

Supplementary Material File 1 – Supplementary Tables

Molecular evolution of α -latrotoxin, the exceptionally potent vertebrate neurotoxin in black widow spider venom.

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Table S1. Specimens sampled for this study, species ID, and NCBI sequence Accession numbers for α -latrotoxin (α -LTX) ~4.1 kb and 618 bp sequences and mitochondrial gene cytochrome oxidase I.

| Specimen | Species | Collection Locality‡ | α -LTX Accession # (~4.1 kb) | α -LTX Accession # (618 bp) | Mt COI Accession # (428-659 bp) |
|----------|----------------------------|--------------------------|--|---------------------------------------|------------------------------------|
| 018 | <i>Latrodectus sp.</i> | Salvador, Bahia, Brazil | NA | KC414040 | AY383073 |
| 010 | <i>Latrodectus sp.</i> | Chile | NA | KC414041 | AY383074 |
| LatDR | <i>Latrodectus sp.</i> | Dominican Republic | KC414035 | NA | KC414075 |
| 009 | <i>L. antheratus</i> | Argentina | NA | KC414042 | AY383048 |
| 050 | <i>L. bishop</i> | Florida, US | NA | KC414043 | AY383060 |
| 013 | <i>L. corallinus</i> | Argentina | NA | KC414044 | AY383061 |
| 020 | <i>L. corallinus</i> | Argentina | NA | KC414045 | AY383062 |
| 002 | <i>L. diaguita</i> | Argentina | NA | KC414046 | AY383063 |
| 003 | <i>L. diaguita</i> | Argentina | NA | KC414047 | AY383064 |
| 097 | <i>L. geometricus</i> | Roodepoort, South Africa | NA | KC414048 | AY383050 |
| 099 | <i>L. geometricus</i> | Roodepoort, South Africa | NA | KC414049 | AY383049 |
| 016 | <i>L. geometricus</i> | Argentina | NA | KC414050 | AY383065 |
| 017 | <i>L. geometricus</i> | Argentina | NA | KC414051 | AY383066 |
| 034 | <i>L. geometricus</i> | Florida, US | NA | KC414052 | AY383067 |
| 033 | <i>L. geometricus</i> | Florida, US | NA | KC414053 | AY383068 |
| geoCA1 | <i>L. geometricus</i> | San Diego, California | KC414036 | NA | KC414076 |
| LhasP4 | <i>L. hasselti</i> | Perth, Western Australia | NA | KC414054 | KC414077 |
| LhasP5 | <i>L. hasselti</i> | Perth, Western Australia | NA | KC414055 | KC414078 |
| LhasP2 | <i>L. hasselti</i> | Perth, Western Australia | KC414033 | NA | KC414079 |
| 049 | <i>L. hesperus</i> | New Mexico, US | NA | KC414056 | AY383071 |
| LhesUCR | <i>L. hesperus</i> | Riverside, California | KC414032 | NA | KC414080 |
| Lhesmar1 | <i>L. hesperus</i> | Yarnell, Arizona | KC414037 | NA | KC414081 |
| 028 | <i>L. katipo</i> | New Zealand | NA | KC414057 | AY383053 |
| 029 | <i>L. katipo</i> | New Zealand | NA | KC414058 | AY383052 |
| 100 | <i>L. mactans</i> | Mississippi, US | NA | KC414059 | AY383072 |
| 014 | <i>L. mirabilis</i> | Argentina | NA | KC414060 | AY383076 |
| 015 | <i>L. mirabilis</i> | Argentina | NA | KC414061 | AY383077 |
| Lpa15 | <i>L. pallidus</i> | Be'er Sheva, Israel | NA | KC414062 | KC414082 |
| Lpa14 | <i>L. pallidus</i> | Be'er Sheva, Israel | KC414038 | NA | KC414083 |
| 098 | <i>L. renivulvatus</i> | Gauteng, South Africa | NA | KC414063 | AY383057 |
| 114 | <i>L. rhodesiensis</i> | Namibia/S. Africa border | NA | KC414064 | AY383058 |
| 091 | <i>L. rhodesiensis</i> | Namibia/S. Africa border | NA | KC414065 | AY383079 |
| Ltre2 | <i>L. tredecimguttatus</i> | | KC414034 | NA | KC414084 |
| 036 | <i>L. tredecimguttatus</i> | Sayeret Shaaed, Israel | NA | KC414066 | AY383081 |
| Ltre4 | <i>L. tredecimguttatus</i> | | NA | KC414067 | KC414085 |

