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# Cecropins as a marker of Spodoptera frugiperda immunosuppression during entomopathogenic bacterial challenge

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#### Abstract :

An antimicrobial peptide (AMP) of the cecropin family was isolated by HPLC from plasma of the insect pest, *Spodoptera frugiperda*. Its molecular mass is 3910.9 Da as determined by mass spectrometry. Thanks to the EST database Spodobase, we were able to describe 13 cDNAs encoding six different cecropins which belong to the sub-families CecA, CecB, CecC and CecD. The purified peptide identified as CecB1 was chemically synthesized (syCecB1). It was shown to be active against Gram-positive and Gram-negative bacteria as well as fungi. Two closely related entomopathogenic bacteria, *Xenorhabdus nematophila* F1 and *Xenorhabdus mauleonii* VC01<sup>T</sup> showed different susceptibility to syCecB1. Indeed, *X. nematophila* was sensitive to syCecB1 whereas *X. mauleonii* had a minimal inhibitory concentration (MIC) eight times higher. Interestingly, injection of live *X. nematophila* into insects did not induce the expression of AMPs in hemolymph. This effect was not observed when this bacterium was heat-killed before injection. On the opposite, both live and heat-killed *X. mauleonii* induced the expression of AMPs in the hemolymph of *S. frugiperda*. The same phenomenon was observed for another immune-related protein lacking antimicrobial activity. Altogether, our data suggest that *Xenorhabdus* strains have developed different strategies to supplant the humoral defense mechanisms of *S. frugiperda*, either by increasing their resistance to AMPs or by preventing their expression during such host-pathogen interaction.

#### **Graphical abstract :**



#### Highlights

▶ We first describe the *Spodoptera frugiperda* cecropin family (*Sf*Cec). ▶ *Sf*Cec family is composed of 12 members. ▶ Two bacteria of *Xenorhabdus* genera show different susceptibility to *Sf*Cec. ▶ *Xenorhabdus* sp. set up different strategies to overcome insect innate immunity.

Keywords : Humoral immunity ; Innate immunity ; Immunosuppression ; Lepidopteran ; Invertebrate

26 Introduction

To fight infection, insects rely on multiple innate defence reactions which include the use of physical barriers together with local and systemic immune responses. These involve phagocytosis and encapsulation by hemocytes (Costa et al., 2005; Russo et al., 1996), proteolytic cascades leading to coagulation (Loof et al., 2011) and melanisation (Kanost et al., 2004), and *de novo* secretion of antimicrobial peptides (AMPs) (Lemaitre and Hoffmann, 2007). AMPs are widely distributed among living organisms (Garcia-Olmedo et al., 1998; Zasloff, 2002).

Since the first report of an inducible defense reaction in Drosophila (Boman et al., 1972), AMPs have been identified and characterized from the main insect orders Diptera (Kylsten et al., 1990), Lepidoptera (Dickinson et al., 1988; Steiner et al., 1981), Coleoptera (Bulet et al., 1991), Hemiptera (Cociancich et al., 1994) and Hymenoptera (Casteels et al., 1989). On the basis of sequence and structural features, these peptides have been classified into three broad classes: (i) linear peptides with amphipathic  $\alpha$ -helices, (ii) cysteine-stabilized  $\alpha$ -helical/ $\beta$ -sheet motif containing peptides and (iii) linear peptides with an overrepresentation in proline and/or glycine residues (Bulet and Stocklin, 2005).

Cecropins are among the best known  $\alpha$ -helical cationic antimicrobial peptides. Mature cecropins are composed of highly basic amino acid residues that can fold into two amphiphatic  $\alpha$ -helices and integrate into the acidic cell membranes of bacteria leading to their disruption (Steiner et al., 1988). Recent works indicate that cecropins seem to be a target of insect pathogens. Indeed, the bacterial-challenged induction of cecropins from the lepidoptera Plutella xylostella was shown to be inhibited by a symbiotic polyDNA virus (CpBV) of the hymenoptera endoparasitoid Cotesia plutellae (Barandoc et al., 2010). On the other hand, Xenorhabdus nematophila, a Gram-negative bacterium belonging to the Enterobacteriaceae family, kills various insects. These bacteria form a species-specific mutualistic association with the entomopathogenic nematode, Steinernema carpocapsae (Thomas and Poinar, 1979) and are transported and released by nematode vectors into the hemocoel (body cavity) of

insect hosts. *Xenorhabdus* initially colonizes the connective tissue surrounding the anterior midgut and hemolymph (bloodstream) of the Lepidoptera *Spodoptera littoralis* (Sicard et al., 2004), leading to the death of the insect, probably due to a combination of the effects of toxins and septicaemia. *X. nematophila* can independently kill insects and grow within their bodies if introduced into the hemolymph by direct injection (Sicard et al., 2004). *X. nematophila* proliferates in the hemolymph before the insect dies and must therefore be able to escape the insect immune response. Hence, it was shown that *Xenorhabdus* inhibits the expression of cecropins (Ji and Kim, 2004). This indicates that different pathogens might use a common strategy in order to establish their pathogenicity. In this work, we purified a cecropin from the hemolymph of bacterial-challenged larvae of the crop pest, *Spodoptera frugiperda*. The analysis of Spodobase

(http://bioweb.ensam.inra.fr/spodobase/), a Spodoptera specific cDNA database (Negre et al., 2006), allowed the identification of 13 distinct nucleotide sequences encoding members of the cecropin family. The deduced amino acid sequences led us to classify the S. frugiperda cecropins in 4 families (CecA, B, C and D) and to identify the purified cecropin as CecB1. CecB1 was chemically synthesized (syCecB1) and its antimicrobial activity was tested against a panel of microorganisms. Thus, we showed that two closely related entomopathogenic bacteria, Xenorhabdus nematophila F1 strain and *Xenorhabdus mauleonii* strain VC01<sup>T</sup>, were not sensitive to the same extent to syCecB1. Additionally, X. nematophila F1 had an inhibitory effect on cecropin expression whereas X. mauleonii VC01<sup>T</sup> did not have such an effect. This difference between the two Xenorhabdus strains could be extended to the global antimicrobial activity present into the hemolymph of infected larvae. Finally, the same results were obtained when we followed the outcome of Spod-11-tox, an immune-related protein lacking antimicrobial activity and mainly expressed by hemocytes (Destoumieux-Garzon et al., 2009). Altogether, these results showed that the two entomopathogenic Xenorhabdus strains use two different strategies in order to circumvent the humoral immune response of their insect host.

#### 1. Materials and Methods

#### 1.1. Insects and Immune Challenge

Spodoptera frugiperda were reared on artificial diet (Pagès and Ginibre, 2006) at 23°C with a photoperiod of 12 h. One day-old sixth-instar larvae were used for the expression studies. Larvae used for antimicrobial peptide purification were bacterial-challenged with Escherichia coli CIP7624 (Gram negative) and *Micrococcus luteus* CIP5345 (Gram positive)(10<sup>6</sup> bacteria/larva). Eight hours post challenge, hemolymph was collected from a cut abdominal proleg into ice-cold anticoagulant buffer (69 mM KCl, 27 mM NaCl, 2 mM NaHCO<sub>3</sub>, 100 mM D-glucose, 30 mM tripotassium citrate, 26 mM citric acid, 10 mM Na<sub>2</sub>-EDTA, pH 4.6, 420 mOsm)(van Sambeek and Wiesner, 1999). This time point was chosen because Girard et al. (2008) previously showed that this was the time at which maximum expression of antimicrobial peptides occurred. 

Experimental infections were also performed by injection of 10<sup>6</sup> PBS-washed *Xenorhabdus nematophila* strain F1 or *Xenorhabdus mauleonii* strain VC01<sup>T</sup> (from the laboratory collection). The
use of this high dose of bacteria was to avoid difference of virulence of the *Xenorhabdus* sp.
In some cases, the bacteria were heat killed by 10 min incubations at 95°C.

1.2. Plasma (cell-free hemolymph) Purification Procedure as described in (Destoumieux-Garzon et al., 2009)

1.2.1. Extraction and pre-purification by solid-phase extraction (SPE)

Hemocytes and plasma were separated by centrifugation at 600 x g during 30 sec at 4°C. Plasma
from bacterial-challenged larvae (28 mL) was acidified to pH 2 with 1 M HCl. The acidic extraction
was performed overnight under gentle shaking at 4°C. After centrifugation (16,000 x g for 30 min at 4
°C), the supernatant was pre-purified by SPE on Sep-Pak C<sub>18</sub> cartridges (Waters) equilibrated with
0.05% trifluoroacetic acid (TFA). Elutions were performed with 10%, 40% and 80% acetonitrile in
acidified water. All fractions were freeze-dried in a vacuum centrifuge (Speed-Vac, Cryo Rivoire) and
subsequently reconstituted with MilliQ water at 1/20 of the initial hemolymph volume.

1.2.2. HPLC purification

The 40% Sep-Pak fractions were subjected to reverse phase chromatography on an
UP5NEC25QS column (Interchim) equilibrated in 0.05% TFA. Separation of the 40% Sep-Pak fractions
was performed with a linear gradient of 0-60% acetonitrile in acidified water over 80 min at a flow
rate of 1 mL/min.

Antimicrobial activity-containing fractions were purified on the same reversed phase column as
above at a controlled temperature of 35°C. The linear biphasic gradient was composed of 0%-28%
acetonitrile in 0.05% TFA over 5 min, and of 28%-45% over 55 min at a flow rate of 1 mL/min.
When needed, the fractions of interest were subjected to a final purification step on a narrowbore reversed phase column (Xbridge BEH130, Waters Associates) at 40°C with a flow rate of 0.25
mL/min using the biphasic gradients described above.

All HPLC purifications were performed on a Waters HPLC system (Waters 600 pump) equipped
 with a photodiode array (Waters 996 PDA). Column effluent was monitored by its UV absorption at
 225 nm. Fractions corresponding to absorbance peaks were hand collected in polypropylene tubes
 (Microsorb 75 mm x 12 mm, Nunc immunotubes), concentrated under vacuum (Savant) and
 reconstituted in MilliQ water (Millipore<sup>™</sup>) before antimicrobial activity testing.

1.3. Antimicrobial Assays.

Antimicrobial activity was assayed against Gram positive bacteria, Gram negative bacteria and fungi (listed in Table 2) based on the liquid growth inhibition assay described previously (Hetru and Bulet, 1997). Poor Broth (PB : 1% bacto-Tryptone, 0.5% NaCl w/v, pH 7.5), and ½ Potato Dextrose Broth (Difco) were used for bacterial and fungal growth, respectively. Growth of bacteria was monitored spectrophotometrically at 600 nm on a multifunctional microplate reader (Tecan infinite 200) while fungal growth was evaluated after 24 and 48 hours at 30°C by optical microscopy and measurement of the culture absorbance at 595 nm. MIC values are expressed as the lowest concentration tested that caused 100% of growth inhibition (micromolar). The bactericidal activity of the peptide was assessed by plating cultures. The absence of CFUs on agar plates, after an overnight
 incubation at 30°C, was considered indicative of a bactericidal effect.

128 1.4. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry

Mass spectrum was acquired on Ultraflex (Bruker) in positive mode. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. A solution of  $\alpha$ -cyano-4-hydroxycinnamic acid (10 mg/ml) used as matrix and a product solution were mixed in a ratio of 1:1. A 1  $\mu$ L aliquot of the matrix/product mixture was deposited and air dried. External mass calibrations were performed with a standard peptide mixture. The analyses were recorded in reflector mode. Mass spectrum was analyzed with Flex Analysis software.

1.5. Dot-blot assay

The presence of cecropins in *Spodoptera frugiperda* plasma from treated or untreated larvae
was evaluated by spotting 3 µL of 40% ACN Sep-Pak fraction on a PVDF membrane (0.22 µm, ImmunBlot<sup>™</sup>, Bio-Rad). Membranes were probed with rabbit anti-*Bombyx mori* CecB antibodies (Acris
Antibodies, Germany) diluted at 300 ng/mL in PBS containing 0.05% Tween-20 and 1% BSA. A
horseradish peroxidase coupled anti-rabbit IgG (GE Healthcare) was used at a 1/5,000 dilution for
detection by chemiluminescence (ECL Western blotting detection, GE Healthcare) and finally, the
PVDF membrane was exposed to Hyperfilm-ECL (GE Healthcare).

143 1.6. Enzyme-linked immunosorbent assay (ELISA)

Spod-11-tox content in the plasma of challenged and unchallenged larvae was measured by
ELISA. Eight hours post-challenge, larvae were bled from a cut abdominal proleg into 150 µL of icecold anti-coagulant buffer. Tubes were weighed before and after bleeding to measure the volume of
hemolymph from each larva. Hemocytes were separated from plasma by centrifugation (600 x g for
30 sec at 4°C). For ELISA, microtiter plates (Maxisorp Nunc-Immuno Plate<sup>™</sup>) were coated with plasma
(50 µL/well), and incubated overnight at room temperature, washed three times with 0.05% Tween-

20 in PBS (PBS-T) and blocked by incubation with 0.25% BSA in PBS-T for 2 h at room temperature. <sup>2</sup> 151 Anti-Spod-11-tox antibodies (100 ng/mL in PBS-T containing 1% BSA) were added to each well and the plates were incubated for 1 h at room temperature. The plates were washed four times with PBS-T and incubated for 1 h with peroxidase-linked donkey anti-rabbit IgG (1/5,000 dilution; GE Healthcare). The plates were washed four times with PBS-T and 100 µL of 1-Step™ Ultra TMB-ELISA (Pierce) solution was added to each well. Color development was stopped after 30 min by the addition of 100  $\mu$ L of 2 M H<sub>2</sub>SO<sub>4</sub>, and absorbance at 450 nm was measured with a microplate reader (Tecan Infinite 200).

1.7. Production of synthetic S. frugiperda CecB1 

> Synthetic CecB1 peptide was produced by NeoMPS S.A. (Strasbourg, France) using the t-Boc solid-phase peptide synthesis technology.

### 162 2. Results

#### 163 2.1. Purification of a Spodoptera frugiperda cecropin

In previous work, we detected several antimicrobial activities in the plasma (cell-free hemolymph) of bacterial-challenged S. frugiperda larvae (Destoumieux-Garzon et al., 2009). To further characterize the molecules responsible for those activities, we used a classical procedure for purification of antimicrobial peptides (AMPs) (as described in Materials and Methods). Nine fractions were found to contain antimicrobial activity against the three microorganisms tested (Figure 1a). A fraction that eluted around 40% acetonitrile (indicated by an arrow on Figure 1a) was further purified (Figure 1b). The molecular weight and the N-terminal sequence of the purified molecule were determined by MALDI-TOF-MS (Figure 1c) and Edman degradation (Figure 1d), respectively. Both, the MS data (one single ion at m/z=3,910.9) and the single N-terminal sequence, Arg-Xaa-Lys-Phe-Phe-Lys-Lys-Ile-Glu-Lys-, showed the purity of the collected peptide. These data are consistent with the isolation of a cecropin.

2.2. Spodoptera frugiperda genome contains genes encoding members of the antimicrobial peptide cecropin family

In order to further characterize the purified peptide, we analyzed the Spodobase, a Spodoptera
ESTs database (http://bioweb.ensam.inra.fr/spodobase/). We identified 189 sequences
corresponding to cecropin transcripts. These sequences were clustered by the use of Seqman
(Lasergene, DNASTAR inc.) into 13 different contigs whose characteristics are summarized in Table 1.
Their lengths ranged from 433 bp up to 992 bp and the number of sequences in each contig varied
from 4 up to 31. On the other hand, the analysis of their nucleotide sequences allowed the
identification of five clusters within which contig sequences differed by few nucleotide substitutions
likely due to polymorphism as in cluster II (Supplementary Figure 2) or by the presence of gaps
mainly in the 3' UTR for clusters I, III and V which might indicate gene duplication (Supplementary

Figures 1, 3 and 5). However, the alignment of the deduced amino acid sequences (Figure 2)
indicated that the nucleotide sequences encode only 6 different polypeptides composed of 62 or 63
amino acids.

