

UC Berkeley

UC Berkeley Previously Published Works

Title

Development and characterization of thirteen microsatellite markers for the Fiscal Flycatcher (*Sigelus silens*) for use in phylogeographic and landscape genetics research

Permalink

<https://escholarship.org/uc/item/9qw2496c>

Journal

Conservation Genetics Resources, 7(1)

ISSN

1877-7252

Authors

Wogan, Guinevere OU
Feldheim, Kevin A
Voelker, Gary
et al.

Publication Date

2015-03-01

DOI

10.1007/s12686-014-0309-2

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives License, availalbe at
<https://creativecommons.org/licenses/by-nc-nd/4.0/>

Peer reviewed

Development and characterization of thirteen microsatellite markers for the Fiscal Flycatcher (*Sigelus silens*) for use in phylogeographic and landscape genetics research

Guinevere O. U. Wogan¹, Kevin A. Feldheim², Gary Voelker³, and Rauri C. K. Bowie¹

¹Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, 3101 Valley Life Sciences Building, Berkeley, CA 94720, USA

²Prizker Laboratory for Molecular Systematics and Evolution, The Field Museum, 1400 South Lake Shore Drive, Chicago IL 60605, USA

³Department of Wildlife and Fisheries Sciences, Biodiversity Research and Teaching Collection, Texas A&M University, College Station, TX 77843, USA

ABSTRACT

The Fiscal Flycatcher, *Sigelus silens*, is the only representative of a monotypic genus, endemic to Southern Africa, and may represent two cryptic species. Here we describe the development of thirteen microsatellite markers, and characterize polymorphism for each one. We found that all but one of our 13 loci were highly variable, each having five or more alleles. This suggests that these markers will have high variability across the species range and will be of utility in understanding the extent of gene flow among populations.

Keywords: Africa, Muscicapidae, Passeriformes

The Fiscal Flycatcher *Sigelus silens* is the only representative of a monotypic genus, and endemic to Southern Africa. Two subspecies are recognized and paleoclimate models suggest that these taxa may have been restricted to distinct refugia in the past (Bowie et al. Unpubl. Data), and hence may represent cryptic species. The Fiscal Flycatcher is unusual in that it closely mimics the plumage of the Common Fiscal Shrike (*Lanius collaris*) a predatory songbird species widespread across much of southern Africa. In order to examine the extent of population structure within the Fiscal Flycatcher, and to better understand the population trends and evolutionary history of this species, as well as to generate a framework for the comparison of phylogeographic and

landscape genetics patterns between the Fiscal Flycatcher and the Common Fiscal Shrike, we developed thirteen tetra or penta-nucleotide microsatellite markers.

Microsatellite enrichment followed the standard protocol of Glenn and Schable (2005). Genomic DNA from a single individual was digested with RsaI and XmnI, and SuperSNX24 linkers were ligated onto the resulting DNA fragments. Five biotin labeled tetranucleotide probes were hybridized to the fragmented DNA: (AAAT)₈; (AACT)₈; (AAGT)₈; (ACAT)₈; (AGAT)₈. The probe-DNA complex was attached to streptavidin-coated magnetic beads (Dynabeads M-270, Invitrogen) and washed twice with 2X SSC, 0.1% SDS and four times with 1X SSC, 0.1% SDS at 52°C. Enriched fragments were separated from the beads through denaturing and standard ethanol precipitation. In total, 88 colonies were sequenced, 69 of which contained repetitive elements.

Enriched fragments that had tetra or penta -nucleotide repeats with at least six but preferably eight repeats were selected for additional processing. Primers were designed for 18 loci in WebSat (Martins et al. 2009). Each forward primer was 5' tagged with either the HEX or FAM flourophore.

