

GENERATION of NOVEL RANDOM MUTAGENESIS LIPASE LIBRARIES  
via  
DIRECTED EVOLUTION

by  
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# Generation of Novel Random Mutagenesis Lipase Libraries via Directed Evolution

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## Abstract

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) function as significant biocatalysts in biotechnological applications. The fact that their mechanism, selectivity and structure is well known make lipases a suitable candidate for studies of protein engineering and directed evolution. Using the merits of DNA shuffling method, directed evolution and random mutagenesis, libraries of mutant lipases are constructed with improved features and functionality of pre-existing ones, which in turn encourages the use of industrial lipases in applications such as biosensors, pharmaceuticals, agrochemicals, bioremediation, etc. With increasing demand on lipase production for commercial use, it has thus become crucial to identify and isolate novel and target-specific lipases, as well as optimizing existing ones for acquisition of desired functionality. The aim of this study is to generate mutant lipase libraries using directed evolution and to screen for a candidate biocatalyst. There are two lipases of interest, the mesophilic *Aspergillus niger* lipase (ANL) and the thermophilic *Bacillus thermocatenuatus* lipase (BTL), which were shuffled in order to obtain a mutant library that would have the desired features such as increased thermostability, pH stability and a broader range of substrate specificity.



# Yönlendirilmiş evrim metodu ile yeni, rastgele mutasyona uğratılmış lipaz kütüphanelerinin oluşturulması.

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Anahtar Kelimeler : DNA karma metodu, Yönlendirilmiş evrim, Endüstriyel lipazlar,  
Rastgele Mutasyon Yöntemi

## Özet

Lipazlar (triasilgliserol asilhidrolaz EC 3.1.1.3), biyoteknolojik uygulamalarda etkili rol oynayan biyokatalizörlerdir. Mekanizmaları, seçicilikleri ve yapıları bilindiği için, protein mühendisliği ve yönlendirilmiş evrim metodlarına uygun adaylardır. DNA karma metodu, yönlendirilmiş evrim ve rastgele mutajenez tekniklerinin bir arada kullanılabilmesi, istenilen özelliklere sahip mutant lipaz kütüphanelerinin oluşturulabilmesine imkan tanımıştır. Bu durum, lipazların aynı zamanda biyosensör, ilaç, tarım endüstrilerinde kullanılmasına olanak sağlamıştır. Lipazların ticari kullanımına karşı artan talep sonucu yeni ve hedefe özgün lipazların tanımlanması ve izole edilmesi kritik önem taşımaktadır. Bu tez, yönlendirilmiş evrim tekniğiyle mutant lipaz kütüphanelerinin oluşturulmasını hedef almakla beraber, aday biyokatalizörlerin analizini yapmayı amaçlamaktadır. Bunun için, fungal ve mezofilik bir lipaz olan *Aspergillus niger* lipazı (ANL) ile bakteriyel ve termofilik bir lipaz olan *Bacillus thermocatenuatus* lipazı (BTL) DNA karma metodu ile karıştırılmıştır. Rastgele mutajene olacak olan enzimlerin, termostabilite, pH stabilite ve sübstrat seçiciliğinde artı göstermesi ve endüstriyel ve biyoteknolojik uygulamalarda önemli bir lipaz olması öngörülmektedir. Elde edilen klonlarda, sübstrat seçiciliği değişikliği görülmemekle beraber, enzimlerin genel aktivitelerinde belirli değişiklikler saptanmış, ve bu özellikler yapısal boyutta tartışılmıştır.

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*To ones who are close to the heart.*



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# **1 Introduction**

## **1.1 Lipases**

Lipases are metabolic enzymes which are involved in every domain of life. Animals, plants and microorganisms are all producing various types of lipases. Lipases were discovered by Eijkmann in 1900s. This discovery was simply the observation of several bacteria that can produce and secrete lipases and the degradation of lipids via these produced enzymes. Microbial enzymes are one of the largest enzyme class due to the large variety of known microbes and therefore lipases are one of the most studied enzyme class as well. Since lipases have been studied sweepingly, their mechanism of action, selectivity and structure are already well known [1, 2].

Lipases (triacylglycerol acylhydrolases; EC 3.1.1.3) function as biocatalysts to hydrolyze triglycerides into glycerol and fatty acids on water-insoluble substrates [3]. They actively have a role on the digestion, transportation and processing of triglycerides. It is well known fact that bacteria, yeast and fungi share a high potential on the production of lipases. Since lipases which are produced from different types of microorganisms have specific features in terms of substrate specificity, heat resistance thresholds and wide pH range, these features make them efficient in terms of selectivity through many industrial



applications such as biosensors, pharmaceuticals, cosmetics, agrochemicals, bioremediation etc. Lipases have been used on numerous studies, ranging from industrial production and immobilization techniques, to the analysis of pure enzymes and their biocatalytical features [4]. For instance, lipase-detergent compounds are used to clean surface fatty residues and clogged drains [5].

As well as being lipolytic, lipases have the capability to perform esterification. This combined feature enables them to be effective under wide substrate range [6]. With increasing demand on lipase production for commercial use, it has become crucial to identify and isolate novel and target-specific lipases, as well as optimizing existing ones for acquisition of desired functionality. Nowadays, this can be achieved by directed evolution techniques, and it is heavily relied upon [7]. By means of directed evolution, it is aimed to improve features and functionality of pre-existing lipases, both with time and cost efficiency. DNA shuffling is a widely used technique, serving as a fundamental method for studies in directed evolution.

Since properties of lipases are mainly strain-dependent, the catalytic properties and functional parameters are crucial for design and application procedures, including thermostability, specificity, optimum pH and enantioselectivity. For instance, *Aspergillus niger* lipase (ANL) is one of the significant biocatalysts used in industrial food processing and production, utilized as food and detergent additive, as well as in cellulose acetylation [8]. ANL shows regio-selectivity on its first and third position, towards glycerol binding site. Because of this, this lipase has proven to be safe for utilization in food and pharmaceutical industry [9]. The thermoalkalophilic lipase, *Bacillus thermocatenuatus* lipase 2 (BTL2), on the other hand, is an enantioselective biocatalyst which shows considerably high resistance and stability at elevated temperatures and organic solvents, making it a hub for industrial and biotechnological applications [10]. Quyen *et.al*, (2002) have successfully produced the recombinant BTL lipase out of both *Pichia pastoris* and *E.coli* and performed enzyme characterization [11].

## 1.2 Reaction

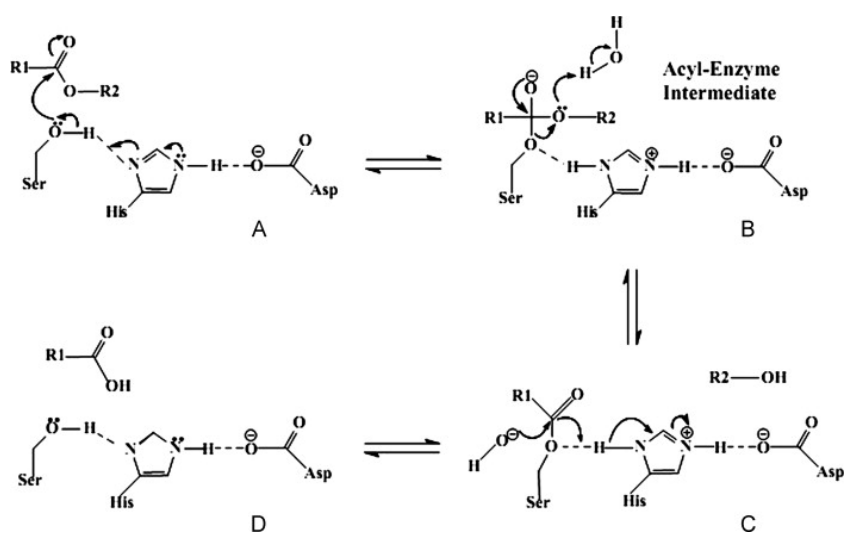
The hydrolysis of triacylglycerols to free fatty acids, diacylglycerols, monoacylglycerols and glycerol is catalyzed by lipases. This breakdown reaction is an equilibrium reaction, which can be disturbed by changing the concentration of substrates or products. One of the reactants of hydrolysis reaction is water. Therefore, changing the hydrolytic conditions of the hydrolysis causes a shift of the equilibrium [12].

Either by breaking as in hydrolysis or by forming as in esterification, lipases act on carboxylic ester bonds. The acyl transfer reactions through esterification are also catalyzed by lipases. Triacylglycerols are commonly their natural substrates, depending on the chemical properties of the reactants and the amount of water in the medium [13]. Under low water conditions, a carboxyl/thiolester or amide can also be formed. An ester group, serving as an acyl donor, can form an acyl-enzyme intermediate by either releasing an acid, in this case acyl acceptor would be water, or by forming a new ester and in that case acyl acceptor would be alcohol/thiol/amine. In addition, acidolysis, alcoholysis, aminolysis and interesterification can be given as examples to transesterification.

## 1.3 Mechanism

Although lipases catalyze many versatile reactions, the reaction mechanisms are distinctive. In all lipases, the catalytic machinery is conserved and it is composed of three residues, which are serine, histidine and aspartate/glutamate [14]. Two residues (histidine and aspartate/glutamate) are aligned in order to lower the pKa of the serine hydroxyl, so that serine can carry out a nucleophilic attack on the ester bond. The acyl donor, the substrate, interacts with the active site of the lipase, forming the enzyme-substrate (ES) complex. The hydroxyl group of serine is activated by the histidine in the catalytic triad. Serine carries out a nucleophilic attack on the carbonyl carbon of the substrate, so that the first tetrahedral intermediate is formed. The main-chain amide groups of two residues generate a hole and the negative charge on the oxyanion is stabilized. The aspartate or glutamate stabilize the positive charge on the histidine. The tetrahedral intermediate is

then decomposed into another intermediate which is determined by the first leaving group of the substrate (an alcohol or an acyl enzyme intermediate). A second tetrahedral intermediate is formed right after the formation of the acyl enzyme intermediate. Newly formed intermediate corresponds to the highest energy barrier in the reaction. In order to yield the deacylated-free form of the enzyme and the hydrolysis of the second substrate, an acid, this intermediate is also collapsed. A proton is transferred from the substrate to histidine during the deacylation step.



Scheme 1: Mechanism of hydrolysis by lipases. During Step A, His residue acts as a general base and removes a proton from the active site of Ser. In Step B, an acyl-enzyme intermediate is formed, followed by the deacetylation (Step C). With a nucleophile attacking the acetylated enzyme, the catalytic site is regenerated and a long-chain fatty acid is formed as a product (Step D). [15]



## 1.4 Selectivity

Lipase selectivity towards Triacylglycerols (TAGs) is generally categorized as regio-selectivity, stereo-selectivity and substrate selectivity [16,17]. Regio-selectivity is related to the position of the ester bond in TAG, whereas stereo-selectivity is related to the chiral center. Substrate selectivity is related to the type and chain-length.

### 1.4.1 Substrate Selectivity

Lipases show selectivity regarding to the type and the chain-length of the acyl groups in their substrates. They can differ in terms of certain fatty acids or groups of fatty acids. For instance, porcine is a lipase with specificity to cis-2 over cis-7 octadecenoyl moiety [2]. Moreover, the lipases most preferred substrate is primary alcohols and the least preferred is tertiary alcohols [5]. Lipases are also able to accommodate cyclic esters, thioesters and amines apart from triacylglycerols and aliphatic esters [18–20]. The chain-length selectivity of lipases has been the subject of many studies [21–24]. Lipases mostly prefer a range of medium (C6) to long (C16) chain-length with respect to the chain-length of fatty acids [2]. However, there are some exceptions to this generalization such as *Penicillium roqueforti* and *Bacillus thermocatenuatus* lipases and they hydrolyze only short chains of C4.

## 1.5 Structure

In 1990, the first lipase structure was crystallized by Brady [14]. In protein data bank, there are more than one hundred 3D lipase structures available. According to these studies, some common features of all lipases are determined. One of the common features is that all lipases are among the members of  $\alpha$ - $\beta$  (hydrolase fold so that lipase structures are composed of central sheets and surrounding helices [25–28]. Another one is that, in a hairpin turn between an  $\alpha$ -helix and  $\beta$ -sheet is placed the catalytic serine in lipases. A highly conserved penta-peptide sequence of G-X-S-X-G is also found in this region. This sequence forms a characteristic turn, which is referred as the nucleophilic elbow [9,26,27]. Moreover, the active sites in lipases consist of three amino acids, ser-

ine, histidine and aspartic/glutamic acid, which is common to another class of hydrolases, serine-protease [14,29]. Compared to proteases, the structural arrangement of the residues in active site is oriented to invert the stereochemistry of the catalytic triad in lipases, although they have the same chemistry for their active sites [30]. There is an amphiphilic lid found covering the active site of lipases [31, 32]. The composition and size of the lid structure differs from lipase to lipase. The lid from the lipase of guinea pig has only five amino acids where the lid from *Bacillus thermocatenulatus* lipase has two  $\alpha$ -helices which corresponds to 20% of the whole lipase structure [28, 33].

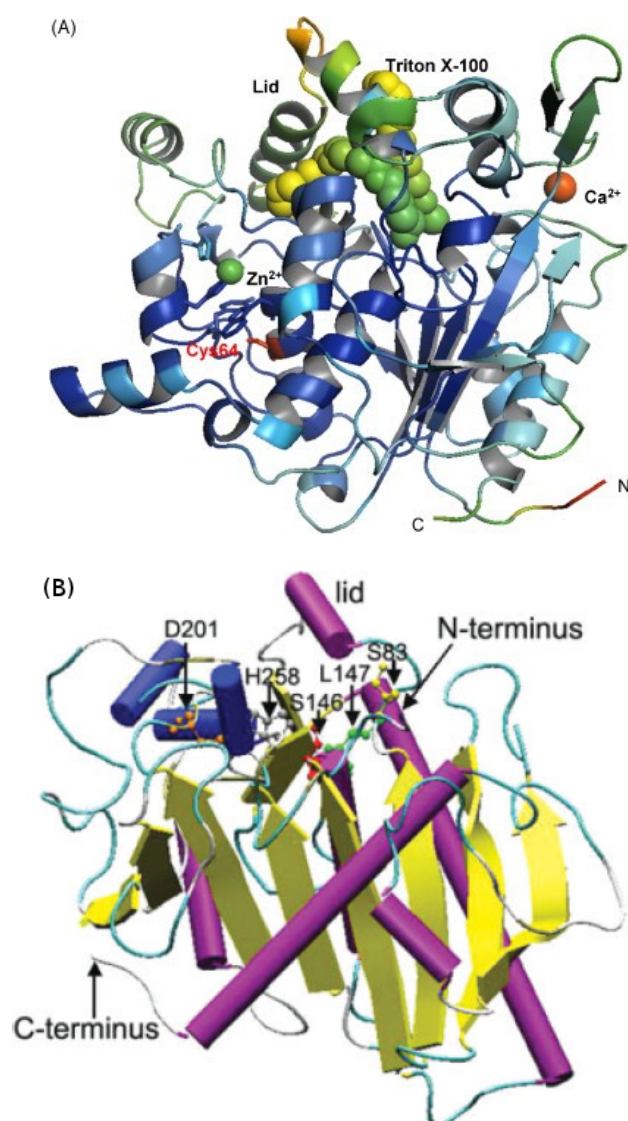
In addition to the given features of lipase structures, the catalytic cleft in lipases are attributed to specificity. The lipase clefts, fatty acid binding sites in particular, have been investigated and it came out that there are three different geometry that lipase structures may exhibit; crevice-like, funnel-like and tunnel-like [34]. These differences in the binding pockets is related to the diverse substrate specificities in lipases. Additionally, stereoselectivity depends on the steric interaction of the cleft with the substrate, so lipase structure is also critical for understanding the stereoselectivity [35].

## **1.6 Lipase in Industry**

Enzymes are generally produced from bio-based elements by fermentation so that the enzymes are entirely biodegradable and are able to be revitalized [38]. Lately, there has been many improvements made in the production processes. These have led us that delivering enzyme concentrates at relatively low costs [39] is possible – encouraging enzyme applications in the bulk industrial process.

Since the hydrolysis or production of many esters can be catalyzed by naturally occurring fats and oils, they are the generally favored substrates of lipases [40–43]. With their far-scaled spectrum of substrates, lipases are among the largest class of biocatalysts considering the two coercing enzyme markets which are food processing and detergent industry, are the mainly operations of lipases [42].

Since lipases are able to catalyze reactions in aqueous and in organic media, they are especially tempting for solving challenging synthesis of organic reactions [44]. Lipases are particularly attractive for protein engineering applications because of their broad use in industry consequent to their important products. The extracellular nature of microbial lipases enables them to be produced easily at large quantities and to be isolated and they are the favored source for many industrial applications. Being stable in organic solvents, at high temperatures and ionic strengths, not requiring cofactors and having a wide substrate selectivity and high enantioselectivity are among the main reasons for the high



**Figure 1:** (A) – Structure of mature *Bacillus thermocatenuulatus* lipase 2, on which amino and carboxyl termini, Zn<sup>2+</sup>, Ca<sup>2+</sup> and cysteine 64 is labeled. [36] (B) – Structure of *Aspergillus niger* lipase by homology modeling [37].

industrial potential of microbial lipases [45]. Large number of articles and reviews studied in the molecular biology, biochemical and structural properties of lipases and their biotechnological applications reflect the tempt in microbial lipases.

Removal of the pitch in pulp industry is also among the known applications of lipases. The hydrophobic component of wood is called pitch and it must be removed before processing. At low costs, the enzymatic removal of pitch is achieved via *C. rugosa* lipase [46]. Lipases are also used in the textile industry in order to bypass a process called stoning, especially for denim production [47]. Abbreviated exposure to toxic chemicals used in chemical process, which requires asbestos, is among the numerous advantages of this enzymatic bypass mentioned above. Pharmaceutical industry also uses lipases because of their ability to synthesize enantio-pure drugs [48–50]. Ibuprofen for pain, Taxol for cancer and Diltiazem for high blood pressure medications are examples to enantio-pure drugs. The costs and drug toxicity in pharmaceutical industry are reduced by the use of lipases in enantioselective production of drugs.

## **1.7 Protein engineering and Directed Evolution**

Directed evolution is an *in vitro* process, where genetic mutations are generated and inserted into a microorganism's genome to analyze specific functional patterns and properties on molecular level. Directed evolution performs under the same fundamental principles as natural evolution: the offsprings vary from the predecessor and selection criteria is the survival of the fittest. In nature, random mutation and recombination lead to genetic diversification. Directed evolution requires a mechanism, for introducing those genetic variations such as error prone PCR, nucleoside analogs [51], degenerate oligonucleotides [52], propagation in strains that lack DNA repair capabilities, growth in the presence of chemical mutagens and DNA shuffling for recombination [53].

Mimicking the evolution *in vitro* would provide a better understanding of natural evolution as well as allow the development of new enzyme activities. Since there is a considerable interest in new biocatalysts, directed evolution became more widely used in

industrial and academic laboratories in order to generate and modify enzymes [54].

Directed evolution in laboratory, necessitates a precise selection of a suitable starting genes. A suitable candidate for molecular evolution is the class of  $\alpha/\beta$ - barrel class of proteins due to their wide spectrum of catalytic functions [54]. Evolution did its job by evolving  $\alpha/\beta$ - barrel to have a substrate binding sites within the barrel and the catalytic residues within the connecting loop regions. These distinct regions, which are responsible for specificity and catalysis, are suitable tools for their semi-autonomous evolution. And this will lead to generate diversity in a combinatorial manner.

Protein engineering has been used as a key concept for producing biotechnologically functional and novel biocatalysts. It has been applied on numerous fields such as oil recovery enhancement, in which cellulosic ethanol has been produced [55], as well as detergents, in which proteases are used [56], and polyester production via enzyme modeling and engineering [57].

Since rational design introduces mutation(s) specifically for the desired properties of certain protein sites and acquires numerous structural and functional parameters, as well as characteristic information about the enzymes, itself and molecular evolution are the fundamental approaches held for protein engineering. Despite the fact that molecular evolution does not necessarily need any structural or functional information of the enzymes, randomly generated mutants need additional screening for establishing desired properties.

To choose a best approach, limitations of both approaches should be considered thoroughly, such as methods for mutagenesis, information intensity that includes further details of structural and functional information, and selection and screening methods for directed evolution techniques. Thereby, various strategies may result in a different outcome, all having their certain advantages or disadvantages. The trade-off between rational design efforts and screening can be given as an example. If X-ray crystal structures are used for the rational design of a well-characterized and understood enzyme, it may limit

screening to a few number of amino acid substitutions. On the contrary, if a powerful screening method is applied, it may disregard the rational design altogether [58]. As a result, choosing the optimal method depends on detailed information about the locations for amino acid substitution, as well as the screening methods, group wise.

Another common target for protein engineering is to increase thermostability of a protein. A rigid and stable enzyme can be engineered, specifically functional at high temperatures, using the X-ray structure as one approach, following a design of stability interactions such as disulfide bonds and/or salt bridges, as well as inserting Proline or removing Glycine for stabilizing loops and focusing specifically on mutagenesis at flexible regions of the desired enzyme [58].

Another improvement can be made on the catalytic activity and the enantioselectivity of the enzyme, by substituting certain amino acid residues on the catalytic site of a target protein that are closely located [57]. Certain strategies for introducing substitution includes shuffling, simultaneous mutagenesis (for multiple amino acid substitution), Error-prone PCR, saturation mutagenesis (single amino acid substitution), and gene synthesis for specific modifications [59]. While establishing multiple amino acid substitutions provides numerous possibilities and design parameters for a target protein, it may lead to a loss in cooperative interactions and create a large library that has many idle variants. To overcome this problem with cooperative interactions of multiple amino acid substitutions, F.H. Arnold has suggested a stepwise accretion of single amino acid substitutions that has each variant superior than the previous one [60].

To be able to locate paths for a specific derivative of a protein with desired properties, all available paths should be tested beforehand. As an example, for an amino acid substitution that has five positions, there are 120 possible paths that leads to a final decisive variant. In a study, the resistance of  $\beta$ -lactamase was enhanced with 18 paths at each stage [61]. Thermostability of the enzyme phytase has been enhanced. After following 9 rounds of optimization, Tyr277Asp mutation has been resulted as the only single base exchange,

while the other was double-base (three mutations) and three-base exchanges [62]. A prior mutation earlier than the final variant being developed may lead to a dead end, as well as random mutations at several sites. Nevertheless, this may cause further screening efforts, therefore a well-designed selection method can be applied beforehand, as well as eliminating unfolded or unstable proteins by reductionist assumptions [63, 64] to diminish the number of variants that needs to be screened [65].

It is probable to detect which mutations are more functionally beneficial for a particular given characteristic by manipulating the data manually, if it's a small dataset. However, as more mutations are evaluated and screened for functional variance through each library, the analysis of the data by hand becomes too complicated as it increases in size. Statistical analysis introduced by ProSar uniquely analyzes the biological evolutionary relationship between protein structure and activity and it compares the data for each variant that has same or similar substitutionary information which in turn provides a clear understanding about whether the particular substitution is functionally significant or non-functional at all [66, 67]. In his study, G.W. Fox has used this statistical approach to successfully observe the evolutionary path of a halohydrin dehalogenase [68].

As easily may be anticipated, there are numerous screening strategies, most of them being either time or labor intensive. Most of the time, a final target variant can be missed out due to a lack of a suitable and efficient screening-selection strategy. However, some limited calorimetric screening methods can be developed for a limited number of enzymes to overcome this complication. To be concise, the best protein engineering approach may be defined as the one that brings the optimal solution with the least amount of effort and with a time efficiency. Because of this reason, certain individual approaches should be combined in order to produce the optimum outcome. For instance, due to the lack of a suitable screening methodology for industrial enzymes used in directed evolution applications, and the difficulty of hitting the optimal substitution for an enzyme with desired functionality in rational design, using these approaches individually is not a unique protein engineering approach. Instead, a combination of both strategies is crucial to provide a

novel and efficient path for enhancing the functional and structural properties of enzymes and thereby leading to rapid improvements in protein engineering.

### 1.7.1 DNA shuffling

Stemmer had introduced DNA shuffling as a technique for *in vitro* recombination of homologous genes, for accelerating evolution rate of certain genes to perform directed evolution. The technology is highly used in applications such as gene therapy, vaccines, small molecule pharmaceuticals, and so on [7]. DNA shuffling techniques mimic diversity due to the merits of meiotic recombination. It is noted that libraries as large as  $10^{15}$  molecules can be constructed by directed evolution. This may be considered as a drawback in terms of challenges at screening procedures; however, it is more of an advantage in terms of obtaining more recombinations that facilitates the production of a targeted enzyme, which is aimed to be utilized in industrial applications. As well as recombining DNA fragments, point mutations are also introduced to the sequence with a low rate, naturally propose a high-throughput methodology – both accelerating rate of evolution and obtaining the desired features for functionality, which leads to a novel advancement in industrial applications [7].

The classical DNA shuffling method is basically performed by digesting specific genes by DNase I enzyme and attaining pieces of different types of genes shuffled together and reassembled under optimized PCR protocol, followed by integration into vectors and transformation into the target organisms [69, 70]. One of the advantages of DNA shuffling is that, after the gene to be improved is introduced with a point mutation, there is a variety of beneficial mutations which are low on frequency relative to deleterious mutations, which then can be added to the cycle one at a time, building more beneficial mutations that eventually give out the best mutant from that given cycle [7]. Suen *et al.* (2004), have used DNA shuffling method on *Candida antarctica* lipase (CALB) to obtain chimeric lipase B. The obtained lipase have shown 20 fold increase in activity and 11 fold increase in the 45°C half life towards the diethyl 3-(3',4'-dichlorophenyl) glutarate (DDG) substrate, compared to its wild type [71]. Yu *et al.* (2012) have also used DNA shuffling method



to increase heat resistance of *Rhizopus chinernsis* lipases. The half life of the obtained mutant at 60°C and 65°C have increased by 46 and 23 fold, respectively [72].

Directed evolution has played a significant role in improving the performance of an enzyme (or create one) through introducing new features that natural selection normally would not necessarily provide [73], as well as enhancing the selection criteria to yield targeted properties for that specific enzyme or microorganism, through custom schemes. A lead enzyme is picked and mutagenized, followed by selection or screening which results in improved variants that contains the target evolved enzyme. Protein molecules can be altered due to their structural and functional properties, which in turn can increase their thermal stability or introduce a new functionality (enzyme engineering), as well as changing the topology or structure, and altering the existing properties for improvement. While creating new enzymes improved for applications in industry and biotechnology, directed evolution methods can also be applied to improve limitations of certain functions of proteins, via accumulating beneficial mutations that lead to an augmentation of the enzyme's activity. In a study, it has been noted that the improved enzyme previously containing 10 amino acid substitution is enhanced by 157-fold [73].

## 2 Materials and Methods

### 2.1 Molecular Cloning

*Bacillus thermacatenulatus* lipase gene(*BTL2*), which is 1,167 bp DNA fragment, and *Aspergillus niger* lipase gene(*ANL*), which is 891 bp, are amplified from the mature lipase clones (pMCSG7 - *BTL2* and *ANL*). Primer sets are containing ligation independent cloning (LIC) sites; for forward (F\_*BTL2*\_LIC : 5'- TACTTCCAATCCAATGCGCGGCATCCCCACGC - 3' and F\_*ANL*\_LIC: 5' - TACTTCCAATCCAATGAAATGTTCTCTGGACGGTTTG - 3') and for reverse (R\_*BTL2*\_LIC: 5' - TTATCCACTTCCAATGTTAAGGCCGCAAACACTCGCC - 3' and R\_*ANL*\_LIC: 5' - TTATCCACTTCCAATGAATAGCAGGCACTCGGAAA - 3'). PCR conditions are the following: 5 min at 94 °C, 35 cycles of 30 sec at 94 °C, 30 sec at 53 °C, 1 min at 72 °C, 10 min at 72 °C. After DNA shuffling procedures, 4µg of expression vector, pMCSG7, is linearized using *SspI* restriction enzyme (see Appendix A.1 for vector map). Linear vector and insert are electroporated at 135 V in 1.5% agarose gels using tris borate EDTA (TBE) buffer system for 20 minutes. Both fragments are extracted from agarose gel and treated with T4 DNA Polymerase. The exonuclease activity is restricted using excess amount of dGTP for vector and dCTP for the inserts according to the given LIC sequences. The reaction is carried on for 50 minutes at 20°C followed by heat-activation at 70°C for 20 minutes. Phenol/chloroform extraction and ethanol precipitation procedures are applied to the T4 DNA polymerase treated DNA samples. Vector (in 5 µl) and shuffled lipase genes(in 3µl) solubilized in distilled water and annealed at 23°C for 16 hours. *E.coli* Shuffle chemically competent cells are prepared according to Maniatis *et al* [74]. All the annealing reaction after 16 hours are transformed into *E.coli* Shuffle cells by using a chemical transformation method [75]. For colony PCR , *ANL*\_LIC and *BTL2*\_LIC primer pairs are used in the given PCR cycle profile for the selected colonies. PCR products, which are amplified by Colony PCR reaction, are run in 1.5% agarose gel using GeneRuler 1kb DNA Ladder SM0311(Fermentas). To the colony PCR positive clones, plasmid purifications are applied. The plasmid purifications are done using Qiagen Plasmid Purification kit .

Plasmids are sequenced using the primer set combinations which are used at the DNA shuffling process by Molecular Cloning Laboratories (MCLAB).

## **2.2 DNA Shuffling**

PCR products of ANL and BTL2 genes, which are amplified with the PCR cycle mentioned above, are purified using Qiagen PCR Purification Kit. 1.2 $\mu$ g DNA from ANL and BTL2 is mixed in a tube and digested with 0.05 U DNase(Roche,10 U/ $\mu$ l) in 10X digestion buffer with MnCl<sub>2</sub> for 75, 90 and 120 min at room temperature. The reaction is inactivated with 2.5 mM EDTA and incubation at 85<sup>o</sup>C for 5 minutes. The mixtures are run in 2% agarose gel. Fragments that are lower than 50 base pairs in size and between 50 and 100 base pairs are extracted from the gel by using QIAEX II gel extraction kit. Extracted fragments are reassembled by the previous PCR cycles but without the primers. Amplification of the reassembled fragments are made through the same PCR reaction using the assembled fragments as the template but the primer combinations of ANL and BTL2 were added. Amplified reassembled fragments are cloned to pMCSG7 expression vector by ligation independent cloning.

## **2.3 Lipase Expression**

Shuffled genes are transformed to Shuffle *E.coli* by chemical transformation. Transformed cells are plated on LB-Agar plates with Ampicillin. Colonies, which survived, inoculated on LB- Rhodamine, which has IPTG (isopropyl- $\beta$  -D-thiogalactopyranoside), plates to check the qualitatively check the lipase activity. All possible mutants are also expressed in suspension culture using 1mM IPTG in LB broth. The expressions are lasted out for eight hours and sampled once at fourth hour. The cells are harvested by centrifugation (for 5 min at top speed) and lysed by using B-PER(Thermoscientific). After centrifugation at maximum speed for 10 min, lipase activity of soluble fractions is determined using fluorescent substrates, 4MU-C8 in 0.1M Tris at pH 7.25. SDS-PAGE gel is run to analyze the soluble fractions and visualized by coomassie staining.

## **2.4 Lipase Assays**

Lipase activity is determined in two different ways:

### **2.4.1 Rhodamine plate assays**

Selected colonies are inoculated on to the Rhodamine-LB agar plates which are containing IPTG (for expression), oil (as substrate), Rhodamine(dye that interacts with free fatty acids) and LB agar. When the expression starts at the inoculated colonies, expressed recombinant lipases start to hydrolyze oil. Fatty acids which yielded from the hydrolysis reaction interacts with the Rhodamine dye and gives light under UV. Preliminary detection of active recombinant enzymes is achieved by this screening technique.

### **2.4.2 Fluorescence assay**

For more quantitative measurement, lipase activity is measured with fluorescent assay methods. For fluorescent assays, lipase activity measured in a 96-well black micro titer plate using 4MU - caprylate as the substrate. Expression medium, which do not contain any cell, assayed in reaction medium of 100 mM Tris-Cl at pH 7.25. 4MU fluorescence is measured by using Gemini XS (Molecular Devices) using wavelength of 355 nm for excitation and an emission wavelength of 460 nm. For one hour, in every minutes, measurements are taken. All assays are made in duplicates and initial velocities are calculated using SoftMax Pro Software (Molecular Devices). Relative Fluorescent Unit obtained from fluorometer is converted to 4MU units with respect to the linear relationship obtained from 4MU standard curve.

## **2.5 Lipase Characterization**

### **2.5.1 Thermostability**

Soluble fractions of the clones that do have expressions, are used in fluorescent assays to profile thermostability by quantifying the residual activity of lipases after 30 minutes of incubation at temperatures 30°C, 40°C, 50°C, 60°C, 70°C, and 80°C is set to 100% activity for calculating the percent activity.

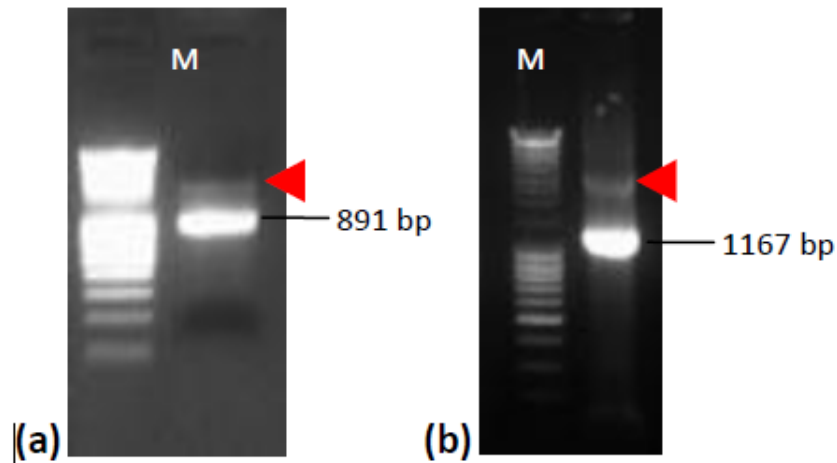
### **2.5.2 Substrate specificity**

Chain length specificity of the mutated lipases are screened by fluorescent enzyme assays using reaction medium of 100 mM Tris-Cl at pH 7.25 as the reaction buffer. From the chain lengths of 2C to 16C the following lipids are used; Acetate(2C), propionate(3C), butyrate(4), caproate(6C), enatiothe(7C), caprylate(8), and palmitate(16C).

### 3 Results

In this study, DNA shuffling method has been successfully performed on ANL and BTL2 genes in order to generate randomly mutated novel lipases. The reason ANL and BTL was chosen as candidates due to their evolutionary distance, in which they share 42% identity. Furthermore, the fact that *Bacillus thermocatenulatus* lipase is thermophilic and *Aspergillus niger* lipase is mesophilic, DNA shuffling on these candidate lipases could lead to obtain the expected features such as wide range of temperature, pH, which remarkably appeals to industrial and biotechnological applications.

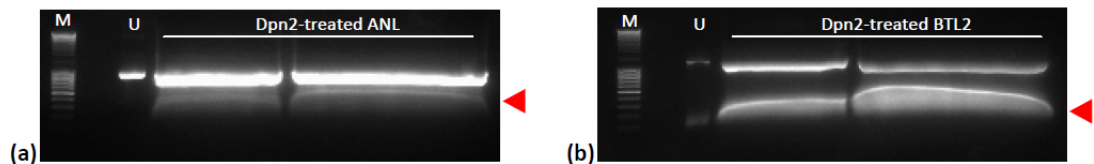
Due to the fact that DNA shuffling introduces random mutagenesis, numerous mutants can be produced in a single run, which in turn enables an increase in the shuffled gene library size. In our experimentation, multiple optimization experiments have been held for DNase digestion to obtain the desired fragment size (50bp), using time, temperature and DNA concentration as parameters. Using regular cloning methodology, randomly mutated library has been transformed into Shuffle type *E.coli* cells. For screening, only qualitative plate assay has been performed, and for sequencing, sequencing data have been obtained and analyzed from libraries to detect random mutations.



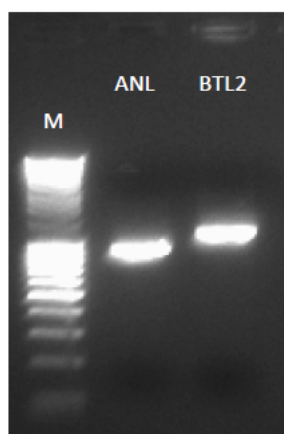
**Figure 2:** PCR amplifications of (a) ANL (891bp), and, (b) BTL2 (1167bp). Arrowheads show the plasmids. M: MassRuler DNA Ladder Mix (Fermentas).

ANL and BTL2 genes were amplified from the mature lipase clones, pMCSG7-ANL and BTL2, by using their specific primers. PCR conditions are the following: 5 min at 94 °C, 35 cycles of 30 sec at 94 °C, 30 sec at 53 °C, 1 min at 72 °C, 10 min at 72 °C. In Figure 2, the bands that appeared above the PCR product indicates presence of the plasmid which is used as the template. This plasmid would spoil the DNase digestion.

In order to eliminate plasmid interference, Dpn2 digestion applied to the PCR products at 37°C for overnight. In Figure 3, digested plasmid fragments are shown with arrowheads. PCR products were extracted from the agarose gel.



**Figure 3:** Dpn2 digestion of the plasmids carrying (a) ANL, and (b) BTL2. Arrowheads show the digested plasmid fragments. U: Uncut PCR products for (a) ANL, and (b) BTL2. M: MassRuler DNA Ladder Mix (Fermentas).

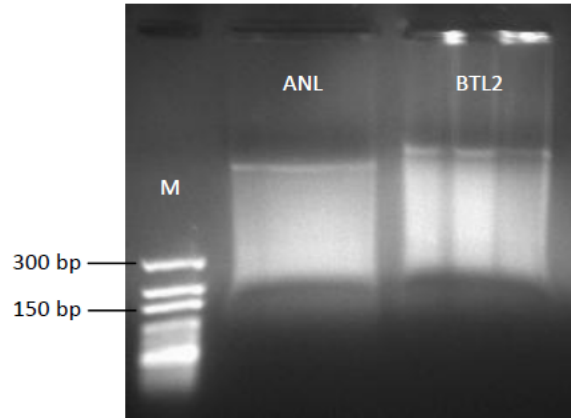


**Figure 4:** Purified PCR products for ANL and BTL2, excised and extracted from the agarose gel shown in Figure 2. M: MassRuler DNA Ladder Mix (Fermentas).