In order to classify the different members of the S. frugiperda cecropin family, deduced amino acid sequences of the mature polypeptides were aligned with a selection of cecropins from two Diptera (Drosophila melanogaster and Anopheles gambiae) and eight Lepidotera (Bombyx mori, Helicoverpa armigera, Hyalophora cecropia, Hyphantria cunea, Plutella xylostella, Spodoptera exigua, Spodotera litura and Trichoplusia ni) (Supplementary Table 1) using Seaview software (Gouy et al., 2010). The alignment was performed by clustalW (Larkin et al., 2007) and the phylogenetic tree (Figure 3) was obtained by Maximum Likelihood method (Guindon et al., 2010). The cladogram shows that all the lepidopteran cecropins cluster apart from the dipteran sequences. The previously described lepidopteran cecropins A, B, C and E cluster together, whereas cecropins D cluster in a separate clade. The S. frugiperda cecropins that are described here for the first time were named (Table 1) according to their positions in the cladogram. The previously described S. litura CecD however appears to be misnamed, clustering with the lepidopteran CecA/B sub-group.

Comparison of the amino acid sequences of *S. frugiperda* Cec with the N-terminal sequence obtained from the purified cecropin showed that it corresponded to CecB1 which is the only one that contains, in the mature peptide obtained after removal of the 26-residue signal peptide, a Phe and an lle at positions 4 and 8, respectively. According to the amino acid sequence deduced from its cDNA, *S. frugiperda* CecB1 should be a 36-residues peptide with a molecular mass of 3,951.7 Da, while the mass of the purified cecropin is 3,910.9 Da (Figure 1d). This strongly suggests that the mature peptide is generated by an enzymatic cleavage of the carboxy-terminal Gly residue and a Cterminal amidation as previously reported (Boman et al., 1989), and therefore, has a final molecular mass of 3,894.7 Da. The 16 Da difference found with the mass determined by MALDI-TOF is likely due to an oxidation of the peptide during the mass spectrometry analysis. The oxidation likely occurred on the Trp-residue in position 2, which gave no standard PTU-amino acid by Edman degradation (Xaain Figure 1d).

## 2.3. Antimicrobial activity of S. frugiperda CecB1

To investigate the antimicrobial activity of S. frugiperda CecB1, we used a chemically synthesized CecB1 (syCecB1) produced by the t-Boc solid-phase peptide synthesis technology. syCecB1 is a 35 amino acid peptide corresponding to the mature peptide formed by deletion of the C-terminal Gly and amidated on the Leu at 35 position (R-35-L-NH<sub>2</sub>). syCecB1 antimicrobial activity was determined against a panel of microorganisms, including Gram-positive and Gram-negative bacteria, and filamentous fungi. The MIC values obtained are reported in Table 2. Like native CecB1, the synthetic peptide was active against M. luteus, E. coli SBS363 and F. oxysporum, the three microorganisms used during the different purification steps of CecB1. syCecB1 was more active against Gram-negative bacteria than against Gram-positive bacteria as described for cecropins characterized in other insects (Bulet et al., 2003). syCecB1 had bactericidal effects against all the Gram-negative bacteria tested (Table 2). Interestingly, entomopathogenic microorganisms such as the Gram negative bacterium S. marcescens, Gram positive bacteria Bacillus sp., and the two fungi Beauveria bassiana and Nomuraea rileyi, appeared to be less susceptible to syCecB1 than non-pathogenic ones. Finally, X. nematophila

F1 and *X. mauleonii* VC01<sup>T</sup>, two closely related entomopathogenic bacteria were not sensitive to the
same extent. Indeed, the MIC value for *X. mauleonii* was eight times higher than the one of *X. nematophila*.

2.4. Effect of X. nematophila F1 and X. mauleonii VC01<sup>T</sup> infection on the induction of humoral
 response in S. frugiperda.

To investigate if the differential sensitivity of the two *Xenorhabdus* sp. to syCecB1 was correlated to different infection strategies, we evaluated the total Cec plasma content of *S*.

frugiperda larvae challenged by X. nematophila or X. mauleonii. Larvae were challenged with PBS or E. coli (CIP7624) as controls, or with live or heat-killed Xenorhabdus. The 40% Sep-Pak fractions (SPE40) (see Materials and Methods) were freeze-dried and reconstituted with MilliQ water at 1/20 of the initial hemolymph volume. Reconstituted SPE40 (3 µl) were spotted on nylon membrane which was then probed by commercial antibodies directed towards Bombyx mori cecropins (Figure 4). Immuno-reactivity was observed within the plasma of larvae challenged with heat-killed bacteria of the two strains, X. nematophila F1 and X. mauleonii VC01<sup>T</sup> as well as with live X. mauleonii VC01<sup>T</sup> and E. coli CIP7624. On the contrary, when insects were challenged with live X. nematophila F1, the level of immune-staining was similar to that found in unchallenged larvae. This indicates that larvae challenged with live *X. nematophila* F1 do not induce cecropins.

The above plasma samples were also used in liquid growth inhibition assays using E. coli SBS363 as bacterial target (Table 3). Results show that plasmas of larvae challenged with either live or heat-killed *X. mauleonii* VC01<sup>T</sup> displayed levels of total antimicrobial activity similar to that found in plasma from E. coli-challenged larvae. In larvae challenged with live X. nematophila F1, total plasma antimicrobial activity was only about 10% of that seen in insects challenged with X. mauleonii or E. coli. However, when X. nematophila bacteria were heat-killed, the antimicrobial activity reached a level comparable to that present in plasma of E. coli-challenged larvae. Again, these results suggest that larvae challenged with live X. nematophila F1 have a reduced expression of antimicrobial peptides. Finally, we quantified the production of Spod-11-tox, an immune-related protein lacking antimicrobial activity (Destoumieux-Garzon et al., 2009), in the plasma of larvae challenged by the two Xenorhabdus sp. (Figure 5). The plasma content of Spod-11-tox in E. coli-challenged larvae was 9 times higher than in unchallenged or PBS-injected larvae. Similar high levels of Spod-11-tox were measured in plasma of *X. mauleonii* VC01<sup>T</sup>-challenged larvae whether the bacteria were alive or dead. Conversely, Spod-11-tox plasma content of larvae injected with live X. nematophila F1 was comparable to that measured in plasma of unchallenged or PBS-challenged larvae whereas in larvae

challenged by heat-killed *X. nematophila*, Spod-11-tox plasma content was similar to that measured
in plasma of *E. coli*-challenged larvae.

Altogether, these results show that while *X. mauleonii* VC01<sup>T</sup> elicits a regular humoral response for insects challenged by bacteria, *X. nematophila* F1 prevents the expression of plasma-soluble immune-related peptides and proteins.

2.5. X. nematophila secretes an inhibitor of S. frugiperda humoral response.

266To test whether X. nematophila was able to prevent humoral immune response, E. coli bacteria267were injected alone or with a culture supernatant of X. nematophila (Table 4). The antimicrobial268activity measured in the hemolymph of such challenged insects was comparable to the one269measured in the hemolymph of insects challenged with PBS or live X. nematophila. In other words,270the presence of X. nematophila culture supernatant was able to inhibit the AMPs expression271observed when E. coli was injected alone. Altogether, these results suggest that the reduced272expression of antimicrobial activity observed above is due to a bacterial factor produced and273secreted by X. nematophila.

#### 3. Discussion

In this study, we have identified a family of cecropins (Cec) encoded by sequences present in Spodobase, a Spodoptera frugiperda specific EST library (Negre et al., 2006). This family appears to be composed of 13 members, 3 CecA, 7 CecB, one CecC and 2 CecD according to their cDNA sequences. Recently, 13 cecropin genes were found in the genome of Bombyx mori (Cheng et al., 2006; Tanaka et al., 2008), the only Lepidopteran genome available to date (The International Silkworm Genome Consortium, 2008). However, the 2 CecA and the 6 CecB silkworm cDNA encode only two amino acid sequences (Tanaka et al., 2008). In our work, we show that a similar situation is present in S. frugiperda, since the 3 CecA give only one protein as do 5 of the CecB cDNA. Therefore, we may suggest that Lepidopteran insects have a high degree of cecropin gene duplication. Moreover, since the database does not represent the whole S. frugiperda transcriptome, we cannot rule out the possibility that S. frugiperda genome might contain more cecropin genes. The accessibility of *S. frugiperda* genome would help to check this hypothesis. S. frugiperda Cec are 62 or 63 amino acids long in their prepro-forms which, after posttranslational modifications, generate mature polypeptides of 36, 37 and 42 amino acids that possess

one feature that is characteristic of most insect cecropins which is the presence of a tryptophan

residue in the first or second position. A TMHMM analysis

(http://www.cbs.dtu.dk/services/TMHMM/) indicates that, as already described for other insect cecropins (Bulet and Stocklin, 2005), all S. frugiperda Cec have a long N-terminal, basic and amphipathic  $\alpha$ -helix (residues 2 to 22 in the mature peptide, positions determined from CecA1 in Figure 2) and a shorter and more hydrophobic C-terminal helix (residues 25 to 34), linked by a highly conserved Gly<sup>23</sup>-Pro<sup>24</sup> hinge region.

A phylogenetic analysis conducted with 51 cecropins from a selection of Dipteran and Lepidopteran allowed the classification of Cec from S. frugiperda as members of the cecropin peptide <sub>57</sub> 299 families CecA, CecB, CecC and CecD. Lepidopteran cecropins were found distributed into two main **300** clades, one containing all types of cecropins except CecD. Lepidopteran CecD present several specific

features such as one highly conserved acidic amino acid, Glu<sup>6</sup> or Asp<sup>6</sup>, and a Ser<sup>20</sup> (positions determined from the CecD1 mature polypeptide in Figure 2). In addition, the highly conserved Ala-Pro-Glu-Pro sequence which precedes the mature peptide and corresponds to a pro-peptide removed in two steps by a dipeptidyl aminopeptidase in other cecropins (Boman et al., 1989), is absent from CecD sequences. This suggests that they are produced as mature active peptide directly after digestion by a signal peptidase at the carboxy-terminus of Ala<sup>-1</sup>. Finally, although this phylogenetic tree does not give a lot of information on the evolutionary history of the cecropin gene, it may indicate that some insects Cec have likely been wrongly named such as for example S. litura CecD that we propose to rename S. litura CecB according to its position in the cladogram. The  $\alpha$ -helical linear antimicrobial peptides of insects, such as cecropins, are mostly active against bacteria, with a higher efficacy on Gram negative than Gram positive strains (Choi et al., 2000; Kylsten et al., 1990; Samakovlis et al., 1990). They have also been shown to be active against some fungi (Ekengren and Hultmark, 1999). As expected, syCecB1 had a similar profile of antimicrobial activity. It is noteworthy that the two strains of entomopathogenic fungi tested here were not susceptible to syCecB1 at concentrations as high as 25  $\mu$ M. By contrast, entomopathogenic Xenorhabdus nematophila strain F1 was as sensitive as the most susceptible strains, Escherichia coli CIP7624 and *Micrococcus luteus*. In contrast, *X. mauleonii* VC01<sup>T</sup>, an insect pathogen closely related to X. nematophila, was much less sensitive to syCecB1. We therefore studied the effect of these two bacteria on the cecropins expression in S. frugiperda. Our results showed that X. nematophila F1 was able to inhibit the expression of cecropins in S. frugiperda. This is reminiscent of two recent studies showing that microorganisms such as a polyDNA bracovirus from Cotesia plutellae or X. nematophila inhibited the expression of cecropins in *Plutella xylostella* (Barandoc et al., 2010) and *Spodoptera* exigua (Ji and Kim, 2004), respectively. Moreover, X. nematophila had also an inhibitory activity on the expression of Spod-11-tox, an immune-related protein inducible by infection (Girard et al., 2008). Because only live X. nematophila controls the expression of host immune-related genes, it is likely that this occurs through the secretion by the bacteria of immunosuppressive products that prevent

the host immune response. This hypothesis is reinforced by the fact that the injection of supernatant from X. nematophila culture prevents the induction of antimicrobial activity by E. coli. It is known that X. nematophila secretes some inhibitory metabolites against insect immune-associated phospholipase A<sub>2</sub> (PLA<sub>2</sub>) to suppress both the cellular and humoral immune responses in the hemocoel of target insects (Kim et al., 2005; Song et al., 2011). However, Ji and Kim (Ji and Kim, 2004) demonstrated that the use of specific  $PLA_2$  inhibitors did not inhibit antimicrobial activity or cecropin gene expression when Spodoptera were infected with heat-killed X. nematophila suggesting that the inhibition of X. nematophila on the expression of the antimicrobial peptide is not related to the inhibition of the eicosanoid pathway. Consequently, the X. nematophila-induced inhibition of two different immune-related genes allows us to raise the hypothesis that the bacterial virulence factor may interact with an insect molecular target upstream of the genes, *cecropins* and *Spod-11-tox*. Over the past years, several works have been performed to study the interactions between insect pathogens and different defense mechanisms of insects. Hence, it was shown in Drosophila melanogaster that during the early steps of infection, the highly virulent Pseudomonas aeruginosa PA14 strain down-regulates not only cecropins but also IMD pathway-dependent AMPs such as attacins and defensin (Apidianakis et al., 2005). We are currently involved in the characterization of the immunosuppressive factor present in supernatant from X. nematophila culture.

Another major result from this study is that two closely related pathogens of insects have evolved divergent strategies to overcome the host defense reactions. Indeed, while *X. nematophila* evades the host antimicrobial response by repressing the expression of AMPs, the other pathogen, *X. mauleonii* strain VC01<sup>T</sup>, induces cecropin expression but is resistant to high concentration of SfCecB1. Therefore, these two species of entomopathogenic bacteria use different strategies to evade the host antimicrobial response either by developing AMP resistance mechanisms or by preventing AMP expression.

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11 12	357	Nucleotide sequences reported in this paper have been submitted to the GenbankTM/EBI Data
13 14 15	358	Bank with the following accession numbers DY773722, DY773924, DY776416, DY774879, DY778990,
16 17	359	DY774910, DY775152, DY779994, DY775699, DY774316 and DY777111 for Sf1F00836, Sf1F01169,
18 19 20	360	Sf1F04795, Sf1F02507, Sf1F09170, Sf1F02553, Sf1F02902, Sf1F11023, Sf1F03731, Sf1F01749 and
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**Figure legends** 

Figure 1: Cecropin purification from fractionated *S. frugiperda* plasma.

(a) a 40% Sep-Pak fraction from the extraction of immunized *S. frugiperda* plasma was subjected to reversed phase HPLC on a UPNEC25QS column (Interchim) using a 0-60% linear gradient (dashed line) of acidified acetonitrile over 80 min. Abs, Absorbance monitored at 225 nm. Antimicrobial activity against *Escherichia coli* SBS363 (black rectangles), *Micrococcus luteus* CIP5345 (grey rectangles) and *Fusarium oxysporum* (white rectangles) was measured by liquid growth inhibition assays. (b) Final purification step with a Xbridge BEH130 narrowbore column with a 0-28-45% biphasic gradient of acidified acetonitrile over 5 and 40 min. (c) The molecular mass of the purified peptide was measured by MALDI-TOF MS (Ultraflex). (d) The N-terminal sequence of the purified peptide was determined by Edman degradation.

**Figure 2**: Alignment of deduced amino acid sequences of mature cecropins from *Spodoptera frugiperda*. Sequences were analysed by ClustalW. Arrow head indicates maturation cleavage site. Asterisks indicate differences between CecD1 (Sf1F01749) and CecD2 (Sf1F05978). Identical residues are indicated by Dots.

**Figure 3**: Phylogenetic analysis of *S. frugiperda* cecropins. 39 amino acid sequences of mature cecropins from two Dipteran (*Drosophila melanogaster* and *Anopheles gambiae*) and eight Lepidopteran (*Bombyx mori, Helicoverpa armigera, Hyalophora cecropia, Hyphantria cunea, Plutella xylostella, Spodoptera exigua, Spodotera litura* and *Trichoplusia ni*) were aligned together with *Spodoptera frugiperda* cecropins using ClustalW and the phylogenetic tree was obtained by the Maximum Likelyhood method. Bootstrap values of 1000 trials are indicated as numbers. Sequences used in this analysis are listed in Supplementary Table 1.