We sampled 23 *Sigelus silens* from South Africa. Blood samples were stored frozen in lysis or DMSO/NACK buffer until extraction using the manufacturer's protocol for the DNeasy Kit (Quigen, USA). Loci were PCR-amplified in 10μl reactions consisting of 1μl DNA (diluted 1:10), 0.12U of Taq polymerase (Invitrogen), 1μl 10×PCR buffer, 0.3μl 50mM MgCl₂, 0.6μl 10 μg/μL BSA, 0.25μl 10mM dNTPs and 0.6μl 10mM of each primer. Thermocycling conditions for all markers were: initial denaturation at 94°C for 3 minutes, then 30 cycles consisting of denaturation at 94°C for 45s, annealing at primer specific temperature for 30s, and extension at 72°C for 45s,

followed by a final 30 minute extension at 72°C. Fragments were suspended in formamide and analyzed using LIZ500 then genotyped on an ABI-3730. We used Genemapper 4.0 (Applied Biosystems, USA) to size and bin the alleles. All individuals were PCR-amplified and genotyped twice to ensure marker consistency and repeatability.

We assessed repeat motif, number of alleles, size range of alleles, expected and observed heterozygosity (with 1000 replicates), and polymorphism information content (PIC) in the PopGenKit R package (Paquette 2013). Departures from Hardy-Weinberg Equilibrium (HWE) were assessed using the exact test in the pegas R package (Paradis 2010). Micro-checker was used to check for the presence of null alleles (van Oosterhout et al. 2004).

Of thirteen microsatellites, twelve were highly variable. Observed heterozygosity values ranged from 0.043 to 0.87 and the number of alleles ranged from two to eleven (Table 1). PIC values indicate that twelve loci were highly polymorphic with high information content (Table 1). The population examined here is in HWE, but the exact test suggests that three loci exhibit a departure: SSI2, SSI24, and SSI34. There was no evidence for scoring errors, although locus SSI2 had a general excess of homozygous individuals, suggesting that it may contain null alleles.

The microsatellite loci developed and characterized in this study will be of utility in assessing the nature of gene flow among populations of this endemic Southern African flycatcher, and will provide insight into the effect of landscape features on population structure.

Acknowledgements

The authors thank Hanneline Smit, Graeme Oatley, Ângela Ribeiro, Dawie de Swardt and Jérôme Fuchs for help with collecting samples. This research was supported by NSF grants to R.C.K.B and G.V. (DEB1120356, DEB1119931). This is publication XX of the Biodiversity Research and Teaching Collections at Texas A&M.

References

- Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci Methods in Enzymology 395:202-222
- Martins WS, Lucas DCS, Neves KFS, Bertioli DJ (2009) WebSat- a web software for microsatellite marker development Bioinformation 3:282-283
- Paquette S (2013) PopGenKit: Useful functions for (batch) file conversion and data resampling in microsatellite datasets. CRAN,
- Paradis E (2010) pegas: an R package for population genetics with an integrated-modular approach Bioinformatics 26:419-420
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data Molecular Ecology Notes 4:535-538

