	265	D	N	P	A	Y	E	G	I	D	V	D	N	T	1000000
254	266	N	P	A	Y	E	G	I	D	V	D	N	T	L	1000000
256	268	A	Y	E	G	I	D	V	D	N	T	L	Y	F	1000000
260	272	I	D	V	D	N	T	L	Y	F	S	S	N	D	1000000
261	273	D	V	D	N	T	L	Y	F	S	S	N	D	N	1000000
262	274	V	D	N	T	L	Y	F	S	S	N	D	N	S	1000000
267	279	Y	F	S	S	N	D	N	S	I	N	I	A	P	1000000
269	281	S	S	N	D	N	S	I	N	I	A	P	D	P	1000000
270	282	S	N	D	N	S	I	N	I	A	P	D	P	D	1000000
272	284	D	N	S	I	N	I	A	P	D	P	D	F	L	1000000
274	286	S	I	N	I	A	P	D	P	D	F	L	D	I	1000000
275	287	I	N	I	A	P	D	P	D	F	L	D	I	V	1000000
276	288	N	I	A	P	D	P	D	F	L	D	I	V	A	1000000
277	289	I	A	P	D	P	D	F	L	D	I	V	A	L	1000000
278	290	A	P	D	P	D	F	L	D	I	V	A	L	H	1000000
279	291	P	D	P	D	F	L	D	I	V	A	L	H	R	1000000
283	295	F	L	D	I	V	A	L	H	R	P	A	L	T	1000000
286	298	I	V	A	L	H	R	P	A	L	T	S	R	R	1000000
287	299	V	A	L	H	R	P	A	L	T	S	R	R	T	1000000
288	300	A	L	H	R	P	A	L	T	S	R	R	T	G	1000000
289	301	L	H	R	P	A	L	T	S	R	R	T	G	I	1000000
290	302	H	R	P	A	L	T	S	R	R	T	G	I	R	1000000
291	303	R	P	A	L	T	S	R	R	T	G	I	R	Y	1000000
293	305	A	L	T	S	R	R	T	G	I	R	Y	S	R	1000000
295	307	T	S	R	R	T	G	I	R	Y	S	R	I	G	1000000
296	308	S	R	R	T	G	I	R	Y	S	R	I	G	N	1000000
297	309	R	R	T	G	I	R	Y	S	R	I	G	N	K	1000000
298	310	R	T	G	I	R	Y	S	R	I	G	N	K	Q	1000000
300	312	G	I	R	Y	S	R	I	G	N	K	Q	T	L	1000000
302	314	R	Y	S	R	I	G	N	K	Q	T	L	R	T	1000000
305	317	R	I	G	N	K	Q	T	L	R	T	R	S	G	1000000
306	318	I	G	N	K	Q	T	L	R	T	R	S	G	K	1000000
307	319	G	N	K	Q	T	L	R	T	R	S	G	K	S	1000000
308	320	N	K	Q	T	L	R	T	R	S	G	K	S	I	1000000
311	323	T	L	R	T	R	S	G	K	S	I	G	A	K	1000000
313	325	R	T	R	S	G	K	S	I	G	A	K	V	H	1000000
314	326	T	R	S	G	K	S	I	G	A	K	V	H	Y	1000000
316	328	S	G	K	S	I	G	A	K	V	H	Y	Y	Y	1000000
319	331	S	I	G	A	K	V	H	Y	Y	Y	D	L	S	1000000
323	335	K	V	H	Y	Y	Y	D	L	S	T	I	D	P	1000000
327	339	Y	Y	D	L	S	T	I	D	P	A	E	E	I	1000000
329	341	D	L	S	T	I	D	P	A	E	E	I	E	L	1000000
330	342	L	S	T	I	D	P	A	E	E	I	E	L	Q	1000000
332	344	T	I	D	P	A	E	E	I	E	L	Q	T	I	1000000
333	345	I	D	P	A	E	E	I	E	L	Q	T	I	T	1000000
334	346	D	P	A	E	E	I	E	L	Q	T	I	T	P	1000000
335	347	P	A	E	E	I	E	L	Q	T	I	T	P	S	1000000
336	348	A	E	E	I	E	L	Q	T	I	T	P	S	T	1000000

340	352	E	L	Q	T	I	T	P	S	T	Y	T	T	T	1000000
343	355	T	I	T	P	S	T	Y	T	T	T	S	H	A	1000000
351	363	T	T	S	H	A	A	S	P	T	S	I	N	N	1000000