**Figure 4**: Effect of *Xenorhabdus nematophila* F1 and *Xenorhabdus mauleonii* VC01<sup>T</sup> on the expression of *Spodoptera frugiperda* cecropins. Fractionated plasma samples (40% ACN eluted fraction after

SEP) of *S. frugiperda* after challenges as indicated (at least 20 larvae per challenge condition) were spotted on nylon membrane. Then, immuno-blot was stained with anti-*Bombyx mori* CecB antibodies (Acris Antibodies, Germany). U.C.: unchallenged, P: PBS-challenged, L: live, H: heat-killed, *X. n.*: *Xenorhabdus nematophila* F1, *X. m.*: *Xenorhabdus mauleonii* VC01<sup>T</sup>, *E. c.*: *Escherichia coli* CIP7624. Samples used were: reconstituted plasmas (RP) or plasmas diluted 1:1 or 1:10 in PBS as indicated to the left.

**Figure 5**: Effect of *Xenorhabdus nematophila* F1 and *Xenorhabdus mauleonii* VC01<sup>T</sup> on Spod-11-tox plasma content. Spod-11-tox content in *S. frugiperda* plasmas was measured by ELISA as described in material and methods section. For each experimental condition, Spod-11-tox content was individually measured into the plasma of at least 20 larvae. Values represent the mean of three independent experiments while error bars show standard deviation. \*p < 0.001 (as determined by Student t-test) compared to PBS injected larvae. U.C.: unchallenged, P: PBS-challenged, L: live, H: heat-killed, *X. n.: Xenorhabdus nematophila* F1, *X. m.: Xenorhabdus mauleonii* VC01<sup>T</sup>, *E. c.: Escherichia coli* CIP7624.

**Supplementary Figures 1 to 5:** Cecropin-encoding contigs (see Table 1) present in the Spodobase (Negre et al., 2006) were aligned using Clustal W. Identical nucleotides in Clusters I, II and V are boxed. In the case of Cluster III, boxes indicate residues which are identical in, at least, 3 sequences. ORFs are indicated by the black line.

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Table 1

Contig	Name	Nbr of EST	Length	Best BLAST	E_value	Score	Deduced peptide (AA)
Sf1F00836	CecA1	14	498	AF142341.1 Antimicrobial protein cecropin A [S. litura]	4e-31	117	62
Sf1F01169	CecA2	16	494	AF142341.1 Antimicrobial protein cecropin A [S. litura]	4e-36	117	62
Sf1F04795	CecA3	31	505	AF142341.1 Antimicrobial protein cecropin A [S. litura]	1e-31	117	62
Sf1F02507	CecB1	25	471	GU182910.1 Antimicrobial protein cecropin 3 [H. armigera]	4e-21	108	62
Sf1F09170	CecB1	4	436	GU182910.1 Antimicrobial protein cecropin 3 [H. armigera]	4e-21	108	62
Sf1F02553-1	CecB4	10	598	AF142342.1 Antibacterial protein cecropin B [S. litura]	2e-17	97	63
Sf1F02553-2	CecB2	9	992	AF142342.1 Antibacterial protein cecropin B [S. litura]	2e-19	94	63
Sf1F02902	CecB3	10	915	AF142342.1 Antibacterial protein cecropin B [S. litura]	3e-18	96	63
Sf1F11023-1	CecB5	12	804	AF142342.1 Antibacterial protein cecropin B [S. litura]	3e-25	123	63
Sf1F11023-2	CecB6	10	858	AF142342.1 Antibacterial protein cecropin B [S. litura]	1e-15	93	63
Sf1F03731	CecC	14	433	GU182909.1 Antibacterial peptide cecropin 2 [H. armigera]	2e-18	97	63
Sf1F01749	CecD1	15	505	EU041763.1 Antimicrobial peptide CecD [H. armigera]	6e-25	121	63
Sf1F05978	CecD2	19	498	EU041763.1 Antimicrobial peptide CecD [H. armigera]	8e-24	117	63

**Table 1:** Analysis of the SpodoBase, a *Spodoptera* EST database (Negre et al., 2006). This database is composed of cDNA sequences from three different tissues (hemocytes, fat body and midgut) as well as the cell line Sf9.

Clusters and deduced amino acid sequences were determined using SeqMan and EditSeq (Software suite, Lasergene, DNASTAR inc.), respectively. tblastx were performed at NCBI (<u>http://blast.ncbi.nlm.nih.gov/Blast.cgi</u>).

Micro-organisms	MIC (µM)	Activity
Gram positive bacteria		
Bacillus cereus CIP14579*	20	Bacteriostatic
Bacillus thuringiensis*	20	Bactericidal
Micrococcus luteus CIP5345 <sup>⊕</sup>	1.25	Bactericidal
Staphylococcus aureus CIP103428	20	Bacteriostatic
Gram negative bacteria		
Escherichia coli CIP7624	1.25	Bactericidal
Escherichia coli SBS363 <sup>⊕</sup>	0.08	Bactericidal
Klebsiella pneumoniae 100633	10	Bactericidal
Salmonella enterica CIP5858	10	Bactericidal
Serratia marcescens 363*	> 20	n. d.
X. mauleonii VC01 <sup>™</sup> *	10	Bactericidal
X. nematophila F1*	1.25	Bactericidal
Fungi and Yeast		
Fusarium oxysporum <sup>⊕</sup>	2.5	Fungicidal
Beauveria bassiana $^{st}$	> 25	n. d.
Nomuraea rileyi <sup>*</sup>	> 25	n. d.

Table 2: Activity of synthetic Cecropin B1 on various micro-organisms.

 $^{\boldsymbol{\vartheta}}$  Used during the different purification steps.

\* Entomopathogenic microorganisms.

n. d., not determined

				Larval t	treatment		
<b>-</b> • .			- "	X. ne	matophila	Х. п	nauleonii
Experiments	<b>U.C.</b>	PBS	E. COli	Live	Heat-killed	Live	Heat-killed
N° 1	1/4	1/16	1/256	1/8	1/128	1/64	1/64
N° 2	1/4	1/2	1/128	1/16	1/128	1/128	1/256
N° 3	1/4	1/8	1/128	1/8	1/64	1/64	1/128

Table 3: Effect of *Xenorhabdus nematophila* F1 and *Xenorhabdus mauleonii* VC01<sup>T</sup> on the expression of *Spodoptera frugiperda* antimicrobial peptides directed towards *Escherichia coli*.

The AMP activity present in the different plasma samples was measured by bacterial growth inhibition assay with *E. coli* SBS363 as bacterial target. Numbers indicate the minimal dilution of the samples that allowed normal bacterial growth. Three independent experiments were performed. U.C.: unchallenged, PBS: PBS-challenged, *E. coli*: *Escherichia coli* CIP7624, *X. nematophila*: *Xenorhabdus nematophila* F1, *X. mauleonii*: *Xenorhabdus mauleonii* VC01<sup>T</sup>.

			Larval treat	ment	
Experiments	U.C.	PBS	E. coli	Live <i>X. n.</i>	<i>E. coli/</i> Sup <sup>(a)</sup> <i>X. n.</i>
N°1	1/1	1/16	1/128	1/16	1/4
N°2	1/2	1/4	1/64	1/4	1/16
N°3	1/2	1/16	1/128	1/16	1/16

Table 4: Effect of supernatant from *Xenorhabdus nematophila* F1 culture on the expression of *Spodoptera frugiperda* antimicrobial peptides.

The AMP activity present in the different plasma samples was measured by bacterial growth inhibition assay with *E. coli* SBS363 as bacterial target. Numbers indicate the minimal dilution of the samples that allowed normal bacterial growth. Three independent experiments were performed. U.C.: unchallenged, PBS: PBS-challenged, *E. coli*: *Escherichia coli* CIP7624, *X. n.*: *Xenorhabdus nematophila* F1. <sup>(a)</sup> Sup: Culture supernatant of *X. nematophila* was obtained after centrifugation and filtration (0.22 µm) of an overnight bacterial culture. 20 µL of *X. nematophila* culture supernatant was injected at the same time than *E. coli*.



Figure 1

			20 I	T	40 I		60 I
CecA1	MKFSRVFLFV	FACLVALS	AVSAAPE	PRWKVFKKI	EKVGRNVRDGI	IKAGPAIGVLGQA	KALG 62
CecA2							62
CecA3							62
CecB1				F		A.	62
CecB2	*********				M I	VVEA.	63
CecB3					M I	VVEA.	63
CecB4					M I	VVEA.	K 63
CecB5					M I	VVEA.	63
CecB6					M I	VVEA.	63
CecC	.N.TKI	F.LMA	T G	F V	/L.Q.I	VA.V.S.	A.I
CecD1	.NKIII.L	CI.FL.V.	Τ	DL E.	.GQRAV	.SVDTK.	.K.AD.SSEED 63
CecD2	.NKIII.L	CI.FL.V.	Т	DL EL		.SVDTK.	.K.AG.SSEED 63
				*	k		*







# Supplementary Table 1: Sequences of insect cecropins used in this study.

Species	Sequences	Acc numbers
A. gambiae Cec1	GRLKKLGKKIEGAGKRVFKAAEKALPVVAGVKALG	<u>XP_311223</u>
A. gambiae Cec2	FKKFLKKVEGAGRRVANAAQKGLPLAAGVKGLVG	<u>XP_311222</u>
A. gambiae Cec3	RWKFGKRLEKLGRNVFRAAKKALPVIAGYKALGA	<u>XP_311224</u>
A. gambiae Cec4	LKKFGKKLEKVGKNVFHAVEKVVPVLQGIQDLRDKKNGQRG	<u>XP_565481</u>
B. mori CecA1	RWKLFKKIEKVGRNVRDGLIKAGPAIAVIGQAKSLGK	ref. [31]
B. mori CecA2	RWKLFKKIEKVGRNVRDGLIKAGPAIAVIGQAKSLGK	ref. [31]
B. mori CecB1	RWKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK	ref. [31]
B. mori CecB2	RWKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK	ref. [31]
B. mori CecB3	RWKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK	ref. [31]
B. mori CecB4	RWKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK	ref. [31]
B. mori CecB5	RWKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK	ref. [31]
B. mori CecB6	RWKIFKKIEKMGRNIRDGIVKAGPAIEVLGSAKAIGK	ref. [31]
B. mori CecC	KRKVFKIIEKIGRNVRGGVITAGPAVVVVGQAASVGM	ref. [31]
B. mori CecD	GNFFKDLEKMGQRVRDAVISAAPAVDTLAKAKALGQG	ref. [31]
B. mori CecE	RWKIFKKIEKVGQNIRDGIIKAGPAVAVVGQAATIAHGK	ABB19289
<i>B. mori</i> Enbocin1	WNFFKEIERAVARTRDAVISAGPAVATVAAASAVASG	AAC02238
<i>B. mori</i> Enbocin2	WNFFKEIERAVARTRDAVISAGPAVATVGAAAAVASG	BAF51563
D. melanogaster CecA1	GWLKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATARG	<u>NP 524588</u>
D. melanogaster CecA2	GWLKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATARG	<u>NP 524589</u>
D. melanogaster CecB	GWLRKLGKKIERIGQHTRDASIQVLGIAQQAANVAATARG	<u>NP 524590</u>
D. melanogaster CecC	GWLKKLGKRIERIGQHTRDATIQGLGIAQQAANVAATARG	<u>NP 524591</u>
H. armigera CecA	RWKVFKKIEKVGRNVRDGVIKAGPAIAVLGEAKALG	<u>AAX51304</u>
H. armigera CecD	WDFFKELEGAGQRVRDAIISAGPAVDVLTKAKGLYDSSEEKD	<u>AAX51193</u>
H. cecropia CecA	KWKLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAKG	<u>CAA29871</u>
H. cecropia CecB	KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKALG	<u>AAA29187</u>
<i>H. cecropia</i> CecD	WNPFKELEKVGQRVRDAVISAGPAVATVAQATALAKGK	<u>AAX51193</u>
<i>H. cunea</i> CecA	RWKIFKKIERVGQNVRDGIIKAGPAIQVLGTAKALGK	<u>P50720</u>
H. cunea CecA1	RWKFFKKIERVGQNVRDGLIKAGPAIQVLGAAKALGK	<u>P50721</u>
H. cunea CecA2	RWKVFKKIEKVGRNIRDGVIKAGPAIAVVGQAKALGK	<u>P50722</u>
H. cunea CecA3	RWKVFKKIEKVGRHIRDGVIKAGPAITVVGQATALGK	<u>P50723</u>
P. xylostella CecA	RWKPFKKLEKVGRNIRNGIIRYNGPAVAVIGQATSIARPTGK	<u>ACX31606</u>
S. exigua CecA	RWKVFKKIEKVGRNVRDGIIKAGPAIGVLGQAKAL	ref. [20]
S. exigua CecB	RWKVFKKIEKVGRNIDGIIKAGPAVEVLGTAKAL	ref. [20]
S. frugiperda CecA1	RWKVFKKIEKVGRNVRDGIIKAGPAIGVLGQAKALG	This study
S. frugiperda CecA2	RWKVFKKIEKVGRNVRDGIIKAGPAIGVLGQAKALG	This study
S. frugiperda CecA3		This study
S. frugiperaa CecB1	RWKFFKKIEKVGRNIRDGIIKAGPAIEVLGAAKALG	This study
S. frugiperda CecB2	RWKVFKKIEKMGRNIRDGIVKAGPAVEVLGAAKALGK	This study
S. frugiperaa CecB3	RWKVFKKIEKMGRNIRDGIVKAGPAVEVLGAAKALGK	This study
S. Jrugiperda CecB4		This study
S. Jrugiperda CecBS	KWKVFKKIEKMCRNIRDCIVKACPAVEVLCAAKALCK	This study
S. Jrugiperua Ceceo		This study
S. Jrugiperda Cecc	KWKFFKKVEKLGQNIKDGIIKAGPAVAVVGSAAAIGK	This study
S. Jrugiperua CecD1		This study
S. Jiugiperuu CecDZ	DMKAEKKIEKAGDUADUUIIKYUDYIUAUUTAGO2255D	
S. IILUIU CECA	DMKAEKKIEKWUDNIDUULAKYUDYIEAIOATOAIUN VMVAEVATUATUA GUAAUDUIIUWOLUIGATOATOATO	
S. IILUIU CECB	BMKAEKKIEKWUBNIDUULIKYUUDYALAI CANAI CA	<u>497400</u> ABO51002
J. mara CECD T. ni CocA	BMKEEKKIEKNCUNIBUCIIKYCDYNYNCUYYGIAC UMUAEUUTEUUGUUTUDGIIUYGEYAEAPOOUUGU	P50724
T ni Coce	RMKALKKIEKAGBUALDGIIKYGGYAAAAGAYYA	<u>1 307 24</u> ΔΒ\/68972
T ni CecD	CNEEKDLEGIGORVRDATESACPAVDVLCRAAALSRCEOOORE	<u>ΔΒV68873</u>

**Legend of Supplementary Figures 2 to 6**: Cecropin-encoding contigs (see Table 1) present in the Spodobase (Negre et al., 2006) were aligned using Clustal W. Identical nucleotides in Clusters I, II and V are boxed. In the case of Cluster III, boxes indicate residues which are identical in, at least, 3 sequences. ORFs are indicated by the black line.

Supplementary Fig. 2. Supplementary material.

Cluster I



Supplementary Fig. 3. Supplementary material.