| Locus | Genbank Accession | Primer Sequence (5'-3'), fluorescent label | Repeat motif | T _a (°C) | Size Range (bp) | N _A | H _O | H _E | HWE (p-value) | PIC |
|--------|-------------------|---|-----------------------|---------------------|-----------------|----------------|----------------|----------------|---------------|-------|
| SSI 2 | KJ580508 | F-(FAM)-TTTCTGATTCTTAGGCCACTGA R-CTATAAACACAGCTCCTCCCC | (ATAG) ₁₆ | 52 | 147-175 | 8 | 0.609 | 0.834 | 0.028 | 0.813 |
| SSI 6 | KJ580509 | F-(FAM)-TCTGGTAACCTGACTCTGAAGAATG R-GAATAACTGCCCTGTCCTTTG | (ATAG) ₉ | 59 | 333-353 | 6 | 0.696 | 0.703 | 0.264 | 0.669 |
| SSI 8 | KJ580510 | F-(HEX)-AGCCGTGCTTATAGATGGAA R-AATCACCACTCTTGCTGAGGT | (ACAT) ₁₄ | 56 | 256-274 | 6 | 0.739 | 0.787 | 0.737 | 0.754 |
| SSI 9 | KJ580511 | F-(FAM)-AGCAGTCATTCTCAGTGGAGGT R-CAGCAAGGAACAGGTTACACAC | (ATCC) ₁₃ | 52 | 188-224 | 10 | 0.87 | 0.834 | 0.667 | 0.813 |
| SSI 14 | KJ580512 | F-(FAM)TTTGTATGGCCCTGTCCTTAGT R-GCTCTCTTGGTCCAAGTATTGG | (TATC) ₉ | 56 | 254-274 | 7 | 0.783 | 0.833 | 0.361 | 0.811 |
| SSI 17 | KJ580513 | F-(HEX)-GCTTGGCTAATCTGAAATGTGA R-GTAGGTAGGAAGGACAATGCC | (TATC) ₁₃ | 53 | 285-333 | 9 | 0.826 | 0.832 | 0.509 | 0.814 |
| SSI 20 | KJ580514 | F-(FAM)-CACCTGGAGAGAAGAAAAGAGAA R-TGACTGTTGCTGAAGGCATATAG | (TATC) ₁₀ | 56 | 263-271 | 5 | 0.696 | 0.768 | 0.576 | 0.726 |
| SSI 22 | KJ580515 | F-(HEX)-CTTGGTTCACTCTCAACACCTG R-GCAGAACATCACAAACACTTGGTC | (TATC) ₁₁ | 53 | 154-186 | 7 | 0.783 | 0.809 | 0.369 | 0.782 |
| SSI 24 | KJ580516 | F-(FAM)-ATTCCCCTGTTCCCTCTA R-GCTGCTGTAGGAGCAGTGAGA | (ATAG) ₁₇ | 58 | 206-226 | 8 | 0.652 | 0.813 | 0.001 | 0.789 |
| SSI 29 | KJ580517 | F-(FAM)-CAACATAACCCAGAGCACAGAAA R-AGGGCTTGACCTAACGCTGTTAAT | (TCTA) ₁₂ | 64 | 372-394 | 8 | 0.773 | 0.823 | 0.053 | 0.801 |
| SSI 33 | KJ580518 | F-(FAM)-TGAACCTCCACTAACATTAGGGT R-GACCTCCGATCTAACAACTGG | (ATGG) ₁₅ | 58 | 320-357 | 9 | 0.87 | 0.837 | 0.400 | 0.816 |
| SSI 34 | KJ580519 | F-(HEX)-GATGTTCCCTCCATCAAAGA R-AAGCCACATGGTATCAAAGGT | (TACCA) ₁₄ | 53 | 187-217 | 11 | 0.783 | 0.886 | 0.024 | 0.875 |
| SSI 40 | KJ580520 | F-(HEX)-GAATCACCATCACTCAACCAA R-CCATCCCAGGAGACAAGAGTAA | (TATG) ₆ | 51 | 384-392 | 2 | 0.043 | 0.043 | 1.000 | 0.042 |

Table 1. Characteristics of microsatellite loci for *Sigelus silens*

>SSI2

TGTTTAAGGCCTAGCTAGCAGAACACTTGCTGTAAATTCTGATTCTTAGGCCACTGAGAGAGAAGAAAAT
ATCATCGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAG
ATTTCTAGGAGTCTTCTGCTTGAACTCAGAAGGGAGGAGCTGGTTTATAGAACACATGAAGACA
TACAGATTATTAGAAATGAAAAAATTTAAAGCATTTGAACAATTGAATATTGAAAAAATTTGAAAAA
ATACTTTGGCCTCTGGTCAATGAAATTTCAGGGAGAGCTGCATTGCCCTCAAGCTTAAGGATGTGAA
AGTCAATGCATCAAGTGACTCCTGGATGTTGCATCCTTACCCAGTCACATTCAAAGCTGAAAGTCTGTCAC
TCCCCACCTCATAAATGCAGTTACTGATGTTCCCTTTAGGTTGATCAGTAGCAAGGCTGAGGGCTATCC
CACAGGCAAAAGATTGAAGTTCATACACCCCTCCTAATCTCTAGTTCACAAATATTCATATTCACTTTCC
TCTAATACCAGTCTGTTCTTGGCACAAATTGGCATTCCGAACATAATACAGCTATAGAGG

