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Development of Anchor Loci to Map a BT Resistance Factor in the Domesticated Silkworm, Bombyx mori

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Development of Anchor Loci to Map a BT Resistance Factor in the Domesticated Silkworm, *Bombyx mori*

The BT toxin is an insecticide produced by the bacterium Bacillus thuringiensis, and millions of acres of genetically modified crops expressing this agent have been planted. However, with such widespread use, there is a great potential for pests resistant to this toxin to develop. An understanding of how BT resistance operates and is inherited is central to ensuring continued use of the transgenic crops. Bombyx mori, the domesticated silkworm, is the model organism for the Lepidoptera, an order of insects containing many important agricultural pests. Screening shows that BT resistance is present in some silkworm strains, and linkage group 15 (LG15) harbors a major BT resistance factor (W. Hara, personal communication). We have found BT resistance in a different silkworm strain, and are testing to determine if the same linkage group is responsible. To do this we chose ribosomal protein (Rp) genes, which also mapped to this chromosome, as candidates for genetic markers. Because these genes are orthologous, meaning that they are present in related species and are derived from the same ancestor, they are considered to be anchor loci, which can be applied to a variety of species. By comparing genomic and cDNA sequences of Rp genes, I identified introns, likely to be the most polymorphic areas of a gene, for amplification by polymerase chain reaction. Using a mapping panel prepared by a graduate of the Goldsmith lab, analysis with one anchor locus suggests that the resistance phenotype does indeed map to LG15. More markers that have been mapped to this chromosome are being scanned in an effort to corroborate this finding. Recent publications have shown that there is strong synteny, the conservation of chromosomal linkage of orthologous genes, within the Lepidoptera. As such, it is expected that these anchor loci will be present on the same linkage group as the putative BT resistance factor in related species, and therefore of use to future studies.

Keywords: Bombyx mori, BT toxin, anchor locus

Faculty Sponsor: Dr. Marian Goldsmith

Introduction

The BT toxin is produced by the bacterium *Bacillus thuringiensis*, and produces its lethal effects on various insects and nematodes, leaving other organisms unharmed. This property has made it a very useful pest-control agent in agriculture, and recently crops of genetically modified cotton, corn, and potatoes expressing BT toxin (eliminating the need to spray the plants with the toxin) have been grown in large numbers (Lawlor, 2004). However, with such widespread use, there is the great potential for strains of pests resistant to this toxin to develop in the wild. An understanding of how this resistance operates and is inherited is central to ensuring its continued use.

Bombyx mori, the domesticated silkworm, is the model organism for the Lepidoptera. This family of insects consists of an estimated 170,000 identified species including moths and butterflies. Aside from the economic importance of the silkworm, many of the Lepidoptera are important agricultural pests, making the study of this organism particularly relevant (Shi et al., 1995). Much investigation of the silkworm has involved linkage mapping using an assortment of different methodologies. Recently, particular attention has been paid to the creation of codominant probes called anchor loci, which can be used to form genetic maps of general utility. Anchored loci are often developed from orthologous genes, which are genes in related species that are derived from the same ancestor. Such anchors are also invaluable for integration of numerous linkage maps that have been published over the years with the genomic sequences and physical maps that are now being published in China and Japan.

Screening shows that BT resistance is present in some silkworm strains, and linkage group 15 (LG15) harbors a major BT resistance factor (W. Hara, personal communication). Carrie A. Lawlor, a graduate of the Goldsmith lab, performed matings between another BT resistant silkworm strain, 404, and a sensitive strain, 516. Her data eliminated 22 chromosomes from carrying the resistance loci, leaving six putative linkage groups, one of them being LG15 (Lawlor, 2004). The goal of this study was to create anchor loci situated on LG15 to map the locus responsible for BT resistance in 404.

Ribosomal protein genes mapped to LG15 were chosen as candidates for genetic markers. The introns were targeted for amplification from primers residing at the edges of exons. Since exons are highly conserved, it is expected that these primer pairs (or very similar ones) should be able to be applied to other Lepidoptera species. In recent months, unpublished

work has shown that there is strong synteny, the conservation of chromosomal arrangement of orthologous genes, in the Lepidoptera. As such, it is expected that these anchor loci will be present on the same linkage group as the putative BT resistance factor in other resistant silkworm strains and Lepidoptera species, and therefore of use to future studies. And since intergenic sequences are non-coding, they are expected to be under less selective pressure, and therefore more polymorphic. PCR products not showing size polymorphism were digested with restriction enzymes to discover differences in restriction sites.

Using the BT mapping panel, the placement of LDH to sex chromosome Z was also attempted.

Materials and Methods

Primer Design
Using the NCBI computer program Splign, genomic and cDNA sequences of the putative anchor loci were compared to determine the positions of introns and exons. The primers were designed to reside in exons, and amplify introns, with the PCR product roughly 2kb. The primers are approximately 20 bp and of similar GC content.

RpS8 Anchor Locus
Genomic: AADK01004780
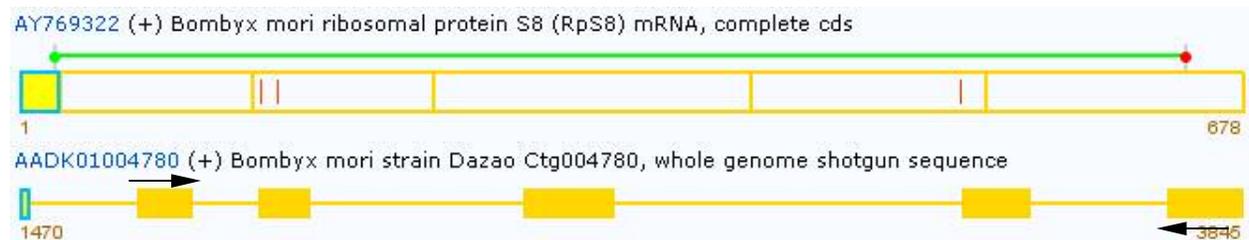cDNA: AY769322

Forward primer: 5 – ATCACTGGCATAAGCGAAGG -3
Binding in exon 2, cDNA 23, genomic1695
Tm60

Reverse primer: 5- ACACTGACCTGGGCGACTC -3
Binding in exon 6, cDNA 540, genomic 3698
Tm61