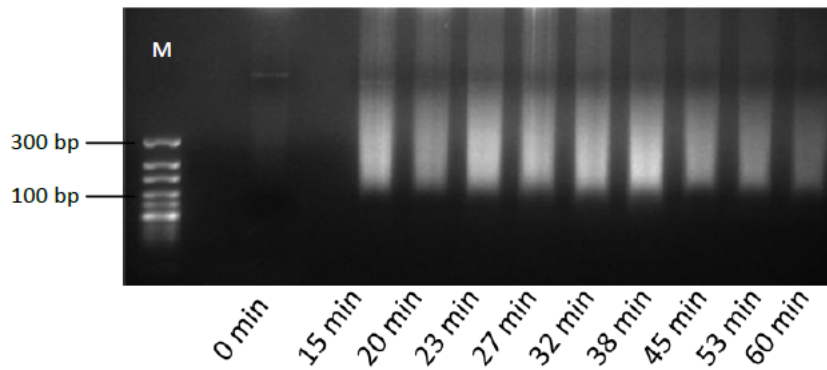
Extracted PCR products are run on 1.2% agarose gel in order to confirm that there is no plasmid left in the mixture. So Figure 4 indicates that the PCR products are plasmid-free and ready to DNase digestion.

DNase digestion is applied to PCR products. 2  $\mu\text{g}$  DNA from ANL and BTL were digested separately with 0.05 unit of DNase I (Roche). As the reaction buffer, 10X digestion buffer which is 500mM Tris-HCl pH 7.4 and 100mM  $\text{MnCl}_2$  is used. The digestion was done at room temperature until it is terminated after 20 minutes by heating at 85°C with the presence of 2.5mM EDTA. Mixture is run on 2% agarose gel. Smear on Figure 5 shows the digested fragments of the particular genes. Fragments from 150 to 300bp are excised and extracted from the gel.





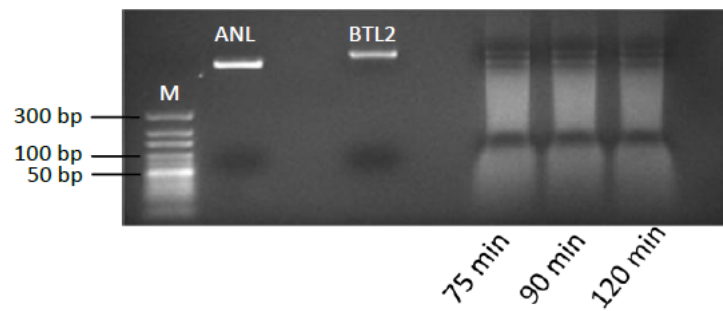
**Figure 5:** DnaseI digestion of ANL and BTL2 PCR Products. DNA smears indicative of digestion products of varying lengths, as low as 200bp, are evident. M: GeneRuler Ultra Low Range DNA Ladder (Fermentas).



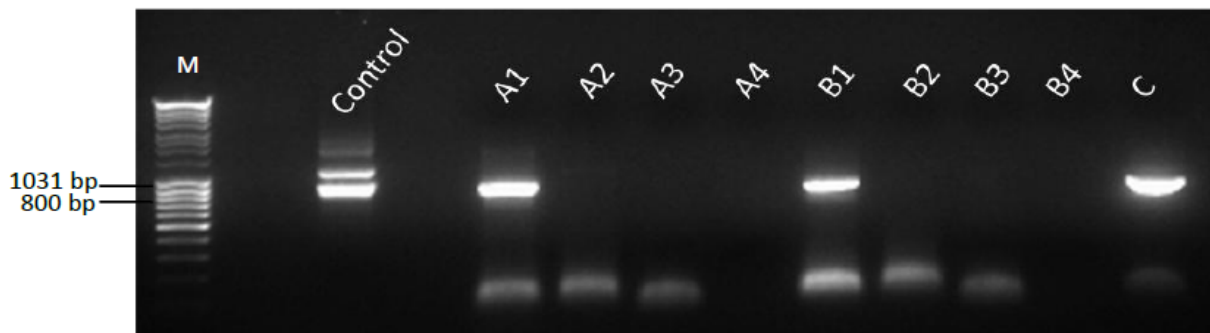
**Figure 6:** Digest products from DnaseI-treated ANL and BTL2 mixtures at different time points as given at the bottom. M: GeneRuler Ultra Low Range DNA Ladder (Fermentas).

20 minutes of digestion was not sufficient for obtaining the desired fragment size which is smaller than 100 base pair. So DNase digestion is applied to PCR products. This time 1,2  $\mu$ g of each gene from were mixed and digested in a tube with 0.05 unit of DNase I (Roche). As the reaction buffer, 10X digestion buffer which is 500mM Tris-HCl pH 7.4 and 100mM MnCl<sub>2</sub> is used. The digestion was done at room temperature. 15, 20, 23, 27, 32, 38, 45, 53, 60 minutes of digestion was done in order to find out the most efficient digestion time point. Inactivation is done by heating the samples at 85C with the presence of 2.5mM EDTA. Mixture is run on 2% agarose gel. Smears on Figure 7 show the digested fragments of the particular genes.

Same protocol used at Figure 5 was applied for 75 minutes, 90 minutes and 120 minutes in order to obtain smaller fragments(smaller than 50bp). Inactivation is done by heating the samples at 85C with the presence of 2.5 mM EDTA. Mixture is run on 2% agarose gel. Smears on Figure 8 show the digested fragments which are in the range of desired fragment size. Fragments below 50bp and between 50 -100bp are excised and extracted from the gel followed by the reassembly PCR.



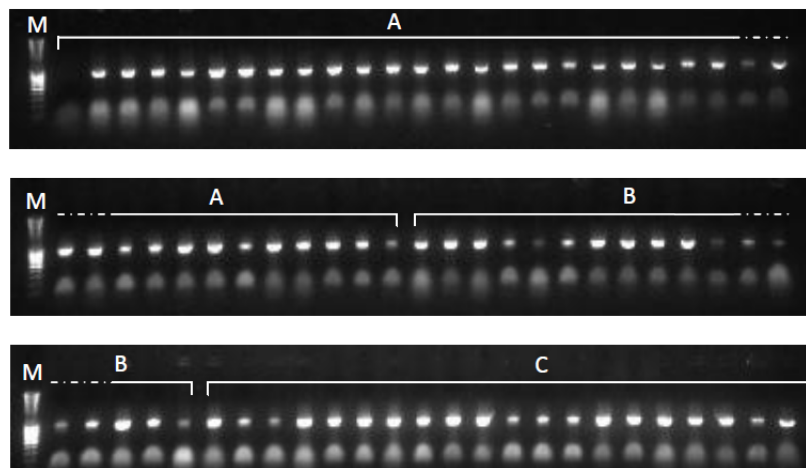
**Figure 7:** Digest products from DnaseI-treated ANL and BTL2 mixtures at different time points as given at the bottom. M: GeneRuler Ultra Low Range DNA Ladder (Fermentas).



**Figure 8:** Amplification PCR with the primer combinations. M: MassRuler DNA Ladder Mix (Fermentas).

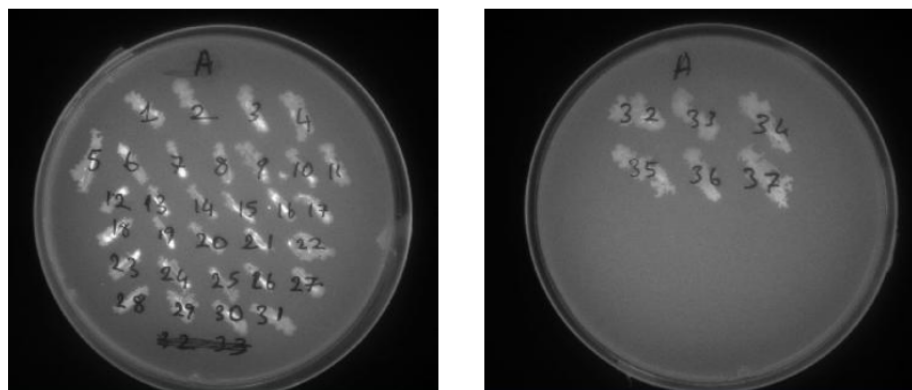
In figure 7, Group A indicates the reassembled 50bp and below fragments, while Group B indicates the reassembled fragments in the range of 50-100 bp. Lastly, Group C indicates the reassembled ANL fragments which are 150-300 bp in length. The primer combinations are; (1) F\_ANL - R\_ANL, (2) F\_BTL, R\_BTL, (3) F\_ANL, R\_BTL, and (4) F\_BTL, R\_ANL. A1, B1 and C1 labeled samples, which are produced by using ANL primer sets, are detected on 1,2% agarose gel. The amplified fragments were excised and extracted from the gel.

Extracted shuffled genes are cloned to expression vector pMCSG7 by ligation independent cloning and transformed into Shuffle *E.coli* competent cells by chemical transformation. As a result of the transformation, 75 colonies (37 colonies from A1, 18 colonies from B1, 20 colonies from C1) were obtained on LB-agar plates. Colony PCR was performed to check the insertion of the shuffled genes into the transformed vectors. It is confirmed that all colonies had the insert, shown in Figure 9.



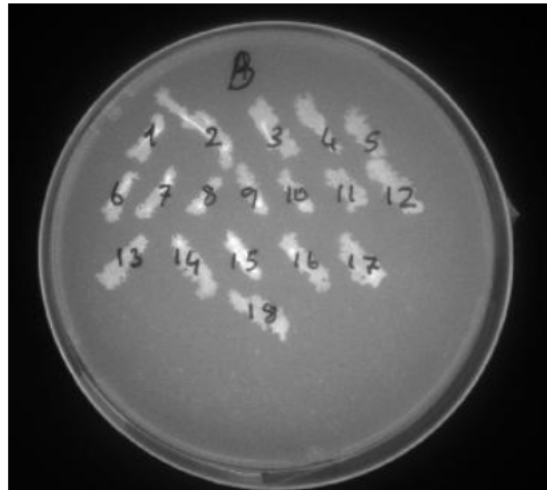
**Figure 9:** Colony PCR of obtained colonies.

Obtained A1 colonies are inoculated to LB - rhodamine activity plates to decide the activity of the possible mutants qualitatively. All the colonies except colony 33 had the lipase activity (Figure 10).



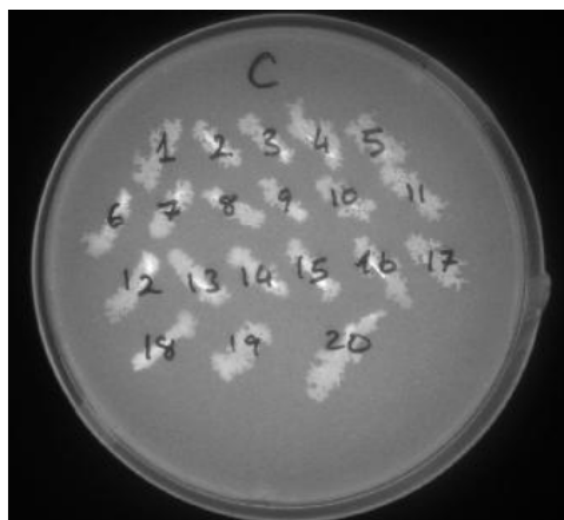
**Figure 10:** LB-Rhodamine activity plates of A colonies.

Obtained B1 colonies are inoculated to LB - rhodamine activity plates to decide the activity of the possible mutants qualitatively. The LB- rhodamine plate of B1 combination showed that most of the colonies have the lipase activity (Figure 11).



**Figure 11:** LB-Rhodamine activity plates of B colonies.

Obtained C1 colonies are inoculated to LB - rhodamine activity plates to decide the activity of the possible mutants qualitatively. Inoculated 14 C1 colonies out 20 have the lipase activity on LB-rhodamine plates (Figure 12).



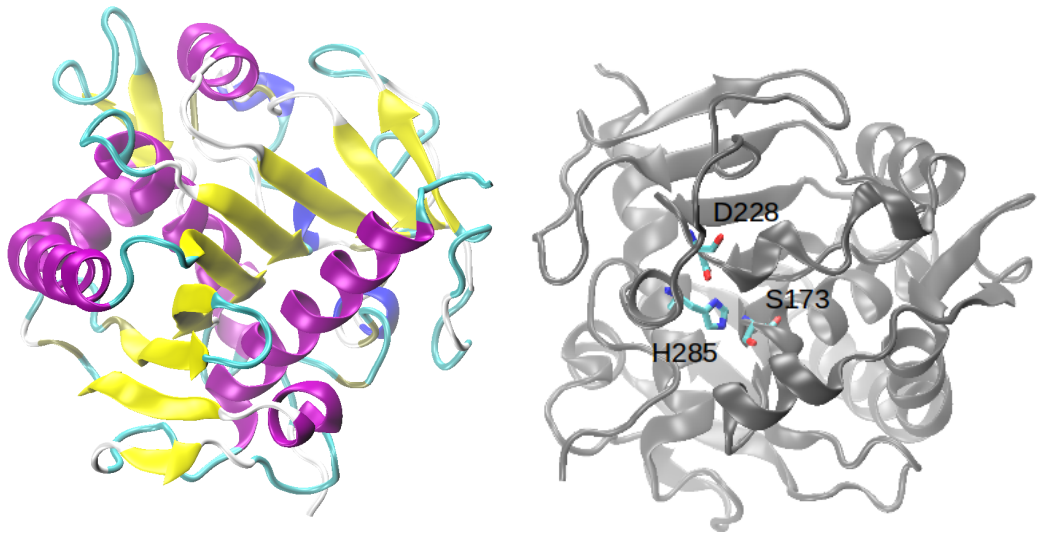
**Figure 12:** LB-Rhodamine activity plates of C colonies.

To screen the mutations which occurred by DNA shuffling, plasmid isolation is applied to all 75 colonies and the purified plasmids are sent to sequencing with R\_ANL primer. Alignment of the samples with ANL and BTL2 native genes shows that samples have similarity more than 90% with ANL gene. Although, samples mostly similar to ANL, eight of the samples have point mutations which may lead to the activity change. In fact, colony A33 has 10 point mutations which lead to the loss of activity. Point mutations at these eight samples are listed at Table 1. There are point mutations in these samples that would normally lead to activity loss but didn't caused any activity loss such as Asp to Gly mutation at colony B15 or Gly to Ser mutation at colony C15 or Thr to Ala at colony C15.

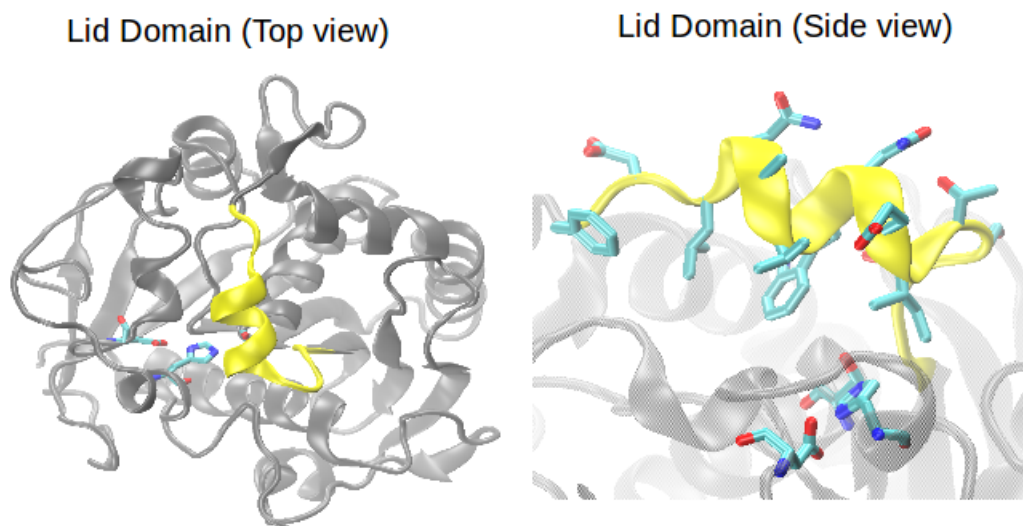
Clone	Location of the Mutation	Amino acid Substitution	Activity
A33	60 <sup>th</sup> amino acid	GUG (Val) → GUA (Val).	(-)
A33	64 <sup>th</sup> amino acid	GCC (Ala) → ACC (Thr)	
A33	68 <sup>th</sup> amino acid	CCU (Pro) → GCU (Ala)	
A33	95 <sup>th</sup> amino acid	GCC (Ala) → GUC (Val)	
A33	106 <sup>th</sup> amino acid	GCC (Ala) → UCC (Ser)	
A33	124 <sup>th</sup> amino acid	CUC (Leu) → CUU (Leu)	
A33	160 <sup>th</sup> amino acid	GCC (Ala) → GAC (Asp)	
A33	184 <sup>th</sup> amino acid	AGC (Ser) → AAC (Asn)	
A33	214 <sup>th</sup> amino acid	ACG (Thr) → GAG (Glu)	
A33	250 <sup>th</sup> amino acid	AGC (Ser) → AAC (Asn)	
B2	3 <sup>rd</sup> amino acid	UCU (Ser) → UAU (Tyr)	(+)
B2	44 <sup>th</sup> amino acid	UCU (Ser) → UCC (Ser)	
B2	46 <sup>th</sup> amino acid	GCA (Ala) → GCG (Ala)	
B8	188 <sup>th</sup> amino acid	AAU (Asn) → AAC (Asn)	(+)
B15	65 <sup>th</sup> amino acid	GAC(Asp) → GGC(Gly)	(+)
B15	61 <sup>st</sup> amino acid	AAU(Asn) → AGU(Ser)	
C6	61 <sup>th</sup> amino acid	ACA(Thr) → GCA(Ala)	(+)
C6	275 <sup>th</sup> amino acid	GGU(Gly) → GAU(Asp)	
C12	137 <sup>th</sup> amino acid	CAC(His) → CGC(Arg)	(+)
C14	86 <sup>th</sup> amino acid	AAC(Asn) → GAC(Asp)	(+)
C15	31 <sup>th</sup> amino acid	ACU(Thr) → GCU(Ala)	(+)
C15	151 <sup>th</sup> amino acid	CUG(Leu) → CCG (Pro)	
C15	176 <sup>th</sup> amino acid	GGC(Gly) → AGC (Ser)	
C15	223 <sup>th</sup> amino acid	GUU(Val) → GCU(Ala)	

**Table 1 :** Types of point mutations and their locations on the sequence.

The locations of the mutations of particular colonies given in table 1 were investigated. Since the colonies have more than 90% identity with ANL, homology model of ANL is used to locate the point mutations which are detected. Homology model of ANL is made by using *Thermomyces lanuginosa* lipase structure as the template. They have 51% sequence similarity with the query coverage of 99%.



**Figure 13:** Homology modeling of *Aspergillus niger* lipase, using 1dt3 (*Thermomyces lanuginosa* lipase) as the template.



**Figure 14:** The Lid domain has been shown from top and side view, respectively. The predicted model corresponds to the inactive lipase form where the lid is in its closed conformation.

The best candidate for the Lid domain is shown in Figure 14 in yellow. The reason for chosen as best candidate is because the catalytic Serine has also been covered. Also, the amino acid content of the lid explains the interfacial properties of the lipase. In the closed conformation, the polar residues like, N, D and T are exposed to solvent. In the open conformation, the non-polar residues like W, I and V should be exposed to the lipid-interface.

Multiple sequence alignment is applied to all mutants (see Appendix A.2), which are obtained from sequencing, with native ANL. Afterwards, locations of the point mutations, which are detected from sequencing data, pointed with arrows (Red = "deadly" mutations, Blue = "compensating" mutations, Grey = "silent" mutations) and in addition, only "deadly" mutations which indicates non polar amino acid - polar amino acid change, are shown on the homology model of ANL by using VMD (Visual Molecular Dynamics).

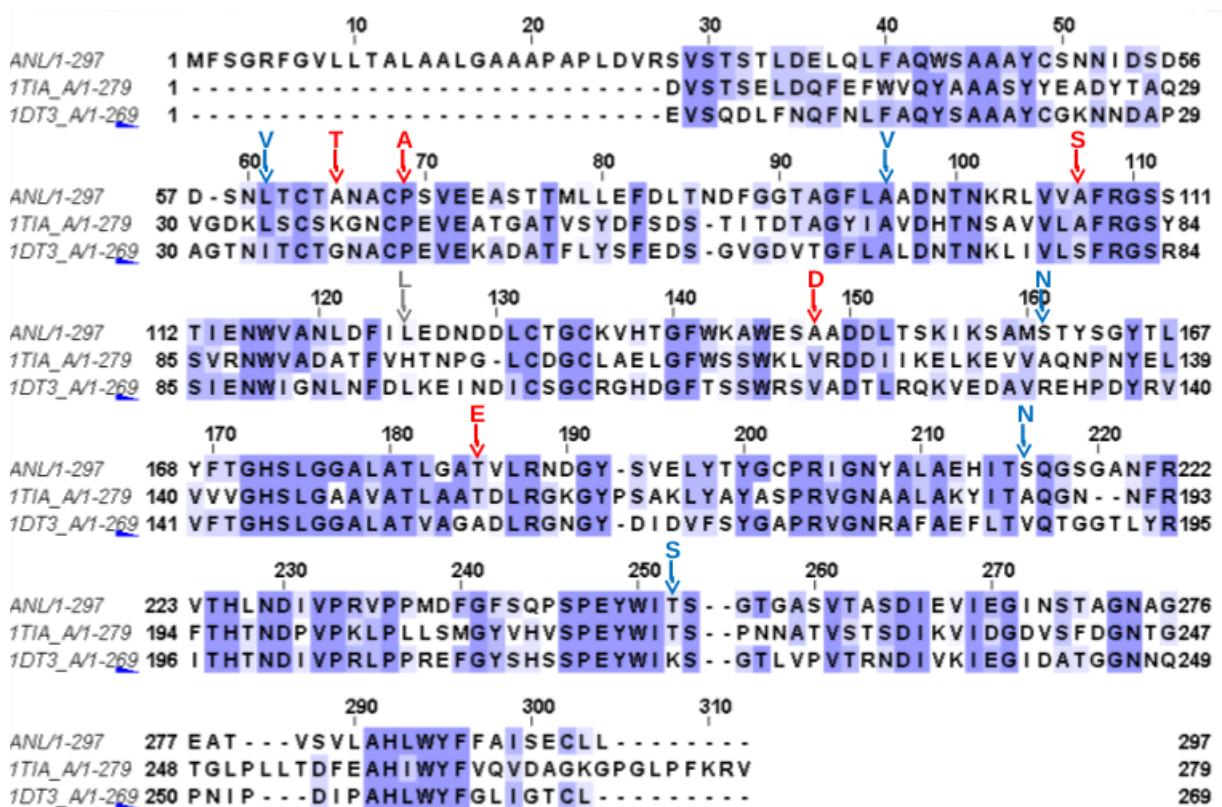
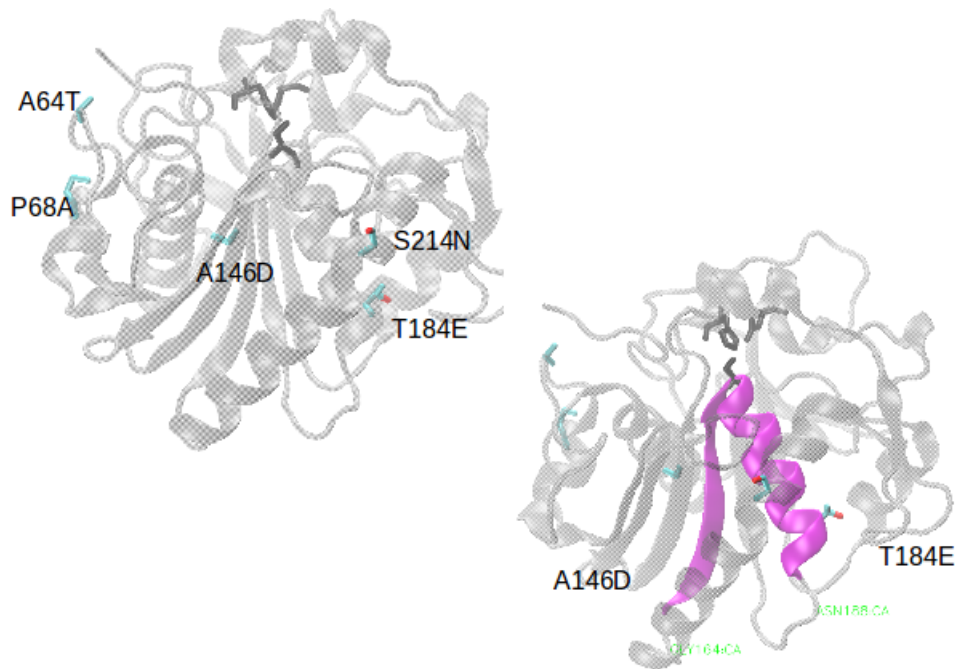


Figure 15: Multiple sequence alignment of Colony A33.



Colony 33 had 5 “deadly”, 5 “compensating” and 1 “silent” mutations. As it can be seen from Figure 15, 2 out of 5 deadly mutations are located at core of the protein. In fact, T184E mutation located on the nucleophilic elbow (in Magenta color) which carries the catalytic serine residue. And the A146D mutation is located at the adjacent  $\beta$ -sheet to the nucleophilic elbow. Locations of these 2 mutations make them important due to their closeness to the catalytic site.



**Figure 16:** Locations of the mutations for Colony A33. The nucleophilic elbow that carries the catalytic serine is shown in magenta.

For the mutations at the colonies B2, B8, B15, C12 and C14, they are either “deadly” but surface exposed or silent mutations which are unlikely to cause a change in activity of the enzyme. At the colony B2, three codon changes have occurred, two of them being silent mutations (Serine to Tyrosine on the 3<sup>rd</sup> amino acid, Serine to Serine on the 44<sup>th</sup> amino acid, and Alanine to Alanine on the 46<sup>th</sup> amino acid, respectively). At the colony B8, Asparagine to Asparagine change has occurred on the 188<sup>th</sup> amino acid. At the colony B15, two codon changes have occurred, an Aspartic acid to Glycine on the 65<sup>th</sup> amino acid, and an Asparagine to Serine on the 61<sup>st</sup> amino acid. At the colony C12, a Histidine to Arginine substitution has occurred on the 137<sup>th</sup> amino acid, and at the colony C14, an Asparagine to Aspartic acid substitution on the 86<sup>th</sup> amino acid.

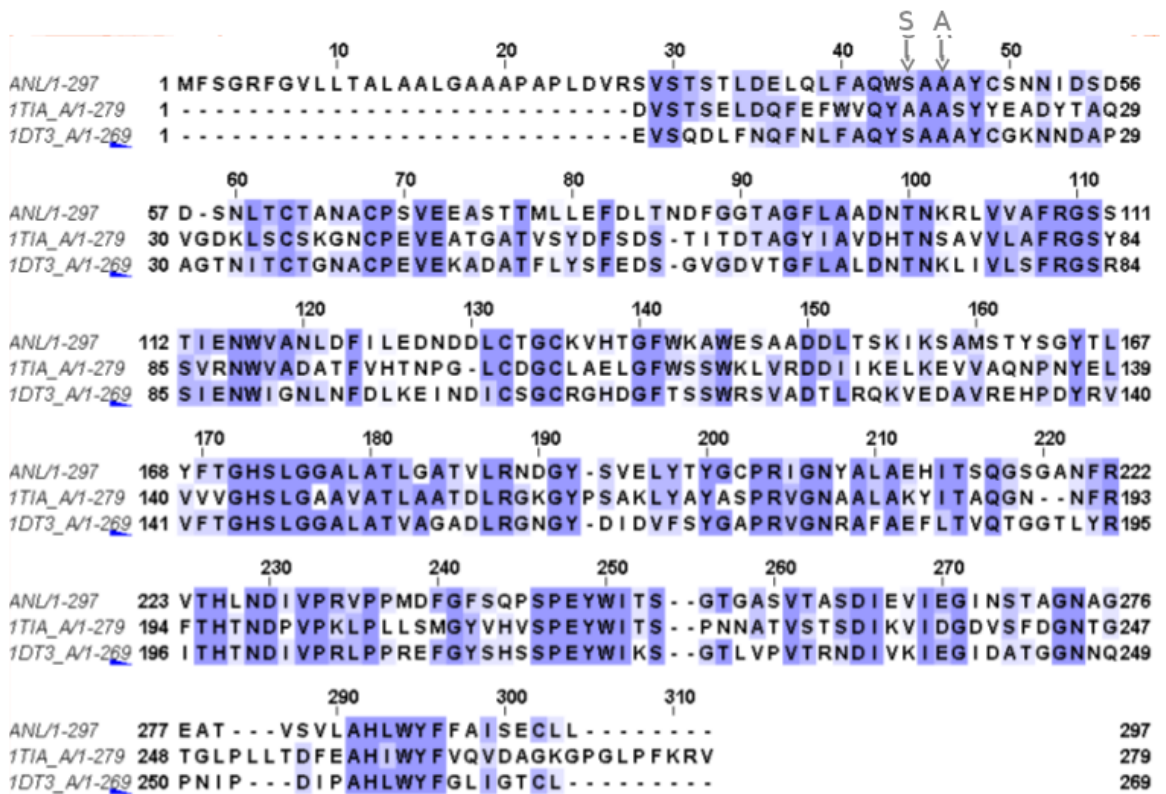


Figure 17: Multiple sequence alignment of Colony 39 (B2).

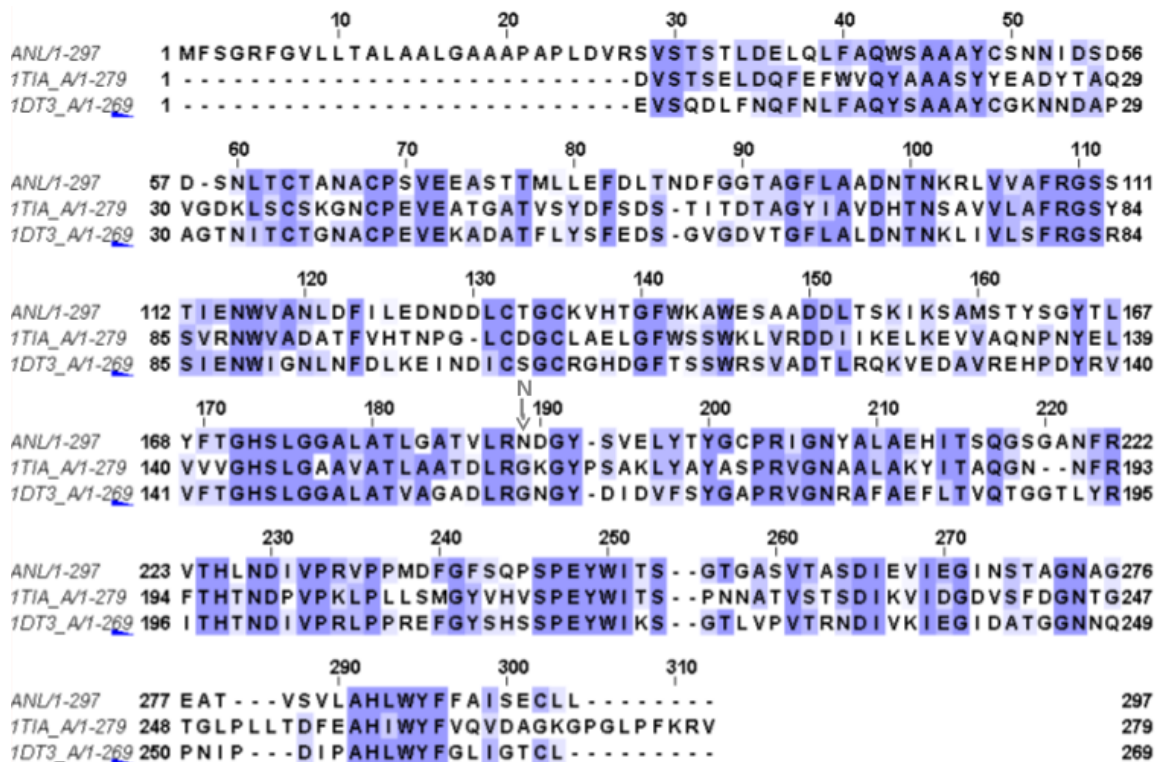


Figure 18: Multiple sequence alignment of Colony 45 (B8).

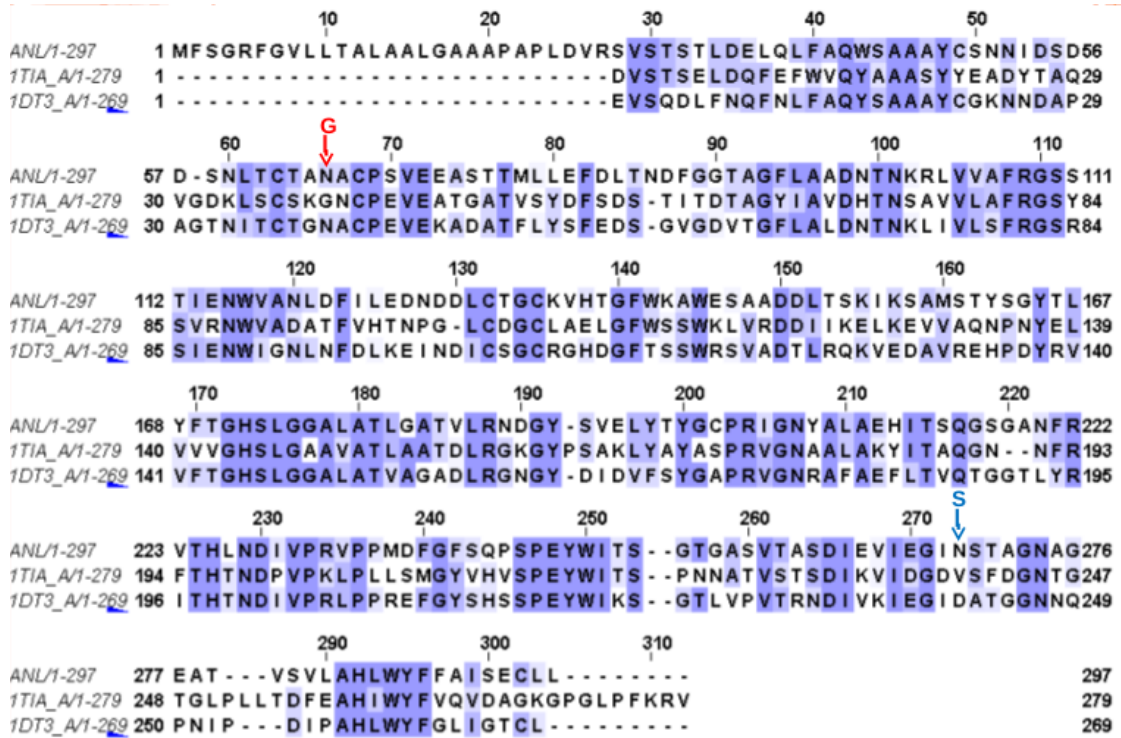


Figure 19: Multiple sequence alignment of Colony 52 (B15).

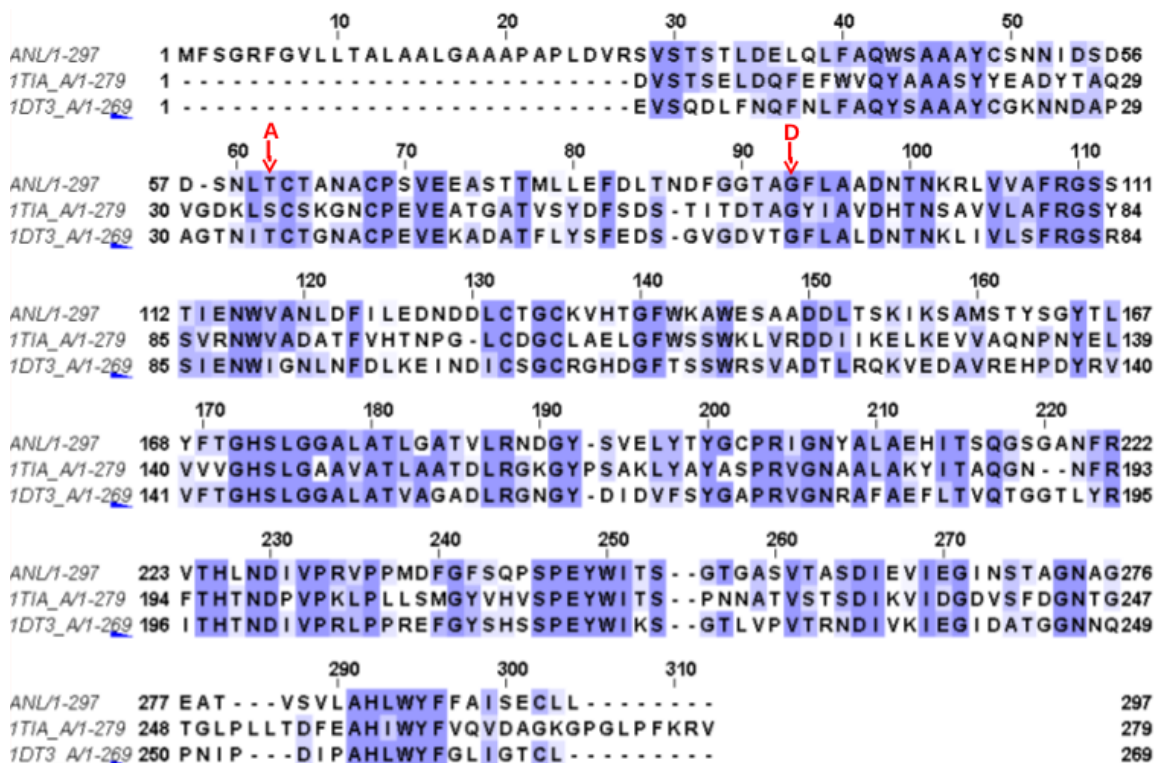
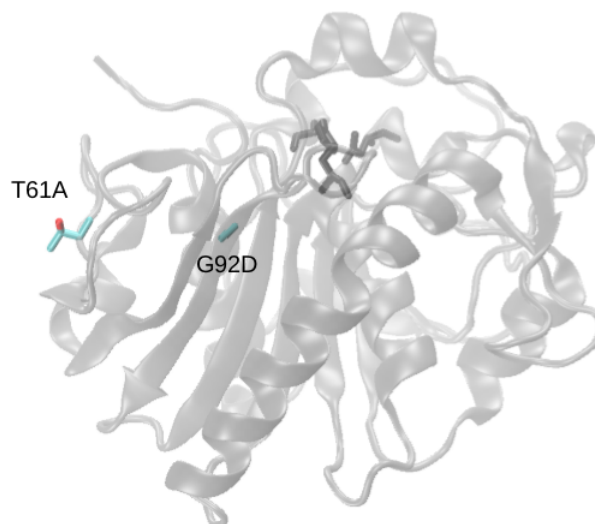


Figure 20: Multiple sequence alignment of Colony 61 (C6).