>SSI6

TGGTAACTTGACTCTGAAGAACATGTATAGATAGATAGATAGATAGATAGATAGATAGATAAACTCATAA
AATTATATTAAAAACTCACTAGCTTAAAGGAGTAGTGGTGTGCAAACACTCAAGCAAAGTTAAAGAATGTGGG
AAAAAAAGTGGCCCAGGCAGCAACTGAGGCAGGACTTAACACTCATATAATTAAATTCACTCCCTAAATT
AGGATTGCAATATGGATTAAATTATGTGATTACATTACTAATTTCCTCCAGGAAATAATCACTCAGTC
CACAACTGAGGTAGATGGTGCCTAACAAAGGAACAGGGCAGTTACAGTATTGTTATTCTCACTGTTCA
GAGAGTGGCCTCCATCTCCTTAGTTCATATTCACTTCAAATGTATTACAGAACACAGAAATTAAATT
ACTTGTGGATAGTCTATTGCACTCCATGGCATAGTATCTGAATAATTCTCAAACATTAATGAATTATCTTC
AGAACAACTCTTGGTTCAATTAAATTGAAACATTACAAGTGGGAAGCTGAGGCACAGAGCTAACAGGT
AAAAAAATCTGTTCATCCTGGTTCTGAGATGCCAACACTGTTCTCCTACTATTCAAGCATCATGCA
ACACTGTATTGTTCAAGTGT

>SSI8

TGCTGGAGAGGTCTTGTAGGGTTGCAAAGCAGGTGATAGCTTTGAATGAAAAGCTATACCGGTATAA
AATGTTGTAATATTAAATTGTGTTCTACTGTGTCAGGCATGATCTAACACCATCATGGTTGCTCTAG
GACTTAATGATAGCTGATCTGTTATTGTGAGGGTAGTTATCCCAAGCCACAGGGCAGGTAGTTGCTCTTA
ATGCCCAACAATTCACTGAATAACAAAATTAAATTAAACTACTACTCTTCTGGTTGCTCAGCTGA
GGTTTCCCTTTCTATTAGATATTGCTCTCTTCTACTGAAATAACTTCCCTGAAGCCATTGCTTCGAT
TATGTGTTATTGCTAACAGGAAATTGACGGCATAAGAGCAAAGATGGAAGTGTCTGTTTACACTTT
TGTAGTGCTTCCATTGAAATACTATCCCAGCTATTCTCAGATGTGGCATTGGTAGATCTCACAGAAC
CACATAAGGTAGTCTACATGCAGTTATATCAGCCGTCTTATAGATGGAAAACAAGGTGGTGGACAT
ACATACATACATACATACATACATACATACATACATACATACATACAGCAATGGAGTTCAAGAC
CAAACAGAAATTCCCTGGCTTTGCTCATATTTCAGACCATTAGATCATACCTCTGTAGATAGATAAAT
AGATAAAATAGTTAACAGATTAGCATTCTCATATGCCATAGGTCTTAGTTGAGAGGGACCTCAGCAAGAA
GTGGT

>SSI9

ACTAGAGATGCTTTATAGACTCTGATTGTAAACAAATGACAAAGTCCAACAACCACAGCAAATCCAACACG
TTTGCCTTTAAGGAAAAACACACACACAAACATAACTAATGTTGCTGAGTGTCAAGCAGTCATTCTCAG
TGGAGGTATCCATCCATCCATCCATCCATCCATCCATCCATCCATCCATCCACAGTGGGGGAA
TGGCAGCATCCATGGAAAATTGGAGGAATTGCACCTGTGATGGCATGCCAGGGTCATCTGCCAAGGAGGAGG
AGGGAGAGTTTCAGAGGTGTAACTGTTCTGCTGCCAGGTGTTCTGAAGCCAGGGTCATCTCCAGG
CTGGAGAAGCAGCGGGACAAGCTGATATCCAGAAAGATGACTCTGCTCAGGCTGCCAAGGGCTTCC
GCCGCCAAAATTCAAGAGACTGAAGGT

>SSI14

GGTCAGAAAGCAGGACATTCTGAACCTCATGACTCAGTGGGAGGGTCTCCTGACAGTTGTATGGCCCTG
TCCTTAGTAAATAATCAAGGAAATCTTGTAAATCACTGTCACATTACATTGAATATTAGCTCATGAATT
TTATTGATACAGCATATGGTGGCTCTTCTGTGAATGAAGATCATCACACTAACAGACACAATAGTCTGTC
TCTATCTATCTATCTATCTATCTATCTATCTATCTGAAAAAACACAGAAAAACCCCCAGAAGGAATGTT