AY769322 (+) Bombyx mori ribosomal protein S8 (RpS8) mRNA, complete cds

AADK01004780 (+) Bombyx mori strain Dazao Ctg004780, whole genome shotgun sequence

Expected size: 2020bp
ATCACTGGCATAAGCGAAGGGCCACCGGTGGGAAACGTG
CGCCCATACGCAAGAAGAGGAAGTATGAGTTAGGGCGTCCCGCTGCAAACACCAGGGTAAGAACATAACC
TAAGTAGATACTACAATTAGACCCCTACTATAACTTTGGAACAGTTGACGTCGTTGAATAAATAAGAGAA

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GCCTTGCACTTCGCGGTTTCATTTTATTTTTTTCACCTTTTCAGCTCGGCCCTCAGCGAATCCACTCCGT
TCGTTCACGTGGTGGAAATACTAAGTACCGTGCGCTGCGTCTGGACACCGGTAACTTCTCTTGGGGATCG
GAATGTGAGTCTGTCGACCTTTTAATTTATTTGTCAATTAGAAAAGTTAAGACTTCACTTAATGTGGCTT
ACAGTAAATTTATGAGCATTTAACTTACTTAATGCACTATCATAGCAAAAATGCATTTTAATATGAGAAG
TTCTTAAAACAGTAAACATTGAAGAATATTGGTGTGATATAAATCCACTTCTGTATCCAAATATTGTGTT
GCCAAATATTCCAATGTCATTAAAATTTGTGTAAGTGTTATTATGTAGAATCGACCTATGTGTGCATATG
AATTCAGTTCTCGTAAAGCACTAATTTATTACTTAAGATCCTTGCCTTAATTTATTATACCTTTAATACA
AGCACCTCACTTTAATTTGTTCACATAAAAATTACATGAACAATCTAATAGATTGGTTTACCCTTTCAGG
TTCAACTCGCAAAACCCGTATCATTGATGTTGTGTATAATGCATCTAACAATGAATTGGTGCGTACAAAG
ACCCTTGTCAAGAATGCAATTGTTGTAGTAGATGCAACTCCATTCAGGCAGTGGTATGAGTCGCATTATA
CGTTGCCTCTTGGGAGGAAGAAGGGAGCGAAACTGGTAAGTGTCACTCTTGTTATGTTTTCTAGTGATTA
TTGATGATAATACTAGATTTTAGGTACTAAAAGTTGTAAATTAGTAATTACAATGTATTAAGAAGAGGAA
TGGAAGGGCTATGGAATTAAGATTAAAATATGAATTCTTGGACATTTGATAATGCATCATTTTCTTTCAT
AATACAAATAATATTTAGTAGGATTCATAGATCGTAGTAGTAGTATTTATAAGTATAATTTATTTAGTAT
TGTTTCACTTCTTCAAATTAACATAAATTAAGAACTAAATTTCAATTAAAAGAGAAATGGTAATGAAAGA
AATGTGTGTTTGTATTTAAACTAAAACACATGGTAAACTTATTTATTATGCTATATACCAGTAGCTATCT
TAGTGTTTGAAGAAATCTTGCAAAAAAATAAATGACACCAAATAAATTTACAAGAGAAGCTTATTTATGT
AGAAATAACCAACTATATTATCAGACTCCCCAGCGGTATATTACCCTCTTGCTGAAATATCCTCATTAGT
TTTGTCCGAAACATGTGTTTTTTTTCAAAATGATGAAGAATTTTTGCGTGTCAAATGATTGAATTCAAAT
GATTCAAGCAAGCTCTGAAATTATATTTTCATATTCAATTTAACTTTTTCCTTAAAAAGTAACAATAAAT
GTTTTTCATTTAGACTGAGGCTGAGGAAGCTATTATCAACAAGAAGAGAAGTCAAAAGACAGCAAGAAAA
TACTTGGCAAGGCAACGTCTTGCTAAGGTTGAGGGTGCTCTAGAAGAGCAATTCCACACAGGGCGTTTGC
TGGGTAATTTATAAATTGTTTCCTAATCATCGTATTTGAACTAGAATACTAATTATATCAGCATGTGGAG
TATTAAAAAAATAAATAAAAAATTAAAAGAAATGATGAAAAATAAGATTTGAGGTTGCATTTAGTGACTG
TTACCGTTTTGGGAACAATCCAGTAGCCATATGATGCACTTTGTCATAGTTACTCTGATTTGGTGAACCG
TGTGCAACCTAATTCAATTCATACCTATGTGCTCTTTTTAATGAGTTCTTTATTGTTTTCAGCTTGCGTG
GCGAGTCGCCCAGGTCAGTGT
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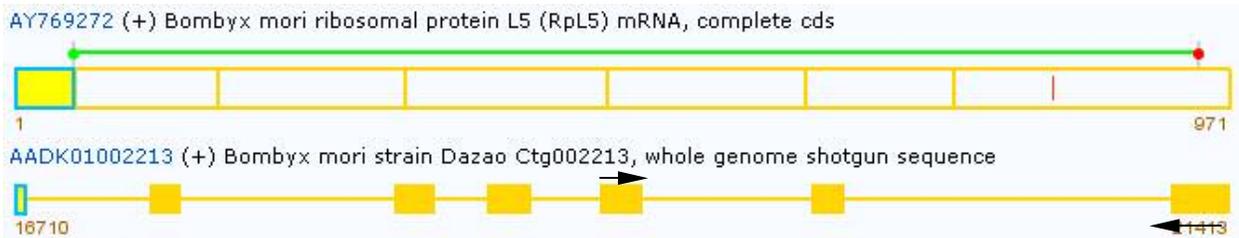
RpL5 Anchor Locus
Genomic: AADK01002213
cDNA: AY769272

Forward primer: 5- ATTCCCTGGCTATGATGCAG -3
Binding in exon 5, cDNA539, genomic19046
Tm60

Reverse primer: 5- CTCTCGGCCAATGTTAGCTT -3
Binding in exon7, cDNA816, genomic21258
Tm59

AY769272 (+) Bombyx mori ribosomal protein L5 (RpL5) mRNA, complete cds

1   971

AADK01002213 (+) Bombyx mori strain Dazao Ctg002213, whole genome shotgun sequence