| | | | | | |
|--------|----------------------------------|-----------------------|----------|-----------------------|----------|
| 004 | <i>L. variegatus</i> | Santa Cruz, Argentina | NA | KC414068 | AY383083 |
| 005 | <i>L. variegatus</i> | Bariloche, Argentina | NA | KC414069 | AY383084 |
| 051 | <i>L. varioulus</i> | Maryland, US | NA | KC414070 | AY383082 |
| PDK270 | <i>Steatoda capensis</i> | Maui, Hawaii | NA | KC414071 | KC414086 |
| SG-SP | <i>Steatoda grossa</i> | Yarnell, Arizona | KC414039 | NA | KC414087 |
| SgroSA | <i>Steatoda grossa</i> | Maui, Hawaii | NA | KC414072 | KC414088 |
| Ptep1 | <i>Parasteatoda tepidariorum</i> | Massachusetts, US | NA | KC414073, KC414074 | KC414089 |

Genbank accessions for *L. hasselti* α -latrotoxin FR851877, for *L. tredecimguttatus* α -latrotoxin X55009.1

‡ Further locality details provided in Garb et al. 2004 for some specimens

Table S2. Sequence variability of α -latrotoxin and mt COI genes across sampled species for pairwise comparisons. Numbers show average in bold, above minimum to maximum values computed for sequences in Figure 2. Wing, body, head and post-trans (post-translationally cleaved) are major domains of α -latrotoxin.

| | α -latrotoxin | | | | | mt COI |
|---|-------------------------------|-------------------------------|------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| | All α -LTX | wing | body | head | post trans. | |
| No. sites (bp) | 4233 | 972 | 2121 | 549 | 591 | 659 |
| Nu distance ave. (min-max)* | | | | | | |
| Across mactans clade | 0.043 0.002-0.058 | 0.045 0.00-0.107 | 0.041 0.002-0.56 | 0.047 0.006-0.081 | 0.053 0.00-0.068 | 0.121 0.007-0.160 |
| Across <i>Latrodectus</i> | 0.061 0.002-0.128 | 0.055 0.00-0.108 | 0.058 0.002-0.126 | 0.062 0.006-0.126 | 0.080 0.000-0.187 | 0.135 0.007-0.186 |
| <i>S. grossa</i> vs. <i>Latrodectus</i> | 0.279 0.276-0.282 | 0.261 0.231-0.244 | 0.285 0.280-0.291 | 0.285 0.280-0.292 | 0.316 0.306-0.323 | 0.193 0.173-0.203 |
| Latrotoxin paralogs | 0.508 0.312-0.524 | 0.472 0.268-0.506 | 0.485 0.329-0.510 | 0.609 0.333-0.765 | 0.563 0.305-0.615 | NA |
| AA distance ave. (min-max) | | | | | | |
| Across mactans clade | 0.060 0.005 - 0.080 | 0.054 0.00 - 0.087 | 0.054 0.004 -0.074 | 0.075 0.006-0.146 | 0.078 0.000-0.115 | 0.012 0.000-0.033 |
| Across <i>Latrodectus</i> | 0.080 0.005 - 0.160 | 0.066 0.00 - 0.131 | 0.072 0.004 -0.149 | 0.097 0.006-0.195 | 0.122 0.000-0.291 | 0.023 0.000-0.057 |
| <i>S. grossa</i> vs. <i>Latrodectus</i> | 0.348 0.342 - 0.356 | 0.294 0.281 - 0.307 | 0.341 0.328-0.346 | 0.378 0.365-0.390 | 0.432 0.414-0.452 | 0.062 0.055-0.068 |
| Latrotoxin paralogs | 0.650 0.347 - 0.670 | 0.609 0.292 - 0.667 | 0.626 0.370-0.673 | 0.786 0.363-0.973 | 0.727 0.395-770 | NA |
| Ka ave. (min-max) | | | | | | |
| Across mactans clade | 0.024 0.001-0.033 | 0.024 0-0.036 | 0.022 0.001-0.032 | 0.035 0-0.089 | 0.027 0-0.053 | 0.008 0-0.021 |
| Across <i>Latrodectus</i> | 0.034 0.001-0.077 | 0.028 0-0.056 | 0.032 0.001-0.076 | 0.053 0-0.138 | 0.049 0-0.141 | 0.018 0-0.049 |
| <i>S. grossa</i> vs. <i>Latrodectus</i> | 0.214 0.210-0.220 | 0.177 0.169-0.185 | 0.232 0.220-0.239 | 0.251 0.209-0.280 | 0.208 0.201-0.218 | 0.048 0.039-0.051 |
| Latrotoxin paralogs | 0.612 0.230-0.679 | 0.568 0.189-0.638 | 0.614 0.253-0.708 | 1.045 0.271-3 | 0.651 0.206-0.702 | NA |
| Ks ave. (min-max) | | | | | | |
| Across mactans clade | 0.113 0.006-0.155 | 0.110 0-0.196 | 0.110 0.007-0.152 | 0.090 0-0.162 | 0.147 0-0.259 | 0.874 0.031-1.419 |
| Across <i>Latrodectus</i> | 0.167 0.006-0.374 | 0.152 0-0.357 | 0.167 0.007-0.402 | 0.125 0-0.277 | 0.204 0-0.582 | 0.961 0.031-1.425 |