GCTGGAGGTATGCCAATACTTCCACCAAGAGAGCATTCTTGGGAGGAGTCAGCTCAAACACCACCTTCAT
TTGGT

>SSI17

ACAGCATGGCTGGGCAGATAGAGCCACTTGGTGGCTGGCTAATCTGAAATGTGACATTTATCTATCTATC
TATCTATCTATCTATCTATCTATCTATCTATCTATCTCTATAGCATATGAATCAATTACTGGAGTAA
TTTAGATGTGGCAGGTGTTATTGTGTTACCATGAAATCCTGAGGCAAGGAAAGCTAGTGTGCAATTATT
AATGGTAGAACACTGAACAGCTGCTATTTTACAATAGTATTAAATATCTTACAGTATGTGCCCTTAATAGA
AAACCCACATGAAATGAGATTCTATCCCTGGCTTTAAAAAAGGAGCTGTGCTCTACCTACTGCAGTG
TGAACTGTGCTGCAAACACTGGCTACTAGTTGGTGAATCATTCTCTAAACTCTATCTCACTTTGCCT
CTCTGGATGTAGAGAAAGGGATTCTTGAGGTAGCTTGGGAAGCTGTCTGAGAGATTGAAACGGTT
GTGTAGTGTAAATGGAATACCAAGCGAGATGTGAATGCTGGCACCTGCTTGT

>SSI20

TTGTTAAGGCCTAGCTAGCAGAACATCACAGCTATTTGTTCTGCTAAAATCCAATGTAGATCAAACGTGAT
TTAAAAAATTGTCATAAATATGGAGCAGCGATATCTTACTGCCATTCTAACGCTATATACATGAAGAATT
TACATAATGTAAGTGTGAAACCCATAAATAGATAAGCATAAACAGATATACTTAAACTACACCTGGAG
AGAAGAAAAGAGAACATGCTATGAAGAAAATAAGGGATTAAAGAGTAATTATCTGATAGCTTCTATATT
TTACCTTCTTCTTCTACTCAAAGAGATCTATCTATCTATCTATCTATCTATCTATCTATCTATC
TACACACATGTGTGGATATGGATATATCTATATCGTCTATCTGTACCTAGAAACACACATTGTGGATA
TGAATATATCTATATGCCTTCAGCAACAGTCACAGAACAGTAAAGTGAATTACATCAGAGCTAAAGTTGCT
CTGAAATGCTAAATTCTAAATATAAAATTCTGAGTTCTTGTGAGGAAATTGAGGTGATTCAAACA
GCTCAATGTCACAAATGAAAAGGCACAACAGTAAAGTGTGCTTGTGAAATTCTGACTCATCTAATTATT
TTGTTAATAACCTCTGTCATTAATAATGGTATATGAGCAAATGATAAAATCACATTGTAGTGCATT
ACACATGACATTTGTCCTGCCAATGGCATATTATGCCAATGAAGTAGGAGAAAATAATATTCTTGC
ATAAGTTGCATAACACTTACTGAAAAAATTACTTCAGCTATGTATTTCTACATGACTGAAATTCTGT
GTCTTAGCAATTCTTGATTTACAGGAAAATTGCTAAAACATCGCAGGGAGTGTCTATTCCACTATTGAA
CTTCACAAAGAAGCTTATAGAAGTCTGTGTCAGTAGGACCAAGCAAGGCTGTGAATTGTCTGAGATGTGAG
TCTGAACAGTGTAGTCAAGCAAATTATGACAATGCAGATGTGACTCCATTGTGGACAAAC
AGTATTCTACATCACCAGTTAGCTTCTATGGTATACATGCACATTACATAGTGTCTGACTTCAAG
TATGATAGAAGTTATTGAAAGGTGATGAGCCACTGACATGAGTTGCTCAACTAAATTGTGTATGCCTCATCCC
TGGAAAGTGGTCAGTGCCAGCTGGATGGGGCCCTGGGAATTGGCATAAGATGATCTTAAGGTCTTACAG
CCAAAACCTTCTATGATTCTGTGATATTCTTAATCTTAACTTATCATAGCAAGATGCAGTGT