16710   1413

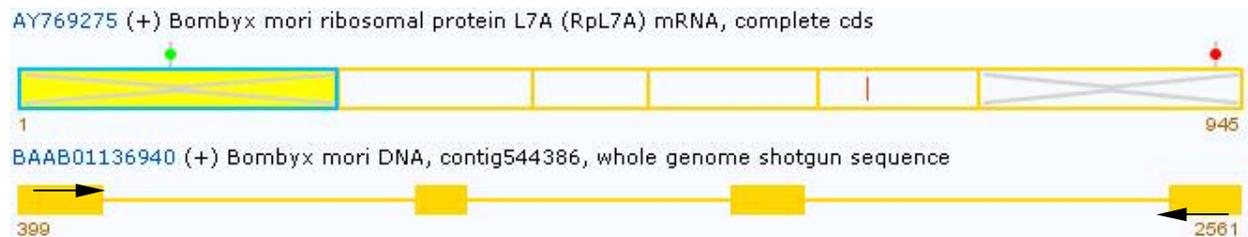*Expected size: 2212bp*
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ATTCCCTGGCTATGATGCAGAA
TCCAAAAAGTTCAATGCTGAAGTACACAGGTAATTTTATACTACCTTTGAAAGCAGCCTATCTGATAGTG
TATGGTTCCTAAAACTCATAAATATGAATAAAATCATAGTGAAATTTCAATAGCTGATATTTAATCAAAT
GCAGTCAGTCAGCCATGTGAATTGATTGAATCATTTTAATTATGAAAAATTATAAGTACCTACATATAAC
ATTGTAATTAGCATACCTGCTTCCTTGTATATTGTAAGGTAGTATTGTAGCAGTGGTTGCTTCATATAAA
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CAGTTCTACTTAAAACAAAATTTGCATAGAAAATTTTTGCTCTTATCATCCTCCAATAAATAGATAAATC
TATTAATTGGACAGCCATTTGAGAGCTAAATTAATGACATGGTTAATGGTTGTGAAAACAAAATAAATAA
ATTGTTGTAACCCATCATGTCAACATACAGTACAAATAATGCTCACTTTTAAATATTAGAATATAAATAA
CTAGTGTAATTATTGCGTCAGTCTGTAAGATAATACCAAAGGCTTGCCTGTCTTGGCCATCATTTGTGAA
TTCTGGATGGAAGAGTAAGTGCAGTCTGAAGCTGCTAGTGCTGGCAATAATGATGTATGGATTCCTGAAA
TAGCGTAGTGGACTGGACTGGTTTGTTTACCCATGTGATTTAACACAAATGTTTAATTTTAGGGCTCACA
TCTTTGGTTTGCATGTTGCTGAATACATGAGAAGTCTTGAACAGGATGATGAGGATTCTTTCAAGAGACA
ATTCAGCAAGTACATAAAACTAGGAGTCACTGCAGATGCTGTAAGTACTTATCATGTTAGGTTGATATAA
AAATTAAAAACAAATGCTATAAACCTAGGATAGCAATTTTTAGGGCAAATAACTAAAAAAAGACGTCCAA
CCAAATTACTAATCCTCTTTAGATAGAGATTAGTGTCGAGCTTGGACTAAATTATTTTGCAATGTAATAT
AAGACTTTGATGTATTTGAGGCATCAAGGACCTGTTGAATGTTGTGACACTCAGCTTACATTACTGCAGC
TTAAAATAATATCTGCCCAATCTACTGAAACTAGCATGTACAGAGATGTTATTTAGCATTATTAAAATGT
TCTGCCGACATCGCCCTAAACACGTCATTACGGATCCTGCCGATCGATTAACGGTGCTTATAGGTACCTC
AAGCAGGGGACACCGCCCTTGCCGAACCCGCCGCATGCGACGTAGGGCTTGACGAGTAAATTAACCCATA
GACACAGCCCACTGAGTTTCTCACTGGATCTTCTCAGTGGGTCGCGTTTCCGATCTAGTGGTAGATTCTG
CGAAGCACTGCTCTTTCTAGGGCCAGTGTTTGCAACACTCCCGGTTTGAGCCATGTGAGCTTACCTACAC
GTCAAGGAGAAGCTGAAATAGTCTCTCAAAGCTATCAGCATAGGGAGGGGGGAAAAAAGCTTGTATAATA
AAACTTAAATAATTTATGATATTTCACCCAAATCAGCTATTTTTGCATGATTGAGTAACAGATAACCAAA
ACTAAACTTTTACAATAATAATCATAGATTATAGTCATTTTATTTGACATCTACTACTAAAAATTCCTGT
TTGAAAGTAATATATATGAATGTAATATTCAATGTTGTATGATGTAAGAAACTCAATCATAAATCAATTT
GTGGAAATTAGAACTAATATGCTTTGAATAATTCATGTTCTTTACCTATTTTTTGCTTAGTATTTTGAAA
GTTATTTACAAAACAATAGGTAGTATCCATATAATACTCTTGTCTGATTCAACAATAAGTATTAATTACA
TTATCCTTATCCCAATTATAAATAGTGATATTTTACATGTTTATGTGAAATTATGCATTTTTTAGAACTT
GAAATAGATTGTAAATTCTTCAATAACCTATTTATCAGAACAGTTTTAAAACAAACAGACCTGCCTTACT
TTGAACCTTTTCAATTAGTCTTTTAAAAACAAAATTTCAATATTGAAGTTTTAATTAGCATTTTGGACAT
TTTTGTGTATTGATTTTTGTTAGTTCCCATTCTGAGATATTTTATTTCATAGATTGAAGCCATCTACAAG
AAAGCCCATGAAGCCATCCGTGCGGATCCATCCCACAAGAAGAAAGAGTTAAAGAAAGACTCTGTCAAAC
AGAAGCGCTGGAACAAACGC
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AAGCTAACATTGGCCGAGAG

RpL7a Anchor Locus
Genomic: BAAB01136940
cDNA: AY769275

Forward Primer: 5- AGGCTGTACTTCAGCGTCGT -3
Binding in exon 2, cDNA 318, genomic 469
Tm60

Reverse Primer: 5- GTCTTGCGGTGTACAAGTGC -3
Binding in exon 5, cDNA 684, genomic 2433
Tm59