# Supplementary Fig. 4. Supplementary material.

# Cluster III

Cluster	<u>r III</u>	122	52	
	20	40 I	60 I	ac 1
Sf1F02553-2	ATCATTGAGAGCAGAGCCACAGAGTCGCGC	AGAACAACACCAGTGCCACACAGC	ATCAGTA	·····CAGCATCAGGA 72
Sf1F02902	ATCATTGAGTGCAGAGCCACAGAGTCGCGC	AGTACCGCACCAGTGCCACACAGC	ATCAGTA	·····CAGTAGCAGAA 72
Sf1F02553-1	ACCATTGAGTGCAGAGCCATAGAGTCGCGC	AGTACATCACCAGTGCCACACAGC	ATCAGTACAGCTCCAGTGCC	ACACAGCATCAGTGCAGCATCAGGA 89
Sf1F11023-1	ATCATTGAGTGCAGAGCCACAGAGTCGCGC	AGTACCGCACCAGTGCCACACAGC	ATCAGTC	CAGCAGCAGGA 72
Sf1F11023-2	ATCATTGAGTGCAGAGCCACAGAGTCGCGC	GGTACAGCTCCAGTGCCACACAGC	GTCAGTA	CAGCAGCAGGA 72
				-
	100 120	140	160	180
Sf1F02553+2	TGAAGTTCTCCCGAGTGTTCCTGTTCGTGT	TCGCGTGCCTGGTCGCGCTGAGCG	CCGTCAGCGCCGCGCCAGAG	CCGAGGTGGAAGGTCTTCAAGAAGA 171
Sf1F02902	TGAAGTTCTCCCGAGTATTCCTGTTCGTGT	TCGCGTGCCTGGTCGCGTTGAGCG	CCGTCAGCGCCGCGCCAGAG	CCGAGGTGGAAGGTCTTCAAGAAGA 171
Sf1F02553-1	TGAAGTTCTCCCGAGTGTTCCTGTTCGTGT	TCGCGTGCCTGGTCGCGCTGAGCG	CCGTCAGCGCCGCGCCAGAG	CCGAGGTGGAAGGTCTTCAAGAAGA 198
Sf1F11023-1	TGAAGTTCTCCCGAGTGTTCCTGTTCGTGT	TCGCGTGCCTGGTCGCGCTGAGCG	CCGTCAGCGCCGCGCCAGAG	CCGCGGTGGAAGGTCTTCAAGAAGA 171
Sf1F11023-2	TGAAGTTCTCCCGAGTGTTCCTGTTCGTGT	TCGCGTGCCTGGTCGCGCTGAGCG	CCGTCAGCGCCGCGCCAGAG	CCGAGGTGGAAGGTCTTCAAGAAGA 171
		in a constraint from the	5 87-0-71	
	200 220	240	290	290
Sf1F02553-2	TTGAGAAGATGGGCCGCAACATCAGAGACG	GTATCGTCAAGGCAGGTCCTGCTG	TCGAGGTGTTGGGTGCAGCC	AAGGCGCTGGGGAAGTAATCAGCAG 270
Sf1F02902	TTGAGAAGATGGGCCGCAACATCAGAGACG	GTATCGTCAAGGCAGGTCCTGCTG	TCGAGGTGTTGGGTGCAGCC	AAGGCGCTGGGGAAGTAATCAGCAG 270
Sf1F02553-1	TTGAGAAGATGGGCCGCAACATCAGAGACG	GTATCGTCAAGGCAGGTCCTGCTG	TCGAGGTGTTGGGTGCAGCC	AAGGCACTGGGGAAGTAATCAGCAG 297
Sf1F11023-1	TTGAGAAGATGGGCCGCAACATCAGAGACG	GTATCGTCAAGGCAGGTCCTGCTG	TCGAGGTGTTGGGTGCAGCC	AAGGCGCTGGGGAAGTAATCAGCAG 270
Sf1F11023-2	TTGAGAAGATGGGCCGCAACATCAGAGACG	GTATCGTCAAGGCAGGTCCTGCTG	TCGAGGTGTTGGGTGCAGCC	AAGGCGCTGGGGAAGTAATCAGCAG 270
	2000 ACC		1.1.1.1	
	300 320	340	360	340
Sf1F02553-2	AATCGTCTTCATC ATCATCACTTA	ATATCATCACAGTCTTATGGTGTG	ACCAGCATATCTGGTGACAT	CAACCTGGTGACCAACAACCCTTAA 363
Sf1F02902	TACCGTCTTTATC ATCAGCACTTA	GTATCATCCCAGTCTTGTGGTGAA	ACCAGCATATCTGGTGACAT	CAACCTGGTGACCAACAACCTTTAA 363
Sf1F02553-1	TACCGTCTTCATC ATCATCACTTA	ATATCATCCCAGTCTTGTAGTGTG	ACCAGTATATCTGGTGAC	······ CAACAACCCTTTA 376
Sf1F11023-1	TATCACCATCAACGGATCTACTGTCACACT	TCAACA-CACGGCAATAGACAATA	GGAAACGTTTACACCAACCT	TTCTTANATGTTANAGAGACCCCTA 368
Sf1F11023-2	CATCACCATCAACTGATCTACTGGCACACT	TCGACA-CATGACGCCAGGCAATA	GGGGACGTTT-TACCAACCT	TTCTTAAATGTTAAAGATACCCCTA 367
	400 420	440	480	480
SHE02553+2		CAACGCACTTGTACCO	CICCICITICAAGTOTCCAT	
Sf1E02902	ATTCCTAACCCACCAAAAGGCTGG	CAACGCACTTGTAACG	CGGGTGTTTCAAGTGTCCAT	GGGCGGAGGCGATTGCTTACCATCA 451
Sf1E02553+1	ATTCCTAAC CCACCAAAAGGCCGG	CAACGCATTTGTAACGCCT	CGGGTGTTTCAAGTGTCCAT	GGGTGGTGGCAATTGCTTACCATCA 464
Sf1E11023-1	TATTCTGATAGATGGACACTTAATGACAAT	TIGGATTITTACCAAGATATACIT	AAGTGGCATCTAAAGTTAAG	AGGTCGGTTTAGTTGCTCCCTCACG 467
Sf1F11023-2	TGTTCAGATAGACGGACACTTAATGACTAT	TTG-ATTTTCACCAAGATATACTT	CAGTGAGTGTTAAG	AGGTCGGTTTAGTTGCTCCCTCACG 459
0.11 11020 2				
0.1000000000		540 I	1	960 I
<b>MAX PRODUCTS</b> 10				and the second sec
ST1F02553-2	GAAGATCCGTCT GATCGTTTA	CCGGCTTATTCCATAAAAAAAAAAA	AATACATGGTGTCTGAAGTA	ATTTGGTAATTAGACCAGGG CGA 539
Sf1F02553+2 Sf1F02902	GAAGATCCGTCTGATCGTTTA GATAATCCGTCTGATCGTTTA	CCGGCTTATTCCATAAAAAAAAAAA CCGGCTTATTCCATAAAAA	AATACATGGTGTCTGAAGTA	ATTTGGTAATTAGACCAGGG - CGA 539 ATTTGGTAATTAGACTAGGA - CGA 530
Sf1F02553+2 Sf1F02902 Sf1F02553+1	GAAGATCCGTCTGATCGTTTA GATAATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA	CCGGCTTATTCCATAAAAAAAAAAAC CCGGCTTATTCCATAAAAA CCGGCTTATTCCATAAAAAA	AATACATGGTGTCTGAAGTA	ATTTOSTAATTAGACCAGGG - CGA 539 ATTTOSTAATTAGACTAGGA - CGA 530 ATTTGTTATTAGACCAGGGTTCGA 546
Sf1F02553-2 Sf1F02902 Sf1F02553-1 Sf1F11023-1	GAAGATCCGTCTGATCGTTTA GATGATCCGTCT	CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAA CCGGCTTATTCCATAAAAA GCTGGCCATAGCCTAACCA	AATACATGGTGTCTGAAGTA CATGGTTTCTGAAGTG CATGGTTTCTGTAGTG GGTGTAAAAA	ATTTGGTAATTAGACGAGGG - CGA 539 ATTTGGTAATTAGACTAGGA - CGA 530 ATTTTGTTATTAGACGAGGTTCGA 546 GTCTTAAAGTCGGACTTTCAACAAG 551
Sf1F02553-2 Sf1F02902 Sf1F02553-1 Sf1F11023-1 Sf1F11023-2	GAAGATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA GATGATCGTCTGATCGTTTA CTAAGTATCTCTCTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTATTAAGGAGATAGTTAA	CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAA  GCTGGCCATATTCCATAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACTA	AATACATGGTGTGTCTGAAGTA ····CATGGTTTCTGAAGTG ····CATGGTTTCTGTAGTG ·····GGTGTAA···AAA ·····GGTGTAACCAAAA	АТТТЮСТААТТАСАССАССС - ССА 530 АТТТОСТАТТАСАСТАССА 530 АТТТТСТТАТТАСАССАССС 546 ОГСТТАААСТСССА 546 СТСТТАААСТСССАСТТТСААСААС 551 СГСТТАААСТСССАСТТТСААСААС 546
ShF02553-2 ShF02502 ShF02553-1 ShF11023-1 ShF11023-2	GAAGATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA GATGATCGTCTGATCGTTTA CTAAGTATCTCTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTATTAAGGAGATAGTTAA 000 800	CCGGCTTATTCCATAAAAAAAAAA CCGGCTTATTCCATAAAAA CCGGCTTATTCCATAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACTA GATGGCCATAGCCTAACTA	AATACATGGTGTCTGAAGTG CATGGTTTCTGAAGTG CATGGTTTCTGTAGTG GGTGTTAAAA GGTGTAACCAAAA 	ATTTOGTAATTAGACCAGGG CGA 530 ATTTOGTAATTAGACTAGGA CGA 530 ATTTTGTTATTAGACCAGGGTTCGA 546 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGG <u>GACT</u> TTCAACAAG 546
ShF02553-2 ShF02902 ShF02553-1 ShF11023-1 ShF11023-2 ShF02553-2	GATGATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA CTAAGTATCTCTTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTACTAAGGAGATAGTTAA 800 820 820 820 820 820 820 820 820 820	CCGGCTTATTCCATAAAAAAAAAAA CCGGCTTATTCCATAAAAA  GCTGGCCATATTCCATAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACTA B40 TTACGACGTTTGGCGTTCTGATTG	ААТА(САТОВСТСТОЙАЮТА САТОВСТГТСТОЙАВСТО САТОВСТГТСТОЙАВСТО СОТОВСТГТСТОТАВСТО СОТОТАА	ATTTIGGTAATTAGACCAGGG CGA 339 ATTTIGGTAATTAGACTAGAG CGA 530 ATTTIGTTATTAGACCAGGGTTCGA 546 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGGACTTTCAACAAG 546 ccaatCagaggGCTAAATGCGGTAA 638
ShF02553-2 ShF02902 ShF02553-1 ShF11023-1 ShF11023-2 ShF02553-2 ShF02553-2	GATGATCCGTCT GATGATCCGTCT GATGATCCGTCT CTAAGTATCCGTCT CTAAGTATCTCTTATTAAGGAGATAGTTAA 00 GTTGATCTCTTACTAAGGAGATAGTTAA 00 GTTTGTTTTACGTTAACGAGATCGAAAAACT GTTTGTTTTACGTTAACGAGATCGAAAAACT	CCGGCTTATTCCATAAAAAAAAA CCGGCTTATTCCATAAAAA  GCTGGCCATAGCCTAACCA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA TACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG	ААТАСАТОВТСТСТОЙАВТА САТОВТПТСТОЙАВТО САТОВТПТСТОТАВТО САТОВТПТСТОТАВТО СОТОТАВТО СОТОТАВТО ССТОЛАВТТААСТТА 	ATTTGGTAATTAGACCAGGG CGA 539 ATTTGGTAATTAGACTAGA CGA 530 ATTTGGTATTAGACCAGGGTTCGA 546 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGGACTTTCAACAAG 546 0 0 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638
SHF02553-2 SHF02902 SHF02553-1 SHF11023-1 SHF11023-2 SHF02553-2 SHF02553-2 SHF02553-1	GATGATCCGTCT GATGATCCGTCT GATGATCCGTCT CTAAGTATCCTCTTATTAAGGAGATAGTTA CTAAGTATCTCTTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTATTAAGGAGATAGTTAA 600 GTTTGTTTTACGTTAACGAGATCGATAGAAACCT GTTTGTTTTATGTTAACGTCATAGAAACCT GTTTNNNNTTACATTAACGCATCGATCGACTCT	CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAA CCGGCTATTCCATAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA MA TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGACGTTCTGATTG	ААТАҚСАТӨӨТСТ СТАЙАӨТА 	ATTTOGTAATTAGACCAGGG CGA 530 ATTTOGTAATTAGACTAGGA)- CGA 530 ATTTTGTATTAGACCAGGGTTCGA 546 GTCTTAAAGTCGBACTTTCAACAAG 551 GTCTTAAAGTCGBACTTTCAACAAG 546 600 CCCAATCAGAGGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGGCTAAATGCGGTAA 638 S98
St1F02953-2 St1F02902 St1F02553-1 St1F11023-1 St1F11023-2 St1F02553-2 St1F02553-2 St1F02902 St1F02553-1 St1F102553-1	GATGATCCGTCT GATGATCCGTCT CTAAGTATCCGTCT CTAAGTATCTCTCTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTATTAAGGAGATAGTTAA 00 GTTTGTTTTACGTTAACGAGATCGAAAACT GTTTGTTTTACGTTAACGAGATCGAAAACT GTTTATTTATGTTAACGACATCGAACAAACT GTTNNNNTTACATTAACGCAATCGAACAAAGTT	CCGGCTTATTCCATAAAAAAAAAA CCGGCTTATTCCATAAAAA CCGGCTATTCCATAAAAA GCTGGCCATAGCCTAACCA SATGGCCATAGCCTAACCA TTACGACGTTGGCCTAACTA TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATT TTGCTTTTAATATTTTTGTT	ААТАСАТОВТСТСТОЙАВТА 	ATTTOGTAATTAGACCAGGG - CGA 339 ATTTOGTAATTAGACTAGGA - CGA 540 ATTTTGTATTAGACTAGGACTTCAACAG 546 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGGACTTTCAACAAG 546 00 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 639 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638
SHF02953-2 SHF02902 SHF02553-1 SHF1023-1 SHF102553-1 SHF02553-2 SHF02902 SHF02902 SHF02953-1 SHF102553-1 SHF11023-2	GARGATCCGTCT GATGATCCGTCT CTAAQTATCCTCTTATTAAGGAGATAGTTAA CTAAQTATCTCTTTATTAAGGAGATAGTTAA CTAAQTATCTCTTTATTAAGGAGATAGTTAA 000 800 GTTTGTTTTACGTTAACGAGATCGAAAAACT GTTTGTTTTACGTTAACGAGATCGATAGAAACT GTTTATTATTATGTTAACGAGATCGATAGAAACT GTTTATGTCTGTCTTAGTATAGAACAAAGTT CTTTAATGTCTGTCTTAGTATAGAACAAAGTT CCTTAATATGTTTCGAGGATAGAACAAAGT	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAA  GCTGGCCATAGCCTAACCA  GATGGCCATAGCCTAACCA  TTACGACGTTGGCCTAACCA TTACGACGTTGGCCTTCTGATTG TACGACGTTGGACGTTCTGATTG TACGACGTTGGACGTTCTGATTG TACGACGTTGGACGTTCTGATTG TACGACGTTGGACGTTCTGATTG TACGACGTTGAACATTTTTGTTT TGTCTTTTAATATTTTTTATTT	ААТАСАТОВТСТСТОЙАВТА 	ATTTGGTAATTAGACCAGGG - CGA 339 ATTTGGTAATTAGACTAGGA - CGA 530 ATTTGGTATTAGACCAGGGTTCGA 546 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGGACTTTCAACAAG 548 600 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638
SHF02953-2 SHF02902 SHF02553-1 SHF1023-1 SHF1023-1 SHF02553-2 SHF02553-2 SHF02902 SHF02553-1 SHF102553-1 SHF11023-2		CCGGCTTATTCCATAAAAAAAAAA CCGGCTTATTCCATAAAAAAAAAA	ААТАСАТОВТСТСТОЙАВТА 	ATTTGGTAATTAGACCAGGG CGA 339 ATTTGGTAATTAGACTAGAA CGA 540 ATTTGGTATTAGACCAGGGTTCGA 546 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGGACTTTCAACAAG 546 60 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 639 598 620 620 620 611 780
SHF02953-2 SHF029553-1 SHF029553-1 SHF11023-2 SHF029523-2 SHF029523-2 SHF02553-2 SHF02553-2 SHF102553-2 SHF102553-2	GAAGATCCGTCT         -GATCGTTTA           GATGATCCGTCT         -GATCGTTTA           GATGATCGTCT         -GATCGTTTA           CTAAGTATCTCTTATTAAGGAGATAGTTAA         -GATCGTTTA           CTAAGTATCTCTTTACTAAGGAGATAGTTAA         -GATCGTTAA           GTTTGTTTTACCTTTACGAGAGATCGATAGTTAA         -GATCGTTAACGAGATCGAAAAACTTAA           GTTTGTTTTACGTTAACGAGATCGAAAAACCT         -GTTNGTTTATACGTTAACGCGATCGAAAAACCT           GTTNGTTTTACGTTAACGCGATCGAACAAACTT         -GTTNATTTACATTAACGCGATCGAACAAAGTT           CTTAATGTGTGTTTGAGATAAGAAAAGTT         -GTTAATATGTTTGCAGATAAGAAAGAAAGTT           CTTAATATCTTTCAGGATAAGAAAGACAAAAGTT         -GTT           700         700         700           700         700         700           700         700         700         700           700	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAA GATGGCCATAGCCTAACCAAAAAA TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGAT TTTGTTTTAATATTTTTTGTTT TGTCTTTTAAATATTTTTTTTTTTTTTTTTTTTTTTT	ΑΑΤΑΚCΑΤGGTGTCTGAAGTG 	АТТТЮЙТААТТАGАС[ДАGG СGA 530 АТТТЮЙТААТТАGАСТАGA] - СGA 530 АТТТЮЙТАТТАGАСДАGGGT[CGA 546 GTCTТАААСТСОВАСТТТСААСААС 551 GTCTTAAAGTCGBACTTTCAACAAC 546 600 CCCATCAGAGGGCTAAATGCGGTAA 638 CCATCAGAGGGCTAAATGCGGTAA 638 CCATCAGGGCTAAATGCGGTAA 638 CCATCAGGGCTAAATGCGGTAA 638 CCATCAGGGCTAAATGCGGTAA 638 CCATCAGGGCTAAATGCGGTAA 638 CCATCAGGGCTAGGCGGCGGCGGGCGGGCGGGCGGGCGGG
SHF02953-2 SHF02952 SHF029553-1 SHF11023-1 SHF11023-2 SHF02553-2 SHF02902 SHF02553-1 SHF11023-1 SHF11023-2 SHF02553-2 SHF02902	GATGATCCGTCT GATGATCCGTCT CTAAGTATCCGTCT CTAAGTATCTCTTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTATTAAGGAGATAGTTAA 600 600 600 600 600 600 600 6	ССGGCTTATTCCATAAAAAAAAAAC ССGGCTTATTCCATAAAAA 	ААТАСАТОВТСТСТОЙАЮТА САТОВТ[ТІСТСТОЙАВТА САТОВТ[ТІСТСТОЙАВТС САТОВТ[ТІСТС]АВТС 	АТТ ТЮСТААТТАСАС (ДАССС - ССА) 530 АТТ ТЮСТААТТАСАСТАССА 540 АТТ ТСТТАТАСС САСС 551 СТТАААСТСОВАСТТТСААСААС 551 ССААТСАСАСССАСТТТСААСААС 546 ВОС ССААТСАСАСССАСТААТССССАА 638 ССААТСАСАСССААСТААТССССАА 638 ССААТСАСАСССААСТААТССССААСТААСТАСССААСТААСТАССААСТААТСССААСТААСТАССААСТАСТ