**Figure 21:** Locations of the mutations for Colony 61 (C6)

Colony C6 has 2 “deadly” mutations which are Threonine to Alanine substitution at 61st amino acid which is surface exposed and far away from the catalytic site. The other one is the Glycine to Asparagine substitution at the 275<sup>th</sup> amino acid which is located more closely to the catalytic site (Figure 20). Since this is also a non polar to polar substitution, the mutation has a role in changing the activity.



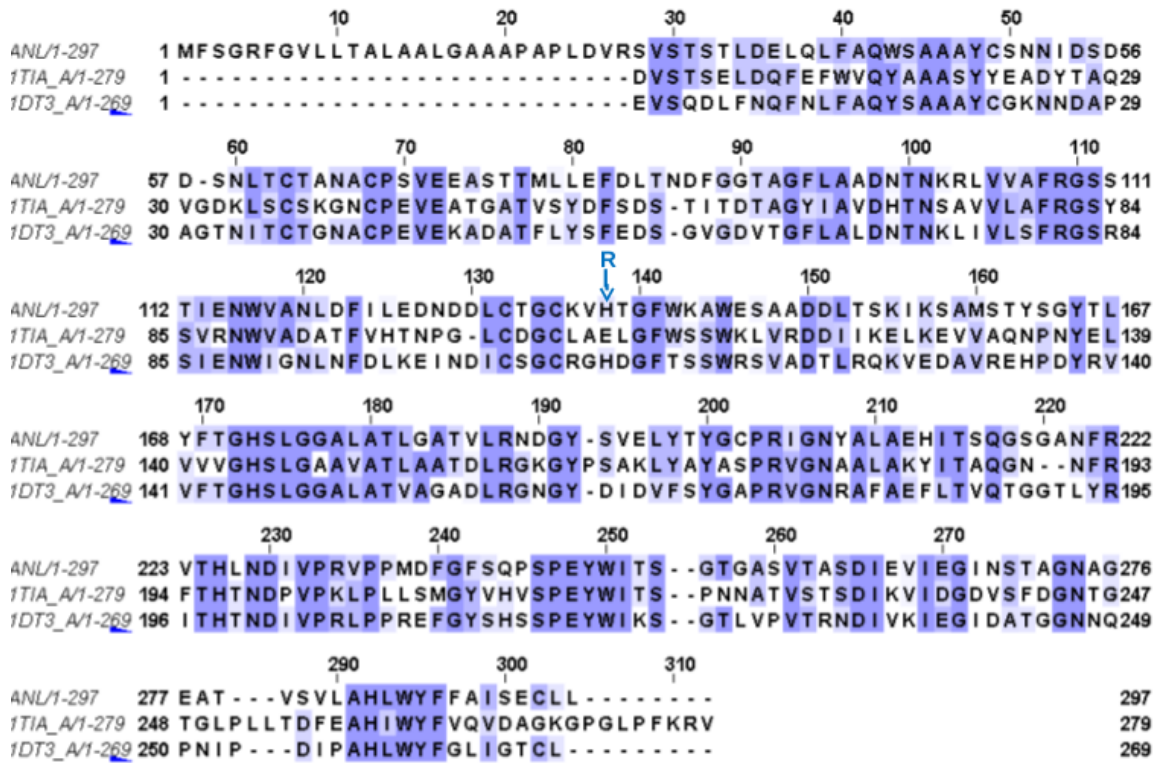


Figure 22: Multiple sequence alignment of Colony 67 (C12).

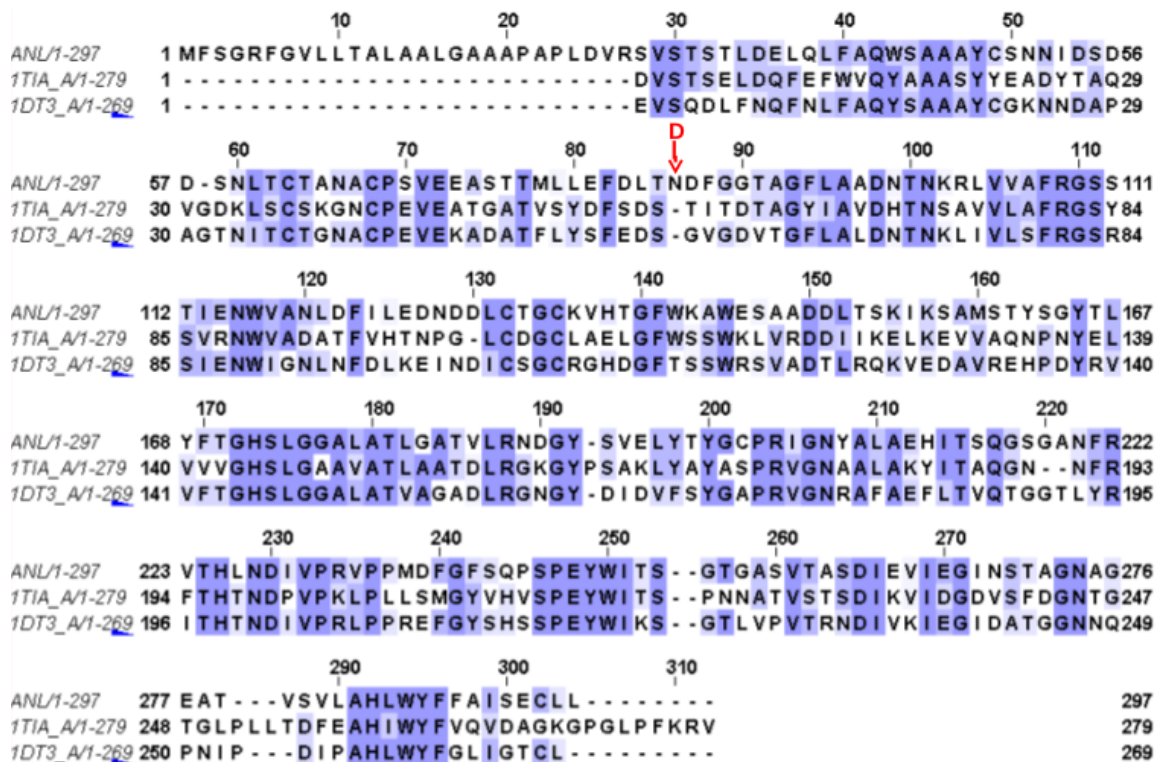
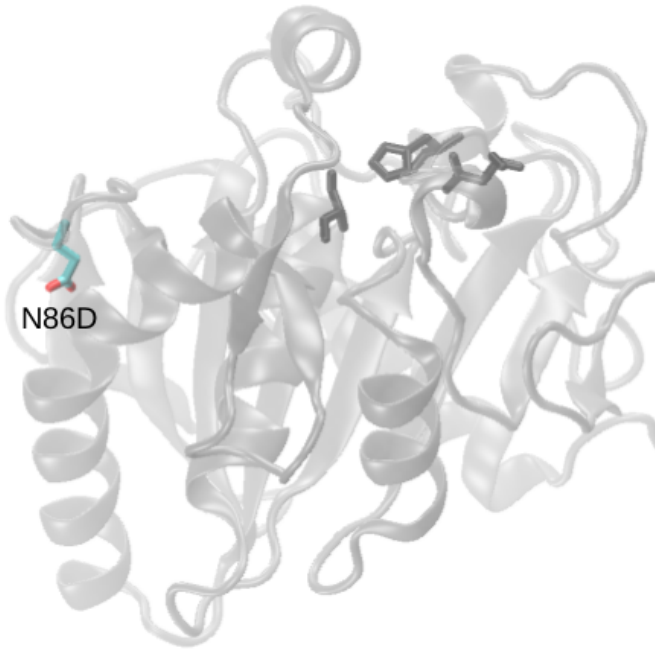
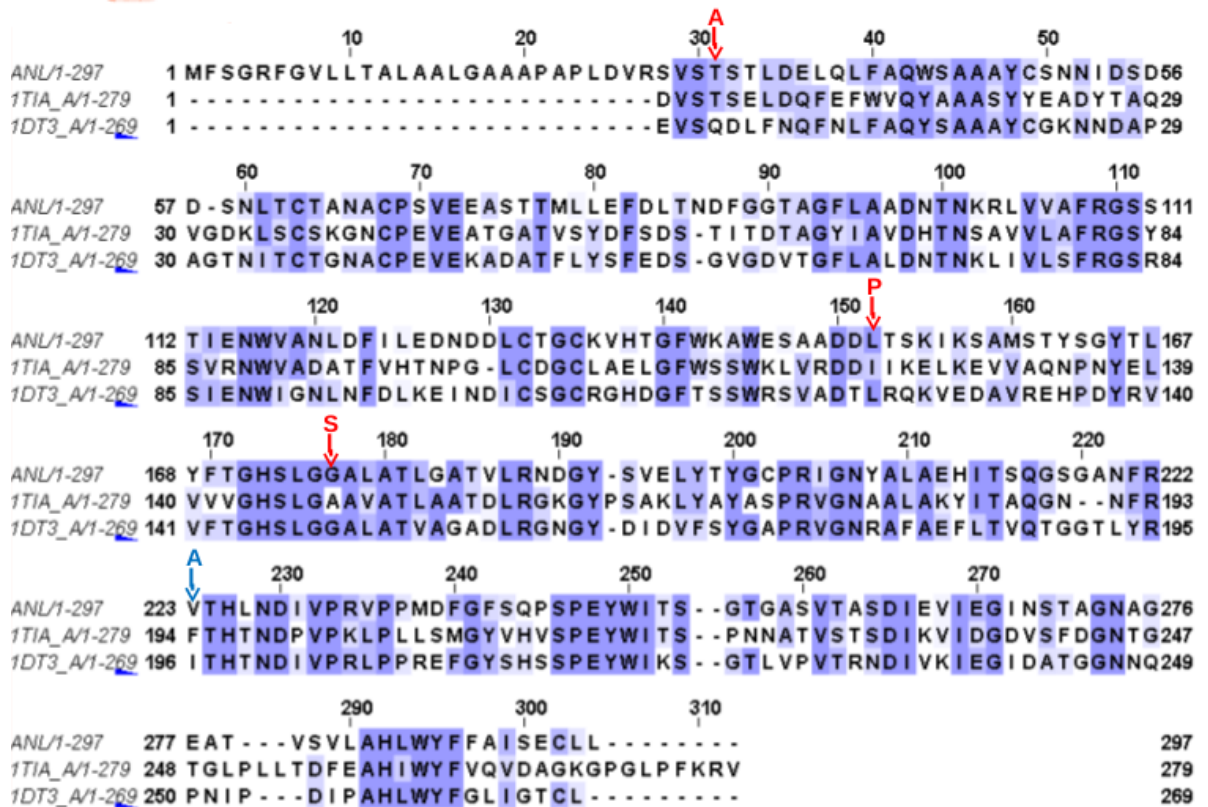


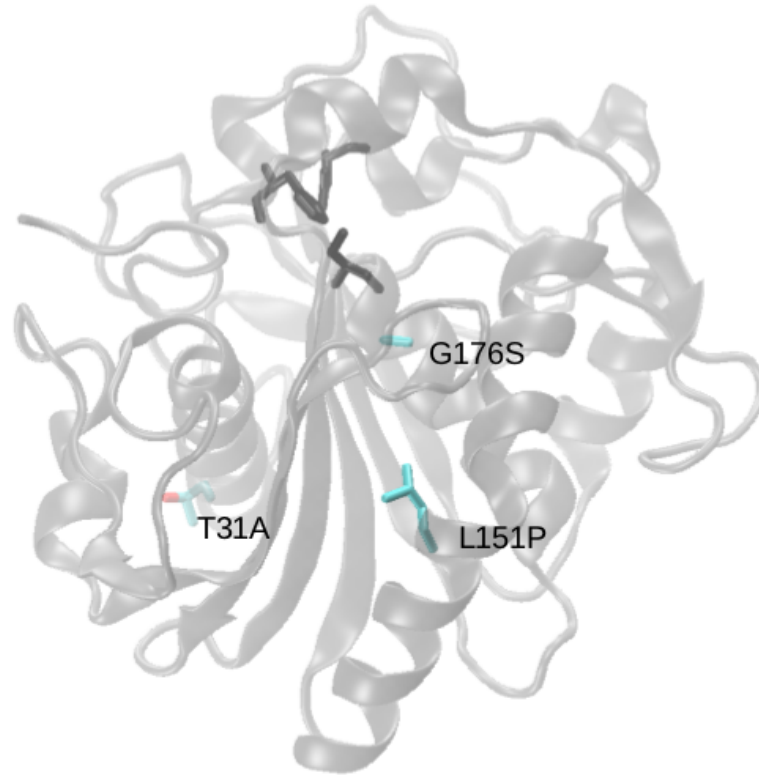
Figure 23: Multiple sequence alignment of Colony 69 (C14).



**Figure 24:** Locations of the mutations for Colony 69 (C14).

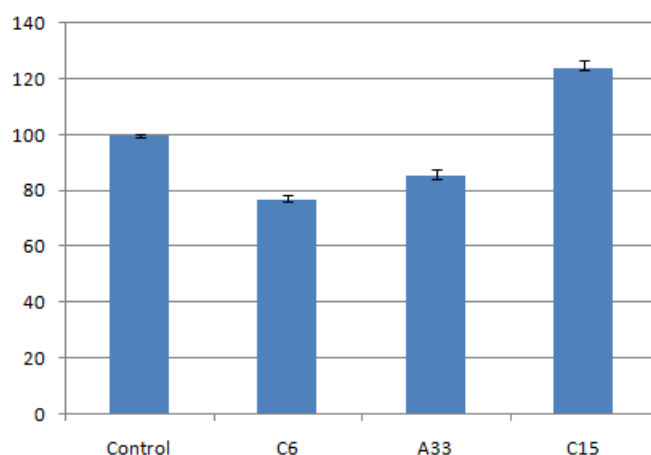


**Figure 25:** Multiple sequence alignment of Colony 70 (C15).



**Figure 26:** Locations of the mutations for Colony 70 (C15).

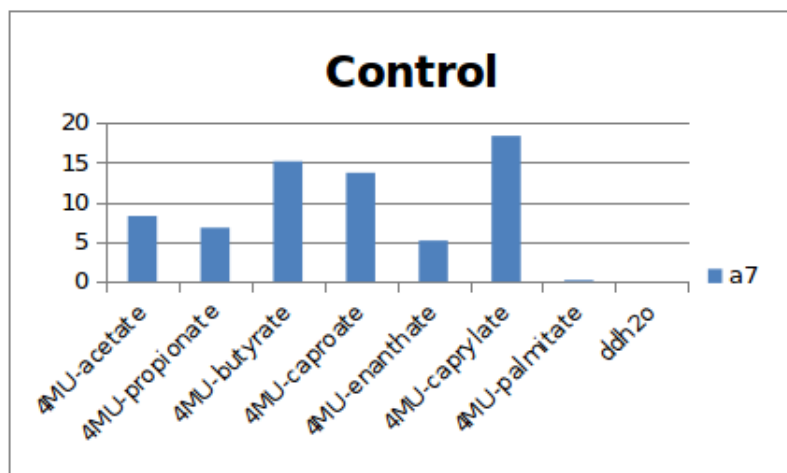
Colony C15 has 3 “deadly” mutations which are Threonine to Alanine substitution at 31st amino acid Leucine to Proline substitution at 151<sup>st</sup> amino acid, Glycine to Serine substitution at 176<sup>th</sup> amino acid (Figure 25). 2 out of these 3 “deadly” mutations are likely to effect the activity of the enzyme. G176S mutation is very much at the core of the protein and is significantly close to the catalytic site. This kind of mutation (non-polar to polar) would be significant since there will be a presentation of a polar amino acid at the core of the protein. Another significant “deadly” mutation would be L151P because a presentation a Proline in to a existing  $\alpha$ -helix would cause rupture the  $\alpha$  helical structure which is also likely to change the activity of an enzyme.



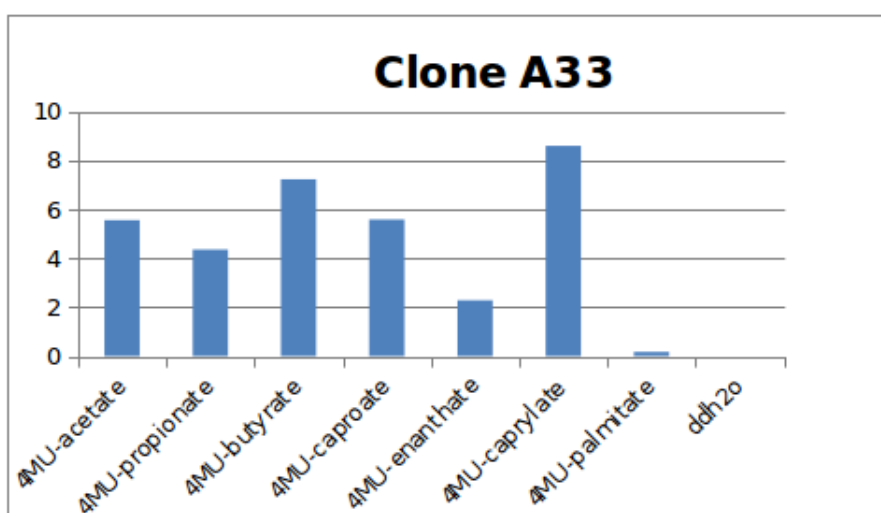
**Figure 27:** The relative activity plot of the colonies, using fluorescence enzyme assays. As a substrate, 4MU-caprylate has been used to detect the activity of lipase from the soluble fraction.

For quantitatively determining the lipase activity, fluorescent lipase assay methodology is applied. 5 micro liters of the soluble fraction from the cultured cells are assayed by using 4MU - caprylate as the substrate. Soluble fraction of the cultured cells, which do not contain any cell, assayed in reaction medium of 100 mM Tris-Cl at pH 7.25. 4MU fluorescence is measured by using Gemini XS (Molecular Devices) using wavelength of 355 nm for excitation and an emission wavelength of 460 nm. As it is shown in Figure 26 the mutations caused a decrease on the lipase activity at clones A33 and C6, as well as increase in activity of clone C15 by 25%. These activity changes may be due to the “deadly” mutations that are presented near the catalytic site. To investigate whether the mutations caused a change in substrate selectivity and due to that the activity loss against caprylate has occurred or it is the general activity loss, a substrate selectivity assay is made. From the chain lengths of 2C to 16C the following lipids are used; Acetate(2C), propionate(3C), butyrate(4), caproate(6C), enatiothe(7C), caprylate(8), and palmitate(16C) for the investigation of the substrate selectivity. As it is shown in Figures 27, 28, 29 and 30, there is no detectable change in the substrate selectivity trend with respect to the control group which is the native ANL.

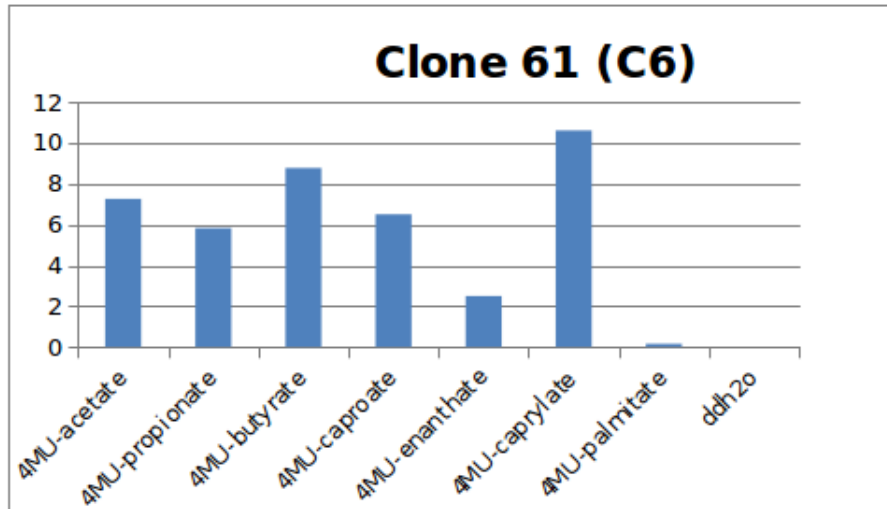




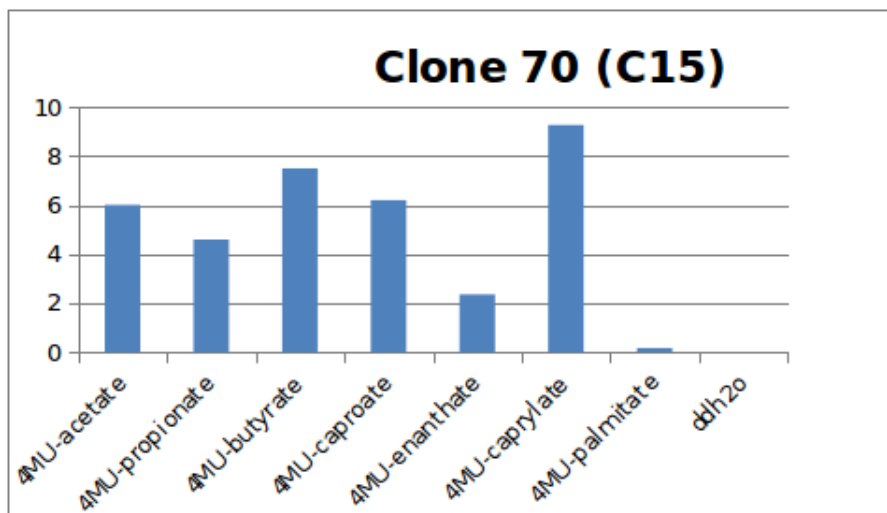
**Figure 28:** Substrate selectivity assay of native ANL. From the chain lengths of 2C to 16C the following lipids are used; Acetate(2C), propionate(3C), butyrate(4), caproate(6C), enanthate(7C), caprylate(8), and palmitate (16C).



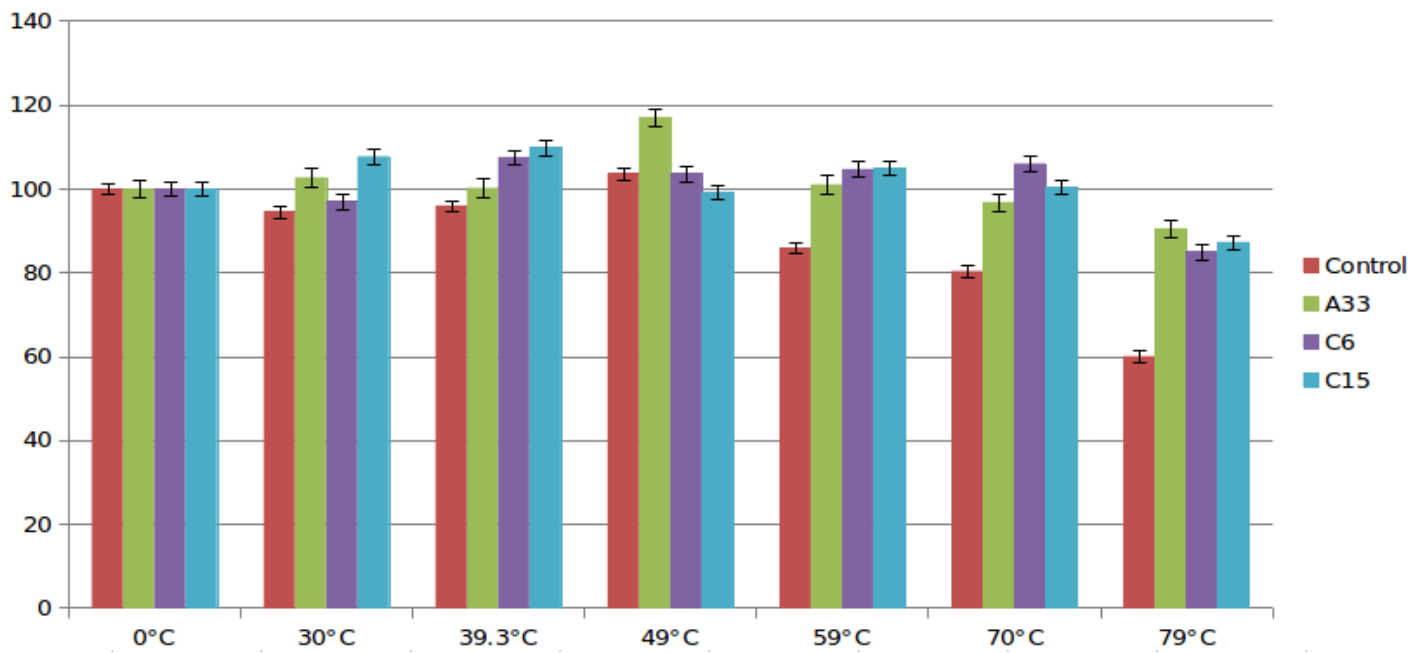
**Figure 29:** Substrate selectivity assay of clone A33. From the chain lengths of 2C to 16C the following lipids are used; Acetate(2C), propionate(3C), butyrate(4), caproate(6C), enanthate(7C), caprylate(8), and palmitate (16C).



**Figure 30:** Substrate selectivity assay of clone 61 (C6). From the chain lengths of 2C to 16C the following lipids are used; Acetate(2C), propionate(3C), butyrate(4), caproate(6C), enanthate(7C), caprylate(8), and palmitate (16C).



**Figure 31:** Substrate selectivity assay of clone 70 (C15). From the chain lengths of 2C to 16C the following lipids are used; Acetate(2C), propionate(3C), butyrate(4), caproate(6C), enanthate(7C), caprylate(8), and palmitate (16C).



**Figure 32:** Thermostability assay of clone 33 (A33), 61 (C6), and 70 (C15).

Soluble fractions of the clones that do have expressions, are used in fluorescent assays to profile thermostability by quantifying the residual activity of lipases after 30 minutes of incubation at temperatures 30°C, 40°C, 50°C, 60°C, 70°C, and 80°C is set to 100% activity for calculating the percent activity. As it is shown at the results, C6's and C15's activity peak shifted to around 40°C whereas A33's has peaked at the same with control but has higher activity on the particular temperatures. This may due to the various mutations located at the different places at the structure. Although, the activity of A33 is decreased 20% , it's thermostability is slightly better.

## 4 Discussion and Conclusion

Here, it is reported that DNA shuffling is a suitable methodology for generating randomly mutated lipase libraries for industrial usage. In this study, instead of family shuffling, which is a common preferred method for random mutagenesis of an enzyme, non-family shuffling has been tried. ANL, which is a fungal lipase, and BTL2, which is a bacterial lipase, were shuffled in order to obtain a mutant library which would have the desired features such as increased thermostability, and broader substrate specificity.

To the best of our knowledge, this study describes the first application of DNA-shuffling of lipases from *B. thermocatenuulatus* and *Aspergillus niger*. As it can be seen from multiple alignments, the clones which are obtained from this study are not chimeric proteins. Multiple sequence alignments of the clones do not produce hits for BTL sequence and moreover, they all have at least 99% sequence identity to ANL sequence. This may be due to the usage of genes from different families. Although ANL and BTL have 41% sequence homology, they didn't shuffle, as can be deduced from the results. It can be speculated that the reason for not seeing any chimeric proteins would be the slight difference in their codon usage. Due to this, the chimeric proteins couldn't have survived during the cloning selection. The reason of not seeing any BTL self-shuffled proteins would be the evolutionary difference between fungi and bacteria, since their codon usage is related to their evolutionary path as well. Evolution of synonymous codon usage is reported to be decided by a balance between mutation, genetic drift and natural selection. However, natural selection on codon usage is considered to be a weak evolutionary force and selection on codon usage is expected to be the strongest force [76]. Reported point mutations may come from self shuffling of ANL fragments which could lead to point mutations as well or it may come from the errors of the DNA polymerase. The second case is not likely since Pfu DNA polymerase is used and Pfu has high fidelity. Therefore, it can be reported that these mutations are coming from the self shuffling of the ANL fragments. There are also studies which indicate that using single genes and random point mutations which are generated by shuffling of the single gene is also a source of diversity [53, 77]. Self

shuffling or family shuffling utilizes naturally occurring nucleotide substitutions as the driving force for the evolution *in vitro*. It is also reported that multiple rounds of shuffling would increase the recombination and the mutations yield [77]. Thereby, in further studies, shuffling of the clones which are obtained from this study can be used to generate different mutations and more evolved clones.

From a single round of DNA-shuffling with these two parent lipases, three mutants (A33, C6, C15) with various point mutations have occurred, which could normally cause an activity loss (non polar - polar /charged amino acid substitution). In this case, only A33 and C6 clones show activity loss around 20% due to the point mutations. Activity loss of these clones may occur due to the mutations that are located at a close proximity to catalytic site. In A33 clone, T184E mutation which is located on the nucleophilic elbow is likely to cause the activity loss and the C6 G92D mutation may be the responsible of the activity loss due to the fact that they are close to the catalytic triad and also located at the core of the protein.

Since the aim of this project was to generate the library that could be investigated on further studies, the mutant proteins are not purified. However, substrate selectivity and thermostability assays are effectuated, with the soluble fraction of the cells to shed light on the features of the lipases as a preliminary examination. As it is mentioned at the results section, there are no changes in the substrate selectivity trend with respect to the control's substrate selectivity trend. In this case, this is expected, since the point mutations are not in close proximity with amphiphilic lid of the lipases. All three clones and the control have the highest activity against caprylate (8C) and the lowest activity against palmitate (16C).

Their thermostability features are more diverse than their substrate selectivity. As it is shown in the results, C6's and C15's activity peak shifted to around 40°C whereas A33's has peaked at the same with control but has higher activity on the particular temperatures. This may be due to the various mutations located at the different places at the structure.

These mutations may have lead to decreased flexibility of A33 clone and therefore it might cause an increase in thermostability. Although the activity of A33 has decreased by 20% , it's thermostability is slightly better. Again this may due to synergistic effect of all point mutations but the A146D and T184E mutations could have a crucial effect on both flexibility and the activity of the protein. The reason for the thermostability shift of clone may again be caused by the G92D mutation because this mutation would lead an increase on flexibility of the protein therefore decrease in thermostability. In clone C15, there is a G176S mutation at the core of the protein and a L151P mutation at a helical structure which would break the helix and would turn it to loop or multiple helices. As it can be seen from the bar graph of thermostability, thermostability trend of clone C15 is shifted around 40°C and it is more likely that this shift is caused by the L151P mutation because since proline mutation breaks the  $\alpha$  helical structure, the flexibility of the protein would increase and therefore again it would lead to a decrease in thermostability. Although the thermostability trend is shifted in C6 and C15, their activity is not dead even in 80°C. It may be caused by the other proteins in the soluble fraction of the cells. To find out the real stability of these enzymes further studies like purification and same characterization experiments should be performed.

These point mutations may affect substrate binding and/or regulate the reaction rate for the hydrolysis of the covalent reaction intermediate. Therefore, it could affect the activity change. Also, synergistic effects of mutations may occur and lead up to the getting the desired functions for the industrial applications.

## References

- [1] Eduardo Busto, Vicente Gotor-Fernandez, and Vicente Gotor. Hydrolases: catalytically promiscuous enzymes for non-conventional reactions in organic synthesis. *Chem. Soc. Rev.*, 39:4504–4523, 2010.
- [2] Manali Kapoor and Munishwar Nath Gupta. Lipase promiscuity and its biochemical applications. *Process Biochemistry*, 47(4):555 – 569, 2012.
- [3] N.R. Kamini, J.G.S. Mala, and R. Puvanakrishnan. Lipase production from *Aspergillus niger* by solid-state fermentation using gingelly oil cake. *Process Biochemistry*, 33(5):505 – 511, 1998.
- [4] Licia M. Pera, Cintia M. Romero, Mario D. Baigori, and Guillermo R. Castro. Catalytic properties of lipase extracts from *Aspergillus niger*. *Food Technol. Biotechnol.*, 44(2):247–252, 2006.
- [5] Fariha Hasan, Aamer Ali Shah, Sundus Javed, and Abdul Hameed. Enzymes used in detergents : Lipases. *African Journal of Biotechnology*, 9(31):4836–4844, August 2010.
- [6] F Beisson, V Arondel, and R Verger. Assaying arabidopsis lipase activity. *Biochem Soc Trans*, 28(6):773–5, 2000.
- [7] P. A. Patten, R. J. Howard, and W. P. Stemmer. Applications of dna shuffling to pharmaceuticals and vaccines. 8:724–33+, 1997.
- [8] Zhengyu Shu, Mojie Duan, Jiangke Yang, Li Xu, and Yunjun Yan. *Aspergillus niger* lipase: Heterologous expression in *pichia pastoris*, molecular modeling prediction and the importance of the hinge domains at both sides of the lid domain to interfacial activation. *Biotechnol. Prog.*, 25(2):409–416, 2009.
- [9] H. Wong and M.C. Schotz. The lipase gene family. *J Lipid Res*, 43(7):993–9, 2002.
- [10] Cesar Carrasco-Lopez, Cesar Godoy, Blanca de las Rivas, Gloria Fernandez-Lorente, Jose M. Palomo, Jose M. Guisan, Roberto Fernandez-Lafuente, Martin Martinez-Ripoll, and Juan A. Hermoso. Crystallization and preliminary X-ray diffraction studies of the BTL2 lipase from the extremophilic microorganism *Bacillus thermocatenuatus*. *Acta Crystallographica Section F*, 64(11):1043–1045, Nov 2008.
- [11] Dinh Thi Quyen, Claudia Schmidt-Dannert, and Rolf D Schmid. High-level expression of a lipase from *Bacillus thermocatenuatus* {BTL2} in *pichia pastoris* and some properties of the recombinant lipase. *Protein Expression and Purification*, 28(1):102 – 110, 2003.
- [12] Alexander M. Klibanov. Enzymatic catalysis in anhydrous organic solvents. *Trends in Biochemical Sciences*, 14(4):141 – 144, 1989.
- [13] Romas J. Kazlauskas and Uwe T. Bornscheuer. Biotransformations with lipases. pages 36–191, 2008.

- [14] L Brady, A M Brzozowski, Z S Derewenda, E Dodson, G Dodson, S Tolley, J P Turkenburg, L Christiansen, B Høge-Jensen, and L Nørskov.
- [15] Adriano A. Mendes, Pedro C. Oliveira, and Heizir F. de Castro. Properties and biotechnological applications of porcine pancreatic lipase. *Journal of Molecular Catalysis B: Enzymatic*, 78(0):119 – 134, 2012.
- [16] Ricardo N. Farias, Merc Torres, and Ramon Canela. Spectrophotometric determination of the positional specificity of nonspecific and 1,3-specific lipases. *Analytical Biochemistry*, 252(1):186 – 189, 1997.
- [17] Laurent Vaysse, Aboubakry Ly, Guy Moulin, and Eric Dubreucq. Chain-length selectivity of various lipases during hydrolysis, esterification and alcoholysis in biphasic aqueous medium. *Enzyme and Microbial Technology*, 31(5):648 – 655, 2002.
- [18] Rolf D. Schmid and Robert Verger. Lipases: Interfacial enzymes with attractive applications. *Angewandte Chemie International Edition*, 37(12):1608–1633, 1998.
- [19] Ching-Shih Chen and Charles J. Sih. General aspects and optimization of enantioselective biocatalysis in organic solvents: The use of lipases [new synthetic methods (76)]. *Angewandte Chemie International Edition in English*, 28(6):695–707, 1989.
- [20] A.L. Gutman and M. Shapira. Synthetic applications of enzymatic reactions in organic solvents. 52:87–128, 1995.
- [21] R.D. Joerger and M.J. Haas. Alteration of chain length selectivity of a rhizopus delemar lipase through site-directed mutagenesis. *Lipids*, 29(6):377–84, 1994.
- [22] G.H. Peters, D.M. van Aalten, A. Svendsen, and R. Bywater. Essential dynamics of lipase binding sites: the effect of inhibitors of different chain length. *Protein Eng*, 10(2):149–58, 1997.
- [23] J. Schmitt, S. Brocca, R.D. Schmid, and J. Pleiss. Blocking the tunnel: engineering of candida rugosa lipase mutants with short chain length specificity. *Protein Eng*, 15(7):595–601, 2002.
- [24] Junhao Yang, Yuichi Koga, Hideo Nakano, and Tsuneo Yamane. Modifying the chain-length selectivity of the lipase from burkholderia cepacia kwi-56 through in vitro combinatorial mutagenesis in the substrate-binding site. *Protein Engineering*, 15(2):147–152, 2002.
- [25] J.D. Schrag and M. Cygler. Lipases and alpha/beta hydrolase fold. *Methods Enzymol*, 284, 1997.
- [26] M. Nardini and B.W. Dijkstra. Alpha/beta hydrolase fold enzymes: the family keeps growing. *Curr Opin Struct Biol*, 9(6):732–7, 1999.
- [27] M. Holmquist. Alpha/beta-hydrolase fold enzymes: structures, functions and mechanisms. *Curr Protein Pept Sci*, 1(2):209–35, 2000.
- [28] C. Carrasco-Lpez, C. Godoy, B. de Las Rivas, G. Fernndez-Lorente, J.M. Palomo, J.M. Guisn, R. Fernndez-Lafuente, M. Martnez-Ripoll, and J.A. Hermoso. Activation of bacterial thermoalkalophilic lipases is spurred by dramatic structural rearrangements. *J Biol Chem*, 2008.