AY769275 (+) Bombyx mori ribosomal protein L7A (RpL7A) mRNA, complete cds
BAAB01136940 (+) Bombyx mori DNA, contig544386, whole genome shotgun sequence

Expected size: 2062bp
AGGCTGTACTTCAGCGTCGTCTGAAAGTGCCCCCTCCGATCAACCAATTTACCCAGACACTGGAC
AAGACTACAGGTTTGTTCTCTTAAGTTATGCTTCACGAATACTTAGAAATGCCAACTGTTAAAGAGAAAG
TCAACAAGAACAGCAAGAGATACAAAGAAAGACTAAATGAGCACTCAAATGAATTGGCAAGGTGCTAGAC

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ACAACTGGAGATGTGAAATGTTTAAAAAGATGGCAAAGTTTAGACTTAGACCATTGAATAATGTCATCCA
CAACTCGAACCCTTTGGAACACTGATTATGACTAAATAGCCGATTGCAGATAAATTAGAAAGAAAAAAAA
AAAGAAAAATGTTATTCTTATGTTGTTACATATTAACAAAGATGTAGTTAATATTTTTTTAAATAGTAT
TTTTTTTAAACTGCACAATTTTGTATTGTTGTCGGTGGTTTCAAAATTTTATGTAAATGTGTCAAAGAAT
TACATGATGATCCTCAAATGCAACTATGAATCACTATGATTATCAAAAAAACATACGAGCTTTTTAACTC
TGATAATTTTTTTAATAGTTAAATTAATGTATTTAACAAAGAATATGTTCTAAAGCATTTGAATTTTTGT
TCTAGCTAAGGGCCTTTTCAAGATTTTGGAGAAATACAGGCCTGAGACTGAAGCAGCCAGGAAAGAGAGG
CTGAGGAAAGCTGCTGAAGCTAAGGTTTGTTCACTCATTATATCGATTATGAATGCTATTTTTTTGTAAT
GTCAAATATTATGAATGCTATTTTTTTGAATTGTCAAATATTATTTTAAAACATTTATTAAAGAATCATT
GGCTATTTGTTACGATGGCAAGTATATAAGCATCATCGTCGTCATCATTCTTTCCTATTACTCTCTGCGG
GCGTGTAGAGCTCGAATCAAATTTTTCAATTTACATTGGTCTTGGGCTGTCTGTTCTAGCTTCTCCCATG
TCATGCCCAAAACCCTAGCTCCTGCTCCACCGAGCGACGCCAAGTTGTTTTGGGGCGGCCTCTCTTATAT
AAGTATATAAGCAAGTGATGCATAAACAATCCAACTCTGAAAGTTGTGATGACTCAACCACCAAGATCTC
TGATGGCATTTCATACAAATGACTACTATTGTAATAAATGCAACGAATATCTAAGAAATCATATGTTTCA
GGTTGCCAAGAAAGATGAGCCTCCACCAAAGAGGCCCAACACCATCCGATCCGGCACAAACACAGTCACC
AAGCTGGTCGAGAAGAAGAAGGCGCAGCTTGTGGTCATCGCTCATGATGTTGATCCTATTGAGGTATGTG
ATAATTCTCTACTGTATTCACAACCTTCTCTATAAACTGATATGGCTTAAAATTGAATTGAGAAATCTCT
GTACGCTGTACGGAAGCTATAATTTTGTGTAAATATAAAAAAAAATATTATTAAATTGTAAGACACCTCA
TTGCATGCTGAAGTAATTTGCCCAAATTTGTGGAAGTCTAGGAAATTTCCAGTATCCATATTAGATTTTTT
TTTGCAAAAAACGTACATGATTACATCTTAGGCTAAGATTCTTAGCTCTGGTATCAGTATGGTAGAATTT
TTATATTTGTATGCTCACAGTGAATAGTTTTGAATATAGCATTTTTATCATTGATCAAGTTGGGAAATAC
TATATGCAATACGATTATCAGATGTTATGTAGATTTGATTTTCTTGATGACACAAATAGTGTGACCATTT
GATAATACGAATTATCAAGCACGTCTTCTTGGCCTAGCTGTGATGTTAGAATTTCTTCACCTGAGGCTGC
AATGTAACATTATACATGTATAATGTTGACAGCTGAATGAAGACAATTGACTCTGAGCTACTTTACATCT
CAAAATCAATATACCAATAACAAATACTGTTAAATTACTTTTAGTTTAGAATTAATTGTAATTTATTGGA
TGTTTACAGCTGGTTCTCTTCCTGCCAGCGTTATGCCGTAAAATGGGTGTACCATACTGCATTGTCAAGG
GCAAGTCCCGCCTCGGT**GCACTTGTACACCGCAAGAC**
```

LDH1

Chris Wheat created this set of primers.

Genomic: AADK01000293
CDNA:
```
ATGGAGTCCCTGAAGAAGCTGTTCCAGCCCGTGCACGAGAAGGTGGACGAAACTTGGAGCAAGGTGACCATTGTCGG
GGTCGGTCAGGTTGGGATGGCCGCAGCTTTCTCTATGCTGACGCAGAATGTTACGAATAACATCGCTCTAGTAGACA
TGATGGCTGACAAATTGAAAGGAGAGATGATGGACCTGCAGCACGGATCAGCATTCATGAGGAACGCCAAGATCCAA
TCTAGTACGGATTATTCTATAACAGCTGGCTCGAAGATCTGCGTGGTTACTGCTGGTGTTCGACAACGCGAAGGTGA
ATCTCGTCTCGATCTCGTGCAGAGAAACACCGATGTTCTTAAACAAATAATCCCGCAGCTGATAAAGTACAGTCCGG
ACACAATATTGGTGATCGCCAGTAACCCCGTGGATATTCTGACGTATGTTACGTGGAAGATTAGCGGGCTGCCYAAG
CACCGCGTCATCGGGTCCGGCACTAACTTGGACTCGGCACGGTTCCGTTACCTGCTGTCGGACAGGCTCGGTATCGC
TACCACGTCCTGCCACGGCTACATCATCGGCGAACACGGAGACAGCAGCGTTCCAGTATGGTCTGCAgTAAACATAG
CGGGAGTACGCCTCAGTGATCTCAACAATCAGATCGGAACCGACGACGATCCTGAGAACTGGAAGGAGCTTCACGAG
AATGTCGTGAAAGTGCTTACGAAGTCATCAAACTGAAGGGATACACTTCCTGGGCTATCGGACTGTCGCTGGCTCA
GATCGTGAGGGCTATACTCACGAACGCTAACAGCGTTCATGCGGTCTCTACTTATCTCAAGGGCGAGCACGGCATCG
AAGACGAAGTATTCTTGTCGCTGCCGTGCGTGTTGAGTCACTGTGGAGTCTCTGACGTCATCCGCCAGCCTCTAACC
GAACTTGAAGTGGCACAGCTTCGGAAATCTGCTAAAGTCATGGCTAAAGTGCAGAACGACATTAAATTCT
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<u>Forward Primer</u>: 5- ATGGACCTGCAGCACGGATCA -3
Binding in exon 2, cDNA 189, genomic 33152
Tm61