| | | | | | | |
|---|-----------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------------|-----------------------------|
| <i>S. grossa</i> vs. <i>Latrodectus</i> | 0.982 0.950-1.05 | 0.882 0.820-0.934 | 1.073 1.018-1.168 | 0.694 0.588-0.784 | 0.931 0.839-0.996 | 1.644 1.253-1.927 |
| Latrotoxin paralogs | 2.216 1.556-3.158 | 2.93 1.36-5.32 | 2.061 1.654-0.137 | 2.155 0.635-3.621 | 2.235 1.006-6.215 | NA |
| ω ave. (min-max) | | | | | | |
| Across mactans clade | 0.210 0.140-0.266 | 0.241 0.088-0.421 | 0.208 0.085-0.389 | 0.482 0-1.696 | 0.208 0.055-0.508 | 0.008 0-0.032 |
| Across <i>Latrodectus</i> | 0.207 0.140-0.266 | 0.220 0.088-0.421 | 0.204 0.085-0.389 | 0.480 0-1.696 | 0.238 0.055-0.508 | 0.017 0-0.046 |
| <i>S. grossa</i> vs. <i>Latrodectus</i> | 0.218 0.203-0.231 | 0.202 0.187-0.226 | 0.216 0.198-0.234 | 0.369 0.267-0.449 | 0.224 0.210-0.260 | 0.029 0.026-0.035 |
| Latrotoxin paralogs | 0.280 0.148-0.358 | 0.206 0.116-0.328 | 0.302 0.137-0.428 | 0.636 0.185-1.384 | 0.348 0.107-0.666 | NA |

* Uncorrected genetic distance; mactans clade includes all *Latrodectus* except *L. geometricus* and *L. rhodesiensis* (see Figure 3). Nu = nucleotides; AA = amino acids. Note number of sites includes all in nucleotide alignment across all sequences.

Table S3. Sequence database matches to complete α -latrotoxin and first 1407 bp (excludes ankyrin repeats) using various Blast programs against UniProtKB, NCBI nr and NCBI dbEST databases. Shown are top matches (excluding latrotoxins) including accession, followed by descriptor, taxon, and E-score, length.