- [29] Variketta M. Haridasan Namboodiri and Rajagopal Chattopadhyaya. Purification and biochemical characterization of a novel thermostable lipase from *Aspergillus niger*. *Lipids*, 35(5):495–502, 2000.
- [30] G.G. Dodson, D.M. Lawson, and F.K. Winkler. Structural and evolutionary relationships in lipase mechanism and activation. *Faraday Discuss*, (93):95–105, 1992.
- [31] A.M. Brzozowski, U. Derewenda, Z.S. Derewenda, G.G. Dodson, D.M. Lawson, J.P. Turkenburg, F. Bjorkling, B. Høge-Jensen, S.A. Patkar, and L. Thim. A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex. *Nature*, 351(6326):491–4, 1991.
- [32] Mirosław Cygler and Joseph D. Schrag. [1] structure as basis for understanding interfacial properties of lipases. In Edward A. Dennis Byron Rubin, editor, *Lipases, Part A: Biotechnology*, volume 284 of *Methods in Enzymology*, pages 3 – 27. Academic Press, 1997.
- [33] Annegrethe Hjorth, Frederic Carriere, Claire Cudrey, Helle Woldike, Esper Boel, David M. Lawson, Francine Ferrato, Christian Cambillau, and Guy G. and Dodson. A structural domain (the lid) found in pancreatic lipases is absent in the guinea pig (phospho)lipase. *Biochemistry*, 32(18):4702–4707, 1993.
- [34] J. Pleiss, M. Fischer, and R.D. Schmid. Anatomy of lipase binding sites: the scissile fatty acid binding site. *Chem Phys Lipids*, 93(1-2):67–80, 1998.
- [35] R.V. Muralidhar, R.R. Chirumamilla, R. Marchant, V.N. Ramachandran, O.P. Ward, and P. Nigam. Understanding lipase stereoselectivity. *World Journal of Microbiology and Biotechnology*, 18(2):81–97, 2002.
- [36] Csar A. Godoy, Blanca de las Rivas, Marco Filice, Gloria Fernandez-Lorente, Jose M. Guisan, and Jose M. Palomo. Enhanced activity of an immobilized lipase promoted by site-directed chemical modification with polymers. *Process Biochemistry*, 45(4):534 – 541, 2010.
- [37] Zhengyu Shu, Mojie Duan, Jiangke Yang, Li Xu, and Yunjun Yan. *Aspergillus niger* lipase: Heterologous expression in *Pichia pastoris*, molecular modeling prediction and the importance of the hinge domains at both sides of the lid domain to interfacial activation. *Biotechnology Progress*, 25(2):409–416, 2009.
- [38] Ariel Louwrier. Industrial products - the return to carbohydrate-based industries. *Biotechnology and Applied Biochemistry*, 27(1):1–8, 1998.
- [39] U.T. Bornscheuer. Trends and challenges in enzyme technology. *Adv Biochem Eng Biotechnol*, 100, 2005.
- [40] Fredrik Bjorkling, Sven Erik Godtfredsen, and Ole Kirk. The future impact of industrial lipases. *Trends in Biotechnology*, 9(1):360 – 363, 1991.
- [41] Uwe T. Bornscheuer. Lipase-catalyzed syntheses of monoacylglycerols. *Enzyme and Microbial Technology*, 17(7):578 – 586, 1995.
- [42] B. Borgström and H.L. Brockman. *Lipases*. Elsevier, 1984.

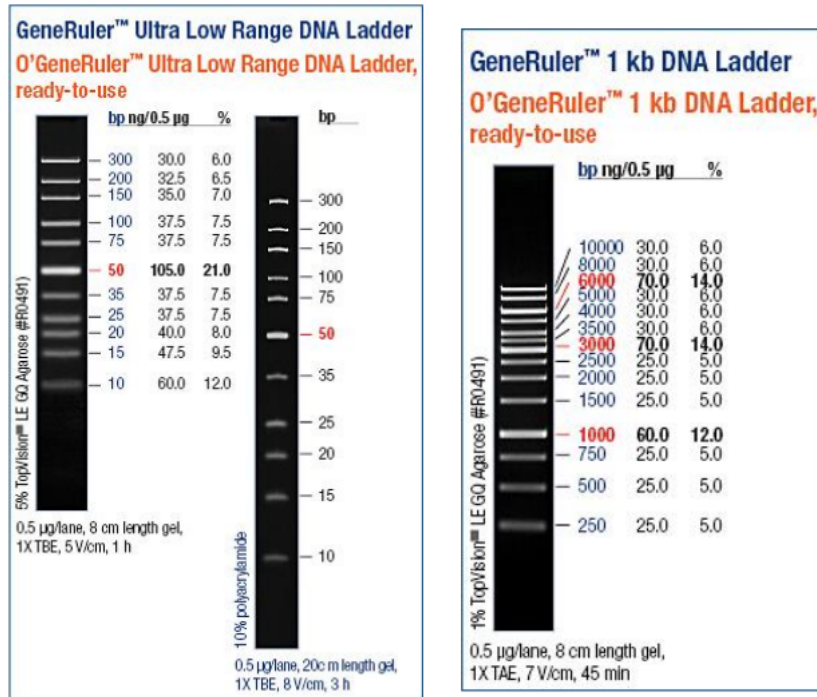
- [43] M.L. Ra, C. Schmidt-Dannert, S. Wahl, A. Sprauer, and R.D. Schmid. Thermoalkalophilic lipase of bacillus thermocatenulatus large-scale production, purification and properties: aggregation behaviour and its effect on activity. *J Biotechnol*, 56(2):89–102, 1997.
- [44] L. Poppe and L. Novak. Selective biocatalysis: a synthetic approach. 1992.
- [45] K. Jaeger and T. Eggert. Lipases for biotechnology. *Curr Opin Biotechnol*, 13(4):390–7, 2002.
- [46] RobertaL. Farrell, Kunio Hata, and MaryBeth Wall. Solving pitch problems in pulp and paper processes by the use of enzymes or fungi. 57:197–212, 1997.
- [47] AdrienneL. Huston. Biotechnological aspects of cold-adapted enzymes. pages 347–363, 2008.
- [48] J.Peter Rasor and Edgar Voss. Enzyme-catalyzed processes in pharmaceutical industry. *Applied Catalysis A: General*, 221(12):145 – 158, 2001. ;ce:title;Hoelderich Special Issue;/ce:title;.
- [49] B. Joseph, P.W. Ramteke, and G. Thomas. Cold active microbial lipases: some hot issues and recent developments. *Biotechnol Adv*, 26(5):457–70.
- [50] R. Piamtongkam, S. Duquesne, F. Bordes, S. Barbe, I. Andr, A. Marty, and W. Chulalaksananukul. Enantioselectivity of candida rugosa lipases (lip1, lip3, and lip4) towards 2-bromo phenylacetic acid octyl esters controlled by a single amino acid. *Biotechnol Bioeng*, 108(8):1749–56, 2011.
- [51] Manuela Zaccolo, David M. Williams, Daniel M. Brown, and Ermanno Gherardi. An approach to random mutagenesis of {DNA} using mixtures of triphosphate derivatives of nucleoside analogues. *Journal of Molecular Biology*, 255(4):589 – 603, 1996.
- [52] Marco A. Mena and Patrick S. Daugherty. Automated design of degenerate codon libraries. *Protein Engineering Design and Selection*, 18(12):559–561, 2005.
- [53] W. P. Stemmer. Rapid evolution of a protein in vitro by dna shuffling. *Nature*, 370(6488):389–391, August 1994.
- [54] Cristina Aguayo Alan R. Fersht Myriam M. Altamirano, Jonathan M. Blackburn. Directed evolution of new catalytic activity using the /-barrel scaffold. *Nature*, (6770):617622, 2000.
- [55] Kevin A Gray, Lishan Zhao, and Mark Emptage. Bioethanol. *Current Opinion in Chemical Biology*, 10(2):141 – 146, 2006. ;ce:title;Bioinorganic chemistry / Biocatalysis and biotransformation;/ce:title;.
- [56] D A Estell, T P Graycar, and J A Wells.
- [57] Krista L. Morley and Romas J. Kazlauskas. Improving enzyme properties: when are closer mutations better? *Trends in Biotechnology*, 23(5):231 – 237, 2005.
- [58] Romas J. Kazlauskas and Uwe T. Bornscheuer. Finding better protein engineering strategies. *Nature Chemical Biology*, 5(8):526–529, August 2009.

- [59] C. Neylon. Chemical and biochemical strategies for the randomization of protein encoding dna sequences: library construction methods for directed evolution. *Nucleic Acids Res*, 32(4):1448–59, 2004.
- [60] J.D. Bloom, S.T. Labthavikul, C.R. Otey, and F.H. Arnold. Protein stability promotes evolvability. *Proc Natl Acad Sci U S A*, 103(15):5869–74, 2006.
- [61] D.M. Weinreich, N.F. Delaney, M.A. Depristo, and D.L. Hartl. Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science*, 312(5770):111–4, 2006.
- [62] J.B. Garrett, K.A. Kretz, E. O’Donoghue, J. Kerovuo, W. Kim, N.R. Barton, G.P. Hazlewood, J.M. Short, D.E. Robertson, and K.A. Gray. Enhancing the thermal tolerance and gastric performance of a microbial phytase for use as a phosphate-mobilizing monogastric-feed supplement. *Appl Environ Microbiol*, 70(5):3041–6, 2004.
- [63] J.D. Bloom, P.A. Romero, Z. Lu, and F.H. Arnold. Neutral genetic drift can alter promiscuous protein functions, potentially aiding functional evolution. *Biol Direct*, 2, 2007.
- [64] R.D. Gupta and D.S. Tawfik. Directed enzyme evolution via small and effective neutral drift libraries. *Nat Methods*, 5(11):939–42, 2008.
- [65] E. Whittle and J. Shanklin. Engineering delta 9-16:0-acyl carrier protein (acp) desaturase specificity based on combinatorial saturation mutagenesis and logical redesign of the castor delta 9-18:0-acp desaturase. *J Biol Chem*, 276(24):21500–5, 2001.
- [66] H. Chen, U. Borjesson, O. Engkvist, T. Kogej, M.A. Svensson, N. Blomberg, D. Weigelt, J.N. Burrows, and T. Lange. Prozar: A new methodology for combinatorial library design. *J Chem Inf Model*, 2009.
- [67] R. Fox, A. Roy, S. Govindarajan, J. Minshull, C. Gustafsson, J.T. Jones, and R. Emig. Optimizing the search algorithm for protein engineering by directed evolution. *Protein Eng*, 16(8):589–97, 2003.
- [68] R.J. Fox, S.C. Davis, E.C. Mundorff, L.M. Newman, V. Gavrilovic, S.K. Ma, L.M. Chung, C. Ching, S. Tam, S. Muley, J. Grate, J. Gruber, J.C. Whitman, R.A. Sheldon, and G.W. Huisman. Improving catalytic function by prozar-driven enzyme evolution. *Nat Biotechnol*, 25(3):338–44, 2007.
- [69] Valrie Abcassis, Denis Pompon, and Gilles Truan. High efficiency family shuffling based on multi-step pcr and in vivo dna recombination in yeast: statistical and functional analysis of a combinatorial library between human cytochrome p450 1a1 and 1a2. *Nucleic Acids Research*, 28(20):e88, 2000.
- [70] W P Stemmer. Dna shuffling by random fragmentation and reassembly: in vitro recombination for molecular evolution. *Proceedings of the National Academy of Sciences*, 91(22):10747–10751, 1994.
- [71] W. Suen, N. Zhang, L. Xiao, V. Madison, and A. Zaks. Improved activity and thermostability of candida antarctica lipase b by dna family shuffling. *Protein Eng Des Sel*, 17(2):133–40, 2004.

- [72] Xiao-Wei Yu, Rui Wang, Meng Zhang, Yan Xu, and Rong Xiao. Enhanced thermostability of a rhizopus chinensis lipase by in vivo recombination in pichia pastoris. *Microbial Cell Factories*, 11(1):102, 2012.
- [73] L. You and F. H. Arnold. Directed evolution of subtilisin E in *Bacillus subtilis* to enhance total activity in aqueous dimethylformamide. *Protein Eng*, 9(1):77–83, January 1994.
- [74] M.R. Green and J. Sambrook. *Molecular cloning: A laboratory manual*. (v. 1), 2012.
- [75] M. Dagert and S.D. Ehrlich. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. *Gene*, 6(1):23 – 28, 1979.
- [76] PrK Ingvarsson. Molecular evolution of synonymous codon usage in *Populus*. *BMC Evolutionary Biology*, 8(1):1–13, 2008.
- [77] A. Cramer, E. A. Whitehorn, E. Tate, and W. P. Stemmer. Improved green fluorescent protein by molecular evolution using dna shuffling. *Nat Biotechnol.*, 14(3):315–9., 1996.

# A Appendix A

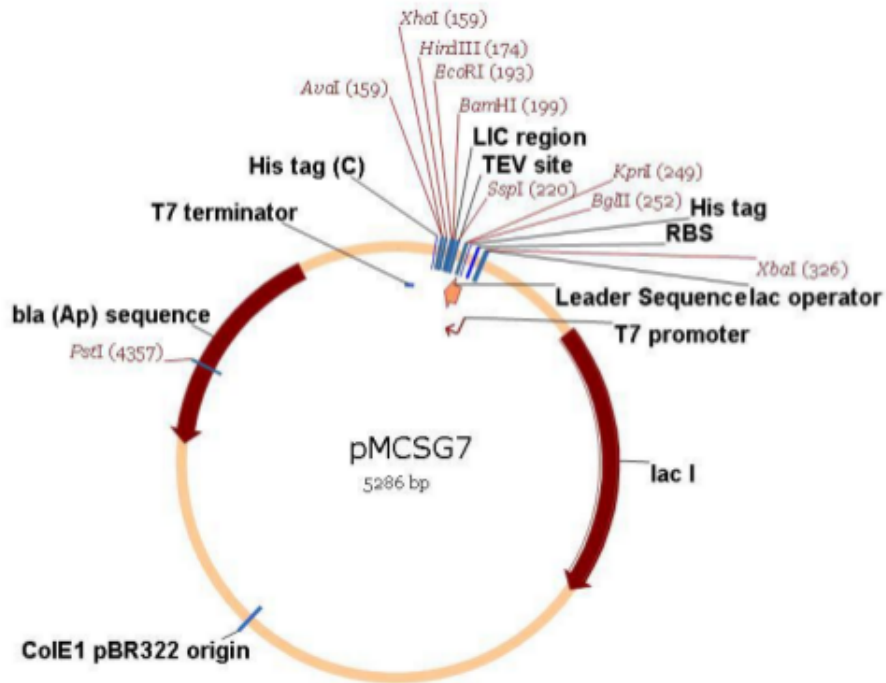
## A.1 DNA ladders and Experimental Protocols



**Figure A.1** : DNA molecular weight markers, Ultra low range and 1kb ladders, respectively.

### A.1.1 Vector (pMCSG-7) Overnight Digest with SspI

pMCSG-7 (his-tagged bacterial expression vector) is a vector that is transformed into *E.coli* and expressed as 6 x His-tagged-protein complex. This vector was digested by restriction enzyme digestion. The restriction enzyme was chosen as SspI.



**Figure A.2 :** pMCSG -7 (his-tagged bacterial expression vector)

The restriction enzyme digestion of pMCSG-7 was performed according to the table below:

pMCSG-7	50 $\mu$ l
SspI	2 $\mu$ l
Green Buffer	6 $\mu$ l
ddH <sub>2</sub> O	2 $\mu$ l

**Table A.1 :** Procedure for the restriction enzyme digestion of pMCSG-7

### **A.1.2 Gel Electrophoresis Procedure**

For the gel electrophoresis the general protocol was applied as indicated below.

- Take 50X TAE buffer, dilute it to 1X by adding 10 ml to 490 ml water.
- Weight 1 gr agarose; dissolve it in 100 ml 1X TAE buffer.
- Heat the solution until full homogeneity is obtained.
- Cool the solution; add 2 l EtBr; mix the solution to solve EtBr.
- Pour solution to the rack; place comb; wait till polymerization.
- Place the rack onto the container; fill the container with 1X TAE buffer.
- Place the samples to the wells.
- Run the machine for 30 min; later on, check the gel under UV light.
- If necessary run for additional minutes.

### **Gel Extraction**

After the agarose gel electrophoresis of PCR products and pMCSG-7 overnight digest with Ssp1, the samples are extracted from the gel for obtaining pure reaction products.

QIAGEN- QIAquick Gel Extraction Kit Protocol was performed as listed.

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
2. Weigh the gel slice in a colorless tube. Add 3 volumes Buffer QG to 1 volume gel (100 mg 100  $\mu$ l). For > 2% agarose gels, add 6 volumes Buffer QG.
3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). Vortex the tube every 2–3 min to help dissolve gel.
4. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose). If the color of the mixture is orange or violet, add 10  $\mu$ l 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
5. Add 1 gel volume of isopropanol to the sample and mix.
6. Place a QIAquick spin column in a provided 2 ml collection tube or into a vacuum manifold.
7. To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min or apply vacuum to the manifold until all the samples have passed through the column. Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of > 800  $\mu$ l, load and spin/apply vacuum again.

8. If the DNA will subsequently be used for sequencing, *in vitro* transcription, or micro injection, add 0.5 ml Buffer QG to the QIAquick column and centrifuge for 1 min or apply vacuum. Discard flow-through and place the QIAquick column back into the same tube.
9. To wash, add 0.75 ml Buffer PE to QIAquick column and centrifuge for 1 min or apply vacuum. Discard flow-through and place the QIAquick column back into the same tube.  
Note: If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), let the column stand 2–5 min after addition of Buffer PE.
10. Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min at 17,900 x *g* (13,000 rpm) to remove residual wash buffer.
11. Place QIAquick column into a clean 1.5 ml micro centrifuge tube.
12. To elute DNA, add 50  $\mu$ l Buffer EB (10 mM Tris · Cl, pH 8.5) or water to the center of the QIAquick membrane and centrifuge the column for 1 min. For increased DNA concentration, add 30  $\mu$ l Buffer EB to the center of the QIAquick Membrane, let the column stand for 1 min, and then centrifuge for 1 min. After the addition of Buffer EB to the QIAquick membrane, increasing the incubation time to up to 4 min can increase the yield of purified DNA.
13. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.



### A.1.3 T4 DNA Polymerase Reaction

For ligation independent cloning vector and insert were modified according to T4 DNA Polymerase reaction which creates guanine overhangs for vector and cytosine overhangs for insert.

Vector	Volume	Insert	Volume
ddH <sub>2</sub> O	-	ddH <sub>2</sub> O	1 $\mu$ L
5X Buffer	16 $\mu$ L	5X Buffer	14 $\mu$ L
T4 Polymerase	3 $\mu$ L	T4 Polymerase	3 $\mu$ L
dGTP	2 $\mu$ L	dCTP	2 $\mu$ L
DNA	60 $\mu$ L	DNA	50 $\mu$ L
V <sub>final</sub>	80 $\mu$ L	V <sub>Final</sub>	70 $\mu$ L

Reaction Condition	20°C	60'
Inactivation Condition	75°C	20'

**Table A.2** : T4 DNA Polymerase Reaction Program

#### **A.1.4 Phenol/Chloroform Extraction and Ethanol Precipitation**

Phenol/Chloroform extraction and ethanol precipitation is carried out for both vector and insert in separate tubes based on the following procedure.

- Product of T4 DNA polymerase reaction for vector (80  $\mu$ l) was completed up to 100  $\mu$ l with ddH<sub>2</sub>O
- Product of T4 DNA polymerase reaction for insert (70  $\mu$ l) was completed up to 100  $\mu$ l with ddH<sub>2</sub>O.
- Addition of 1:1 ratio phenol/chloroform (100  $\mu$ l) into both tubes.
- Vortex
- 5' of top speed centrifuge (13.2 rpm)
- Take supernatant
- Addition of 4  $\mu$ l NaOAc, 10  $\mu$ l LPA and 250  $\mu$ l EtOH (%100)
- Keep the tubes at -80°C for 20'
- 15' Top speed centrifuge (13,2 rpm)
- Discard the supernatant
- Addition of 250  $\mu$ l EtOH (%70) onto the pellet
- 10' Top speed centrifuge
- Discard the supernatant
- Re-suspend the pellet with 10  $\mu$ l of ddH<sub>2</sub>O

### A.1.5 Annealing Reaction

Purified vector and insert were combined together by annealing reaction at 22°C for 45'– 60'.

	Control	Sample
Vector	150 ng	150 ng
Insert	-----	100 ng

**Table A.3** : Annealing reaction

### A.1.6 Transformation to Shuffle Competent Cells

For the transformation of plasmid into the competent cell, the following procedure was carried out.

- Mix 2  $\mu\text{L}$  product of annealing reaction with 200  $\mu\text{L}$  of each competent cells
- Keep on ice for 20'
- Heat shock for 1' at strictly at 42°C
- Keep on ice for 10'
- Addition of 800  $\mu\text{L}$  SOC
- Incubation at 37°C for 60'
- Centrifuge at 7000 rpm for 2'
- Discard the supernatant until 100  $\mu\text{L}$  of supernatant remains
- Re-suspend the pellet in 100  $\mu\text{L}$  of supernatant
- Spread on LB agar plate with beads.
- Incubation at 37°C for 24–48 hours.

### A.1.7 Colony PCR and Mini-prep Protocol

Taq Polymerase MM	7,5 $\mu$ L
Reverse Primer	0,75 $\mu$ L
Forward Primer	0,75 $\mu$ L
ddH <sub>2</sub> O	6 $\mu$ L
V <sub>final</sub>	15 $\mu$ L

**Table A.4 :** Colony PCR

Positive colonies are taken from the previously streaked plate and inoculated into 5 mL of LB broth. Overnight incubation was performed at 37°C shaker. The 5 mL of cultures were centrifuged for 5' at the top speed. The supernatants were discarded and QIAGEN-Plasmid DNA Purification Kit was performed on pellets.

#### QIAGEN - Plasmid DNA Purification Kit Protocol:

1. Re-suspend pelleted bacterial cells in 250  $\mu$ l Buffer P1 and transfer to a 1.7ml micro-centrifuge tube. No cell clumps should be visible after resuspension of the pellet. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
2. Add 250  $\mu$ l Buffer P2 and mix thoroughly by inverting the tube 4–6 times. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min. If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.
3. Add 350  $\mu$ l Buffer N3; mix immediately and thoroughly by inverting the tube 4–6 times. Keep on ice for 10 mins. To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. The solution should become cloudy. If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless.
4. Centrifuge for 10 min at 13,000 rpm ( 17,900 x g) in a table-top micro-centrifuge. A compact white pellet will form.
5. Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.
6. Centrifuge for 30 – 60 sec. Discard the flow-through.
7. Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30 – 60 sec.

8. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.  
Important: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
9. Place the QIAprep column in a clean 1.5 ml micro-centrifuge tube. To elute DNA, add 50  $\mu$ l Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

#### **A.1.8 Expression**

- Take positive clones from each transformation plates (Shuffle) by tips.
- Add them to 5 ml LB Broth with 5  $\mu$ l Ampicillin (1000X)
- After overnight growth at 37°C, take glycerol stocks of each cell culture (200  $\mu$ l 60% Glycerol + 600  $\mu$ l cell culture)
- Transfer cell cultures to 30 ml LB Broth with 30  $\mu$ l Ampicillin (1000X)
- Take 1 ml sample from each of the cultures and label them as  $t_0$
- Add IPTG when efficient optimal density is reached.
- For expression of the proteins wait for 8 hours.
- Centrifuge samples for 15 minutes at 4000 rpm.
- Add sufficient amount of B-PER according to the pellet amount (between 100  $\mu$ l -250  $\mu$ l)
- Centrifuge samples for 5 minutes at 13.2 rpm.
- Take 20  $\mu$ l sample from supernatant and mix with 4  $\mu$ l dye mix (loading dye + DTT )
- Load the samples and run SDS-PAGE electrophoresis and carry out the characterization step.

## A.2 Multiple Sequence Alignments

	01	02	03	04	05	06	07	08	09	10	11	12
A	Strip .. 864/869..	Strip .. 862/864..	Strip .. 861/863..	Strip .. 862/863..	Strip .. 677/712..	Strip .. 0/0/895	Strip .. 20/20/894	Strip .. 859/863..	Strip .. 0/0/895	Strip .. 857/859..		
B	2 Reve.. 865/867..	10 Reve.. 858/858..	18 Reve.. 858/859..	26 Reve.. 858/858..	34 Reve.. 855/858..	42 Reve.. 0/0/895	50 Reve.. 0/0/895	58 Reve.. 858/858..	66 Reve.. 0/0/895	74 Reve.. 0/0/895		
C	3 Reve.. 862/862..	11 Reve.. 862/867..	19 Reve.. 859/860..	27 Reve.. 859/860..	35 Reve.. 864/865..	43 Reve.. 859/860..	51 Reve.. 860/865..	59 Reve.. 0/0/895	67 Reve.. 855/862..	75 Reve.. 849/859..		
D	4 Reve.. 854/857..	12 Reve.. 853/866..	20 Reve.. 855/857..	28 Reve.. 817/824..	36 Reve.. 0/0/895	44 Reve.. 778/789..	52 Reve.. 860/863..	60 Reve.. 860/860..	68 Reve.. 851/863..			
E	5 Reve.. 861/863..	13 Reve.. 863/863..	21 Reve.. 865/865..	29 Reve.. 0/0/895	37 Reve.. 833/836..	45 Reve.. 857/859..	53 Reve.. 0/0/895	61 Reve.. 859/863..	69 Reve.. 850/857..			
F	6 Reve.. 860/862..	14 Reve.. 790/797..	22 Reve.. 860/862..	30 Reve.. 858/860..	38 Reve.. 862/863..	46 Reve.. 857/858..	54 Reve.. 860/860..	62 Reve.. 858/859..	70 Reve.. 860/864..			
G	7 Reve.. 860/860..	15 Reve.. 0/0/895	23 Reve.. 854/858..	31 Reve.. 0/0/895	39 Reve.. 856/860..	47 Reve.. 0/0/895	55 Reve.. 0/0/895	63 Reve.. 862/863..	71 Reve.. 859/860..			
H	8 Reve.. 857/858..	16 Reve.. 861/862..	24 Reve.. 0/0/895	32 Reve.. 863/864..	40 Reve.. 0/0/895	48 Reve.. 0/0/895	56 Reve.. 0/0/895	64 Reve.. 853/859..	72 Reve.. 0/0/895			

Color Overview (Alignment Percentage)	Identities >= 90%	90% > Identities >= 75%	75% > Identities >= 60%	Identities < 60%
--	-------------------	-------------------------	-------------------------	------------------

**Figure A.2:** The scheme of multiple sequence alignments of the clones against native ANL.







Score = 1709 bits (862), Expect = 0.0  
Identities = 862/862 (100%)  
Strand = Plus / Minus

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Query: 18  accacaagtgaagccaaaaacgtccaccggttgcctcgccatcccccgcgcgcaattga 77
      |
Sbjct: 862  accacaagtgaagccaaaaacgtccaccggttgcctcgccatcccccgcgcgcaattga 803

Query: 78  ttccctcgatgagttcaatatccgacgcgctgacaactggctccggtgccaactggatgac 137
      |
Sbjct: 802  ttccctcgatgagttcaatatccgacgcgctgacaactggctccggtgccaactggatgac 743

Query: 138  agtattctggacttggtggctggaatccaaagtcacatgggtggcaaccgggggacgagtg 197
      |
Sbjct: 742  agtattctggacttggtggctggaatccaaagtcacatgggtggcaaccgggggacgagtg 683

Query: 198  cgttcaagtgtgtaacgcggaagttcgctccagatccctggctggtgatgtgctcggcca 257
      |
Sbjct: 682  cgttcaagtgtgtaacgcggaagttcgctccagatccctggctggtgatgtgctcggcca 623

Query: 258  ggcacatagtttccgactcaggacatccataggtgtacagttcaacgctataaacctcat 317
      |
Sbjct: 622  ggcacatagtttccgactcaggacatccataggtgtacagttcaacgctataaacctcat 563

Query: 318  ttgcgaagaccgttgctccagtgtagccaaatgcgccccaagctgtgcccggtgaagt 377
      |
Sbjct: 562  ttgcgaagaccgttgctccagtgtagccaaatgcgccccaagctgtgcccggtgaagt 503

Query: 378  agagggtatagcccgaatcgtgctcatcgcggacttgatcttgctcgtcagatgtctg 437
      |
Sbjct: 502  agagggtatagcccgaatcgtgctcatcgcggacttgatcttgctcgtcagatgtctg 443

Query: 438  cagcggcttcccatgcctccagaatccagtgtaaccttgccagccagtaacagaggtcat 497
      |
Sbjct: 442  cagcggcttcccatgcctccagaatccagtgtaaccttgccagccagtaacagaggtcat 383

Query: 498  cgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgctc 557
      |
Sbjct: 382  cgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgctc 323

Query: 558  ggaaggcgaccacagaccgcttggtggtgtgctcgcggccaggaaaccggtgtgctc 617
      |
Sbjct: 322  ggaaggcgaccacagaccgcttggtggtgtgctcgcggccaggaaaccggtgtgctc 263

Query: 618  caaagtatttgcagggtcaaacccagcagcatcttggtgctcgcctcccgactgatg 677
      |
Sbjct: 262  caaagtatttgcagggtcaaacccagcagcatcttggtgctcgcctcccgactgatg 203

Query: 678  gacaggcgtcgccgtgcatgtoacgttagagtgctcagatcgatattgttcgagcaat 737
      |
Sbjct: 202  gacaggcgtcgccgtgcatgtoacgttagagtgctcagatcgatattgttcgagcaat 143

Query: 738  aagctgcggcagaccattgcgagaacaattgcagctcatccaacgtggagtgagacac 797
      |
Sbjct: 142  aagctgcggcagaccattgcgagaacaattgcagctcatccaacgtggagtgagacac 83

Query: 798  tccgcacatcaagtggtgctggtgcccgcagcactcagcgcagcgtgcccgtcaaaaagca 857
      |
Sbjct: 82  tccgcacatcaagtggtgctggtgcccgcagcactcagcgcagcgtgcccgtcaaaaagca 23

Query: 858  ctccaaaccgtccagagaacat 879
      |
Sbjct: 22  ctccaaaccgtccagagaacat 1
```

Figure A.5: Clone A3



Score = 1699 bits (857), Expect = 0.0  
Identities = 861/863 (99%)  
Strand = Plus / Minus

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Query: 16  tacnacaagtgngccaaaacgtccaccgttgcttgcctgcattcccgcgcgtcgaattg 75
      ||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 863  taccacaagtgagccaaaacgtccaccgttgcttgcctgcattcccgcgcgtcgaattg 804

Query: 76  attccctcgatgagttcaataatccgacgccgtgacac tggctccgggtgccactggtgatc 135
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 803  attccctcgatgagttcaataatccgacgccgtgacac tggctccgggtgccactggtgatc 744

Query: 136  cagtattctggacttggctggctgaatccaaagtcac tgggtggcaaccgggggacgatg 195
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 743  cagtattctggacttggctggctgaatccaaagtcac tgggtggcaaccgggggacgatg 684

Query: 196  tcgttcaagtggtgtaacgcggaagtccgctcagatc cctggctggtgatgtgctcggcc 255
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 683  tcgttcaagtggtgtaacgcggaagtccgctcagatc cctggctggtgatgtgctcggcc 624

Query: 256  agcgcatagtttccgactcgaggacatccataggtgtac agttcaacgctataaccgta 315
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 623  agcgcatagtttccgactcgaggacatccataggtgtac agttcaacgctataaccgta 564

Query: 316  ttctgcaagaccgttgctcccagtgtagccaatgcgc cggcccaagctgtgcccgtggaag 375
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 563  ttctgcaagaccgttgctcccagtgtagccaatgcgc cggcccaagctgtgcccgtggaag 504

Query: 376  tagagggtatagcccgaaacgtgctcctcgcggacttg atcttgcctcgtcagattgtct 435
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 503  tagagggtatagcccgaaacgtgctcctcgcggacttg atcttgcctcgtcagattgtct 444

Query: 436  gcagcggcttcccatgcttccagaatccagtggtgaa ccttgcagccagtagcagaggta 495
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 443  gcagcggcttcccatgcttccagaatccagtggtgaa ccttgcagccagtagcagaggta 384

Query: 496  tcgttattctgcaggatgaagtcagatcagcaatcc agttcttgatggtgctaetgct 555
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 383  tcgttattctgcaggatgaagtcagatcagcaatcc agttcttgatggtgctaetgct 324

Query: 556  cggaaaggc gaccacgagccgcttggttggtgtgtg tccggcggcaggaaaccggctgtgct 615
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 323  cggaaaggc gaccacgagccgcttggttggtgtgtg tccggcggcaggaaaccggctgtgct 264

Query: 616  ccaaagtatttgtcaggtcaaac tccagcagcatcttggtgctcgcctcctcgactgat 675
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 263  ccaaagtatttgtcaggtcaaac tccagcagcatcttggtgctcgcctcctcgactgat 204

Query: 676  ggacaggcgtcggc cgtgcatgtcacgtagagtcgt ccgagtcgatattgttcgagcaa 735
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 203  ggacaggcgtcggc cgtgcatgtcacgtagagtcgt ccgagtcgatattgttcgagcaa 144

Query: 736  taagctgcggcagaccat tgcgagaacaattgcagctc atc caa cgtggaagtcgagaca 795
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 143  taagctgcggcagaccat tgcgagaacaattgcagctc atc caa cgtggaagtcgagaca 84

Query: 796  ctccgcacatcaagtgggtgtcgggtgccgcagcactc agcgcagcgtgcccgcgtcaaaagc 855
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 83  ctccgcacatcaagtgggtgtcgggtgccgcagcactc agcgcagcgtgcccgcgtcaaaagc 24

Query: 856  actccaaaacggtccagagaacat 878
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct: 23  actccaaaacggtccagagaacat 1
```

Figure A.7: Clone A5

Score = 1697 bits (856), Expect = 0.0  
Identities = 860/862 (99%)  
Strand = Plus / Minus

Query: 18 accacaagtnggccanaacgtccacogttgcttgcctgcaatcccccgcgctogaat tga 77  
||||||||| ||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 862 accacaagtgagccaaaaacgtccacogttgcttgcctgcaatcccccgcgctogaat tga 803

Query: 78 ttccctcgatgagt tcaatatccgacgcegtgacactggctccggtgccaactggtgatcc 137  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 802 ttccctcgatgagt tcaatatccgacgcegtgacactggctccggtgccaactggtgatcc 743

Query: 138 agtattctggacttggctggctgaaaccaaagtc catgggtggcaac cggggga cagatgt 197  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 742 agtattctggacttggctggctgaaaccaaagtc catgggtggcaac cggggga cagatgt 683

Query: 198 cgttcaagtgtgtaacgcggaagt tgcctcagatccctggctggatgtgctcggcca 257  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 682 cgttcaagtgtgtaacgcggaagt tgcctcagatccctggctggatgtgctcggcca 623

Query: 258 gcgcatagtttccgactcggagacatccataggtgta cagt tcaacgctataaacgct cat 317  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 622 gcgcatagtttccgactcggagacatccataggtgta cagt tcaacgctataaacgct cat 563

Query: 318 ttogcaagaccggtgctccagtgtagccaatgcccgc cccaagctgtgccggtgaaat 377  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 562 ttogcaagaccggtgctccagtgtagccaatgcccgc cccaagctgtgccggtgaaat 503

Query: 378 agagggatagcccgaatacgtgctcctcggactt gatc ttgctcgtcagat tgtctg 437  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 502 agagggatagcccgaatacgtgctcctcggactt gatc ttgctcgtcagat tgtctg 443

Query: 438 cagcggcttcc catgcttccagaatc cagtgtgaaaccttgccagcca gta cagaggt cat 497  
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Sbjct: 442 cagcggcttcc catgcttccagaatc cagtgtgaaaccttgccagcca gta cagaggt cat 383

Query: 498 cgttatcttgaggatgaagtcagatcagcaatccagttc ttgatgggtgctactgccc 557  
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Sbjct: 382 cgttatcttgaggatgaagtcagatcagcaatccagttc ttgatgggtgctactgccc 323

Query: 558 ggaaggcagaccagagccgcttgttggtgtgtcgcggccaggaaa cggctgtgccc 617  
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Sbjct: 322 ggaaggcagaccagagccgcttgttggtgtgtcgcggccaggaaa cggctgtgccc 263

Query: 618 caaagtattttgctcaggtcaaactccagcagcatcttggtgctcgcctcctcagactgatg 677  
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Sbjct: 262 caaagtattttgctcaggtcaaactccagcagcatcttggtgctcgcctcctcagactgatg 203

Query: 678 gacagcgcteggcctgcatgtcacttagagtgctcagagtcgatatgttccagcaat 737  
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Sbjct: 202 gacagcgcteggcctgcatgtcacttagagtgctcagagtcgatatgttccagcaat 143

Query: 738 aagctgoggcagaccattgcgagaacaattgcagctcatccaacgtggaagtcgagacac 797  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 142 aagctgoggcagaccattgcgagaacaattgcagctcatccaacgtggaagtcgagacac 83

Query: 798 tccgcacatcaagtgggtgctggcgcagcactcagcgcagcgtgcgccgtcaaaa gca 857  
||||||||| |||||||||||||||||||||||||||||||||||||||||  
Sbjct: 82 tccgcacatcaagtgggtgctggcgcagcactcagcgcagcgtgcgccgtcaaaa gca 23

Query: 858 ctccaacogtccagagaacat 879  
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Sbjct: 22 ctccaacogtccagagaacat 1

Figure A.8: Clone A6

Score = 1705 bits (860), Expect = 0.0  
Identities = 860/860 (100%)  
Strand = Plus / Minus

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Query: 20  cacaagtgagccaaaaagtcaccggtgcttcgectgcattcccgcgctgcaattgatt 79
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Sbjct: 860  cacaagtgagccaaaaagtcaccggtgcttcgectgcattcccgcgctgcaattgatt 801

Query: 80  ccctcgatgagttcaataccgacgccgtgacac tggctccgggtgccactggtgatccag 139
      |||
Sbjct: 800  ccctcgatgagttcaataccgacgccgtgacac tggctccgggtgccactggtgatccag 741

Query: 140  tattctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgtcg 199
      |||
Sbjct: 740  tattctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgtcg 681

Query: 200  ttcaagtgtgtaacgcggaagtccgctccagatccctggetggtgatgtgctcggccagc 259
      |||
Sbjct: 680  ttcaagtgtgtaacgcggaagtccgctccagatccctggetggtgatgtgctcggccagc 621

Query: 260  gcatagttccgactcagggacatccataggtgtacagttcaacgctataaccgctcatt 319
      |||
Sbjct: 620  gcatagttccgactcagggacatccataggtgtacagttcaacgctataaccgctcatt 561

Query: 320  cgcaagaccgttgc tcccagtgtagccaatgcgcgcgccaagctgtgccgggtag 379
      |||
Sbjct: 560  cgcaagaccgttgc tcccagtgtagccaatgcgcgcgccaagctgtgccgggtag 501