>SSI22

TTGTTAAGGCCTAGCTAGCAGAACATCTGTTCTGCTCTGTTGCCATGGGACAGTTGGAATACACTGAAGA
ACAGTTCCACTCTATTCTCAGCATATGCAGCAATGTTCTCAATTGAAAGGGCACGTTCTGAGTCAGTCTCATG
AACTTGAACTGTCCACACTGGCAACTGGGGAAAGACTTTCTTGGTGTCCATAAATTAGGGAGAGAAC
CCTAACGCCTGGTTCACTCTCAACACCTGTGTTGGTAGAACAGCAATCAGGTCAACTCTCCTGTGCCACAT
CTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTGTTCTGGAACGTGGCTTCTGTC
CTGACCAAGTGTGT

>SSI24

ACCATCCCCTGTTGTCAGCTGCCTCAGTGCATTTAACAGAAGTGTGGAGGACACTCGATCTGAG
CCACACTTCAATTGCCATGAGATAGCATGTATTCTGTTCATAGATGCAAGAGATAAAGCTCAGCCAAA
GTCATCCTCTGGCCCCACAGAGTTACCACTGGGATCTGATTCCGTGTTCCCTCTAGACATGTAGAAAT
AGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATATG
CCAGATAGAGGAAGGGACAAGAGCTCAGCTCATTCCACATGCAAGTTGATTACTGCAATTCCCTGAGTGTATG
CTGCAGCAGAGAGCCTCACTGCTCCTACAGACAGCTGT

>SSI29

ACAACACCAAGTGGTAGTGAGAGAACAGCAATTCTGACTGAGAGCAGCAATTCTGACTGAGACATGATCC
CACCCAATCCTGGCAAATGGACCTCCCCAGTTCAACACACATTACAGCAGCATCCTCTAATGAAAATCT
ATTTATTATTAGGTGTAGCCACAAAGAACATGCAAATATCTTCCATGTGCCTGTATTAAATATTGTTATTA
ATTTCTTTTCATTTAAGGCTTGGTTATGGCAGCTAGCAGTATATTTCCTTTAAATGAGA
ACCACACCAAAACAGAGTCACACCGACCCTCAAAGACAATTGATGTGCTATAAAAAAGCAGAAATCTTTA
TTGGTTAATCATAATGTAGTATTGTAGGTTCTGCCTAAAACAGCTAACATACCCAGAGCACAGAAAAAA
AAAATCTGAAAAAGAAGAAAGTATGGAAAAAAAGCATATATATGTCCATATACCTAGGTATATAGACATA
TATCTATATAACATATAGACATACATATCTATATCTAGGTAACTATCTATCTATCTATCTATCTAT
CTATCTATCTATCTACCTCCCCATAACCAGGAAGTAGTGGCTTTAAAACTAATTGTCTATTAGAAAG
CCAAACATTGTAATTCAATTAGAAAAGTCCAAAATAGATAATTATTATAAGACCATACACAGTATTGATC
AACTACTAACTCAAATAACACTATTCTGAGACTTTAAAGAACATTAAACAGCTTAGGTCAAGCCCTGCAA
AATCACCAGGTTACTAAAGGGCTCATCCTATTCTGACACAAAGAACATTCTGTAACCTGAGATATGTTCTGC
TTAATTCTTAACAGGAACATTCTGTGAAATGCCAGACTGGGCTGCTGAGCTCCCCACGCTGCCTCATCTGC
TCAGGGGATGTTGAAATGTATTTATAGTAAATTGAACACTCCACACTGAGCCATGGCATCTCCCTCATCTTC
TGGTCTCTAACTACTCTGCTGATGTTCTTCACATTAGCGGTAAAGGCCTATGGGACCAGGACAGGGAAT
GCTTACTCATAGTGTGACTGAGCAATGAATCATGAATGCTCTGCTCCAAAATAATTAGCATTCCTGTA
ACAAATGAGAGATCTCTATTGATTCAAGCCTTCTCCCTCTTCAGCCTCACTCTGCACTTCTGT
CCAAATACCTGATTGACTGATGCTCCACACATCCCTCCACTGGTATGTCATAGCAGAAGAACACAG
ATTTCAAACAAAAATATGCAAATGAGAACAGCTTGCCTAGGTCTGTCAGATTCTTGAACCTCCTTTT
TATGGCAGCCTGTATTTAATTCTGGGATCGTAAAGCTGAAAGATTCTGCTAGCTAGGCCTTAA
CAA

