DISSERTATION ON MOLECULAR SCREENING OF MYOCILIN GENE IN PATIENTS WITH POSITIVE FAMILY HISTORY OF GLAUCOMA

Submitted in partial fulfillment of requirements of

M.S.OPHTHALMOLOGY

BRANCH – III

REGIONAL INSTITUTE OF OPHTHALMOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003

THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI

APRIL 2014

CERTIFICATE

This is to certify that the dissertation titled, **"MOLECULAR SCREENING OF MYOCILIN GENE IN PATIENTS WITH POSITIVE FAMILY HISTORY OF GLAUCOMA"** is a bonafide record of the research work done by DR.SINDHUSHREE.R, post graduate in the Regional Institute of Ophthalmology & Government Ophthalmic Hospital, Madras Medical College and Research Institute,Chennai-03,submitted in partial fulfillment of the regulations laid down by the Tamil Nadu Dr. M.G.R. Medical University, Chennai for the award of M.S.Ophthalmology Branch III, under my guidance and supervision during the academic years 2011-2014.

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I hereby declare this dissertation entitled **"MOLECULAR SCREENING OF MYOCILIN GENE IN PATIENTS WITH POSITIVE FAMILY HISTORY OF GLAUCOMA"** is a bonafide and genuine research work carried out by me under the guidance of Prof.Dr.Waheeda Nazir and Prof.Dr.M.R.Chitra.

DATE :

PLACE: DR.SINDHUSHREE.R

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INSTITUTIONAL ETHICS COMMITTEE
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CERTIFICATE OF APPROVAL

To

Dr.R.Sindushree ,
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Madras Medical College, Chennai -3

Dear Dr.R.Sindushree,

The Institutional Ethics committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled "Molecular screening of Myocilin gene in patients with positive family history of galucoma" No.22022013.

The following members of Ethics Committee were present in the meeting held on 05.02.2013 conducted at Madras Medical College, Chennai -3.

1.	Dr.SivaKumar, MS FICS FAIS	--- Chairperson
2.	Prof. R. Nandhini MD Director, Instt. of Pharmacology ,MMC, Ch-3	-- Member Secretary
3.	Prof. Shyamraj MD Director i/c , Instt. of Biochemistry , MMC, Ch-3	-- Member
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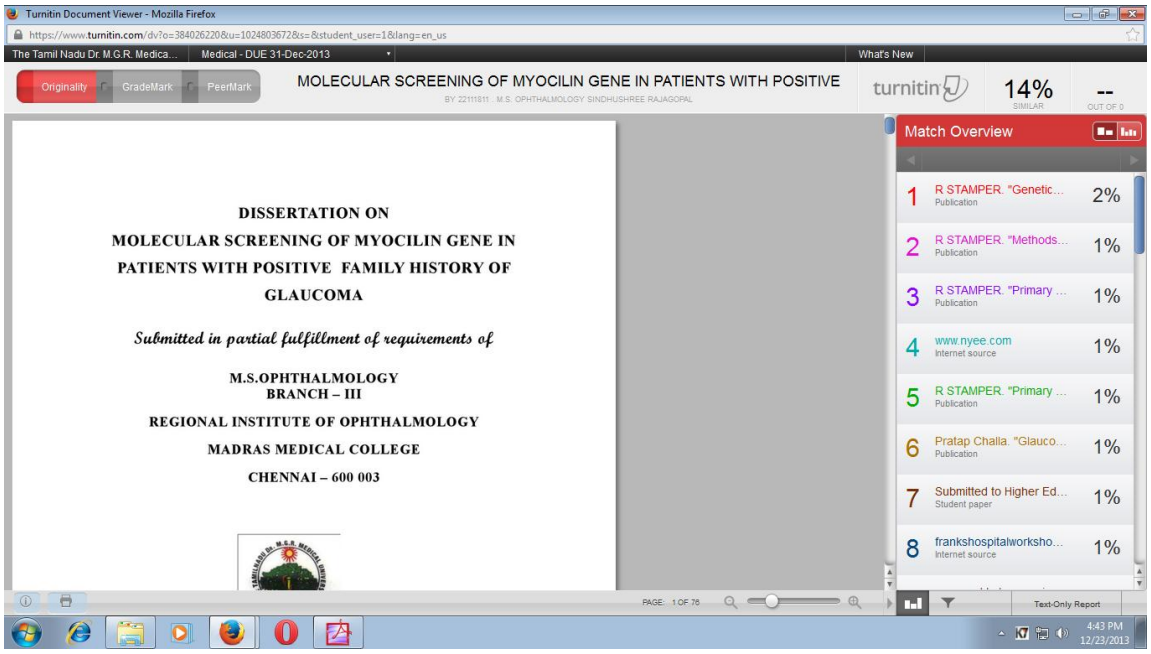
6.	Prof. S. Deivanayagam MS Prof of Surgery, MMC, Ch-3	-- Member
7.	Thiru. S. Govindsamy. BABL	-- Lawyer
8.	Tmt. Arnold Soulina MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/ Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

R Nedi 22/2/13
Member Secretary, Ethics Committee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DISSERTATION ON MOLECULAR SCREENING OF MYOCILIN GENE IN PATIENTS WITH POSITIVE FAMILY HISTORY OF GLAUCOMA Submitted in partial fulfillment of requirements of M.S.OPHTHALMOLOGY BRANCH – III REGIONAL INSTITUTE OF OPHTHALMOLOGY MADRAS MEDICAL COLLEGE CHENNAI – 600 003 THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI APRIL 2014 CERTIFICATE This is to certify that the dissertation titled, “MOLECULAR SCREENING OF MYOCILIN GENE IN PATIENTS WITH POSITIVE FAMILY HISTORY OF GLAUCOMA” is a bonafide record of the research work done by DR.SINDHUSHREE.R, post graduate in the Regional Institute of Ophthalmology & Government Ophthalmic Hospital, Madras Medical College and Research...

MOLECULAR SCREENING OF MYOCILIN GENE IN PATIENTS WITH POSITIVE FAMILY HISTORY OF GLAUCOMA

ABSTRACT:

AIM: To screen myocilin gene for known/unknown variations in patients with positive family history of glaucoma.

METHODOLOGY: All patients underwent a thorough ophthalmological examination after obtaining a detailed family history with custom designed questionnaire. On securing informed consent, the blood samples were collected from probands and their available family members and subjected to DNA analysis. DNA was isolated using Phenol-Chloroform-iso amyl alcohol method and amplified using polymerase chain reaction. Purified PCR product was then directly sequenced using forward primer. The sequences were aligned for homology with their respective reference sequences using NCBI BLASTN and analysed for probable sequence variations.

RESULTS: A total of 35 subjects were included in this study. Majority of the probands were females in the age group of 50-70 years. DNA analysis was done which showed exonic variations in six of the probands of which three had variations in exon 1, two in exon 3 and one had in both exon 1 and exon 3. All these exonic variations were found to be heterozygous except two that were homozygous. The proband DKEG12A had four exonic variations one in exon 1 viz., R76K which was homozygous and other three were in exon 3 as T325M , L349L, R470R all were heterozygous. The change T325M is a novel variation. The proband DKEG11A had Q368X in heterozygous state which is a truncated mutation. Intronic variations were noted in proband DKEG3A, DKEG4A, DKEG5A, DKEG7A, DKEG9A, DKEG10A, and DKEG12A of which some of them were homozygous and some were of heterozygous.

CONCLUSION: The truncated mutation Q368X has been found for the first time in south Indian population, though previous studies have documented this mutation in western as well as in north Indian population. Two novel synonymous variations L349L, R470R are being documented for the first time to be in association with glaucoma. The novel variation T325M has to be functionally characterised. Thus molecular screening may open an exciting frontier in the future to aid in early diagnosis and treatment of glaucoma.

KEYWORDS: Hereditary,POAG,Myocilin,Genetic testing

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PART- II

PART- III

PART - I

INTRODUCTION

Glaucoma is defined as chronic progressive optic neuropathy characterized by structural changes in the optic nerve head with corresponding visual field defects for which increased intraocular pressure, the only modifiable parameter, is the most important risk factor.

Detection of glaucoma at its earlier stage and monitoring its progression is critically important for successful management of glaucoma patients. Often there occurs visual loss which goes unnoticed and patients may present only in the late stages when significant damage has already occurred. As the damage occurred is not reversible with medications or any other mode of treatment, identification in the early stages and appropriate treatment may prevent further progression of the disease and helps in retaining useful vision for the patient.

Several gene mutations have been known to be associated with glaucoma. To date there have been over 20 genetic loci and 3 genes MYOC (Myocilin), OPTN (Optineurin) and WDR36 that have been linked to POAG. A positive family history was found as an important risk factor for primary open angle glaucoma (POAG) in population based studies[1]. So identification of gene mutations among siblings and parents

of those with positive family history will help in early detection and thus appropriate management can be done before significant visual loss occurs and also broaden our understanding in the field of molecular genetics in relation to glaucoma helping us to diagnose this dreadful disease even before it sets in to manifest clinically.

REVIEW OF LITERATURE

Glaucoma, the second leading cause of blindness, is characterized by changes in the optic nerve head with corresponding visual field defects. It is characterized by loss of retinal ganglion cells (RGC) and their axons. The intraocular pressure (IOP) was considered to be the prime risk factor responsible for the glaucomatous optic neuropathy and it is the only modifiable parameter towards which all treatment protocols are addressed to.

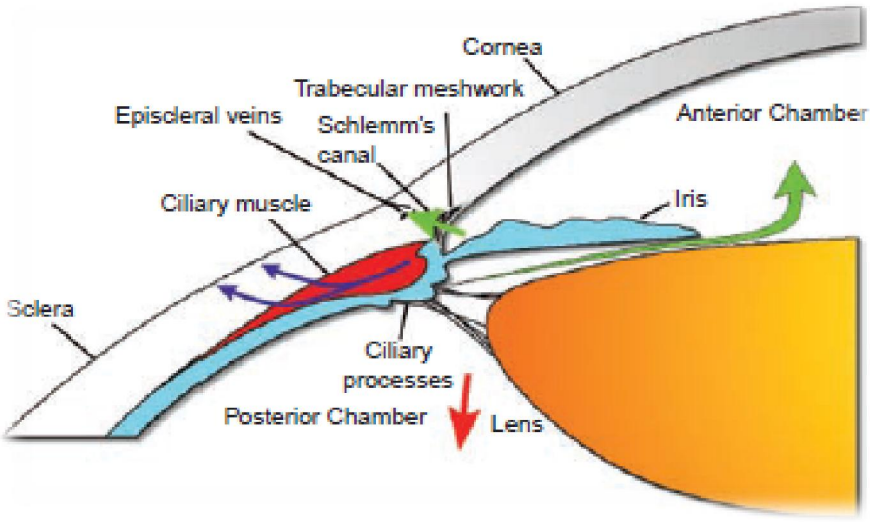
ANATOMICAL STRUCTURES RELATED TO GLAUCOMA:

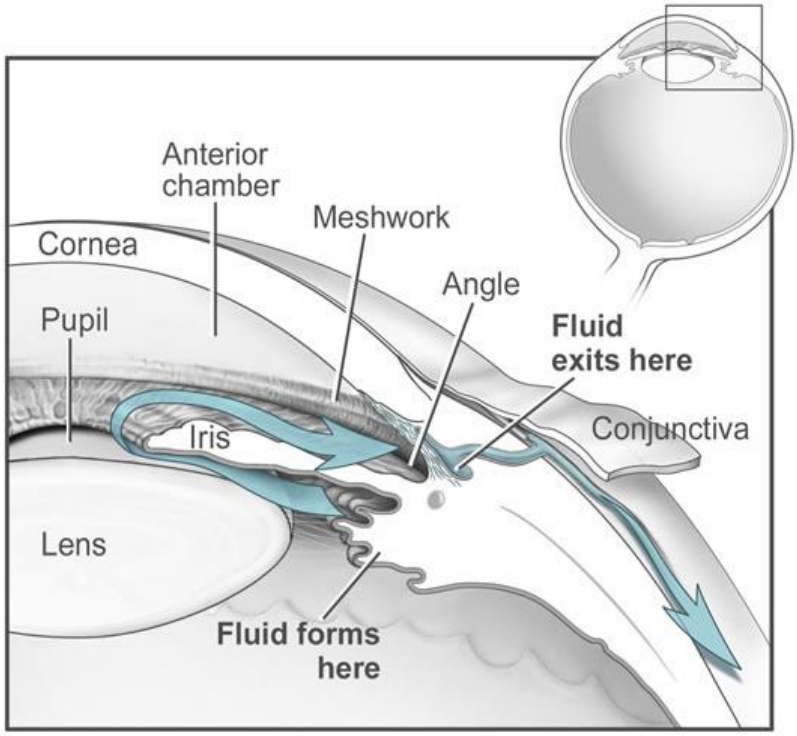
CILIARY BODY:

It is the portion of the uveal tract that lies between the iris and the choroid. It attaches to the scleral spur and creates a potential space, the supraciliary space, between itself and the sclera.

Ciliary body is composed of two parts – the innermost and anterior most regions is called the pars plicata comprising the ciliary processes which is the actual site of aqueous humor production and a posterior portion called the pars plana.

ANATOMY OF ANGLE OF ANTERIOR CHAMBER





The pars plicata region is composed of smooth muscle, which serves the important functions of accommodation and uveoscleral outflow. The pars plana, has a flatter inner surface and joins the choroid at the ora serrata. The lens is suspended from the ciliary body by zonules.

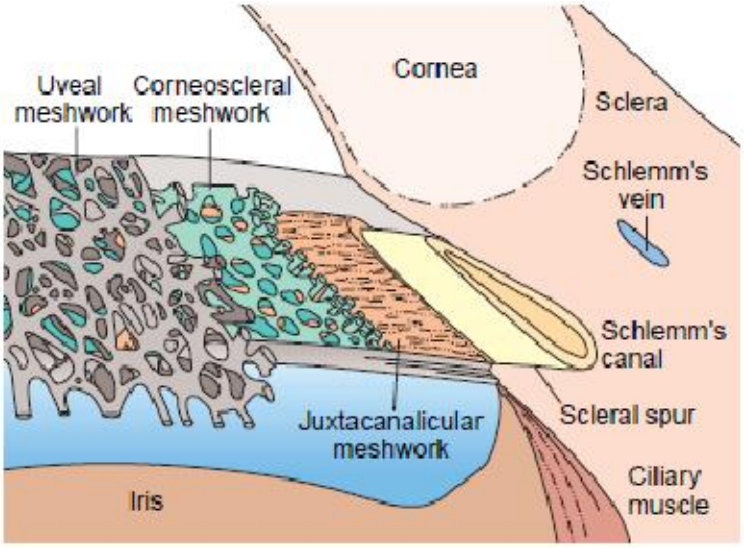
TRABECULAR MESHWORK:

Trabecular meshwork is a sponge like connective tissue which lies in the angle of the anterior chamber with the canal of schlemm's on its outer aspect. It is responsible for draining the aqueous humour from the eye through the anterior chamber. The trabecular meshwork is anatomically divided into 3 parts , each part having a different ultrastructure.

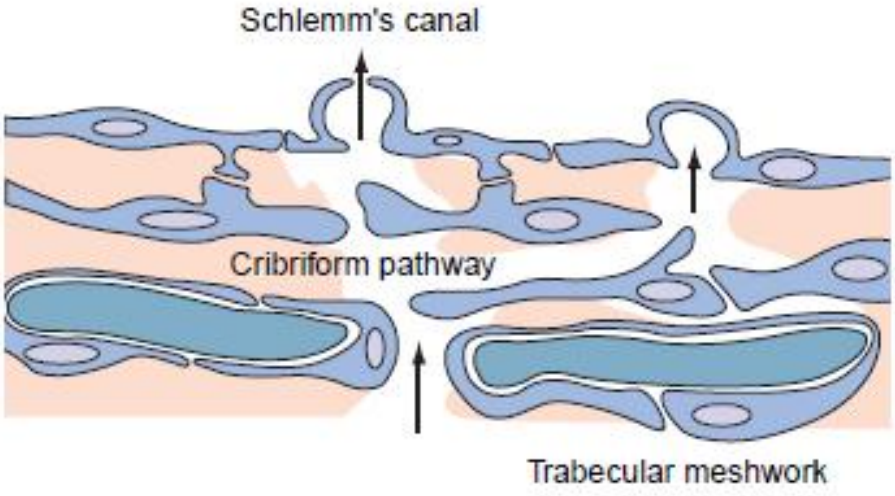
- Inner uveoscleral meshwork
- The corneoscleral meshwork
- The outermost juxtacanalicular portion that lies beneath the inner wall of Schlemm's canal and this layer is considered to be the main site of resistance to aqueous humor outflow.

Aqueous humour passes from anterior chamber through inter and intra trabecular spaces. These spaces are lined by trabeculocytes which maintain the state of hydration of the connective tissue core and also has

LAYERS OF THE TRABECULAR MESHWORK



INNER WALL OF SCHLEMM'S CANAL AND JUXTACANALICULAR MESHWORK



a phagocytic action that helps in trapping and removing debris from the aqueous humour as it percolates through these spaces which narrows down as the Schlemm’s canal is approached.

SCHLEMM'S CANAL:

It is an endothelial lined oval channel present circumferentially in the scleral sulcus. The endothelial cells lining the inner wall are irregular; spindle shaped and contains giant vacuoles while those lining the outer wall are smooth and flat. The outer wall has numerous openings of collector channels which ultimately drain into the episcleral veins via direct and indirect system.

POSTERIOR CHAMBER

It is a triangular space bounded anteriorly by posterior surface of the iris and part of ciliary body and posteriorly by the lens and its zonules and contains around 0.06ml of aqueous humor.

ANTERIOR CHAMBER

It is bounded anteriorly by the back of the cornea and posteriorly by the anterior surface of iris and part of ciliary body. It is about 2.5mm deep and contains about 0.25ml of the aqueous humor. Its peripheral

recess is called the angle of the anterior chamber which is mainly formed by the trabecular meshwork.

ANGLE OF THE ANTERIOR CHAMBER

From posterior to anterior the angle recess is formed by the following structures:-

- Ciliary body band :

It is the posterior most landmark in the angle recess formed by the anterior most part of the ciliary body between its attachment to the scleral spur and insertion of the iris. It appears as a grey or dark brown band in gonioscopy.

- Scleral spur :

It is the posterior portion of the scleral sulcus which usually appears as a prominent white line on gonioscopy.

- Trabecular meshwork

It is seen as a band just anterior to scleral spur . It has no pigment at birth and develops pigment with increasing age.

- Schwalbe’s line :

It marks the anterior limit of the structures forming the angle of the anterior chamber formed by the termination of the descemet’s membrane of the cornea seen as a glistening white line.

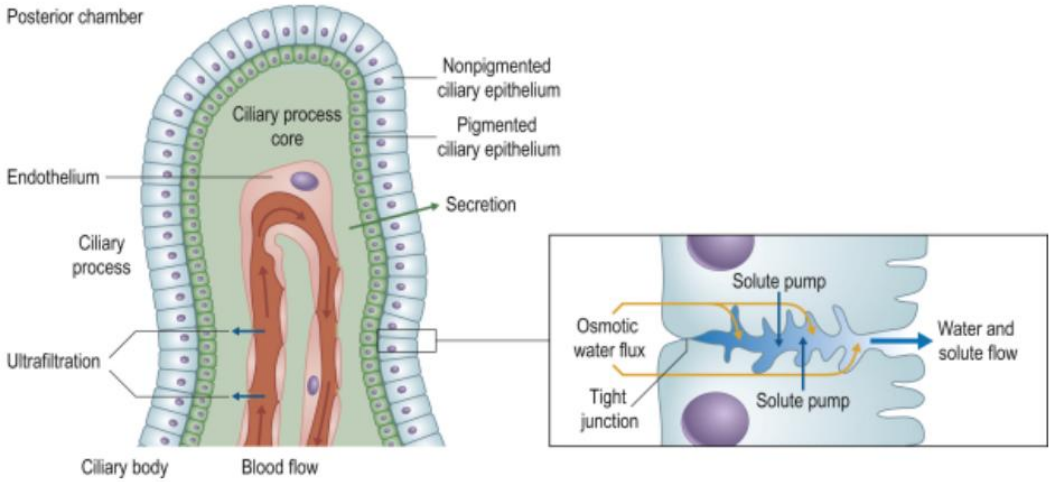
AQUEOUS HUMOUR:

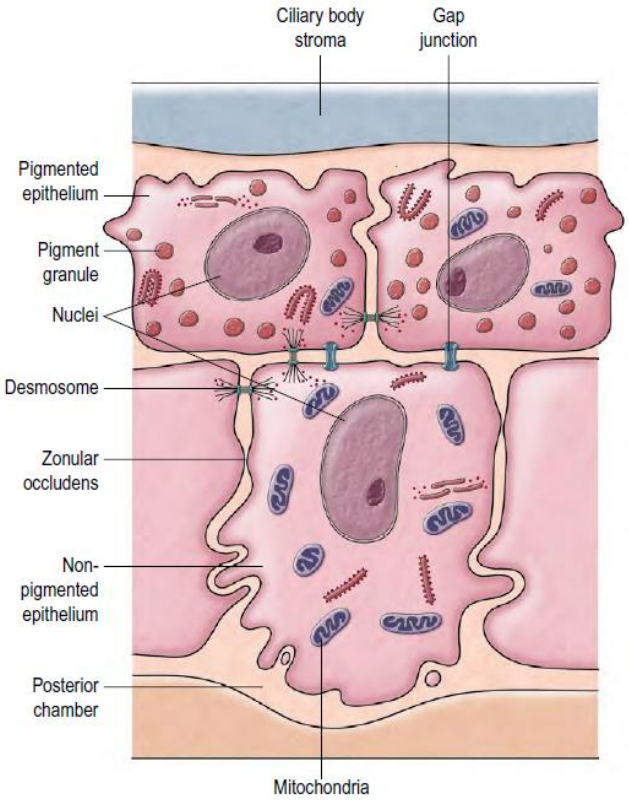
Aqueous humour is transparent, clear, slightly alkaline fluid which occupies the anterior and posterior chambers of the eye. It resembles blood plasma in composition but composed of less protein and glucose and more lactic acid and ascorbic acid. It has multiple physiologic functions throughout the various ocular structures. It provides nutrients and oxygen to ocular tissues that lack a direct blood supply such as the lens and cornea and also removes their metabolic waste products. Additionally, it is also responsible for maintaining the intraocular pressure (IOP) which is needed to maintain the integrity of the eyeball.

AQUEOUS HUMOUR DYNAMICS

There exists a dynamic balance between aqueous humor production and its outflow which helps to maintain the normal intraocular pressure. The two main structures related to aqueous humor dynamics are the

AQUEOUS HUMOR DYNAMICS





ciliary body, the site of aqueous humor production and the trabecular meshwork the principal site of aqueous humor outflow.

SECRETION OF AQUEOUS HUMOUR:

Aqueous humour is derived from blood plasma within the capillary network of the ciliary processes by three mechanisms:

• **Diffusion:** It is the process wherein there is a passive movement of ions across a membrane related to charge and concentration.

• **Ultra filtration:** Water and water-soluble substances, limited by size and charge, are transported across the cell membrane pores in response to an osmotic gradient or hydrostatic pressure which in turn is influenced by IOP, blood pressure in the ciliary capillaries, and plasma oncotic pressure.

Diffusion and ultrafiltration are both passive mechanisms, with lipid and water soluble substances from the capillary core traversing the stroma and passing through pigmented epithelial cells and limited by the tight junctions of the non-pigmented epithelial cells.

• **Active transport (secretion):** Water-soluble substances of larger size or greater charge are actively transported across the cell membrane .This

process requires the expenditure of energy which is derived from the Na-K ATPase pump and glycolytic enzymes present in nonpigmented epithelial cells. Active transport accounts for the majority of aqueous production.

AQUEOUS OUTFLOW PATHWAYS:

There are two main pathways of aqueous humor outflow which is involved in control of IOP. 70-90% of aqueous humor leaves the anterior chamber through the conventional outflow pathway (trabecular meshwork and Schlemm's canal) and enters the episcleral veins. A proportion of aqueous humour (10-30%) drains via the non conventional outflow path way (intercellular spaces between ciliary muscle fibers and the loose connective tissue of the suprachoroidal space).

PATHOPHYSIOLOGY OF GLAUCOMA

Glaucoma is a chronic progressive disease characterized by slow and progressive degeneration of retinal ganglion cells (RGC's).This results in decrease in the neuroretinal rim width with concomitant enlargement of the cup.

The pathophysiological mechanisms underlying the glaucomas are not fully understood.

There are two main mechanisms proposed for glaucomatous damage of retinal ganglion cells.[2]

1. **Mechanical theory** – Raised IOP causing mechanical compression of the retinal ganglion cell axons thereby interrupting the axonal flow and causing cell death

2. **Ischemic theory** – Development of intraneuronal ischemia due to decreased ocular perfusion and thereby causing death of the ganglion cell axons.

Several other possible mechanisms have been proposed which are unrelated to IOP. These include excessive retinal glutamate, deprivation of neuronal growth factors, free nitrate radicals, immune mediated nerve damage and oxidative stress which may also contribute to the initiation and progression of glaucoma.[2]Nevertheless, the molecular mechanisms responsible for impaired aqueous outflow in glaucoma are still not clear.[3]

CLASSIFICATION OF GLAUCOMA

There are several ways in which glaucoma can be classified such as based on anatomic, gonioscopic, biochemical and molecular and genetic features.[4]

However traditionally they are classified into either open angle or closed angle and whether primary or secondary. Glaucomas are classified as primary when they occur with no known etiology or as secondary when they are associated with other ocular and systemic disorders. In general, glaucomas may be categorized into three major types:

• Primary open-angle glaucoma (POAG)

• Primary congenital glaucoma (PCG)

• Primary angle closure glaucoma (PACG)

EPIDEMIOLOGY OF GLAUCOMA

Among 285 million people who are visually impaired worldwide, 39 million are blind and 246 million have low vision. Glaucoma is recognized to be the second most common cause for bilateral blindness and constitutes about 8% of the causes for global blindness.[5]

According to the World Health Organization statistics which was published in 2010, glaucoma accounts for blindness in 4.5 million persons behind only cataracts at 18 million person[6]. In India glaucoma accounts for blindness in about 1.5 million people .[7]The number of

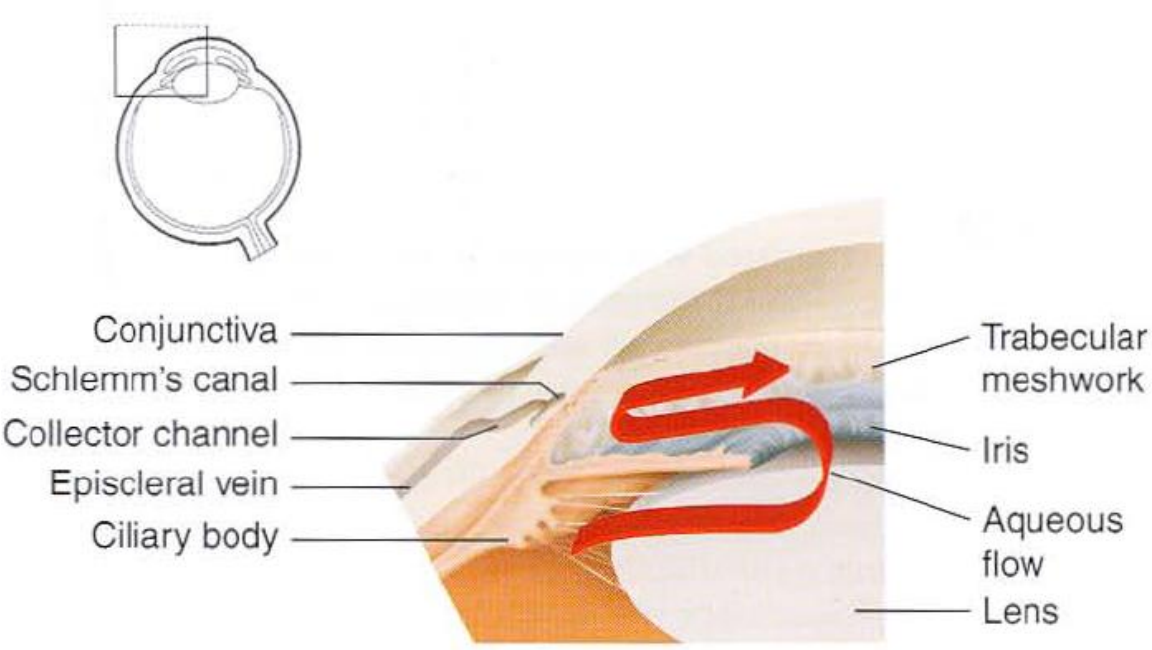
people affected with glaucoma is supposed to increase to almost 80 million by 2020.[6]

PRIMARY OPEN ANGLE GLAUCOMA

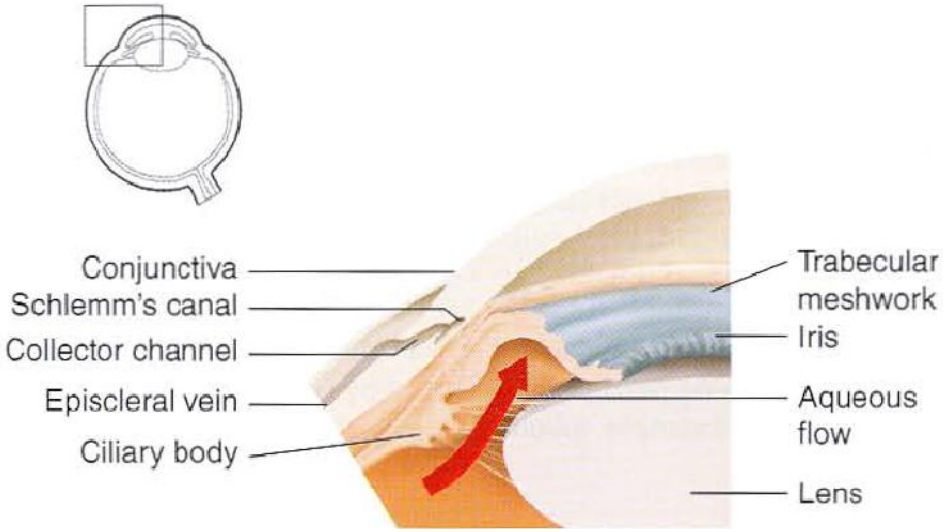
Primary open angle glaucoma (POAG) is the most common type of glaucoma. Its incidence increases with increasing age affecting 1-2% of all individuals over the age of 40 years which increases to 8-10% in 65 years and above age group. POAG is found to be more prevalent in Africans than in Caucasian and Asians. Several population-based studies done in southern India have reported various prevalence rates of primary open-angle glaucoma. The prevalence of POAG in the Chennai Glaucoma study was 1.62%.[8]

Primary open-angle glaucoma (POAG) is defined as chronic, progressive, anterior optic neuropathy which is accompanied by characteristic optic disc changes, visual field loss, and open angles, having no obvious causative ocular or systemic conditions[9]. The most important risk factor, though not the only cause of all damage, being elevated intraocular pressure (IOP) probably reflecting a reduced aqueous humor outflow facility. There are several other risk factors other than elevated IOP such as age, race, gender, heredity, socioeconomic status,

PRIMARY OPEN ANGLE GLAUCOMA



PRIMARY ANGLE CLOSURE GLAUCOMA



myopia, diabetes etc... Of all this heredity i.e. family history has been found to be significantly associated with POAG.[9, 10]

Around 5–50% of cases of POAG are hereditary, the risk of developing POAG in first degree relatives being 4–16%[9]. The likelihood of relatives of patients with POAG developing glaucoma was 10 times more than relatives of those without glaucoma. Furthermore, siblings of those individuals with glaucoma have been found to have a higher IOP and a larger cup-to-disc ratio than siblings of those without glaucoma.

The onset of this disease is usually insidious and has a slowly progressive course which may present as either progressive loss of vision or may remain completely asymptomatic being detected incidentally on routine clinical examination. Intraocular pressure is elevated in most of the patients and is found mostly to be in the range of 22-40mmhg. Gonioscopy shows open angles. Fundus examination shows characteristic optic disc cupping with nasalization, bayoneting, neuroretinal rim thinning etc. along with corresponding visual field defects.

The main goal of treatment is to improve the patient's life by improving or preserving visual function without causing undue problems occurring due to therapy. Treatment has to be individualized depending on the degree of the glaucomatous damage, rate of change of patient's

clinical condition and the presence of associated risk factors. The target pressure should be estimated for each patient in order to prevent further damage. Main modality of treatment is lowering the IOP with topical antiglaucoma medications although argon laser trabeculoplasty is also an appropriate initial treatment. Filtration surgery is usually preferred if patient is not responding to medical/laser treatment or is intolerant to topical medications .

PRIMARY ANGLE CLOSURE GLAUCOMA

Primary angle closure glaucoma is due to appositional or synechial closure of the angle of the anterior chamber angle caused by pupillary block in the absence of other causes of angle closure.

Classification of angle closure glaucoma

- **Primary angle closure suspect:** Greater than 270 degree of irido-trabecular contact plus absence of peripheral anterior synechiae plus normal IOP, disc, and visual field. The angle is at risk.
- **Primary angle closure:** Greater than 270 degree of irido-trabecular contact with elevated IOP and/ or peripheral anterior synechiae plus normal disc and visual field examinations. The angle is abnormal in structure or function.

- **Primary angle closure glaucoma:** Greater than 270 degree of irido-trabecular contact plus elevated IOP plus optic nerve and visual field damage. The angle is abnormal in structure and function with optic neuropathy.

Commonly presents in sixth and seventh decades of life being more common in women compared to men. Most of the cases are sporadic in nature though few studies have shown autosomal dominant and recessive mode of inheritance. Prevalence is more in hyperopic eyes.

Several ocular risk factors have been proposed which include

- Shallow anterior chamber
- Decreased anterior chamber volume
- Short axial length
- Small corneal diameter
- Increased posterior corneal curvature
- Decreased corneal height
- Increased thickness and anterior curvature of the lens
- More anterior insertion of iris into the ciliary body

The underlying pathophysiology is the development of relative pupillary block in patients with above risk factors leading to impairment

of aqueous outflow and thereby a raise in intraocular pressure. Several factors can trigger a relative pupillary block resulting in an acute attack in patients with predisposing risk factors which include dim illumination, emotional stress, topical or systemic use of cycloplegic or mydriatic, etc…

Symptoms include sudden development of redness, pain, haloes and diminished vision. On examination, IOP is elevated associated with corneal edema, mid-dilated pupil with poor reaction and a shallow anterior chamber. Gonioscopy done once corneal edema clears shows presence of closed angles. Optic disc is edematous and hyperemic in case of acute attack. But patients who have had recurrent attacks and have chronic angle closure usually show characteristic cupping and vessel changes. Examination of the fellow eye shows shallow anterior chamber with occludable angles. Treatment includes laser peripheral iridotomy in patients with occludable angles especially those not expected to turn up for regular follow ups. Acute elevations of IOP can be controlled by topical and systemic antiglaucoma medications. Definitive treatment is filtering surgery.