Query: 380  agggatatagccggaatacgtgctcatcgggacttga tcttgcctcagattgtctgca 439
      |||
Sbjct: 500  agggatatagccggaatacgtgctcatcgggacttga tcttgcctcagattgtctgca 441

Query: 440  gcggettccatgcttc cagaatccagtgtagaaccttgca gccagtagaggtag 499
      |||
Sbjct: 440  gcggettccatgcttc cagaatccagtgtagaaccttgca gccagtagaggtag 381

Query: 500  ttatcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgctcgg 559
      |||
Sbjct: 380  ttatcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgctcgg 321

Query: 560  aaggcgaccacgagccgc tttgtgtgttgcgcgccaggaaaccggctgtgctcca 619
      |||
Sbjct: 320  aaggcgaccacgagccgc tttgtgtgttgcgcgccaggaaaccggctgtgctcca 261

Query: 620  aagtatttgtcaggtcaaac tccagcagcatcttgggtgctcgcctcctcactgatgga 679
      |||
Sbjct: 260  aagtatttgtcaggtcaaac tccagcagcatcttgggtgctcgcctcctcactgatgga 201

Query: 680  caggcgtggcgtgcatgtcagttagagtcgtccagtc gatattgttcgagcaataa 739
      |||
Sbjct: 200  caggcgtggcgtgcatgtcagttagagtcgtccagtc gatattgttcgagcaataa 141

Query: 740  gctgcggcagaccattgc gagaacaatgcagctcatccaa cgtggaagtcgagacactc 799
      |||
Sbjct: 140  gctgcggcagaccattgc gagaacaatgcagctcatccaa cgtggaagtcgagacactc 81

Query: 800  cgcaatcaagtggtgctgggtgccgcagcactcagcgcagcgtgcgcctcaaaagcact 859
      |||
Sbjct: 80  cgcaatcaagtggtgctgggtgccgcagcactcagcgcagcgtgcgcctcaaaagcact 21

Query: 860  ccaaaccgtccagagaacat 879
      |||
Sbjct: 20  ccaaaccgtccagagaacat 1
```

Figure A.9: Clone A7

Score = 1695 bits (855), Expect = 0.0  
Identities = 857/858 (99%)  
Strand = Plus / Minus

```
Query: 22 caagtgagccaaaaagtcaccggtgcttgcctgcattcccgcgctcgaattgatcc 81
      |||
Sbjct: 858 caagtgagccaaaaagtcaccggtgcttgcctgcattcccgcgctcgaattgatcc 799

Query: 82 ctcgatgagttcaatatacgcgcgctgacactggctccgggtgcacatggatccagta 141
      |||
Sbjct: 798 ctcgatgagttcaatatacgcgcgctgacactggctccgggtgcacatggatccagta 739

Query: 142 ttctggacttggctggtgaaatccaaagtccatgggtggcaaccgggggacgatgctgtt 201
      |||
Sbjct: 738 ttctggacttggctggtgaaatccaaagtccatgggtggcaaccgggggacgatgctgtt 679

Query: 202 caagtgtgtaacgcggaaagtgcctccagatcccggctgggtgatgtgctcgccagcgc 261
      |||
Sbjct: 678 caagtgtgtaacgcggaaagtgcctccagatcccggctgggtgatgtgctcgccagcgc 619

Query: 262 atagtttcgactcaggacatccatagggtgtacagttcaacgcataaacgctcatttcg 321
      |||
Sbjct: 618 atagtttcgactcaggacatccatagggtgtacagttcaacgcataaacgctcatttcg 559

Query: 322 caagaccgttgcctccagtgtagc caatgctgcgcccgaagctgtgccgggtgaaatagag 381
      |||
Sbjct: 558 caagaccgttgcctccagtgtagc caatgctgcgcccgaagctgtgccgggtgaaatagag 499

Query: 382 ggtagtagccgaatacgtgctcatcgcggacttgatcttgcctcagatgtgctgcagc 441
      |||
Sbjct: 498 ggtagtagccgaatacgtgctcatcgcggacttgatcttgcctcagatgtgctgcagc 439

Query: 442 ggcttcccagcttccagaatccagtgtaaccttgccagccagtcagaggatcctgtt 501
      |||
Sbjct: 438 ggcttcccagcttccagaatccagtgtaaccttgccagccagtcagaggatcctgtt 379

Query: 502 atcttgcaaggatgaagtcgagatcagcaatccagttcttgatgggtgctacgtcc 561
      |||
Sbjct: 378 atcttgcaaggatgaagtcgagatcagcaatccagttcttgatgggtgctacgtcc 319

Query: 562 ggccgaccaagagccgcttgttgggtgttgcctgcggccaggaaaccggctgtgctccaaa 621
      |||
Sbjct: 318 ggccgaccaagagccgcttgttgggtgttgcctgcggccaggaaaccggctgtgctccaaa 259

Query: 622 gttatttgcaggtcaaacctcagcagcactcttgggtgctgcctcctcagactgaggaca 681
      |||
Sbjct: 258 gttatttgcaggtcaaacctcagcagcactcttgggtgctgcctcctcagactgaggaca 199

Query: 682 ggctgctggcctgcatgtcacgttagatgctccagtgatgatttgtcgagcaataagc 741
      |||
Sbjct: 198 ggctgctggcctgcatgtcacgttagatgctccagtgatgatttgtcgagcaataagc 139

Query: 742 tgcggcagaccattgcgagaaatcagcagcctcagcgcagngtgcgctcggcctcggcctcc 801
      |||
Sbjct: 138 tgcggcagaccattgcgagaaatcagcagcctcagcgcagngtgcgctcggcctcggcctcc 79

Query: 802 cacatcaagtgtgtcgggtgcgcagcactcagcgcagngtgcgctcggcctcggcctcc 861
      |||
Sbjct: 78 cacatcaagtgtgtcgggtgcgcagcactcagcgcagngtgcgctcggcctcggcctcc 19

Query: 862 aaaccgtccagagaacat 879
      |||
Sbjct: 18 aaaccgtccagagaacat 1
```

Figure A.10: Clone A8

Score = 1681 bits (848), Expect = 0.0  
Identities = 862/864 (99%), Gaps = 2/864 (0%)  
Strand = Plus / Minus

```
Query: 18  accacaagtgaaccaaaacgtccaccggttgccttcgctgcattccccgccgtggaattga 77
      |
Sbjct: 862  accacaagtgaaccaaaacgtccaccggttgccttcgctgcattccccgccgtggaattga 803

Query: 78  ttccctcgatgagttcaatatccgacgcggtgacactggctccggtgccactgggtgatcc 137
      |
Sbjct: 802  ttccctcgatgagttcaatatccgacgcggtgacactggctccggtgccactgggtgatcc 743

Query: 138  agtattctggacttggctggctggaatccaaagtcacatgggtggcaaccgggggacgatgt 197
      |
Sbjct: 742  agtattctggacttggctggctggaatccaaagtcacatgggtggcaaccgggggacgatgt 683

Query: 198  cgttcaagtgtgtaacgcggaagtccgctccagatccctggctgggtgatgtgctcggcca 257
      |
Sbjct: 682  cgttcaagtgtgtaacgcggaagtccgctccagatccctggctgggtgatgtgctcggcca 623

Query: 258  gcgcatagtttccgactcaggacatccataggtgtacagttcaacgctataaccgctcat 317
      |
Sbjct: 622  gcgcatagtttccgactcaggacatccataggtgtacagttcaacgctataaccgctcat 563

Query: 318  ttccgaagaccggttgcctccagtgtagccaatgcccgccccaagctgtgcccggtgaagt 377
      |
Sbjct: 562  ttccgaagaccggttgcctccagtgtagccaatgcccgccccaagctgtgcccggtgaagt 503

Query: 378  agagggtatagcccgaatacgtgctcactccggacttgatcttgctcgtcagattgtctg 437
      |
Sbjct: 502  agagggtatagcccgaatacgtgctcactccggacttgatcttgctcgtcagattgtctg 443

Query: 438  cagcggcttcccatgctctccagaatccagtggaaccttgccagccagtaacagaggtcat 497
      |
Sbjct: 442  cagcggcttcccatgctctccagaatccagtggaaccttgccagccagtaacagaggtcat 383

Query: 498  cgttatcttgcaggatgaagtccagatcagcaatccagttcttgatggtgctactgctc 557
      |
Sbjct: 382  cgttatcttgcaggatgaagtccagatcagcaatccagttcttgatggtgctactgctc 323

Query: 558  ggaaggcgaccacgagccgcttgttggtgttgctcgcggccaggaaaaccggtgtgctc 617
      |
Sbjct: 322  ggaaggcgaccacgagccgcttgttggtgttgctcgcggccaggaaaaccggtgtgctc 263

Query: 618  caaagtatttgcaggtcaaactccagcagcatcttggtgctcgcctctctcgactgatg 677
      |
Sbjct: 262  caaagtatttgcaggtcaaactccagcagcatcttggtgctcgcctctctcgactgatg 203

Query: 678  gacagggtcgccgctgcatgtcagcttagagtgctcagagtcgatattgttcagagcaat 737
      |
Sbjct: 202  gacagggtcgccgctgcatgtcagcttagagtgctcagagtcgatattgttcagagcaat 143

Query: 738  aagctgcgccagaccattgcgagaacaattgcagctcatccaacgtggaagtcgagacac 797
      |
Sbjct: 142  aagctgcgccagaccattgcgagaacaattgcagctcatccaacgtggaagtcgagacac 83

Query: 798  tccgcacatcaagtgggtgctgggtgcccagcactcagcgccagcgtgcccgtcaaaaacn 857
      |
Sbjct: 82  tccgcacatcaagtgggtgctgggtgcccagcactcagcgccagcgtgcccgtcaaaaacn- 24

Query: 858  actccaaaccgtcccagagaacat 881
      |
Sbjct: 23  actccaaaccgt-ccagagaacat 1
```

Figure A.11: Clone A9

Score = 1701 bits (858), Expect = 0.0  
Identities = 858/858 (100%)  
Strand = Plus / Minus

```
Query: 20 caagtgaagccaaaacgctcacccgttgccttcgctgcattcccgccgctcgaattgatcc 79
      |||
Sbjct: 858 caagtgaagccaaaacgctcacccgttgccttcgctgcattcccgccgctcgaattgatcc 799

Query: 80 ctcgatgagttcaatatacgaagcgcgctgacaactggctccgggtgcactgggtgatccagta 139
      |||
Sbjct: 798 ctcgatgagttcaatatacgaagcgcgctgacaactggctccgggtgcactgggtgatccagta 739

Query: 140 ttctggacttggtggctgaaatccaaagtcacatgggtggcaaccgggggacgatgctggt 199
      |||
Sbjct: 738 ttctggacttggtggctgaaatccaaagtcacatgggtggcaaccgggggacgatgctggt 679

Query: 200 caagtgtgtaacgcggaaagttcgtccagatcccggctgggtgatgtgctcggccagcgc 259
      |||
Sbjct: 678 caagtgtgtaacgcggaaagttcgtccagatcccggctgggtgatgtgctcggccagcgc 619

Query: 260 atagtttcgactcaggacatccataggtgtacagtccaacgcataaacgctcatttcg 319
      |||
Sbjct: 618 atagtttcgactcaggacatccataggtgtacagtccaacgcataaacgctcatttcg 559

Query: 320 caagaccggttgctccagtgtagcacaatgcgcgcccaagctgtgccgggtgaaagtag 379
      |||
Sbjct: 558 caagaccggttgctccagtgtagcacaatgcgcgcccaagctgtgccgggtgaaagtag 499

Query: 380 ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagatgtctcgcagc 439
      |||
Sbjct: 498 ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagatgtctcgcagc 439

Query: 440 ggttcccattgcttccagaaatcagtggaaccttgccagccagtacagaggtcattcgt 499
      |||
Sbjct: 438 ggttcccattgcttccagaaatcagtggaaccttgccagccagtacagaggtcattcgt 379

Query: 500 atcttgcaaggatgaagtcgagatcagcaatcagttcttgatgggtgctactgccctggaa 559
      |||
Sbjct: 378 atcttgcaaggatgaagtcgagatcagcaatcagttcttgatgggtgctactgccctggaa 319

Query: 560 ggcgaccaagagccgcttgttggtgtgtgctcgggcccaggaaccggctgtgctccaaa 619
      |||
Sbjct: 318 ggcgaccaagagccgcttgttggtgtgtgctcgggcccaggaaccggctgtgctccaaa 259

Query: 620 gttatttgcaggtcaaacctcagcagcattcttggtgctcgcctcctcgactgatggaca 679
      |||
Sbjct: 258 gttatttgcaggtcaaacctcagcagcattcttggtgctcgcctcctcgactgatggaca 199

Query: 680 ggcgtcggccgctgcatgtcacggttagagtgctccgagtcgatattgttcgagcaataagc 739
      |||
Sbjct: 198 ggcgtcggccgctgcatgtcacggttagagtgctccgagtcgatattgttcgagcaataagc 139

Query: 740 tgcggcagaccattgcgagaaatcgcagctcaatccaaactggaagtcgagacactccg 799
      |||
Sbjct: 138 tgcggcagaccattgcgagaaatcgcagctcaatccaaactggaagtcgagacactccg 79

Query: 800 cacatcaagtgggtgcgggtgcgcagcactcagcgcagcgtgcgcgctcaaaagcactcc 859
      |||
Sbjct: 78 cacatcaagtgggtgcgggtgcgcagcactcagcgcagcgtgcgcgctcaaaagcactcc 19

Query: 860 aaaccgtcagagaacat 877
      |||
Sbjct: 18 aaaccgtcagagaacat 1
```

Figure A.12: Clone A10



Score = 1649 bits (832), Expect = 0.0  
Identities = 862/867 (99%), Gaps = 4/867 (0%)  
Strand = Plus / Minus

```
Query: 14  taccacaagtgagc caaaacgtccaccgttgettgcctgcattcccgcgcgtcgaattg 73
      |||
Sbjct: 863  taccacaagtgagc caaaacgtccaccgttgettgcctgcattcccgcgcgtcgaattg 804

Query: 74  attccctc gatgagttcaataccgacgccgtgacac tggctccgggtgccactggtgatc 133
      |||
Sbjct: 803  attccctc gatgagttcaataccgacgccgtgacac tggctccgggtgccactggtgatc 744

Query: 134  cagtattctggact tggctggctgaatccaaagtc atgggtggcaaccgggggacgatg 193
      |||
Sbjct: 743  cagtattctggact tggctggctgaatccaaagtc atgggtggcaaccgggggacgatg 684

Query: 194  tcggtcaagtggttaacgcggaagttcgctc cagatccctggctggtgatgtgc tggcc 253
      |||
Sbjct: 683  tcggtcaagtggttaacgcggaagttcgctc cagatccctggctggtgatgtgc tggcc 624

Query: 254  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgta 313
      |||
Sbjct: 623  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgta 564

Query: 314  tttcgcaagaccgttgctcccagtgtagccaatgcgcgcgcc caagctgtgcccggtgaag 373
      |||
Sbjct: 563  tttcgcaagaccgttgctcccagtgtagccaatgcgcgcgcc caagctgtgcccggtgaag 504

Query: 374  tagagggtatagccgaa tacgtgctcatcgcggaacttgatcttgcctcgtcagattgtct 433
      |||
Sbjct: 503  tagagggtatagccgaa tacgtgctcatcgcggaacttgatcttgcctcgtcagattgtct 444

Query: 434  gcagcggcttcccatgcc ttc cagaatccagtg tgaaccttgacgccagtcacagaggta 493
      |||
Sbjct: 443  gcagcggcttcccatgcc ttc cagaatccagtg tgaaccttgacgccagtcacagaggta 384

Query: 494  tcggtatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgcct 553
      |||
Sbjct: 383  tcggtatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgcct 324

Query: 554  cggaaaggc gaccacgagcgccttggttggtgtgtccgcggccaggaaccggctgtgcct 613
      |||
Sbjct: 323  cggaaaggc gaccacgagcgccttggttggtgtgtccgcggccaggaaccggctgtgcct 264

Query: 614  ccaaagttatttgtcaggtcaaactccagcagcagatcttggtgctgcctcctcgactgat 673
      |||
Sbjct: 263  ccaaagttatttgtcaggtcaaactccagcagcagatcttggtgctgcctcctcgactgat 204

Query: 674  ggacaggcgtcggc cgtgcatgacgcttagagtcgtccgagtcgatattgttcgagcaa 733
      |||
Sbjct: 203  ggacaggcgtcggc cgtgcatgacgcttagagtcgtccgagtcgatattgttcgagcaa 144

Query: 734  taagctgcggcagaccat tgcgagaacaattgcagctcatccaa cgtggaagtcgagaca 793
      |||
Sbjct: 143  taagctgcggcagaccat tgcgagaacaattgcagctcatccaa cgtggaagtcgagaca 84

Query: 794  ctccgcacatcaagtgg tgcgggtgccgcagcactcaggcgcagcgtgcgcgctcaana 853
      |||
Sbjct: 83  ctccgcacatcaagtgg tgcgggtgccgcagcactcaggcgcagcgtgcgcgctcaana-a 26

Query: 854  gcactcccaaacggttcagagaaacat 880
      |||
Sbjct: 25  gcact-ccaaaccg-tccagagaaacat 1
```

Figure A.13: Clone A11

Score = 1540 bits (777), Expect = 0.0  
Identities = 853/866 (98%), Gaps = 10/866 (1%)  
Strand = Plus / Minus

```
Query: 23 agtgnGCCAAAacgtccaccgttgcttcgcctgcattccccgcgcggaattgattccct 82
      ||| |
Sbjct: 856 agtgagccAAAacgtccaccgttgcttcgcctgcattccccgcgcggaattgattccct 797

Query: 83 cgatgagttcaataccgaacgcctgacactggctccgggtgccactggtgatccagatt 142
      ||| |
Sbjct: 796 cgatgagttcaataccgaacgcctgacactggctccgggtgccactggtgatccagatt 737

Query: 143 ctggacttggctggctgaatcCAAAGTccatgggtggcaacCGGGGacgatgtcgttca 202
      ||| |
Sbjct: 736 ctggacttggctggctgaatcCAAAGTccatgggtggcaacCGGGGacgatgtcgttca 677

Query: 203 agtgtgtaacgcggaagtTcgctcCAGATccctggtggatgtgcTGGCCagcgcat 262
      ||| |
Sbjct: 676 agtgtgtaacgcggaagtTcgctcCAGATccctggtggatgtgcTGGCCagcgcat 617

Query: 263 agttccgactcgaggacatccataggtgtacagttcaacgctataaCCGTCatTtcgca 322
      ||| |
Sbjct: 616 agttccgactcgaggacatccataggtgtacagttcaacgctataaCCGTCatTtcgca 557

Query: 323 agaccggttgcTcccagtgtagccaatgcgcgccCAAGGTgtgcCCGGTgaagtagaggg 382
      ||| |
Sbjct: 556 agaccggttgcTcccagtgtagccaatgcgcgccCAAGGTgtgcCCGGTgaagtagaggg 497

Query: 383 tatagcccgaatacgtgctcAtcgcggacttgatcttgetcgtcagattgtctgcagcgg 442
      ||| |
Sbjct: 496 tatagcccgaatacgtgctcAtcgcggacttgatcttgetcgtcagattgtctgcagcgg 437

Query: 443 ctcccatgcccttcCAGAATCagtgTgaaccttgcagccagtaCAGAGTcatcgttat 502
      ||| |
Sbjct: 436 ctcccatgcccttcCAGAATCagtgTgaaccttgcagccagtaCAGAGTcatcgttat 377

Query: 503 cttgcaggatgaagtcagatcagcaatccagttcttgatggtgctaCtgcctcggaaag 562
      ||| |
Sbjct: 376 cttgcaggatgaagtcagatcagcaatccagttcttgatggtgctaCtgcctcggaaag 317

Query: 563 cgaccacgagccgcTtTgtTggtTgtTgcTcgcggccagGAAAcggctgtgcctcCAAAAG 622
      ||| |
Sbjct: 316 cgaccacgagccgcTtTgtTggtTgtTgcTcgcggccagGAAAcggctgtgcctcCAAAAG 258

Query: 623 ttatttTgtcaggtCAAactcCagcagcatctTggtgctcgcctccctcgactgatggac 682
      ||| |
Sbjct: 257 tta-tTgtcaggtCAAactcCagcagcatctTggtgctcgcctccctcgactgatggac 200

Query: 683 aggcgtoggccgtgcatgtcaCGTtagagtcgtcCagtgatattgntcagcaataag 742
      ||| |
Sbjct: 199 aggcgtoggccgtgcatgtcaCGTtagagtcgtcCagtgatattgntcagcaataag 140

Query: 743 ctgcggcagaccatTgcgagaacaattTgcagctcatccaaCGTggaagtcagacactc 802
      ||| |
Sbjct: 139 ctgcggcagaccatTgcgagaacaattTgcagctcatccaaCGTggaagtcagacactc 81

Query: 803 cgccacatctaagtggTgtcggTgcccgcagcactcaGnGcagcgtgcgcCGTcaaaag 862
      ||| |
Sbjct: 80 cg-caacatc-aagtggTgtcggTg-ccgcagcactcaGcGcagcgtgcg-cCGTcaaaag 25

Query: 863 ccactcccaaaCCGTccagagaacat 888
      ||| |
Sbjct: 24 -cact-ccaaaCCGTccagagaacat 1
```

Figure A.14: Clone A12

Score = 1711 bits (863), Expect = 0.0  
Identities = 863/863 (100%)  
Strand = Plus / Minus

```
Query: 16  taccacaagtgagccaaaacgtccaccggttgcttgcctgcattcccgcgcgtogaattg 75
          |||
Sbjct: 863  taccacaagtgagccaaaacgtccaccggttgcttgcctgcattcccgcgcgtogaattg 804

Query: 76  attccctcgatgagttcaatatccgacgccgtgacactggctccgggtgccactggtgatc 135
          |||
Sbjct: 803  attccctcgatgagttcaatatccgacgccgtgacactggctccgggtgccactggtgatc 744

Query: 136  cagtattctggacttggctggctgaatccaaagtcctgggtggcaaccgggggacgatg 195
          |||
Sbjct: 743  cagtattctggacttggctggctgaatccaaagtcctgggtggcaaccgggggacgatg 684

Query: 196  tcgttcaaagtgtgtaacgcggaagttcgctcagatccctggctggtgatgtgctcggcc 255
          |||
Sbjct: 683  tcgttcaaagtgtgtaacgcggaagttcgctcagatccctggctggtgatgtgctcggcc 624

Query: 256  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgtca 315
          |||
Sbjct: 623  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgtca 564

Query: 316  ttctgcaagaccgttgctcccagtgtagccaatgcgcgcgccaaagctgtgcccgggtgaag 375
          |||
Sbjct: 563  ttctgcaagaccgttgctcccagtgtagccaatgcgcgcgccaaagctgtgcccgggtgaag 504

Query: 376  tagagggtatagcccgaatacgtgctcctcgcggacttgatcttgcctcgtcagattgtct 435
          |||
Sbjct: 503  tagagggtatagcccgaatacgtgctcctcgcggacttgatcttgcctcgtcagattgtct 444

Query: 436  gcagcggcttccatgcccttcagaatccagtggtgaaacctgcagccagtagcagaggta 495
          |||
Sbjct: 443  gcagcggcttccatgcccttcagaatccagtggtgaaacctgcagccagtagcagaggta 384

Query: 496  tcgttatcttgcaggatgaagtcgagatcagcaatccagttcttgatggtgctactgcct 555
          |||
Sbjct: 383  tcgttatcttgcaggatgaagtcgagatcagcaatccagttcttgatggtgctactgcct 324

Query: 556  cggaaaggcgaccacgagccgcttggttggtgtgtccgcggccaggaaaccggctgtgcct 615
          |||
Sbjct: 323  cggaaaggcgaccacgagccgcttggttggtgtgtccgcggccaggaaaccggctgtgcct 264

Query: 616  ccaaagttatttgtcaggtcaaactccagcagcatcttggtgctcgcctcctcgactgat 675
          |||
Sbjct: 263  ccaaagttatttgtcaggtcaaactccagcagcatcttggtgctcgcctcctcgactgat 204

Query: 676  ggacaggcgtcggcctgcatgtcaagtttagagtcgtccgagtcgatattgttcgagcaa 735
          |||
Sbjct: 203  ggacaggcgtcggcctgcatgtcaagtttagagtcgtccgagtcgatattgttcgagcaa 144

Query: 736  taagctgcggcagaccattgctgagaacaattgcaagctcctcctcctcctcctcctcct 795
          |||
Sbjct: 143  taagctgcggcagaccattgctgagaacaattgcaagctcctcctcctcctcctcctcct 84

Query: 796  ctccgcacatcaagtgggtgctgggtgccgcagcac tcaagcagcgtgctcctcctcctc 855
          |||
Sbjct: 83  ctccgcacatcaagtgggtgctgggtgccgcagcac tcaagcagcgtgctcctcctcctc 24

Query: 856  actccaaaaccgtccagagaacat 878
          |||
Sbjct: 23  actccaaaaccgtccagagaacat 1
```

Figure A.15: Clone A13

```

Score = 1509 bits (761), Expect = 0.0
Identities = 790/797 (99%), Gaps = 3/797 (0%)
Strand = Plus / Minus

Query: 22  caagtgngccaaaaacgtccacogtctgcttcgcctgcattcccgcogctogaattgatccc 81
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 858  caagtgngccaaaaacgtccacogtctgcttcgcctgcattcccgcogctogaattgatccc 799

Query: 82  ctogatgagttcaatatacgaacgcogtgacactggctccggtgcactggatccagta 141
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 798  ctogatgagttcaatatacgaacgcogtgacactggctccggtgcactggatccagta 739

Query: 142  ttctggacttgctggctgaatccaaagtcacatgggtggcaaccgggggacgatgctgt 201
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 738  ttctggacttgctggctgaatccaaagtcacatgggtggcaaccgggggacgatgctgt 679

Query: 202  caagtgtgtaacgcggaagtctcctccagatcccctggctgggtgagtgtgctcgccagcgc 261
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 678  caagtgtgtaacgcggaagtctcctccagatcccctggctgggtgagtgtgctcgccagcgc 619

Query: 262  atagtttcgactcgaggacatccataggtgtacagttcaacgcataaacctcatttcg 321
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 618  atagtttcgactcgaggacatccataggtgtacagttcaacgcataaacctcatttcg 559

Query: 322  caagaccgttgctccagtgtagcacaatgcgcgcccaagctgtgcccggtgaaagtag 381
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 558  caagaccgttgctccagtgtagcacaatgcgcgcccaagctgtgcccggtgaaagtag 499

Query: 382  ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagattgctcgcgc 441
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 498  ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagattgctcgcgc 439

Query: 442  ggcttcccattgcttccagaaaccagtgatgaaccctgcagccagctacagaggctcatt 501
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 438  ggcttcccattgcttccagaaaccagtgatgaaccctgcagccagctacagaggctcatt 379

Query: 502  atcttgcaaggatgaagtcgagatcagcaatecagttcttgatgggtgctactgcccgaa 561
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 378  atcttgcaaggatgaagtcgagatcagcaatecagttcttgatgggtgctactgcccgaa 319

Query: 562  ggcgaccacgagccgcttggtgtgtgccggggccaggaaccggctgtgctcccaaa 621
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 318  ggcgaccacgagccgcttggtgtgtgccggggccaggaaccggctgtgctcccaaa 259

Query: 622  gttattgtcaggtcaaacctcagcagcagccttggtgctcgcctcctcagactgatggaca 681
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 258  gttattgtcaggtcaaacctcagcagcagccttggtgctcgcctcctcagactgatggaca 199

Query: 682  ggtcgtcgccgctgcatgtcactgtagatgctcagatcgatattgttcgagcaataag 741
        ||  |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 198  gg-cgtcgccgctgcatgtcactgtagatgctcagatcgatattgttcgagcaataag 140

Query: 742  ctgcggcagaccattgcgagaacaattgcagctcaccnaoctgggaagtgcagcact 801
        |||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||  |||||||
Sbjct: 139  ctgcggcagaccattgcgagaacaattgcagctcaccnaoctgggaagtgcagcact 82

Query: 802  ccgcacatcaagnggtg 818
        |||||  ||||
Sbjct: 81  ccgcacatcaagtgtg 65

```

Figure A.16: Clone A14

Score = 1703 bits (859), Expect = 0.0  
Identities = 861/862 (99%)  
Strand = Plus / Minus

```
Query: 18  accacaagtgnccaaaaagtcacccgttgettcgctgcattcccgccgtogaattga 77
      |||
Sbjct: 862  accacaagtgaacaaaaagtcacccgttgettcgctgcattcccgccgtogaattga 803

Query: 78  ttccctcgatgagttcaatatccgaagccgtgacactggctccgggtgccactggtgatcc 137
      |||
Sbjct: 802  ttccctcgatgagttcaatatccgaagccgtgacactggctccgggtgccactggtgatcc 743

Query: 138  agtattctggaacttggtggc tgaatc caaagtc catgggtggcaaccgggggacgagt 197
      |||
Sbjct: 742  agtattctggaacttggtggc tgaatc caaagtc catgggtggcaaccgggggacgagt 683

Query: 198  cgttcaagtgtgtaacgcggaagttcgctccagatccctggctggtgatgtgctcggcca 257
      |||
Sbjct: 682  cgttcaagtgtgtaacgcggaagttcgctccagatccctggctggtgatgtgctcggcca 623

Query: 258  ggcgatagttccgactcaggacatccataggtgtacagttcaacgctataaacgctcat 317
      |||
Sbjct: 622  ggcgatagttccgactcaggacatccataggtgtacagttcaacgctataaacgctcat 563

Query: 318  ttgcgaagaccggtgctccagtgtagccaatgcccggccagctgtgcccgggtgaagt 377
      |||
Sbjct: 562  ttgcgaagaccggtgctccagtgtagccaatgcccggccagctgtgcccgggtgaagt 503

Query: 378  agagggtatagcccgaatacgtgctcactcgcggaacttgatcttgcctgctcagatgtctg 437
      |||
Sbjct: 502  agagggtatagcccgaatacgtgctcactcgcggaacttgatcttgcctgctcagatgtctg 443

Query: 438  cagcggcttcccatgcttccagaatccagtgtaaccttgccagccagta cagaggctcat 497
      |||
Sbjct: 442  cagcggcttcccatgcttccagaatccagtgtaaccttgccagccagta cagaggctcat 383

Query: 498  cgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgctc 557
      |||
Sbjct: 382  cgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgctc 323

Query: 558  ggaaaggcgaccacagaccgcttgttggtgtgtgcgcggccaggaaaacggctgtgctc 617
      |||
Sbjct: 322  ggaaaggcgaccacagaccgcttgttggtgtgtgcgcggccaggaaaacggctgtgctc 263

Query: 618  caaagtattttgacaggtcaaacccagcagcatcttggtgctcgcctcccgactgatg 677
      |||
Sbjct: 262  caaagtattttgacaggtcaaacccagcagcatcttggtgctcgcctcccgactgatg 203

Query: 678  gacaggcgtcgccgctgcatgtcaagcttagagtgctccagtgatgataattgttagcaat 737
      |||
Sbjct: 202  gacaggcgtcgccgctgcatgtcaagcttagagtgctccagtgatgataattgttagcaat 143

Query: 738  aagctgcggcagaccattgcgagaacaattgcagctcatccaacgtggaa gtcgagacac 797
      |||
Sbjct: 142  aagctgcggcagaccattgcgagaacaattgcagctcatccaacgtggaa gtcgagacac 83

Query: 798  tccgcacatcaagtgggtgctgggtgccgcagcactcagcgcagcgtgcccgtcaaaa gca 857
      |||
Sbjct: 82  tccgcacatcaagtgggtgctgggtgccgcagcactcagcgcagcgtgcccgtcaaaa gca 23

Query: 858  ctccaaaccgtccagagaacat 879
      |||
Sbjct: 22  ctccaaaccgtccagagaacat 1
```

Figure A.17: Clone A16

```

Score = 1699 bits (857), Expect = 0.0
Identities = 861/863 (99%)
Strand = Plus / Minus

Query: 16  tacnacaagtgngccaaaaagtcaccggttgettcgcctgcattcccgcgcgtcgaattg 75
      ||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 863  taccacaagtgagccaaaaagtcaccggttgettcgcctgcattcccgcgcgtcgaattg 804

Query: 76  attccctcgatgagttcaataccgacgcgcgtgacactggctccgggtgccactggtgatc 135
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 803  attccctcgatgagttcaataccgacgcgcgtgacactggctccgggtgccactggtgatc 744

Query: 136  cagtattctggacttggctggctgaatccaaagtcacatgggtggcaaccgggggaacgatg 195
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 743  cagtattctggacttggctggctgaatccaaagtcacatgggtggcaaccgggggaacgatg 684

Query: 196  tcgttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgggc 255
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 683  tcgttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgggc 624

Query: 256  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgtca 315
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 623  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgtca 564

Query: 316  ttctgcaagaccgttgctcccagtgtagcaatgcgcgcgcccaagctgtgccgggtgaag 375
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 563  ttctgcaagaccgttgctcccagtgtagcaatgcgcgcgcccaagctgtgccgggtgaag 504

Query: 376  tagagggtatagcccgaatacgtgctcctcgcggacttgatcttgcctcgtcagattgtct 435
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 503  tagagggtatagcccgaatacgtgctcctcgcggacttgatcttgcctcgtcagattgtct 444

Query: 436  gcagcggcttccatgcccttcagaatccagtgtgaaacctgcagccagtcagagggtca 495
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 443  gcagcggcttccatgcccttcagaatccagtgtgaaacctgcagccagtcagagggtca 384

Query: 496  tcgttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgcct 555
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 383  tcgttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgcct 324

Query: 556  cggaaaggcgaaccacgagccgcttggttggtgttgtccgcggccaggaaaccggctgtgct 615
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 323  cggaaaggcgaaccacgagccgcttggttggtgttgtccgcggccaggaaaccggctgtgct 264

Query: 616  ccaaagttatttgtcaggtcaaacccagcagcaatcttggtgctcgcctcctcagactgat 675
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 263  ccaaagttatttgtcaggtcaaacccagcagcaatcttggtgctcgcctcctcagactgat 204

Query: 676  ggacaggcgtcggcctgcatgtcacgtagagtctcagagtcgatattgttcagagcaa 735
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 203  ggacaggcgtcggcctgcatgtcacgtagagtctcagagtcgatattgttcagagcaa 144

Query: 736  taagctgcggcagaccattgcgagaacaattgcagctcatccaaagtcggaagtcgagaca 795
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 143  taagctgcggcagaccattgcgagaacaattgcagctcatccaaagtcggaagtcgagaca 84

Query: 796  ctcgcacatcaagtgggtgctgggtgccgcagcacccagcgcagcgtgctgcgcgtcaaaagc 855
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 83  ctcgcacatcaagtgggtgctgggtgccgcagcacccagcgcagcgtgctgcgcgtcaaaagc 24

Query: 856  actcctcaaacggtccagagaacat 878
      ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||
Sbjct: 23  actcctcaaacggtccagagaacat 1

```

**Figure A.18:** Clone A17

```

Score = 1697 bits (856), Expect = 0.0
Identities = 858/859 (99%)
Strand = Plus / Minus

Query: 18   acaagtgngccaaaacgtccaccgttgettcgocctgcattcccgccgctcgaattgatc 77
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 859   acaagtgagccaaaacgtccaccgttgettcgocctgcattcccgccgctcgaattgatc 800

Query: 78   cctcgatgagttcaatatccgacgccgtgacactggctccggtgccactggtgatccagt 137
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 759   cctcgatgagttcaatatccgacgccgtgacactggctccggtgccactggtgatccagt 740

Query: 138  attctggaacttggctggctgaatccaaagtcacatgggtggcaaccgggggacgagtgtcgt 197
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 739  attctggaacttggctggctgaatccaaagtcacatgggtggcaaccgggggacgagtgtcgt 680

Query: 198  tcaagtgtgtaacgcggaagtctgctccagatcccctggctggtgatgtctcggccagcg 257
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 679  tcaagtgtgtaacgcggaagtctgctccagatcccctggctggtgatgtctcggccagcg 620

Query: 258  catagttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcatttc 317
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 619  catagttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcatttc 560

Query: 318  gcaagaccgttgcctccagtgtagccaatgcccgcgccaagctgtgcccgggtgaagtaga 377
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 559  gcaagaccgttgcctccagtgtagccaatgcccgcgccaagctgtgcccgggtgaagtaga 500

Query: 378  gggatagcccgaatacgtgctcactcgggacttgatcttctcgcagatctgtcagattgtctgcag 437
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 499  gggatagcccgaatacgtgctcactcgggacttgatcttctcgcagatctgtcagattgtctgcag 440

Query: 438  cggcttcccatgccctccagaatccagtgtaaccttgcagccagtaacagaggtcatcgt 497
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 439  cggcttcccatgccctccagaatccagtgtaaccttgcagccagtaacagaggtcatcgt 380

Query: 498  tacccttgaggatgaagtgcagatcagcaatccagttcttgatggtgctactgctcggg 557
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 379  tacccttgaggatgaagtgcagatcagcaatccagttcttgatggtgctactgctcggg 320

Query: 558  aggcgaccacgagccgcttgttgggtgtgtcgcggcaggaaccgggtgtgctcctcaa 617
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 319  aggcgaccacgagccgcttgttgggtgtgtcgcggcaggaaccgggtgtgctcctcaa 260

Query: 618  agttatttgtcagggtcaaaactccagcagcatcttggtgctcgcctccctcgactgatggac 677
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 259  agttatttgtcagggtcaaaactccagcagcatcttggtgctcgcctccctcgactgatggac 200

Query: 678  aggcgtcgccgtgcatgtcaacttagagtcgctcagagtcgataattgttcgagcaataag 737
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 159  aggcgtcgccgtgcatgtcaacttagagtcgctcagagtcgataattgttcgagcaataag 140

Query: 738  ctgcggcagaccatgctgagaacaattgcagctcctcccaacgtggaagtcgagacactcc 797
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 139  ctgcggcagaccatgctgagaacaattgcagctcctcccaacgtggaagtcgagacactcc 80

Query: 798  gcacatcaagtgggtgctgggtgcccgcagcactcagcgcagcgtgcccgtcaaaagcactc 857
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 79   gcacatcaagtgggtgctgggtgcccgcagcactcagcgcagcgtgcccgtcaaaagcactc 20