SITF02563-2 SITF02502 SITF02553-1 SITF102553-2 SITF02553-2 SITF02553-2 SITF02553-1 SITF102553-2 SITF02553-2 SITF02553-2 SITF02553-2	GATGATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA CTAAQTATCTCTTATTAAGGAGATAGTTAA CTAAQTATCTCTTTATTAAGGAGATAGTTAA 000 800 GTTTGTTTTACGTTAACGAGATCGGAAAAC GTTTGTTTTACGTTAACGACATCGGAAAAC GTTTGTTTTACGTTAACGACATCGAAAAC GTTNNNTTACATTAACGCAATCGAACAAAGT CCTTAATGTCTGTCTTAGTATAGGAACAAAGT CCTTAATGCTGTCTTAGTATAGGAACAAAGT CCTTAATGCTGTCTTAGTATAGGAACAAAGT CCTTAATGCTGTCTTAGTATAGGAACAAAGT CCTTAATGCTGTCTTAGTATAGGAACAAAGT CCTTAATGCTGTCTTAGTATAGGAACTAACTC	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAA	ААТАСАТОВТСТСТОЙАЮТА 	ATTTGGTAATTAGACGAGGG - CGA 539 ATTTGGTAATTAGACTAGAGA - CGA 540 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGGACTTTCAACAAG 546 600 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 620 611 780 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 620 611 780 780 780 780 780 780 780 780 780 780
ShiFu2553-2 ShiFu2553-1 ShiF102553-1 ShiF11023-1 ShiF11023-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-1 ShiF11023-1	GAAGATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA GATGATCCGTCTGATCGTTTA CTAAGTATCTCTTATTAAGGAGATAGTTAA TO GTTGTTTTACGTTAACGAGATCGAAAACT GTTTGTTTTACGTTAACGAGATCGAAAACT GTTTGTTTTACGTTAACGCAAGATCGAAAACT GTTGTTTATGTTTACGTTAACGAAGAACAAAACT CTTAATCTGTCTTACTAACGAAGAACAAAAGT CCTTAATATATGTTAACGAAGAAGAAAACT TO TO CATATCGATCGATTAAAGTAGAACTAACTC	CCGGCTTATTCCATAAAAAAAAAA CCGGCTTATTCCATAAAAAAAAAA	ААТАСАТОВТСТСИЙАВТА 	АТТ ТЮСТААТТАСАС (ДАССО - ССА) 539 АТТ ТЮСТААТТАСАС ТАССА 530 АТТ ТОСТГАТТАСАС САССА 546 СТСТ ТАААСТССВАСТТТСААСААС 551 СССАТСАСАСССТТТСААСААС 548 ССААТСАСАСССТААСААТССССТА 638 СССАТСАСАСССТАСАСААТССССТА 638 СССАТСАСАССССТАТАСТАТТТААТС 737 АСТСТАААТААСТТТАСТАТАТТТТААТС 738 ССААТСАСАСССТАСАСААТСТАС 738 АСТСТАААТААСТТАСТАТАТТТТААТС 738 2000 2000 2000 2000 2000 2000 2000 200
Sh1F02553-2 Sh1F02553-1 Sh1F102553-1 Sh1F11023-1 Sh1F11023-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2	GAAGATCCGTCTGATCGTTTA GATAATCCGTCTGATCGTTTA CTAAGTATCTCTTATTAAGGAGATAGTTAA CTAAGTATCTCTTATTAAGGAGATAGTTAA GTTGTTTTACCTCTTACTAAGGAGATGGATAACT GTTGTTTTACCTTAACGAGATCGAAAACT GTTTGTTTTACGTTAACGAGATCGAACAACT GTTJGTTTATGTTAACGTGATAGAAAAAGT CTTTGTTTAGTCTGTCTAACGAGATAGGAACAAAGT CCTTAATGTGTGTTTGAGGATAGGAACAAAGT 700 720 700 720 CATATCGATCGATTAAAGTAGAACTAACTC CATATCGATCGATTAAAGTAGAACTAACTC	CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTATTCCATAAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAAA	ААТАСАТОВТСТСТОЙАЮТА 	ATT TIGGTAATTAGAC[AGGG CGA 530 ATT TIGGTAATTAGACTAGA] CGA 530 ATT TIGTATTAGAC[AGGGTTCGA 546 GTCTTAAAGTCGBACTTTCAACAAG 546 600 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAAATGCGGTAA 638 CCCAACCTTACTTTAATTTTAATG 737 ACTGTAAATAACTTTAATTTTAATG 737 ACTGTAAATAACTTTAATTTTAATG 737 CCAAAGTCTTACTATATTTTAATG 655
Sh1F02553-2 Sh1F02553-1 Sh1F102553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F102553-2 Sh1F102553-2 Sh1F02553-2 Sh1F02553-1 Sh1F102553-1 Sh1F10253-1 Sh1F10253-2	GATGATCCGTCT GATGATCCGTCT CTAAGTATCCGTCT CTAAGTATCTCTTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTATTAAGGAGATAGTTAA CTAAGTATCTCTTTACTAAGGAGATAGTTAA CTAAGTATCTCTTACTAAGGAGATAGTTAA CTAAGTATCTCTTACGTTAACGAGATCGATAGAAACT GTTTGTTTTACGTTAACGAGATCGACACAAAGTT GTTTMINNNTTACATTAACGCAATCGACTCTT CTTTAGTCTGTGTTTACGTTAAGAAGAAGTAAGTT CCTTAATGTTGTTTCAGGATAGAACAAAGTT CCTTAATGTTGTTTCAGGATAGAACAAAGTT CCTTAATGTCGATCGATTAAGAAGAACTAACTC CATATCGATCGATTAAAGTAGAACTAACTC CATATCGATCGATTAAAGTAGAACTAACTC SSO SSO SSO	CCGGCTTATTCCATAAAAAAAAAC           CCGGCTTATTCCATAAAAA           CCGGCTATTCCATAAAAA           GCTGGCCATAGCCTAACCA           GATGGCCATAGCCTAACCA           GATGGCCATAGCCTAACCA           TACGACGTTGGCCTAACCA           B40           TTACGACGTTGGCGTCTGATTG           TACGACGTTGGCGTTCTGATTG           TACGACGTTGGCGTTCTGAT           TTGTCTTTTAAATATTTTTGTTT           GCGGACTAGGCACTTCTAATTATT           GCGACTAGGCACTTCTAATAATAG           B40	ΑΑΤΑζ ΑΤΘΟ ΤΕ ΤΟ ΓΑΛΑΤΕ	АТТ ТЮСТААТТАСАС (САССО - ССА) 530 АТТ ТЮСТААТТАСАСТАССАССО - ССА) 530 АТТ ТСОГТААСТАССАССО 546 СТТААСТГАТТАССССАССТТТСАССАС 546 ССААТСАСАСССАСТТТСАССАС 546 ССААТСАСАСССАСТТТСАССАС 546 ССААТСАСАСССАСТАСАССАС 546 ССААТСАСАСССАСТАСАССАС 546 ССААТСАСАСССАСТАСАССАС 546 ССААТСАСАСССАСТАСАССАС 546 ССААТСАСАСССАСТАСАССАС 546 ССААТСАСАСССАСТАСАССАС 546 ССААТСАСАСССАССАССАС 546 ССААСССТТАСТАСАСАССАС 546 ССААССТТГАСТАСАТТТТААТС 728 ССАААСТСТГАСТАТАТТТТААТА 5464 ССАААСТСТГАСТАТАТТТТААТА 565 В10
SITF02563-2 SITF02502 SITF02553-1 SITF102553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF102553-2 SITF02553-2 SITF02553-1 SITF11023-2 SITF102553-2 SITF102553-2	GATGATCCGTCT	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAAA	ААТАСАТОВОТСТОЙАЮТА САТОВОТПТСТОЙАЮТА САТОВОТПТСТОЙАВОТО САТОВОТПТСТОЙАВОТО 	ATT TIGGTAATTAGAC[AGGG CGA 539 ATT TIGGTAATTAGACTAGGA] CGA 530 ATT TIGGTAATTAGACTAGAGA CGA 540 GTCTTAAAGTCGGACTTTCAACAAG 551 GTCTTAAAGTCGGACTTTCAACAAG 546 60 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAATTGCGGTAA 638 CCAATCAGAGGCTAATTGCGTAA CCGAAGTCTTACTTTAATG 780 780 CCAAGTCTTACTTACTTTAATG 655 780 100 100 100 100 100 100 100 100 100 1
Sh1F02553-2 Sh1F02553-1 Sh1F102553-1 Sh1F11023-1 Sh1F11023-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-3 Sh1F11023-3 Sh1F11023-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2 Sh1F02553-2	GAAQATCCGTCT         -GATCGTTTA           GATGATCCGTCT         -GATCGTTTA           GATGATCCGTCT         -GATCGTTTA           CTAAQTATCTCTTATTAAGGAGATAGTTAA         -GATCGTTAA           CTAAQTATCTCTTATTAAGGAGATAGTTAA         -GTGTTGTTTACGTTAACGAGATCGAAAACT           GTTTGTTTTACGTTAACGAGATCGAAAACT         -GTAGTATTAACGCAGATCGAAAACT           GTTTGTTTTACGTTAACGCAGATCGAAAACT         -GTTGTTTACGTTAACGCAGATCGAAAACT           GTTTGTTTTACGTTAACGCAGATCGAAAACT         -GTAGTCGATGGATAACTGCAAAAGT           GTTGTTTTACGTTAACGCAGATCGAACAAAGT         -GTTAGTCTGTGTCTTAGTATAGAAAACTAACTC           CCTTAATGTTGTGTTAACGAGATGGAACTAACTC	ССGGCTTATTCCATAAAAAAAAAA ССGGCTTATTCCATAAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACTA 040 TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATT TTTGTC 740 GCGACTAGGCACTAATTATAATAG 740 740 740 740 740 740 740 740	ААТАҚСАТӨВТГІТСТСІ ҚАҚАБТА 	АТТ ТЮСТААТТАСАС (САССО - ССА) 539 АТТ ТЮСТААТТАСАС ТАССАС (САССО) - ССА) 530 АТТ ТОСТТААТТАСАС (САССО) - ССА) 546 ОГСТ ТАААСТССВАСТТТСААСААС 551 СССАТСАСОСССТААТСССВАСТТТСААСААС 546 ССАТСАСОСССТААТССССТААСААС 546 СССАТСАСОСССТААТССССТААСААС 546 СССАТСАСОСССТААТССССТААСААС 546 СССАТСАСОСССТААТССССТААСААС 546 СССАТСАСОСССТААСТАССССТААСААС 546 СССАТСАСОСССТААСТАССССТААСААС 546 СССАТСАСОСССТАСТАСТААСТАССССТААСААС 546 СССАТСАСОСССТАСТАССССТААСААС 546 СССАТСАСОСССТАСТАСТАСТАССССТААСААС 546 СССАТСАССССТАСТАСТАСТАТТТААТС 737 АСТСТАААТААСТАТСТАТАТТТТААТС 738 СССАААСССТТСАСТАСТАТТТТААТС 738 СССАААСТСТТСАСТАСТАТТТТААТС 738 СССАААСТСТТСАСТАСТАТТТТААТС 738 СССАААСТСТТСАСТАСТАТТТТААТС 738 СССАААСТСТТСАСТАСТАТТТТААТС 548 СССАААСТСТТСАСТАСТАТТТТААТС 548 СССАААСТСТТСАСТАСТАТТТТААТА 546 СССАААСТСАСТТСАСТАСТАТТТТААТА 645 СССАААСТАСТТСАСТАСТАТТТТААТА 645 СССАААСТААТААСТТТТААТТТТААТА 645 СССАААСТАСТТСАСТАСТАСТАТТТААТА 645 СССАААСТАСТТСАСТАСТАТТТААТТТАСТА 836 ААТСТАААТААСТТТСАСТАТАСТАТА 836
ShTP02553-2 SHTF02553-1 SHTF102553-1 SHTF102553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2 SHTF02553-2	GATAGICCGTCT         -GATCGTTA           GATGATCCGTCT         -GATCGTTA           CTAAGTATCCGTCT         GATCGTTA           CTAAGTATCTCTTATTAAGGAGATAGTTA           CTAAGTATCTCTTATTAAGGAGATAGTTA           00         600           1         GTGTTGTTTACGTTAACGAGATCGAAAAGTA           01         600         600           1         GTTTGTTTTACGTTAACGAGATCGAAAAGCT           1         GTTTGTTTTACGTTAACGAGATCGAAAAGCT           1         GTTTGTTTTACGTTAACGCGATAGAAAGCACT           1         GTTTGTTTTACGTTAACGCGATAGAAAGCACT           1         GTTTGTTTTACGTTAACGAGATCGACACAAAGT           1         GTTAGTCTGTGTTAACGCAAAAGTAGACAAAAGT           1         GTTAGTCTGTTATACGCAAAACGAACAAAAGT           1         GTTAGTCTGTTAACGTCAACTAGAAAAGTA           1         700         720           1         700         720           1         700         720           1         700         720           1         700         720           1         720         720           1         700         720           1         700         720           1         700         720           1	ССGGCTTATTCCATAAAAAAAAAAC ССGGCTTATTCCATAAAAAA ССGGCTATTCCATAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GAT TACGACGTTGGCGTTCTGATTG TTACGACGTTGGCGTTCTGATTG TTACGACGTCGCACGTCTGAT TTTCTTTAAATATTTTTGTTT TGTCTTTAAATATTTTTTTTTTTTTT GCGACTAGGCACTAATATAATAG GCAAATAGGCATTCTTAATAATAG GCAAATAGGCATTCTTAATAATAG TTTTCATTCAGATCATTAATAGTT TTTTTCATTCAGATCATTAATGTT	ААТАСАТОВТСТСИЙАВТА 	ATTTGGTAATTAGACGAGGG CGA 530 ATTTGGTAATTAGACTAGAJ CGA 530 ATTTTGGTATTAGACGAGGTTCGA 546 GTCTTAAAGTCGBACTTTCAACAAG 546 600 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCATCAGAGGGCTAAATGCGGTAA 638 CCCATCAGAGGGCTAAATGCGGTAA 638 CCCATCAGAGGGCTAAATGCGGTAA 638 CCCATCAGAGGGCTAAATGCGGTAA 638 CCCATCAGAGGGCTAAATGCGGTAA 638 CCCATCAGAGGCTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 655 CCAAAGTCTTACTATATTTTAATG 655 AGTGTAAATAAGTTTTAATTTTATG 655 AGTGTAAATAAGTTTTAATTTTATG 856 S98
Sh1F02553-2 Sh1F02553-1 Sh1F102553-2 Sh1F02555-2 Sh1F02555-2 Sh1F02555-2 Sh1F02555-2 Sh1F02555-2 Sh1F0	GAAGATCCGTCT         - GATCGTTTA           GATGATCCGTCT         - GATCGTTTA           GATGATCCGTCT         - GATCGTTTA           CTAAGTATCTCTTATTAAGGAGATAGTTAA         GATCGTTTA           CTAAGTATCTCTTTATTAAGGAGATAGTTAA         600           GTTTGTTTTACCTTAACGAGATCGATAGATAG         600           GTTTGTTTTACCTTAACGAGATCGAAACTTAA         600           GTTTGTTTTACGTTAACGAGATCGAAAACTTAA         600           GTTTGTTTTACGTTAACGAGATCGAAAACTT         600           GTTTMGTTTTACGTTAACGAGATCGAAAACTT         600           GTTTAGTCTTGTACTAAGGAACAAAGTT         700           T00         700         700           CATACGATCGATTAAAGTAGAACTAACTC         700         700           CATACGATCGATCAATTAAAGTAGAACTAACTC         700         700           CATACGATCGATCAATTAAAGTAGAACTAACTC         700         700           CATACGATCGATCAATTAAAGTAGAACTAACTC         700         700           CAAAAATCTGTAATTAAAGTAGAACTAACTC         700         700         700           CAAAAAATCGATCGATTAAAGTAGAACTAACTC         700         700         700         700         700         700         700         700         700         700         700         700         700         700         700         700         700         700 </td <td>CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA TACGACGTTGGCCTAACCA TACGACGTTGGCCTTCTGATTG TTACGACGTTGGCGTTCTGATTG TACGACGTCGCACGTCTGAT TTTCCTTTTAATATTTTTTGTTT TGTCTTTTAAATATTTTTTTTTTTTT GTCGTTTTAAATATTTTTTTTTTTTTTT GCGACTAGGCACTAATATTATATAG GCAAATAGGCATTCTTAATAATAG GCAAATAGGCATTCTTAATAATAG</td> <td>АЛТАСАТОВТСТСИЙАВТА САТОВТІТІСТСИЙАВТО САТОВТІТІСТСИЙАВТО </td> <td>ATT TIGGTAATTAGAC[AGGG [CGA] 539 ATT TIGGTAATTAGACTAGGA]- [CGA] 540 ATT TIGGTAATTAGACTAGGA] 546 GTCTTAAAGT[CGBACTTTCAACAAG 546 GTCTTAAAGT[CGBACTTTCAACAAG 546 GTCTTAAAGTCGGGACTTTCAACAAG 546 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAAATGCGGTAA 638 CCAATGTAAATAAGTTTAATG CGAAAGTCTTACTTAATAGCTATTAATG 737 ACTGTAAATAAGTTTAATTAGCTATTA 836 AATGTAAATAAGTTTAATGCGTTATAA 736 CTAAATAAGTATAAGTTTAATG 737 CTGTAAATAAGTAATAAGTTTAATG 836 CCAATGTAAATAAGTAAGTATTAAGCTATTA 836 CCAATGTAAATAAGTAAGTATTAAGCTATTA 736</td>	CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA TACGACGTTGGCCTAACCA TACGACGTTGGCCTTCTGATTG TTACGACGTTGGCGTTCTGATTG TACGACGTCGCACGTCTGAT TTTCCTTTTAATATTTTTTGTTT TGTCTTTTAAATATTTTTTTTTTTTT GTCGTTTTAAATATTTTTTTTTTTTTTT GCGACTAGGCACTAATATTATATAG GCAAATAGGCATTCTTAATAATAG GCAAATAGGCATTCTTAATAATAG	АЛТАСАТОВТСТСИЙАВТА САТОВТІТІСТСИЙАВТО САТОВТІТІСТСИЙАВТО 	ATT TIGGTAATTAGAC[AGGG [CGA] 539 ATT TIGGTAATTAGACTAGGA]- [CGA] 540 ATT TIGGTAATTAGACTAGGA] 546 GTCTTAAAGT[CGBACTTTCAACAAG 546 GTCTTAAAGT[CGBACTTTCAACAAG 546 GTCTTAAAGTCGGGACTTTCAACAAG 546 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAA 638 CCAATCAGAGGCTAAATGCGGTAA 638 CCAATGTAAATAAGTTTAATG CGAAAGTCTTACTTAATAGCTATTAATG 737 ACTGTAAATAAGTTTAATTAGCTATTA 836 AATGTAAATAAGTTTAATGCGTTATAA 736 CTAAATAAGTATAAGTTTAATG 737 CTGTAAATAAGTAATAAGTTTAATG 836 CCAATGTAAATAAGTAAGTATTAAGCTATTA 836 CCAATGTAAATAAGTAAGTATTAAGCTATTA 736