| Query Sequence | Database/operation | Accession | Descriptor | Taxon | E-value | Length |
|--|-----------------------------------|--------------|--|---|---------|---------|
| Descriptor: α-latrotoxin Taxon: <i>L. tredecimguttatus</i> Accession: X55009.1 Length: 4110 bp; 1370 aa | UniProtKB/ blastx | P16157.3 | Ankyrin 1 | <i>Homo sapiens</i> | 2e-60 | 1881 aa |
| As above | UniProtKB (Arthropoda)/ blastx | Q7Z020.3 | dTRPA1* | <i>D. melanogaster</i> | 4e-36 | 1296 aa |
| As above | NCBI nr/ Psi-blast | XP_001193670 | ankyrin 2,3/unc44 | <i>Aedes aegypti</i> | 2e-83 | 2069 aa |
| As above | NCBI dbEST/ tblastx | DV253213 | cDNA clone A2FNK62 | <i>Aedes aegypti</i> | 1e-39 | 900 bp |
| Descriptor: α-latrotoxin Taxon: <i>L. tredecimguttatus</i> Accession: X55009.1 Length: first 1407 bp | UniProtKB / blastx | P7522 | 16S rRNA m7G methyltransferase | <i>Mycoplasma pneumoniae</i> M129 | 0.001 | 191 aa |
| As above | UniProtKB (Arthropoda)/ blastx | Q8IT89 | Tropomyosin | <i>Haemaphysalis longicornis</i> | 3e-04 | 284 aa |
| As above | NCBI nr/ Psi-blast | ZP_09676930 | hypothetical protein PDENDC454_13370 | <i>Paenibacillus dendritiformis</i> C454 | 0.15 | 176 aa |
| As above | NCBI dbEST/ tblastx | FY374455 | House spider embryo cDNA library early stage 6 | <i>Parasteatoda tepidariorum</i> | 2e-04 | 778 bp |

* dTRPA1 is short for *Drosophila* Transient receptor potential cation channel subfamily A member 1

Table S4. Summary of nucleotide substitution models for datasets analyzed in this study as selected by jModeltest (jMT) using the Akaike Information Criteria and estimated model parameters utilized in Maximum Likelihood tree construction or with Mr.Modeltest (MMT) for partitioned Bayesian analyses (Est. = model parameters were estimated in Mr.Bayes).

| Data/Program | Model | Freq A | Freq C | Freq G | R(a) [AC] | R(b) [AG] | R(c) [AT] | R(d) [CG] | R(e) [CT] | gamma | ncat | pinvar |
|--|--------------|--------|--------|--------|-----------|-----------|-----------|-----------|-----------|--------|------|--------|
| 4.2 kb ALTX jMT - Fig. 2 | GTR+G | 0.3515 | 0.1654 | 0.1949 | 1.9199 | 4.2581 | 1.2165 | 1.3969 | 4.8488 | 1.1960 | 4 | 0 |
| 642 bp ALTX jMT - Fig. 4 | TPM3uf+ G | 0.3850 | 0.1561 | 0.1657 | 2.1953 | 5.5257 | 1.0000 | 2.1953 | 5.5257 | 0.8780 | 4 | 0 |
| mtCOI jMT - Fig.3 | GTR+I+G | 0.3264 | 0.0654 | 0.1664 | 0.3302 | 17.3613 | 0.7000 | 3.8951 | 28.8099 | 0.6100 | 4 | 0.5580 |
| mtCOI 1 st & 2 nd positions only jMT | TIM1+I+ G | 0.2089 | 0.1664 | 0.2460 | 1.0000 | 23.1869 | 14.4349 | 14.4349 | 93.7231 | 3.8050 | 4 | 0.8080 |
| 4.2 kb ALTX MMT 1st pos. Fig. 2 | GTR+G | est | est | est | est | est | est | est | est | est | est | est |
| 4.2 kb ALTX MMT 2nd pos. Fig. 2 | GTR+G | est | est | est | est | est | est | est | est | est | est | est |
| 4.2 kb ALTX MMT 3rd pos. Fig. 2 | GTR+G | est | est | est | est | est | est | est | est | est | est | est |
| 642 bp ALTX MMT 1st pos. Fig. S4 | GTR+G | est | est | est | est | est | est | est | est | est | est | est |
| 642 bp ALTX MMT 2nd pos. Fig. S4 | GTR+I | est | est | est | est | est | est | est | est | est | est | est |
| 642 bp ALTX MMT 3rd pos. Fig. S4 | GTR+G | est | est | est | est | est | est | est | est | est | est | est |
| mtCOI - Fig. S3 MMT – 1st pos. | GTR+G | est | est | est | est | est | est | est | est | est | est | est |
| mtCOI - Fig. S3 MMT – 2nd pos. | GTR+I | est | est | est | est | est | est | est | est | est | est | est |
| mtCOI- Fig. S3 MMT – 3rd pos. | GTR+G | est | est | est | est | est | est | est | est | est | est | est |