Query: 858  caaaccgctccagagaacat 876
          |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct: 19   caaaccgctccagagaacat 1

```

**Figure A.19: Clone A18**





Score = 1677 bits (846), Expect = 0.0  
 Identities = 855/857 (99%), Gaps = 1/857 (0%)  
 Strand = Plus / Minus

```

Query: 20  caagtgagccaaaaagtcacacgttgccttgcctgcattcccgccgctogaattgatcc 79
          |||
Sbjct: 858  caagtgagccaaaaagtcacacgttgccttgcctgcattcccgccgctogaattgatcc 799

Query: 80  ctcgatgagttcaatatacgcgcgctgacactggctccgggtgcacatggatccaagta 139
          |||
Sbjct: 798  ctcgatgagttcaatatacgcgcgctgacactggctccgggtgcacatggatccaagta 739

Query: 140  ttctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgctgtt 199
          |||
Sbjct: 738  ttctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgctgtt 679

Query: 200  caagtgtgtaacgcggaaattcgtccagatccctggctgggtgatgtgctcgccagcgc 259
          |||
Sbjct: 678  caagtgtgtaacgcggaaattcgtccagatccctggctgggtgatgtgctcgccagcgc 619

Query: 260  atagtttcgcactcgaggacatccatagggtgtacagtccaacgcataaacgctcatttcg 319
          |||
Sbjct: 618  atagtttcgcactcgaggacatccatagggtgtacagtccaacgcataaacgctcatttcg 559

Query: 320  caagaaccgttgcctccagtgtagc caatgcccggcccaagctgtgcccggtgaagtagag 379
          |||
Sbjct: 558  caagaaccgttgcctccagtgtagc caatgcccggcccaagctgtgcccggtgaagtagag 499

Query: 380  ggtagtagccgaatacgtgctcatcgcggacttgatcttgctcgtcagattgctcgcagc 439
          |||
Sbjct: 498  ggtagtagccgaatacgtgctcatcgcggacttgatcttgctcgtcagattgctcgcagc 439

Query: 440  ggcttcccagcctccagaaaccagtgtaaccttgccagccagtagagaggtcaccgtt 499
          |||
Sbjct: 438  ggcttcccagcctccagaaaccagtgtaaccttgccagccagtagagaggtcaccgtt 379

Query: 500  atcttgcaaggatgaagtcagatcagcaatccagttcttgatgggtgctac tgcctcgaa 559
          |||
Sbjct: 378  atcttgcaaggatgaagtcagatcagcaatccagttcttgatgggtgctac tgcctcgaa 319

Query: 560  ggccaccaagcgcctgtgtggtgttgcgcggccaggaaaccggctgtgctccaaa 619
          |||
Sbjct: 318  ggccaccaagcgcctgtgtggtgttgcgcggccaggaaaccggctgtgctccaaa 259

Query: 620  gttatttgcaggtcaaacctcagcagcattcttggtgctcgcctcctcagactgaggaca 679
          |||
Sbjct: 258  gttatttgcaggtcaaacctcagcagcattcttggtgctcgcctcctcagactgaggaca 199

Query: 680  ggcgtggccgctgcatgtcaogttagatgctccgagtgatatttgtcgagcaataagc 739
          |||
Sbjct: 198  ggcgtggccgctgcatgtcaogttagatgctccgagtgatatttgtcgagcaataagc 139

Query: 740  tgcggcagaccattgcgagaaacaattgcagcctcctccaaagctgnaagtcagacactccg 799
          |||
Sbjct: 138  tgcggcagaccattgcgagaaacaattgcagcctcctccaaagctgnaagtcagacactccg 79

Query: 800  cacatcaagtggtgctgggtgcgcagcactcagcgcagcgtgcgcgctcaaaagcactcc 859
          |||
Sbjct: 78  cacatcaagtggtgctgggtgcgcagcactcagcgcagcgtgcgcgctcaaaagcactcc 19

Query: 860  aaaaacgtccagagaac 876
          |||
Sbjct: 18  -aaaacgtccagagaac 3
  
```

Figure A.21: Clone A20

Score = 1715 bits (865), Expect = 0.0  
Identities = 865/865 (100%)  
Strand = Plus / Minus

```
Query: 13 agtaccacaagtga gccaacacgtccaacgttgccttcgctgcaattccccgocgtcgaat 72
      |||
Sbjct: 865 agtaccacaagtga gccaacacgtccaacgttgccttcgctgcaattccccgocgtcgaat 806

Query: 73 tgattccctcgatgagttcaatatccgaagcgcgtgacactggctccgggtgccactgggtga 132
      |||
Sbjct: 805 tgattccctcgatgagttcaatatccgaagcgcgtgacactggctccgggtgccactgggtga 746

Query: 133 tccagtatcttggaacttggtggctgaaatccaaagtcacatgggtggcaaccgggggacga 192
      |||
Sbjct: 745 tccagtatcttggaacttggtggctgaaatccaaagtcacatgggtggcaaccgggggacga 686

Query: 193 tgcgttcaagtgtgtaacgcggaagtgcctccagatccccggctgggtgatgtgctcgg 252
      |||
Sbjct: 685 tgcgttcaagtgtgtaacgcggaagtgcctccagatccccggctgggtgatgtgctcgg 626

Query: 253 ccagcgcataagtttccgactcaggacatccataggtgtacagttcaacgctataacgct 312
      |||
Sbjct: 625 ccagcgcataagtttccgactcaggacatccataggtgtacagttcaacgctataacgct 566

Query: 313 catttcgcaagaccgttgctccagtgtagc caatgcgcgcccgaagctgtgcccgggtga 372
      |||
Sbjct: 565 catttcgcaagaccgttgctccagtgtagc caatgcgcgcccgaagctgtgcccgggtga 506

Query: 373 agtagagggtatagcccgaatacgtgctcatcgcggacttgatcttgcctgctcagattgt 432
      |||
Sbjct: 505 agtagagggtatagcccgaatacgtgctcatcgcggacttgatcttgcctgctcagattgt 446

Query: 433 ctgcagcggcttcccatgcttccagaatccagtgtaaccttgacagccagtcacagaggt 492
      |||
Sbjct: 445 ctgcagcggcttcccatgcttccagaatccagtgtaaccttgacagccagtcacagaggt 386

Query: 493 catcgttatcttgcaggatgaaagtcagatcagcaatccagttcttgatggtgctactgc 552
      |||
Sbjct: 385 catcgttatcttgcaggatgaaagtcagatcagcaatccagttcttgatggtgctactgc 326

Query: 553 ctcggaaggcgaccacgagccgcttgttggtgttgcctcgccaggaacccggctgtgc 612
      |||
Sbjct: 325 ctcggaaggcgaccacgagccgcttgttggtgttgcctcgccaggaacccggctgtgc 266

Query: 613 ctccaaagttatcttgcaggatcaaaactccagcagcatcttggtgctcgcctcctcgaactg 672
      |||
Sbjct: 265 ctccaaagttatcttgcaggatcaaaactccagcagcatcttggtgctcgcctcctcgaactg 206

Query: 673 atggacaggcgtcgccgctgcatgtcaagttagagtcgtccgagtcgatattgttcgagc 732
      |||
Sbjct: 205 atggacaggcgtcgccgctgcatgtcaagttagagtcgtccgagtcgatattgttcgagc 146

Query: 733 aataagctgcggcagaccattgcgagaacaattgcagctcaaccaacgtggaagtcgaga 792
      |||
Sbjct: 145 aataagctgcggcagaccattgcgagaacaattgcagctcaaccaacgtggaagtcgaga 86

Query: 793 cactccgcacatcaagtggtgctcgggtgcgcagcactcagcgcagcgtgcgcctcctcaaaa 852
      |||
Sbjct: 85 cactccgcacatcaagtggtgctcgggtgcgcagcactcagcgcagcgtgcgcctcctcaaaa 26

Query: 853 gcactccaaacgctccagagaacat 877
      |||
Sbjct: 25 gcactccaaacgctccagagaacat 1
```

Figure A.22: Clone A21

Score = 1695 bits (855), Expect = 0.0  
Identities = 860/862 (99%)  
Strand = Plus / Minus

```
Query: 18 accacaagtgnccaaaaagtcacacgtttgcttcgccatgcccccggcgtcgattga 77
      |||
Sbjct: 862 accacaagtga gcaaaaaagtcacacgtttgcttcgccatgcccccggcgtcgattga 803

Query: 78 ttccctcgatgagttcaatatccgacgcggtgacactggctccggtgccactggtgatcc 137
      |||
Sbjct: 802 ttccctcgatgagttcaatatccgacgcggtgacactggctccggtgccactggtgatcc 743

Query: 138 agtattctggacttggtggctgaaacaaaagtcacatgggtggcaaccgggggacgatgt 197
      |||
Sbjct: 742 agtattctggacttggtggctgaaacaaaagtcacatgggtggcaaccgggggacgatgt 683

Query: 198 cgttcaagtgtgtaacgcggaagtccgctccagatcccctggctggtgatgtgctcggcca 257
      |||
Sbjct: 682 cgttcaagtgtgtaacgcggaagtccgctccagatcccctggctggtgatgtgctcggcca 623

Query: 258 gcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcat 317
      |||
Sbjct: 622 gcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcat 563

Query: 318 ttcgcaagaccgttgcttccagtgtagccaatgcgcccccaggctgtgcccggatgaagt 377
      |||
Sbjct: 562 ttcgcaagaccgttgcttccagtgtagccaatgcgcccccaggctgtgcccggatgaagt 503

Query: 378 agagggtatagcccgaatacgtgctcactcgcggacttgatcttgctcgtcagattgtctg 437
      |||
Sbjct: 502 agagggtatagcccgaatacgtgctcactcgcggacttgatcttgctcgtcagattgtctg 443

Query: 438 cagcggcttcacatgcttccagaatccagtgtaaccttgagcagcaagtcagaggtcat 497
      |||
Sbjct: 442 cagcggcttcacatgcttccagaatccagtgtaaccttgagcagcaagtcagaggtcat 383

Query: 498 cgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgctc 557
      |||
Sbjct: 382 cgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgctc 323

Query: 558 ggaaggcgaccacgagccgcttgttgggtgtgctcgcggccaggaaaccggctgtgctc 617
      |||
Sbjct: 322 ggaaggcgaccacgagccgcttgttgggtgtgctcgcggccaggaaaccggctgtgctc 263

Query: 618 caaagtatttgcagggtcaaaactccagcagcatcttgggtgctcgcctcccgactgatg 677
      |||
Sbjct: 262 caaagtatttgcagggtcaaaactccagcagcatcttgggtgctcgcctcccgactgatg 203

Query: 678 gacagggctcgccgtgcatgtcaagcttagagtcgtccagtgatattgttcgagcaat 737
      |||
Sbjct: 202 gacagggctcgccgtgcatgtcaagcttagagtcgtccagtgatattgttcgagcaat 143

Query: 738 aagctgoggcgaccattgcgagaacaattgcagctcattcaacgtggaagtcgagacac 797
      |||
Sbjct: 142 aagctgoggcgaccattgcgagaacaattgcagctcattcaacgtggaagtcgagacac 83

Query: 798 tcggcacatcaagtggtgtcggtgcggcagcactcagcgcaaggtgcgccgtcaaaaagca 857
      |||
Sbjct: 82 tcggcacatcaagtggtgtcggtgcggcagcactcagcgcaaggtgcgccgtcaaaaagca 23

Query: 858 ctccaaaccgtccagagaacat 879
      |||
Sbjct: 22 ctccaaaccgtccagagaacat 1
```

Figure A.23: Clone A22

Score = 1677 bits (846), Expect = 0.0  
Identities = 854/858 (99%)  
Strand = Plus / Minus

```
Query: 18 caagtgngccaaaaacgtcacacggtgcttgcctgcattcccgcgctogaattgatcc 77
      ||||| |
Sbjct: 858 caagtgngccaaaaacgtcacacggtgcttgcctgcattcccgcgctogaattgatcc 799

Query: 78 ctogatgagttcaatatacgaacgcgctgacactggctccgggtgcacatggatccagta 137
      ||||| |
Sbjct: 798 ctogatgagttcaatatacgaacgcgctgacactggctccgggtgcacatggatccagta 739

Query: 138 ttctggacttggtggctgaatccaaagtcacatgggtggcaaccgggggacgatgctgtt 197
      ||||| |
Sbjct: 738 ttctggacttggtggctgaatccaaagtcacatgggtggcaaccgggggacgatgctgtt 679

Query: 198 caagtgtgtaacgcggagttcgcctccagatcccggctggatgtgtcctggccagcgc 257
      ||||| |
Sbjct: 678 caagtgtgtaacgcggagttcgcctccagatcccggctggatgtgtcctggccagcgc 619

Query: 258 atagtttcgcactcaggacatccataggtgtacagttcaacgcctataaccgctcatttcg 317
      ||||| |
Sbjct: 618 atagtttcgcactcaggacatccataggtgtacagttcaacgcctataaccgctcatttcg 559

Query: 318 caagaccgttgctccagtgtagc caatgcgcgcgcccaagctgtgcccggtgaaagtaag 377
      ||||| |
Sbjct: 558 caagaccgttgctccagtgtagc caatgcgcgcgcccaagctgtgcccggtgaaagtaag 499

Query: 378 ggtatagccgaatacgtgctcatcgcggacttgatcttgcctgctcagatgtctgcagc 437
      ||||| |
Sbjct: 498 ggtatagccgaatacgtgctcatcgcggacttgatcttgcctgctcagatgtctgcagc 439

Query: 438 ggttcccatgctctccagaatccagtgtaacccttgccagccagtacagaggctcctcgtt 497
      ||||| |
Sbjct: 438 ggttcccatgctctccagaatccagtgtaacccttgccagccagtacagaggctcctcgtt 379

Query: 498 atcttgcaggatgaagtcgagatcagcaatccagttcttgatgggtgctactgctcggaa 557
      ||||| |
Sbjct: 378 atcttgcaggatgaagtcgagatcagcaatccagttcttgatgggtgctactgctcggaa 319

Query: 558 ggcgaccacgagccgcttggtggtgtccgcccaggaaccggctgtgctccaaa 617
      ||||| |
Sbjct: 318 ggcgaccacgagccgcttggtggtgtccgcccaggaaccggctgtgctccaaa 259

Query: 618 gttatgttcagggtcaaacctcagcagcctcttgggtgctcgcctcctcgaactgatggaca 677
      ||||| |
Sbjct: 258 gttatgttcagggtcaaacctcagcagcctcttgggtgctcgcctcctcgaactgatggaca 199

Query: 678 gggtcggcggctgcatgtcacgttagagtgctccgagtcgatattgttcgagcaataagc 737
      ||||| |
Sbjct: 198 gggtcggcggctgcatgtcacgttagagtgctccgagtcgatattgttcgagcaataagc 139

Query: 738 tgcggcagaccattgcgagaacaattgcagctcatccaacgtggaagtcgagacactccg 797
      ||||| |
Sbjct: 138 tgcggcagaccattgcgagaacaattgcagctcatccaacgtggaagtcgagacactccg 79

Query: 798 cacatcaagtggtgctgggtgcgcagcactcagcgcannngcgcgctcaaaagcactcc 857
      ||||| |
Sbjct: 78 cacatcaagtggtgctgggtgcgcagcactcagcgcagcgtgcgcgctcaaaagcactcc 19

Query: 858 aaaccgtccagagaacat 875
      ||||| |
Sbjct: 18 aaaccgtccagagaacat 1
```

Figure A.24: Clone A23

Score = 1705 bits (860), Expect = 0.0  
Identities = 862/863 (99%)  
Strand = Plus / Minus

```
Query: 15  taccacaagtgnccaaaacgtccaccgttgcttcgctgcattcccgcgcgtcgaattg 74
          |||
Sbjct: 863  taccacaagtgagccaaaacgtccaccgttgcttcgctgcattcccgcgcgtcgaattg 804

Query: 75  attccctc gatgagttcaata tccgacgccgtgacac tggctccgggtgccactggtgatc 134
          |||
Sbjct: 803  attccctc gatgagttcaata tccgacgccgtgacac tggctccgggtgccactggtgatc 744

Query: 135  cagtattc tggacttggctggctgaat ccaaagtcca tgggtggcaaccgggggacgatg 194
          |||
Sbjct: 743  cagtattc tggacttggctggctgaat ccaaagtcca tgggtggcaaccgggggacgatg 684

Query: 195  tcgttcaa gtgtgtaacgcggaagt ttcgctcagatcc cctggctggtgatgtgc tggcc 254
          |||
Sbjct: 683  tcgttcaa gtgtgtaacgcggaagt ttcgctcagatcc cctggctggtgatgtgc tggcc 624

Query: 255  agcgcata gtttccgactcgaggacat ccataggtgtac agttcaacgctataa ccgtca 314
          |||
Sbjct: 623  agcgcata gtttccgactcgaggacat ccataggtgtac agttcaacgctataa ccgtca 564

Query: 315  tttcgcaa gaccgttgctcccagtgta gccaatgcgc cggcccaagctgtgcccgggtgaag 374
          |||
Sbjct: 563  tttcgcaa gaccgttgctcccagtgta gccaatgcgc cggcccaagctgtgcccgggtgaag 504

Query: 375  tagagggt atagcccgaatacgtgctc atcgcggaactt gatettgetcgtcagattgtct 434
          |||
Sbjct: 503  tagagggt atagcccgaatacgtgctc atcgcggaactt gatettgetcgtcagattgtct 444

Query: 435  gcagcggc tcccca tgccttc cagaatccagtg tgaaccttgcagccagtagcagaggtca 494
          |||
Sbjct: 443  gcagcggc tcccca tgccttc cagaatccagtg tgaaccttgcagccagtagcagaggtca 384

Query: 495  tcgttate ttgcaggatgaagtogaga tcagcaatcc agttcttgatggtgctactgcct 554
          |||
Sbjct: 383  tcgttate ttgcaggatgaagtogaga tcagcaatcc agttcttgatggtgctactgcct 324

Query: 555  cggaaagg caccac gagcgcctt gttggtgtgtg tccgcggccaggaaccggctgtgcct 614
          |||
Sbjct: 323  cggaaagg caccac gagcgcctt gttggtgtgtg tccgcggccaggaaccggctgtgcct 264

Query: 615  ccaaagtt atttgtcagg tcaaac tccagcagca tcttggtgctcgcctcctcgactgat 674
          |||
Sbjct: 263  ccaaagtt atttgtcagg tcaaac tccagcagca tcttggtgctcgcctcctcgactgat 204

Query: 675  ggacaggc gtcggc cgtgcatgtcac gtttagagt cgtccagatog atattgttcgagcaa 734
          |||
Sbjct: 203  ggacaggc gtcggc cgtgcatgtcac gtttagagt cgtccagatog atattgttcgagcaa 144

Query: 735  taagctgc ggcagacc at tgcgagaacaatt gcagct catc caacgtggaagtcgagaca 794
          |||
Sbjct: 143  taagctgc ggcagacc at tgcgagaacaatt gcagct catc caacgtggaagtcgagaca 84

Query: 795  ctccgcac atcaagt ggtgtc ggtgcc gcagcactc a gcgcagcgtgcgc cgtcaaaaagc 854
          |||
Sbjct: 83  ctccgcac atcaagt ggtgtc ggtgcc gcagcactc a gcgcagcgtgcgc cgtcaaaaagc 24

Query: 855  actc caaac cgtccagagaacat 877
          |||
Sbjct: 23  actc caaac cgtccagagaacat 1
```

Figure A.25: Clone A25

Score = 1701 bits (858), Expect = 0.0  
Identities = 858/858 (100%)  
Strand = Plus / Minus

```

Query: 19  caagtgagccaaaacgtccacogttgcttgcctgcattcccgcogtogaattgatcc 78
      |||
Sbjct: 858 caagtgagccaaaacgtccacogttgcttgcctgcattcccgcogtogaattgatcc 799

Query: 79  ctogatgagttcaatatacgaagcogtgacactggctccggtgcccactgggtgatccagta 138
      |||
Sbjct: 798 ctogatgagttcaatatacgaagcogtgacactggctccggtgcccactgggtgatccagta 739

Query: 139  ttctggacttggtggctgaaatccaaagtccatgggtggcaaccgggggacgatgctgtt 198
      |||
Sbjct: 738 ttctggacttggtggctgaaatccaaagtccatgggtggcaaccgggggacgatgctgtt 679

Query: 199  caagtgtgtaacgcggaaagttcgtccagatcccggctgggtgatgtgctcggccagcgc 258
      |||
Sbjct: 678 caagtgtgtaacgcggaaagttcgtccagatcccggctgggtgatgtgctcggccagcgc 619

Query: 259  atagtttcgactcgaggacatccataggtgtacagttcaacgcataaacogtcatctcg 318
      |||
Sbjct: 618 atagtttcgactcgaggacatccataggtgtacagttcaacgcataaacogtcatctcg 559

Query: 319  caagaccggttgctcccagtgtagc caatgcgcgcgccaaagctgtgcccggtgaaagtagag 378
      |||
Sbjct: 558 caagaccggttgctcccagtgtagc caatgcgcgcgccaaagctgtgcccggtgaaagtagag 499

Query: 379  ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagatgtctcgcagc 438
      |||
Sbjct: 498 ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagatgtctcgcagc 439

Query: 439  ggcttccatgccttccagaaatccagtggaaccttgccagcagctacagaggctcatggt 498
      |||
Sbjct: 438 ggcttccatgccttccagaaatccagtggaaccttgccagcagctacagaggctcatggt 379

Query: 499  atcttgccaggtgaaagtcgagatcagcaatccagttcttgatgggtgctactgcccggaa 558
      |||
Sbjct: 378 atcttgccaggtgaaagtcgagatcagcaatccagttcttgatgggtgctactgcccggaa 319

Query: 559  ggcgaccaagagccgcttgttggtgtgtccgcggccaggaaacggctgtgctccaaa 618
      |||
Sbjct: 318 ggcgaccaagagccgcttgttggtgtgtccgcggccaggaaacggctgtgctccaaa 259

Query: 619  gttatttgcaggtcaaacctcagcagcatcttggtgctgcctcctcgactgatggaca 678
      |||
Sbjct: 258 gttatttgcaggtcaaacctcagcagcatcttggtgctgcctcctcgactgatggaca 199

Query: 679  ggctgagccogtgeatgtcacgttagagtgctccagtgatgatattgttcgagcaataagc 738
      |||
Sbjct: 198 ggctgagccogtgeatgtcacgttagagtgctccagtgatgatattgttcgagcaataagc 139

Query: 739  tgcggcagaccattgcgagaa caattgcagctcaatccaaagtggaagtcgagacactccg 798
      |||
Sbjct: 138 tgcggcagaccattgcgagaa caattgcagctcaatccaaagtggaagtcgagacactccg 79

Query: 799  cacatcaagtggtgctgggtgcgcagcactcagcgcagcgtgcgcccgtcaaaagcactcc 858
      |||
Sbjct: 78  cacatcaagtggtgctgggtgcgcagcactcagcgcagcgtgcgcccgtcaaaagcactcc 19

Query: 859  aaaccgtccagagaacat 876
      |||
Sbjct: 18  aaaccgtccagagaacat 1

```

Figure A.26: Clone A26

Score = 1699 bits (857), Expect = 0.0  
Identities = 859/860 (99%)  
Strand = Plus / Minus

```
Query: 18  cacaagtgngccaaaaagtcaccggttgettgcctgcattcccgcgctcgaaattgatt 77
      |||
Sbjct: 860  cacaagtgagccaaaaagtcaccggttgettgcctgcattcccgcgctcgaaattgatt 801

Query: 78  cctcgcgatgagttcaataccgacgccgtgacacggctccgggtgccactggtgatccag 137
      |||
Sbjct: 800  cctcgcgatgagttcaataccgacgccgtgacacggctccgggtgccactggtgatccag 741

Query: 138  tattctggaacttggtgctgaatccaaagtcattgggtggcaaccgggggacgatgctg 197
      |||
Sbjct: 740  tattctggaacttggtgctgaatccaaagtcattgggtggcaaccgggggacgatgctg 681

Query: 198  ttcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggccagc 257
      |||
Sbjct: 680  ttcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggccagc 621

Query: 258  gcatagttccgactcagaggaacatccataggtgtacagttcaacgctataaccgctcatt 317
      |||
Sbjct: 620  gcatagttccgactcagaggaacatccataggtgtacagttcaacgctataaccgctcatt 561

Query: 318  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgccgggtagtag 377
      |||
Sbjct: 560  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgccgggtagtag 501

Query: 378  agggatatagccagaatacgtgctcatcgggaacttgatcttgcctcagattgtctgca 437
      |||
Sbjct: 500  agggatatagccagaatacgtgctcatcgggaacttgatcttgcctcagattgtctgca 441

Query: 438  gggcttccatgcttcagaaatccagtgtgaaacctgcagccagtagagaggtcatcg 497
      |||
Sbjct: 440  gggcttccatgcttcagaaatccagtgtgaaacctgcagccagtagagaggtcatcg 381

Query: 498  ttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgctcgg 557
      |||
Sbjct: 380  ttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgctcgg 321

Query: 558  aaggcgaccacgagccgcttgttgggtgttgcgcggccaggaaaccgctgtgctcca 617
      |||
Sbjct: 320  aaggcgaccacgagccgcttgttgggtgttgcgcggccaggaaaccgctgtgctcca 261

Query: 618  aagtatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcgaactgatgga 677
      |||
Sbjct: 260  aagtatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcgaactgatgga 201

Query: 678  caggcgctggcctgcatgtcagcttagagtcgtccgagtcgatattgttcgagcaataa 737
      |||
Sbjct: 200  caggcgctggcctgcatgtcagcttagagtcgtccgagtcgatattgttcgagcaataa 141

Query: 738  gctgcgccagaccattgcgagaacaattgcagctcatccaaagtggaagtcgagacactc 797
      |||
Sbjct: 140  gctgcgccagaccattgcgagaacaattgcagctcatccaaagtggaagtcgagacactc 81

Query: 798  cgcaatcaagtggtgtcgggtgccgcagcactcagcgcagcgtgctcgcctcaaaagcact 857
      |||
Sbjct: 80  cgcaatcaagtggtgtcgggtgccgcagcactcagcgcagcgtgctcgcctcaaaagcact 21

Query: 858  ccaaacctccagagaacat 877
      |||
Sbjct: 20  ccaaacctccagagaacat 1
```

Figure A.27: Clone A27

Score = 1550 bits (782), Expect = 0.0  
Identities = 817/824 (99%), Gaps = 5/824 (0%)  
Strand = Plus / Minus

```
Query: 29   aaaaacgtccacccgttgccttgcctgcattcccgccgctogaattgatccctcgatgagt 88
          |||
Sbjct: 848  aaaaacgtccacccgttgccttgcctgcattcccgccgctogaattgatccctcgatgagt 789

Query: 89   tcaatatacgaacgcctgacactggctccgggtgcactggatccagtaattctggactt 148
          |||
Sbjct: 788  tcaatatacgaacgcctgacactggctccgggtgcactggatccagtaattctggactt 729

Query: 149  ggctggctgaaatccaaagtcctgggtggcaaccgggggacgatgctggtcaagtgtgta 208
          |||
Sbjct: 728  ggctggctgaaatccaaagtcctgggtggcaaccgggggacgatgctggtcaagtgtgta 669

Query: 209  acgcggaaattcgctccagatccctggctggtagtgctcggccagcgcacatagtttccg 268
          |||
Sbjct: 668  acgcggaaattcgctccagatccctggctggtagtgctcggccagcgcacatagtttccg 609

Query: 269  actcgaggacatccataggtgtacagtccaacgcataaccgctatttcgcaagaccgtt 328
          |||
Sbjct: 608  actcgaggacatccataggtgtacagtccaacgcataaccgctatttcgcaagaccgtt 549

Query: 329  gctccagtgtagc caatgcgcgcccaagctgtgccgggtgaaatagagggtatagccc 388
          |||
Sbjct: 548  gctccagtgtagc caatgcgcgcccaagctgtgccgggtgaaatagagggtatagccc 489

Query: 389  gaatacgtgctcatcgccgacttgatcttgcctcagattgctcgcagcggctcccat 448
          |||
Sbjct: 488  gaatacgtgctcatcgccgacttgatcttgcctcagattgctcgcagcggctcccat 429

Query: 449  gccttccagaatccagtggtgaaccttgccagccagtcacagaggtcacgttatcttgcagg 508
          |||
Sbjct: 428  gccttccagaatccagtggtgaaccttgccagccagtcacagaggtcacgttatcttgcagg 369

Query: 509  atgaagtcgagatcagcaatccagtttcttgatggtgctactgcccggaggcgcacac 568
          |||
Sbjct: 368  atgaagtcgagatcagcaatccag-ttcttgatggtgctactgcccggaggcgcacac 310

Query: 569  gagccgcttgttgggtgttgcgcggccaggaaaacggctgtgcccacaaagttatttgt 628
          |||
Sbjct: 309  gagccgcttgttgggtgttgcgcggccaggaaaacggctgtgcccacaaagttatttgt 250

Query: 629  caggtcaaaactccagcagcatcttggctgctcgcctcccgactgatggacaggcgtcggc 688
          |||
Sbjct: 249  caggtcaaaactccagcagcatcttggctgctcgcctcccgactgatggacaggcgtcggc 190

Query: 689  cgtgcatgtcaacttagagtcctcagagtcgataatggttcgagcaataagctcggcaga 748
          |||
Sbjct: 189  cgtgcatgtcaacttagagtcctcagagtcgataatggttcgagcaataagctcggcaga 130

Query: 749  ccatgcgagaacaattgcagctcctc caacgtggaaatcgagacaccccgccacatca 808
          |||
Sbjct: 129  ccatgcgagaacaattgcagctcctc caacgtggaaatcgagacaccccgccacatca 72

Query: 809  agtgggtgcgggtgcggcagcactcagcgcagncgngcgccgctc 852
          |||
Sbjct: 71   agtgggtgcgggtgcggcagcactcagcgcag-cgtgcccgctc 30
```

Figure A.28: Clone A28



Score = 1683 bits (849), Expect = 0.0  
Identities = 858/860 (99%), Gaps = 1/860 (0%)  
Strand = Plus / Minus

```

Query: 22  acaagtgngccaaaacgtccaccgttgettcgectgcattccccgccgtcgaattgatc 81
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 859  acaagtgagccaaaacgtccaccgttgettcgectgcattccccgccgtcgaattgatc 800

Query: 82  cctcgatgagttcaatatccgacgcctgacactggctccgggtgccactggtgatccagt 141
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 799  cctcgatgagttcaatatccgacgcctgacactggctccgggtgccactggtgatccagt 740

Query: 142  attctggacttggtggtggaatccaaagtc catgggtggcaaccgggggacgatgtcgt 201
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 739  attctggacttggtggtggaatccaaagtc catgggtggcaaccgggggacgatgtcgt 680

Query: 202  tcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggccagcg 261
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 679  tcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggccagcg 620

Query: 262  catagttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcatc 321
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 619  catagttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcatc 560

Query: 322  gcaagaccgttgetcccagtgtagccaatgcgccgcccaagctgtgccgggtgaagtaga 381
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 559  gcaagaccgttgetcccagtgtagccaatgcgccgcccaagctgtgccgggtgaagtaga 500

Query: 382  gggatatagcccgaatacgtgctcatcgcggacttgatcttgcctcgcagattgtctgcag 441
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 499  gggatatagcccgaatacgtgctcatcgcggacttgatcttgcctcgcagattgtctgcag 440

Query: 442  cggcttcccatgcttccagaatccagtggtgaaccttgcagccagta cagaggtcatcgt 501
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 439  cggcttcccatgcttccagaatccagtggtgaaccttgcagccagta cagaggtcatcgt 380

Query: 502  tate ttgcaggatgaagt cga gatcagcaatccagttcttgatggtgctactgcctcggga 561
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 379  tate ttgcaggatgaagt cga gatcagcaatccagttcttgatggtgctactgcctcggga 320

Query: 562  aggcgaccacgagccgcttgttggtgtgttcgcggccaggaaaccggctgtgcctc 621
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 319  aggcgaccacgagccgcttgttggtgtgttcgcggccaggaaaccggctgtgcctc 260

Query: 622  agttatttgcagggtcaaaactccagcagcatcttggtgctcgcctccctcagactgatggac 681
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 259  agttatttgcagggtcaaaactccagcagcatcttggtgctcgcctccctcagactgatggac 200

Query: 682  aggcgtcggcctgcatgtcaacttagagtcgctcagagtcgataattgttcgagcaataag 741
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 199  aggcgtcggcctgcatgtcaacttagagtcgctcagagtcgataattgttcgagcaataag 140

Query: 742  ctgcggcagaccatttgcgagaacaattgcagctcctc caacgtggaaagtcgagacactcc 801
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 139  ctgcggcagaccatttgcgagaacaattgcagctcctc caacgtggaaagtcgagacactcc 80

Query: 802  gcacatcaagtgggtgctgggtgccgagcactcagcgcagcgtgcgccgttcaaaagcact 861
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 79  gcacatcaagtgggtgctgggtgccgagcactcagcgcagcgtgcgccgttcaaaagcact 21

Query: 862  ccaaaccgtccagagaacat 881
          ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| ||||||| |||||||
Sbjct: 20  ccaaaccgtccagagaacat 1

```

Figure A.29: Clone A30

Score = 1707 bits (861), Expect = 0.0  
Identities = 863/864 (99%)  
Strand = Plus / Minus

```
Query: 15  gtaccacaagtgngccaaaacgtcacacggtgcttcgcctgcatcccgcgctcgaatt 74
      |||
Sbjct: 864  gtaccacaagtgagccaaaacgtcacacggtgcttcgcctgcatcccgcgctcgaatt 805

Query: 75  gattccctcgatgagttcaatatacgaacgcctgacactggctccggtgcactggtgat 134
      |||
Sbjct: 804  gattccctcgatgagttcaatatacgaacgcctgacactggctccggtgcactggtgat 745

Query: 135  ccagtattctggacttggctggctgaaatccaaagtcacatgggtggcaaccggggacgat 194
      |||
Sbjct: 744  ccagtattctggacttggctggctgaaatccaaagtcacatgggtggcaaccggggacgat 685

Query: 195  gtogttcaagtgtgtaacgcggaaagttcgctccagatccctggctggtgagtgtctcggc 254
      |||
Sbjct: 684  gtogttcaagtgtgtaacgcggaaagttcgctccagatccctggctggtgagtgtctcggc 625

Query: 255  cagcgcatagtttcgcactcaggacataccataggtgtacagttcaacgcataaacgctc 314
      |||
Sbjct: 624  cagcgcatagtttcgcactcaggacataccataggtgtacagttcaacgcataaacgctc 565

Query: 315  atttcgcaagaccggttgcctccagtgtagccaatgcgcgcaccaagctgtgcccggtaa 374
      |||
Sbjct: 564  atttcgcaagaccggttgcctccagtgtagccaatgcgcgcaccaagctgtgcccggtaa 505

Query: 375  gtagaggtatagccgaatacgtgctcctcgcggacttgatcttgcctcagattgtc 434
      |||
Sbjct: 504  gtagaggtatagccgaatacgtgctcctcgcggacttgatcttgcctcagattgtc 445

Query: 435  tgcagcggcttccatgcttccagaaatcagtggtgaaccttgcagccagtcagaggtc 494
      |||
Sbjct: 444  tgcagcggcttccatgcttccagaaatcagtggtgaaccttgcagccagtcagaggtc 385

Query: 495  atogttatcttgcaggatgaaagtcgagatcagcaatccagttcttgatggtgctactgcc 554
      |||
Sbjct: 384  atogttatcttgcaggatgaaagtcgagatcagcaatccagttcttgatggtgctactgcc 325

Query: 555  tcggaaggcagccacgagccgcttgttggtgtgtccgcccaggaaacggcctgtgcc 614
      |||
Sbjct: 324  tcggaaggcagccacgagccgcttgttggtgtgtccgcccaggaaacggcctgtgcc 265

Query: 615  tccaaagtatttgcagggtcaaacctcagcagcactcttggtgctcgcctcctcagctga 674
      |||
Sbjct: 264  tccaaagtatttgcagggtcaaacctcagcagcactcttggtgctcgcctcctcagctga 205

Query: 675  tggacagcgcctcggcctgcatgtcacgttagagtcgctcagagtcgatattggtcagaca 734
      |||
Sbjct: 204  tggacagcgcctcggcctgcatgtcacgttagagtcgctcagagtcgatattggtcagaca 145

Query: 735  ataagctgcggcagaccattgcgagaacaattgcagctcatccaacgtggaagtcagac 794
      |||
Sbjct: 144  ataagctgcggcagaccattgcgagaacaattgcagctcatccaacgtggaagtcagac 85

Query: 795  actccgcatcaagtgggtgcggctgcgacgcaactcagcgcagcgtgcccgtcaaaag 854
      |||
Sbjct: 84  actccgcatcaagtgggtgcggctgcgacgcaactcagcgcagcgtgcccgtcaaaag 25

Query: 855  cactccaaaccgctcagagaacat 878
      |||
Sbjct: 24  cactccaaaccgctcagagaacat 1
```

Figure A.30: Clone A32



Score = 1673 bits (844), Expect = 0.0  
Identities = 855/858 (99%), Gaps = 1/858 (0%)  
Strand = Plus / Minus

```
Query: 19 ccacaagtngccanaacgtccaccggttgcctgcattcccgcgcgtogaattgat 78
      |||
Sbjct: 861 ccacaagtgagccaaaacgtccaccggttgcctgcattcccgcgcgtogaattgat 802

Query: 79 tccctcgatgagttcaatatacgcagcgcgtgacaactggctcgggtgccactgggtgatcca 138
      |||
Sbjct: 801 tccctcgatgagttcaatatacgcagcgcgtgacaactggctcgggtgccactgggtgatcca 742

Query: 139 gtattctggacttggctggctgaatccaaagtcacatgggtggcaaccgggggacgatgtc 198
      |||
Sbjct: 741 gtattctggacttggctggctgaatccaaagtcacatgggtggcaaccgggggacgatgtc 682

Query: 199 gttcaagtgtgtaacgcgggaagtgcctccagatccctggctgggtgatgtctcggccag 258
      |||
Sbjct: 681 gttcaagtgtgtaacgcgggaagtgcctccagatccctggctgggtgatgtctcggccag 622

Query: 259 cgcatagtttcgcactcgaggacatccataggtgtacagttcaacgcgtataaccgctcatt 318
      |||
Sbjct: 621 cgcatagtttcgcactcgaggacatccataggtgtacagttcaacgcgtataaccgctcatt 562

Query: 319 tcgcaagaccggttgcctccagtgtagc caatgcgcgcccaagctgtgcccggtgaagta 378
      |||
Sbjct: 561 tcgcaagaccggttgcctccagtgtagc caatgcgcgcccaagctgtgcccggtgaagta 502