PRIMARY CONGENITAL GLAUCOMA

This is a rare condition which is the most common form of glaucoma in infants and more than 80% of cases manifest within the first year of life. The exact cause is unknown. It is possibly due to maldevelopment of angle structures during development. The clinical findings typically consist of epiphora, photophobia, corneal edema and buphthalmos. Goniotomy or trabeculotomy is the first line treatment, second choice is guarded filtering procedure and third choice is tube shunt surgery. Management of corneal haziness, refractive changes and efforts to prevent amblyopia is essential.

DIAGNOSIS OF GLAUCOMA

Single test is not sufficient to diagnose glaucoma. Currently diagnosis is based on correlating several factors mainly IOP, optic disc damage with corresponding visual field defects. Close attention is given to elevated IOP and a positive family history of the disease. Diagnosis of glaucoma especially POAG requires comprehensive history taking and examination including visual acuity, slit lamp biomicroscopy, CCT corrected Goldmann applanation tonometry, gonioscopy, optic nerve head assessment, retinal nerve fiber layer assessment and visual field testing .

TONOMETRY

Tonometry is defined as the measurement of intraocular pressure. A tonometer is an instrument used for measuring tension or pressure.

Classification of Tonometers

All clinical tonometers measure the IOP by relating a deformation of the globe to the force responsible for the deformation.

The two basic types of tonometers differ according to the shape of the deformation: indentation and applanation (flattening).

Indentation Tonometers

In indentation tonometry, a known weight is placed on the cornea, and the IOP is estimated by measuring the deformation or indentation of the globe. The Schiotz tonometer is the prototype for this class of instruments.

Applanation tonometers

The applanation tonometers are further differentiated on the basis of the variable that is measured.

GOLDMANN APPLANATION TONOMETER

Variable Force

This type of tonometer measures the force that is required to applanate (flatten) a standard area of the corneal surface. The prototype is the Goldmann applanation tonometer, which was introduced in 1954.

Variable Area

This type of tonometer measures the area of the cornea that is flattened by a known force (weight). The prototype in this group is the Maklakoff tonometer, which was introduced in 1885.

Goldmann type tonometers have relatively minimal displacement, whereas that with Maklakoff-type tonometers is sufficiently large to require the use of conversion tables.

Goldmann tonometry is considered to be the gold standard in tonometry. It is based on the principle of the Imbert-Fick law. This law states that an external force (W) against a sphere equals the pressure in the sphere (P_t) multiplied by the area flattened (applanated) by the external force (A):

$$W=P_t \times A$$

The procedure consists of adjusting the force applied by a tonometer tip on the central cornea to anesthetized eye until the observer is satisfied with the pattern produced by the visible fluorescence of the precorneal tear film. The force (in grams) is multiplied by ten and is assumed to be IOP expressed as mmHg. Perkins tonometry is used for examination of infants under anesthesia. Perkins tonometer is a special type of portable applanation tonometer, which allows measurement of IOP in children, patients unable to cooperate for slit lamp examination, and in anaesthetised patients who may be in a supine position.

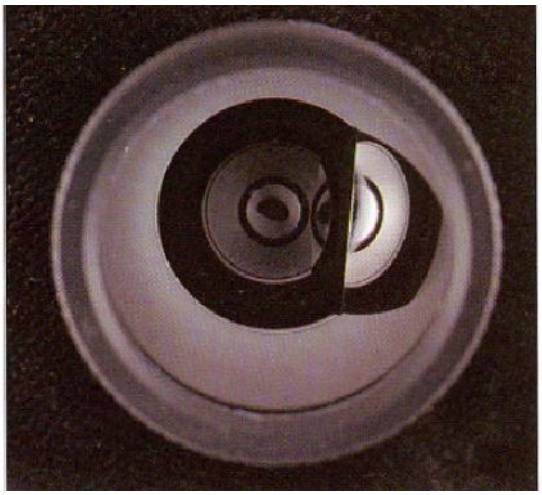
Noncontact Tonometer

A third type of tonometer uses a puff of air to deform the cornea and measures the time or force of the air puff that is required to create a standard amount of corneal deformation.

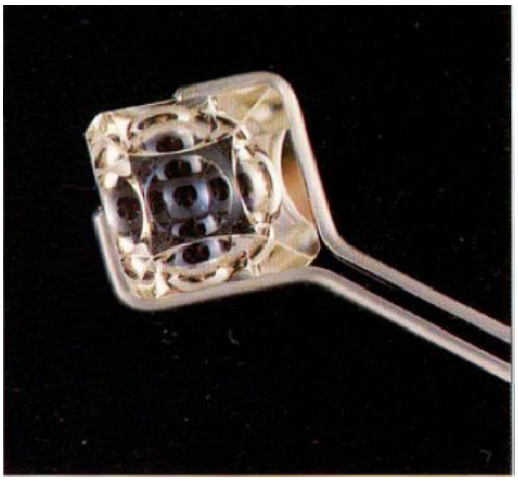
GONIOSCOPY:

Gonioscopy is the method of visualizing the anterior chamber angle to know whether it is open or closed. It is essential in diagnosing the type and etiology of glaucoma and also is helpful prognostically and therapeutically in glaucoma.

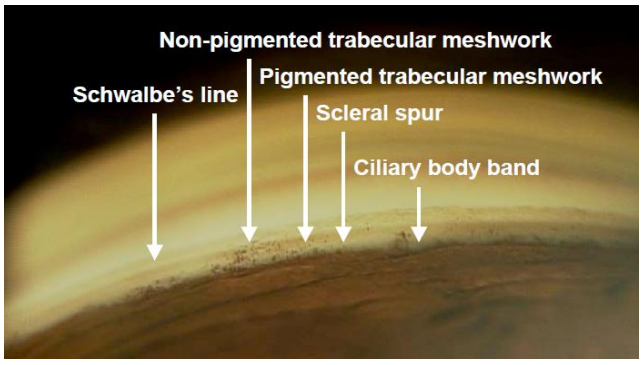
GOLDMANN SINGLE MIRROR



ZEISS FOUR MIRROR



NORMAL ANGLE IN GONIOSCOPY



There are 2 types of gonioscopy – Direct and Indirect

Direct gonioscopy:

Uses Koeppe lens to visualize the angles. The surface of this lens is quite large and needs the use of saline or similar fluid as a coupling agent, and this necessitates that the patient should lie supine. However has the advantage of giving a panoramic view of the angle structures. The curvature of this lens adds 1.53 to the magnification of the angle image. Lighting is usually obtained by a Barkan hand held illuminator or fiber optic light source, and magnification is obtained by a supported, counterbalanced microscope having 1.63 objective lenses.

Indirect gonioscopy:

Two types of gonioscopic contact lenses are available: (1) those whose surface is slightly larger than the cornea and that require a gonioscopic coupling gel (e.g., Goldmann lens) (2) those whose surface is smaller than the cornea and that use the patient's tear film as a coupling agent (e.g., Zeiss or Sussman four-mirror lens)

Goldmann and Zeiss lenses:

These types of lenses are termed *indirect gonioscopic lenses* because they have mirrors by which the angle is examined with reflected light

VISUAL FIELDS

Evaluation of visual fields plays an important role in the diagnosis of glaucoma. Visual fields can be evaluated using two types of techniques

1. Kinetic perimetry
2. Static perimetry

Kinetic perimetry:

In this case the stimulus is of the same intensity and only the location of the stimulus is changed.

Static perimetry:

Here the stimulus is stationary or static whereas the size and intensity of the stimulus can be varied

The static automated perimetry is available in

1. Octopus perimeter
2. Humphrey field analyzer

ASSESSMENT OF FIELDS BY AUTOMATED PERIMETRY

Automated perimetry is the standard method of measuring the visual field. The standard protocol of static white on white stimuli is called standard automated perimetry. A major limitation of tangent screen and arc perimeters was a lack of standardization of test objects and the background as well as the patient's fixation.

Since automated perimetry is a computerized method of evaluating the visual fields it has provided the following advantages over manual perimetry

1. Random presentation of targets to avoid patient anticipation of the next presentation sites
2. Estimation of patient's reliability
3. Reduced variability
4. Ability to determine the extent of decrease in the retinal sensitivity at each point

TESTING PATTERNS

The central 24-30 degrees field with 6 degree separation between test locations is commonly used test pattern. In case of tubular fields as occurs in advanced glaucoma the central 10 degree of field is tested to know how much of central field is preserved and this also helps in deciding whether the patient can be taken up for ant glaucoma surgery in order to prevent the snuff out phenomenon post operatively.

TESTING STRATEGIES:

Several strategies are available namely full threshold, Tendency oriented perimetry (TOP), FASTPAC, Swedish interactive threshold algorithm (SITA).

Full threshold strategy tests more points and takes a longer time to assess the visual fields. This may cause fatigability of the patients thereby making the field analysis unreliable. To overcome this problem and to make the test simpler and more reliable as well as comfortable for the patients, faster strategies were introduced. These include TOP, FASTPAC and SITA strategies of which the former one belongs to Octopus and latter two strategies were incorporated in Humphrey. TOP uses a computational approach to estimate threshold values by

extrapolating information from the surrounding test points. FASTPAC reduces the testing time by using a single threshold crossing in 3 db increments instead of standard double threshold crossing to estimate the visual field defects.

INTERPRETATION OF RESULTS

Reliability of the test is assessed by determining the number of false positives and false negatives as well as the frequency of fixation loss and number of stimuli required to complete the test.

Octopus provides printout with probability, corrected probability and comparison, corrected comparison.

Global indices are mathematically analyzed data allowing detection of more subtle visual field abnormalities. It comprises of mean sensitivity which is the average of the patient's responses for all the points tested. Mean defect/deviation refers to the measurement of how the mean of the patient's responses varies from the mean of the responses of a series of normal patients of similar age under similar testing conditions. These indices primarily reflect diffuse changes. The way to detect localized defect is to calculate the number of threshold values that deviate

significantly from the age corrected normal which is called loss variance. Corrected loss variance takes into account the short term fluctuation.

Field defects commonly seen in glaucoma are:

1) Para central Scotoma / Bjerrum Scotoma
2) Seidel scotoma
3) Arcuate Scotoma
4) Isolated Arcuate scotoma
5) Ronne's Nasal Step
6) Peripheral temporal breakthrough
7) Central or temporal island

OPTICAL COHERENCE TOMOGRAPHY

Optical coherence tomography (OCT) is an imaging modality that employs near-infrared light to create cross sectional images of the retina and optic nerve, thereby allowing analysis of the optic nerve head, macula, and retinal nerve fiber layer (RNFL).It is based on the principle of Michelson's laser interferometry and is a non-invasive and rapidly obtained imaging test that provides detailed information about the posterior segment. It shows cross sectional living histology of retina with high resolution and reproducibility.

OCT has been shown to have good reproducibility of RNFL thickness measures, suggesting it may be a useful clinical tool to monitor glaucomatous disease progression.OCT also helps in early detection of glaucoma even before visual field defects become apparent and hence is an important tool in the diagnosis of pre perimetric stage of glaucoma

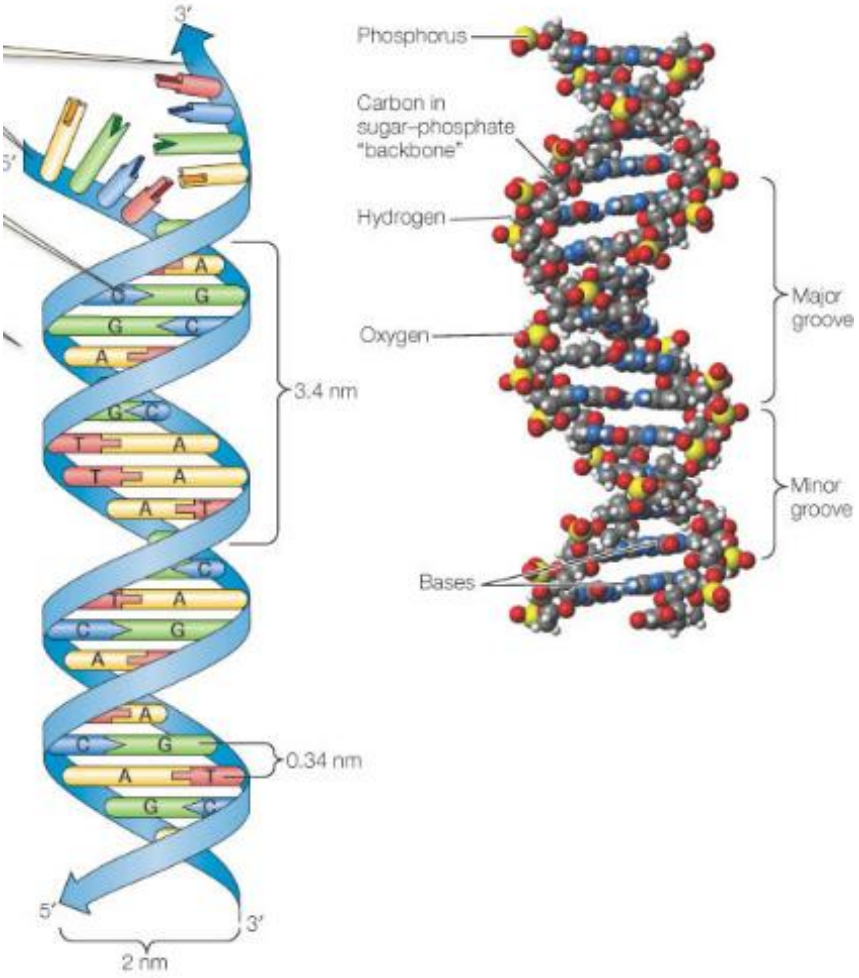
GENETICS IN GLAUCOMA

The molecular etiology of glaucoma is largely unknown, but there are numerous studies establishing a genetic etiology for this disorder. Understanding the molecular basis of glaucoma is important to several aspects of glaucoma diagnosis and management. Genetic testing could be used to identify individuals who are at high risk for the development or progression of glaucoma. Identifying novel pathways could be used to design more specific and effective therapies.

BASIC GENETICS

Every individual has about 23 pairs of chromosomes which comprises of 22 autosomal pairs and one sex pair which contain either two X chromosomes (female) or one X and one Y chromosome (male). Each human chromosome is made up of thousands of genes which in turn are arrayed along one molecule of DNA. Each chromosome pair

STRUCTURE OF NORMAL DNA



consists of one DNA molecule from each parent. The DNA is made up of nucleotides. Each nucleotide is formed by varying sequences of four nucleic acid bases. The nucleic acid bases are divided into two groups purines (adenine and guanine) and pyrimidines (thymidine and cytosil/uracil).

A codon refers to series of three nucleic acid base pairs. It is the sequence of the base pairs and codons that determine the nature of the amino acid and protein synthesis directed by the DNA nucleotide, and it is the protein which determines the phenotypic trait of an individual. DNA is the template from which RNA is made by a process known as transcription and RNA governs protein synthesis inside the cell. Groups of DNA bases that are transcribed into messenger RNA which results in protein synthesis are called exons and the DNA strands that form inactive mRNA in between the exons are called introns.

GENETIC NOMENCLATURE

The human genome organization (HUGO) designates the locus of the gene. The first three letters usually indicate the broad category of the disease state. For example, GLC indicates it is a primary glaucoma rather than a secondary glaucoma. The numbers 1, 2, 3, etc., following the first three letters indicates the type of glaucoma. 1 denotes open angle, 2

denotes closed angle and 3 denotes congenital glaucoma. Depending on the chronological order of identification, each newly mapped gene is given a sequential letter such as ‘a’ is for the first, ‘b’ for the second gene identified, and so on

GENES AND LOCI ASSOCIATED WITH GLAUCOMA[9]

Locus	Location or reference	Gene orproteinname	Phenotype	Age of onset	Inheritance	Per cent of phenotype withgene
GLC1A	1q23-q25	TIGR/myocilin	JOAG/POAG	Juvenile/adult	Dominant	3
GLC1B	2cen-q13	-	POAG	Adult	Dominant	Very low
GLC1C	3q21-24		POAG	Adult	Dominant	Very low
GLC1D	8q23		POAG	Adult	Dominant	Very low
GLC1E	10p15-14	Optineurin	POAG/NTG	Adult	Dominant	5
GLC1F	7q35		POAG	Adult	Dominant	Very low
OPA1	3q28	OPA1	NTD	Adult	?	~30%
GLC3A	2p21	CYP1B1	Congenital	Infant	Recessive	Majority
GLC3B	1p36		Congenital	Infant	Recessive	
NNOS	11		Angle closure/ Nanopthalmos	Young–older adult	Dominant	Majority
RIEG1	4q25	PITX2	Rieger syndrome	Infant–childhood	Dominant	
RIEG2	13q14	FOXC1	Rieger syndrome	Infant–childhood	Dominant	
IRID1	6p25 7q35	FKHL7 -	Iridogoniodysgenesis Pigment dispersion	Infant–child Young adult	Dominant	
NPS	9q34	LMX1B	Nail-patella syndrome	Young adult	Dominant	
PAX6			Aniridia	Congenital		
LOXL1	15q21	Lysyl oxidase	Exfoliation syndrome and glaucoma	Late adult	?	5–10%

JOAG, juvenile-onset open-angle glaucoma; NTD, neural tube defect; POAG, primary open-angle glaucoma.

To date there have been over 20 genetic loci and 3 genes MYOC (myocilin) , OPTN (optineurin) and WDR36 that have been linked to POAG.[11]

MYOC

This was the first gene to be associated with the development of POAG.It was previously known as TM inducible glucocorticoid response protein or TIGR which was initially localized to GLC1A locus. It was then subsequently linked to the MYOC gene. Over 70 different point mutations have been identified in MYOC gene in multiple ethnic groups worldwide[11]. Most of them were located in the coding regions of the gene and are associated with 3-5% of POAG cases throughout the world. In India MYOC gene mutations has been identified in only 2% of the population with POAG.[12]

It is expressed in multiple ocular and non ocular tissues. Its exact function is not yet known. It consists of 3 exons and contains 504 amino acids and has a predicted molecular weight of approximately 57kDa.Has 2 major domains with an N-terminal myosin-like domain and C-terminal olfactomedin –like domain. The N-terminal is encoded by exon 1 and C-terminal by exon 3.The myosin-like domain seems to be more variable

between species except for a highly conserved leucine-zipper element and the olfactomedin domain is also well preserved across the species.

Myocilin is found in many tissues and organs of the body and throughout most ocular structures, the highest being in iris, sclera and trabecular meshwork.

Most common mutations include Glu229Lys, Arg368His, Gln368Stop, etc. The common mutation Gln368Stop found in the western population was not observed in the POAG cases screened in Indian population. Mutation frequency of the MYOC gene is only 2% in the Indian population[12]. But another study states Gln48His is the prevalent myocilin mutation in POAG and primary congenital glaucoma phenotypes in India.[13]

OPTINEURIN

This gene has been localised to the GLC1E locus on chromosome 10p14 in the year 1998. It was then subsequently linked to OPTN gene in 2002. It was previously known as FIP-2. Its expression has been localised to several non ocular and ocular tissues. Because optineurin in expressed in the retina and because it is associated with cellular apoptosis, it was thought that mutations in this gene could possibly explain why the optic

nerves of the patients with normal pressure glaucoma are more susceptible to optic nerve deterioration. It consists of 16 exons and its exact function and how its mutations lead on to glaucoma is unknown. Overall it accounts for <1% of open angle glaucoma. Studies done using this as a candidate gene does not suggest a significant involvement of OPTN in POAG patients of Indian origin[14]. Further studies are required to determine the exact function of OPTN and to find out the exact mechanism through which mutations in OPTN lead to development of glaucoma

WDR36

This gene was initially localised to GLC1G locus at chromosome 5q22.1 and then subsequently linked to WDR36 in 2006. It is a 23 exon gene. It is found in several intraocular structures and several organs including the heart, liver and kidneys. Initial studies showed an association of this gene in 5% of POAG cases. But subsequent studies have shown low and no associations of WDR36 and open angle glaucoma. The exact role of this gene in pathogenesis of POAG needs further investigations.

GENE ASSOCIATED WITH PRIMARY CONGENITAL GLAUCOMA (PCG)

Inheritance of PCG is usually sporadic in most of the cases but sometimes can present with autosomal recessive mode of inheritance. It is more common in the Middle East and in Gypsies probably because of the higher prevalence of consanguinity in these cultures[9]. There are 3 different genetic loci which have been linked to date in PCG. The first two loci identified (GLC3A and GLC3B) were mapped to chromosome 2 (2p21) and chromosome 1 (1p36.2-p36.1) respectively. The third locus GLC3C was mapped to chromosome locus 14q24.3.Only GLC3A locus has been linked to a specific gene. This gene is called CYP1B1, the largest known enzyme of the human cytochrome –p 450 pathways. It is the first gene in this well known family to result in primary developmental defect.

Several mutations have been identified in CYP1B1 which primarily consists of insertions or deletions of the gene. Studies have identified CYP1B1 as a causative gene in primary congenital glaucoma and as a modifier gene in POAG. Rarely may it act as a causative factor for several anterior segment dysgenesis disorders.

GENES ASSOCIATED WITH PRIMARY ANGLE CLOSURE GLAUCOMA (PACG)

Most of the cases of PACG are sporadic in nature and don't have a positive family history. There are no specific genes which have been linked to the development of PACG, though several pedigrees are reported to have a high prevalence of PACG.

Some pedigrees showed autosomal dominant and some showed autosomal recessive pattern of inheritance. The shallower the anterior chamber more is the risk for primary angle-closure glaucoma. Certain other factors like increasing thickness of lens with age also leads to shallowing of the anterior chamber further increasing the risk of angle closure glaucoma. However recent studies have reported a single nucleotide pleomorphism in MMP-9 gene to be associated with angle closure glaucoma.[10]

Having gone through various studies , it is very interesting to know that molecular screening of genes may open an exciting frontier in the field of glaucoma .We have seen that open angle glaucoma may be associated with several different genes, each of which may produce a different time of onset and clinical course. Additionally, similar phenotypes can be seen with different mutations of different

chromosomes even within the same family. Probably there might be much more clarity about the genes and the way they may be responsible for different types of glaucoma in the near future.

PART - II

AIMS AND OBJECTIVES

AIM:

To screen myocilin gene for pathogenic/non-pathogenic mutations in patients with positive family history of glaucoma

OBJECTIVE:

To analyze the role of myocilin gene variants and its association in Indian families attending our glaucoma clinic.

MATERIALS AND METHODS

This is a prospective study consisting of patients who attended glaucoma services of Regional Institute of Ophthalmology in the period of June 2011to June 2013 (2 years). The study enrolled 12 families comprising of 35 subjects which included the proband as well as the siblings and parents.

INCLUSION CRITERIA:

Patients with positive family history of glaucoma

EXCLUSION CRITERIA:

Glaucoma patients without positive family history

Secondary glaucomas

Congenital glaucoma

MATERIALS REQUIRED FOR GENE ANALYSIS

Chemicals:

Chemical used in the molecular study were purchased from standard chemical companies unless otherwise mentioned.

EDTA, Ethanol, sodium chloride, sodium hydroxide pellets, Tris base, 100bp ladder DNA.

Agarose, Ethidium Bromide, primers

PCR purification kit

Amplifier Profile for Myocilin:

The details of oligonucleotide primers used for the amplification of myocilin (Exon1, Exon2, Exon3)

Primer	Sequence (5'- 3')	Tm (C)	Ta (C)	Amplicon Size(bp)
Exon 1 Forward	ACAGCAGAGCTTTCCAGAGG	63.7	63.7	927
Exon 1 Reverse	CATCTCACCCGGTCCTTTTA	63.7		
Exon 2 Forward	GCCCAACTGTTATCAGCACAGTC	66.8	69	770
Exon 2 Reverse	TCCCCTCCCTCTGCTCCCAG	72.2		
Exon 3 Forward	CTGAAAGTCACACAGCCAGCG	68.3	68.7	1103
Exon 3 Reverse	GGTGACCATGTTCATCCTTCTGG	68.7		

Reagents:

RBC Lysis Buffer

Ammonium chloride - 155mM (8.29g)

EDTA -0.1mM (1.00g)

NaHCO3 -12mM (0.034g)

Adjust pH to 7.4 with 1M HCl or NaOH; make up to 1000ml with distilled water. Autoclave and store at room temperature.

WBC Lysis Buffer

NA_2EDTA -25mM (8.14g)

NaCl -200mM (11.69g)

Adjust pH to 8.0 with 1M NaOH, make up to 1000ml with distilled water, autoclave and store at room temperature

Proteinase K (10 mg/ml)

Proteinase K - 100mg

TE(Tris EDTA) -10ml

Dissolve 100mg proteinase K in 10ml TE for 30min at room temperature and store it at -20°C. Proteinase K is the enzyme commonly

employed for digestion of proteins. It is a highly active protease from the mold *Tritirachium album.*

Sodium dodecyl sulphate (SDS) 10%SDS – 10 gram.(Add water to make up to 100 ml, stir on a magnetic stirrer, filter and store at room temperature.)

Phenol (Saturated,pH 8)

Phenol: Chloroform: Isoamyl alcohol mixture

To prepare Phenol: Chloroform: Isoamyl alcohol mixture mix 25 parts of phenol, 24 parts of Chloroform and 1 part of isoamyl alcohol.

Isopropyl Alcohol

The action of isopropyl alcohol is to precipitate the DNA leaving RNA and polysaccharides in the solution.

70% Ethanol

Ethanol – 70 ml

Distilled water – 30 ml

It removes residual salt and moisture in the precipitated DNA.

Tris-EDTA (1 X TE) Buffer (pH – 8.0)

Tris base – 1.2114 gram

EDTA – 0.0372 gram

Dissolve in 900 ml distilled water and adjust the pH to 8.Make up the volume to 1000 ml. Filter autoclave and store at 4°C.

METHODOLOGY

CLINICAL EVALUATION:

All subjects who were enrolled in the study underwent a detailed historical evaluation which included age of onset of the disease, number of family members affected, detailed medical and surgical history, history of consanguineous marriage and pedigree details.

They were then subjected to a comprehensive ophthalmic examination including refraction, estimation of best corrected visual acuity, and measurement of intraocular pressure (IOP) by Goldmann applanation tonometry, measurement of central corneal thickness (CCT) by ultrasonic pachymetry.The CCT corrected IOP was taken for analysis.

Testing of visual acuity, slit lamp examination of the anterior segment, examination of the fundus by 90D, gonioscopy using four mirror , fields by octopus perimetry, RNFL and optic nerve head topographic analysis by SD-OCT was done.

The blood samples were collected from the probands and the siblings and parents whoever were available after written informed

consent.This study was approved by Institutional Human Ethical committee, Madras Medical College, Chennai

PROCEDURE FOR GENOMIC DNA ISOLATION:

Using Phenol-Chloroform-iso amyl alcohol method

- 5 ml of the patient's blood sample as well as their family members who were available was collected using EDTA containing vacutainer.
- The collected blood sample was first centrifuged at 3500 rpm for 30 minutes.
- After centrifugation the sample gets separated into 3 different phases, first layer containing plasma, middle layer containing WBC and the last layer containing RBC. The WBC layer appears like a Buffy coat.
- The WBC layer is taken carefully and transferred to a fresh tube.
- To the separated layer add double the amount of RBC lysis buffer and vortex it. Repeat the steps until the pellet is devoid of red colour.

- Add working WBC lysis buffer (500-1000µl), with 1% SDS and Proteinase K, after adding the buffer do not disturb the tubes and keep it for overnight incubation in the water bath (37˚C).
- After overnight incubation in water bath at 37˚C , add equal volume of Phenol-Chloroform-Isoamyl alcohol(24:24:1) and shake it well till the whole solution becomes milky white.
- Centrifuge the sample at 11000rpm for 20minutes at 25˚C.
- The sample gets separated into three phases-aqueous, organic and white.
- Take the aqueous phase and dissolve it in ice cold absolute alcohol (2.5ml).Shake it well.
- The DNA will be visible like a thread and will assume the shape of a cotton ball.
- Add 1 ml of 70% ethanol to the labeled eppendorff tubes. Suspend the DNA into it and spin it at 4˚C with a speed of 11000rpm for about 20 minutes.
- The Ethanol was discarded and the pellet is air-dried in a sterile place for 3 hours to remove any trace of residual ethanol.
- Approximate amount of 1 X TE buffer was added according to the size of the pellet, allowed to dissolve and stored at -20°C

Preparation of primers:

The stock solution of primers was having concentration of 100 pm/µl. working solution of 10 pmol/µl was prepared by adding 2µl of stock solution of primers 18µl of sterile milliQ water.

Concentration and purity were checked using Nano drop (Thermo scientific, Germany)

Concentration of DNA:

Concentration of double stranded DNA sample (µg/µl) = A260 × 50

Purity of DNA:

Pure DNA= A260/A280 ≥ 1.8

<1.8 indicates protein and phenol contamination

>2.0 indicates the possible contamination of RNA

Polymerase Chain Reaction (PCR):

Principle:

The PCR is a process in which minuscule amounts of DNA can be amplified to enormous amounts in a matter of hours. The original DNA strand is used as a template to synthesize a new strand, each of which

then serves as a template for a new strand, and so forth geometrically. The process is highly automated and results in sufficient DNA to give a reliable test when gene probes are used.

PCR Conditions for MYOC

PCR Conditions- ***MYOC Ex-1***

Steps	Temperature (^{0}C)	Time	Cycles (N)
Initial denaturation	95^{0}C	5 min	1
Denaturation	94^{0}C	45sec	29
Annealing	63.7ºC	45sec	29
Extension	72^{0}C	45sec	29
Final extension	72^{0}C	7 min	1

PCR Conditions – ***MYOC Ex-2***

Steps	Temperature (^{0}C)	Time	Cycles (N)
Initial denaturation	95^{0}C	5 min	1
Denaturation	95^{0}C	45sec	29
Annealing	69ºC	45sec	29
Extension	72^{0}C	45sec	29
Final extension	72^{0}C	7 min	1

PCR Conditions- ***MYOC Ex-3***

Steps	Temperature (^{0}C)	Time	Cycles (N)
Initial denaturation	95^{0}C	5 min	1
Denaturation	95^{0}C	45sec	29
Annealing	68.7ºC	45sec	29
Extension	72^{0}C	45sec	29
Final extension	72^{0}C	7 min	1

PCR Product Purification:

The PCR product is purified by using "Qiagen PCR purification kit"

- Add 5 volume of buffer PB to 1 volume of the PCR reaction and mix. If the colour at the mixture is orange or violet add 10 µl 3M sodium acetate, pH 5 and mix. The colour of the mixture turns yellow.
- Place QIA quick column in a provided 2ml collection tube.
- To bind DNA apply the sample to the QIA quick column and the centrifuge for 30-60 seconds at 8000 rpm. Discard flow through and place the QIA column back into the same tube.

- To wash add 0.75 ml buffer PE to the QIA quick column. Centrifuge for 30-60 seconds at 8000 rpm. Discard flow through and place the QIA column back into the same tube.
- Centrifuge the QIA quick column once more in the provided 2 ml collection tube for 1 minute to remove residual wash buffer.
- Place each QIA quick column in a clean 1.5 ml micro centrifuge tube.
- To elute DNA, add 50µl EB buffer (10mM Tris cl, pH 8.5) or water(pH 7-8.5) to the centre of the QIA quick membrane and centrifuge the column for 1 minute for increased DNA concentration, add 20µl of the elution to the centre of the QIA quick membrane, let the column stand for 1 minute and then centrifuge at 5000 rpm for one minute.
- If the purified DNA is to be analyzed on a gel add 1volume of loading dye to 5 volume of purified DNA. Mix the solution by pipetting up and down before loading the gel.

Agarose Gel Electrophoresis;

The amplicons were visualized in a 2% agarose gel in TBE buffer stained with Ethidium Bromide. The 2% gel was prepared by melting 0.60 g of agarose powder in 30 ml of TBE (0.5X, pH 8.0) buffer in

microwave oven. The molten agarose was then cooled to 600^0c; 2µl of EtBr was added to it and poured in to a gel casting unit after both the edges were sealed with cellophane tapes. When the gel is set, about 4µl of PCR sample and 1µl of gel loading dye (3X) were loaded and electrophoresed at a constant voltage of 75 volt and 125 mA for approximately 30 to 45 minutes or till the dye reaches two thirds of the gel. After the electrophoresis the gel was visualized in UV-transilluminator

Sequencing of the purified PCR products:

Purified PCR product is commercially sequenced with the forward primer at Amnion Biosciences Pvt.Ltd, Bangalore.

BLASTN analysis:

The sequences were aligned for homology with their respective reference sequences using NCBI BLASTN and analyzed for probable sequence variations depictive of either polymorphism or putative mutation.

OBSERVATION AND RESULTS

4906 patients who attended our glaucoma clinic during the period June 2011 to June 2013 were evaluated for the presence of glaucoma and any family history of glaucoma if present was noted. Among these patients, 59 patients were having a history of glaucoma in their families and among them, 32 patients were found to be having established glaucoma, 21 were found to be glaucoma suspects and 6 of them had no evidence of glaucoma. Among 32 patients, around 12 of them were diagnosed to have primary open angle glaucoma which constitutes around 37.5% of the patients with POAG having positive family history of glaucoma.

12 families were included in this study comprising of 35 subjects In each family all available family members were evaluated clinically and their blood samples subjected to DNA analysis after getting consent of the patients.

AGE DISTRIBUTION

Onset of primary open angle glaucoma is usually in the 4^{th}-6^{th} decade in most of the patients except for a few who present early who are termed as having juvenile onset open angle glaucoma.

In our study among the 12 families, each family consisted of a proband whose siblings and parents whoever available was included. Among the 12 probands majority were in the age group of 50-70 years.