Table S5. Distribution of sites undergoing positive selection as determined from TreeSAAP analyses (magnitude categories 6-8, 8 being most extreme) along *Latrodectus* α -latrotoxin branches for properties where Z-score ≥ 3.09 (magnitude category 8 (red text), category 7 (blue text), category 6 (black text)).

| Property | Wing | Body | Head | Post-trans. cleaved |
|--|---------------|---------------------|--------------|---------------------|
| Coil tendencies | | | 1116 | 1289 |
| Composition | | 496 | | |
| Equilibrium constant (ionization of COOH) | 90, 286 | 734, 758, 774 | | 1340 |
| Isoelectric point | 137, 203 | 406, 760* | | 1287 |
| Total non-bonded energy | 203 | | | 1195 |
| Average number of surrounding residues | | 774 | | |
| Buriedness | | | | 1195 |
| Chromatographic index | | 496 | | 1195 |
| Compressibility | | 467, 554*, 596 | | 1116 |
| Hydropathy | | | | 1195 |
| Long-range non-bonded energy | | | | 1195 |
| Mean r.m.s. fluctuation displacement | | | | 1195 |
| Normalized consensus hydrophobicity | 203 | | | |
| Polar requirement | | | | 1195 |
| Polarity | | | | 1195 |
| Power to be at the C-terminal | | 760* | | 1116, 1287 |
| Refractive index | 203 | | | |
| Solvent accessible reduction ratio | | 774 | | |
| Surrounding hydrophobicity | | 774 | | |
| Thermodynamic transfer hydrophobicity | | 467, 554*, 596, 774 | | 1353 |
| Turn tendencies | | 328 | | 1116, 1289, 1332 |
| Affected residues per domain | 4/320 1.2% | 10/694 1.4% | 1/61 1.6% | 7/295 2.3% |

*Note - Residues in TreeSAAP analyses correspond to sites in codeml/HyPhy and ADAPTSITE analyses as follows (TreeSAAP left of equal sign; PAML and others to right): 90=83; 137=127; 203=189; 286=267; 328=308; 406=384; 467=443; 496=470; 554=524; 596=563; 734=688; 760=711; 774=724; 1287=1000; 1289=1002; 1332=1039; 1340=1047 (sites 758, 1116, 1195 and 1353 were not included in codeml analyses because they contain gaps (cleandata=1 option)). Sites 554 and 760 identified as positively selected along the *Latrodectus* α -latrotoxin branch in the TreeSAAP analysis were also identified as positively selected in the codeml M8 model, albeit with weak support (BEB posterior probability $\omega > 1$ being 0.596 and 0.587, respectively).

Table S6. Primer combinations used to amplify ~4.1 kb α -latrotoxin sequences from genomic data, with primer sequences listed in Supplementary Table 7. External primer set was used in touchdown PCR and internal primer set was a nested PCR, using product obtained with external primer set as template. Primer to left of ampersand in each pair is in forward direction, to right of ampersand is in reverse direction. NA indicates a single primer combination was used without subsequent nested PCR.