<u>Reverse Primer</u>: 5- CTGCACGAGATCGAGACGAGA -3
Binding in exon 3, cDNA 307, genomic 34139
Tm58

LDH (+) cDNA chris

AADK01000293 (+) Bombyx mori strain Dazao Ctg000293, whole genome shotgun sequence

Expected size: 1016bp

ATGGACCTGCAGCACGGATCAGCATTCATGAGGA
ACGCCAAGATCCAATCTAGTACGGGTAAGCGGAGATTTATTAATTTACAATTTTATTAGTCCCTAAGAAT
TTTATTTATTTGTTTAATATCATTTTAGATTGAATGCTACGATCGGCGAACCGTAGTATTTTGTTATTCT
AGTTGATATGTTTTCCAAGACGTAGTATAGTATAATATAGTATAGATATAGTATAACATATACTTAGTTA
TTTTCTCTATATTTTACTGTATATTATGAATTAGGTATACCAATCGAAAAATACTTCATATTTTAAATTT
ATATCGGAGAGTCCAAAGGTGATCTATGGGATAATAAAATCTACGCGCGATTTCGTATTGTCTCTGCAAG
CTTGGTTGCAAGAAACTAGGGCTTATTTCGAAGTACAACTGTGTCGTATTTTTTAAAAATCAAATTGAAT
TTGAGTATCCACATTATGTGATATATGAAGAGATGCAGATATGCGCGGTTGAATAATTCGACAGTAACAA
AGATAGATTCTTTCACTAGATAAATCATCAGTAATAAATATAAATGAATTTTATTATTTGAATAGTATGC
TTCTTATAACACGTAAATCTAAGATATTTTACTGGTGTTTGGAGTCTAGTGGACTGACACGGGTAGAACA
CTCCGTTCTGCCTATTTATCCTGCGAAAAAATAATACATTTCGTTTTGAACGTTAGGGCAACCATAGGAC
TATATAACTTATTATTATATCTCAAGGCGATTGTTCGCACTCATCACGTAAAAAAAGTGAAAAAAACACT
CAACAGAGACCTTTAAATGCTTAATGAATAGAAAAATAGTTTTATGTTTAGAAATCGTTGTGCATAAAA
TTTAAAATGCAAAAACTTGGCGACTTAAATTTAATGTTAATGTTTTTTCGCAGATTATTCTATAACAGCT
GGCTCGAAGATCTGCGTGGTTACTGCTGGTGTTCGACAACGCGAAGGTGAA**TCTCGTCTCGATCTCGTGC
AG**

LDH2

Chris Wheat created this set of primers.

<u>Forward Primer</u>: 5- GCGTCATCGGGTCCGGCACTA -3
Binding in exon 4, cDNA 432, genomic 36850
Tm64

<u>Reverse Primer</u>: 5- ATGTCGTTCTGCACTTTAGCC -3
Binding in exon 6, cDNA 967, genomic 38307
Tm55

LDH (+) cDNA chris

AADK01000293 (+) Bombyx mori strain Dazao Ctg000293, whole genome shotgun sequence

Expected size: 1442bp

GCGTCATCGGGTCCGGCACTAACTTGGACTCGGCACGGTTCCGTTACCTGCTGTCGGACAGGCTCGGTATCGCTAC
CACGTCCTGCCACGGCTACATCATCGGCGAACACGGAGACAGCAGCGGTAAGGGGAATTTATCGAGCGAA
TATTGCTTACATTTTAACTTTTTTTTTTATTTCTACCCACAGGCAAACAACGAAGTTTGCTTACGTTCCTA

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TGATCATAAAAAGATAGGGACATTTTAATTTGTCGGTATAAATATAAACAATATAGGTGTTATCAACCAA
TGGATAAACACATATCGTCCTCTTGCATTAAAACCGTATAATCTCAAAACTTACTAATTTTACAGGTGAG
TGTTTTAAAGGTTTACTACCATGTGATATTTTTAATATTAATCATCTTTGCAACATTCATTTTTTACCAG
TTACATTATCTGTCTTTTAAAGTAAGATAAAATTATGTATTAATTTTATTAATACAGTCAAAACATAAAT
AACTAAAAAAAAACTATATCTAAACTACGCTCTTTTGACAGTATTTATGAGAAAAATACTTACTGGTGAT
AGCAAATGATTCCGCATATTAACTCAATTTCGAATATTTTTGGAAATTGTACACTTTTAATGCGAAAAGA
TCATATTTCGTGTATAATAATAATAATAATTGTACCCCCGTTTAATCCTAACGTAAATTTTTGGACACAG
TTCCAGTATGGTCTGCAGTAAACATAGCGGGAGTACGCCTCAGTGATCTCAACAATCAGATCGGAACCGA
CGACGATCCTGAGAACTGGAAGGAGCTTCACGAGAATGTCGTGAAAGTGCTTACGAAGTCATCAAACTG
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ACAGCGTTCACGCGGTCTCTACTTATCTCAAGGTAATCTGGGTTATGGGCTATGATGAATCATTAGATAG
TATAAACAAAGTCGCTTTCTCTGTCCCTATGTATGCTTAAATCTTTAAAACTACGTAACTGATTTTGAT
GCGGTTTTTTTTAATAGATAGTGATTGAAGAGGAAGGTTTATATGTATAATACCATCCATTAAATAGTGG
AGAAATACTGTTATTTTTGAGGTTTCTAATGGGATGTCGTAAAAAATCAAGAATAAAATTTCATGTTTCC
GAAGCAAAGCGAGGGTGGGTCGCTAGTGTTTTATATAATACTGTAATCTTGATGATATGTAAATTGGCCT
AATGTCACCGTTTCTCCTTAGGGCGAGCACGGCATCGAAGACGAAGTATTCTTGTCGCTGCCGTGCGTGT
TGAGTCACTGTGGAGTCTCTGACGTCATCCGCCAGCCTCTAACCGAACTTGAAGTGGCACAGCTTCGGAA
ATCTGCTAAAGTCAT**GGCTAAAGTGCAGAACGACAT**
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Diluting Primers
•On the blue top of the lyophilized primer tube, put name of primer and date.
•Spin down tubes for at least 30 seconds.
•Add the amount of TE required to make a 100μM stock. For example, add 304.2μl of TE to 30.42 nMoles of lyophilized primer. Flick tubes and spin down.
•Take 1.5mL tubes, label with primer name, date, and '10μM working stock.' Add 90μl of ddH20 and 10μl of 100μM stock. Flick and spin down.