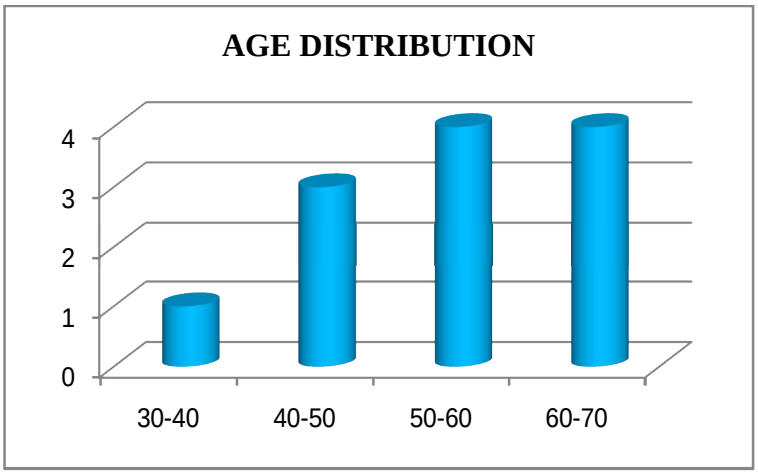


Figure 1 – Age distribution among the probands

SEX DISTRIBUTION

There is no gender predilection for development of primary open angle glaucoma and among 12 probands included in our study 8 of them were females and 4 were males.

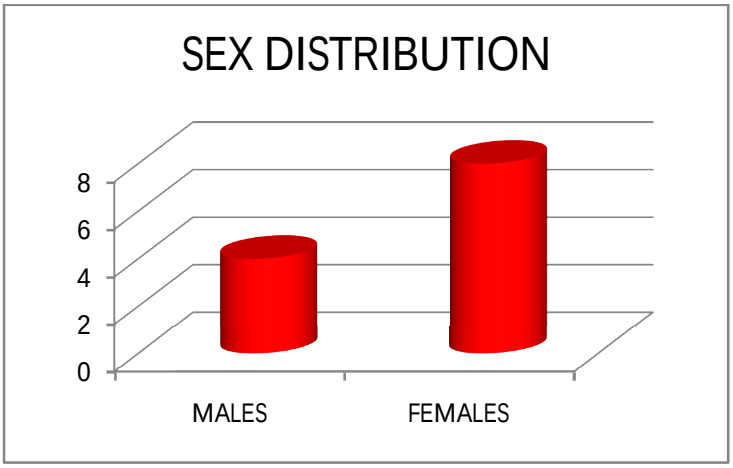


Figure 2- Sex distribution among the probands

IOP DISTRIBUTION

Intraocular pressure was measured using Goldmann applanation tonometry and the IOP measured was corrected for central corneal thickness. The mean IOP in normal population is 15.8 +/- 2.8 mmhg. Majority of patients had their intraocular pressure within the normal range. Only 5 persons had IOP above the normal range. Few of them were already on treatment with topical medications, few were just glaucoma suspects and few patients had already undergone antiglaucoma surgery.

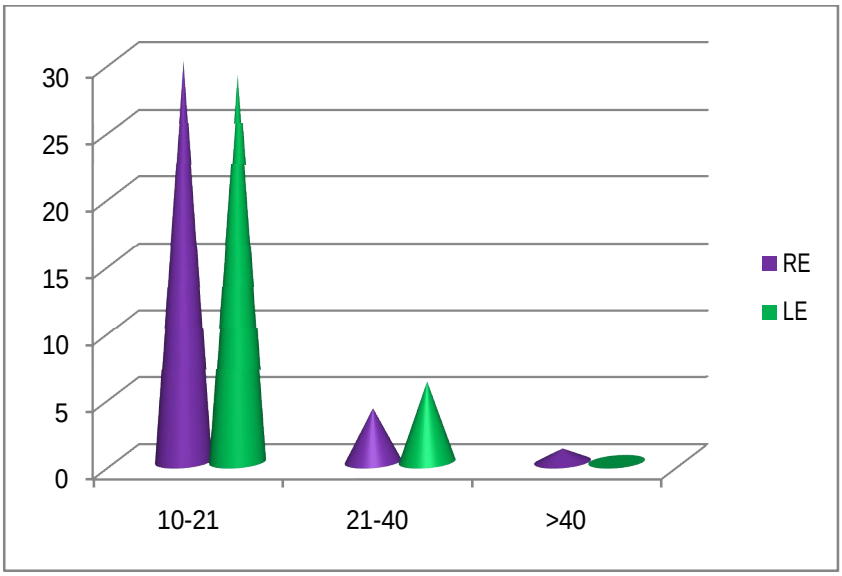


Figure 3 – IOP distribution

CUP: DISC RATIO

Normal cup: disc ratio for a normal size disc is around 0.3-0.5. They are larger in eyes with larger cups and smaller in eyes with small cups. Glaucoma is suspected in case of an asymmetry of CD ratio > 0.2 between 2 eyes, presence of other characteristic vessel changes along with presence of NRR thinning. Slit lamp biomicroscopy with 90D was done to assess the disc size and all patients in our study had disc size within the normal range of 1.5+/-0.5mm. Around 16 patients had moderate glaucomatous damage with cup: disc ratio of 0.5-0.7 whereas 8 patients had advanced cupping with CD ratio of 0.8-0.9.

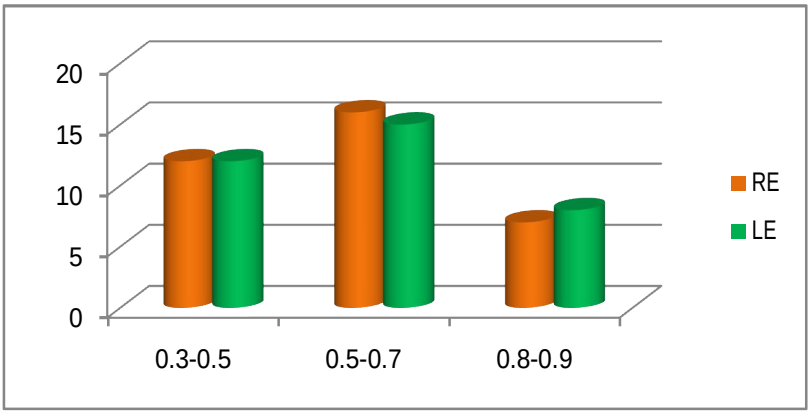


Figure 4 – Distribution of Cup: Disc Ratio

GONIOSCOPY

Gonioscopy was done in all the patients using posner 4 mirror gonioscope and grading of angles was done based on the modified

Shaffer's grading. Grade1 or less in more than 2 quadrants were categorised as having narrow angles. Our study was comprised of predominantly patients with open angles except for 2 of them who had closed angles.

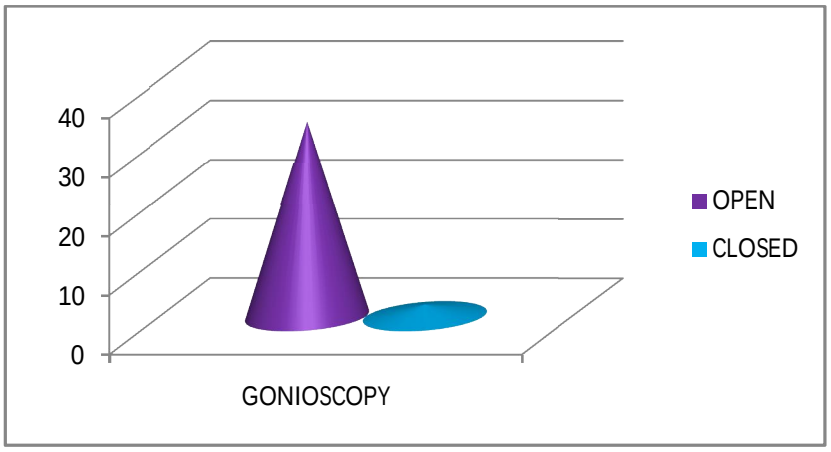


Figure 5 Gonioscopy

VISUAL FIELDS

Visual fields were assessed using OCTOPUS perimeter (301). A minimum of 3 fields were recorded, if any field defect was noted to determine if the field defect is persistent or not. The field defects were considered to be reliable if false positives and false negatives were less than 33% and fixation losses were below 20%. Majority of patients had their visual fields within normal limits. Tubular fields were noted in 2 patients who had advanced glaucomatous damage. Visual fields could not be assessed in 2 patients. One of them had very poor vision in both the

eyes while the other had poor vision only in 1 eye and hence field assessment could not be carried out.

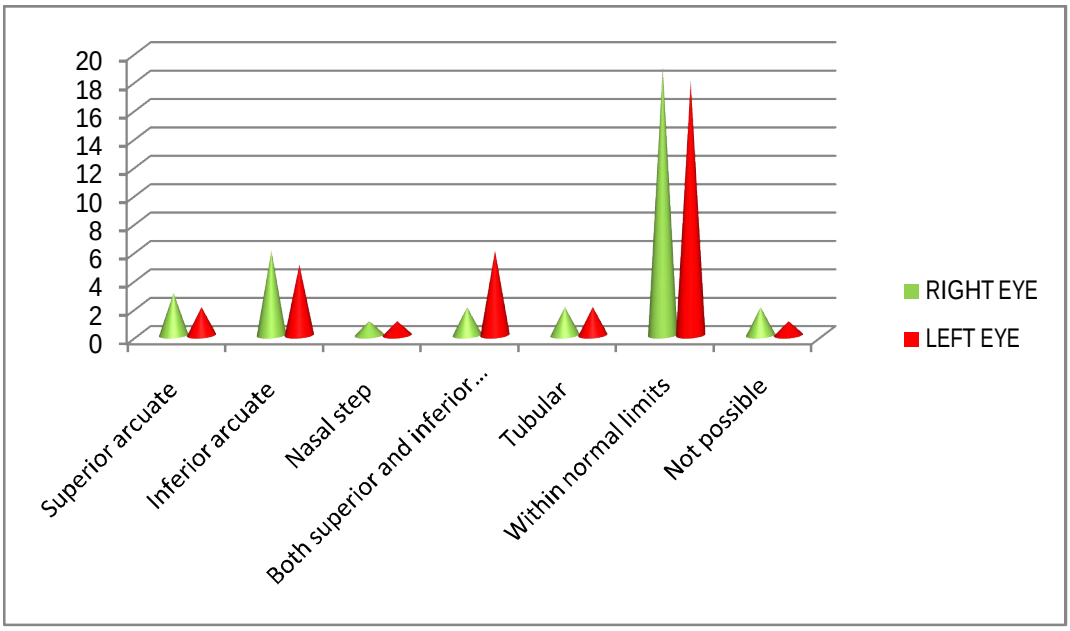


Figure 6 Visual fields by Octopus perimeter

RNFL THICKNESS IN OCT

Optical coherence tomography is a tool used to assess the retinal nerve fibre layer thickness (RNFL) and study the optic nerve head topography as well as macular topography. In glaucoma it is predominantly used to evaluate the RNFL thickness and to analyse whether the disease is progressing or not. Mainly helps in detecting pre perimetic stage of glaucoma as field defects arise only if a minimum of 30% of RNFL is damaged. All patients underwent imaging by spectral domain optical coherence tomography in both eyes. (OTI ophthalmic technologies OCT / SLO COMBINATION IMAGING SYSTEM V 1.37

was used for all patients).Average RNFL thickness in normal population varies from 103.07±10.13µ (Archana Malik et al, 2010). In our study, RNFL thickness was found to be below 100 µ in 12 patients with 2 patients having a thickness of lesser than 90µ.

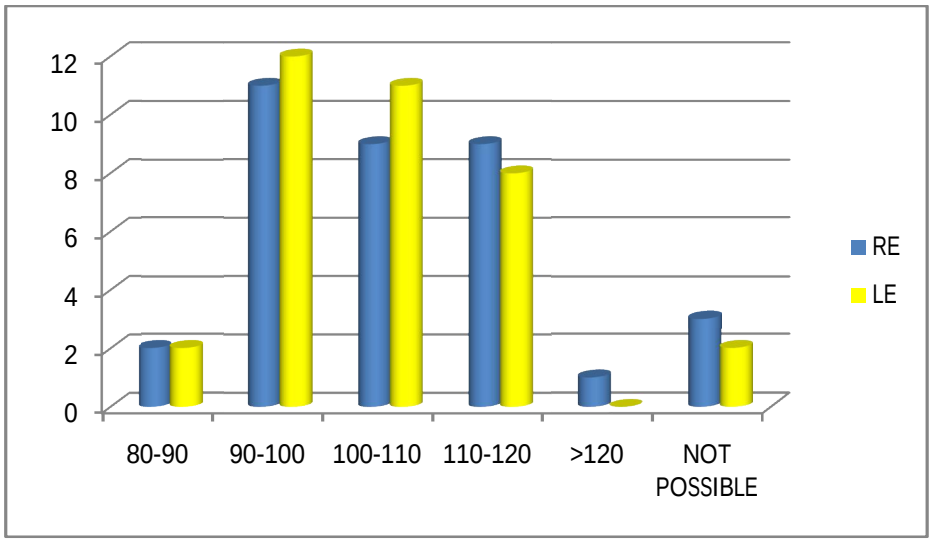


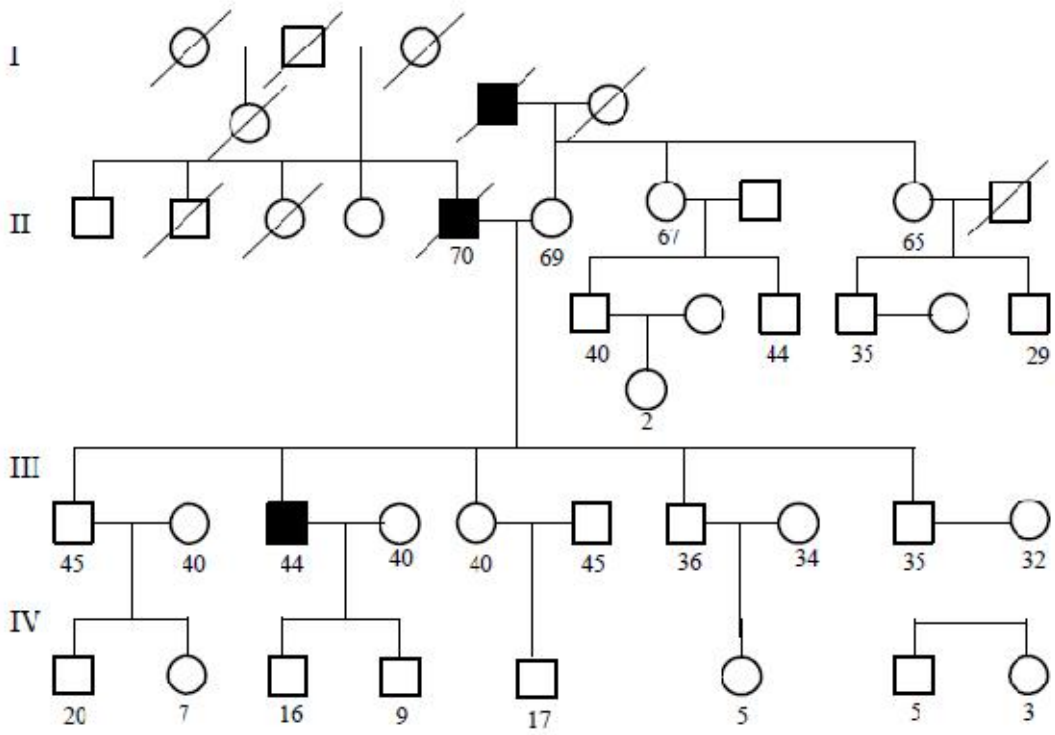
Figure 7. Distribution of RNFL thickness assessed using OCT

MODE OF TREATMENT IF ANY

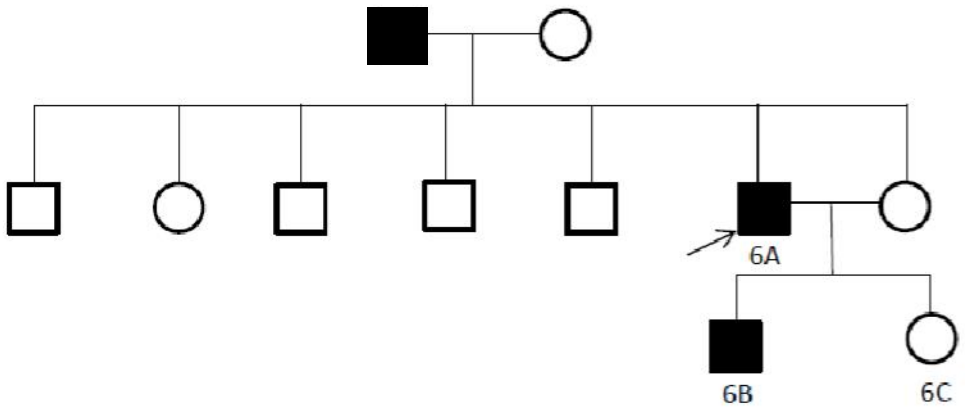
Once primary open angle glaucoma is diagnosed medical treatment with topical antiglaucoma medications is the first line of treatment. If the disease progresses despite maximal tolerated medical therapy the patient is taken up for antiglaucoma surgery namely trabeculectomy which is the gold standard. In our study 17 patients were just observed and were on regular follow up and were not on any mode of treatment. 11 patients

PEDIGREE CHARTS

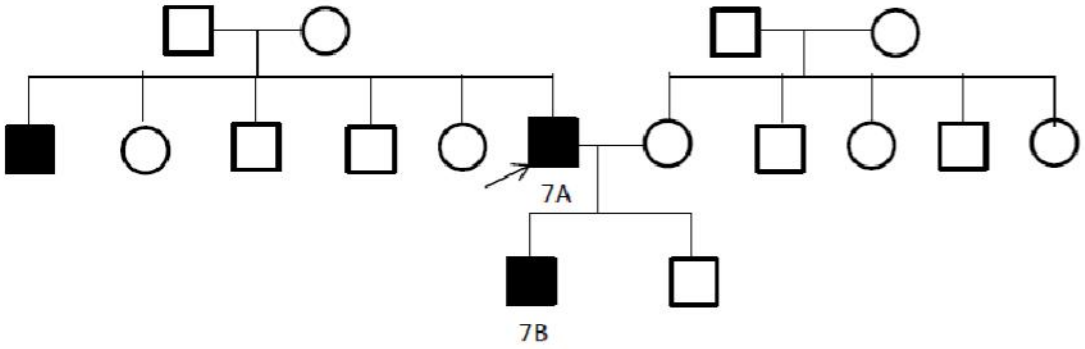
DKEG 1



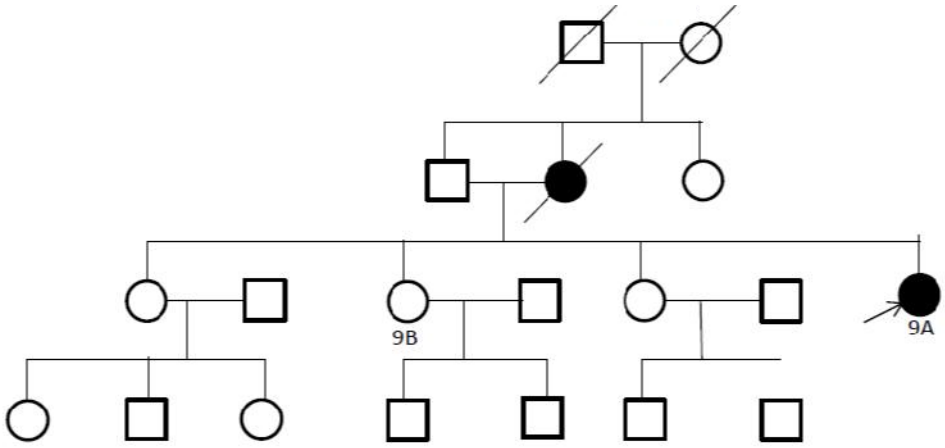
DKEG 6



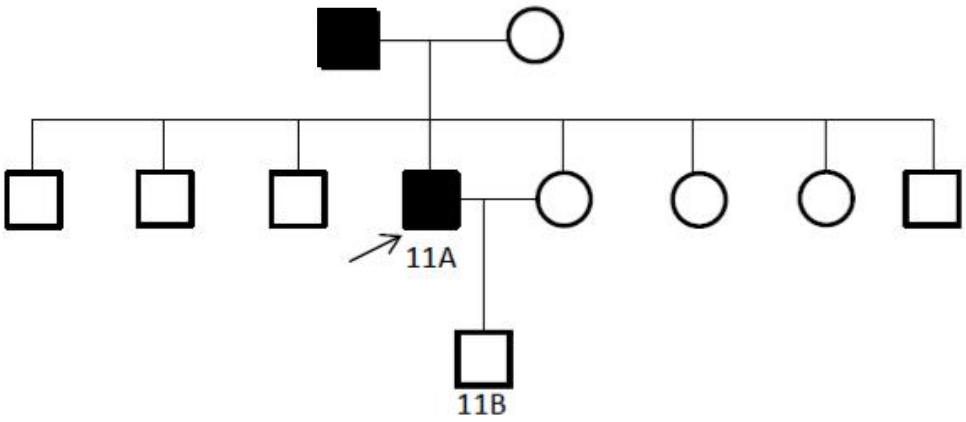
DKEG7



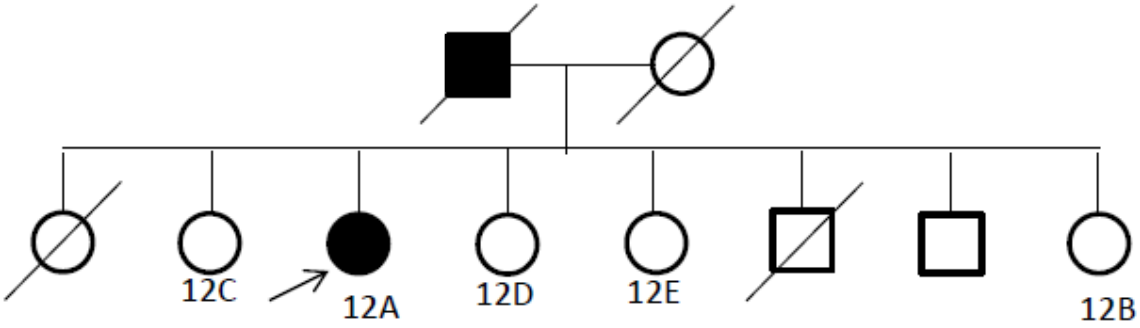
DKEG 9



DKEG 11



DKEG 12



were on topical antiglaucoma medications. 6 patients had undergone surgical treatment of which 2 patients had their IOP under surgical control while the remaining 4 were started on topical antiglaucoma medications due to poor surgical control of IOP.

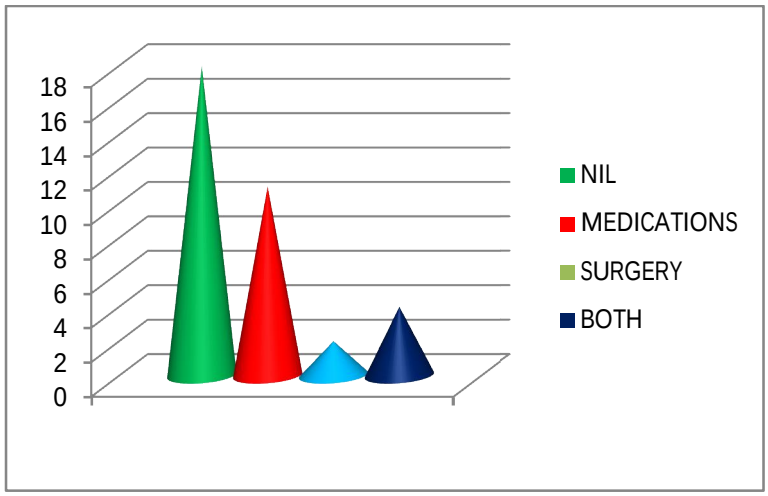


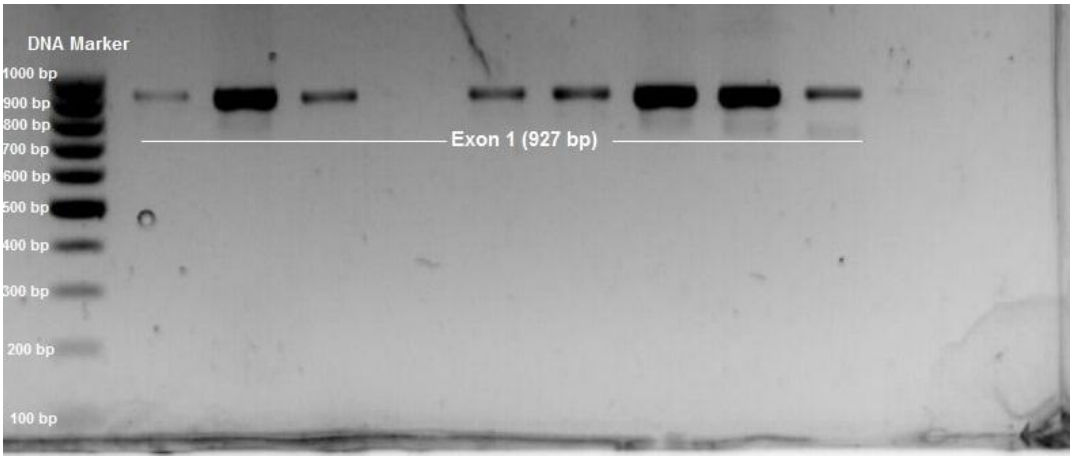
Figure 8 – Different modes of treatment the patients had underwent

DNA QUANTIFICATION DATA (Using nanodrop)

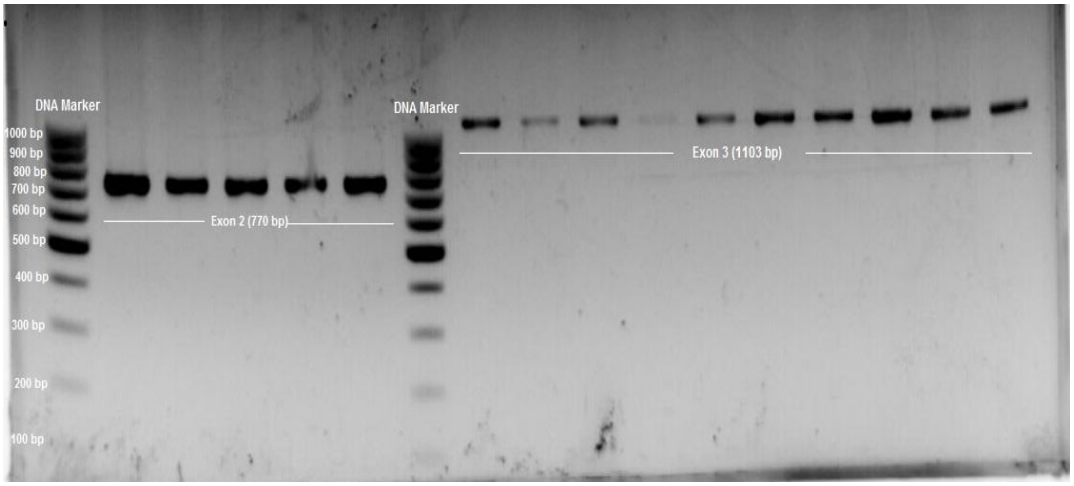
Sample ID	Nucleic acid concentration (ng/µl)	Absorbance ratio 260/280
DKEG1A	2468.0	1.81
DKEG1B	2055.9	1.87
DKEG1C	3480.0	1.80
DKEG1D	2046.2	1.88
DKEG2A	1655.8	1.84
DKEG2B	408.2	1.83
DKEG3A	1791.5	1.84
DKEG3B	1216.6	1.86

DKEG3C	1181.7	1.87
DKEG3D	360.5	1.81
DKEG4A	258.2	1.82
DKEG4B	1506.4	1.85
DKEG4C	2932.6	1.82
DKEG4D	1283.4	1.83
DKEG5A	271.0	1.84
DKEG5B	875.8	1.81
DKEG6A	1858.3	1.84
DKEG6B	733.8	1.82
DKEG6C	718.2	1.86
DKEG7A	3440.0	1.85
DKEG7B	1069.5	1.81
DKEG8A	4046.8	1.84
DKEG8B	2322.0	1.82
DKEG8C	3013.2	1.86
DKEG9A	2377.7	1.81
DKEG9B	2351.4	1.83
DKEG10A	2269.3	1.83
DKEG10B	121.5	1.78
DKEG11A	371.5	1.88
DKEG11B	1484.1	1.84
DKEG12A	2120.4	1.84
DKEG12B	2868.4	1.88
DKEG12C	1844.0	1.86
DKEG12D	2574.2	1.81
DKEG12E	2270.1	1.84

ELECTROPHEROGRAM OF MYOCILIN EXON 1



ELECTROPHEROGRAM OF MYOCILLIN EXON 2 AND EXON 3



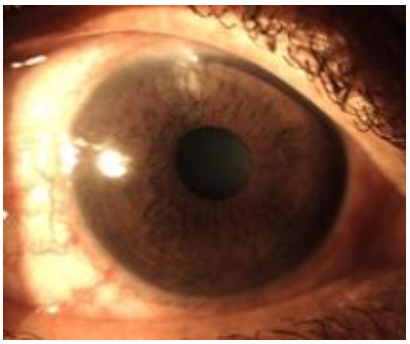
MUTATIONS/SNP'S IN MYOC IN SOUTH INDIAN PATIENTS ATTENDING OUR GLAUCOMA CLINIC

FAMILY ID	NUCLEOTIDE CHANGE	EXON	AMINO ACID CHANGE	GENOTYPE	INTRONIC VARIATIONS
DKEG1A	c.227G>A	Exon 1	R76K	Homozygous	NO
DKEG1A	c.605-374 C>G	-	-	Homozygous	YES
DKEG1A	c.605-280 T>G	-	-	Homozygous	YES
DKEG3A	c.605-374 C>G	-	-	Homozygous	YES
DKEG3A	c.605-332 G>A	-	-	Heterozygous	YES
DKEG3A	c.730+35 G>A	-	-	Homozygous	YES
DKEG4A	c.605-374 C>G	-	-	Homozygous	YES
DKEG4A	c.605-332 G>A	-	-	Heterozygous	YES
DKEG4A	c.605-280 T>G	-	-	Heterozygous	YES
DKEG4A	c.730+35 G>A	-	-	Homozygous	YES
DKEG5A	c.605-374 C>G	-	-	Homozygous	YES
DKEG5A	c.605-303 C>G	-	-	Heterozygous	YES
DKEG5A	c.605-280 T>G	-	-	Heterozygous	YES
DKEG5A	c.730+35 G>A	-	-	Heterozygous	YES
DKEG6A	c.227G>A	Exon 1	R76K	Heterozygous	NO
DKEG6A	c.605-374 C>G	-	-	Homozygous	YES
DKEG6A	c.605-280 T>G	-	-	Homozygous	YES
DKEG6A	c.730+35 G>A	-	-	Homozygous	YES

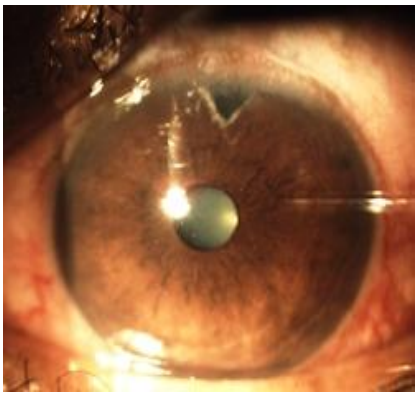
DKEG7A	c.227G>A	Exon 1	R76K	Heterozygous	NO
DKEG7A	c.604+136G>A	-	-	Homozygous	YES
DKEG7A	c.605-374 C>G	-	-	Homozygous	YES
DKEG7A	c.605-332 G>A	-	-	Heterozygous	YES
DKEG7A	c.605-280 T>G	-	-	Heterozygous	YES
DKEG7A	c.730+35 G>A	-	-	Homozygous	YES
DKEG9A	c.605-374 C>G	-	-	Homozygous	YES
DKEG9A	c.605-280 T>G	-	-	Heterozygous	YES
DKEG9A	c.730+35 G>A	-	-	Homozygous	YES
DKEG9A	c.1041 T>C	Exon 3	Y347Y	Heterozygous	NO
DKEG10A	c.605-374 C>G	-	-	Heterozygous	YES
DKEG10A	c.605-332 G>A	-	-	Heterozygous	YES
DKEG10A	c.730+35 G>A	-	-	Heterozygous	YES
DKEG11A	c.1102 C>T	Exon 3	Q368X	Heterozygous	NO
DKEG12A	c.227G>A	Exon 1	R76K	Homozygous	NO
DKEG12A	c.974 C>T	Exon 3	T325M	Heterozygous	NO
DKEG12A	c.1045 C>T	Exon 3	L349L	Heterozygous	NO
DKEG12A	c.1410 C>T	Exon 3	R470R	Heterozygous	NO