>SSI33

ACAATACCTGATATCTCTCTGTTATTGTTGAAGATAAAGCCCACAAGACCCACCCCTGTAATAAATCTGT
ATGTCACAAATTAGAGCAAGCAGTTCTCATTATGAATAATTGTTGCTCCTCCATGACTTCACTGACAGA
ACTTAGAGTTCTCTGTTCAATTCAAAGAGAAAATGTGTTCACTGTTGCTGACTTCACTGGCTTGAACCTCCACTAA
TTCAGGGTAAGAGCAATGTGAAGCAGAGATCCTGTATCACTCATTCACTAGTAGTAGGGCAGCTCCAAAATGGGAGA
AGCCAAGGTGATTCCAGTTATGTTAAAGCAGCTTCACACTAGAAGATGGATGGATGGATGGATGGATGGAT
GATGGATGGATGGATGGATGGATGGATGGATGGCTCTCTGACTCCAAGGGTTCCAAGCACAAAGGGCT
GATAAAAACCAAGGATGCAACAAATGTTCTGAACATTATTAAAAGGCTTGTAGTTGCTTGTATTAA
GTAGATCCAGTTGTAAGATGGAGGTCAAGATAAATTTAAAAGTATATATAATGATGCTCCATAGG
AGTTGCTAAAAGCTTCTGCTTCCATCTGATATGGCATAGGAATT

>SSI34

ACAAGGAGTTCTAGTCTGGAAAAGCAGAACATGCAATTAGACATTCTGTTGGCAAAGTGCAGCAAGGACT
AAGCTGGATATCAAAACACATTGCTGAAGAACAGTGTGCTTATATCATGTGTTAGGTTGGATTTA
AAAACCTAAAGGGATGTTCCCTCATCAAAGAAAATTTCATTGAACATAGATGTGTAAGTGGCAGCTAA
ATACTTAATATGAAGATTACATACCATAACCATAACCATAACCATAACCATAACCATAACCATA
CCATACCATACCATAACCAGTTGGATTCTCTGTAACACAAGGCAGTCAGAACTACCTTGTACCAT
GTGGCTTATTACTTTAGTTTACAGTAGAGGAAAGCACTAAAAGCAAACCATAAAGATTCCACAGTGT
CTTCTGTTCAAAGGTCTGACTGGATGAATTGCTGCACTGAAGTGTAAATTACTAGCTGTGAGATGGA
ACAAGGGTGGCTGGCATAGCAGATAGAAGAAAATTGCTGCAAGGATGCACAGATGAATGGCCATGCTGG
AGGAGCACTAGTGTGCTGTCTGTTCTGATAACACGAATTGCTGCTCCCTTGTACTCATCTGAGTTCT
AGTAGTTTCAGGTGATGAGGGAGGCTGCTCCCTGCTTGTCAACTGTAACGTATTCTGCTTGTAGAAG
AGATAATGGTAAAGAGTAATGGTAGTATTACTAATCATTTGTCTTCATTCATCCTCAGT

>SSI40

ACCATCACTCAACCAAGGGACATCTAACGCTGAATGATCAAGACTCATGGAATGTTGACAGGGTGGTAGAGAT
TTTCATTCACTTTCTCTCTTCTCTTCCCTACAGAGTCCTGGGTGATACTTTGTGCTTCACTTAA
CAAGGCTTCAATTATTACTATAGATAATAACAAACAAACTACACATACCTGCTTCTTGTCAACCAAA
TTGTTCTAAAATAACCATATAACATTATATGTGTTGTATGTATGTATGTATGT

TTTACAAACATCAGTCATTCACTGTTGTCTCACTCTAATCCACTCCAAGAAATCTATTAACAAGAACCTGGG
TTACTCTTGTCTCCTGGATGGATCATGACAAAGCACA