SITF02553-2 SITF02553-1 SITF102553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-2 SITF02553-1 SITF11023-2	GATGATCCGTCT         -GATCGTTTA           GATGATCCGTCT         -GATCGTTTA           GATGATCCGTCT         -GATCGTTTA           CTAAGTATCTCTTATTAAGGAGATAGTTAA         GATCGTTTA           CTAAGTATCTCTTTATTAAGGAGATAGTTAA         GATCGTTAA           600         600           GTTTGTTTTACCGTTAACGAGATCGATAGTTAA           600         600           GTTTGTTTTACGTTAACGAGATCGAAAACT           GTTTGTTTTACGTTAACGAGATCGAAAACT           GTTTGTTTTACGTTAACGAGATCGACCAAAACT           GTTNNNNTTACATTAACGCAATCGACTAAAACT           GTTTAGTCTGTGTTAACGAAGACAAAGTT           CCTTAATGTGTGTGTTAACGAAGACAAAGTACCC           700         720           700         720           CATATCGATCGATTAAAGTAGAACTAACTC           700         720           700         720           CATATCGATCGATTAAAGTAGAACTAACTC           700         720           CATATCGATCGATTAAAGTAGAACTAACTC           700         720           700         720           CATATCGATCGATTAAAGTAGAACTAACTC           6         6           6         7           CAAAAATCTGTAATTAATTAATTAATTAATTAA           TAAAAA-TCAGTAATTATATTATTATTAATTAA           TAAAAA-TCAGTAATTAATTAATAGTATATAGTATATTAA     <	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAA GCTGGCCATAGCCTAACCAA GATGGCCATAGCCTAACCAA TTACGACGTTTGGCGTTCTGATTG TTACGACGTTGGACGTTCTGATTG TTACGACGTTGGACGTTCTGAT TTGCTTTTAATATTTTTTGTTT TGCTTTTAAATATTTTTTTTTTTTTTT GCGACTAGGCATTAATAATAG GCAAATAGGCATTCTTAATAATAG GCAAATAGGCATTCTTAATAATAG	ААТАСАТОВОТСТОЙАЮТА 	ATTTQGTAATTAGAC[AGGG - CGA 539 ATTTQGTAATTAGACTAGA] - CGA 530 ATTTTGTATTAGACTAGA] - CGA 540 GTCTTAAAQTCGGACTTTCAACAAG 551 GTCTTAAAQTCGGACTTTCAACAAG 546 600 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAATGCGGTAA 638 CCAATCAGAGGCTAATTGCGTATTAATG 100 100 100 100 100 100 100 10
Shi Fu2553-2 Shi Fu2553-1 Shi F102553-1 Shi F102553-2 Shi F02553-2 Shi F02553-1 Shi F102553-1 Shi F1023-2	GAAQATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           CTAAQTATCTCGTCT         GATCGTTA           CTAAQTATCTCTTATTAAGGAGATAGTTA         GATGGTTA           000         GOO           GTTGTTTACGTTAACGAGATCGAAAACT         GTAGTTA           000         GOO           GTTGTTTACGTTAACGAGATCGAAAACT         GTTGTTTACGTTAACGCAGATCGAAAACT           GTTGTTTTACGTTAACGCAGATCGACAAAGT         CCTAAGTCGTGTCTAACGAGATGGAACAAAGT           CCTTAATGTTGTCTTAGTATAGGAACAAAAGT         CCTTAATAGTGTGTCTAGGATAGGAACAAAAGT           700         730           CAAAAAATCTGTAATTAAAGTAGAAACTAACTO         730           CAAAAAATCTGTAATTAATTAAGTAGAAAAATAACTG         730           CAAAAAATCTGTAATTAATTAATTTATTGTAATTAAA         730           CAAAAAATCTGTAATTAATTAATTAATTAATTAATTAATT	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAAA	ΑΑΤΑΚCΑΤGGTGTCTGAAGTG          CATGGT[TCTGAAGTG          CATGGT[TCTGAAGTG          CATGGT[TCTGAAGTG          GGTGTAAAAA          GGTGTAAAAA          GGTGTAAAAA          GGTGTAAAAA	ATT TIGGTAATTAGAC[GAGG - CGA 530 ATT TIGGTAATTAGACTAGA] - CGA 530 ATT TIGGTAATTAGACTAGA] - CGA 546 GTCTTAAAGTCGBACTTTCAACAAG 546 GTCTTAAAGTCGBACTTTCAACAAG 546 GTCTTAAAGTCGBACTTTCAACAAG 546 GTCTAAAGTCGBACTTTCAACAAG 546 GTCTAAAGTCGBACTTTCAACAAG 546 GTCTAAAGTCGBACTAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 GCAATGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 655 GCAAAGTCTTACTATAATTTTAATA 654 CCAAATGAATAAGTTTTAATTTTATG 655 GTTGTAAATAAGTTTTAATTTTATG 759 AATGTAAATAAGTTTTAATTTTAATA 654 CCGAAAGTCTTGCAATAAGTTTTAATG 759 ACTGTAAATAAGTTTTAATTTAATA 654 CCGAAAGTCTTAATAAGTTTTAATTTTAATG 759 - TGTAAATAAGTAATAAGTTTAATG 7598
Shi Fu2553-1 Shi Fu2553-1 Shi Fu2553-1 Shi Fu2553-1 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-1 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-1 Shi Fu2553-2 Shi Fu2553-1 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2 Shi Fu2553-2	GATGATCCGTCT         -GATCGTTA           GATGATCCGTCT         -GATCGTTA           GATGATCCGTCT         -GATCGTTA           CTAAGTATCTCTTATTAAGGAGATAGTTA           CTAAGTATCTCTTTATTAAGGAGATAGTTA           00         600           10         600           GTTTGTTTTACCGTTAACGAGATCGAAAACTA           10         600           11         11           12         11           13         11           14         11           15         11           16         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         11           17         12           17         12           17         12           17         12           <	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC CCGGCTATTCCATAAAAAAAAAA	ААТАСАТОВТСТСИЙАВТА 	ATT TIGGTAATTAGAC[GAGGG CGA 530 ATT TIGGTAATTAGACTAGA] CGA 530 ATT TIGGTAATTAGACTAGA] CGA 540 GTCTTAAAGTCGBACTTTCAACAAG 546 600 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATCAGAGGGCTAAATGCGGTAA 638 CCCAATGCAAATAAGGTTTAATTTTAATG 640 CCCAAAGTCTTAGCTATAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 665 CCCAAAGTCTTACTTTAATTTTAATG 665 CCCAAAGTCTTACTTTAATTTTAATG 655 CCCAAAGTCTTAATAAGTTTTAATA 654 CCCAAAGTCTTTACTTTTAATA 654 CCCAAAGTCTTTACTTTAATTTTATG 759 AATGTAAATAAGTTTTAATTTTAATG 759 CTGTAAATAATAATAATAATAATAATAATAAGTTTATA 654 CTGTAAATAATAATAATAATAATAAGTTTTAATA 654 CTGTAAATAATAATAATAATAATAATAA
ShiF02553-2 ShiF02553-1 ShiF102553-1 ShiF102553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2 ShiF02553-2	GATAATCCGTCTGATCGTTTA GATAATCCGTCTGATCGTTTA CTAAGTATCCTCTTATTAAGGAGATAGTTAA CTAAGTATCTCTTATTAAGGAGATAGTTAA CTAAGTATCTCTTACTATAAGGAGATAGTTAA GTAGTATCTCTTACCTTAACGAGATCGAAAACTTA CTAAGTATCTCTTACGTTAACGAGATCGAAAACTTA CTTTGTTTTACCTTAACGTGATAGGAACAAAGTT GTTTMUTTACATTAACGCGATCGAAAACTT CTTTAGTCTGTGTTTACGTTAAGAAGAAGAAGTA CCTTAATATGTTTCAGTATAAGGAGACTAACTC CTTTAGTCTGTATCTAAGGAGACTAACTC CATATCGATCGATCGATTAAAGTAGGAACTAACTC CATATCGATCGATTAAAGTAGAAGACTAACTC CATATCGATCGATTAAAGTAGAAGACTAACTC CATATCGATCGATTAAAGTAGAAGACTAACTC CATATCGATCGATTAAAGTAGAAGACTAACTC CATATCGATCGATTAAAGTAGAAGACTAACTC CATATCGATCGATTAAAGTAGAAGACTAACTC CATATCGATCGATTGATTTATTTGTGTAATTAA TAAAA.TCAGTAATTTATTATTGTGTAATTAATTA T	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAAA	АЛТАСАТОВТСТСТОЙАЮТА 	ATT TIGGTAATTAGAC[AGGG [CGA] 539 ATT TIGGTAATTAGACTAGA] [CGA] 530 ATT TIGGTAATTAGACTAGAGA] [CGA] 546 GTCTTAAAGT[CGBACTTTCAACAAG 546 GTCTTAAAGT[CGBACTTTCAACAAG 546 GTCTTAAAGTCGGGACTTTCAACAAG 546 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATGGGAGGCTAAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAATGCGGTAA 638 CCAATGGGGCTAAATGCGGTAA CTGTAAATAAGTTTTAATG 737 CTGTAAATAAGTAGCTTTAATG 655 S80 CCAATGGGAGGCTTAATTTAATG 737 CTGTAAATAAGTAAGTATGGGTTATTA 706 TGTAAATAATAATAATAAGTTTAATG 737 CGGAAGGCTTAATAATAAGTTTAATG 737 CTGTAAATAATAAGTAGCTTTAATG 655 S80 CCATGGAAATAAGTAATAAGTTTAATG 737 CTGTAAATAATAAGCTTTAATGGGTTTATA 706 TGTAAATAATAATAAGTATAATAACGTTTATA 706 TGTAAATAATAATAATAAGTATAATAACGATTTAATG 698 S80 CATTTTTTAAGGATCATAAAAAAAAAAAA 932 CAATGGAAAAAAAAAAAA 932 CAATGGAAAAAAAAAAAAA 932 CTGTAAATAAGATAATAATAATAAGTATAATAAAAAAAAA
ShiFu2553-2 ShiFu2553-1 ShiFu2553-1 ShiFu2553-1 ShiFu2553-2 ShiFu2553-2 ShiFu2553-2 ShiFu2553-2 ShiFu2553-2 ShiFu2553-2 ShiFu2553-1 ShiFu2553-2 ShiFu2553-1 ShiFu2553-1 ShiFu22553-1 ShiFu22553-2 ShiFu22553-2 ShiFu2553-2 ShiFu2553-2	GATGATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTTA           GATGATCCGTCT         GATCGTTTA           CTAAGTATCTCTTATTAAGGAGATAGTTAA           CTAAGTATCTCTTTATTAAGGAGATAGTTAA           GTT         GTTTGTTTACCGTTAACGAGATAGTTAA           600         600           GTTTGTTTACCGTTAACGAGATCGATAGAAACT           GTTTGTTTACGTTAACGAGATCGATAGAAACT           GTTTGTTTATATGTTAACGCAATCGACCAAAGT           GTTTAGTTTAACGTCGATAGAAACT           GTTTAGTTTATATGTTAACGCAATCGACCAAAGT           GTTTAGTCTGTGTTAACGACGATCGACCAAAGT           GTTTAGTCTGTGTAACTAACGAAGACAAAGT           CCTTAATGATCGTCGATTAAAGTAGAACTAACTC           700         720           700         720           CATATCGATCGATTAAAGTAGAACTAACTC           CATATCGATCGATTAAAGTAGAACTAACTC           700         720           700         720           CATATCGATCGATTAAAGTAGAACTAACTC           700         720           CATATCGATCGATTAAAGTAGAACTAACTC           6         720           700         720           CATATCGATCGATTAAAGTAGTAGTAACTACTC           AAAAATCTGTAATTAATTAATTAATTAATTAACGAACTAACT	ССGGCTTATTCCATAAAAAAAAAAAC ССGGCTTATTCCATAAAAAA ССGGCTTATTCCATAAAAA GCTGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA GATGGCCATAGCCTAACCA B40 TTACGACGTTGGCGCTCTGATTG TTACGACGTTGGCGCTCTGAT TITCTTTTAATATTTTTGTTT GCGACTAGCCATAATATTTTTTTTTTTTTTTTTTTTTTT	АЛТАСАТОВОТСТОЙАЮТА 	ATTTQGTAATTAGAC[AGGG - CGA 539 ATTTGGTAATTAGACTAGA] - CGA 530 ATTTTGTATTAGACTAGA] - CGA 540 GTCTTAAAQTCGGACTTTCAACAAG 551 GTCTTAAAQTCGGACTTTCAACAAG 546 60 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAAAGTCTTAATGTTAATGCGTA CTGTAAATAATAAGTTTAATA 664 CCGAAAGTCTTACTATAATATAGCTATTA S50 TATTTTTAAGGTCATAAAAAAAAA 832 TATTTTTTAAGGTCATAAAAAAAAA 832 TATTTTTTAAGGTCATAAAAAAAAAA 832 TATTTTTTAAGGTCATAAAAAAAAAA 832 TATTTTTTAAGGTCATAAAAAAAAAA 832
Shi Fu2553-2 Shi Fu2553-1 Shi F102553-1 Shi F11023-1 Shi F11023-1 Shi F11023-2 Shi F02503-2 Shi F02503-1 Shi F11023-1 Shi F11023-2 Shi F02553-2 Shi	GAAQATCCGTCT         -GATCGTTA           GATGATCCGTCT         -GATCGTTA           GATGATCCGTCT         -GATCGTTA           CTAAQTATCTCCTTATTAAGGAGATAGTTA         GATCGTTA           CTAAQTATCTCTTATTAAGGAGATAGTTA         -GATCGTTA           000         000         000           GTTGTTTACCGTTAACGAGATCGAAAACT         GTTGTTTACCGTTAACGAGATCGAAAACT           GTTGTTTTACGTTAACGCAGATCGAAAACT         GTTGTTTACGTTAACGCAAAACTAGAAAACT           GTTGTTTAACGTGTAACGAGATCGAAAAGT         CCTAAGTCGATGGATAAACGAAAAGTA           CCTTAATATGTGTGTTAACGGAATAGGAACAAAAGT         700         730           CAAAAAATCTGTAATTAAAGTAGAAAAATAACTG         730         730           CAAAAAATCTGTAATTAATTAAGAAAAATAACTG         730         730           CAAAAAATCTGTAATTAATTAATTAAGTAGAACTAACTG         730         730           CAAAAAATCTGTAATTAATTATTTTTTTTATTGTAATTGAAATTAAA         730         730           CAAAAAATCTGTAATTAATTAATTTTTTTTTTTTTTTTT	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAAA	ААТАҚСАТӨВТӨТСТ СТ СТ САЙАБТА 	ATT TIGGTAATTAGAC[GAGGG [CGA] 539 ATT TIGGTAATTAGACTAGA] [CGA] 530 ATT TIGGTAATTAGACTAGA] [CGA] 546 OTCT TAAAGT[CGBACTTTCAACAAG 546 GTCTTAAAGT[CGBACTTTCAACAAG 546 GTCTAAAGTGGACTTTCAACAAG 546 GTCTAAAGTGGACTAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCGAAAGTCATAAGTTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTTAATTTTAATG 737 ACTGTAAATAAGTTTTTAATTTTAATG 655 CCAAAGTCTTACTATATTTTAATA 654 CCAAAGTCTTACTTTAATTTTATG 759 CTGTAAATAAGTTTTAATTTTACTG 759 TATTTTTAAGATCCTTAAAAAAAAAAA 932 TATTTTTAAGATCCTATAAAAAAAAAA 932 TATTTTTAAGTTCCAAAAAAAAAAA 932 TATTTTTAAGTTCCAAAAAAAAAAA
ShiFu2553-2 ShiFu2553-1 ShiF102553-1 ShiF102553-2 ShiF02553-1 ShiF102553-1 ShiF102553-1 ShiF102553-1 ShiF102553-1 ShiF102553-1 ShiF102553-1 ShiF102553-1	GAAGATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           CTAAGTATCTCTTATTAAGGAGATAGTTA         GATCGTTA           CTAAGTATCTCTTATTAAGGAGATAGTTA         GATCGTTA           000         GOT           GTTTGTTTACCGTTAACGAGATCGAAAACTA         GTA           010         GOT           GTTTGTTTTACGTTAACGAGATCGAAAACCT         GTA           GTTTGTTTTACGTTAACGAGATCGAAAACCT         GTA           GTTTGTTTACGTTAACGAGATCGACAAAAGTT         CCTAAATAACTTGTCTTACTAAGGAAAGAAAAGTT           CCTTAATGTTGTTGAGGATAGGAACTAACTC         700         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700         720         720           700	CCGGCTTATTCCATAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAAA	ААТАСАТОВТСТСИЙАЮТА 	ATTTIGGTAATTAGACCAGGG CGA       530         ATTTIGGTAATTAGACTAGA] CGA       530         ATTTIGGTAATTAGACTAGA] CGA       540         GTCTTAAAGTCGBACTTTCAACAAG       551         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       548         CCAATCAGAGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       598         CCAATCAGAGGGCTAAATGCGGTAA       620         GTGTGTAAATAAGTTTTAATG       737         ACTGTAAATAAGTTTTAATTTTAATG       737         ACTGTAAATAAGTTTTAATTTTAATG       655         CGAAAGTCTTACTTTAATTTTAATA       654         CCAAATAATAAGTTTTAATTTTAATG       655         AATGTAAATAAGTTTTAATTTTAATG       655         AATGTAAATAATAATAATTAATAATAATAATAATAATAAT