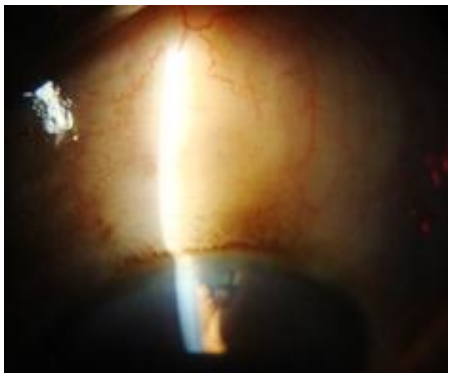
PHENOTYPIC PICTURE OF DKEG11A

RIGHT EYE WITH EXPRESS SHUNT



LEFT EYE SHOWING BLEB WITH PERIPHERAL IRIDECTOMY





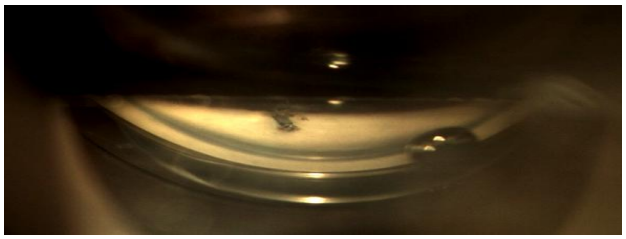
GONIOSCOPY

RIGHT EYE

I

N

T



S

I - Inferior angle as seen in superior mirror

S - Superior angle as seen in inferior mirror

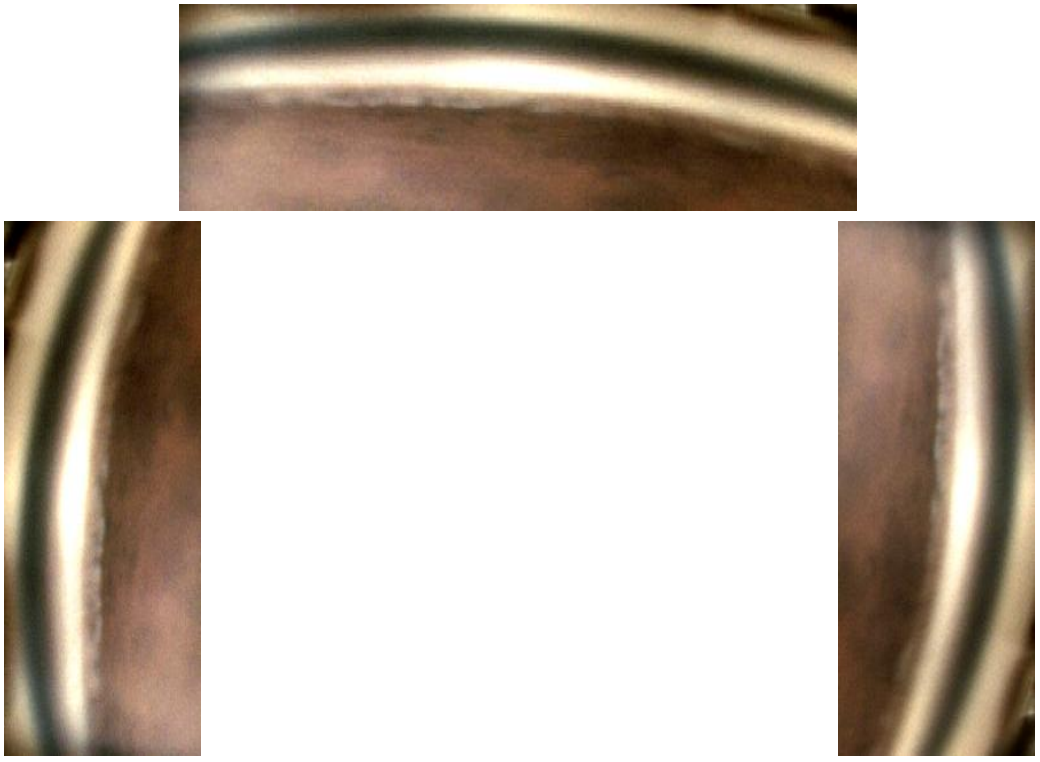
N - Nasal angle as seen in temporal mirror

T - Temporal angle as seen in nasal mirror

GONIOSCOPY

LEFT EYE

I



T

N

S

I - Inferior angle as seen in superior mirror

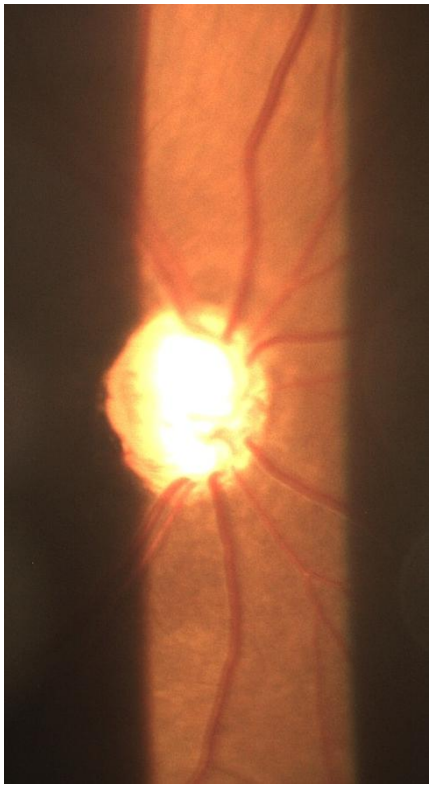
S - Superior angle as seen in inferior mirror

N - Nasal angle as seen in temporal mirror

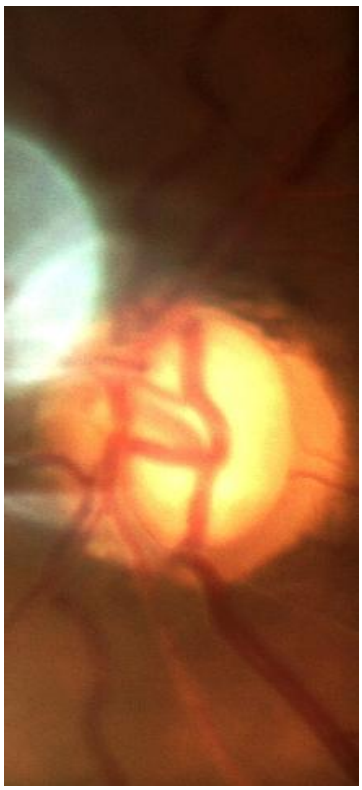
T - Temporal angle as seen in nasal mirror

OPTIC NERVE HEAD

RIGHT EYE



LEFT EYE



OPTICAL COHERENCE TOMOGRAPHY

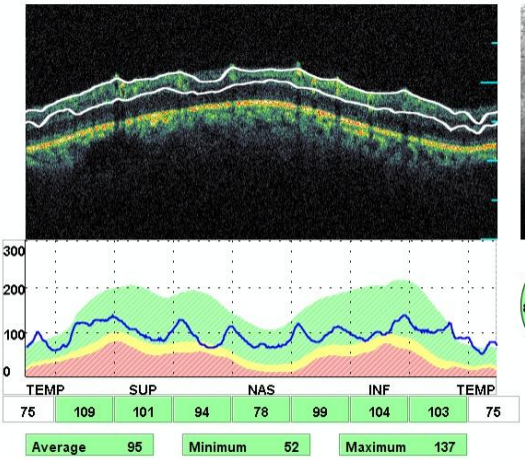
RETINAL NERVE FIBRE LAYER THICKNESS

RIGHT EYE

RIO-GOH ,EGMORE,CHENNAI

Patient Name: PREMANATHAN, R
Patient ID: GOP4803/13 GC NO 152/12
Description: **OD**

D.O.B.: Nov 30, 1955
Date: Dec 14, 2013

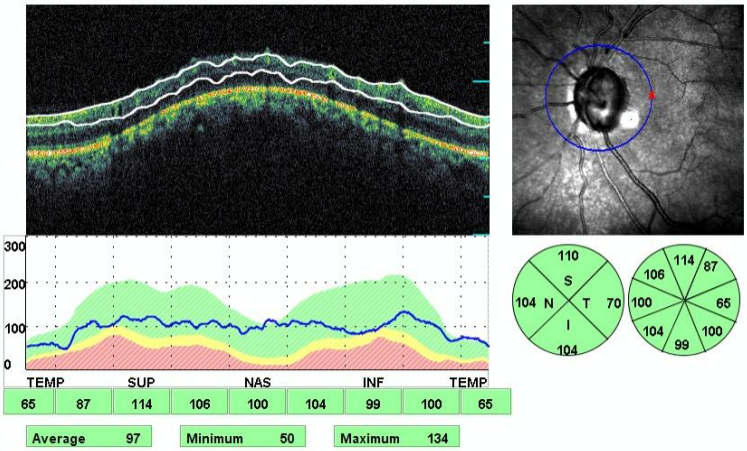


LEFT EYE

RIO-GOH ,EGMORE,CHENNAI

Patient Name: PREMANATHAN, R
Patient ID: GOP4803/13 GC NO 152/12
Description: **OS**

D.O.B.: Nov 30, 1955
Date: Dec 14, 2013



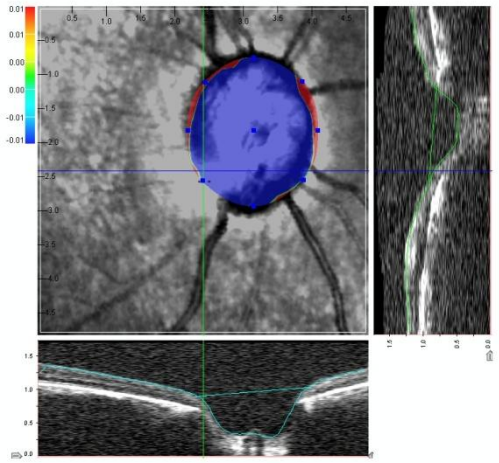
OPTIC NERVE HEAD TOPOGRAPHY

RIGHT EYE

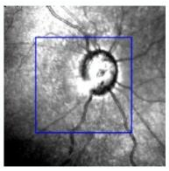
RIO-GOH ,EGMORE,CHENNAI

Patient Name: PREMANATHAN, R
Patient ID: GOP4803/13 GC NO 152/12
Description: **OD**

D.O.B.: Nov 30, 1955
Date: Dec 14, 2013



Full Field Image



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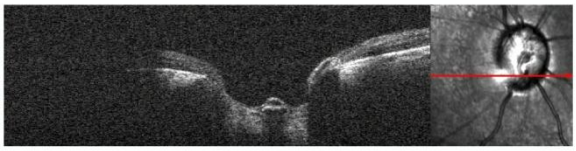
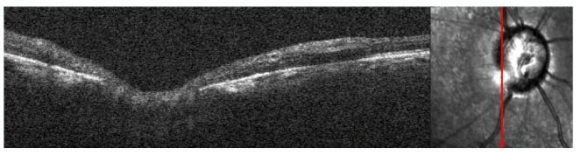
Disk area (mm²):	3.261	0.738	0.844	0.768	0.911
Cup area (mm²):	3.050	0.636	0.810	0.702	0.902
Rim area (mm²):	0.210	0.102	0.034	0.066	0.009
Cup/Disk horizontal ratio:	0.960				
Cup/Disk vertical ratio:	1.000				
Cup/Disk area ratio:	0.936	0.861	0.960	0.915	0.990
Mean cup depth (mm):	0.445				
Max cup depth (mm):	0.770				





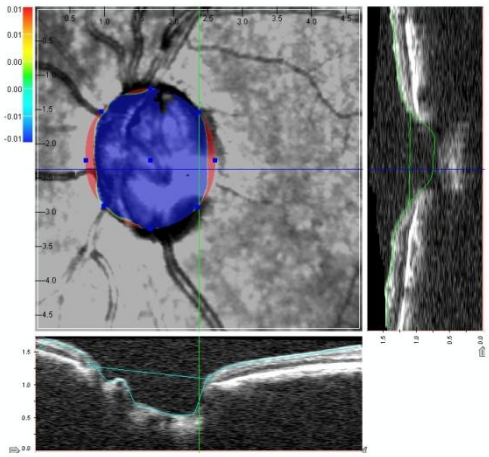
Comments:

LEFT EYE

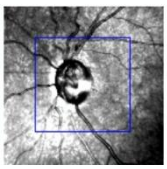
RIO-GOH ,EGMORE,CHENNAI

Patient Name: PREMANATHAN, R
Patient ID: GOP4803/13 GC NO 152/12
Description: **OS**

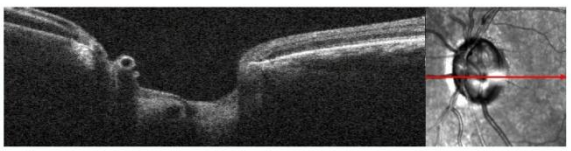
D.O.B.: Nov 30, 1955
Date: Dec 14, 2013

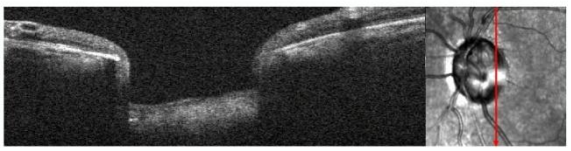


Full Field Image



	Global	N	I	T	S
Disk area (mm²):	2.959	0.699	0.808	0.704	0.748
Cup area (mm²):	2.651	0.572	0.736	0.610	0.732
Rim area (mm²):	0.308	0.126	0.072	0.094	0.016
Cup/Disk horizontal ratio:	0.881				
Cup/Disk vertical ratio:	1.000				
Cup/Disk area ratio:	0.896	0.819	0.911	0.866	0.979
Mean cup depth (mm):	0.396				
Max cup depth (mm):	0.825				





Comments:

Applied Biosystems

VIII114_1AE1_E1F_B06.ab1

VIII114_1AE1_E1F

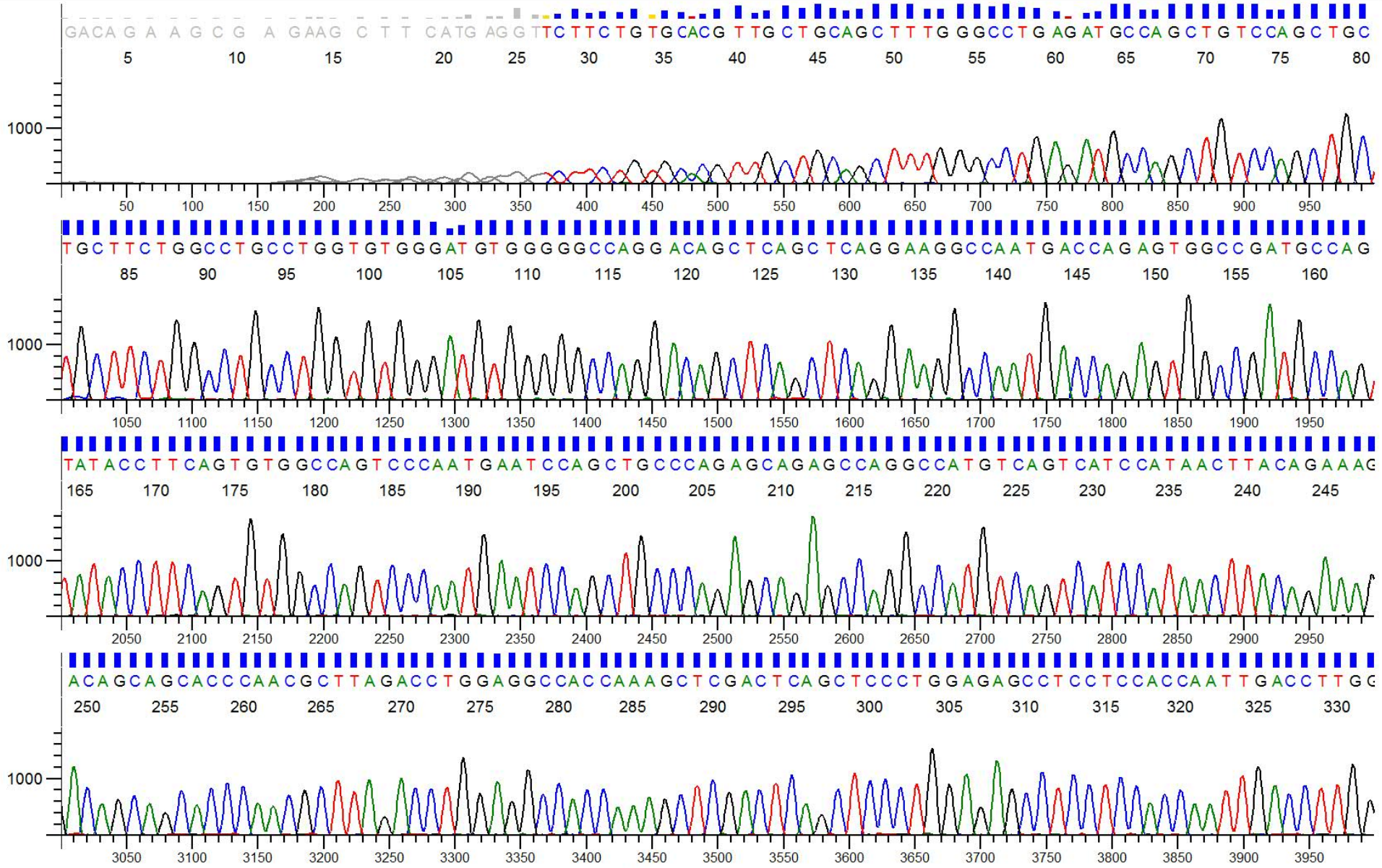
KB 1.2 KB.bcp

KB_3130_POP7_BDTv3.mob

Signal: G:1782 A:1248 T:877 C:1240 AvgSig: 1286

C#:4 W:B6 Plate Name:3008AMXL01

TS:52 CRL:853 QV20+:842



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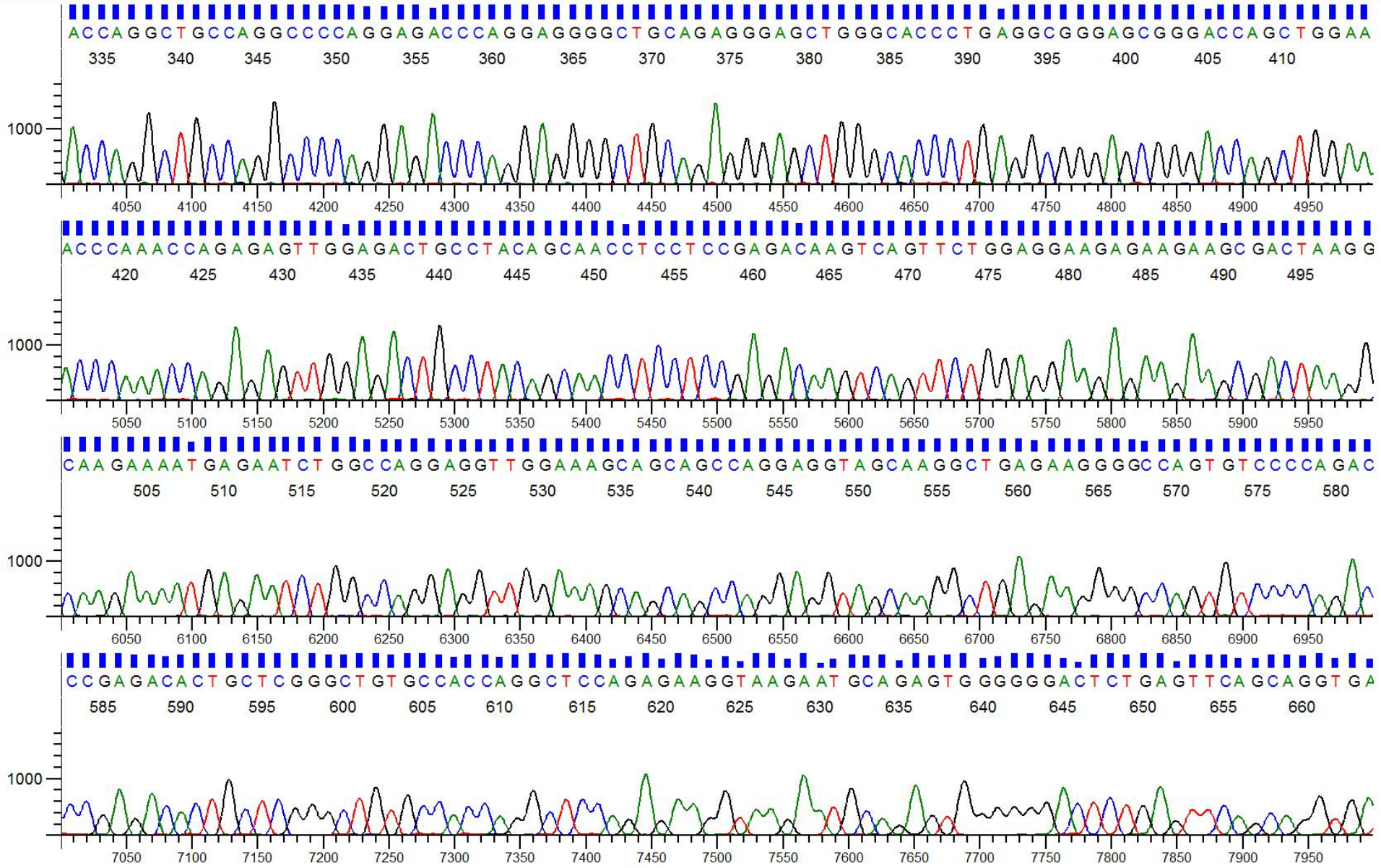
Pure Base QVs: 15 20

Mixed Base QVs: 10 16

Printed on: Aug 31,2013 04:30:37 GMT

Sequence Scanner v1.0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5050 5100 5150 5200 5250 5300 5350 5400 5450 5500 5550 5600 5650 5700 5750 5800 5850 5900 5950
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6050 6100 6150 6200 6250 6300 6350 6400 6450 6500 6550 6600 6650 6700 6750 6800 6850 6900 6950
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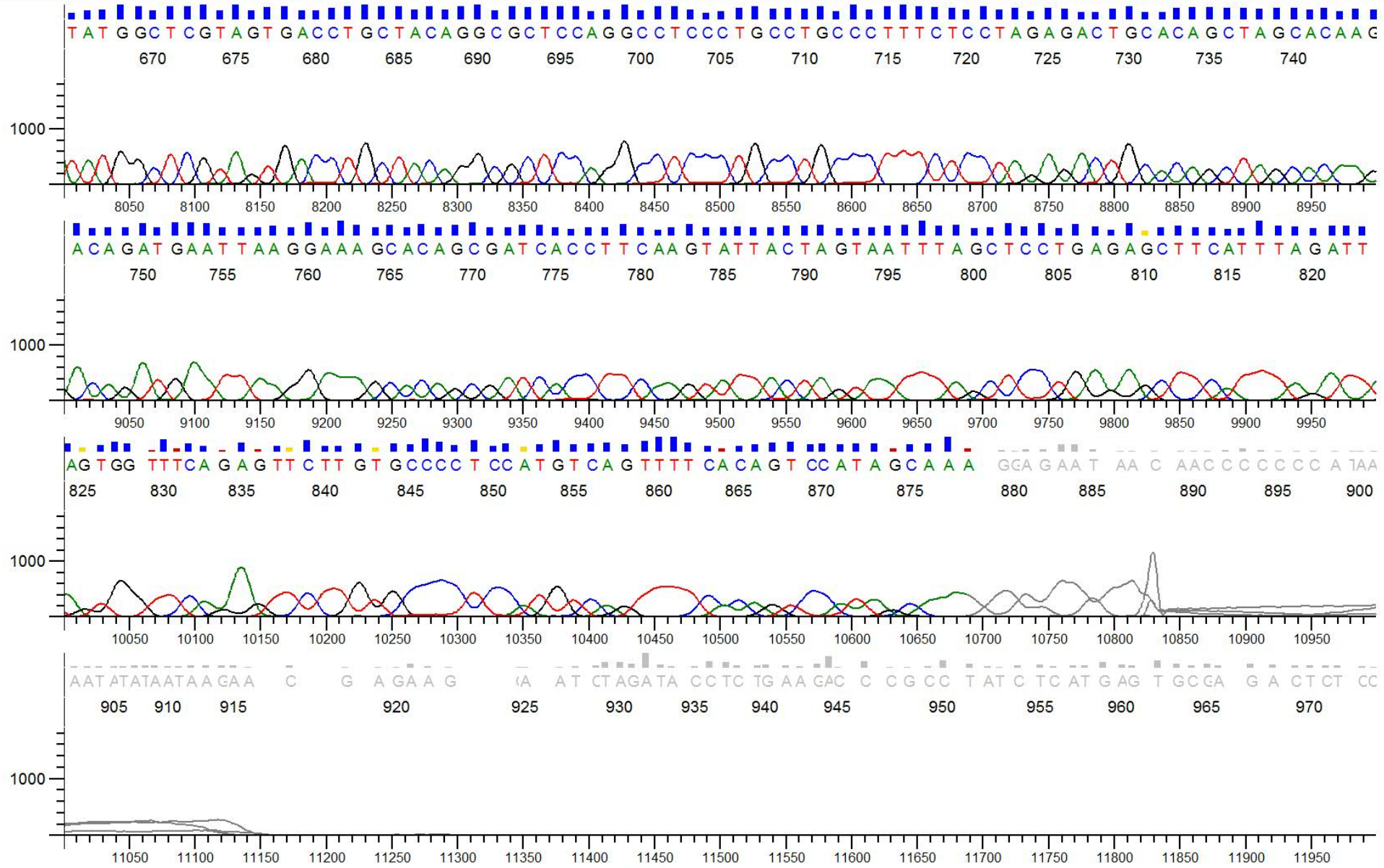
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1000
8050 8100 8150 8200 8250 8300 8350 8400 8450 8500 8550 8600 8650 8700 8750 8800 8850 8900 8950
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9050 9100 9150 9200 9250 9300 9350 9400 9450 9500 9550 9600 9650 9700 9750 9800 9850 9900 9950
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1000
10050 10100 10150 10200 10250 10300 10350 10400 10450 10500 10550 10600 10650 10700 10750 10800 10850 10900 10950
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905 910 915 920 925 930 935 940 945 950 955 960 965 970
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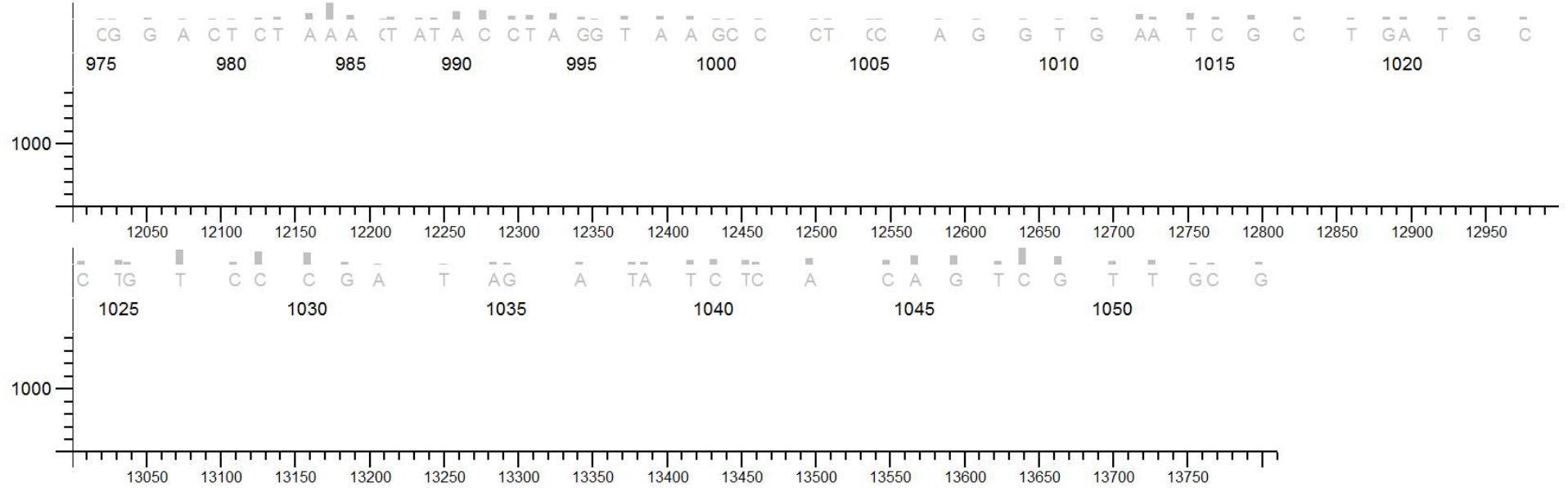
CG G A CT CT AAA CT ATA C CTA GG T A A GC C CT CC A G G T G AA T C G C T GA T G C
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C TG T C C C G A T AG A TA T C TC A C A G T C G T T GC G
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Applied Biosystems

VIII114_6AE1_E1F_F06.ab1

VIII114_6AE1_E1F

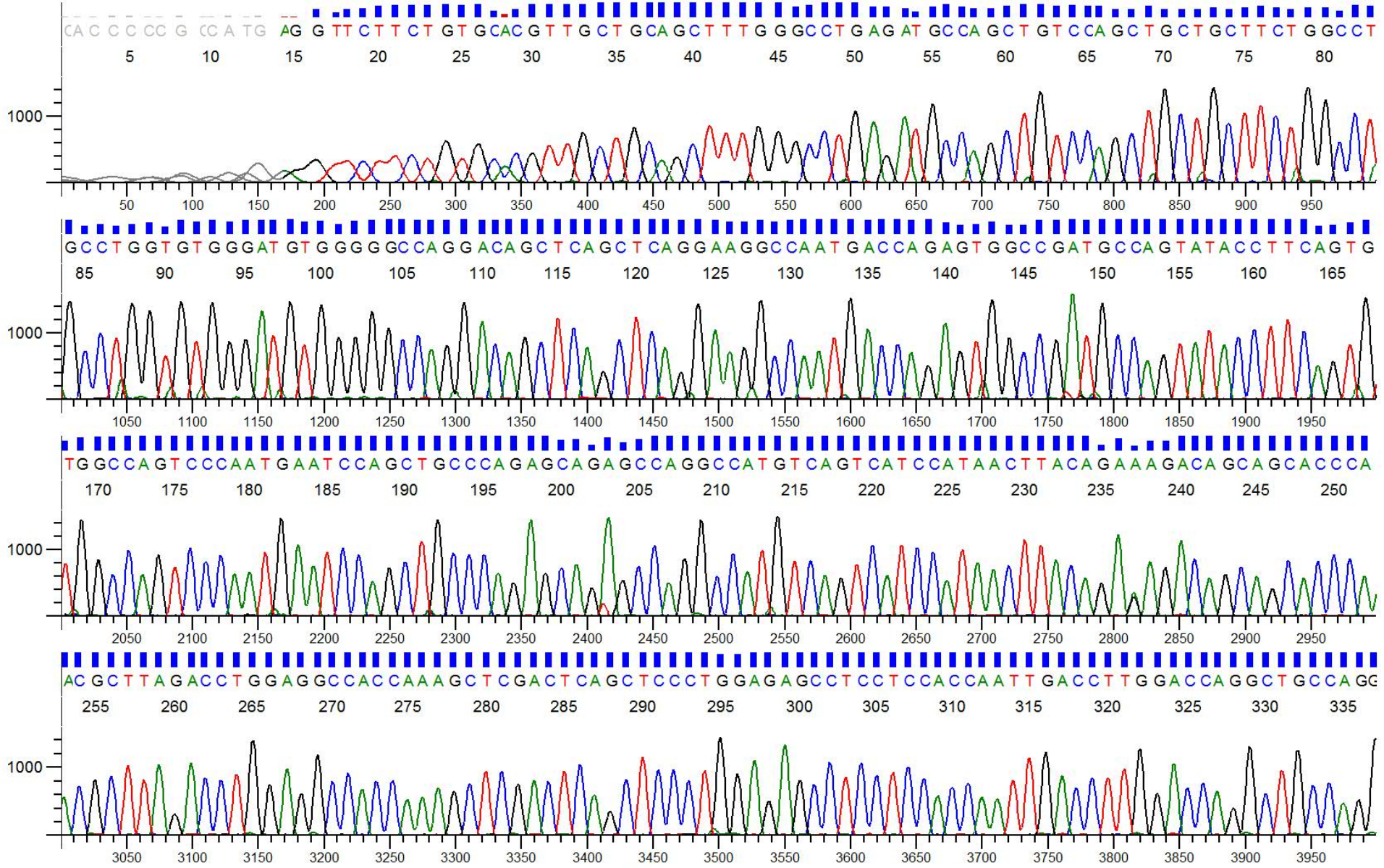
KB 1.2 KB.bcp

KB_3130_POP7_BDTv3.mob

Signal: G:2916 A:2223 T:1744 C:2377 AvgSig: 2315

C#:12 W:F6 Plate Name:3008AMXL01

TS:50 CRL:807 QV20+:823



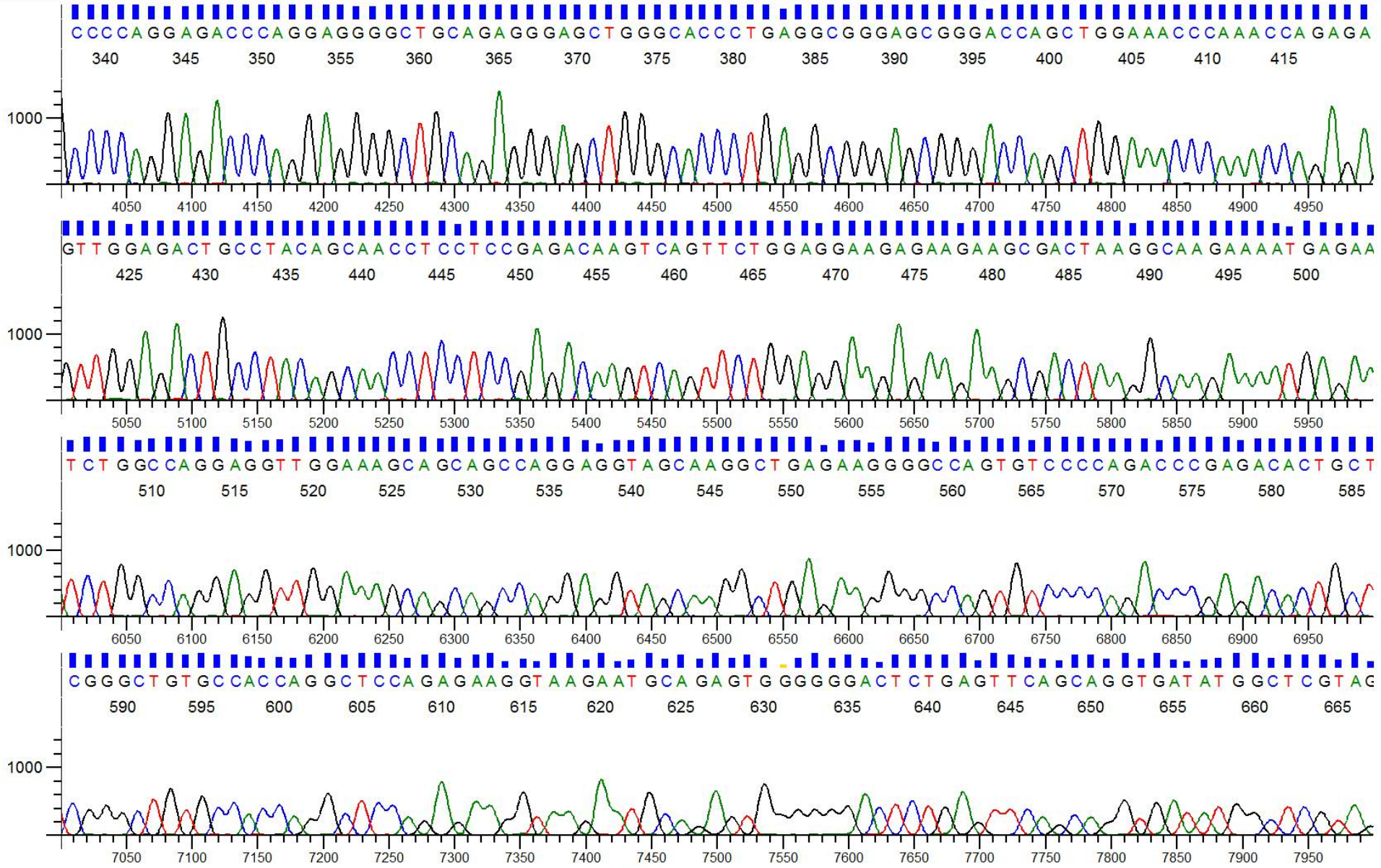
Inst Model/Name:3130xl/3130xlGA-1203-019