PCR
NOTE: Make note of any errors made during this procedure on the PCR template sheet.
• Write down on the PCR template sheet your name, the date, the primers used, the PCR kit used, the PCR program used, and the cycles this program directs.
NOTE: The switch for the thermocycler is on the back near the plug. If you turn the apparatus on and the screen doesn't go to the main menu (show 5 blocks instead), the self-test has failed. Turn off and turn back on.   To review the cycles the apparatus undergoes for a particular program, at the first screen scroll to enter and press <u>proceed</u>, scroll to list and press <u>proceed</u>, scroll through the list to the desired program and click <u>proceed</u>. Scroll through the steps and you will be brought back to the main menu.
• On the PCR template sheet, write down the DNA samples you intend on using, as well as the μl of sample and water to be added to each tube. The template assumes that 5 μl DNA sample will be used (which has a concentration of 5-10 ng/μl). Include a blank (0 μl DNA and 5μl water). Count the number of PCR reactions you plan to undertake, and add one (for pipetting errors). Write this number on the rows underneath the column <u># of Reactions</u> and calculate the total μl of each reagent to be used.
•Retrieve the following reagents from the Epicentre Core PCR kit in the freezer (a green box) and put in a rack: 10X dNTP, $MgCl_2$, Mol H_2O, and Enhancer (if using). Also retrieve your DNA samples from the freezer and put in another rack.
•Let the reagents thaw on the bench and get ice from A128 using a bucket next to the sink.
NOTE: After the dNTPs have thawed, place on ice and leave in ice at all times.
•Take a blue rack and pound into ice.

•Pour out from the beaker containing autoclaved microcentrifuge tubes the quantity you will need (one for each PCR rxn). Label them by number (only touching the sides of the tubes when doing so), and make note of this number on the DNA template sheet. The tubes may be left open on the rack.

NOTE: When getting microcentrifuge tubes put aluminum lid facing up on the counter and pour out onto the lid. Excess tubes are put back into the beaker without touching.

•Once the PCR reagents have thawed, mix well by tapping or shaking, picofuge, and place in ice.

•Tap the DNA samples to mix, picofuge, and place back in rack.

•Aliquot the DNA samples first. Use the p20 to transfer 5 μl of DNA sample to the autoclaved microcentrifuge tubes—put the tip right to the bottom of the tube, discharge, and pull the tip up without dragging against the side of the tube. Dump the tip after each use. When done put the tubes (still opened) in the ice.

•Obtain and autoclaved 1.5 mL tube for the master mix and label MM and put in bucket.

•Move to the PCR bench and take three Pipetmen out of the drawer: P200, P20, and P10 (which uses white tips).

•Add to the MM tube the quantity of reagents that you calculated in the Total μl column of the PCR template sheet. Add the reagents in order and check them off as you go down the list. When adding the water, discharged into the bottom of the tube. When adding the subsequent reagents, discharge into the water and suck up and down between the UP position and the first stop.

NOTE: When getting adding to the Master Mix, it is helpful to have MM tube and reagent tube in one hand and the Pipetman in the other. Do not touch the MM tube at the bottom, as you do not want the contents to warm up.

•Before taking the Taq Polymerase out of the freezer, set the p20 to 20 μl and p10 to the amount of Taq being added to the MM tube.

•Mix MM tube by tapping and picofuge. Retrieve Taq from freezer, add using the p10, and immediately return reagent tube to the freezer. The Taq is very viscous, so one will have to pipet up and down several times.

NOTE: If there is Taq on the side of the reagent tube, then picofuge it first. There is a balance tube for this at the bench.

•Mix well (until the solution is uniform) and hold tube to the light. It should look like a sugar solution—waves should be visible. Picofuge.

•Before aliquotting MM, turn on thermocycler and set to program you will use

•Using the p20, aliquot 20 μl of the master mix to each PCR tubes. Since you are using the same pipette tip for each tube, pipet against the side of the tube between the liquid and where the cap closes (avoid contact with the DNA).

•Picofuge tubes, open the lid of the thermocycler, place the PCR tubes in the same orientation in the thermocycler rack, and close the lid, and start program.

•Once the thermocycler is finished, it will remain indefinitely at 4°C. Hit proceed, it will say "complete," hit proceed again, and one will be returned to the main menu. Turn off the thermocycler, take the samples out the rack, and place in fridge (for 1-2 day storage) or freezer (for long-term storage) before electrophoresis.

• Run PCR products on a 1% agarose gel.

Restriction Endonuclease Digestion

•Compare band with the ladder to determine the concentration of the PCR product. Generally use approximately 100ng of DNA for digestions.

•Use NEB catalog or website to determine NEBuffer to be used and if BSA needed. On a sheet of paper, determine the content of your MM: PCR product, 1µl of NEBuffer, .5µl enzyme, .1µl of 100X BSA (if needed) and q.s. to 10µl with ddH20. The MM should consist of enough reagents for all the digestions plus 1 extra for pipetting error.

•Aliquot DNA directly into the bottom of thin-walled PCR tube; then prepare your MM on ice. Use same pipet tip to aliquot MM into each of the tubes.

•Allow reaction to incubate at 37˚C in water bath for 4 hours. Every hour spin down tubes to bring down condensation to reaction mixture.