Query: 379 gagggatagccgaatacgtgctcatcgggacttgatcttgcctcagattgtctgc 438
      |||
Sbjct: 501 gagggatagccgaatacgtgctcatcgggacttgatcttgcctcagattgtctgc 442

Query: 439 agcggttcccattgcctccagaaaccagtggaacctgcaagcagtagcagaggtcacc 498
      |||
Sbjct: 441 agcggttcccattgcctccagaaaccagtggaacctgcaagcagtagcagaggtcacc 382

Query: 499 gttatcttgcaggatgaaatcgcagatcagcaatccagttcttgatgggtgctactgctcg 558
      |||
Sbjct: 381 gttatcttgcaggatgaaatcgcagatcagcaatccagttcttgatgggtgctactgctcg 322

Query: 559 gaaggcgaccacgagccgcttgggtgtgtgctccgcggccaggaaaccggctgtgctcc 618
      |||
Sbjct: 321 gaaggcgaccacgagccgcttgggtgtgtgctccgcggccaggaaaccggctgtgctcc 262

Query: 619 aaagttatttgcaggtaaaatccagcagcactcttgggtgctcgcctcctcgactgatgg 678
      |||
Sbjct: 261 aaagttatttgcaggtaaaatccagcagcactcttgggtgctcgcctcctcgactgatgg 202

Query: 679 acaggcgtcggcgtgcatgtcacggttagagtgtccgagtcgatatttgttcagcaata 738
      |||
Sbjct: 201 acaggcgtcggcgtgcatgtcacggttagagtgtccgagtcgatatttgttcagcaata 142

Query: 739 agctgcggcagaccattgcgagaacaattgcagctcaatccacgtggaagtgcagacact 798
      |||
Sbjct: 141 agctgcggcagaccattgcgagaacaattgcagctcaatccacgtggaagtgcagacact 82

Query: 799 ccgcacatcaaagtgggtcgggtgcgcagcactcagcgcagcgtgcgcgtcaaaaagca 858
      |||
Sbjct: 81 ccgcacatc-aagtggtgcgggtgcgcagcactcagcgcagcgtgcgcgtcaaaaagca 23

Query: 859 ctccaaaaccgtccagaga 876
      |||
Sbjct: 22 ctccaaaaccgtccagaga 5
```

Figure A.32: Clone A34

Score = 1709 bits (862), Expect = 0.0  
Identities = 864/865 (99%)  
Strand = Plus / Minus

```
Query: 18 agtaccacaagttagccaaaaagtcacacogtgccttcgctgcaatcccccgcgctogaat 77
      |||
Sbjct: 865 agtaccacaagttagccaaaaagtcacacogtgccttcgctgcaatcccccgcgctogaat 806

Query: 78 tgattccctcgatgagttcaatatccgacgcogtgacactggctccgggtgccactggtga 137
      |||
Sbjct: 805 tgattccctcgatgagttcaatatccgacgcogtgacactggctccgggtgccactggtga 746

Query: 138 tccagtatctggacttggtggctgaaatccaaagtcacatgggtggcaacccgggggacga 197
      |||
Sbjct: 745 tccagtatctggacttggtggctgaaatccaaagtcacatgggtggcaacccgggggacga 686

Query: 198 tgtcgttcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcgg 257
      |||
Sbjct: 685 tgtcgttcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcgg 626

Query: 258 ccagcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaacgct 317
      |||
Sbjct: 625 ccagcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaacgct 566

Query: 318 catttcgcaagaccgttgcctccagtgtagc caatgcgcgcccagctgtgcccgggtga 377
      |||
Sbjct: 565 catttcgcaagaccgttgcctccagtgtagc caatgcgcgcccagctgtgcccgggtga 506

Query: 378 agtagagggatagcccgaatacgtgcctatcgcggacttgatcttgctcgtcagatgt 437
      |||
Sbjct: 505 agtagagggatagcccgaatacgtgcctatcgcggacttgatcttgctcgtcagatgt 446

Query: 438 ctgcagcggcttcccatgcctccagaatccagtgtaaccctgcagccagtacagaggt 497
      |||
Sbjct: 445 ctgcagcggcttcccatgcctccagaatccagtgtaaccctgcagccagtacagaggt 386

Query: 498 catcgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgc 557
      |||
Sbjct: 385 catcgttatcttgcaggatgaagtcagatcagcaatccagttcttgatggtgctactgc 326

Query: 558 ctccgaagcggaccacgagccgcttgttggtgttgcgcggccaggaaaccggctgtgc 617
      |||
Sbjct: 325 ctccgaagcggaccacgagccgcttgttggtgttgcgcggccaggaaaccggctgtgc 266

Query: 618 ctccaaagtattttgtcaggtcaaacctccagcagcatcttggtgctcgcctcctcagctg 677
      |||
Sbjct: 265 ctccaaagtattttgtcaggtcaaacctccagcagcatcttggtgctcgcctcctcagctg 206

Query: 678 atggacaggcgtcgccogtgcacatgcaagttagagtcgtccagtcgataattgttcgagc 737
      |||
Sbjct: 205 atggacaggcgtcgccogtgcacatgcaagttagagtcgtccagtcgataattgttcgagc 146

Query: 738 aataagctgoggcagaccattgcgagaacaaatgcagctcattcaaacgtggaagtcgaga 797
      |||
Sbjct: 145 aataagctgoggcagaccattgcgagaacaaatgcagctcattcaaacgtggaagtcgaga 86

Query: 798 cactccgcacataaagtggtgctcggtgcgcgagcactcagcgcagcgtgcgncgtcaaaa 857
      |||
Sbjct: 85 cactccgcacataaagtggtgctcggtgcgcgagcactcagcgcagcgtgcgncgtcaaaa 26

Query: 858 gcactccaaaccgtccagagaacat 882
      |||
Sbjct: 25 gcactccaaaccgtccagagaacat 1
```

Figure A.33: Clone A35

Score = 1629 bits (822), Expect = 0.0  
Identities = 833/836 (99%), Gaps = 1/836 (0%)  
Strand = Plus / Minus

```
Query: 19  cacaagtgagccaaaaagtcaccggttgettgcctgcattcccgcgctcgaaattgatt 78
      |||
Sbjct: 860  cacaagtgagccaaaaagtcaccggttgettgcctgcattcccgcgctcgaaattgatt 801

Query: 79  ccctcgatgagttcaataccgacgccgtgacactggctccgggtgccactggtgatccag 138
      |||
Sbjct: 800  ccctcgatgagttcaataccgacgccgtgacactggctccgggtgccactggtgatccag 741

Query: 139  tattctggacttggctggctgaatccaaagtcattgggtggcaaccgggggacgatgtcg 198
      |||
Sbjct: 740  tattctggacttggctggctgaatccaaagtcattgggtggcaaccgggggacgatgtcg 681

Query: 199  ttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgccagc 258
      |||
Sbjct: 680  ttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgccagc 621

Query: 259  gcatagttccgactcagggacatccataggtgtacagttcaacgctataacgctcatt 318
      |||
Sbjct: 620  gcatagttccgactcagggacatccataggtgtacagttcaacgctataacgctcatt 561

Query: 319  cgcaagaccgttgcctccagtgtagccaatgcccgcgcgccaagctgtgcccggtgaagtag 378
      |||
Sbjct: 560  cgcaagaccgttgcctccagtgtagccaatgcccgcgcgccaagctgtgcccggtgaagtag 501

Query: 379  agggatagcccgaaatcgtgctcctcagggacttgatcttgcctcagattgtctgca 438
      |||
Sbjct: 500  agggatagcccgaaatcgtgctcctcagggacttgatcttgcctcagattgtctgca 441

Query: 439  gggcttccatgcttcagaaatccagtgtagaaccttgcagccagtagaggtagctcg 498
      |||
Sbjct: 440  gggcttccatgcttcagaaatccagtgtagaaccttgcagccagtagaggtagctcg 381

Query: 499  ttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgcctcgg 558
      |||
Sbjct: 380  ttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgcctcgg 321

Query: 559  aaggcgaccacgagccgcttgttgngttgtccgcccagggaaaccggctgtgcctcca 618
      |||
Sbjct: 320  aaggcgaccacgagccgcttgttgngttgtccgcccagggaaaccggctgtgcctcca 261

Query: 619  aagttatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcagctgatgga 678
      |||
Sbjct: 260  aagttatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcagctgatgga 201

Query: 679  caggcgtcggcgtgcatgtcagttagagtcgtccagtcgatattgttcgagcaataa 738
      |||
Sbjct: 200  caggcgtcggcgtgcatgtcagttagagtcgtccagtcgatattgttcgagcaataa 141

Query: 739  gctgcccagaccattgagagaacaattgcagctcatccaaagtggaagtcgagacatc 798
      |||
Sbjct: 140  gctgcccagaccattgagagaacaattgcagctcatccaaagtggaagtcgagacatc 81

Query: 799  cgcaatcaagtggtgctgggtgncgcagcactcagcgcagcgtgcgcccgtcaaaa 854
      |||
Sbjct: 80  cgcaatcaagtggtgctgggtgncgcagcactcagcgcagcgtgcgcccgtcaaaa 26
```

Figure A.34: Clone A37



Score = 1675 bits (845), Expect = 0.0  
Identities = 856/860 (99%)  
Strand = Plus / Minus

```
Query: 18  cacaagtgngccaaaaagtcaccggtgcttcgectgcattcccgcgctgcaattgatt 77
      |||
Sbjct: 860  cacaagtgagccaaaaagtcaccggtgcttcgectgcattcccgcgctgcaattgatt 801

Query: 78  ccctcgatgagttcaataccgacgccgtgacac tggctccgggtgccactggtgatccag 137
      |||
Sbjct: 800  ccctcgatgagttcaataccgacgccgtgacac tggctccgggtgccactggtgatccag 741

Query: 138  tattctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgtcg 197
      |||
Sbjct: 740  tattctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgtcg 681

Query: 198  ttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgccagc 257
      |||
Sbjct: 680  ttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgccagc 621

Query: 258  gcatagttccgactcagggacatccataggtgtacagttcaacgctataaccgctcatt 317
      |||
Sbjct: 620  gcatagttccgactcagggacatccataggtgtacagttcaacgctataaccgctcatt 561

Query: 318  cgcaagaccgttgc tcccagtgtagccaatgcgcgcgccaagctgtgccgggtgaagtag 377
      |||
Sbjct: 560  cgcaagaccgttgc tcccagtgtagccaatgcgcgcgccaagctgtgccgggtgaagtag 501

Query: 378  agggatatagccggaatacgtgctcatcgggacttga tcttgcctcagattgtctgca 437
      |||
Sbjct: 500  agggatatagccggaatacgtgctcatcgggacttga tcttgcctcagattgtctgca 441

Query: 438  gcggttcctcagatccagaaatccagtgtagaaccttgcagccagtagaggtcactcg 497
      |||
Sbjct: 440  gcggttcctcagatccagaaatccagtgtagaaccttgcagccagtagaggtcactcg 381

Query: 498  ttatcttgaggatgaagtcagagtcagcaatccagttcttgatggtgctactgcctcgg 557
      |||
Sbjct: 380  ttatcttgaggatgaagtcagagtcagcaatccagttcttgatggtgctactgcctcgg 321

Query: 558  aaggcgaccacgagccgcttgttgggttgtccgcgccagggaaaccggctgtgcctcca 617
      |||
Sbjct: 320  aaggcgaccacgagccgcttgttgggttgtccgcgccagggaaaccggctgtgcctcca 261

Query: 618  aagtatttgtcaggtcaaac tccagcagca tcttgggtgctcgcctcctcagctgatgga 677
      |||
Sbjct: 260  aagtatttgtcaggtcaaac tccagcagca tcttgggtgctcgcctcctcagctgatgga 201

Query: 678  caggcgtggcgtgcatgtcagttagagtcgtccagtcgatattgttcgagcaataa 737
      |||
Sbjct: 200  caggcgtggcgtgcatgtcagttagagtcgtccagtcgatattgttcgagcaataa 141

Query: 738  gccggcggaaccttgccagaaatgcagctcatccaaagtggaagtcgagacactc 797
      |||
Sbjct: 140  gccggcggaaccttgccagaaatgcagctcatccaaagtggaagtcgagacactc 81

Query: 798  cgcaatcaagtggtgctgggtgccgagcactcagcgcagcgtgcgcctcaaaagcact 857
      |||
Sbjct: 80  cgcaatcaagtggtgctgggtgccgagcactcagcgcagcgtgcgcctcaaaagcact 21

Query: 858  ccaaaccgtccatagaacat 877
      |||
Sbjct: 20  ccaaaccgtccatagaacat 1
```

Figure A.36: Clone B2



Score = 1699 bits (857), Expect = 0.0  
Identities = 859/860 (99%)  
Strand = Plus / Minus

```
Query: 19  cacaagtgnccaaaacgtccaccgttgcttcgectgcattcccgcgctcgaattgatt 78
      |||
Sbjct: 860  cacaagtgagcaaaacgtccaccgttgcttcgectgcattcccgcgctcgaattgatt 801

Query: 79  ccctcgatgagttcaataccgacgccgtgacactggctccgggtgccactggtgatccag 138
      |||
Sbjct: 800  ccctcgatgagttcaataccgacgccgtgacactggctccgggtgccactggtgatccag 741

Query: 139  tattctggacttggttggtgaatccaaagtccaagggtggcaaccgggggacgatgtcg 198
      |||
Sbjct: 740  tattctggacttggttggtgaatccaaagtccaagggtggcaaccgggggacgatgtcg 681

Query: 199  ttcaagtgtgtaacgcggaagttcgctccagatccctggctggtgatgtgctcgccacg 258
      |||
Sbjct: 680  ttcaagtgtgtaacgcggaagttcgctccagatccctggctggtgatgtgctcgccacg 621

Query: 259  gcatagttccgactcgaggacatccataggtgtacagttcaacgctataaccgtcattt 318
      |||
Sbjct: 620  gcatagttccgactcgaggacatccataggtgtacagttcaacgctataaccgtcattt 561

Query: 319  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgcccggtagtag 378
      |||
Sbjct: 560  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgcccggtagtag 501

Query: 379  agggatatagccgaatacgtgctcaccgagcttgatcttgcctcagattgtctgca 438
      |||
Sbjct: 500  agggatatagccgaatacgtgctcaccgagcttgatcttgcctcagattgtctgca 441

Query: 439  gcggcttccatgcttccagaatccagtggtgaaacctgcaagccagtagaggtcactg 498
      |||
Sbjct: 440  gcggcttccatgcttccagaatccagtggtgaaacctgcaagccagtagaggtcactg 381

Query: 499  ttatcttgaggatgaagtcgagatcagcaatccagtcttgatggtgctactgctcgg 558
      |||
Sbjct: 380  ttatcttgaggatgaagtcgagatcagcaatccagtcttgatggtgctactgctcgg 321

Query: 559  aaggcgaccacgagccgcttggttggtgtgtccgcgccaggaaccggctgtgctcca 618
      |||
Sbjct: 320  aaggcgaccacgagccgcttggttggtgtgtccgcgccaggaaccggctgtgctcca 261

Query: 619  aagtattttgtcaggtcacaactccagcagcatcttggtgctcgcctcctcgaactgatgga 678
      |||
Sbjct: 260  aagtattttgtcaggtcacaactccagcagcatcttggtgctcgcctcctcgaactgatgga 201

Query: 679  caggcgctggcctgcatgtcactgtagagtcgctccgagtcgatattggtcgagcaataa 738
      |||
Sbjct: 200  caggcgctggcctgcatgtcactgtagagtcgctccgagtcgatattggtcgagcaataa 141

Query: 739  gctgcggcagaccattgcgagaacaattgcaagctcatccaacgtggaagtcgagacactc 798
      |||
Sbjct: 140  gctgcggcagaccattgcgagaacaattgcaagctcatccaacgtggaagtcgagacactc 81

Query: 799  cgcaatcaagtggtgctgggtgccgcaaaccaagcagcgtgcgcgctcaaaagcact 858
      |||
Sbjct: 80  cgcaatcaagtggtgctgggtgccgcaaaccaagcagcgtgcgcgctcaaaagcact 21

Query: 859  ccaaaccgtccagagaacat 878
      |||
Sbjct: 20  ccaaaccgtccagagaacat 1
```

Figure A.37: Clone B6



Score = 1679 bits (847), Expect = 0.0  
Identities = 857/859 (99%), Gaps = 1/859 (0%)  
Strand = Plus / Minus

```
Query: 21 caagtgagccaaaaagtcacccgttgcttctgacctgcattecccgccgtggaattgat tcc 80
      |||
Sbjct: 858 caagtgagccaaaaagtcacccgttgcttctgacctgcattecccgccgtggaattgat tcc 799

Query: 81 ctctgatgagttcaatatacgaagccgtgacactggctccgggtgcactgggtgatccagta 140
      |||
Sbjct: 798 ctctgatgagttcaatatacgaagccgtgacactggctccgggtgcactgggtgatccagta 739

Query: 141 ttctggacttggtggctggaatccaaagtcacatgggtggcaaccgggggacgatgtcgtt 200
      |||
Sbjct: 738 ttctggacttggtggctggaatccaaagtcacatgggtggcaaccgggggacgatgtcgtt 679

Query: 201 caagtgtgtaacgcggaaagtcctccagatccctggctgggtgatgtcctggccagcgc 260
      |||
Sbjct: 678 caagtgtgtaacgcggaaagtcctccagatccctggctgggtgatgtcctggccagcgc 619

Query: 261 atagtttcgactcaggacatccataggtgtacagtccaaagctataaccgctgttctg 320
      |||
Sbjct: 618 atagtttcgactcaggacatccataggtgtacagtccaaagctataaccgctgttctg 559

Query: 321 caagaccggttgctccagtgtagc caatgcgcgcccaagctgtgccgggtgaaagtag 380
      |||
Sbjct: 558 caagaccggttgctccagtgtagc caatgcgcgcccaagctgtgccgggtgaaagtag 459

Query: 381 ggtatagccgaatacgtgctcatcgcggacttgatcttgctcgtcagatgtctgcagc 440
      |||
Sbjct: 498 ggtatagccgaatacgtgctcatcgcggacttgatcttgctcgtcagatgtctgcagc 439

Query: 441 ggcttcccatgctctccagaaaccagtggaaccttgccagccagtacagaggctcatcgtt 500
      |||
Sbjct: 438 ggcttcccatgctctccagaaaccagtggaaccttgccagccagtacagaggctcatcgtt 379

Query: 501 atcttgaggatgaagtcgagatcagcaatccagttcttgatgggtgctactgccctggaa 560
      |||
Sbjct: 378 atcttgaggatgaagtcgagatcagcaatccagttcttgatgggtgctactgccctggaa 319

Query: 561 ggcgaccaagagccgcttggtggtgctccgcggccaggaaaccggctgtgctcccaaa 620
      |||
Sbjct: 318 ggcgaccaagagccgcttggtggtgctccgcggccaggaaaccggctgtgctcccaaa 259

Query: 621 gttattgtcagggtcaaaactcagcagcactcttggtgctcgcctcctcgaactgatggaca 680
      |||
Sbjct: 258 gttattgtcagggtcaaaactcagcagcactcttggtgctcgcctcctcgaactgatggaca 199

Query: 681 ggctggccgctgcatgtcacgttagagtgctccgagtcgatattgttcgagcaataagc 740
      |||
Sbjct: 198 ggctggccgctgcatgtcacgttagagtgctccgagtcgatattgttcgagcaataagc 139

Query: 741 tgcggcagaccattgcgagaa caattgcagctcaatccaaagtggaagtcgagacactccg 800
      |||
Sbjct: 138 tgcggcagaccattgcgagaa caattgcagctcaatccaaagtggaagtcgagacactccg 79

Query: 801 cacatcaagtgggtgctgggtgcgcagcactcagcgcagcgtgcgccgtcaaaaagcactcc 860
      |||
Sbjct: 78 cacatcaagtgggtgctgggtgcgcagcactcagcgcagcgtgcgccgtcaaaaagcactcc 19

Query: 861 aaaccgctccagagaacat 879
      |||
Sbjct: 18 aaa-ccgtccagagaacat 1
```

Figure A.39: Clone B8



Score = 1655 bits (835), Expect = 0.0  
Identities = 860/865 (99%), Gaps = 3/865 (0%)  
Strand = Plus / Minus

```
Query: 17  accacaagtgnccaaaaacgtccaacggttgettcgctgcattccccgccgtogaat tga 76
          |||
Sbjct: 862  accacaagtgaaccaaaacgtccaacggttgettcgctgcattccccgccgtogaat tga 803

Query: 77  ttcctcogatgagttcaatatccgaacgctgacactggctcgggtgccactggtgatcc 136
          |||
Sbjct: 802  ttcctcogatgagttcaatatccgaacgctgacactggctcgggtgccactggtgatcc 743

Query: 137  agtattctggacttggctggc tgaatc caaagtc catgggtggcaac cgggggacgatgt 196
          |||
Sbjct: 742  agtattctggacttggctggc tgaatc caaagtc catgggtggcaac cgggggacgatgt 683

Query: 197  cgttcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggcca 256
          |||
Sbjct: 682  cgttcaagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggcca 623

Query: 257  ggcgatagtttccgactcaggacatccatagggtgacagttcaacgctataacgctcat 316
          |||
Sbjct: 622  ggcgatagtttccgactcaggacatccatagggtgacagttcaacgctataacgctcat 563

Query: 317  ttccgaagaccggttgcctccagtgtagccaatgcgccccaagctgtgcccggtgaagt 376
          |||
Sbjct: 562  ttccgaagaccggttgcctccagtgtagccaatgcgccccaagctgtgcccggtgaagt 503

Query: 377  agagggtatagcccgaatacgtgctcaatcgcggacttgatcttgcctcagatgtctg 436
          |||
Sbjct: 502  agagggtatagcccgaatacgtgctcaatcgcggacttgatcttgcctcagatgtctg 443

Query: 437  cagcggcttcccatgcctccagaatccagtgtaaccttgccagccagtaacagaggtcat 496
          |||
Sbjct: 442  cagcggcttcccatgcctccagaatccagtgtaaccttgccagccagtaacagaggtcat 383

Query: 497  cgttatcttgcaggatgaagtccagatcagcaatccagttcttgatggtgctactgctc 556
          |||
Sbjct: 382  cgttatcttgcaggatgaagtccagatcagcaatccagttcttgatggtgctactgctc 323

Query: 557  ggaaggcgaccacagagccgcttgttggtggtgctcgcggccaggaaaacggctgtgctc 616
          |||
Sbjct: 322  ggaaggcgaccacagagccgcttgttggtggtgctcgcggccaggaaaacggctgtgctc 263

Query: 617  caaagtatttgtcaggtcaaacccagcagcatcttggtgctcgcctccctcgaactgatg 676
          |||
Sbjct: 262  caaagtatttgtcaggtcaaacccagcagcatcttggtgctcgcctccctcgaactgatg 203

Query: 677  gacaggcgtcggcctgcatgtcagcttagagtcgctccagatcgatattgttcgagcaat 736
          |||
Sbjct: 202  gacaggcgtcggcctgcatgtcagcttagagtcgctccagatcgatattgttcgagcaat 143

Query: 737  aagctgcggcagaccattgcgagaacaattgcagctcattcaacgtggaagtcgagacac 796
          |||
Sbjct: 142  aagctgcggcagaccattgcgagaacaattgcagctcattcaacgtggaagtcgagacac 83

Query: 797  tccgcacatccaagtgggtgctggngccgacgactcagcgcagcgtgcccgtccaaaa 856
          |||
Sbjct: 82  tccgcacatccaagtgggtgctggngccgacgactcagcgcagcgtgcccgtccaaaa 26

Query: 857  gcactccaaaccgctccagagaacat 881
          |||
Sbjct: 25  gcactccaaaccgctccagagaacat 1
```

Figure A.41: Clone B14

Score = 1689 bits (852), Expect = 0.0  
Identities = 860/863 (99%)  
Strand = Plus / Minus

```
Query: 17  taccacaagtgagc caaaacgtccaccgttgcttcgctgcaactcccgcgcgto gaattg 76
          |||
Sbjct: 863  taccacaagtgagc caaaacgtccaccgttgcttcgctgcaattcccgcgcgto gaattg 804

Query: 77  attccctc gatgagttcaata tccgacgccgtgacac tggctccgggtgccactggtgatc 136
          |||
Sbjct: 803  attccctc gatgagttcaata tccgacgccgtgacac tggctccgggtgccactggtgatc 744

Query: 137  cagtattctggact tggctggctgaatccaaagtcca tgggtggcaaccgggggacgatg 196
          |||
Sbjct: 743  cagtattctggact tggctggctgaatccaaagtcca tgggtggcaaccgggggacgatg 684

Query: 197  tcgttcaagtgtgta acgcggaagttcgctcagatcc ctggctggtgatgtgctcgccc 256
          |||
Sbjct: 683  tcgttcaagtgtgta acgcggaagttcgctcagatcc ctggctggtgatgtgctcgccc 624

Query: 257  agcgcatagtttcc gactcga gga catccataggtgtac agttcaac gctataa ccgtca 316
          |||
Sbjct: 623  agcgcatagtttcc gactcga gga catccataggtgtac agttcaac gctataa ccgtca 564

Query: 317  ttctcgcaagac cgttgetccc agt gtagccaatg cgcgcgcc caagctgtgcccgg tgaag 376
          |||
Sbjct: 563  ttctcgcaagac cgttgetccc agt gtagccaatg cgcgcgcc caagctgtgcccgg tgaag 504

Query: 377  tagagggtatagcc gaa tacgtgctc atcgcggaacttg atcttgetcgtc agattgtct 436
          |||
Sbjct: 503  tagagggtatagcc gaa tacgtgctc atcgcggaacttg atcttgetcgtc agattgtct 444

Query: 437  gcagcggcttccca tgccttc cagaatccagtg tgaaccttgc agccagtac agagg tca 496
          |||
Sbjct: 443  gcagcggcttccca tgccttc cagaatccagtg tgaaccttgc agccagtac agagg tca 384

Query: 497  tcgttate ttgcaggat gaagtogaga tcagcaatcc agtctt gatggtgcta ctgcoct 556
          |||
Sbjct: 383  tcgttate ttgcaggat gaagtogaga tcagcaatcc agtctt gatggtgcta ctgcoct 324

Query: 557  cggaaaggc gaccac gagc cgc ttgttggtgt tgcgcggc caggaaacc ggctgtgcoct 616
          |||
Sbjct: 323  cggaaaggc gaccac gagc cgc ttgttggtgt tgcgcggc caggaaacc ggctgtgcoct 264

Query: 617  ccaaagtattttgt cagggtcaaactccagcagca tettggtgctcgcctcctcgactgat 676
          |||
Sbjct: 263  ccaaagtattttgt cagggtcaaactccagcagca tettggtgctcgcctcctcgactgat 204

Query: 677  ggacaggc gccggc cgtgcatg tcaacttagagtcgt ccgagtc gatattgttc gagcaa 736
          |||
Sbjct: 203  ggacaggc gtcggc cgtgcatg tcaacttagagtcgt ccgagtc gatattgttc gagcaa 144

Query: 737  taagctgc ggcagacc at tgcgagaacaattgc agctc atccaa cgtggaagtc gagaca 796
          |||
Sbjct: 143  taagctgc ggcagacc at tgcgagaacaattgc agctc atccaa cgtggaagtc gagaca 84

Query: 797  ctccgcacatca agtgggtgc ggtgccgcagc ac tca ggcagc gngcgc cgtcaaaagc 856
          |||
Sbjct: 83  ctccgcacatca agtgggtgc ggtgccgcagc ac tca ggcagc gngcgc cgtcaaaagc 24

Query: 857  actccaaac cgtccagaga acat 879
          |||
Sbjct: 23  actccaaac cgtccagaga acat 1
```

Figure A.42: Clone B15

Score = 1705 bits (860), Expect = 0.0  
Identities = 860/860 (100%)  
Strand = Plus / Minus

```
Query: 20  cacaagtgagc caaaaagctccaccggtgcttcgectgcattcccgcgctcgaattgatt 79
      |||
Sbjct: 860  cacaagtgagc caaaaagctccaccggtgcttcgectgcattcccgcgctcgaattgatt 801

Query: 80  ccctcgatgagttcaataccgacgccgtgacactggctccgggtgccactggtgatccag 139
      |||
Sbjct: 800  ccctcgatgagttcaataccgacgccgtgacactggctccgggtgccactggtgatccag 741

Query: 140  tattctggacttggtggctgaatccaaagtccaagggtggcaaccgggggacgatgtcg 199
      |||
Sbjct: 740  tattctggacttggtggctgaatccaaagtccaagggtggcaaccgggggacgatgtcg 681

Query: 200  ttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgccagc 259
      |||
Sbjct: 680  ttcaagtgtgtaacgcggaagtccgctccagatccctggctggtgatgtgctcgccagc 621

Query: 260  gcatagttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcatt 319
      |||
Sbjct: 620  gcatagttccgactcgaggacatccataggtgtacagttcaacgctataaccgctcatt 561

Query: 320  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgcccggtagtag 379
      |||
Sbjct: 560  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgcccggtagtag 501

Query: 380  agggatatagccgaatacgtgctcaccggaacttgatcttgcctcagattgtctgca 439
      |||
Sbjct: 500  agggatatagccgaatacgtgctcaccggaacttgatcttgcctcagattgtctgca 441

Query: 440  gcggcttccatgcttccagaatccagtgtagcccttgcaagccagtagagaggtcactg 499
      |||
Sbjct: 440  gcggcttccatgcttccagaatccagtgtagcccttgcaagccagtagagaggtcactg 381

Query: 500  ttatcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgctcgg 559
      |||
Sbjct: 380  ttatcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgctcgg 321

Query: 560  aaggcgaccacgagccgcttgggtgtgtccgcccaggaaccggcgtgctcctcca 619
      |||
Sbjct: 320  aaggcgaccacgagccgcttgggtgtgtccgcccaggaaccggcgtgctcctcca 261

Query: 620  aagtatttggcaggtcacaactccagcagcatcttgggtgctcgcctcctcgaactgatgga 679
      |||
Sbjct: 260  aagtatttggcaggtcacaactccagcagcatcttgggtgctcgcctcctcgaactgatgga 201

Query: 680  caggcgctggcctgcatgtaacgttagagtcgctccgagtcgatattggtcgagcaataa 739
      |||
Sbjct: 200  caggcgctggcctgcatgtaacgttagagtcgctccgagtcgatattggtcgagcaataa 141

Query: 740  gctgcccagacattgagagaacaattgcaagctcatccaaagtggaagtcgagacatc 799
      |||
Sbjct: 140  gctgcccagacattgagagaacaattgcaagctcatccaaagtggaagtcgagacatc 81

Query: 800  cgcaatcaagtggtgctgggtgccgcaaacctcagcagcgtgcccgcgctcaaaagcact 859
      |||
Sbjct: 80  cgcaatcaagtggtgctgggtgccgcaaacctcagcagcgtgcccgcgctcaaaagcact 21

Query: 860  ccaaaccgtccagagaacat 879
      |||
Sbjct: 20  ccaaaccgtccagagaacat 1
```

Figure A.43: Clone B17

Score = 1657 bits (836), Expect = 0.0  
Identities = 859/863 (99%), Gaps = 3/863 (0%)  
Strand = Plus / Minus

```
Query: 22  cacaagtgagccaaaaagtcaccggttgettgcctgcattcccgcgctcgaaattgatt 81
      |||
Sbjct: 860  cacaagtgagccaaaaagtcaccggttgettgcctgcattcccgcgctcgaaattgatt 801

Query: 82  ccctcgatgagttcaataccgacgccgtgacacggctccgggtgccactggtgatccag 141
      |||
Sbjct: 800  ccctcgatgagttcaataccgacgccgtgacacggctccgggtgccactggtgatccag 741

Query: 142  tattctggacttggtgctgaatccaaagtcattgggtggcaaccgggggacgatgctg 201
      |||
Sbjct: 740  tattctggacttggtgctgaatccaaagtcattgggtggcaaccgggggacgatgctg 681

Query: 202  ttcaagtgtgtaacgcggaagttcgtccagatccctggctggtgatgtgctcggccagc 261
      |||
Sbjct: 680  ttcaagtgtgtaacgcggaagttcgtccagatccctggctggtgatgtgctcggccagc 621

Query: 262  gcatagttccgactcagagacatccataggtgtacagttcaacgctataaccgctcattt 321
      |||
Sbjct: 620  gcatagttccgactcagagacatccataggtgtacagttcaacgctataaccgctcattt 561

Query: 322  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgccgggtagtag 381
      |||
Sbjct: 560  cgcaagaccgttgcctccagtgtagccaatgcgcgcgccaagctgtgccgggtagtag 501

Query: 382  agggatatagccagaatacgtgctcatcgggacttgatcttgcctcagattgtctgca 441
      |||
Sbjct: 500  agggatatagccagaatacgtgctcatcgggacttgatcttgcctcagattgtctgca 441

Query: 442  gggcttccatgcttcagaaatccagtgtgaaacctgcagccagtagagaggtcatcg 501
      |||
Sbjct: 440  gggcttccatgcttcagaaatccagtgtgaaacctgcagccagtagagaggtcatcg 381

Query: 502  ttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgctcgg 561
      |||
Sbjct: 380  ttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgctcgg 321

Query: 562  aaggcgaccacgagccgcttgttgggtttgtccggcgccaggaaaccggctgtgctcca 621
      |||
Sbjct: 320  aaggcgaccacgagccgcttgttgggtttgtccggcgccaggaaaccggctgtgctcca 261

Query: 622  aagtatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcgaactgatgga 681
      |||
Sbjct: 260  aagtatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcgaactgatgga 201

Query: 682  caggcgctggcgtgcatgtcaggttagagtcgtccgagtcgatattgttcgagcaataa 741
      |||
Sbjct: 200  caggcgctggcgtgcatgtcaggttagagtcgtccgagtcgatattgttcgagcaataa 141

Query: 742  gctgcggcagaccattgcgagaacaattgcagctcatccaaagtggaagtcgagacactc 801
      |||
Sbjct: 140  gctgcggcagaccattgcgagaacaattgcagctcatccaaagtggaagtcgagacactc 81

Query: 802  cgcaatcaagtggtgtcgtgccgacgacacagcgcagcgtgcgcctgcaaaagcac 861
      |||
Sbjct: 80  cgcaatcaagtggtgtcgtgccgacgacacagcgcagcgtgcgcctgcaaaagcac 22

Query: 862  tccaaaaccgtccagagaacat 884
      |||
Sbjct: 21  tcc-aaaccgt-ccagagaacat 1
```

Figure A.44: Clone C2



Score = 1701 bits (858), Expect = 0.0  
Identities = 858/858 (100%)  
Strand = Plus / Minus

```
Query: 23 caagtgagccaaaaagtcacccgttgcttgcctgcattcccgccgtogaattgat tcc 82
      |||
Sbjct: 858 caagtgagccaaaaagtcacccgttgcttgcctgcattcccgccgtogaattgat tcc 799

Query: 83 ctogatgagttcaatatacgacgcgctgacactggctccgggtgcacactggatccagta 142
      |||
Sbjct: 798 ctogatgagttcaatatacgacgcgctgacactggctccgggtgcacactggatccagta 739

Query: 143 ttctggacttgctggtgaatccaaagtcacatgggtggcaaccgggggacgatgtcgtt 202
      |||
Sbjct: 738 ttctggacttgctggtgaatccaaagtcacatgggtggcaaccgggggacgatgtcgtt 679

Query: 203 caagtgtgtaacgcggaagttcgtccagatcccggctggatgtgctcggccagcgc 262
      |||
Sbjct: 678 caagtgtgtaacgcggaagttcgtccagatcccggctggatgtgctcggccagcgc 619

Query: 263 atagttccgactcgaggacatccataggtgtacagttcaacgcataaacgctcatttcg 322
      |||
Sbjct: 618 atagttccgactcgaggacatccataggtgtacagttcaacgcataaacgctcatttcg 559

Query: 323 caagaccgttgctccagtgtagc caatgcgcgcccaagcgtgcccgggtgaa gta gag 382
      |||
Sbjct: 558 caagaccgttgctccagtgtagc caatgcgcgcccaagcgtgcccgggtgaa gta gag 499

Query: 383 ggtatagccogaatacgtgctcatcgcggacttgatcttgcctgca gat t g t c t g c a g c 442
      |||
Sbjct: 498 ggtatagccogaatacgtgctcatcgcggacttgatcttgcctgca gat t g t c t g c a g c 439

Query: 443 ggcttcccagcctccagaatccagtgtaacc ttgcagc cagtacagaggtcac t g t t 502
      |||
Sbjct: 438 ggcttcccagcctccagaatccagtgtaacc ttgcagc cagtacagaggtcac t g t t 379

Query: 503 atcttgaggatgaagtcgagatcagcaatccagttcttgatgggtgc tactgcccog gaa 562
      |||
Sbjct: 378 atcttgaggatgaagtcgagatcagcaatccagttcttgatgggtgc tactgcccog gaa 319

Query: 563 ggcgaccacgagccgctt g t t g g t g t t g t c c g c g g c c a g g a a a c c g g c t g t g c c t c c a a a 622
      |||
Sbjct: 318 ggcgaccacgagccgctt g t t g g t g t t g t c c g c g g c c a g g a a a c c g g c t g t g c c t c c a a a 259

Query: 623 gttattgtcagggtcaaacctcagcagcactcttggtgctcgcctcctcgactgatggaca 682
      |||
Sbjct: 258 gttattgtcagggtcaaacctcagcagcactcttggtgctcgcctcctcgactgatggaca 199

Query: 683 ggcgtggccgctgcatgtcacggttagagtgctccagtgatgat t g t t o g a g c a a t a a g c 742
      |||
Sbjct: 198 ggcgtggccgctgcatgtcacggttagagtgctccagtgatgat t g t t o g a g c a a t a a g c 139

Query: 743 tgcggcagaccattgcgagaacaa ttgcagctca tccaacgtggaagtcgagacactccg 802
      |||
Sbjct: 138 tgcggcagaccattgcgagaacaa ttgcagctca tccaacgtggaagtcgagacactccg 79

Query: 803 cacatcaagtggtgctcgggtgcgcgacactcagcgcagcgtgcgcgctcaaaagcactcc 862
      |||
Sbjct: 78 cacatcaagtggtgctcgggtgcgcgacactcagcgcagcgtgcgcgctcaaaagcactcc 19