Sequence Scanner v1.0

Pure Base QVs: 15 20

Mixed Base QVs: 10 16

Printed on: Aug 31,2013 04:33:26 GMT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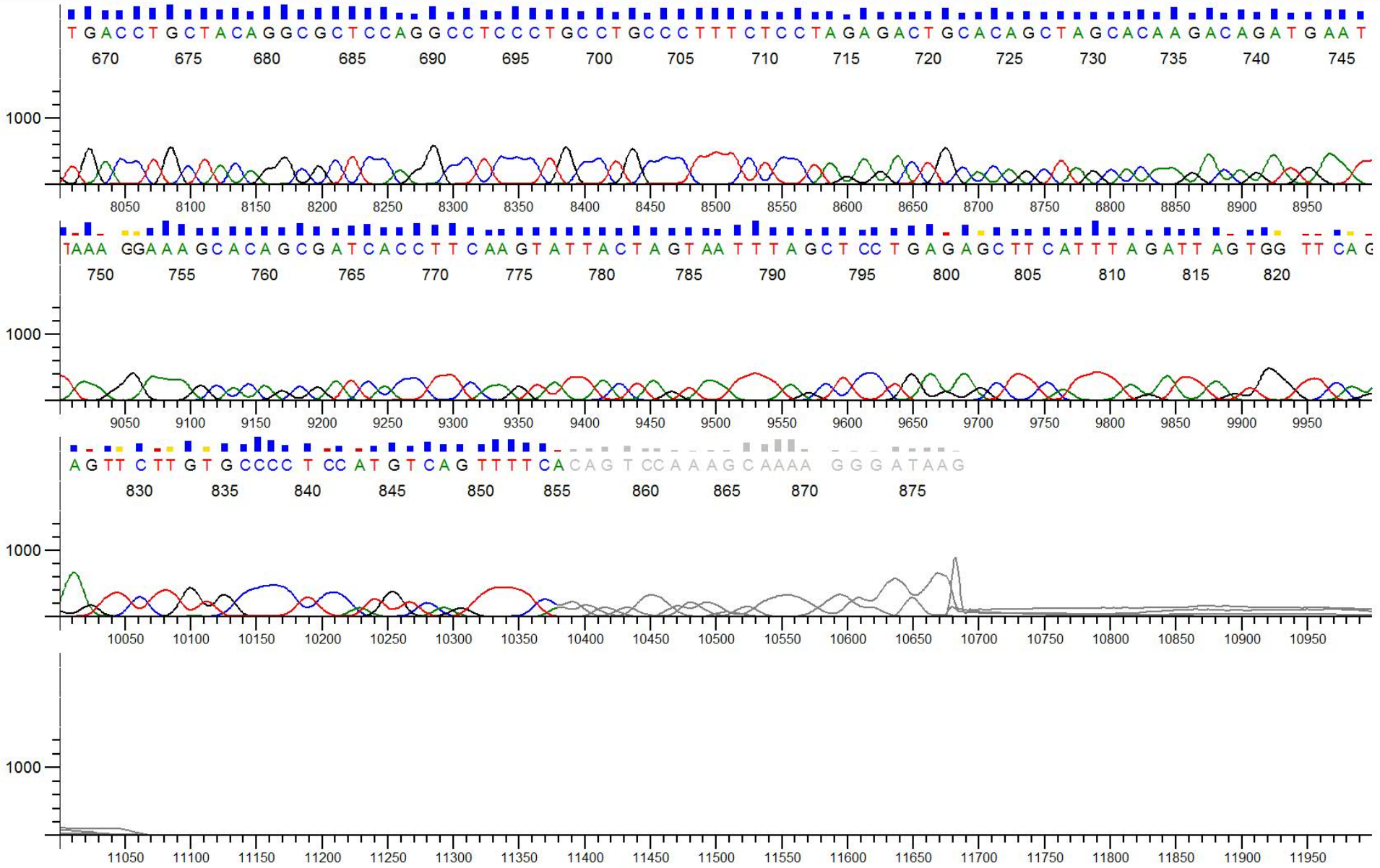
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670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745
1000
8050 8100 8150 8200 8250 8300 8350 8400 8450 8500 8550 8600 8650 8700 8750 8800 8850 8900 8950
T A A A G G A A A G C A C A G C G A T C A C C T T C A A G T A T T A C T A G T A A T T T A G C T C C T G A G A G C T T C A T T T A G A T T A G T G G T T C A G
750 755 760 765 770 775 780 785 790 795 800 805 810 815 820
1000
9050 9100 9150 9200 9250 9300 9350 9400 9450 9500 9550 9600 9650 9700 9750 9800 9850 9900 9950
A G T T C T T G T G C C C C T C C A T G T C A G T T T T C A C A G T C C A A A G C A A A A G G G A T A A G
830 835 840 845 850 855 860 865 870 875
1000
10050 10100 10150 10200 10250 10300 10350 10400 10450 10500 10550 10600 10650 10700 10750 10800 10850 10900 10950
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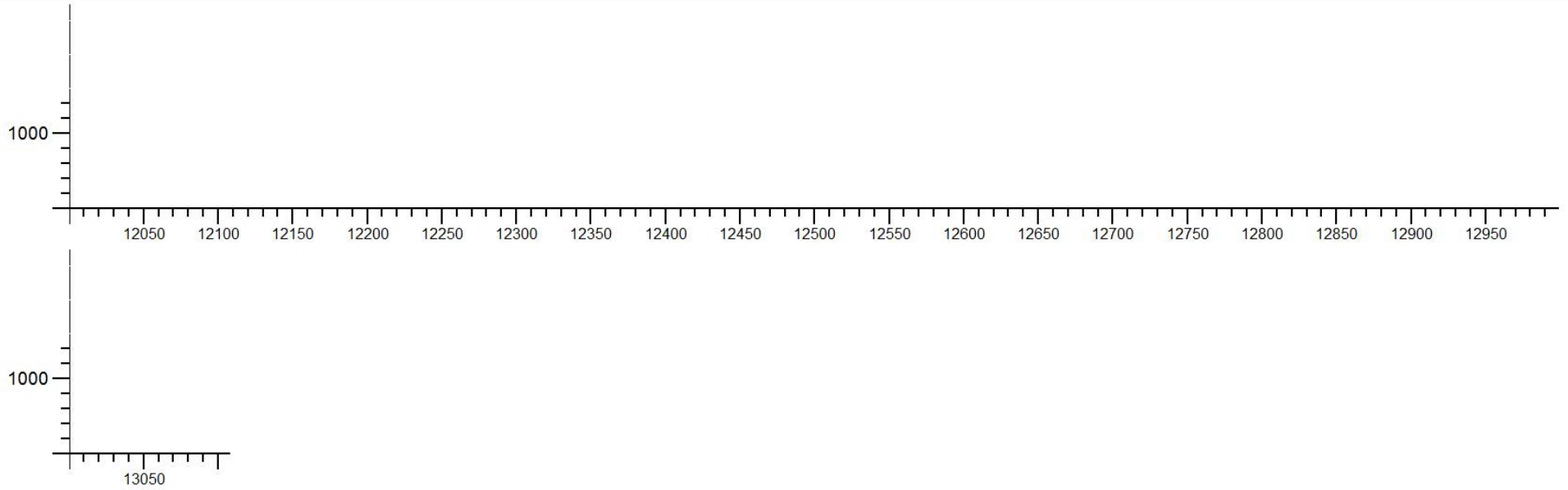
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Applied Biosystems

VIII114_7AE1_E1F_A08.ab1

VIII114_7AE1_E1F

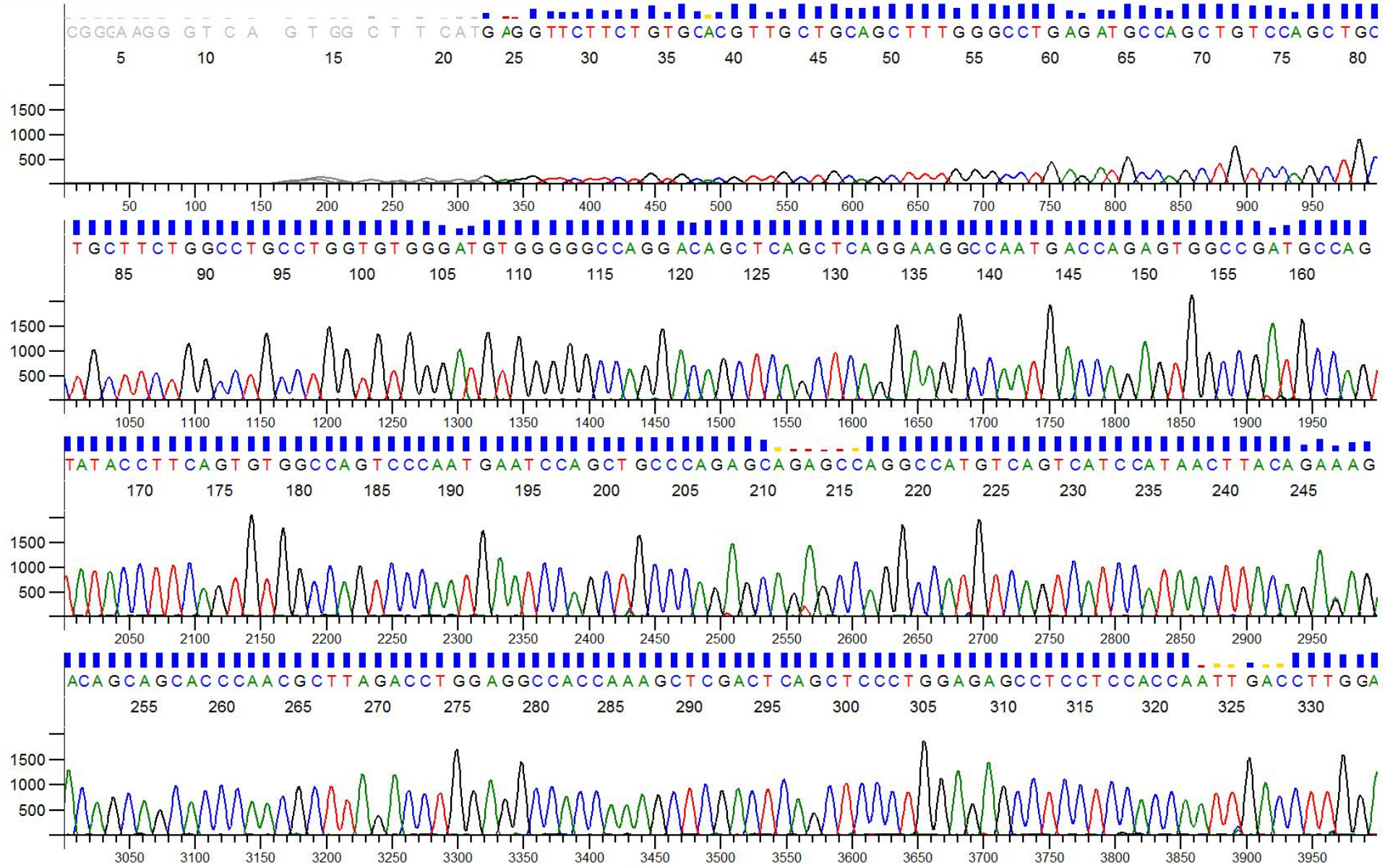
KB 1.2 KB.bcp

KB_3130_POP7_BDTv3.mob

Signal: G:1827 A:2308 T:2041 C:2750 AvgSig: 2231

C#:2 W:A8 Plate Name:0609AMXL02

TS:50 CRL:824 QV20+:808



Inst Model/Name:3130xl/3130xlGA-1203-019

Sequence Scanner v1.0

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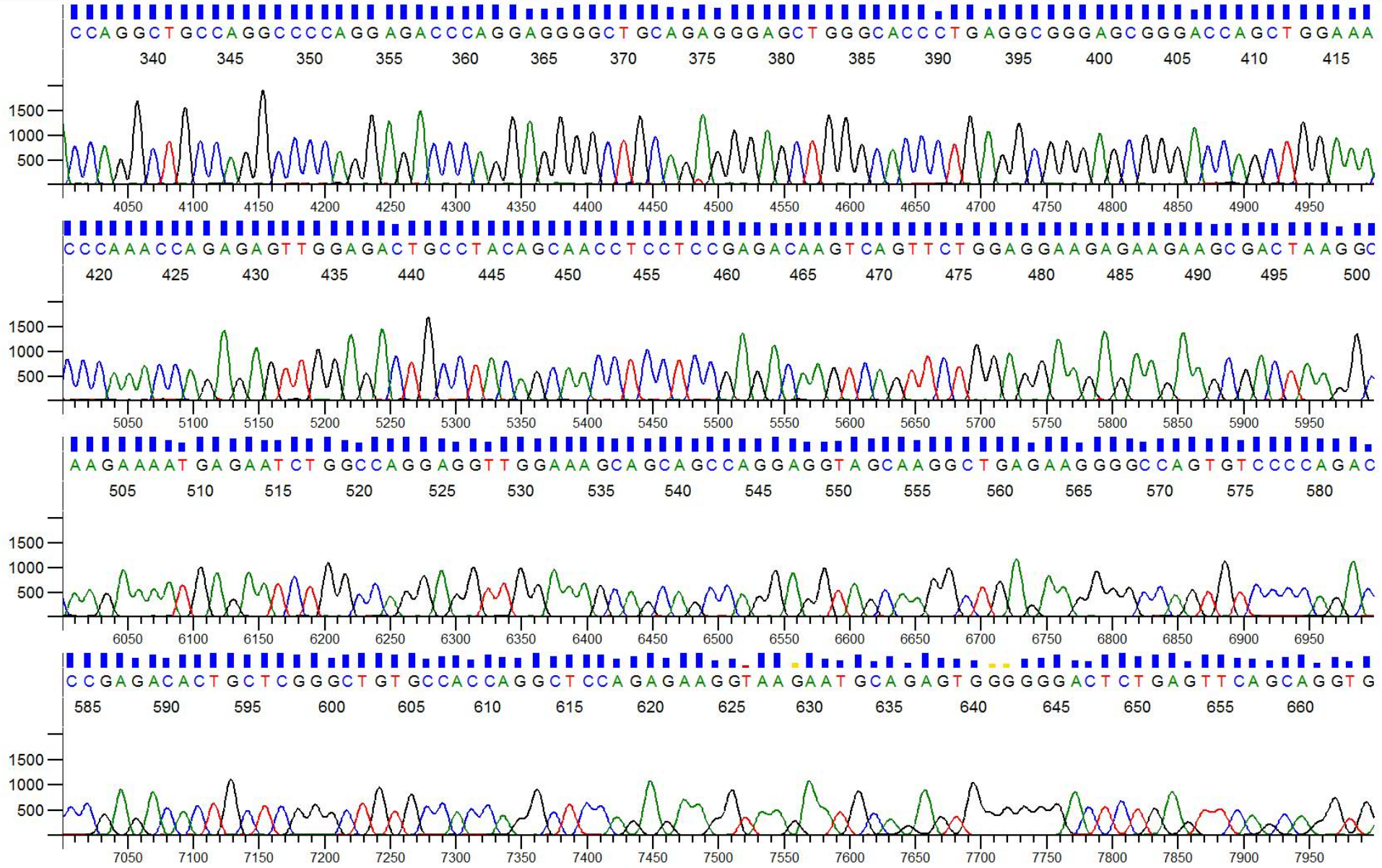
Mixed Base QVs: 10 16

Printed on: Sep 10,2013 10:06:58 GMT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1000
500
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1000
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5050 5100 5150 5200 5250 5300 5350 5400 5450 5500 5550 5600 5650 5700 5750 5800 5850 5900 5950
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1000
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6050 6100 6150 6200 6250 6300 6350 6400 6450 6500 6550 6600 6650 6700 6750 6800 6850 6900 6950
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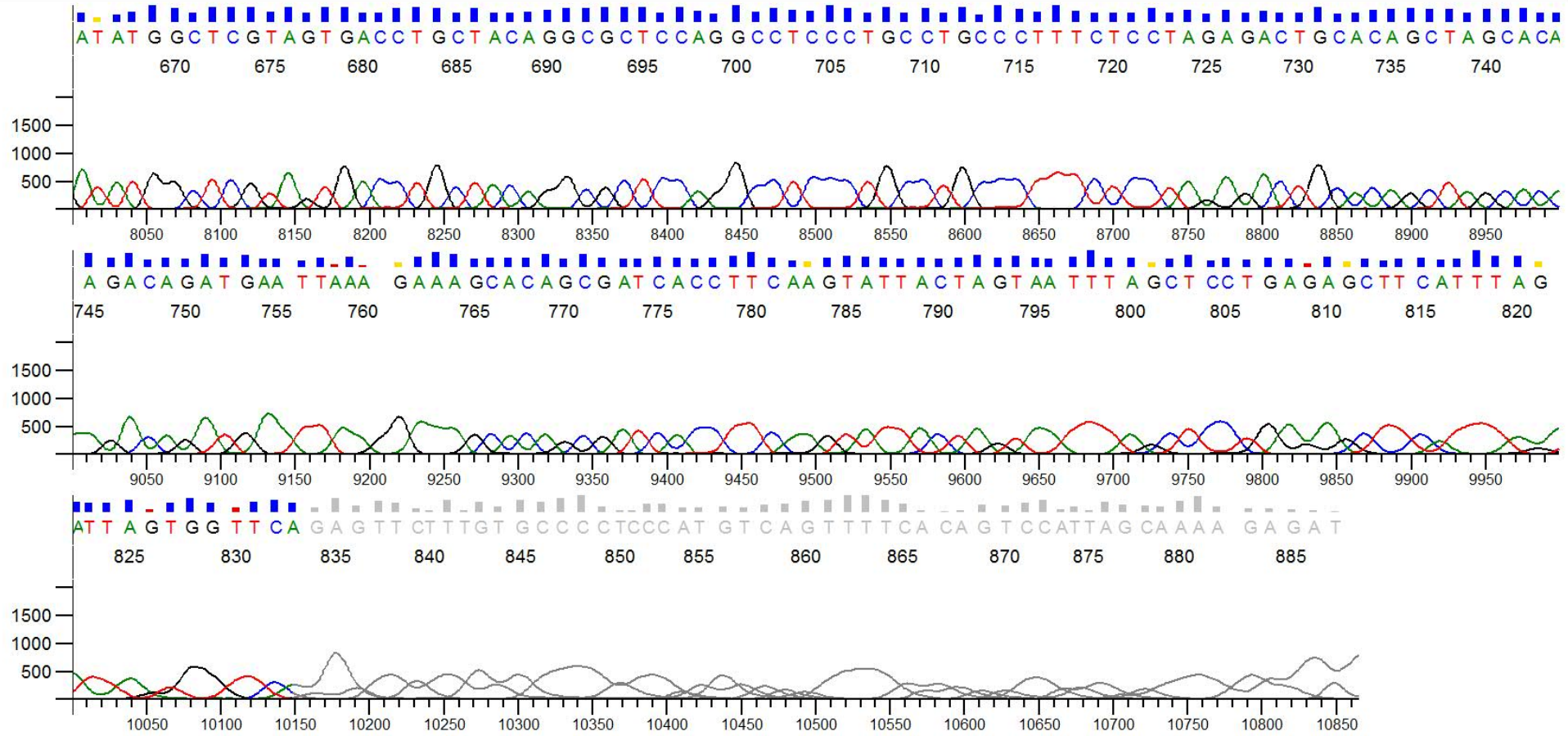
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1500
1000
500
8050 8100 8150 8200 8250 8300 8350 8400 8450 8500 8550 8600 8650 8700 8750 8800 8850 8900 8950
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745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820
1500
1000
500
9050 9100 9150 9200 9250 9300 9350 9400 9450 9500 9550 9600 9650 9700 9750 9800 9850 9900 9950
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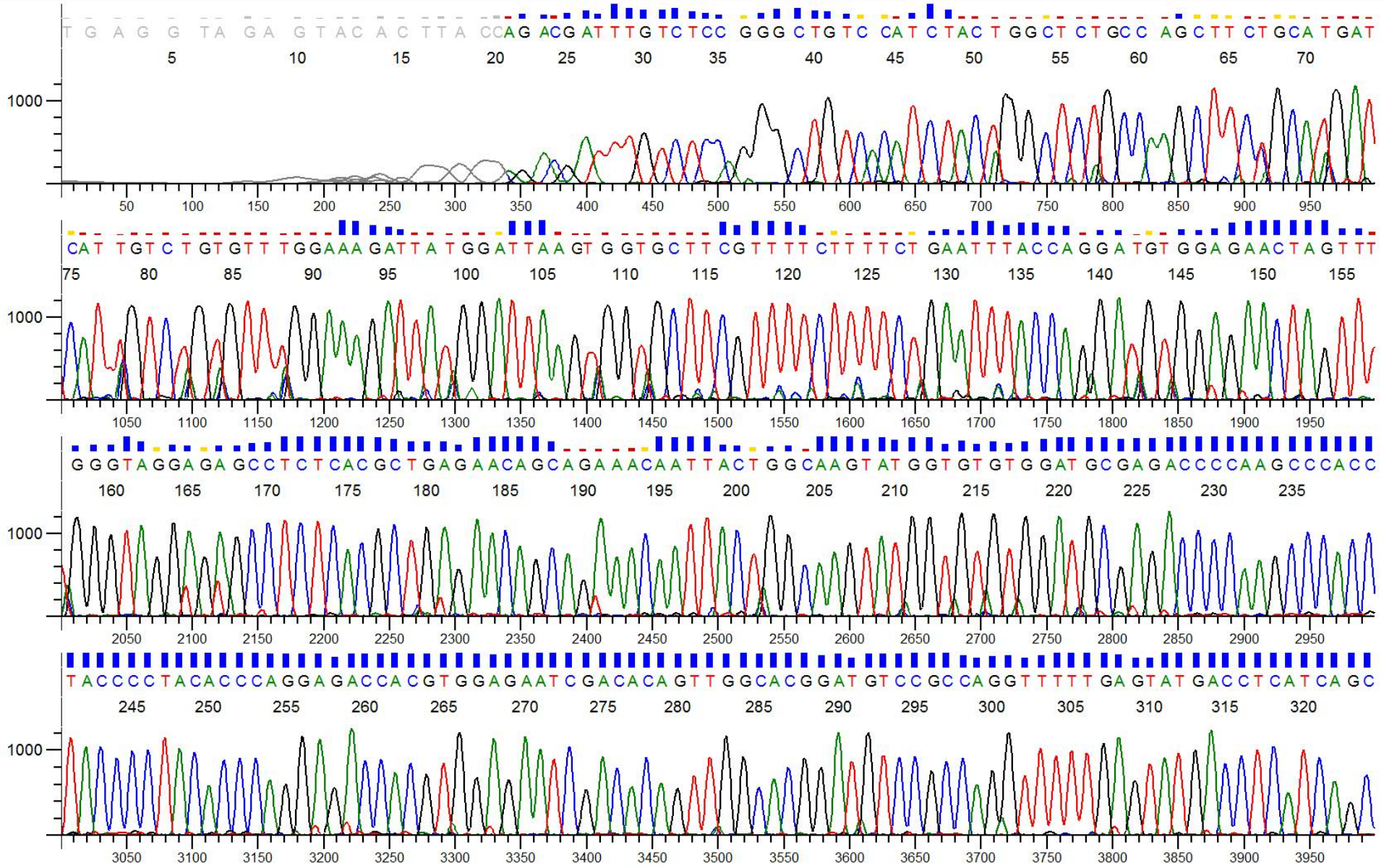
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75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155
1000
1050 1100 1150 1200 1250 1300 1350 1400 1450 1500 1550 1600 1650 1700 1750 1800 1850 1900 1950
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2050 2100 2150 2200 2250 2300 2350 2400 2450 2500 2550 2600 2650 2700 2750 2800 2850 2900 2950
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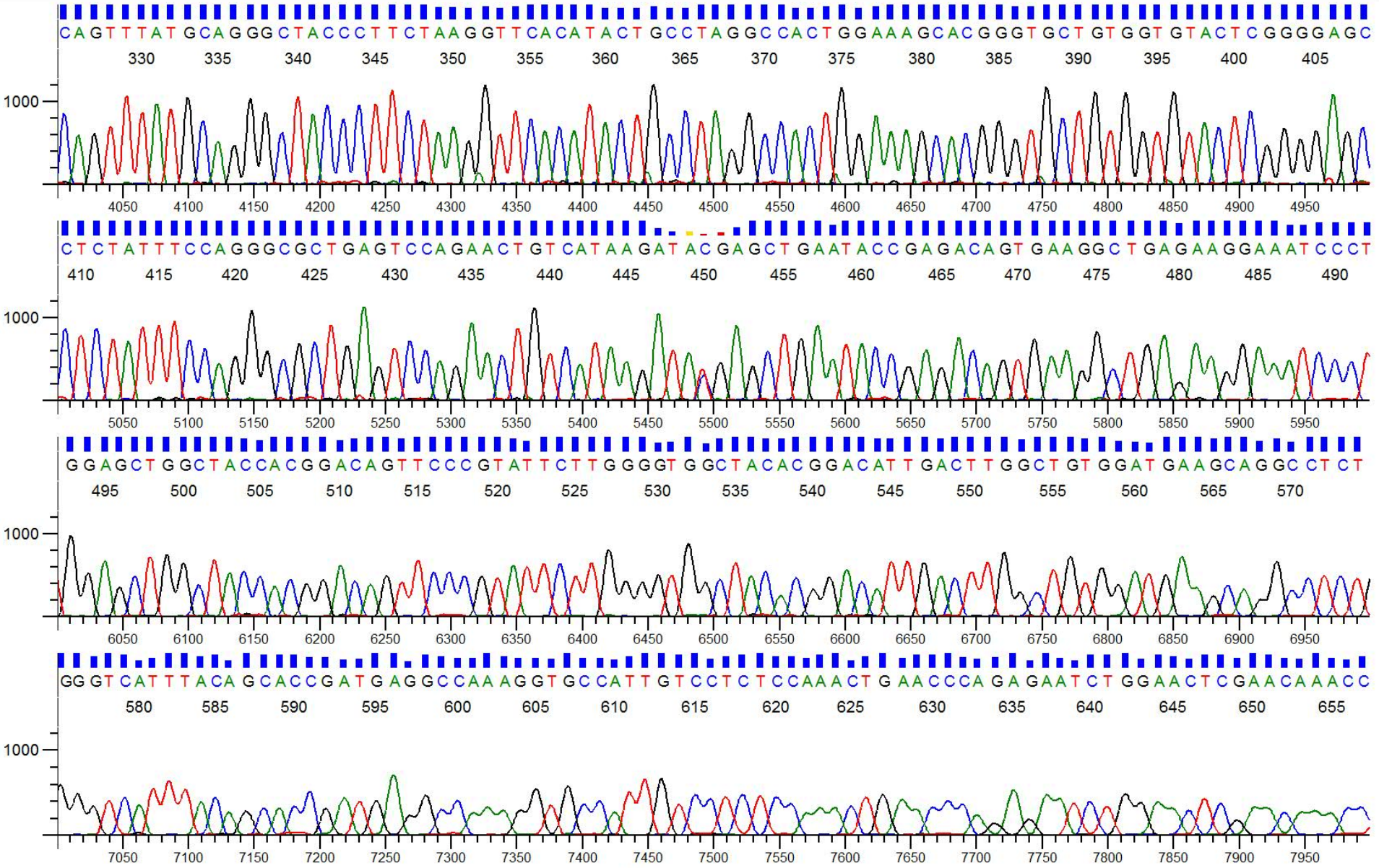
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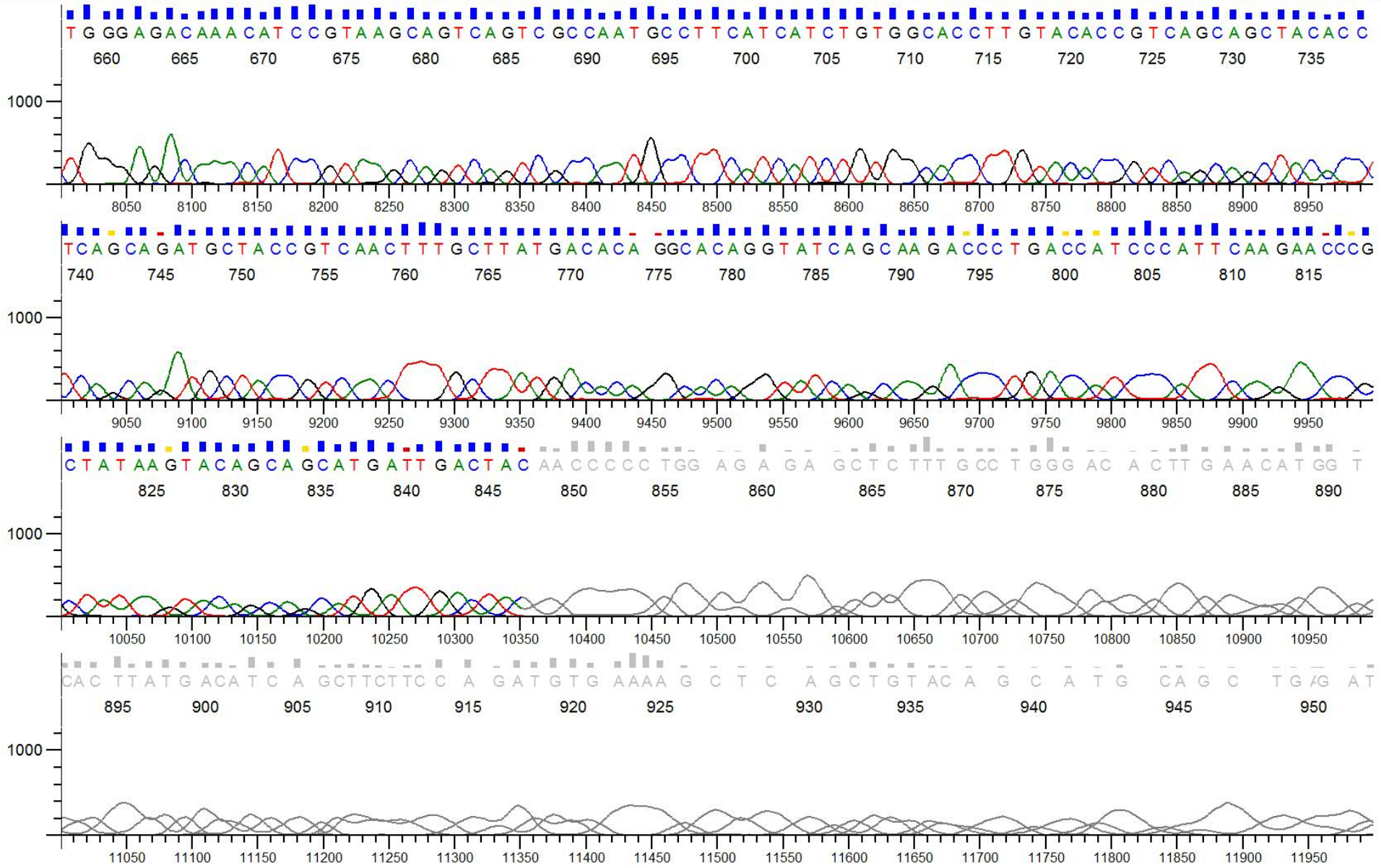
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660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735
1000
8050 8100 8150 8200 8250 8300 8350 8400 8450 8500 8550 8600 8650 8700 8750 8800 8850 8900 8950
T C A G C A G A T G C T A C C G T C A A C T T T G C T T A T G A C A C A GG C A C A G G T A T C A G C A A G A C C C T G A C C A T C C C C A T T C A A G A A C C C G
740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815
1000
9050 9100 9150 9200 9250 9300 9350 9400 9450 9500 9550 9600 9650 9700 9750 9800 9850 9900 9950
C T A T A A G T A C A G C A G C A T G A T T G A C T A C AA C C C C C TGG A G A G A GC T C TTT G CC T G GG AC A C TT G A A C A T GG T
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1000
10050 10100 10150 10200 10250 10300 10350 10400 10450 10500 10550 10600 10650 10700 10750 10800 10850 10900 10950
CA C TT A T G A CA T C A GC T TC T TC C A G A T G T G AAAA G C T C A G C T G T AC A G C A T G CA G C TG G A T
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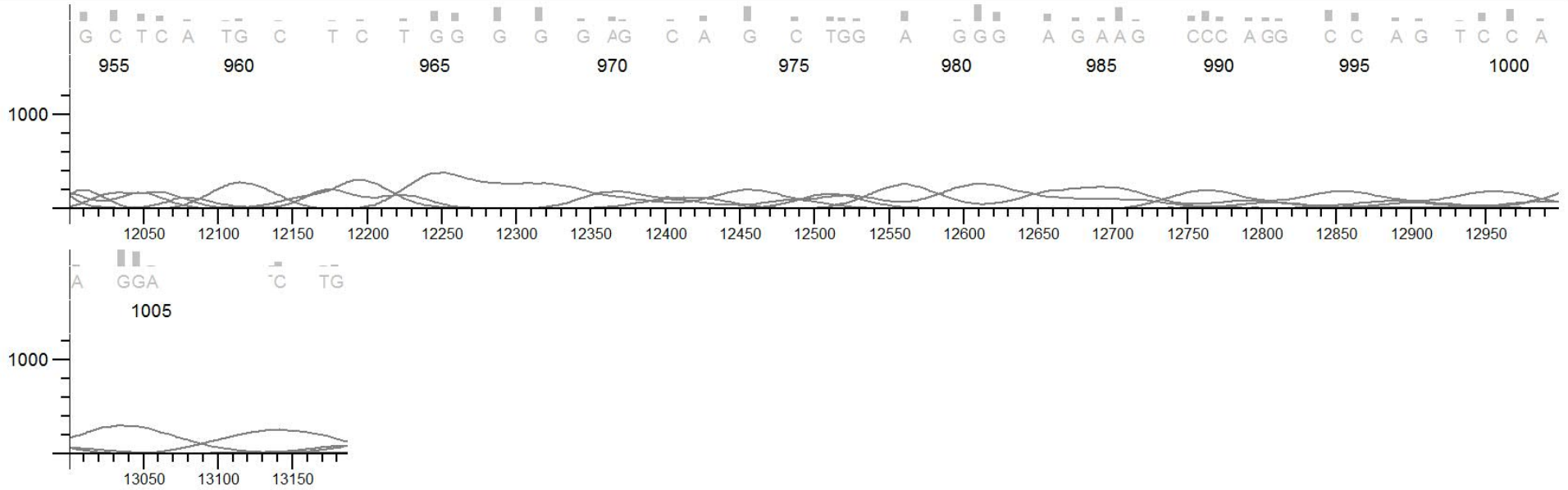
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12950
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Applied Biosystems