Making an Agarose Gel

• A 2% Agarose gel requires 1.0g agarose per 50mL .5X TBE and a 1% gel requires 0.5g agarose per 50mL .5X TBE.

•Clean balance with brush before use.

•Fold weighing paper in half and place on balance.

•Turn knob to the right to put on half-release and tare using the course and fine adjustment knobs on the top of the apparatus. The line should be between the two triangles.

•Turn the "one knob" once so the display shows 1g. Begin adding agarose by tapping the bottle over the weighing paper. Once floating numbers appear, stop adding agarose and turn the knob over to the left to switch to full release. Add agarose until the display reads 1.0000g. Turn off the apparatus, remove weighing paper, and clean with brush.

•Add agarose to 150mL Erlenmeyer flask along with 50mL of .5X TBE working stock and a small stirring bar. Cover with 25mL flask to prevent H20 loss.

•Place on hot plate and stir at 3.5 and heat at 6. In the interim, level a clean gel tray on the bench and place in comb with small teeth. Once the agarose has been completely dissolved, remove from hot plate. Cool the flask to the point where it can be touched without mittens, then pour into gel tray. Allow approximately 30 minutes to harden.

Running and Visualizing an Agarose Gel

•Make wells by taking strip of parafilm, pushing it down on a rack, and removing the paper.

•The loading dye is 6X, so approximately 1µl should be used when adding 3µl of PCR product per well (on a mini-gel) and 2µl dye when running 10µl digestion reaction. First spin down the dye, then use a p20 to take the amount needed for all the wells and make a small drop in each well. When doing this, touch the pipette tip to the parafilm. *NOTE: When using picofuge, make sure tabs sticking out, and use balance tubes.*

•Place the gel in the apparatus, making sure that the wells are on the cathode end. Add .5X TBE (on the shelf on bench) to the apparatus till one no longer sees indentations on top of the wells. One should make sure that the gel is firmly on the bottom (there should be no air bubbles underneath) to ensure no floating/sliding during operation. *NOTE: If gel slides, use pipette tip to adjust.*

•Record on a sheet what samples will be put into each lane. If possible try to leave the side lanes empty. *NOTE: also use this sheet to record errors that one makes (pipetting losses, etc.)*

•Use p20 to take the desired amount of sample, squirt into dot, and suck up and down between the UP position and the first stop. Suck up the entire sample, make sure there is no air bubble in the pipette tip, place tip into the well, and push plunder down to the first stop. Keep plunger steady when removing tip from well. *NOTE: If liquid is stuck in middle of tip, remove tip, place it back on, and air pressure should push liquid down.*

•Place the top on the apparatus, plug it in, make sure it reads 100V and ⊞ ⊟ and turn on. Record the start time. Throw parafilm in trash.

•Run the gel within two lines of the bottom (approximately 30 minutes).

•Turn off device, record stop time, remove lid, take out gel, pour off liquid, and place on a paper towel.

•Make a tic-mark on the tape on the apparatus to indicate that you used it. Put saran wrap (in drawer behind you) on top of the device. The buffer should be replaced with .5X working stock after every five uses.

•Take the gel to the backroom and put on an apron and gloves.

•Slide the gel off the plastic case and onto the spatula. Open the EtBr bath and place inside. Use the top of the bath to prevent dripping as you bring the spatula to the sink and rinse it.

•Place the lid back on the bath, but do not seal it. And allow it to soak for 10-15 minutes, depending on the age of the solution.

•When finished, take dry box and transfer gel to it with spatula, draining off as much EtBr as possible. Add water to the pitcher inside the sink and transfer water to box with gel. Drain out the box using the spatula to ensure that the gel doesn't fall out. Repeat 2-3 times, and then rinse the spatula.

•Take a piece of used X-ray film, paper towels, and dH_20 (found under the hood) with you to B104. This can be opened with the Alice in Wonderland keys found in the desk drawer. Enter from the front door, leave from the back door, and when leaving ensure that lights are on/off as they were when entered.

•Open bottom slot, place the gel in middle of the transilluminator, and close slot. Open the top lid and turn the camera on.

•Place a paper towel on the mouse, and click on application <u>KODAK DLE 306</u>. *NOTE: If problems, restart computer clicking <u>restart</u>, under the <u>special</u> menu.*

•Click on the box in the top left that says <u>DC290 Capture</u>.

•If it is a minigel, put on the smallest size setting. If a larger gel, adjust as necessary.

•Click <u>Preview</u> and quickly turn on the switch of the large timer. After you hear the camera take the picture turn switch off. *NOTE: Do not touch the switch on the transilluminator. To give oneself more time, one can move the minute hand on the timer from 5 to 10.*

•If the preview looks good, then click <u>Take Picture</u> and repeat above procedure.

•Straighten the image using the box that looks like this ----------- >

•Highlight the area that is not black, go to <u>edit</u>, and click <u>crop</u>.

•Adjust the contrast of the image using the image display window. If under/over stained, fine-tune with vertical bar. Clicking on + or – will send you back and forth with histogram window.

•Save in MacHD→DC290→Goldsmith lab alias. Name image as date-sampletype.jpg and switch the file format to jpg.

•Turn on the printer and click <u>print</u>.

•When finished, click the box in the upper left hand corner of the window within the program.

•When it asks you if you want to save changes to untitled, click <u>no</u>.

- Turn off camera and printer. The Computer will turn off itself.
- Return the gel to the dry box, clean transilluminator with dH_20 and paper towel. Return to hood with dH_20, used paper towels, box, and printed page.
- Put towel in wastebasket and gel in waste tray under hood.
- Add water to pitcher in sink, and use to clean the gel box and X-ray film.
- Place used gloves in waste bag in hood.