Query: 863 aaaccgtccagagaacat 880
      |||
Sbjct: 18 aaaccgtccagagaacat 1
```

Figure A.45: Clone C3





Score = 1687 bits (851), Expect = 0.0  
Identities = 858/859 (99%), Gaps = 1/859 (0%)  
Strand = Plus / Minus

```
Query: 23 caagtgagccaaaaagtcacacgttgccttegcctgcattcccgccgctogaattgatcc 82
      |||
Sbjct: 858 caagtgagccaaaaagtcacacgttgccttegcctgcattcccgccgctogaattgatcc 799

Query: 83 ctogatgagttcaatatacgaagcgcgtgacactggctccgggtgcactgggtgatccagta 142
      |||
Sbjct: 798 ctogatgagttcaatatacgaagcgcgtgacactggctccgggtgcactgggtgatccagta 739

Query: 143 ttctggacttggtggctggctgaatccaaagtccatgggtggcaaccgggggacgatgctggt 202
      |||
Sbjct: 738 ttctggacttggtggctggctgaatccaaagtccatgggtggcaaccgggggacgatgctggt 679

Query: 203 caagtgtgtaacgcggaaagttcgcctccagatcccggctggtgatgtgctcggccagcgc 262
      |||
Sbjct: 678 caagtgtgtaacgcggaaagttcgcctccagatcccggctggtgatgtgctcggccagcgc 619

Query: 263 atagtttcgaactcaggacaatccataggtgtacagttcaacgcataaacgctcatttcg 322
      |||
Sbjct: 618 atagtttcgaactcaggacaatccataggtgtacagttcaacgcataaacgctcatttcg 559

Query: 323 caagaccggttgctccagtgtagc caatgcgcgcccaagcgtgcccgggtgaagtagag 382
      |||
Sbjct: 558 caagaccggttgctccagtgtagc caatgcgcgcccaagcgtgcccgggtgaagtagag 499

Query: 383 ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagattgctcgcagc 442
      |||
Sbjct: 498 ggtatagccgaatacgtgctcatcgcggacttgatcttgcctcagattgctcgcagc 439

Query: 443 ggettcccattgcttccagaatccagtggaaccttgccagcagtacagaggctcagctt 502
      |||
Sbjct: 438 ggettcccattgcttccagaatccagtggaaccttgccagcagtacagaggctcagctt 379

Query: 503 atcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgccctggaa 562
      |||
Sbjct: 378 atcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgccctggaa 319

Query: 563 ggcgaccaagagccgcttggtggtgctgcccggccaggaaccggctggtgccccaaa 622
      |||
Sbjct: 318 ggcgaccaagagccgcttggtggtgctgcccggccaggaaccggctggtgccccaaa 259

Query: 623 gttattgtcaggtcaaacctcagcagcatcttggtgctcgcctcctcgactgatggaca 682
      |||
Sbjct: 258 gttattgtcaggtcaaacctcagcagcatcttggtgctcgcctcctcgactgatggaca 199

Query: 683 ggcgtggcgcgtgcatgtcacgttagagtgctccgagtgatattgttcgagcaataagc 742
      |||
Sbjct: 198 ggcgtggcgcgtgcatgtcacgttagagtgctccgagtgatattgttcgagcaataagc 139

Query: 743 tgcggcagaccattgcgagaa caattgcagctcaatccaaagtgaggagtcgagacactcc 802
      |||
Sbjct: 138 tgcggcagaccattgcgagaa caattgcagctcaatccaaagtgaggagtcgagacactcc 80

Query: 803 gcacatcaagtggtgtcggtgcgcgagcactcagcgcagcgtgcccgtcaaaagcactc 862
      |||
Sbjct: 79 gcacatcaagtggtgtcggtgcgcgagcactcagcgcagcgtgcccgtcaaaagcactc 20

Query: 863 caaacgctccagagaacat 881
      |||
Sbjct: 19 caaacgctccagagaacat 1
```

Figure A.48: Clone C7

Score = 1703 bits (859), Expect = 0.0  
Identities = 862/863 (99%)  
Strand = Plus / Minus

```
Query: 16  taccacaagtgagc caaaaacgtccaacgttgettgcctgcattcccgcgctegaattg 75
      |
Sbjct: 863  taccacaagtgagc caaaaacgtccaacgttgettgcctgcattcccgcgctegaattg 804

Query: 76  attccctcgatgagttcaataatccgacgccgtgacactggctccgggtgccactggtgatc 135
      |
Sbjct: 803  attccctcgatgagttcaataatccgacgccgtgacactggctccgggtgccactggtgatc 744

Query: 136  cagtattctggacttggttggtgtaatacaaaagccaatgggtggcaaccgggggacgatg 195
      |
Sbjct: 743  cagtattctggacttggttggtgtaatacaaaagccaatgggtggcaaccgggggacgatg 684

Query: 196  gcgttcaagtgtgtaacgcggaagttcgtccagatccctggctggtgatgtgctcggcc 255
      |
Sbjct: 683  tcgttcaagtgtgtaacgcggaagttcgtccagatccctggctggtgatgtgctcggcc 624

Query: 256  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgta 315
      |
Sbjct: 623  agcgcatagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgta 564

Query: 316  tttcgcaagaccgttgetcccagtgtagcaatgcccgcgcccaagctgtgcccggggaag 375
      |
Sbjct: 563  tttcgcaagaccgttgetcccagtgtagcaatgcccgcgcccaagctgtgcccggggaag 504

Query: 376  tagagggtatagcccgaatacgtgctcatcgggaacttgatcttgetcgtcagattgtct 435
      |
Sbjct: 503  tagagggtatagcccgaatacgtgctcatcgggaacttgatcttgetcgtcagattgtct 444

Query: 436  gcagcggcttccatgcccaccagaatccagtgtaacettgcaagccagtcacagaggta 495
      |
Sbjct: 443  gcagcggcttccatgcccaccagaatccagtgtaacettgcaagccagtcacagaggta 384

Query: 496  tcgttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgct 555
      |
Sbjct: 383  tcgttatcttgaggatgaagtcagatcagcaatccagttcttgatggtgctactgct 324

Query: 556  cggaggcgaccacgagccgcttgggtggtgtgctccggcagcagaaaccggctgtgct 615
      |
Sbjct: 323  cggaggcgaccacgagccgcttgggtggtgtgctccggcagcagaaaccggctgtgct 264

Query: 616  ccaaagttatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcgactgat 675
      |
Sbjct: 263  ccaaagttatttgtcaggtcaaacccagcagcatcttggtgctcgcctcctcgactgat 204

Query: 676  ggacaggcgtcggcctgcatgtcacgtagagtcgtccagatcgatattgttcgagcaa 735
      |
Sbjct: 203  ggacaggcgtcggcctgcatgtcacgtagagtcgtccagatcgatattgttcgagcaa 144

Query: 736  taagctggcgcagaccatgcgagaacaattgcagetcatccaaagtggaagtcgagaca 795
      |
Sbjct: 143  taagctggcgcagaccatgcgagaacaattgcagetcatccaaagtggaagtcgagaca 84

Query: 796  ctcgcacatcaagtgggtgctgggtgccgcagcactcagcgcagcgtgcgcctcaaaagc 855
      |
Sbjct: 83  ctcgcacatcaagtgggtgctgggtgccgcagcactcagcgcagcgtgcgcctcaaaagc 24

Query: 856  actccaaaaccgtccagagaacat 878
      |
Sbjct: 23  actccaaaaccgtccagagaacat 1
```

Figure A.49: Clone C8

Score = 1655 bits (835), Expect = 0.0  
Identities = 853/859 (99%), Gaps = 1/859 (0%)  
Strand = Plus / Minus

```
Query: 22 caagtgnGCCAAAACGTCCACCGTTGCTTGCCTGCATTCCCGCGTGAATTGATTC 81
      |||||
Sbjct: 858 caagtGAGCCAAAACGTCCACCGTTGCTTGCCTGCATTCCCGCGTGAATTGATTC 799

Query: 82 CTCGATGAGTTCAAATATCCGACGCGTGCACCTGGCTCCGGTGCACCTGGTGATCCAGTA 141
      |||||
Sbjct: 798 CTCGATGAGTTCAAATATCCGACGCGTGCACCTGGCTCCGGTGCACCTGGTGATCCAGTA 739

Query: 142 TCTGGACTTGGCTGGCTGAATCCAAAAGTCCATGGGTGGCAACCGGGGACGATGTCGTT 201
      |||||
Sbjct: 738 TCTGGACTTGGCTGGCTGAATCCAAAAGTCCATGGGTGGCAACCGGGGACGATGTCGTT 679

Query: 202 CAAGTGTGTAACGCGGGAAGTCGCTCCAGATCCCCTGGCTGGTGTGTGCTCGGCAGCGC 261
      |||||
Sbjct: 678 CAAGTGTGTAACGCGGGAAGTCGCTCCAGATCCCCTGGCTGGTGTGTGCTCGGCAGCGC 619

Query: 262 ATAGTTTCGACTCGAGGACATCCATAGGTGTACAGTCCAAAGCTATAACCGTCATTTCG 321
      |||||
Sbjct: 618 ATAGTTTCGACTCGAGGACATCCATAGGTGTACAGTCCAAAGCTATAACCGTCATTTCG 559

Query: 322 CAAGACCGTTGCTCCAGTGTAGCAATGCGCCGCCAAGCTGTGCCCGGTGAAGTAGAG 381
      |||||
Sbjct: 558 CAAGACCGTTGCTCCAGTGTAGCAATGCGCCGCCAAGCTGTGCCCGGTGAAGTAGAG 499

Query: 382 GGTA TAGCCGAATACGTGCTCATCGCGGACTTGATCTTGCCTGTCAGATTGCTGCAGC 441
      |||||
Sbjct: 498 GGTA TAGCCGAATACGTGCTCATCGCGGACTTGATCTTGCCTGTCAGATTGCTGCAGC 439

Query: 442 GGCTTCCCATGCCTCCAGAAATCCAGTGTGAACCTTGCAGCCAGTACAGAGGTCATCGTT 501
      |||||
Sbjct: 438 GGCTTCCCATGCCTCCAGAAATCCAGTGTGAACCTTGCAGCCAGTACAGAGGTCATCGTT 379

Query: 502 ATCTTGCAAGGATGAAGTCGAGATCAGCAATCCAGTCTTGTGTTGGTGCCTACTGCCCGGAA 561
      |||||
Sbjct: 378 ATCTTGCAAGGATGAAGTCGAGATCAGCAATCCAGTCTTGTGTTGGTGCCTACTGCCCGGAA 319

Query: 562 GCGACCA CGAGCCGTTGTTGGTGTGTCGCGGCCAGGAAACCGGTGTGCC TCCAAA 621
      |||||
Sbjct: 318 GCGACCA CGAGCCGTTGTTGGTGTGTCGCGGCCAGGAAACCGGTGTGCC TCCAAA 259

Query: 622 GTTATTTGTCAGGTCAAACCTCAGCAGCATCTTGGTGTCTGCTCCTCGACTGATGGACA 681
      |||||
Sbjct: 258 GTTATTTGTCAGGTCAAACCTCAGCAGCATCTTGGTGTCTGCTCCTCGACTGATGGACA 199

Query: 682 GGCCTGGCCGTCATGTCACGTTAGAGTCTCCAGTCGATATGTCGAGCAATAAGC 741
      |||||
Sbjct: 198 GGCCTGGCCGTCATGTCACGTTAGAGTCTCCAGTCGATATGTCGAGCAATAAGC 139

Query: 742 TCGCGCAGACCATTGCGAGAACAAATTGCAGCTCATCCAACTGGAAGTCGAGACACTCCG 801
      |||||
Sbjct: 138 TCGCGCAGACCATTGCGAGAACAAATTGCAGCTCATCCAACTGGAAGTCGAGACACTCCG 79

Query: 802 CACATCAAAGTGGTGTGGTGCCTGCGCAGCACTCAGCGCAGGGGTGCCTGCAAAAACACTC 861
      |||||
Sbjct: 78 CACATCAAAGTGGTGTGGTGCCTGCGCAGCACTCAGCGCAGGGGTGCCTGCAAAAACACTC 20

Query: 862 CAAAACCGTCCAGAGAACAT 880
      |||||
Sbjct: 19 CAAAACCGTCCAGAGAACAT 1
```

Figure A.50: Clone C9

```

Score = 1616 bits (815), Expect = 0.0
Identities = 855/862 (99%), Gaps = 5/862 (0%)
Strand = Plus / Minus

Query: 21  aagtgagccaaaacgtccaccgttgcttegcctgcatccccgcgcgtogaattgattccc 80
      |||
Sbjct: 857  aagtgagccaaaacgtccaccgttgcttegcctgcatccccgcgcgtogaattgattccc 798

Query: 81  tcgatgagttcaatatccgacgccgtgacactggctcgggtgccactggtgatccagtat 140
      |||
Sbjct: 797  tcgatgagttcaatatccgacgccgtgacactggctcgggtgccactggtgatccagtat 738

Query: 141  tctggacttggctggctgaatccaaagtccatgggtggcaacgggggacgatgtcgttc 200
      |||
Sbjct: 737  tctggacttggctggctgaatccaaagtccatgggtggcaacgggggacgatgtcgttc 678

Query: 201  aagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggccagcgca 260
      |||
Sbjct: 677  aagtgtgtaacgcggaagtgcctccagatccctggctggtgatgtgctcggccagcgca 618

Query: 261  tagtttccgactcgaggacatcca taggtgtacagttcaacgctataaccgtcatttcgc 320
      |||
Sbjct: 617  tagtttccgactcgaggacatcca taggtgtacagttcaacgctataaccgtcatttcgc 558

Query: 321  aagaaccgttgc tcc cagtgtagccaatgcgcgc ccaagctgtgccc ggtgaagtagagg 380
      |||
Sbjct: 557  aagaaccgttgc tcc cagtgtagccaatgcgcgc ccaagctgtgccc ggtgaagtagagg 498

Query: 381  gtatagcccgaatacgtgctc atcgcggacttgatctt gctcgtcagattgtctgcagcg 440
      |||
Sbjct: 497  gtatagcccgaatacgtgctc atcgcggacttgatctt gctcgtcagattgtctgcagcg 438

Query: 441  gettcccattgccttccagaatccagtgcgaaaccttgcagccagtagaggtcatctgta 500
      |||
Sbjct: 437  gettcccattgccttccagaatccagtggtgaaaccttgcagccagtagaggtcatctgta 378

Query: 501  tcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgcctcggaag 560
      |||
Sbjct: 377  tcttgaggatgaagtcgagatcagcaatccagttcttgatggtgctactgcctcggaag 318

Query: 561  gcgaccacgagccgcttggttggtgtgctccgcggccaggaaaccggtgtgctccaaa 620
      |||
Sbjct: 317  gcgaccacgagccgcttggttggtgtgctccgcggccaggaaaccggtgtgctccaaa 258

Query: 621  ttatttgcaggtcaaac tccagcagcatcttgggtgc tgcctcctc gactgatggacag 680
      |||
Sbjct: 257  ttatttgcaggtcaaac tccagcagcatcttgggtgc tgcctcctc gactgatggacag 198

Query: 681  gcgtcgccgctgcatgtca cgttagagtcgtccgagtcgatattgttcgagcaataaget 740
      |||
Sbjct: 197  gcgtcgccgctgcatgtca cgttagagtcgtccgagtcgatattgttcgagcaataaget 138

Query: 741  gcggcagaccattgcgagaacaatttgcagctca tccaactggaagtcgagacactccg 800
      |||
Sbjct: 137  gcggcagaccattgcgagaaca--ttgcagctca tccaactggaagtcgagacactccg 79

Query: 801  cacatcaagtgggtgcgggtgc cgcagcactcagcgcagcgtgc gccgttcaaaangcac 860
      |||
Sbjct: 78  cacatcaagtgggtgcgggtgc cgcagcactcagcgcagcgtgc gccg-tcaaaa-gcac 22

Query: 861  tccaaaccggnccagagaacat 882
      |||
Sbjct: 21  tccaaaccg-tccagagaacat 1

```

**Figure A.51:** Clone C12

Score = 1586 bits (800), Expect = 0.0  
Identities = 851/863 (98%), Gaps = 5/863 (0%)  
Strand = Plus / Minus

```
Query: 22 caagtngcacaacgctcacggttgccttgcctgcattcccgccgctcgaattgatcc 81
      ||||| |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 858 caagtgagcacaacgctcacggttgccttgcctgcattcccgccgctcgaattgatcc 799

Query: 82 ctogatgagttcaatatacgacgctgacactggctccgggtgcactggtgatccagta 141
      |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 798 ctogatgagttcaatatacgacgctgacactggctccgggtgcactggtgatccagta 739

Query: 142 ttctggacttggtggctgaatccaaagtccatgggtggcaaccgggggacgatgtcgtt 201
      |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 738 ttctggacttggtggctgaatccaaagtccatgggtggcaaccgggggacgatgtcgtt 679

Query: 202 caagtgtgtaacgcgggaagtgcctccagatccctggctggtgatgtgctcggcagcgc 261
      |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 678 caagtgtgtaacgcgggaagtgcctccagatccctggctggtgatgtgctcggcagcgc 619

Query: 262 atagtttcgactcgaggacatccatagggtgtacagtcacgcgcnncncogtcatttcg 321
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 618 atagtttcgactcgaggacatccatagggtgtacagtcacgcgctataaacgctatttcg 559

Query: 322 caagaccgttgctccagtgtagc caatgctgcgcccgaagctgtgcccggtgaa gtagag 381
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 558 caagaccgttgctccagtgtagc caatgctgcgcccgaagctgtgcccggtgaa gtagag 499

Query: 382 ggtatagccgaatacgtgctcatcgccgacttgatcttgcctgctcagattgtctgcagc 441
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 498 ggtatagccgaatacgtgctcatcgccgacttgatcttgcctgctcagattgtctgcagc 439

Query: 442 ggcctcccagcctccagaatccagtgtaaccttgccagccagtcacagaggtcacgtt 501
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 438 ggcctcccagcctccagaatccagtgtaaccttgccagccagtcacagaggtcacgtt 379

Query: 502 atcttgcaaggatgaagtcgagatcagcaatecagttcttgaagggtgctactgccctggaa 561
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 378 atcttgcaaggatgaagtcgagatcagcaatecagttcttgaagggtgctactgccctggaa 319

Query: 562 ggcgaccacagagccgcttgttgggtgttgcgcccaggaaacggctgtgccctccaaa 621
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 318 ggcgaccacagagccgcttgttgggtgttgcgcccaggaaacggctgtgccctccaaa 259

Query: 622 gttatttgcagggtcaaacctccagcagcattctgggtgctgcctcctcgactgatggaca 681
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 258 gttatttgcagggtcaaacctccagcagcattctgggtgctgcctcctcgactgatggaca 199

Query: 682 ggcgtcggccgctgcatgtcacggttagagtgctccgagtcgatattgttcgagcaataagc 741
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 198 ggcgtcggccgctgcatgtcacggttagagtgctccgagtcgatattgttcgagcaataagc 139

Query: 742 tgcggcagaccattgcgagaa caattgcagctcatccaacgtgggaa gtcgagacactcc 801
      |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 138 tgcggcagaccattgcgagaa caattgcagctcatccaacgt-ggaa gtcgagacactcc 80

Query: 802 gcaccatcaagtgggtgtcggtgcgcagcactcagcgcaggcgtgcgcccgtaaaaagc 861
      || ||||| |||||||||||||||||||||||||||||||
Sbjct: 79 gca-catcaagt-ggtgtcggtgcgcagcactcagcgcaggcgtgcgcccgtaaaaagc 24

Query: 862 actccaaaccgtccagagaacat 884
      |||||||||||||||||||
Sbjct: 23 actccaaaccgtccagagaacat 1
```

Figure A.52: Clone C13  
108



```

Score = 1616 bits (815), Expect = 0.0
Identities = 850/857 (99%), Gaps = 4/857 (0%)
Strand = Plus / Minus

Query: 26  gtgngccaaaaacgtccacogttgcttcgectgcattecccgccgtogaattgatccctc 85
      ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 855  gtgagccaaaaacgtccacogttgcttcgectgcattecccgccgtogaattgatccctc 796

Query: 86  gatgagttcaatatccgacgcogtgacactggctccgggtgc cactgggtgatccagtatte 145
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 795  gatgagttcaatatccgacgcogtgacactggctccgggtgc cactgggtgatccagtatte 736

Query: 146  tggacttggetggetgaaatccaaagtc catgggtggcaaccgggggacgatgtcgttcaa 205
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 735  tggacttggetggetgaaatccaaagtc catgggtggcaaccgggggacgatgtcgttcaa 676

Query: 206  gtgtgtaacgcggaagttogctccagatccctggctgggtgatgtgctcggccagcgcata 265
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 675  gtgtgtaacgcggaagttogctccagatccctggctgggtgatgtgctcggccagcgcata 616

Query: 266  gtttccgactcgaggacatccatagggtgtacagt tcaacgc tataaacogtcatttcgcaa 325
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 615  gtttccgactcgaggacatccatagggtgtacagt tcaacgc tataaacogtcatttcgcaa 556

Query: 326  gaccggttgcctccagtgtagc caatgcgcgccccaagctgt gcccggtgaagtagagggt 385
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 555  gaccggttgcctccagtgtagc caatgcgcgccccaagctgt gcccggtgaagtagagggt 496

Query: 386  atagccogaatacogtgcctcatcgcggacttgatcttgcctcgcagattgtctgcagcggc 445
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 495  atagccogaatacogtgcctcatcgcggacttgatcttgcctcgcagattgtctgcagcggc 436

Query: 446  ttcccatgcctccagaatccagtggaaccttgacagccagtacagaggctcatcgttattc 505
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 435  ttcccatgcctccagaatccagtggaaccttgacagccagtacagaggctcatcgttattc 376

Query: 506  ttgcaggatgaagtcgagatcagcaatccagttcttgatgggtgc tactgcctcggaaagge 565
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 375  ttgcaggatgaagtcgagatcagcaatccagttcttgatgggtgc tactgcctcggaaagge 316

Query: 566  gaccacgagccgcttggtggtggtgctcgcggccaggaaaccggctgtgcctccaaagtc 625
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 315  gaccacgagccgcttggtggtggtgctcgcggccaggaaaccggctgtgcctccaaagtt 256

Query: 626  atttgtcaggtcaaaactcagcagcatcttggtgctcgcctcctcgaactgatggacaggc 685
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 255  atttgtcaggtcaaaactcagcagcatcttggtgctcgcctcctcgaactgatggacaggc 196

Query: 686  gtggccggtgcatgtcacggttagagtcgtccgagtcgatattgttcgagcaataagctgc 745
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 195  gtggccggtgcatgtcacggttagagtcgtccgagtcgatattgttcgagcaataagctgc 136

Query: 746  gggcagaccat tgcgagaacaattgcagctcctc caacgtggaaagtcgagacactccgca 805
      ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 135  -ggcagaccat tgcgagaacaattgcagctcctc caacgtggaaagtcgagacactccgca 77

Query: 806  catcaagtggntgtcgggtgccgcgacactcagcgcagcgtgcccgtcaaaagcactcc 865
      ||||||||| ||||||| ||||||||||||||||||||||||||||||||
Sbjct: 76  catcaagtgg-tgtcgggtg-cgcgacactcagcgcagcgtgcccgtcaaaagcact-c 20

Query: 866  caaanogtccagagaac 882
      |||| |||||||||||
Sbjct: 19  caaacogtccagagaac 3

```

**Figure A.53:** Clone C14

Score = 1681 bits (848), Expect = 0.0  
Identities = 860/864 (99%)  
Strand = Plus / Minus

```
Query: 14  gtaccacaagt gagccaaaacgtc caccggtgct tegcctgcat tcccgccgtcgaatt 73
          |||
Sbjct: 864  gtaccacaagt gagccaaaacgtc caccggtgct tegcctgcat tcccgccgtcgaatt 805

Query: 74  gattccctcgatgagttcaatatacgcgcgctgacactggctccgggtgccactggatgat 133
          |||
Sbjct: 804  gattccctcgatgagttcaatatacgcgcgctgacactggctccgggtgccactggatgat 745

Query: 134  ccagtattctggacttggctggetgaatccaaagtcacatgggtggcaaccgggggacgat 193
          |||
Sbjct: 744  ccagtattctggacttggctggetgaatccaaagtcacatgggtggcaaccgggggacgat 685

Query: 194  gtggttcaagtgtgtagcgcgggaagttcgetccagatccctggctgggtgatgtgctcggc 253
          |||
Sbjct: 684  gtggttcaagtgtgtaacgcgggaagttcgetccagatccctggctgggtgatgtgctcggc 625

Query: 254  cagcgcatagtttccgactcgaggacatccataggtgtacagttcaacgcataaaccgtc 313
          |||
Sbjct: 624  cagcgcatagtttccgactcgaggacatccataggtgtacagttcaacgcataaaccgtc 565

Query: 314  atttcgcaagaccggttgc tccagtgtagccaatgcgcgc ccaagctgtgccggatgaa 373
          |||
Sbjct: 564  atttcgcaagaccggttgc tccagtgtagccaatgcgcgc ccaagctgtgccggatgaa 505

Query: 374  gtagagggatagccgaatacgtgctcctcgcggacttgatcttgcctgctcaggattgct 433
          |||
Sbjct: 504  gtagagggatagccgaatacgtgctcctcgcggacttgatcttgcctgctcaggattgct 445

Query: 434  tgcagcggcttccatgcctccagaatccaagtgtgaaccttgcagccagttacagaggtc 493
          |||
Sbjct: 444  tgcagcggcttccatgcctccagaatccaagtgtgaaccttgcagccagttacagaggtc 385

Query: 494  atcgttatcttgccaggtgaaagtcgagatcagcaatccagttcttgatgggtgctactgcc 553
          |||
Sbjct: 384  atcgttatcttgccaggtgaaagtcgagatcagcaatccagttcttgatgggtgctactgcc 325

Query: 554  tcggaaggcgcacacgagccgcttgttgggtgtgtccgcggccaggaaaccggctgtgcc 613
          |||
Sbjct: 324  tcggaaggcgcacacgagccgcttgttgggtgtgtccgcggccaggaaaccggctgtgcc 265

Query: 614  tccaaagtatttgtcaggtaaaactccagcagcctccttgggtgctcgcctcctcagctga 673
          |||
Sbjct: 264  tccaaagtatttgtcaggtaaaactccagcagcctccttgggtgctcgcctcctcagctga 205

Query: 674  tggacaggcgtcggcctgcatgtcacgtagagtcgtccagtcgatattggtcagagca 733
          |||
Sbjct: 204  tggacaggcgtcggcctgcatgtcacgtagagtcgtccagtcgatattggtcagagca 145

Query: 734  ataaactcggcagaccattgcgagaacaatgcagctcatccaactggaagcagagac 793
          |||
Sbjct: 144  ataaactcggcagaccattgcgagaacaatgcagctcatccaactggaagcagagac 85

Query: 794  actcgcacatcaagtgggtcggtgcgcagcactcagcgcagcgtgcccgtcaaaag 853
          |||
Sbjct: 84  actcgcacatcaagtgggtcggtgcgcagcactcagcgcagcgtgcccgtcaaaag 25

Query: 854  cactccaaaccgtccagagaacat 877
          |||
Sbjct: 24  cactccaaaccgtccagagaacat 1
```

Figure A.54: Clone C15

Score = 1699 bits (857), Expect = 0.0  
 Identities = 859/860 (99%)  
 Strand = Plus / Minus

Query: 17 cacaagtgagc caaaaagtcaccgttgettegcctgcattcccgcgctegaattgatt 76  
 |||  
 Sbjct: 860 cacaagtgagc caaaaagtcaccgttgettegcctgcattcccgcgctegaattgatt 801

Query: 77 ccctcgatgagttcaataccgacgccgtgacac tggctccggg gccact ggtgatccag 136  
 |||  
 Sbjct: 800 ccctcgatgagttcaataccgacgccgtgacac tggctccggg gccact ggtgatccag 741

Query: 137 tattctggacttggctggctggaatccaaaagtcac tgggtggcaaccgggggacgatgtcg 196  
 |||  
 Sbjct: 740 tattctggacttggctggctggaatccaaaagtcac tgggtggcaaccgggggacgatgtcg 681

Query: 197 ttcaagtgtgtaacgccgaagttcgcctccagatccct ggct ggt gat gtgctcggccagc 256  
 |||  
 Sbjct: 680 ttcaagtgtgtaacgccgaagttcgcctccagatccct ggct ggt gat gtgctcggccagc 621

Query: 257 gcatagttccgac tcgaggacatcca taggtgtacagttcaacgctataacogtcattt 316  
 |||  
 Sbjct: 620 gcatagttccgac tcgaggacatcca taggtgtacagttcaacgctataacogtcattt 561

Query: 317 cgcaagaccgt tgc tcccagtgtagccaatgcgc cgc ccaagctgtgccgggtagtag 376  
 |||  
 Sbjct: 560 cgcaagaccgt tgc tcccagtgtagccaatgcgc cgc ccaagctgtgccgggtagtag 501

Query: 377 agggatagcc cgaatacgtgctcaccgcggacttgatctt gctcgtcagattgtctgca 436  
 |||  
 Sbjct: 500 agggatagcc cgaatacgtgctcaccgcggacttgatctt gctcgtcagattgtctgca 441

Query: 437 gggcttcccatgcttc cagaatccagtgtaacct tgcagccagtagaggtcatcg 496  
 |||  
 Sbjct: 440 gggcttcccatgcttc cagaatccagtgtaacct tgcagccagtagaggtcatcg 381

Query: 497 ttatcttgaggatgaagtcgagatcagcaatccagttctt gat ggtgctactgctcgg 556  
 |||  
 Sbjct: 380 ttatcttgaggatgaagtcgagatcagcaatccagttctt gat ggtgctactgctcgg 321

Query: 557 aaggcgaccacgagccgcttgttgggtgttgcgcggccaggaaccggctgtgctcca 616  
 |||  
 Sbjct: 320 aaggcgaccacgagccgcttgttgggtgttgcgcggccaggaaccggctgtgctcca 261

Query: 617 aagtatttgtcaggtcaaac tccagcagcatcttgggtgctcgcctcctc gactgatgga 676  
 |||  
 Sbjct: 260 aagtatttgtcaggtcaaac tccagcagcatcttgggtgctcgcctcctc gactgatgga 201

Query: 677 caggcgtcggc cgtgcatgcaacgttagagtcgtccagtcgatattgttcgagcaataa 736  
 |||  
 Sbjct: 200 caggcgtcggc cgtgcatgcaacgttagagtcgtccagtcgatattgttcgagcaataa 141

Query: 737 gctgcggcagaccattgc gagaacaat tgcagctcatccaa cgtggaagtcgagacactc 796  
 |||  
 Sbjct: 140 gctgcggcagaccattgc gagaacaat tgcagctcatccaa cgtggaagtcgagacactc 81

Query: 797 cgcaatcaagtggtgtc ggtgccgcagcactcagcgcagcgtgcgc cgtcaaaagcact 856  
 |||  
 Sbjct: 80 cgcaatcaagtggtgtc ggtgccgcagcactcagcgcagcgtgcgc cgtcaaaagcact 21

Query: 857 ccaaaccgtccagagaacat 876  
 |||  
 Sbjct: 20 ccaaaccgtccagagaacat 1

Figure A.55: Clone C16

Score = 1689 bits (852), Expect = 0.0  
 Identities = 857/859 (99%)  
 Strand = Plus / Minus

```

Query: 20  acaagtgnccaaaacatccacggttgettcgectgcattccccgccgtogaattgattc 79
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 859  acaagtgaagccaaaacgtccacggttgettcgectgcattccccgccgtogaattgattc 800

Query: 80  cctcgatgagtcaaatatccgacgccgtgacactggctccgggtgccactggtgatccagt 139
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 799  cctcgatgagtcaaatatccgacgccgtgacactggctccgggtgccactggtgatccagt 740

Query: 140  attctggacttggtggctgaatc caaagtc catgggtggcaac cgggggacgatgtcgt 199
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 739  attctggacttggtggctgaatc caaagtc catgggtggcaac cgggggacgatgtcgt 680

Query: 200  tcaagtgtgtaacgcggaagt tgcctccagatccctggctggtgatgtgctcggccagcg 259
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 679  tcaagtgtgtaacgcggaagt tgcctccagatccctggctggtgatgtgctcggccagcg 620

Query: 260  catagtttcgactcgaggacatccataggtgtacagttcaacgctaataccgtcatttc 319
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 619  catagtttcgactcgaggacatccataggtgtacagttcaacgctaataccgtcatttc 560

Query: 320  gcaagaccggttgcctccagtgtagccaatgcccgcgccaagctgtgcccgggtgaagtaga 379
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 559  gcaagaccggttgcctccagtgtagccaatgcccgcgccaagctgtgcccgggtgaagtaga 500

Query: 380  gggatagcccgaatacgtgctcaatgcggacattgatcttgctcgtcagattgtctgcag 439
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 499  gggatagcccgaatacgtgctcaatgcggacattgatcttgctcgtcagattgtctgcag 440

Query: 440  cggcttcccatgcttccagaatccagtgtaaccttgagccagtaacagaggtcatcgt 499
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 439  cggcttcccatgcttccagaatccagtgtaaccttgagccagtaacagaggtcatcgt 380

Query: 500  tatcttgcaggatgaagtgcagatcagcaatccagttcttgatggtgctactgctcggg 559
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 379  tatcttgcaggatgaagtgcagatcagcaatccagttcttgatggtgctactgctcggg 320

Query: 560  aggcgaccacagagccgcttgttggtgtgtgctcgcggccaggaaaacggctgtgctc 619
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 319  aggcgaccacagagccgcttgttggtgtgtgctcgcggccaggaaaacggctgtgctc 260

Query: 620  agttatttgcaggtcaaaactccaagcagcatcttgggtgctcgcctccctcactgatggac 679
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 259  agttatttgcaggtcaaaactccaagcagcatcttgggtgctcgcctccctcactgatggac 200

Query: 680  aggcgtcggccgtgcatgtcagcttagagtgctccagtgatattgttcgagcaataag 739
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 199  aggcgtcggccgtgcatgtcagcttagagtgctccagtgatattgttcgagcaataag 140

Query: 740  ctgcggcagaccatgcgagaacaattgcagctcctccaacgtggaagtcgagacactcc 799
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 139  ctgcggcagaccatgcgagaacaattgcagctcctccaacgtggaagtcgagacactcc 80

Query: 800  gcacatcaagtgggtgctgggtgcgagcactcagcgagcgtgctcgcgtcaaaaacac 859
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 79  gcacatcaagtgggtgctgggtgcgagcactcagcgagcgtgctcgcgtcaaaaacac 20

Query: 860  caaacgctccagagaacat 878
         |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct: 19  caaacgctccagagaacat 1

```

Figure A.56: Clone C18

Score = 1624 bits (819), Expect = 0.0  
Identities = 849/859 (98%), Gaps = 2/859 (0%)  
Strand = Plus / Minus

```
Query: 23  aagtgagccaaaacgtccaccgntgnttcgectgcatcccccgccgtcgaattgattccc 82
          |||
Sbjct: 857  aagtgagccaaaacgtccaccgntgnttcgectgcatcccccgccgtcgaattgattccc 798

Query: 83  tcgatgagttcaatatccgacgccgtgacactggctccgggtgccactggtgatccagtat 142
          |||
Sbjct: 797  tcgatgagttcaatatccgacgccgtgacactggctccgggtgccactggtgatccagtat 738

Query: 143  tctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgtcgttc 202
          |||
Sbjct: 737  tctggacttggctggctgaatccaaagtccatgggtggcaaccgggggacgatgtcgttc 678

Query: 203  aagtgtgtaacgcggaagtccgctccagatccctggctgggtgatgtgctcggccagcgc 262
          |||
Sbjct: 677  aagtgtgtaacgcggaagtccgctccagatccctggctgggtgatgtgctcggccagcgc 618

Query: 263  tagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgtcaatttcgc 322
          |||
Sbjct: 617  tagtttccgactcgaggacatccataggtgtacagttcaacgctataaccgtcaatttcgc 558

Query: 323  aagaccgttgcctccagtgtagccaatgcccgcgccaagctgtgcccggtagtagagg 382
          |||
Sbjct: 557  aagaccgttgcctccagtgtagccaatgcccgcgccaagctgtgcccggtagtagagg 498

Query: 383  gtatagccogaatacgtgctcatcgccgacttgatcttgctcgtcagattgtctgcagcg 442
          |||
Sbjct: 497  gtatagccogaatacgtgctcatcgccgacttgatcttgctcgtcagattgtctgcagcg 438

Query: 443  gcttcccatgcttccagaatccagtgtagaaccttgcagccagtacagaggtcatcgta 502
          |||
Sbjct: 437  gcttcccatgcttccagaatccagtgtagaaccttgcagccagtacagaggtcatcgta 378

Query: 503  tcttgcaggatgaaatcgagatcagcaatccagttcttgatggtgctactgcctcggaag 562
          |||
Sbjct: 377  tcttgcaggatgaaatcgagatcagcaatccagttcttgatggtgctactgcctcggaag 318

Query: 563  gcgaccacgagccgcttgttgggttgtcccgccgcaaggaaaccggctgtgcctccaaag 622
          |||
Sbjct: 317  gcgaccacgagccgcttgttgggttgtcccgccgcaaggaaaccggctgtgcctccaaag 258

Query: 623  ttatttgcaggtcaaaatccagcagcatcttgggtgctcgcctcctcagctgatggacag 682
          |||
Sbjct: 257  ttatttgcaggtcaaaatccagcagcatcttgggtgctcgcctcctcagctgatggacag 198

Query: 683  gcgtcggccgtgcatgtcagtttagtgctccagtcgatattgttcgagcaataagct 742
          |||
Sbjct: 197  gcgtcggccgtgcatgtcagtttagtgctccagtcgatattgttcgagcaataagct 138

Query: 743  gcggcagaccattgcgagaacaattgcagctcatccaacgtgnaagtcgagacactccgc 802
          |||
Sbjct: 137  gcggcagaccattgcgagaacaattgcagctcatccaacgtgnaagtcgagacactccgc 78

Query: 803  acatcaantnnngtcggtgccgagcactcagcgcagcgtgcgcgctcaaaaagcactcc 862
          |||
Sbjct: 77  acatcaagtggtgctcggtgccgagcactcagcgcagcgtgcgcgctcaaaaagcactcc 19

Query: 863  aaaancgtccagagaacat 881
          |||
Sbjct: 18  -aaaccgtccagagaacat 1
```

Figure A.57: Clone C20