VIII114_11AE3_E3F_H08.ab1

VIII114_11AE3_E3F

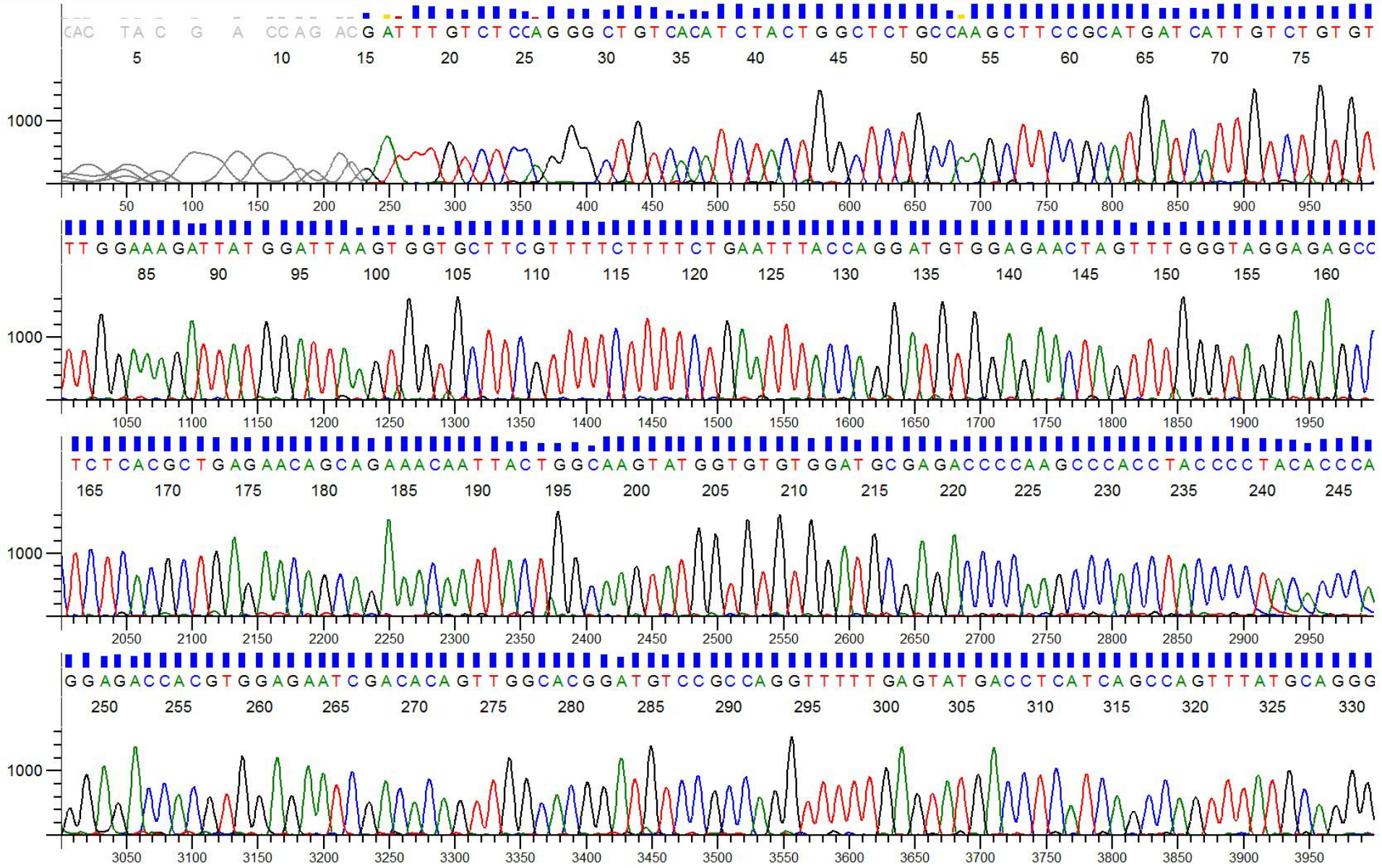
KB 1.2 KB.bcp

KB_3130_POP7_BDTv3.mob

Signal: G:2279 A:1509 T:1375 C:1463 AvgSig: 1656

C#:16 W:H8 Plate Name:3008AMXL01

TS:50 CRL:749 QV20+:728



Inst Model/Name:3130xl/3130xlGA-1203-019

Sequence Scanner v1.0

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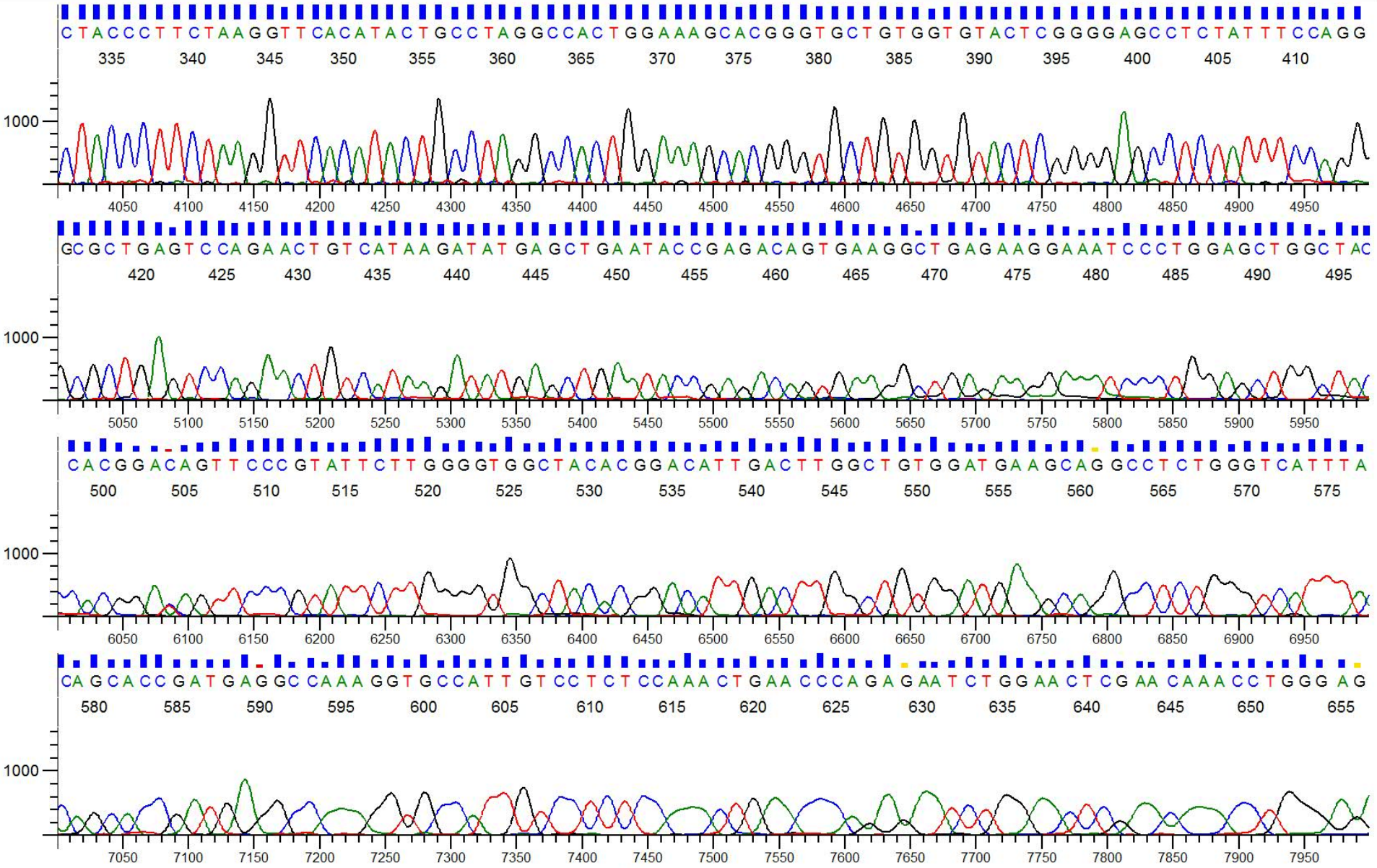
Mixed Base QVs: 10 16

Printed on: Aug 31,2013 04:30:19 GMT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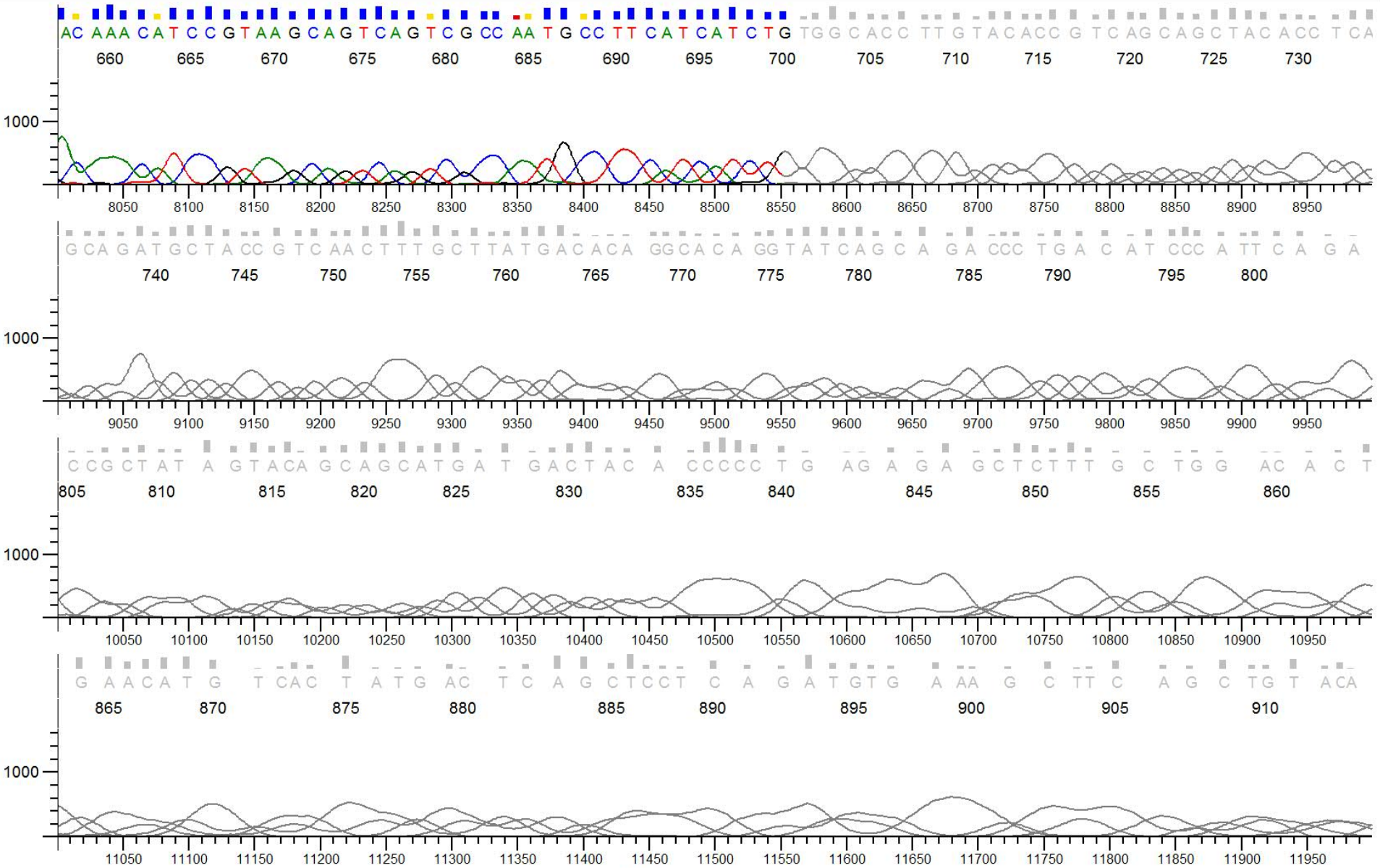
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9050 9100 9150 9200 9250 9300 9350 9400 9450 9500 9550 9600 9650 9700 9750 9800 9850 9900 9950
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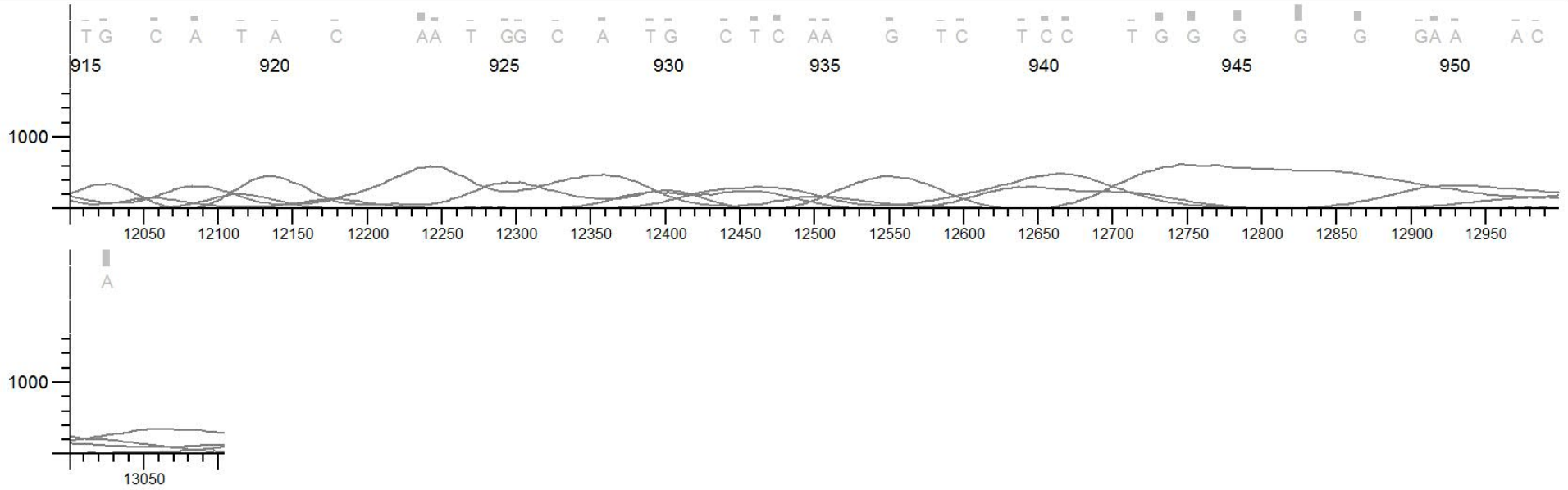
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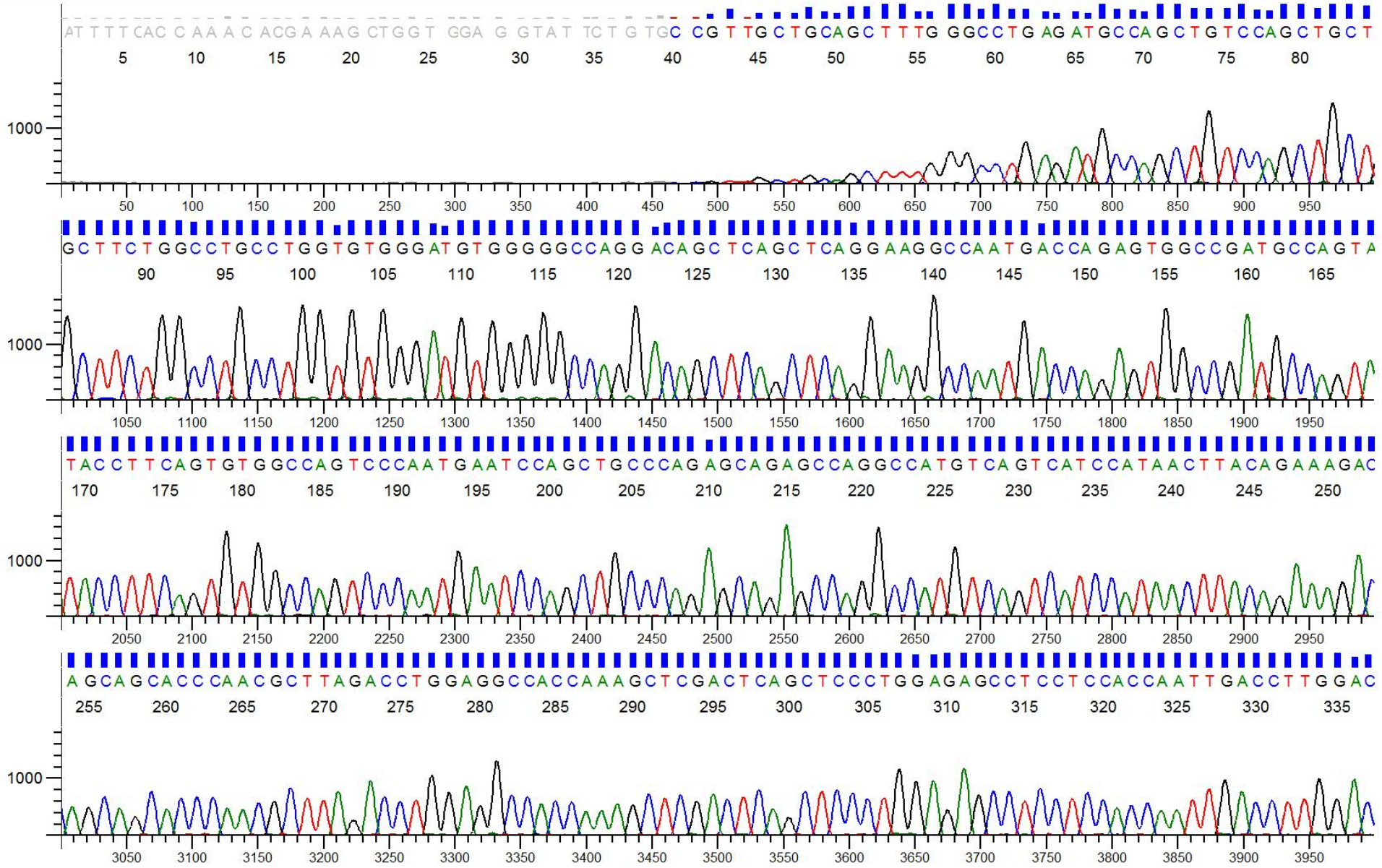
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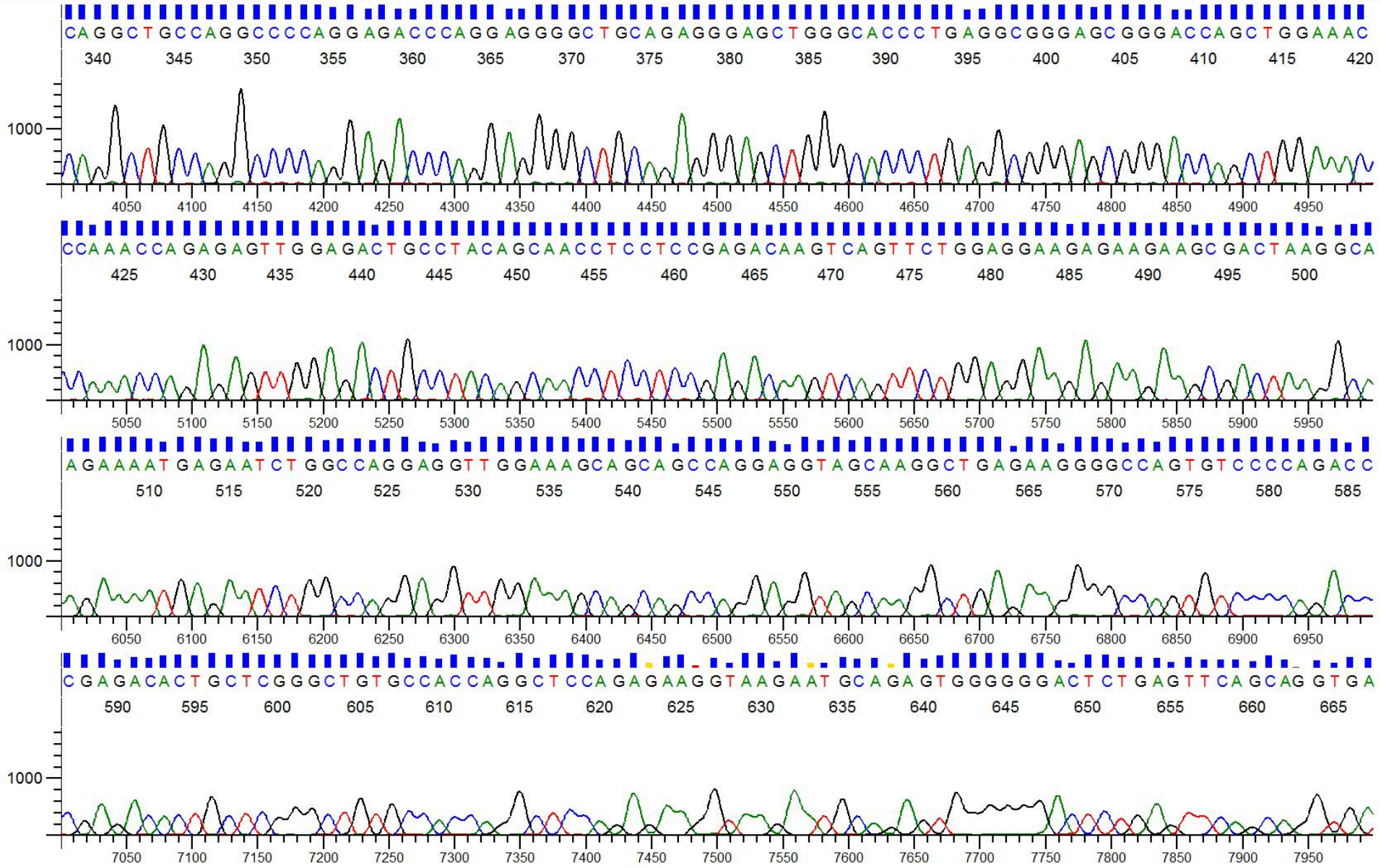

Inst Model/Name:3130xl/3130xlGA-1203-019

Sequence Scanner v1.0

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Mixed Base QVs: 10 16

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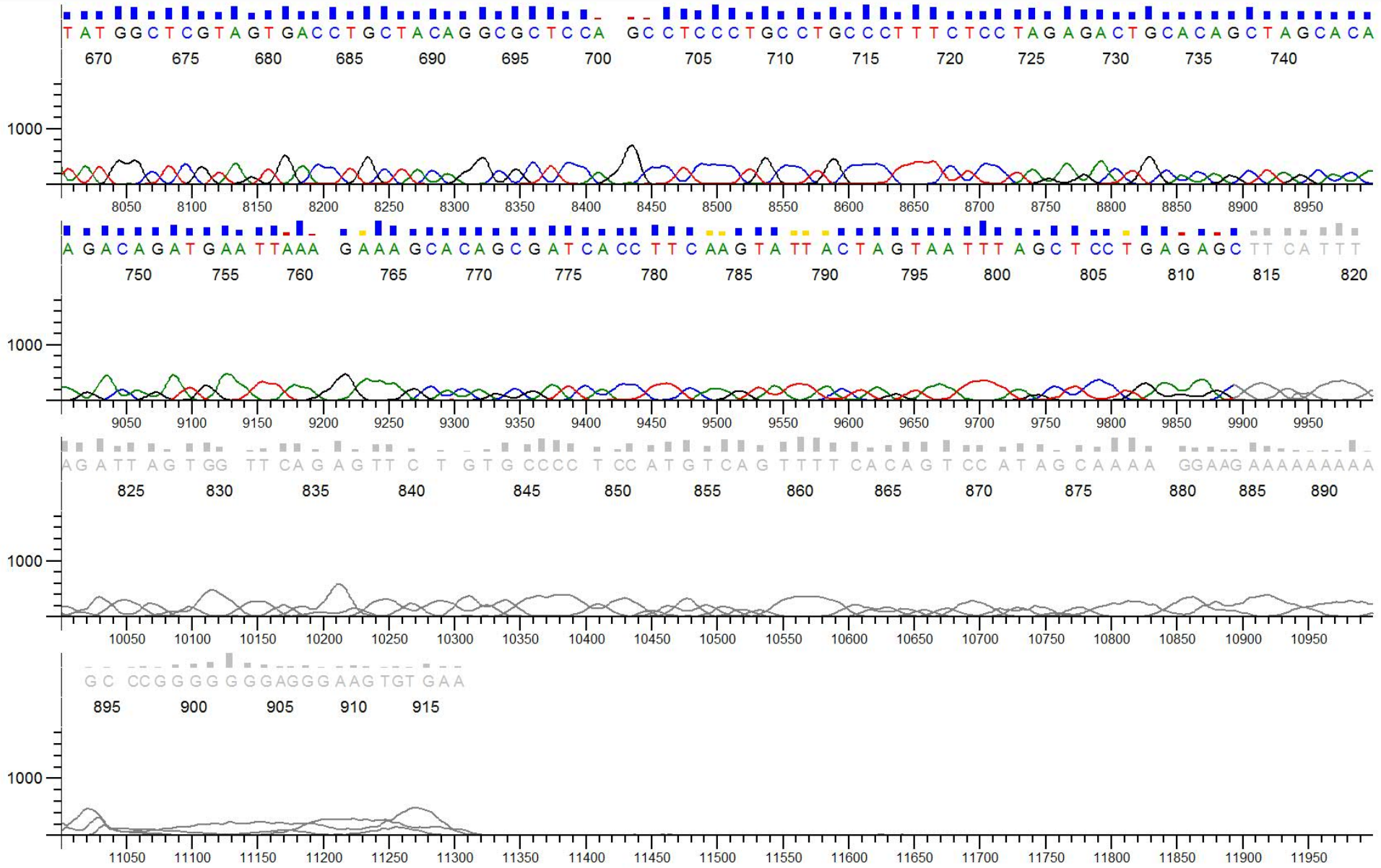
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670 675 680 685 690 695 700 705 710 715 720 725 730 735 740
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8050 8100 8150 8200 8250 8300 8350 8400 8450 8500 8550 8600 8650 8700 8750 8800 8850 8900 8950
A G A C A G A T G A A T T A A A G A A A G C A C A G C G A T C A C C T T C A A G T A T T A C T A G T A A T T T A G C T C C T G A G A G C T T C A T T T
750 755 760 765 770 775 780 785 790 795 800 805 810 815 820
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A G A T T A G T G G T T C A G A G T T C T G T G C C C C T C C A T G T C A G T T T T C A C A G T C C A T A G C A A A A G G A A G A A A A A A A A A
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10050 10100 10150 10200 10250 10300 10350 10400 10450 10500 10550 10600 10650 10700 10750 10800 10850 10900 10950
G C C C G G G G G G A G G G A A G T G T G A A
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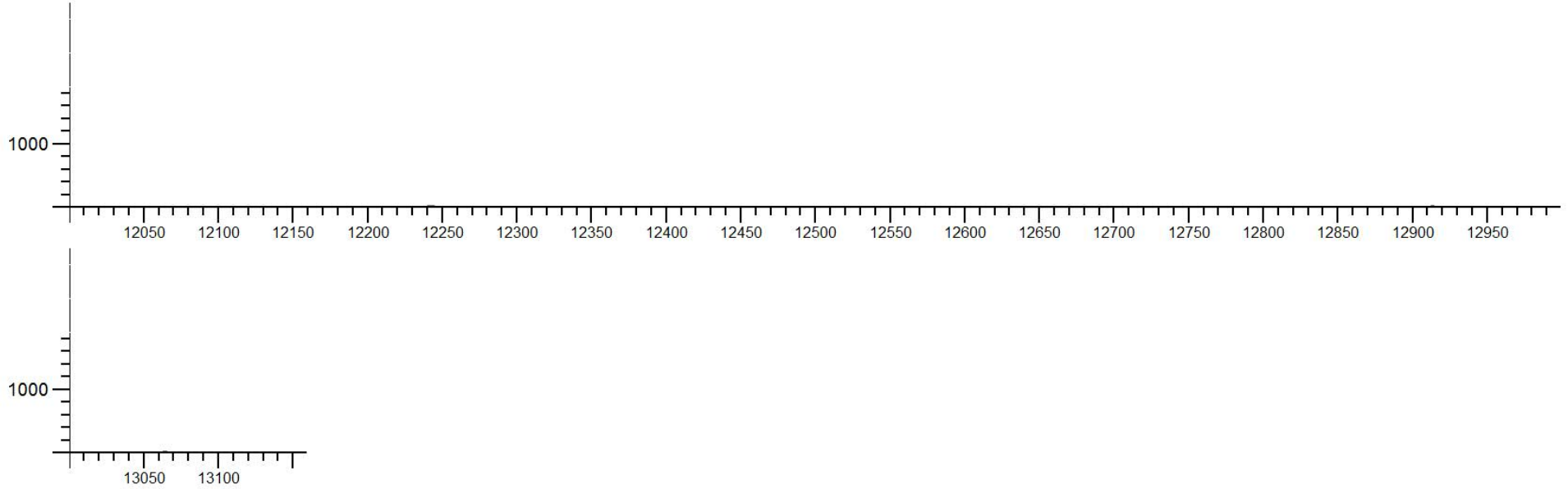
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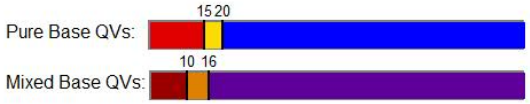
15 20
Pure Base QVs:
10 16
Mixed Base QVs:

Applied Biosystems

IX44_12AE3_E3R_G08.ab1

IX44_12AE3_E3R

KB 1.2 KB.bcp

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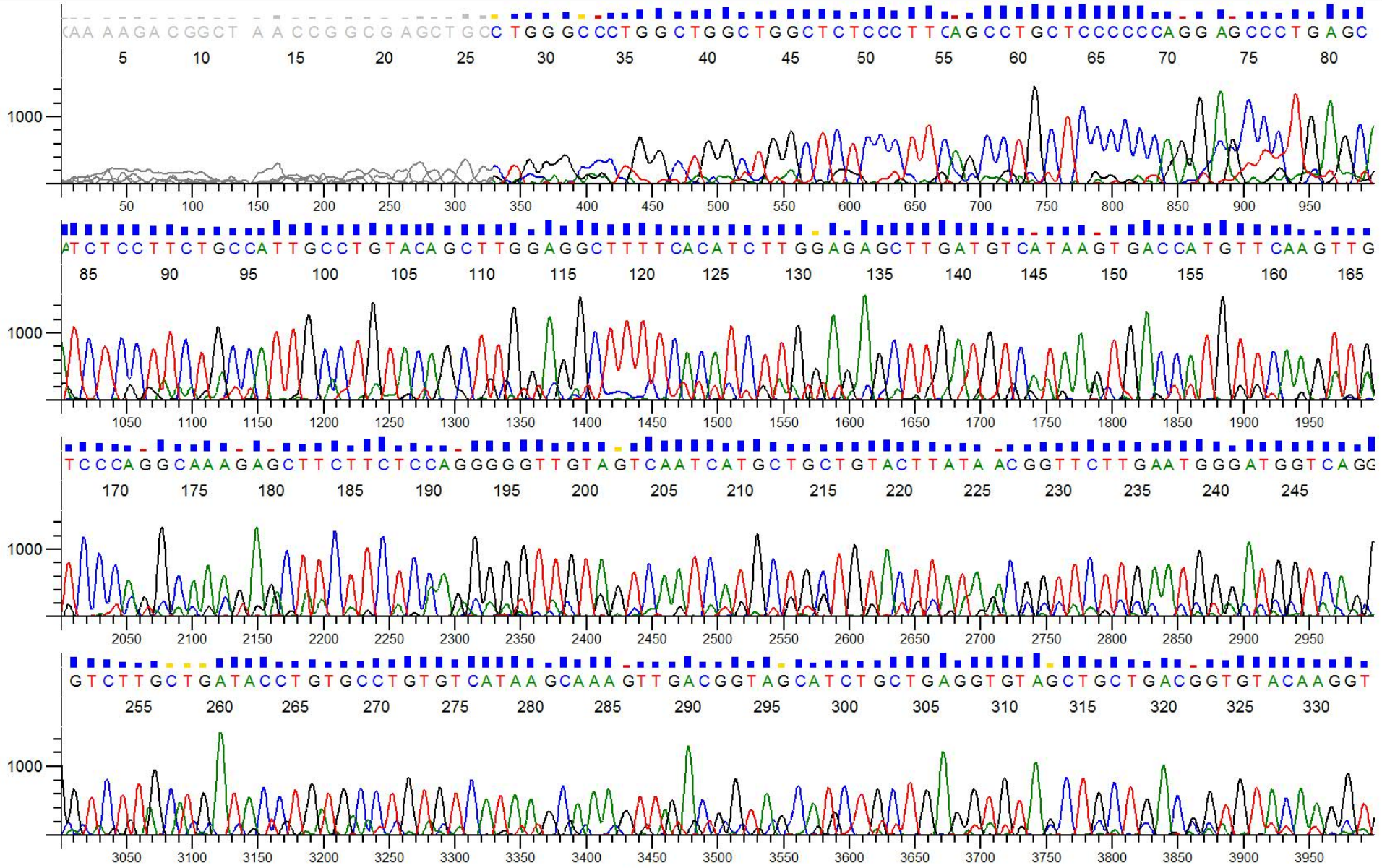