352	364	T	S	H	A	A	S	P	T	S	I	N	N	G	1000000
353	365	S	H	A	A	S	P	T	S	I	N	N	G	L	1000000
355	367	A	A	S	P	T	S	I	N	N	G	L	Y	D	1000000
361	373	I	N	N	G	L	Y	D	I	Y	A	D	D	F	1000000
365	377	L	Y	D	I	Y	A	D	D	F	I	T	D	T	1000000
368	380	I	Y	A	D	D	F	I	T	D	T	S	T	T	1000000
369	381	Y	A	D	D	F	I	T	D	T	S	T	T	P	1000000
370	382	A	D	D	F	I	T	D	T	S	T	T	P	V	1000000
376	388	D	T	S	T	T	P	V	P	S	V	P	S	T	1000000
378	390	S	T	T	P	V	P	S	V	P	S	T	S	L	1000000
380	392	T	P	V	P	S	V	P	S	T	S	L	S	G	1000000
381	393	P	V	P	S	V	P	S	T	S	L	S	G	Y	1000000
383	395	P	S	V	P	S	T	S	L	S	G	Y	I	P	1000000
389	401	S	L	S	G	Y	I	P	A	N	T	T	I	P	1000000
390	402	L	S	G	Y	I	P	A	N	T	T	I	P	F	1000000
394	406	I	P	A	N	T	T	I	P	F	G	G	A	Y	1000000
395	407	P	A	N	T	T	I	P	F	G	G	A	Y	N	1000000
396	408	A	N	T	T	I	P	F	G	G	A	Y	N	I	1000000
399	411	T	I	P	F	G	G	A	Y	N	I	P	L	V	1000000
403	415	G	G	A	Y	N	I	P	L	V	S	G	P	D	1000000
405	417	A	Y	N	I	P	L	V	S	G	P	D	I	P	1000000
407	419	N	I	P	L	V	S	G	P	D	I	P	I	N	1000000
410	422	L	V	S	G	P	D	I	P	I	N	I	T	D	1000000
411	423	V	S	G	P	D	I	P	I	N	I	T	D	Q	1000000
412	424	S	G	P	D	I	P	I	N	I	T	D	Q	A	1000000
413	425	G	P	D	I	P	I	N	I	T	D	Q	A	P	1000000
415	427	D	I	P	I	N	I	T	D	Q	A	P	S	L	1000000
419	431	N	I	T	D	Q	A	P	S	L	I	P	I	V	1000000
420	432	I	T	D	Q	A	P	S	L	I	P	I	V	P	1000000
421	433	T	D	Q	A	P	S	L	I	P	I	V	P	G	1000000
422	434	D	Q	A	P	S	L	I	P	I	V	P	G	S	1000000
424	436	A	P	S	L	I	P	I	V	P	G	S	P	Q	1000000
427	439	L	I	P	I	V	P	G	S	P	Q	Y	T	I	1000000
429	441	P	I	V	P	G	S	P	Q	Y	T	I	I	A	1000000
430	442	I	V	P	G	S	P	Q	Y	T	I	I	A	D	1000000
432	444	P	G	S	P	Q	Y	T	I	I	A	D	A	G	1000000
434	446	S	P	Q	Y	T	I	I	A	D	A	G	D	F	1000000
439	451	I	I	A	D	A	G	D	F	Y	L	H	P	S	1000000
442	454	D	A	G	D	F	Y	L	H	P	S	Y	Y	M	1000000
447	459	Y	L	H	P	S	Y	Y	M	L	R	K	R	R	1000000
448	460	L	H	P	S	Y	Y	M	L	R	K	R	R	K	1000000
449	461	H	P	S	Y	Y	M	L	R	K	R	R	K	R	1000000
452	464	Y	Y	M	L	R	K	R	R	K	R	L	P	Y	1000000
453	465	Y	M	L	R	K	R	R	K	R	L	P	Y	F	1000000
454	466	M	L	R	K	R	R	K	R	L	P	Y	F	F	1000000
455	467	L	R	K	R	R	K	R	L	P	Y	F	F	S	1000000
456	468	R	K	R	R	K	R	L	P	Y	F	F	S	D	1000000
457	469	K	R	R	K	R	L	P	Y	F	F	S	D	V	1000000

458	470	R	R	K	R	L	P	Y	F	F	S	D	V	S	1000000
459	471	R	K	R	L	P	Y	F	F	S	D	V	S	L	1000000
461	473	R	L	P	Y	F	F	S	D	V	S	L	A	A	1000000