| Specimen ID | Species | External Primer Pair | Internal Primer Pair |
|-------------|--|----------------------|----------------------|
| | <i>Latrodectus hesperus</i> (California, US) | 113 & 778 | NA |
| | | 440 & 1967 | Deg356 & 1402 |
| | | 440 & 1967 | 1316 & 1967 |
| | | 1871 & 2537 | NA |
| | | 2452 & 3188 | NA |
| | | 2943 & 3697 | NA |
| | | 3616 & 4252 | NA |
| | <i>Latrodectus hesperus</i> (Arizona, US) | 113 & 778 | NA |
| | | 440 & 1967 | Deg356 & 1402 |
| | | 440 & 1967 | 1316 & 1967 |
| | | 1232 & 2551 | 1871 & 2537 |
| | | 2378 & 3398 | 2452 & 3188 |
| | | 2452 & 3700 | 2943 & 3697 |
| | | 3518 & 4262 | 3616 & 4252 |
| | <i>Latrodectus hasselti</i> (Perth, Australia) | 113 & 778 | 140 & 619 |
| | | 1658 & 2551 | 1700 & 2537 |
| | | 440 & 1967 | Deg356 & 1402 |
| | | 440 & 1967 | 1232 & 1783 |
| | | 2378 & 3398 | 2452 & 3188 |
| | | 2378 & 3398 | 2452 & 3188 |
| | | 1871 & 3700 | 1894 & 3398 |
| | | 2378 & 3700 | 2943 & 3697 |
| | | 1871 & 3700 | 3368 & 3697 |
| | | 3368 & 3700 | 3386 & 3697 |
| | 3548 & 4262 | 3613 & 4252 | |
| | <i>Latrodectus tredecimguttatus</i> (Israel) | 113 & 778 | 140 & 619 |
| | | 440 & 1967 | Deg356 & 1402 |
| | | 440 & 1967 | 1232 & 1967 |
| | | 1658 & 2551 | 1700 & 2537 |
| | | 1871 & 3700 | 1894 & 3398 |
| | | 1871 & 3700 | 2470 & 3398 |
| | | 3368 & 3700 | 3386 & 3697 |
| | | 2943 & 3700 | 3386 & 3697 |
| | | 3518 & 4262 | 3518 & 4229 |
| | | 3518 & 4262 | 3548 & 4252 |
| | <i>Latrodectus sp.</i> (Dominican Rep.) | 113 & 778 | 140 & 619 |
| | | 140 & 1136 | 440 & 1136 |
| | | F284 & 1304 | Deg 356 & 1136 |
| | | 847 & 1484 | NA |
| | | 439 & 1967 | 1232 & 1967 |
| | | 1871 & 3398 | 1894 & 3398 |
| | | 1871 & 3700 | 1885 & 2537 |
| | 1871 & 3700 | 1894 & 2551 | |

| | | |
|--|--------------------|------------------|
| | 1232 & 2551 | 1871 & 2537 |
| | 1871 & 3700 | 2789 & 3401 |
| | 3368 & 3700 | 3386 & 3697 |
| | 3518 & 4262 | 3548 & 4252 |
| <i>Latrodectus pallidus</i> (Israel) | 113 & 4262 | 140 & 4252 |
| <i>Latrodectus geometricus</i> (California) | 113 & 778 | 140 & 619 |
| | F284 & 1304 | Deg 356 & 1136 |
| | 439 & 1967 | 284 & 1304 |
| | 1658 & 2551 | 1700 & 2537 |
| | 2378 & 3398 | 2452 & 3188 |
| | 2378 & 3398 | 2452 & 3188 |
| | 3001 & 3692 | 3017 & 3496 |
| | 3001 & 3496 | 3017 & 3496 |
| | 3308 & 4262 | 3395 & 4252 |
| <i>Steatoda capensis</i> (Maui, HI, US) | 1781 & 2569 | NA |
| | 989 & 1484 | NA |
| | 440 & Saltx1006r | NA |
| | 1412 & 1967 | NA |
| <i>Steatoda grossa</i> (Arizona, US) | 5'R-OUT & 3'F-OUT* | 5'R-In & 3'F-In* |
| | 5'R-OUT & 3'F-OUT* | 5'R-In & 3'F-In* |
| | 1412 & 1967 | NA |
| | 1781 & 2569 | NA |
| | 2588 & 4050 | NA |
| | SGaLTXF1 & 1484 | NA |

* Primers used in inverse PCR reactions (see article Methods for further detail).

Table S7. Sequences of primers used in this study. Refer to Table S6 or article text for combinations of primers used to obtain sequence data in this study.