Inst Model/Name:3130xl/3130xlGA-1203-019

Sequence Scanner v1.0

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Mixed Base QVs: 10 16

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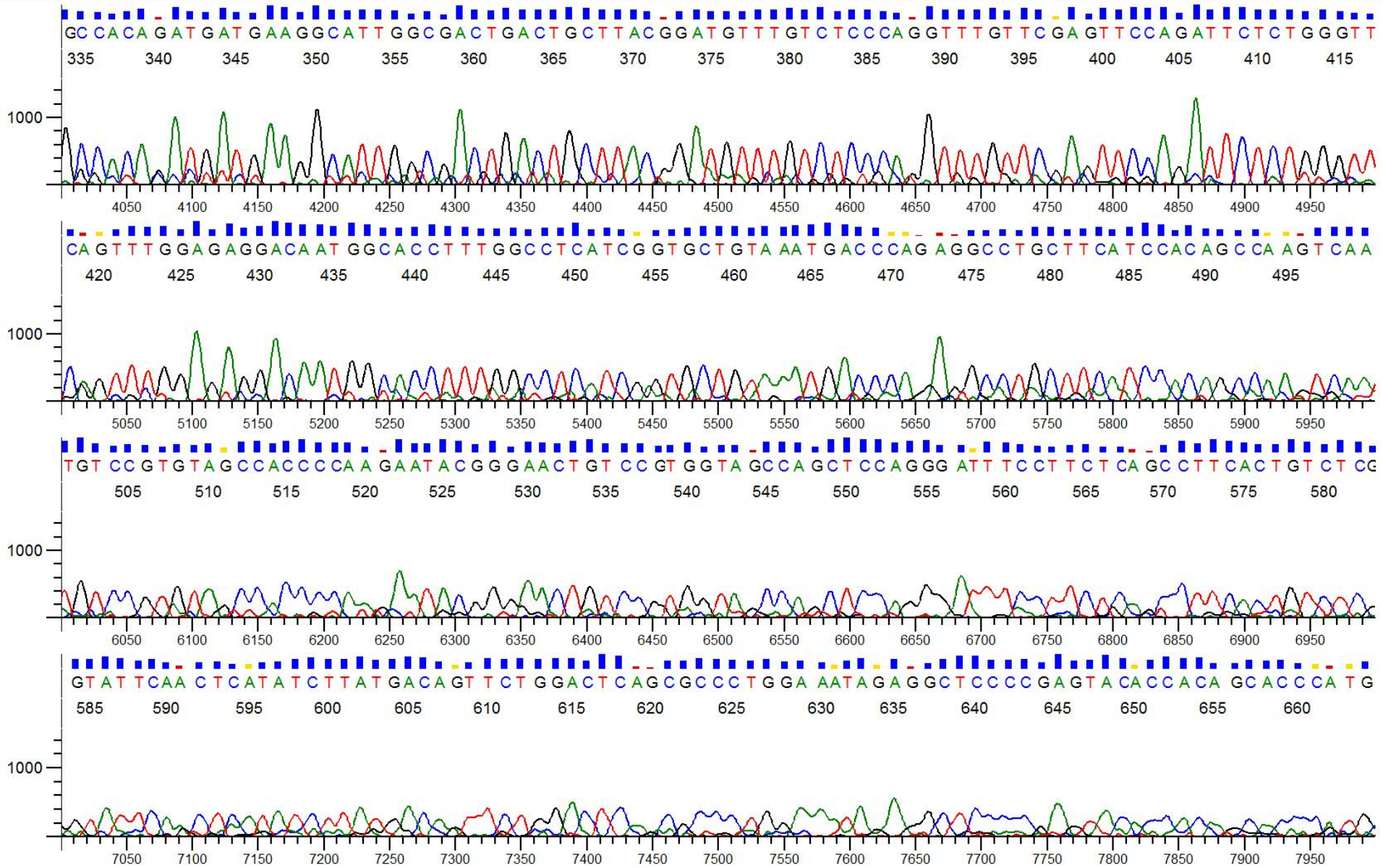
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1000
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CA G T T T G G A G A G G A C A A T G G C A C C T T T G G C C T C A T C G G T G C T G T A A A T G A C C C A G A G G C C T G C T T C A T C C A C A G C C A A G T C A A
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T G T C C G T G T A G C C A C C C C A A G A A T A C G G G A A C T G T C C G T G G T A G C C A G C T C C A G G G A T T T C C T T C T C A G C C T T C A C T G T C T C G
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G T A T T C A A C T C A T A T C T T A T G A C A G T T C T G G A C T C A G C G C C C T G G A A A T A G A G G C T C C C C G A G T A C A C C A C A G C A C C C A T G
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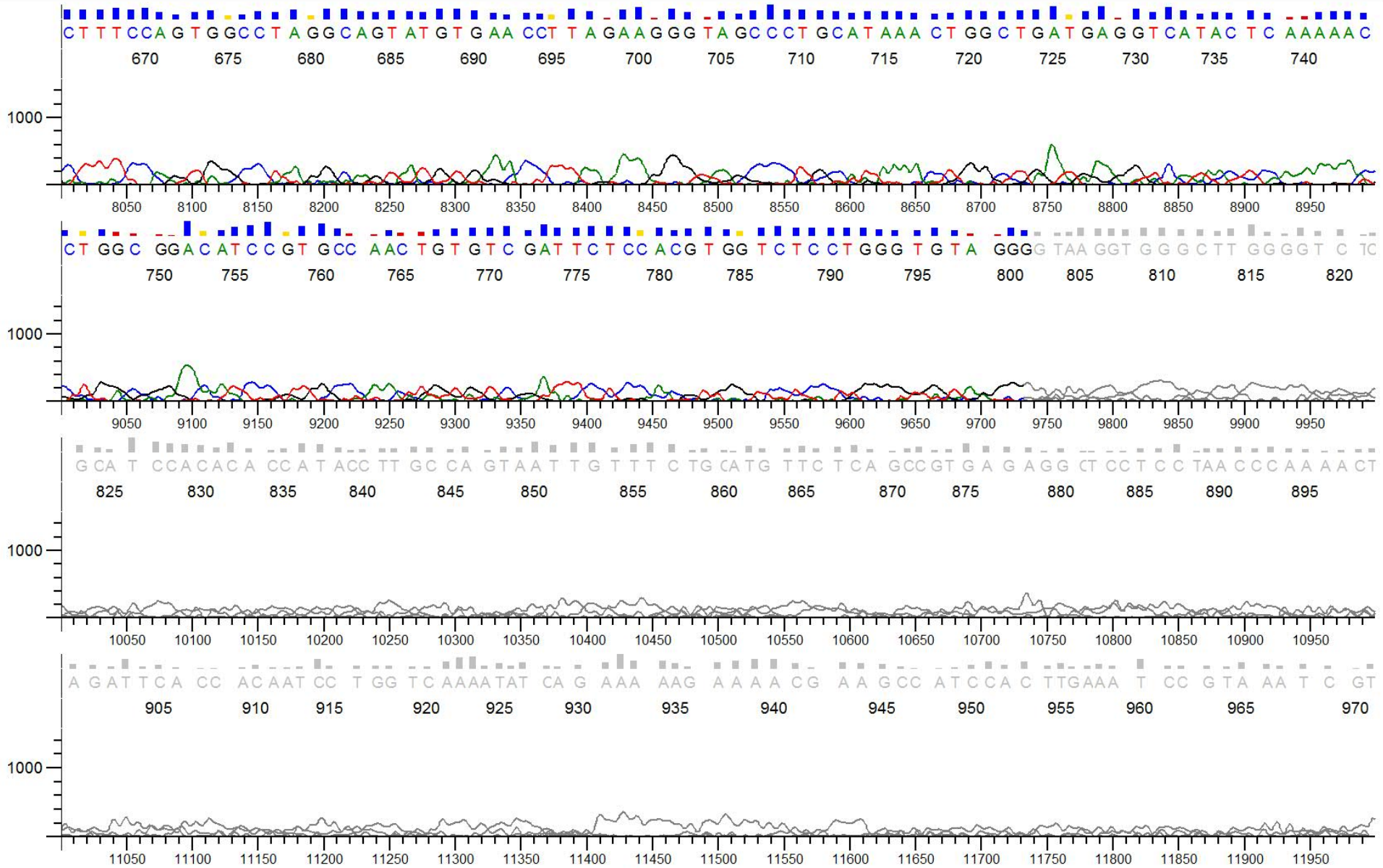

Inst Model/Name:3130xl/3130xlGA-1203-019

Sequence Scanner v1.0

Pure Base QVs: 15 20

Mixed Base QVs: 10 16

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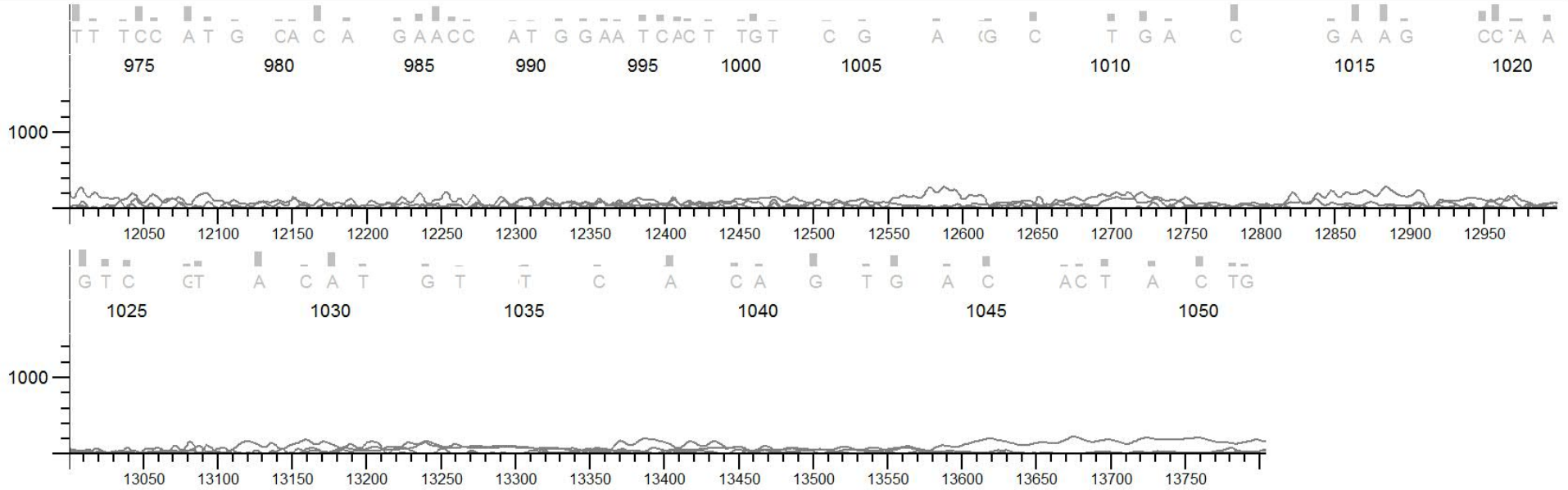
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G T C GT A C A T G T T C A C A G T G A C AC T A C TG
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DKEG1A Exon 1 BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1

Sequence ID: **ref|NG_008858.1|** Length: 24217 Number of Matches: 1
Range 1: 5023 to 5892

Score	Expect	Identities	Gaps	Strand	Frame
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Features:

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Sbjct  5023  ATGAGGTTCTTCTGTGCACGTTGCTGCAGCTTTGGGCCTGAGATGCCAGCTGTCCAGCTG  5082

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Sbjct  5083  CTGCTTCTGCCCTGCCTGGTGTGCGATGTGGGGGCCACCACAGCTCAGCTCACCAAGGCC  5142

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Sbjct  5143  AATGACCAGAGTGGCCGATGCCAGTATACCTTCAGTGTGGCCAGTCCCAATGAATCCAGC  5202

Query  200   TGCCCAGAGCAGAGCCAGGCCATGTCAGTCATCCATAACTTACAGAGAGACAGCAGCACC  259
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Sbjct  5203  TGCCCAGAGCAGAGCCAGGCCATGTCAGTCATCCATAACTTACAGAGAGACAGCAGCACC  5262

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Sbjct  5323  CAATTGACCTTGGACCAGGCTGCCAGGCCCCAGGAGACCCAGGAGGGGCTGCAGAGGGAG  5382

Query  380   CTGGGCACCCTGAGGCGGGAGCGGGACCAGCTGGAAACCCAAACCAGAGAGTTGGAGACT  439
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Sbjct  5383  CTGGGCACCCTGAGGCGGGAGCGGGACCAGCTGGAAACCCAAACCAGAGAGTTGGAGACT  5442

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Sbjct  5443  GCCTACAGCAACCTCCTCCGAGACAAGTCAGTTCTGGAGGAAGAGAAGAAGCGACTAAGG  5502

Query  500   CAAGAAAATGAGAATCTGGCCAGGAGGTTGGAAAGCAGCAGCCAGGAGGTAGCAAGGCTG  559
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Sbjct  5503  CAAGAAAATGAGAATCTGGCCAGGAGGTTGGAAAGCAGCAGCCAGGAGGTAGCAAGGCTG  5562

Query  560   AGAAGGGGCCAGTGTCCTCCAGACCCGAGACACTGCTCGGGCTGTGCCACCAGGCTCCAGA  619
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Sbjct  5623  GAAGGTAAGAATGCAGAGTGGGGGGACTCTGAGTTCAGCAGGTGATATGGCTCGTAGTGA  5682

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Sbjct  5802  AGCTCCTGAGAGCTTCATTTAGATTAGTGG-TTCAGAGTTCTTGTGCCCCTCCATGTCAG  5860

Query  860   TTTTCACAGTCCATAGCAAAAGGAG-AATAAA  890
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Sbjct  5861  TTTTCACAGTCCATAGCAAAAGGAGAAATAAA  5892
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DKEG6A Exon 1 BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1
Sequence ID: **ref|NG_008859.1|** Length: 24217 Number of Matches: 1
Range 1: 5020 to 5883

Score	Expect	Identities	Gaps	Strand	Frame
1539 bits(1706)	0.0()	861/865(99%)	1/865(0%)	Plus/Plus	

Features:

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Sbjct  5020  GCAATGAGGTTCTTCTGTGCACGTTGCTGCAGCTTTGGGCCTGAGATGCCAGCTGTCCAG  5079

Query  68    CTGCTGCTTCTGGCCTGCCTGGTGTGGGATGTGGGGGCCAGGACAGCTCAGCTCAGGAAG  127
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Sbjct  5080  CTGCTGCTTCTGGCCTGCCTGGTGTGGGATGTGGGGGCCAGGACAGCTCAGCTCAGGAAG  5139

Query  128   GCCAATGACCAGAGTGGCCGATGCCAGTATACCTTCAGTGTGGCCAGTCCCAATGAATCC  187
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Sbjct  5140  GCCAATGACCAGAGTGGCCGATGCCAGTATACCTTCAGTGTGGCCAGTCCCAATGAATCC  5199

Query  188   AGCTGCCCAGAGCAGAGCCAGGCCATGTCAGTCATCCATAACTTACAGAAAGACAGCAGC  247
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Sbjct  5200  AGCTGCCCAGAGCAGAGCCAGGCCATGTCAGTCATCCATAACTTACAGAGAGACAGCAGC  5259

Query  248   ACCCAACGCTTAGACCTGGAGGCCACCAAAGCTCGACTCAGCTCCCTGGAGAGCCTCCTC  307
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Sbjct  5260  ACCCAACGCTTAGACCTGGAGGCCACCAAAGCTCGACTCAGCTCCCTGGAGAGCCTCCTC  5319

Query  308   CACCAATTGACCTTGGACCAGGCTGCCAGGCCCCAGGAGACCCAGGAGGGGCTGCAGAGG  367
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Sbjct  5320  CACCAATTGACCTTGGACCAGGCTGCCAGGCCCCAGGAGACCCAGGAGGGGCTGCAGAGG  5379

Query  368   GAGCTGGGCACCCTGAGGCGGGAGCGGGACCAGCTGGAAACCCAAACCAGAGAGTTGGAG  427
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Sbjct  5380  GAGCTGGGCACCCTGAGGCGGGAGCGGGACCAGCTGGAAACCCAAACCAGAGAGTTGGAG  5439

Query  428   ACTGCCTACAGCAACCTCCTCCGAGACAAGTCAGTTCTGGAGGAAGAGAAGAAGCGACTA  487
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Sbjct  5440  ACTGCCTACAGCAACCTCCTCCGAGACAAGTCAGTTCTGGAGGAAGAGAAGAAGCGACTA  5499

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Query  608   AGAGAAGGTAAGAATGCAGAGTGGGGGGACTCTGAGTTCAGCAGGTGATATGGCTCGTAG  667
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Sbjct  5620  AGAGAAGGTAAGAATGCAGAGTGGGGGGACTCTGAGTTCAGCAGGTGATATGGCTCGTAG  5679

Query  668   TGACCTGCTACAGGCGCTCCAGGCCTCCCTGCCTGCCCTTTCTCCTAGAGACTGCACAGC  727
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Sbjct  5680  TGACCTGCTACAGGCGCTCCAGGCCTCCCTGCCTGCCCTTTCTCCTAGAGACTGCACAGC  5739

Query  728   TAGCACAAGACAGATGAATTAAAGGAAAGCACAGCGATCACCTTCAAGTATTACTAGTAA  787
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Sbjct  5740  TAGCACAAGACAGATGAATT-AAGGAAAGCACAGCGATCACCTTCAAGTATTACTAGTAA  5798

Query  788   TTTAGCTCCTGAGAGCTTCATTTAGATTAGTGGTTCAGAGTTCTTGTGCCCCTCCATGTC  847
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Sbjct  5859  AGTTTTCACAGTCCATAGCAAAAGG  5883
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DKEG7A Exon 1 BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1
Sequence ID: **ref|NG_008859.1|** Length: 24217 Number of Matches: 1
Range 1: 5023 to 5882

Score	Expect	Identities	Gaps	Strand	Frame
1523 bits(1688)	0.0()	858/863(99%)	3/863(0%)	Plus/Plus	

Features:

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Query  441   GCCTACAGCAACCTCCTCCGAGACAAGTCAGTTCTGGAGGAAGAGAAGAAGCGACTAAGG  500
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Query  501   CAAGAAAATGAGAATCTGGCCAGGAGGTTGGAAAGCAGCAGCCAGGAGGTAGCAAGGCTG  560
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Query  621   GAAGGTAAGAATGCAGAGTGGGGGGACTCTGAGTTCAGCAGGTGATATGGCTCGTAGTGA  680
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Sbjct  5623  GAAGGTAAGAATGCAGAGTGGGGGGACTCTGAGTTCAGCAGGTGATATGGCTCGTAGTGA  5682

Query  681   CCTGCTACAGGCGCTCCAGGCCTCCCTGCCTGCCCTTTCTCCTAGAGACTGCACAGCTAG  740
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Sbjct  5683  CCTGCTACAGGCGCTCCAGGCCTCCCTGCCTGCCCTTTCTCCTAGAGACTGCACAGCTAG  5742

Query  741   CACAAGACAGATGAATTAAAGAAAGCACAGCGATCACCTTCAAGTATTACTAGTAATTTA  800
             |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  5743  CACAAGACAGATGAATTAAGGAAAGCACAGCGATCACCTTCAAGTATTACTAGTAATTTA  5802

Query  801   GCTCCTGAGAGCTTCATTTAGATTAGTGGTTCAGAGTTCTTTGTGCCCCTCCCATGTCAG  860
             |||||||||||||||||||||||||||||||||||||||||| |||||||| ||||||||
Sbjct  5803  GCTCCTGAGAGCTTCATTTAGATTAGTGGTTCAGAGTTC-TTGTGCCCCT-CCATGTCAG  5860

Query  861   TTTTCACAGTCCATTAGCAAAAG  883
             ||||||||||| ||||||||
Sbjct  5861  TTTTCACAGTCCA-TAGCAAAAG  5882
```

DKEG9A Exon 3 BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1

Sequence ID: ref|NG_008859.1| Length: 24217 Number of Matches: 1

Range 1: 20801 to 21783

Score	Expect	Identities	Gaps	Strand	Frame
1584 bits(1756)	0.0()	952/984(97%)	18/984(1%)	Plus/Plus	

Features:

```
Query  19     CCAGACGATTTGTCTCC-GGGCTGTC-CATCTACTGGCTCTGCCA-GCTTCTGCATGATC  75
Sbjct  20801  CCAGACGATTTGTCTCCAGGGCTGTCACATCTACTGGCTCTGCCAAGCTTCCGCATGATC  20860

Query  76     ATTGTCTGTGTTTGGAAAGATTATGGATTAAGTGGTGCTTCGTTTTCTTTTCTGAATTTA  135
Sbjct  20861  ATTGTCTGTGTTTGGAAAGATTATGGATTAAGTGGTGCTTCGTTTTCTTTTCTGAATTTA  20920

Query  136    CCAGGATGTGGAGAACTAGTTTGGGTAGGAGAGCCTCTCACGCTGAGAACAGCAGAAACA  195
Sbjct  20921  CCAGGATGTGGAGAACTAGTTTGGGTAGGAGAGCCTCTCACGCTGAGAACAGCAGAAACA  20980

Query  196    ATTACTGGCAAGTATGGTGTGTGGATGCGAGACCCCAAGCCCACCTACCCCTACACCCAG  255
Sbjct  20981  ATTACTGGCAAGTATGGTGTGTGGATGCGAGACCCCAAGCCCACCTACCCCTACACCCAG  21040

Query  256    GAGACCACGTGGAGAATCGACACAGTTGGCACGGATGTCCGCCAGGTTTTTGAGTATGAC  315
Sbjct  21041  GAGACCACGTGGAGAATCGACACAGTTGGCACGGATGTCCGCCAGGTTTTTGAGTATGAC  21100

Query  316    CTCATCAGCCAGTTTATGCAGGGCTACCCTTCTAAGGTTCACATACTGCCTAGGCCACTG  375
Sbjct  21101  CTCATCAGCCAGTTTATGCAGGGCTACCCTTCTAAGGTTCACATACTGCCTAGGCCACTG  21160

Query  376    GAAAGCACGGGTGCTGTGGTGTACTCGGGGAGCCTCTATTTCCAGGGCGCTGAGTCCAGA  435
Sbjct  21161  GAAAGCACGGGTGCTGTGGTGTACTCGGGGAGCCTCTATTTCCAGGGCGCTGAGTCCAGA  21220

Query  436    ACTGTCATAAGATACGAGCTGAATACCGAGACAGTGAAGGCTGAGAAGGAAATCCCTGGA  495
Sbjct  21221  ACTGTCATAAGATATGAGCTGAATACCGAGACAGTGAAGGCTGAGAAGGAAATCCCTGGA  21280

Query  496    GCTGGCTACCACGGACAGTTCCCGTATTCTTGGGGTGGCTACACGGACATTGACTTGGCT  555
Sbjct  21281  GCTGGCTACCACGGACAGTTCCCGTATTCTTGGGGTGGCTACACGGACATTGACTTGGCT  21340

Query  556    GTGGATGAAGCAGGCCTCTGGGTCATTTACAGCACCGATGAGGCCAAAGGTGCCATTGTC  615
Sbjct  21341  GTGGATGAAGCAGGCCTCTGGGTCATTTACAGCACCGATGAGGCCAAAGGTGCCATTGTC  21400

Query  616    CTCTCCAAACTGAACCCAGAGAATCTGGAACTCGAACAAACCTGGGAGACAAACATCCGT  675
Sbjct  21401  CTCTCCAAACTGAACCCAGAGAATCTGGAACTCGAACAAACCTGGGAGACAAACATCCGT  21460

Query  676    AAGCAGTCAGTCGCCAATGCCTTCATCATCTGTGGCACCTTGTACACCGTCAGCAGCTAC  735
Sbjct  21461  AAGCAGTCAGTCGCCAATGCCTTCATCATCTGTGGCACCTTGTACACCGTCAGCAGCTAC  21520

Query  736    ACCTCAGCAGATGCTACCGTCAACTTTGCTTATGACACAGGCACAGGTATCAGCAAGACC  795
Sbjct  21521  ACCTCAGCAGATGCTACCGTCAACTTTGCTTATGACACAGGCACAGGTATCAGCAAGACC  21580

Query  796    CTGACCATCCCATTCAAGAACCCGCTATAAGTACAGCAGCATGATTGACTACAACCCCCT  855
Sbjct  21581  CTGACCATCCCATTCAAGAA-CCGCTATAAGTACAGCAGCATGATTGACTACAACCCCCT  21639

Query  856    GGAGA--GAGCTCTTTGCCTGGGAC-ACTTGAACATGGTCACTTATGACATCAGCTTCTT  912
Sbjct  21640  GGAGAAGAAGCTCTTTGCCTGGGACAACTTGAACATGGTCACTTATGACATCAAGCTCTC  21699

Query  913    CCAGATGTGAAAAG-CTC--AGCTGTACA-GCA--TGCAG-CTGAGATGCTCA-TGCTC-  963
Sbjct  21700  CAAGATGTGAAAAGCCTCCAAGCTGTACAGGCAATGGCAGAAGGAGATGCTCAGGGCTCC  21759

Query  964    -TGGGGGAGCA-GCTGGAGGGAGA  985
Sbjct  21760  TGGGGGGAGCAGGCTGAAGGGAGA  21783
```

DKEG9A Exon 3 REVERSE BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1
Sequence ID: ref|NG_008859.1| Length: 24217 Number of Matches: 1
Range 1: 20839 to 21818

Score	Expect	Identities	Gaps	Strand	Frame
1649 bits(1828)	0.0()	961/981(98%)	12/981(1%)	Plus/Minus	

Features:

```
Query  21     AGCAGTCAA-GCTGCCTGGGCCCTGGCTGGCTGGCTCTCCCTTCAGCCTGCTCCCCCCAG  78
              ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21818  AGCAGTCAAAGCTGCCTGGGCCCTGGCTGGCTGGCTCTCCCTTCAGCCTGCTCCCCCCAG  21759

Query  80     GAGCCCTGAGCATCTCCTTCTGCCATTGCCTGTACAGCTTGGAGGCTTTTCACATCTTGG  139
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21758  GAGCCCTGAGCATCTCCTTCTGCCATTGCCTGTACAGCTTGGAGGCTTTTCACATCTTGG  21699

Query  140    AGAGCTTGATGTCATAAGTGACCATGTTCAAGTTGTCCCAGGCAAAGAGCTTCTTCTCCA  199
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21698  AGAGCTTGATGTCATAAGTGACCATGTTCAAGTTGTCCCAGGCAAAGAGCTTCTTCTCCA  21639

Query  200    GGGGGTTGTAGTCAATCATGCTGCTGTACTTATAGCGGTTCTTGAATGGGATGGTCAGGG  259
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21638  GGGGGTTGTAGTCAATCATGCTGCTGTACTTATAGCGGTTCTTGAATGGGATGGTCAGGG  21579

Query  260    TCTTGCTGATACCTGTGCCTGTGTCATAAGCAAAGTTGACGGTAGCATCTGCTGAGGTGT  319
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21578  TCTTGCTGATACCTGTGCCTGTGTCATAAGCAAAGTTGACGGTAGCATCTGCTGAGGTGT  21519

Query  320    AGCTGCTGACGGTGTACAAGGTGCCACAGATGATGAAGGCATTGGCGACTGACTGCTTAC  379
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21518  AGCTGCTGACGGTGTACAAGGTGCCACAGATGATGAAGGCATTGGCGACTGACTGCTTAC  21459

Query  380    GGATGTTTGTCTCCCAGGTTTGTTCGAGTTCCAGATTCTCTGGGTTCAGTTTGGAGAGGA  439
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21458  GGATGTTTGTCTCCCAGGTTTGTTCGAGTTCCAGATTCTCTGGGTTCAGTTTGGAGAGGA  21399

Query  440    CAATGGCACCTTTGGCCTCATCGGTGCTGTAAATGACCCAGAGGCCTGCTTCATCCACAG  499
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21398  CAATGGCACCTTTGGCCTCATCGGTGCTGTAAATGACCCAGAGGCCTGCTTCATCCACAG  21339

Query  500    CCAAGTCAATGTCCGTGTAGCCACCCCAAGAATACGGGAACTGTCCGTGGTAGCCAGCTC  559
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21338  CCAAGTCAATGTCCGTGTAGCCACCCCAAGAATACGGGAACTGTCCGTGGTAGCCAGCTC  21279

Query  560    CAGGGATTTCCTTCTCAGCCTTCACTGTCTCGGTATTCAGCTCATATCTTATGACAGTTC  619
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21278  CAGGGATTTCCTTCTCAGCCTTCACTGTCTCGGTATTCAGCTCATATCTTATGACAGTTC  21219

Query  620    TGGACTCAGCGCCCTGGAAATAGAGGCTCCCCGAGTACACCACAGCACCCGTGCTTTCCA  679
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21218  TGGACTCAGCGCCCTGGAAATAGAGGCTCCCCGAGTACACCACAGCACCCGTGCTTTCCA  21159

Query  680    GTGGCCTAGGCAGTATGTGAACCTTAGAAGGGTAGCCCTGCATAAACTGGCTGATGAGGT  739
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21158  GTGGCCTAGGCAGTATGTGAACCTTAGAAGGGTAGCCCTGCATAAACTGGCTGATGAGGT  21099

Query  740    CATACTCAAAAACCTGGCGGACATCCGTGCCAACTGTGTCGATTCTCCACGTGGTCTCCT  799
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21098  CATACTCAAAAACCTGGCGGACATCCGTGCCAACTGTGTCGATTCTCCACGTGGTCTCCT  21039

Query  800    GGGTGTAGGGGTAGGTGGGCTTGGGGTCTCGCATCCACACACCATACTTGCCAGTAATTG  859
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21038  GGGTGTAGGGGTAGGTGGGCTTGGGGTCTCGCATCCACACACCATACTTGCCAGTAATTG  20979

Query  860    TTTCTGCTGTTCTCAGCGTGAGAGGCTCTCCTACCCAAACTAG-TCTCCACATCTGGGTA  918
              |||||||||||||||||||||||||||||||||||||||||| |||||||||| ||||
Sbjct  20978  TTTCTGCTGTTCTCAGCGTGAGAGGCTCTCCTACCCAAACTAGTTCTCCACATCCTGGTA  20919

Query  919    AATTCAGAAAAG-AAACG-AGCAACACTGA--TCAT-ATCTTTC--AACACAGAACATTG  971
              |||||||||||| ||||| |||| |||| |   ||| |||||||  |||||||| ||| ||
Sbjct  20918  AATTCAGAAAAGAAAACGAAGCACCACTTAATCCATAATCTTTCCAAACACAG-ACAATG  20860

Query  972    ATCATGCG--AGCCTGACAGA  990
              |||||||  |||  || ||||
Sbjct  20859  ATCATGCGGAAGCTTGGCAGA  20839
```

DKEG11A Exon 3 BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1
Sequence ID: **ref|NG_008859.1|** Length: 24217 Number of Matches: 1
Range 1: 20801 to 21767

Score	Expect	Identities	Gaps	Strand	Frame
1501 bits(1664)	0.0()	926/967(96%)	26/967(2%)	Plus/Plus	

Features:

```
Query  9      CCAGACGATTTGTCTCCAGGGCTGTCACATCTACTGGCTCTGCCAAGCTTCCGCATGATC  68
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  20801  CCAGACGATTTGTCTCCAGGGCTGTCACATCTACTGGCTCTGCCAAGCTTCCGCATGATC  20860

Query  69     ATTGTCTGTGTTTGGAAAGATTATGGATTAAGTGGTGCTTCGTTTTCTTTTCTGAATTTA  128
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  20861  ATTGTCTGTGTTTGGAAAGATTATGGATTAAGTGGTGCTTCGTTTTCTTTTCTGAATTTA  20920

Query  129    CCAGGATGTGGAGAACTAGTTTGGGTAGGAGAGCCTCTCACGCTGAGAACAGCAGAAACA  188
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  20921  CCAGGATGTGGAGAACTAGTTTGGGTAGGAGAGCCTCTCACGCTGAGAACAGCAGAAACA  20980

Query  189    ATTACTGGCAAGTATGGTGTGTGGATGCGAGACCCCAAGCCCACCTACCCCTACACCCAG  248
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  20981  ATTACTGGCAAGTATGGTGTGTGGATGCGAGACCCCAAGCCCACCTACCCCTACACCCAG  21040

Query  249    GAGACCACGTGGAGAATCGACACAGTTGGCACGGATGTCCGCCAGGTTTTTGAGTATGAC  308
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21041  GAGACCACGTGGAGAATCGACACAGTTGGCACGGATGTCCGCCAGGTTTTTGAGTATGAC  21100

Query  309    CTCATCAGCCAGTTTATGCAGGGCTACCCTTCTAAGGTTCACATACTGCCTAGGCCACTG  368
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21101  CTCATCAGCCAGTTTATGCAGGGCTACCCTTCTAAGGTTCACATACTGCCTAGGCCACTG  21160

Query  369    GAAAGCACGGGTGCTGTGGTGTACTCGGGGAGCCTCTATTTCCAGGGCGCTGAGTCCAGA  428
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21161  GAAAGCACGGGTGCTGTGGTGTACTCGGGGAGCCTCTATTTCCAGGGCGCTGAGTCCAGA  21220

Query  429    ACTGTCATAAGATATGAGCTGAATACCGAGACAGTGAAGGCTGAGAAGGAAATCCCTGGA  488
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21221  ACTGTCATAAGATATGAGCTGAATACCGAGACAGTGAAGGCTGAGAAGGAAATCCCTGGA  21280

Query  489    GCTGGCTACCACGGACAGTTCCCGTATTCTTGGGGTGGCTACACGGACATTGACTTGGCT  548
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21281  GCTGGCTACCACGGACAGTTCCCGTATTCTTGGGGTGGCTACACGGACATTGACTTGGCT  21340

Query  549    GTGGATGAAGCAGGCCTCTGGGTCATTTACAGCACCGATGAGGCCAAAGGTGCCATTGTC  608
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21341  GTGGATGAAGCAGGCCTCTGGGTCATTTACAGCACCGATGAGGCCAAAGGTGCCATTGTC  21400

Query  609    CTCTCCAAACTGAACCCAGAGAATCTGGAACTCGAACAAACCTGGGAGACAAACATCCGT  668
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21401  CTCTCCAAACTGAACCCAGAGAATCTGGAACTCGAACAAACCTGGGAGACAAACATCCGT  21460

Query  669    AAGCAGTCAGTCGCCAATGCCTTCATCATCTGTGGCACCTTGTACACCGTCAGCAGCTAC  728
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  21461  AAGCAGTCAGTCGCCAATGCCTTCATCATCTGTGGCACCTTGTACACCGTCAGCAGCTAC  21520

Query  729    ACCTCAGCAGATGCTACCGTCAACTTTGCTTATGACACAGGCACAGGTATCAGC-AGACC  787
              |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  21521  ACCTCAGCAGATGCTACCGTCAACTTTGCTTATGACACAGGCACAGGTATCAGCAAGACC  21580