Sh1F02553-2 Sh1F02553-1 Sh1F102553-1 Sh1F102553-2 Sh1F025553-2 Sh1F025553-2 Sh1F0255555555	GATQATCCGTCT         GATCGTTTA           GATQATCCGTCT         GATCGTTTA           GATQATCCGTCT         GATCGTTTA           CTAAQTATCTCTTATTAAGGAGATAGTTAA           CTAAQTATCTCTTTATTAAGGAGATAGTTAA           GTTTGTTTTACCTTAACGAGATCGATAGTTAA           GTTTGTTTTACCTTAACGAGATCGAAAACTA           GTTTGTTTTACCTTAACGAGATCGAAAACTA           GTTTGTTTTACGTTAACGAGATCGAAAACCT           GTTTGTTTTACGTTAACGCGATCGAAAACCT           GTTTGTTTTACGTTAACGCGATCGAAAACCT           GTTTGTTTTACTTACGTTAACGCGATCGAACAAGTT           GTTTGTTTACGTTGACGATAAGAAAGGT           GTTTGTTTACGTTGCACTAACTAGGAACTAACTC           CATAATCGATCGATTAAAGTAGAACTAACTC           CATAACGATCGATTAAAGTAGAACTAACTC           CATAACGATCGATTAAAGTAGAACTAACTC           CATAACGATCGATCGATTAAAGTAGAACTAACTC           CATAACGATCGATCAATTTATTATTGTGTAATTCAACTACTC           CAAAAAATCTGTAATTTATTATTATTGTGTAATTAATTAA	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAAA	ААТАСАТАВТСТСТАЙАВТА 	ATTTQGTAATTAGACCAGGGCGA 530 ATTTGGTAATTAGACTAGAGACGA 546 551 GTCTTAAAGTCGBACTTTCAACAAG 551 GTCTTAAAGTCGBACTTTCAACAAG 546 600 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 CCAATCAGAGGGCTAAATGCGGTAA 638 620 611 700 ACTGTAAATAAGTTTTAATG 737 ACTGTAAATAAGGTTTAATTTAATG 737 ACTGTAAATAAGGTTTAATTTTAATG 737 ACTGTAAATAAGGTTTAATTTTAATG 737 ACTGTAAATAAGGTTTAATTTTAATG 737 ACTGTAAATAAGGTTTAATTTTAATG 738 CCAAAGTCTTACTAAATTTTAATG 655 730 730 74 75 75 75 75 75 75 75 75 75 75 75 75 75
SITF02553-2 SITF02553-1 SITF1023-1 SITF11023-1 SITF11023-1 SITF11023-2 SITF02553-2 SITF025	GATAATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTTA           GATGATCCGTCT         GATCGTTTA           CTAAGTATCCTCTTATTAAGGAGATAGTTA           CTAAGTATCTCTTTATTAAGGAGATAGTTAA           GTTTGTTTACCTTTATTAAGGAGATAGTTAA           GTTTGTTTTACCTTTAACGAGATCGATAGATAACT           GTTTGTTTTACCTTAACGAGATCGAAAACT           GTTTGTTTTACGTTAACGAGATCGAAAACT           GTTTGTTTTACGTTAACGAGATCGAAAAACT           GTTTGTTTTACGTTAACGAGATCGAAAAAACT           GTTTAGTCTTACGTTAACGAGATCGAACAAAAGT           GTTTAGTCTGTGTTAACGACAAAGTACCC           GTTTAGTCTGTGTTAACGACAAAGTACCC           GTTACGATCGATTAAAGTAGAAACTAACTC           700         70           700         70           CATACGATCGATTAAAGTAGAGAACTAACTC           CATATCGATCGATTAAAGTAGAAACTAACTC           CAAAAATCTGTAATTTATTATTTATTGTAAATAGAACTAACT	CCGGCTTATTCCATAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTATTCCATAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAAA	АЛТАСАТОВОТСТОДАЮТА 	ΑΤΤΤΙΟΙGTΑΑΤΤΑGΑCΙC/AGGG CGA       530         ΑΤΤΤΙΟΙGTΑΑΤΤΑGΑCΤΑGΑ] CGA       530         ΑΤΤΤΙΟΙTΑΑΔΟΤΙCOBACTITCAACAAG       551         GTCTTAAAQCICOBACTITCAACAAG       551         GTCTTAAAQCICOBACTITCAACAAG       546         600       600         CCAATCAGAGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       639         CCAATCAGAGGGCTAAATGCGGTAA       639         CCAATCAGAGGGCTAAATGCGGTAA       639         CCAATCAGAGGGCTAAATGCGGTAA       639         CCAATCAGAGGGCTAAATGCGGTAA       639         CCAATCAGAGGGCTAAATGCGGTAA       639         CCAATCAGAGGGCTAAATGCGTA       737         ACTGTAAATAATAATTITAATG       737         ACTGTAAATAAGCTTTAATTTAATG       738         ATGTAAATAAGTTTTAATGTATTATAGCTATA       636         CCAAAGTCTTACTAATAATTTAATGTAGTATTA       636         CCAAAGTCTTACTAATAATAATTAAGTATTATATG       736         AATGTAAATAAGTTTTAATGTATATTATAGCATTAA       635         CTGTAAATAATAATAATTAATTTAATGTATATA       636         CTGTAAATAATAATAATTAATTAAGTATTATAGTATATA       736         TATTTTTAAATAATAATAATTAAATAATAATAAAAAAAA
Shi Fu2553-2 Shi Fu2553-1 Shi F102553-1 Shi F102553-1 Shi F102553-2 Shi F02553-2 Shi F02553-2	GAAQATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           CTAAQTATCTCGTCT         GATCGTTA           CTAAQTATCTCGTTATTAAGGAGATAGTTA         GATCGTTA           000         GOO           GTTGTTTACGTTAACGAGATCGAAAACT         GTAGTTA           000         GOO           GTTGTTTACGTTAACGAGATCGAAAACT         GAAAAACT           GTTGTTTTACGTTAACGAGATCGAAAACT         GCAAAAACT           CCTAACGATCGATGATAAACGAGATCGACAAAGTT         CCTAATCGATCGATCGATAAAGTAGAAACTA           700         730           CAAAAAATCTGTAATTAATTAAGAAGAACTAACTO         730           CAAAAAATCAGTAATTAATTATTATTGTAGAAAATTAAC           TAACGATCGATCGATTAAAGTAGAACTAACTO           100         730           CAAAAAATCAGTACGATTAAAGTAGAACTAACTO           100         730           CAAAAAATCAGTACGATTAAAGTAGAACTAACTO           100         100           100         730           100         730           100         730           CAAAAAATCAGTAGAATTATTATTATTATTATTATTATTATTATTATTA	CCGGCTTATTCCATAAAAAAAAAAAAAACCCCGGCTTATTCCATAAAAAAGCCGGCTATTCCATAAAAAAGCTGGCCTATTCCATAACAAAAGCTGGCCATAGCCTAACCAAGATGGCCATAGCCTAACCAAGATGGCCATAGCCTCTGATGGCGTTCTGATGGCGTTCTGATGGCGTTCTGATGGCGTCTGATGGCGACTAGTGATTATATAGGCAAATAAGGCATACTTATATTTTTTTT	ААТАСАТАВТСТСАЙАВТА 	ATTTIGGTAATTAGACCAGGG CGA       530         ATTTIGGTAATTAGACTAGA] CGA       530         ATTTIGGTAATTAGACTAGA] CGA       530         ATTTTGTTATAGACCAGGTTCGA       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGGACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       548         CCAATCAGAGGGGCTAAATGCGGTAA       638         CCAATCAGAGGGGCTAAATGCGGTAA       638         CCAATCAGAGGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       638         CCGAAAGTCTTAATATTTAATTTTAATG       737         ACTGTAAATAAGTTTTAATTTTAATG       737         AATGTAAATAAGTTTTAATTTAATATAGTTAAGTTTTAATTTAGTG       655         CCAAAGTCTTAATAAGTTTTAATAGTTATATGTG       656         AATGTAAATAAGATTTTAATATTTAATATAGTTATATG       656         CGAAAGTCTTTAATAATAAGTTTTAATATATATATATATA
ShiFu2553-2 ShiFu2553-1 ShiF102553-1 ShiF102553-2 ShiF02553-2	GATQATCCGTCT         -GATCGTTTA           GATQATCCGTCT         -GATCGTTTA           GATQATCCGTCT         -GATCGTTTA           CTAAQTATCTCCTTATTAAGGAGATAGTTAA         GATCGTTA           CTAAQTATCTCCTTATTAAGGAGATAGTTAA         600           GTTGTTTACCGTTAACGAGATCGAAAACTA         600           GTTTGTTTTACCGTTAACGAGATCGAAAACTA         600           GTTTGTTTTACGTTAACGAGATCGAAAACCT         600           GTTTGTTTTACGTTAACGAGATCGAAAACCT         600           GTTTGTTTTACGTTAACGAGATCGAAAACCT         600           GTTTGTTTACGTTAACGAGATCGACAAAAGT         700           CATAATCGATCGATTAAAGTAGAACTAACTC         700           700         720           700         720           CATAATCGATCGATTAAAGTAGAACTAACTC           CATATCGATCGATTAAAGTAGAAACTAACTC           CATAACGATCGATTAAAGTAGAAACTAACTC           700         720           700         720           700         720           700         720           700         720           700         720           700         720           700         720           700         720           700         720           700         720	ССGGCTTATTCCATAAAAAAAAAAAAC ССGGCTTATTCCATAAAAAA 	ААТАСАТАВТСТСЙАЮТА 	ATTTIQGTAATTAGACCAGGG CGA       530         ATTTIQGTAATTAGACTAGA] CGA       530         ATTTIQGTAATTAGACTAGA] CGA       530         ATTTIGGTAATTAGACCAGGTTCCAACAAG       530         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGBACTTTCAACAAG       546         GTCTTAAAGTCGGACTAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       638         CCAATCAGAGGGCTAAATGCGGTAA       639         CCAATCAGAGGGCTAAATGCGGTAA       639         ACTGTAAATAAGTTTTAATG       737         ACTGTAAATAAGTTTTAATTTTAATG       737         ACTGTAAATAAGTTTTAATTTTAATG       655         AATGTAAATAAGTTTTAATTTTAATA       656         900       700         TATTTTTAATAATAATAATAATAATAATAA       932         TATTTTTAATTAATAA       598         CTGTAAATAATAATAATAATAATAATAATAATAATAA       932         TATTTTTAATAATAATAATAATAATAATAATAA       932         TATTTTTAAGATTCAAAAAAAAA       778         TATTTTAAGTTCAAAAAAAAAAA       778         TATTTTAAGTTCAAAAAAAAAAAA       778         CAAAGTCTTAAGTTCAAAAAAAAAAAAAA       778
Shi Fu2553-2 Shi Fu2553-1 Shi F102553-1 Shi F102553-2 Shi F02553-2 Shi	GATAGATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           GATGATCGCGTCT         GATCGTTA           CTAAGTATCCTCTTATTAAGGAGATAGTTAA           GTAGTCGTCT         GATCGTTAA           CTAAGTATCTCTTTATTAAGGAGATAGTTAA           GTTTGTTTTACCTTTACTAAGGAGATCGATAAAACT           GTTTGTTTTACCTTTACTAAGGAGATCGAAAACT           GTTTGTTTTACCTTAACGAGATCGAAAACT           GTTTGTTTTACCTTAACGAGATCGAAAAACT           GTTTGTTTTACCTTAACGAGATCGACAAAAGT           GTTTGTTTTACCTTACTAAGGAGATCGACAAAAGT           GTTTGTTTACCTGTAACTAACGCAAAAGGAT           GTTTGTTTACGTGTGTTAACGCAAAAGAAAAGT           CCTTAATGTTTCAGTAGAACTAACTC           CATAACGATCGATCGATTAAAGTAGAACTAACTC           CATATCGATCGATCGATTAAAGTAGAACTAACTC           CATATCGATCGATCGATTAAAGTAGAACTAACTC           CATATCGATCGATTAAAGTAGAACTAACTC           CAAAAAATCTGTAATTATTATTATTGTAGTAATTAA           TTTGTAATATCGATCGATTAAAGTAGAACTAACTC           CAAAAAATCAGATCGATCAATTATTTATTGTGTAATTATTTGTAG           TTTTGTAATAATAACGATTACTGTAATTTGTAC           TTTTGTAATAATAACGATTACTGTAC           TTTTGTAATAATAACGATTAATTTGTAC           TTTTTGTAATAATAACGATTAATTGTAC           TTTTTGTAATAAAAAAAAAAAAAAAAAAAAAAAAAAAA	CCGGCTTATTCCATAAAAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC CCGGCTTATTCCATAAAAAAAAC GCTGGCCATAGCCTAACCAAAAAAAAA GCTGGCCATAGCCTAACCAAAAAAAAAA	ААТАСАТАВТСТСТАЙАВТА 	ATTTIQGTAATTAGAC[AGGG CGA] 530         ATTTIQGTAATTAGACTAGA] CGA] 530         ATTTIGGTAATTAGAC[AGGG] CGA] 546         GTCTTAAAGT[CGBACTTTCAACAAG 551         GTCTTAAAGT[CGBACTTTCAACAAG 546         GTCTTAAAGT[CGBACTTTCAACAAG 546         GTCTTAAAGT[CGBACTTTCAACAAG 546         GTCTTAAAGT[CGBACTTTCAACAAG 546         GTCTTAAAGT[CGBACTTTCAACAAG 546         GTCTTAAAGT[CGBCTAAATGCGGTAA 638         CCAATCAGAGGGCTAAATGCGGTAA 638         CCAATCAGAGGCTTAATGCGTAA         CCAAAGTCTTACATAATAATTTTAATA         CCAAAGTCTTACTACGTTATATA         CCAAAGTCTTTAATAATAATTTAATAATTAATAATAATTATATA         CCAAAATAATAATAATTTTAATAATTATATA         CCAAAATAATAATAATTTTAATTAATAA         CCAAAATAATAATAATTTTAATTAATAA         CCAAAATAATAATAATTTTAATTTAATA         CCAAAATAATAATAATTTTAATTAATAA         S80         TTTTTAAGATCA
SITF02553-2 SITF02553-1 SITF1023-1 SITF11023-1 SITF11023-1 SITF11023-2 SITF02553-1 SITF11023-1	GAAQATCCGTCT         GATCGTTA           GATGATCCGTCT         GATCGTTA           CTAAQTATCCGTCT         GATCGTTA           CTAAQTATCTCCTTATTAAGGAGATAGTTAA         GATCGTTA           CTAAQTATCTCTTATTAAGGAGATAGTTAA         GTG           GTTGTTTACCGTTAACGAGATCGAAAACT         GTG           GTTTGTTTTACCGTTAACGAGATCGAAAACT         GTTGTTTACCGTTAACGAGATCGAAAACT           GTTTGTTTTACGTTAACGCAGATCGAAAACT         GTTGTTTATCGTTAACGCAAAACTAACTC           GTTTGTTTACGTTAACGGAATAGAAAACT         CCTTAATAGTGTGTTAACGAAAAGTAACTC           GTTTGTTTACGATTAAAGTAGAAAAGTAACTC         TCTTAGTCTGTGTATAAAGTAGAACTAACTC           CATATCGATCGATGAATTAATTATTGTAGAAAATTAACTG         TCTTAGTATAGAAAATTAACTAACTC           700         723           CATATCGATCGATTAAAGTAGAAACTAACTC         TAAAAAAAACGATTAACTGATTAAGTAGAACTAACTC           700         723           CAAAAAAATCGTGTAATTAATTTTTTTTTTTTTTTTTTT	CCGGCTTATTCCATAAAAAAAAAAAAAACC CCGGCTTATTCCATAAAAAAAAAA	АЛТАСАТАВТСТСТАЙАВТА САТАВТІТІСТСТАЙАВТА САТАВТІТІСТСТАЛАВТА 	ATTTIQGTAATTAGAC[AGGG CGA] 530         ATTTIQGTAATTAGACTAGA] CGA] 530         ATTTIQGTAATTAGACTAGA] CGA] 546         BTTTTGTATAGAC[AGGGTTCAACAAG 551         GTCTTAAAGTCGGACTTTCAACAAG 551         GTCTTAAAGTCGGACTTTCAACAAG 546         BTCTTAAAGTCGGACTTTCAACAAG 546         CCAATCAGAGGGCTAAATGCGGTAA 638         CCAATCAGAGGGCTAATTGCGTA         ACTGTAAATAATAAGTTTAATTTAATG         CGAAAGTCTTACTTACTTAATAGTATTTAATA         CGAAAGTCTTACTTAATTTTAATA         CGAAAGTCTTACTTAATTTAATAGTATTTAATA         CGAAAGTCTTACTTAATTTAATAGTATTA         S80         AATGTAAATAAGTTTTAATTAAGTATTA         S80         CTGTAAATAATAATAATTTAATAGTATTA         S80         TATTTTTAAGATTTCAAAAAAAAAA         S80         TATTTTAAGTTTCAAAAAAAAAAAAAAAAAAAAAAAAAA