| Primer Name | Direction | Sequence |
|--------------------|------------------|---------------------------|
| 113 | Forward | AGGAGAAATTATGGAACGCGC |
| 140 | Forward | ATTCGTTAGTGCGAATGAGACG |
| 392 | Forward | GTACCTTTYGATCAGATYAAGGA |
| 439 | Forward | GGAAGTTGGATAGTCATAGTGCGG |
| 440 | Forward | GAAGTTGGATAGTCATAGTGCGGC |
| 640 | Forward | TCCGTGGTTTAAAGTTAAATGATT |
| 847 | Forward | GCTGTATGGWACYCAGACWT |
| 989 | Forward | GAYTTYAAATCRTCMCTTGTGGG |
| 1232 | Forward | ATTGGAGAGATGGTCGGGAAGT |
| 1316 | Forward | GTCAGTGTGAGAGAGAAAGC |
| 1412 | Forward | ARCCMCAATTAGTAGGTTCCGT |
| 1658 | Forward | AACCAACCAGATAAAAAGGG |
| 1700 | Forward | TGCAGATAGTGGAAACGCTGG |
| 1781 | Forward | CTTCAAACACCDYTGCACTTGGC |
| 1871 | Forward | AAAGAGATAAAGATGGATTCACGCC |
| 1885 | Forward | TTCACGCCGCTACATTATGCTA |
| 1894 | Forward | CTACATTATGCTATTCGGGGCG |
| 2378 | Forward | AGACAGCTGAAAAATATACTCC |
| 2452 | Forward | GATCAAGGTTTCAATTTGGAGG |
| 2470 | Forward | GAGGCAAAAACAAATTCTGGTG |
| 2588 | Forward | CAAATGCCTATTCATGGAGC |
| 2789 | Forward | TTGCTCCGTTGCTTGCCTT |
| 2943 | Forward | ACGCTATGTCAGAAAAAGATAAG |
| 3001 | Forward | TGGATGCTCTGGAGAATGCG |
| 3017 | Forward | GAATGCGCAATCTCTCATTTTGC |
| 3308 | Forward | GTATGACTGCTATAGACAAGGC |
| 3368 | Forward | TGAAAATGGAGTTGACTTCCG |
| 3386 | Forward | CCGTCGTAAAAATAGTCGTGG |
| 3395 | Forward | AAAATCGTCTTGATGCTACTCC |
| 3518 | Forward | GGACACTGCCTTACATTTAGCCGT |
| 3548 | Forward | GAAACCTACAGATGATAAAACT |
| 3613 | Forward | GATAAAACAGCTTTGGATATTG |
| 5'R-In | Forward | GCTGCCAGCTTGGTAATTCCT |
| 5'R-OUT | Forward | GCATCTGCTTTCGTGTCTTTTCTAC |
| Deg356 | Forward | TCCGTIGTIGARAARACNMG |
| F284 | Forward | ATAGTCATAGTGCGGCGCTTG |
| SGaLTXF1 | Forward | GTGTTTCTAAATAACCCATATG |
| 619 | Reverse | AATCATTTAACTTTAAACCACG |
| 778 | Reverse | GAGAACTTTAAAAGCTAATTC |
| 1136 | Reverse | TCACGGGATCAGACCAATGAC |
| 1304 | Reverse | AGCGGCATTATAAAGATCTC |

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| 1402 | Reverse | GGTATGGAGTGATTTTCGCCAAC |
| 1484 | Reverse | GAGCRGCATTATAWAGATCTCTAT |
| 1783 | Reverse | AGTTACAAATCCGCGCTGAGC |
| 1967 | Reverse | ATGATTGCCAGATGGAAAGGAG |
| 2537 | Reverse | TCGTCTGTGTCTCTCCAGTTCATT |
| 2551 | Reverse | CATTTGACCGTTTTTCGTCTGTGTC |
| 2569 | Reverse | GCTYCATGAATAGGCATTTG |
| 3188 | Reverse | CGTAACACAAGGGAGTATCAG |
| 3398 | Reverse | TTCAGCCACTGCCGTGAGAA |
| 3401 | Reverse | GTTTTAGCCACTGCCGTGA |
| 3496 | Reverse | AACGGCTAAATGTAAGGCAGTGTC |
| 3692 | Reverse | TGTAAGAGACTGCGTTCACCATACG |
| 3697 | Reverse | CGTTTGTAAGAGACTGCGTTCACC |
| 3700 | Reverse | GATTCGTTTGTAAGAGACTGCGTTC |
| 4040 | Reverse | CCACTCTTTAAGGCTGCGTAGA |
| 4050 | Reverse | GCTMTTYCTKCCACTCTTTAAG |
| 4229 | Reverse | AGACAGGACTCGCCGAACAAGT |
| 4252 | Reverse | GGTCAAATACCGTCAGAATG |
| 4262 | Reverse | ATAAGCTTCTTGGTCAAATAC |
| 3'F-OUT | Reverse | GCATTGCTATATGGTACTCAGACTT |
| 3'F-In | Reverse | GACTTTAAATCGTCACTTGTGG |
| saltx1006r | Reverse | CMRCARYTAAACTTGAAGTAC |