Query  788    CTGA-CATCCCATTCA--GACCGCTAT-AGTACAGCAGCATGA-TGACTAC-ACCCCCT-  840
              |||| ||||||||||   |||||||| ||||||||||||||| ||||||| |||||||
Sbjct  21581  CTGACCATCCCATTCAAGAACCGCTATAAGTACAGCAGCATGATTGACTACAACCCCCTG  21640

Query  841    GAGA--GAGCTCTTTGC--TGGACA--CTGAACAT-GTCAC-TATGAC-TC-AGCTC-CT  889
              ||||  |||||||||   |||||  |||||||| |||| |||||| || ||||| ||
Sbjct  21641  GAGAAGAAGCTCTTTGCCTGGGACAACTTGAACATGGTCACTTATGACATCAAGCTCTCC  21700

Query  890    CAGATGTG-AAAGCTTC--AGCTGTACATGCA----TACAATGGCATGCTCAAGTCTCCT  942
              ||||||| |||| ||   |||||||| |||    |||||||| | |||||| |||||||
Sbjct  21701  AAGATGTGAAAAGCCTCCAAGCTGTACAGGCAATGGCAGAAGGAGATGCTCAGGGCTCCT  21760

Query  943    GGGGGGA  949
              |||||||
Sbjct  21761  GGGGGGA  21767
```

DKEG11A Exon 3 REVERSE BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1
Sequence ID: ref|NG_008859.1| Length: 24217 Number of Matches: 1
Range 1: 20810 to 21818

Score	Expect	Identities	Gaps	Strand	Frame
1671 bits(1852)	0.0()	987/1012(98%)	13/1012(1%)	Plus/Minus	

Features:

```
Query  25     AGCAGTCAA-GCTGCCTGGGCCCTGGCTGGCTGGCTCTCCCTTCAGCCTGCTCCCCCCAG  83
              ||||||||| ||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  21818  AGCAGTCAAAGCTGCCTGGGCCCTGGCTGGCTGGCTCTCCCTTCAGCCTGCTCCCCCCAG  21759

Query  84     GAGCCCTGAGCATCTCCTTCTGCCATTGCCTGTACAGCTTTGGAGGCTTTTCACATCTTGG  143
              ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  21758  GAGCCCTGAGCATCTCCTTCTGCCATTGCCTGTACAGCTTTGGAGGCTTTTCACATCTTGG  21699

Query  144    AGAGCTTGATGTCATAAGTGACCATGTTCAAGTTGTCCCAGGCAAAGAGCTTCTTCTCCA  203
              ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  21698  ACACCTTCATCTCATAACTCACCACATGTTCAACTTGTCCCACGCCAAAGACCTTCTTCTCCA  21639

Query  204    GGGGGTTGTAGTCAATCATGCTGCTGTACTTATAGCGGTTCTTGAATGGGATGGTCAGGG  263
              ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  21638  GGGGGTTGTAGTCAATCATGCTGCTGTACTTATAGCGGTTCTTGAATGGGATGGTCAGGG  21579

Query  264    TCTTGCTGATACCTGTGCCTGTGTCATAAGCAAAGTTGACGGTAGCATCTGCTGAGGTGT  323
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21578  TCTTGCTGATACCTGTGCCTGTGTCATAAGCAAAGTTGACGGTAGCATCTGCTGAGGTGT  21519

Query  324    AGCTGCTGACGGTGTACAAGGTGCCACAGATGATGAAGGCATTGGCGACTGACTGCTTAC  383
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21518  AGCTGCTGACGGTGTACAAGGTGCCACAGATGATGAAGGCATTGGCGACTGACTGCTTAC  21459

Query  384    GGATGTTTGTCTCCCAGGTTTGTCCGAGTTCCAGATTCTCTGGGTTCAGTTTGGAGAGGA  443
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21458  GCATCTTTCTCTCCCACCTTTCTTCCACTTCCACATTCTCTGCCTTCACTTTCCACACCA  21399

Query  444    CAATGGCACCTTTGGCCTCATCGGTGCTGTAAATGACCCAGAGGCCTGCTTCATCCACAG  503
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21398  CAATGGCACCTTTGGCCTCATCGGTGCTGTAAATGACCCAGAGGCCTGCTTCATCCACAG  21339

Query  504    CCAAGTCAATGTCCGTGTAGCCACCCCAAGAATACGGGAACTGTCCGTGGTAGCCAGCTC  563
              ||||||||||||||||||||| ||||||||||||||||| ||||||||||||||||||||
Sbjct  21338  CCAAGTCAATGTCCGTGTAGCCACCCCAAGAATACGGGAACTGTCCGTGGTAGCCAGCTC  21279

Query  564    CAGGGATTTCCTTCTCAGCCTTCACTGTCTCGGTATTCAGCTCATATCTTATGACAGTTC  623
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21278  CAGGGATTTCCTTCTCAGCCTTCACTGTCTCGGTATTCAGCTCATATCTTATGACAGTTC  21219

Query  624    TGGACTCAGCGCCCTGGAAATAGAGGCTCCCCGAGTACACCACAGCACCCGTGCTTTCCA  683
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21218  TGCACTCACCCCCCTCCAAATACACGCCTCCCCCACTACACCACAGCACCCCTCCTTTCCA  21159

Query  684    GTGGCCTAGGCAGTNTGTGAACCTTAGAAGGGTAGCCCTGCATAAACTGGCTGATGAGGT  743
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21158  GTGGCCTAGGCAGTATGTGAACCTTAGAAGGGTAGCCCTGCATAAACTGGCTGATGAGGT  21099

Query  744    CATACTCAAAAACCTGGCGGACATCCGTGCCAACTGTGTCGATTCTCCACGTGGTCTCCT  803
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21098  CATACTCAAAAACCTGGCGGACATCCGTGCCAACTGTGTCGATTCTCCACGTGGTCTCCT  21039

Query  804    GGGTGTAGGGGTAGGTGGGCTTGGGGTCTCGCATCCACACACCATACTTGCCAGTAATTG  863
              ||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  21038  GGGTGTAGGGGTAGGTGGGCTTGGGGTCTCGCATCCACACACCATACTTGCCAGTAATTG  20979

Query  864    TTTCTGCTGTTCTCAGCGTGAGAGGCTCTCCTACCCAAACTAG-TCT-CACATCCTGGTA  921
              ||||||||||||||||||||| ||||||||||||||||||| ||| ||||||||||||
Sbjct  20978  TTTCTGCTCTTCTCACCCTCACACCCTCTCCTACCCAAACTACTTCTCCACATCCTGCTA  20919

Query  922    AATTCAGAAAAG-AAAAG-AGCACAACTGA--TCAT-ATCTTTCC-AACACAGACCATTG  976
              |||||||||||| |||| ||| | |||||||||||| | ||||| || ||
Sbjct  20918  AATTCAGAAAAGAAAAGTGAAGCACCACTTAATCCATAATTTTCCAAACACAGA-CAATG  20860

Query  976    ATCATCCCCCAGCTT GCACACTCACTTCATCGTGACACCTCCTCCCCACAA  1026
              |||||| | ||||| |||| ||| ||| ||||| | ||||| || ||
Sbjct  20859  ATCATGCGGAAGCTTGGCAGAGCCAGTAGAT-GTGACA-GCCCTGGAGACAA  20810
```

DKEG12A Exon 1 BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1
Sequence ID: **ref|NG_008859.1|** Length: 24217 Number of Matches: 1
Range 1: 5032 to 5901

Score	Expect	Identities	Gaps	Strand	Frame
1516 bits(1680)	0.0()	**862**/**871**(99%)	**4**/871(0%)	Plus/Plus	

Features:

```
Query  33    TTCTGTGC-CGTTGCTGCAGCTTTGGGCCTGAGATGCCAGCTGTCCAGCTGCTGCTTCTG  91
Sbjct  5032  TTCTGTGCACGTTGCTGCAGCTTTGGGCCTGAGATGCCAGCTGTCCAGCTGCTGCTTCTG  5091

Query  92    GCCTGCCTGGTGTGGGATGTGGGGGCCAGGACAGCTCAGCTCAGGAAGGCCAATGACCAG  151
Sbjct  5092  GCCTGCCTGGTGTGGGATGTGGGGGCCAGGACAGCTCAGCTCAGGAAGGCCAATGACCAG  5151

Query  152   AGTGGCCGATGCCAGTATACCTTCAGTGTGGCCAGTCCCAATGAATCCAGCTGCCCAGAG  211
Sbjct  5152  AGTGGCCGATGCCAGTATACCTTCAGTGTGGCCAGTCCCAATGAATCCAGCTGCCCAGAG  5211

Query  212   CAGAGCCAGGCCATGTCAGTCATCCATAACTTACAGAAAGACAGCAGCACCCAACGCTTA  271
Sbjct  5212  CAGAGCCAGGCCATGTCAGTCATCCATAACTTACAGAGAGACAGCAGCACCCAACGCTTA  5271

Query  272   GACCTGGAGGCCACCAAAGCTCGACTCAGCTCCCTGGAGAGCCTCCTCCACCAATTGACC  331
Sbjct  5272  GACCTGGAGGCCACCAAAGCTCGACTCAGCTCCCTGGAGAGCCTCCTCCACCAATTGACC  5331

Query  332   TTGGACCAGGCTGCCAGGCCCCAGGAGACCCAGGAGGGGCTGCAGAGGGAGCTGGGCACC  391
Sbjct  5332  TTGGACCAGGCTGCCAGGCCCCAGGAGACCCAGGAGGGGCTGCAGAGGGAGCTGGGCACC  5391

Query  392   CTGAGGCGGGAGCGGGACCAGCTGGAAACCCAAACCAGAGAGTTGGAGACTGCCTACAGC  451
Sbjct  5392  CTGAGGCGGGAGCGGGACCAGCTGGAAACCCAAACCAGAGAGTTGGAGACTGCCTACAGC  5451

Query  452   AACCTCCTCCGAGACAAGTCAGTTCTGGAGGAAGAGAAGAAGCGACTAAGGCAAGAAAAT  511
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Query  572   CAGTGTCCCCAGACCCGAGACACTGCTCGGGCTGTGCCACCAGGCTCCAGAGAAGGTAAG  631
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Query  632   AATGCAGAGTGGGGGGACTCTGAGTTCAGCAGGTGATATGGCTCGTAGTGACCTGCTACA  691
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Query  692   GGCGCTCCA-GCCTCCCTGCCTGCCCTTTCTCCTAGAGACTGCACAGCTAGCACAAGACA  750
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Query  751   GATGAATTAAAGAAAGCACAGCGATCACCTTCAAGTATTACTAGTAATTTAGCTCCTGAG  810
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Query  811   AGCTTCATTTAGATTAGTGGTTCAGAGTTC-TGTGCCCCTCCATGTCAGTTTTCACAGTC  869
Sbjct  5812  AGCTTCATTTAGATTAGTGGTTCAGAGTTCTTGTGCCCCTCCATGTCAGTTTTCACAGTC  5871

Query  870   CATAGCAAAAGGAAGaaaaaaaaaGCCCggg  900
Sbjct  5872  CATAGCAAAAGG-AGAAATAAAAGGACCGGG  5901
```

DKEG12A Exon 3 BLASTN result

Homo sapiens myocilin, trabecular meshwork inducible glucocorticoid response (MYOC), RefSeqGene on chromosome 1

Sequence ID: ref|NG_008850.1| Length: 24217 Number of Matches: 1

Range 1: 20866 to 21809

Score	Expect	Identities	Gaps	Strand	Frame
1561 bits(1730)	0.0()	935/965(97%)	20/965(2%)	Plus/Minus	

Features:

```
Query  21     AGCTGCCTGGGCCCTGGCTGGCTGGCTCTCCCTTCAGCCTGCTCCCCCCAGGAGCCCTGA  80
              ||||||||||||||| |||| |||||||| |||||||||||||||||||| |||| |||
Sbjct  21809  AGCTGCCTGGGCCCTGGCTGGCTGGCTCTCCCTTCAGCCTGCTCCCCCCAGGAGCCCTGA  21750

Query  81     GCATCTCCTTCTGCCATTGCCTGTACAGCTTGGAGGCTTTTCACATCTTGGAGAGCTTGA  140
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Sbjct  21749  GCATCTCCTTCTGCCATTGCCTGTACAGCTTGGAGGCTTTTCACATCTTGGAGAGCTTGA  21690

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Query  201    AGTCAATCATGCTGCTGTACTTATAACGGTTCTTGAATGGGATGGTCAGGGTCTTGCTGA  260
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Sbjct  21329  TGTCCGTGTAGCCACCCCAAGAATACGGGAACTGTCCGTGGTAGCCAGCTCCAGGGAATT  21270

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Query  921    AAAATATCNGAAAAAGAAAACGAAGCCATCCACTTGAAATCCGTAAATCGTTTCCAAGC  980
              |||| |||| ||| |||||||||  |||||| |||| | |||| ||||||| |||||
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Sbjct  20869  ACAGA  20865
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DISCUSSION

Glaucoma is the second leading cause of blindness worldwide and is characterized by progressive optic nerve head damage for which intraocular pressure is one of the most important risk factor. It usually begins in middle aged individuals around 4^{th} decade and progresses slowly but relentlessly. The disease process is insidious and most of the patients remain asymptomatic unless the damage is advanced leading to deterioration of vision. Hence most of the patients remain undiagnosed or untreated. Although the clinical course of the disease is well known the molecular events responsible for glaucoma are currently poorly understood and current therapeutic strategies are not curative.

Globally this disease affects 60 million people, among them 8.4 million people have bilateral blindness due to glaucoma. This is predicted to increase to 80 million and 11.2 million respectively by the year 2020[15]. In India, approximately 11.2 million persons aged 40yrs and older are estimated to be suffering from glaucoma of which POAG is estimated to affect 6.48 million people.[16] In general globally 1.12% among the whole population is suffering from glaucoma. This data is not a true projection as this does not include north Indian population. When that is taken into

account it might be hiked even more. In the present study out of 4906 patients, 59 (1.2%) of them have a family history of glaucoma.

Primary open angle glaucoma (POAG) is the most common type of glaucoma, affecting almost 2% of the world's population. The prevalence of POAG in south Indian population is about 1.6% which is similar to that observed in Western population [17]. In this study among the 59 patients with positive family history, 32 were affected with glaucoma, out of which 37.5% (N=12) of them have primary open angle glaucoma.

Over the past one decade of work in molecular genetics of glaucoma 29 genetic loci have been identified for various forms of glaucoma through linkage analysis[18, 19]. Over 12 candidate loci were reported, of which myocilin is the first gene identified to be associated with glaucoma. It is formerly called as TIGR (Trabecular meshwork–Induced Glucocorticoid Response protein).[20] It was presumed that myocilin is one of the aqueous outflow regulators in cells. Earlier it was known to express in the ciliary body and trabecular meshwork however its presence in the trabecular cells of the uveal, corneoscleral, and juxtacanalicular regions and the endothelial cells of Schlemm's canal were documented through functional studies.[21,22,23]Hence this explains as to how it regulates the aqueous flow in and out of the cells. Till now the

only gene that causes elevated IOP with normal development of the ocular anterior segment is myocilin[24]. Gene-specific pathophysiology is not well characterized for candidate genes of glaucoma. Hence this calls for more molecular studies in glaucoma to validate its role in the pathophysiology of the disease.

Globally myocilin mutations in primary open angle glaucoma are approximately about 2-5%[25,26,27]. Whereas in Indian population, a frequency of roughly 7.1% and 2% has been documented[28,12,29].

In the present study, 12 families comprising of 35 patients were investigated. Most of the affected individuals were in the age group of 50-70yrs. Two of the patients had juvenile onset of the disease and majority of them were females. In the present study two missense variations viz., R76K (c.227G>A), that was found to be homozygous in two of the probands (DKEG1A and DKEG12A), while heterozygous in two other probands (DKEG6A; DKEG7A) and T325M (c.974 C>T) in the proband DKEG12A and a nonsense mutation as Q368X c.1102 C>T in the proband DKEG11A were observed. A couple of variations in the intronic region of myocilin gene, which includes c.604+136G>A as a homozygous allele in the proband DKEG7A were also documented. The intronic variation c.605-374 C>G was found in most of the probands in a

homozygous state (DKEC1A; DKEG3A; DKEG4A; DKEG5A; DKEG6A; DKEG7A; DKEG9A) and in one control subject (DKC150), whereas this was found to be heterozygous in another proband (DKEG10A). The intronic variation c.605-332 G>A occurs in heterozygous condition in some of the probands (DKEG3A; DKEG4A; DKEG7A; DKEG10A), whereas the intronic variation c.605-303 C>G is found only in one proband DKEG5A. The intronic variation c.605-280 T>G in a heterozygous state is found in most of the probands (DKEG4A; DKEG5A; DKEG7A; DKEG9A) and in one control subject (DKC150),and the same variation was found to be homozygous in two of the proband studied. The intronic variation c.730+35 G>A was found to be in homozygous form in most of the probands (DKEC1A; DKEG3A; DKEG4A; DKEG6A; DKEG7A; DKEG9A) and in one control subject (DKC150) studied.

Three synonymous variations have been documented such as Y347Y (c.1041 T>C) in the proband DKEG9A and two other as L349L c.1045 C>T R470R c.1410 C>T in a single proband (DKEG12A).

The documented mutation Q368X poses a 4.7-fold increased risk of POAG in the global population[30]. For the first time this mutation was identified in a patient of south Indian origin and who had advanced

glaucoma. Express shunt was performed in the right eye whereas in the left eye trabeculectomy, the gold standard procedure was done. The IOP was not under surgical control and patient was put on topical antiglaucoma medications. The CD ratio in both the eyes was 0.9 and visual fields showed presence of advanced field defects with only tubular field remaining.

The proband DKEG 12 A is of interest as she was found to be a compound heterozygote carrying two variations (R76K; T325M), amongst which one is a novel mutation (T325M) and two were of synonymous snps (L349L; R470R).She was also an established case of primary open angle glaucoma. Her fundus showed 0.9 cupping in both the eyes and she was on topical antiglaucoma medications.

More than 50 mutations have been reported in myocilin gene causing POAG, Q368X mutation occurs most frequently in patients with POAG. Functional study of this mutation, gives us an insight into the pathology caused by this variation[31]. It is a well known fact that as age advances the outflow resistance increases, accompanied by reduction in the trabecular meshwork cells and alterations in the juxtacanalicular extracellular matrix[32, 33]. This fact was supported by the work of Alvarado et al. (1986)[34], wherein they have reported that glaucomatous eyes exhibit

fewer TM cells and an abnormal appearance of ECM in the juxtacanalicular region.

Sohn et al (2002) has also reported an aggregated expression of the mutant myocilin, thereby causing proteolytic degradation[31]. The accumulation of the degraded protein causes cytotoxic effect and contributes to degenerative disease marked by the formation of insoluble protein plaques, which is characteristic of neurodegenerative diseases.

Myocilin normally exists either as a dimer or as an oligomer, wherein oligomerisation between wild type and truncated myocilins, leads to colocalisation of truncated myocilin (Q368X) with wild type myocilin[31]. They have also shown that secretion of wild-type myocilin is suppressed when co expressed with truncated myocilin. Localization of wild type myocilin to microtubules of mitochondria, or to cross-linked actin networks was not observed, which is in contradictory to the previous work[35, 36, 37, 38].

The other variation (T325M) has to be characterized functionally to deduce its effect on the pathology of POAG. This variation was reported in NCBI SNP database, as observed in an individual documented from a population based cohort study of exome variation in cardiovascular disease, of north American origin. But for the first time we have

identified this in a patient with POAG. There is a lacunae of population data about this variation. Hence this has to be characterized both by population study and functional analysis before finding association with POAG.

SUMMARY

This prospective study was carried out enrolling 12 families comprising of 35 subjects over a period of two years from June 2011 to June 2013. 35 subjects comprised of the proband and their siblings or parents if available. All patients underwent a thorough ophthalmological examination after obtaining a detailed history covering the pedigree details. Ethical clearance for this study was obtained from Institutional Human Ethical Committee, Madras medical College, Chennai. On securing informed consent, the blood samples were collected and subjected to DNA analysis. We collected a minimum of two samples and a maximum of five samples per family depending on the availability.

In our study majority of the probands were females in the age group of 50-70 years. The intraocular pressures recorded were normal in majority of the patients while five of them had their intraocular pressures above the normal range.

Few (DKEG1A, DKEG2A, DKEG2B, DKEG6A, DKEG6C, DKEG7A, DKEG7B, DKEG8A, DKEG9A, DKEG10A, DKEG10B, DKEG12A) were already on treatment with topical antiglaucoma medication, while some (DKEG1B, DKEG3A, DKEG4A, DKEG5A,

DKEG5B, DKEG11A) had undergone antiglaucoma surgery and others were just glaucoma suspects. Around 16 patients had moderate glaucomatous damage and eight of them had advanced glaucoma. Gonioscopy when performed showed closed angles in two patients, rest had open angles. Retinal fibre layer thickness (RNFL) was found to be below 100 µ in 12 patients and in two of the patients thickness was found to be lesser than 90µ.

Amongst the 35 subjects, 17 were just glaucoma suspects and hence were kept under observation. Eleven patients were put on topical antiglaucoma medications and six patients had undergone antiglaucoma surgery out of which two of them had their IOP under surgical control while remaining four were on topical antiglaucoma medications post operatively.

DNA analysis was done which showed exonic variations in six of the probands of which three had variations in exon 1, two in exon 3 and one had in both exon 1 and exon 3. All these exonic variations were found to be of heterozygous nature except two that were homozygous.

The proband DKEG12A had four exonic variations one in exon 1 viz., R76K which was homozygous and other three were in exon 3 as T325M, L349L, R470R all were heterozygous. The change T325M is a

novel variation, that has been reported in a cohort study of exome sequencing (NCBI database). Its precise role in causing glaucoma is as yet undetermined, as there is no report of this variation in POAG. Two of the novel synonymous variations viz., L349L, R470R are being documented for the first time to be in association with glaucoma.

The proband DKEG11A had Q368X in heterozygous state which is a truncated mutation. This mutation has earlier been reported in western populations as well as in another study involving the north Indian population.

There were many intronic variations noted in probands DKEG3A, DKEG4A, DKEG5A, DKEG7A, DKEG9A, DKEG10A, and DKEG12A. Some were heterozygous and others were homozygous variations.

CONCLUSION

In this study, patients having positive family history of glaucoma were evaluated to assess the role of genetic screening in early detection of this dreaded disease.

For the first time a truncated mutation viz., Q368X was detected in one of the patients (DKEG11A) which has previously been reported in a western population. Similar mutation has also been reported in a study from north India. But this is the first time this mutation is being reported in a south Indian population. Studies have indicated a 4.7 fold increased risk of POAG in patients carrying this mutation.[30] This patient had an advanced glaucomatous damage in both eyes and had undergone shunt surgery (EXPRESS SHUNT) in his right eye while trabeculectomy in his left eye. However his intraocular pressures were not under surgical control and had to be started on topical antiglaucoma medications post operatively. This probably suggests that this variation might also be responsible for the severity of the disease presentation.

The variation (T325M) was identified in a proband DKEG12A who was clinically documented to have open angles. This patient also had a tubular field of vision with advanced glaucomatous changes in the optic

disc and was on topical antiglaucoma medications. A perusal of the literature pointed out documentation of this in a population based cohort study of exome variation in a north American population (NCBI-dbSNP database). It has to be characterized functionally to deduce its effect on the pathology of primary open angle glaucoma.

In conclusion molecular screening of myocilin gene in glaucoma patients might aid in early diagnosis and treatment there by imparting a better visual prognosis to the patients for efficient clinical management and lessening the burden of morbidity.

PART – III

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ABBREVIATION

CCT	-	Central corneal thickness
ECM	-	Extracellular matrix
HUGO	-	Human genome organisation
IOP	-	Intraocular pressure
JOAG	-	Juvenile onset open angle glaucoma
MMP	-	Matrix metalloproteinase
MYOC	-	Myocillin
OCT	-	Optical coherence tomography
OPTN	-	Optineurin
PACG	-	Primary angle closure glaucoma
PCG	-	Primary congenital glaucoma
PCR	-	Polymerase chain reaction
POAG	-	Primary open angle glaucoma
RGC	-	Retinal ganglion cells
RNFL	-	Retinal nerve fibre layer
SITA	-	Swedish interactive threshold algorithm
SNP'S	-	Small nuclear polymorphisms
TIGR	-	Trabecular meshwork induced glucocorticoid response protein
TOP	-	Tendency oriented perimetry

GENETICS

A - Adenine

G - Guanine

C - Cytosine

T - Thymidine

CODON FOR AMINO ACIDS

	T			C			A			G		
T	TTT	Phe	F	TCT	Ser	S	TAT	Tyr	Y	TGT	Cys	C
	TTC	Phe	F	TCC	Ser	S	TAC	Tyr	Y	TGC	Cys	C
	TTA	Leu	L	TCA	Ser	S	TAA	stop	*	TGA	stop	*
	TTG	Leu	L	TCG	Ser	S	TAG	stop	*	TGG	Trp	W
C	CTT	Leu	L	CCT	Pro	P	CAT	His	H	CGT	Arg	R
	CTC	Leu	L	CCC	Pro	P	CAC	His	H	CGC	Arg	R
	CTA	Leu	L	CCA	Pro	P	CAA	Gln	Q	CGA	Arg	R
	CTG	Leu	L	CCG	Pro	P	CAG	Gln	Q	CGG	Arg	R
A	ATT	Ile	I	ACT	Thr	T	AAT	Asn	N	AGT	Ser	S
	ATC	Ile	I	ACC	Thr	T	AAC	Asn	N	AGC	Ser	S
	ATA	Ile	I	ACA	Thr	T	AAA	Lys	K	AGA	Arg	R
	ATG	Met	M	ACG	Thr	T	AAG	Lys	K	AGG	Arg	R
G	GTT	Val	V	GCT	Ala	A	GAT	Asp	D	GGT	Gly	G
	GTC	Val	V	GCC	Ala	A	GAC	Asp	D	GGC	Gly	G
	GTA	Val	V	GCA	Ala	A	GAA	Glu	E	GGA	Gly	G
	GTG	Val	V	GCG	Ala	A	GAG	Glu	E	GGG	Gly	G

PROFORMA

Case No :	
Date :	Unit :
O.P. No :	GC NO :

Name of the Proband

Date of Birth

Age & Sex

Fathers Name &
Age:_____
Affected: Yes ☐ No ☐

Mothers Name &
Age:_____
Affected: Yes ☐ No ☐

Siblings if any : _____
Affected: Yes ☐ No ☐

Address :
_____

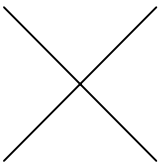
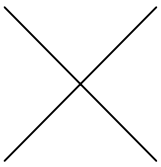
_____

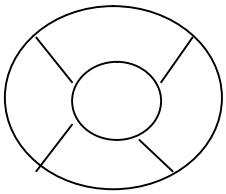
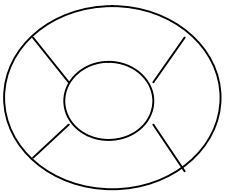
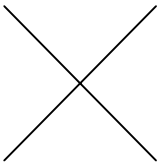
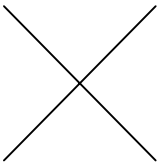
_____
Contact No:
_____

Pedigree details:

SLIT LAMP EXAMINATION

Right Eye		Left Eye
	Lids	
	Conjunctiva	
	Cornea	
	Anterior Chamber	
	Iris	
	Pupil	
	Lens	

	VISION	
	IOP	
	CCT	
	FUNDUS CD RATIO NASALISATION BAYONETTING NRR THINNING	
	GONIOSCOPY (SHAFFER'S GRADING)	
	AUTOMATED PERIMETRY	

RE	OCT	LE
	MACULAR TOPOGRAPHY	
	RNFL	
	ONH DISC AREA CUP AREA RIM AREA CD H RATIO CD V RATIO CD AREA RATIO	

MASTER CHART

S.NO	Sample Number	Name	Age	Sex	Affected or not	BCVA		IOP mmhg		CCT µ		Gonioscopy		Fundus(CD Ratio)		Fields		OCT- RNFL	
						RE	LE	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
1	DKEG1A	Padmalatha	39	F	YES	6/9	6/6	16	18	552	561	Open	Open	0.8	0.7	SA	SA	109	118
2	DKEG1B	Meenakshi	69	F	YES	6/6	6/6	14	14	540	545	Open	Open	0.6	0.7	WNL	SA IA	113	106
3	DKEG1C	Srinivasan	35	M	NO	6/6	6/12	12	14	535	544	Open	Open	0.4	0.4	WNL	WNL	115	101
4	DKEG1D	Ganesh	36	M	NO	6/6	6/6	14	16	565	560	Open	Open	0.4	0.4-0.5	WNL	WNL	105	110
5	DKEG2A	Shanthi	62	F	YES	6/9	6/9	12	12	570	572	Open	Open	0.6	0.6	IA	IA	88	93
6	DKEG2B	Subbulakshmi	51	F	YES	6/9	6/9	20	22	576	578	Open	Open	0.6	0.6-0.7	IA	NS	96	98
7	DKEG3A	Vasantha Gokila	66	F	YES	PL	6/12	14	14	520	540	Open	Open	0.9	0.6	NP	IA	NP	98
8	DKEG3B	Karthikeyan	47	M	NO	6/6	6/6	18	16	528	524	Closed	Closed	0.5	0.4	WNL	WNL	122	112
9	DKEG3C	Bhuvaneshwari.P	45	F	NO	6/6	6/6	18	18	529	520	Open	Open	0.4	0.4	WNL	WNL	115	118
10	DKEG3D	Rajeshwari	43	F	NO	6/6	6/6	16	16	520	520	Open	Open	0.4	0.5	WNL	WNL	120	101
11	DKEG4A	Ramakrishnan	63	M	YES	NO PL	1/2 / 60	42	34	521	534	Open	Open	0.9	0.9	NP	NP	NP	NP
12	DKEG4B	Muthu Chella Bharathi	24	M	NO	6/6	6/6	12	18	570	576	Open	Open	0.3	0.3	WNL	WNL	105	113
13	DKEG4C	Muthukala	24	F	NO	6/6	6/6	12	12	525	579	Open	Open	0.3	0.3	WNL	WNL	116	118
14	DKEG4D	Malathi	31	F	NO	6/6	6/6	12	12	533	530	Open	Open	0.3	0.3	WNL	WNL	103	111
15	DKEG5A	Ulagammal	43	F	YES	6/6	6/6	18	18	545	540	Open	Open	0.6	0.8	SA	SA,IA	98	88
16	DKEG5B	Somasundaram	40	M	YES	6/6	6/6	18	16	534	530	Open	Open	0.7	0.8	SA,IA	SA,IA	96	90
17	DKEG6A	Vignesh	18	M	YES	6/6	6/6	24	24	593	623	Open	Open	0.7	0.6	NS	IA	96	98
18	DKEG6B	Subashree	9	F	NO	6/6	6/6	14	16	520	540	Open	Open	0.5	0.4	WNL	WNL	100	103

S.NO	Sample Number	Name	Age	Sex	Affected or not	BCVA		IOP mmhg		CCT µ		Gonioscopy		Fundus(CD Ratio)		Fields		OCT- RNFL	
						RE	LE	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
19	DKEG6C	Rajendran	47	M	YES	6/12	6/12	12	12	540	520	Open	Open	0.7	0.7	SA	SA	94	98
20	DKEG7A	Selvaraj	60	M	YES	6/6	6/6	36	22	520	580	Open	Open	0.7	0.8	IA	IA	81	96
21	DKEG7B	Surendar	24	M	YES	6/6	6/6	20	34	574	578	Open	Open	0.6	0.6-0.8	IA	SA,IA	98	88
22	DKEG8A	Malini	53	F	YES	6/6	6/6	22	18	531	522	Open	Open	0.5	0.5	IA	IA	100	98
23	DKEG8B	Rekha	33	F	NO	6/12	6/9	18	18	548	540	Open	Open	0.4	0.4	WNL	WNL	112	106
24	DKEG8C	Krishnakumar	27	M	NO	6/12	6/6	12	12	540	540	Open	Open	0.5	0.3	WNL	WNL	106	104
25	DKEG9A	Bhuvaneshwari.A	53	F	YES	6/6	6/6	16	14	520	525	Open	Open	0.4	0.7	IA	SA,IA	100	96
26	DKEG9B	Banumathy	58	F	NO	6/9	6/12	12	12	560	560	Open	Open	0.3	0.3	WNL	WNL	116	110
27	DKEG10A	Suganthi	50	F	YES	6/6	6/6	12	14	540	535	Open	Open	0.8	0.8	SA,IA	SA,IA	98	94
28	DKEG10B	Rajkumar	21	M	YES	6/6	6/6	18	20	520	540	Open	Open	0.8	0.7	WNL	WNL	104	110
29	DKEG11A	Premanathan	57	M	YES	6/6	6/12	32	38	560	574	Open	Open	0.9	0.9	Tubular	Tubular	95	97
30	DKEG11B	Dinesh Kumar	22	M	NO	6/6	6/6	12	12	540	540	Open	Open	0.3	0.3	WNL	WNL	104	106
31	DKEG12A	Leelavathy	48	F	YES	6/6	6/6	18	12	520	540	Open	Open	0.9	0.9	Tubular	Tubular	NP	NP
32	DKEG12B	Rajathi	32	F	NO	6/6	6/6	14	14	530	545	Open	Open	0.6	0.5	WNL	WNL	104	100
33	DKEG12C	Shanthi	50	F	NO	6/6	6/6	14	14	535	520	Open	Open	0.4	0.4	WNL	WNL	113	114
34	DKEG12D	Varalakshmi	48	F	NO	6/6	6/6	14	14	540	545	Open	Open	0.5	0.5	WNL	WNL	120	115
35	DKEG12E	Krishnakumari	42	F	NO	6/6	6/6	14	12	520	530	Closed	Closed	0.6	0.6	WNL	WNL	106	108

KEY TO MASTER CHART

BCVA	-	Best corrected visual acuity
IOP	-	Intraocular pressure
CCT	-	Central corneal thickness
CD Ratio	-	Cup: Disc ratio
SA	-	superior arcuate defect
IA	-	Inferior arcuate defect
NS	-	Nasal step defect
WNL	-	Within normal limits
OCT	-	Optical coherence tomography
RNFL	-	Retinal nerve fibre layer thickness
NP	-	Not possible