Supplementary Fig. 5. Supplementary material.

# Cluster IV



Supplementary Fig. 6. Supplementary material.

## Cluster V

	20	40 I	60 I	80	
Sf1F01749	GGTACTTGTTCATAGATCAGTTT	CATTTGAGCTTTCTCCAAG	TAACAAGGTGCACGGTAGAGG	TAAAGCCAAGTTGCAAAA	AAAGCAAAAATGAATTC 99
Sf1F05978	GACATCAGTTT	CATTCTGAGCTTTCTCCAAG	TAACAAGGTGCACGGTAGAGG	TAAAGCCAAGTTGCAAAA	AAATCTAAAATGAATTC 87
	100 120	140	160	180	
Sf1E01749	CAAAATCATAATTTTCCTGTGCA	TOTOTOTOTOTOTOTOTOTOTOT		TTTAAAGAAATTGAAGGA	GTTGGCCAAAGGGTCCG 198
Sf1E05078	CAAAATCATAATTTTCCTGTGCA	TCTGCTTCCTGGCTGTGTCT	CAGTATCAGCGTGGGACCTC	TTTAAAGAACTTGAAGGA	GTTGGCCAAAGGGTCCG 186
5111 05370					IST TOCCAAAOGOTCCO ISC
	200 220	240	250	29	
			*		
Sf1F01749	TGATGCTGTCATCAGTGCAGGAC	CTGCAGTAGACGTACTAACT	AAGGCTAAAAAGCTGGCTGAT	GGATCCAGCGAAGAAGAC	TAGAAGACCATCA - GGT 296
Sf1F05978	TGATGCTGTCATCAGTGCAGGAC	CAGCAGTGGATGTGCTAACT	AAAGCTAAAAAGCTGGCTGGA	GGATCTAGCGAAGAGGGAC	TAGAAACCCATCATGGT 285
	-				
	300 320	340	360 1		380
Sf1F01749	300 320 I CAAACTACCCTGATGGGAGTATT	340 I GTCAAAGAAAATTATACCAAA		AGTTTTGTAAATACACAT	380 1 CTCATGTTGGTAATCAA 395
Sf1F01749 Sf1F05978	300 I CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT	3400 I GTCAAAGAAAATTATACCAA/ GTCAAAGAAAATTATACCAA/	360 ATGTTTATTTGTATACGATAC	AGTTTTGTAAATACACAT AGTTTTGTAAATACCCAT	CTCATGTTGGTAATCAA 395
Sf1F01749 Sf1F05978	300 320 I CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT 400 40	340 J GTCAAAGAAAATTATACCAAJ GTCAAAGAAAATTATACCAAJ 20 4	300 ATGTTTATTTGTATACGATAC ATGTTTATTTGTATACGATCC	AGTTTTGTAAATACACAT	CTCATGTTGGTAATCAA 395
Sf1F01749 Sf1F05978	300 320 I CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT 400 4	3400 GTCAAAGAAAATTATACCAA GTCAAAGAAAATTATACCAA 20 4 1	300 TGTTTATTTGTATACGATAC ATGTTTATTTGTATACGATCC	AGTTTTGTAAATACACAT AGTTTTGTAAATACCCAT 0	CTCATGTTGGTAATCAA 395 CTCATGTTGTTAATCAA 384
Sf1F01749 Sf1F05978 Sf1F01749	300 320 CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT 400 4 CTGCATTTAATCCTTTATTGTAT	3400 GTCAAAGAAAATTATACCAAA GTCAAAGAAAATTATACCAAA 20 4 TTTTTTTAGTATTTTTTTTTTTTTTTTTTTTTTTTTTT	300 ATGTTTATTTGTATACGATAC ATGTTTATTTGTATACGATCC 10 10 10 10 10 10 10 10 10 10 10 10 10	agttttgtaaataCaCat agttttgtaaataCcCat 0 tatgtattaagcttaaaa	380           I           CTCATGTTGGTAATCAA           395           CTCATGTTGTTAATCAA           480           1
Sf1F01749 Sf1F05978 Sf1F01749 Sf1F05978	200 1 CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT 400 CTGCATTTAATCCTTTATTGTAT CTGCATTTAATCCTTTATTGTAT	3400 GTCAAAGAAAATTATACCAAA GTCAAAGAAAATTATACCAAA GTCAAAGAAAATTATACCAAA 4 1 1 1 1 1 1 1 1 1 1 1 1 1	300 TGTTTATTTGTATACGATAC ATGTTTATTTGTATACGATCC 10 46 TGTGAATCATTTCATTAAACG TGTAAATCTTTTCATTAAACG	AGTTTTGTAAATACACAT AGTTTTGTAAATACCCAT 0 TATGTATTAAGCTTAAAA TATGTAATAAGCTTAAAA	380         395           CTCATGTTGGTAATCAA         395           CTCATGTTGTTAATCAA         384           480         480           AAAAAAAAAAAAAAAAAAAAAA         494           AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Sf1F01749 Sf1F05978 Sf1F01749 Sf1F05978	300 320 CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT 400 4 CTGCATTTAATCCTTTATTGTAT CTGCATTTAATCCTTTATTGTAT 500 5	340 Б Б С С С С С С С С С С С С С	300 ATGTTTATTTGTATACGATAC ATGTTTATTTGTATACGATAC TGTGAATCATTTCATT	AGTTTTGTAAATACACAT AGTTTTGTAAATACCCAT TATGTATTAAGCTTAAAA TATGTAATAAGCTTAAAA	380         395           CTCATGTTGGTAATCAA         395           CTCATGTTGTTAATCAA         384           480         384           1         384           480         484           1         484           1         484           1         484           1         484           1         484
Sf1F01749 Sf1F05978 Sf1F01749 Sf1F05978 Sf1F01749	300 320 1 CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT 400 4 CTGCATTTAATCCTTTATTGTAT CTGCATTTAATCCTTTATTGTAT 500 AAAAAAAAAAA	340           GTCAAAGAAAATTATACCAAA           GTCAAAGAAAATTATACCAAA           GTCAAAGAAAATTATACCAAA           GTCAAAGAAAATTATACCAAA           1           1           1           1           1           1           1           1           520           1           505	300 ATGTTTATTTGTATACGATAC ATGTTTATTTGTATACGATCC 10 10 10 10 10 10 10 10 10 10 10 10 10	AGTTTTGTAAATACACAT AGTTTTGTAAATACCCAT o TATGTATTAAGCTTAAAA TATGTAATAAGCTTAAAA	380         395           CTCATGTTGGTAATCAA         395           CTCATGTTGTTAATCAA         384           480         384           480         494           АААААААААААААААААААА         494           АААААААААААААААААААА         469
Sf1F01749 Sf1F05978 Sf1F01749 Sf1F05978 Sf1F01749 Sf1F01749 Sf1F01749	300 320 1 CAAACTACCCTGATGGGAGTATT CAAACTACCCTGATGGGAGTATT 400 4 CTGCATTTAATCCTTTATTGTAT CTGCATTTAATCCTTTATTGTAT 500 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	3400         3400           GTCAAAGAAAATTATACCAA         1           GTCAAAGAAAATTATACCAA         4           20         4           TTTTTTTAGTATTTTTTTA         4           TTTTTT         -         -           520         -         -           -         -         505           AAAAAAA         498         -	300 ATGTTTATTTGTATACGATAC ATGTTTATTTGTATACGATCC 10 10 10 10 10 10 10 10 10 10 10 10 10	AGTTTTGTAAATACACAT AGTTTTGTAAATACCCAT TATGTATTAAGCTTAAAA TATGTATTAAGCTTAAAA	380         395           CTCATGTTGGTAATCAA         395           CTCATGTTGTTAATCAA         384           480         1           АААААААААААААААААА         494           ААААААААААААААААААА         469