Results

A number of different loci were successfully amplified on different templates (table 1). Most of the PCR products did not show size differences, and had to be digested to discover polymorphism (table 2)

Table1: Loci Successfully Amplified

Date	Locus	Template	Amplicon Size (kb)	Polymorphism
9/19/05	G6PDH	C,P,C/P, 555	0.45	None
10/6/05	Btr175	C,P,C/P, 555	3.5	None
10/27/05	Rcf526	4, 5, 4/5, 555	0.7	None
11/4/05	RpS8	4, 5, 4/5	2	None
11/27/05	RpL5	4, 5, 4/5	2.2	None
1/24/06	RpL7a	4, 5, 4/5, 4/5//4	2.2, 3.0, 3.5	4, 5, and 4/5 specific bands
3/30/06, 4/4/06	Sco14.54	4, 5, 4/5, 4/5//4	0.2, 0.3, 0.4, 0.58, 0.69, 1, 1.5	Polymorphism
4/12/06	LDH1	4, 5, 4/5	1	None
4/12/06	LDH2	4, 5, 4/5	1.5	5 cannot be amplified
4/30/06	LDH1	C,P,C/P	1	None
4/30/06	LDH2	C,P,C/P, C/P//C	1.5	Possible doublet

Table 2: Digests Performed

Date	Restriction Enzyme	Locus	Template	Polymorphism
9/22/05	MseI	G6PDH	C,P,C/P, 555	None
10/4/05	EcoRI	G6PDH	C,P,C/P, 555	555-specific band; 1.5kb
10/13/05	EcoRI	Btr175	C,P,C/P, 555	555-specific band; 1.5kb
10/13/05	HindIII	Btr175	C,P,C/P, 555	None
11/8/05	AluI	RpS8	4, 5, 4/5	None
11/10/05	EcoRI	RpS8	4, 5, 4/5	None
11/29/05	EcoRI	RpL5	4, 5, 4/5	None
11/29/05	HindIII	RpL5	4, 5, 4/5	None
3/2/06	AluI	Rcf526	4, 5, 4/5	None
3/2/06	DdeI	Rcf526	4, 5, 4/5	None
3/2/06	MseI	Rcf526	4, 5, 4/5	None
3/9/06	AluI	RpL5	4, 5	None
3/9,21,23/06	DdeI	RpL5	4, 5	None
3/9/06	DraI	RpL5	4, 5	None
3/9/06	MseI	RpS8	4, 5	None
3/9,21/06	DdeI	RpS8	4, 5	404-specific band; 1kb
3/9/06	DraI	RpS8	4, 5	None
3/28/06	DdeI/DpnI	RpS8	4, 5, 4/5	404-specific band; 300bp
3/28/06	DdeI/EcoRI	RpS8	4, 5, 4/5	404-specific band; 1kb
4/13/06	TaqI	LDH1	4, 5, 4/5	None
4/13/06	DraI	LDH1	4, 5, 4/5	None
4/13/06	DdeI	LDH1	4, 5, 4/5	None
4/13/06	AluI	LDH1	4, 5, 4/5	None
5/7/06	DdeI	LDH1	C,P,C/P	None
5/7/06	DraI	LDH1	C,P,C/P	None
5/7/06	DdeI	LDH2	C,P,C/P	P-specific band; 420bp
5/7/06	DraI	LDH2	C,P,C/P	None
5/12/06	MseI	LDH1	C,P,C/P	P-specific band; 400bp
5/12/06	MseI	LDH2	C,P,C/P	None

Anchor Loci

The RpL5 anchor locus did not show polymorphism in either PCR or digestion products. RpS8 locus did show size polymorphism when digested with DdeI; however, it is not useful, as it is a 404-specific band (Figure 1A). A 516-specific band is required so one may determine if the backcross survivors are 404 homozygous or heterozygous. DdeI/EcoRI and DdeI/DpnI double digests were performed in an attempt to reveal a 516-specific band, but these were not successful (Figure 1B).
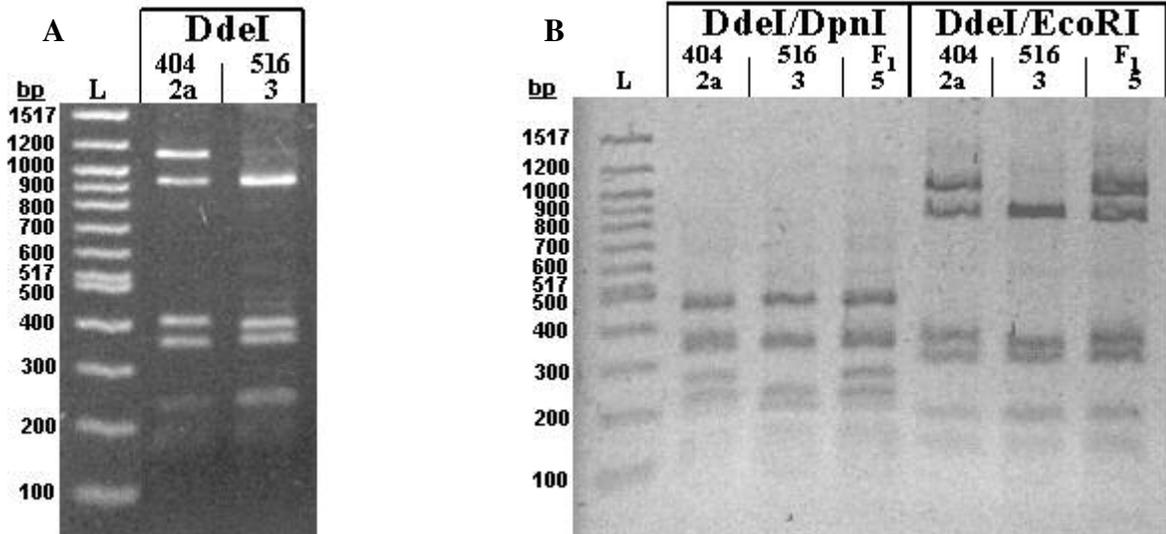

Figure 1. RpS8 Digestions
3μL of 100bp ladder (L) and 10μL of digestion products run on 2% agarose gel at 100V for 30 minutes. A, 3/9/06. B, 3/22/06.

When the RpL7a locus was initially amplified, there was a strong 404 band (2.2kb) and a very faint 516 band (3kb). After reading a paper detailing that short extension times can mask size polymorphism in a locus (Maekawa et al., 2004), I decided to repeat the experiment with an increase in the extension time from 1 to 3 minutes. This PCR gave the same 404 products, a strong 516 band, and a hybrid with both bands plus an additional band running at roughly 3.5kb. Since this additional band was consistently found in all of the heterozygotes, it was ruled to be a PCR artifact (Figure 2A). The PCR was repeated for all of the backcrossed individuals (Figure 2B-E), and it was found that all 17 of the backcross BT-treated survivors were 404 homozygotes while the backcross control population consisted of 6 heterozygotes and 15 404 homozygotes.
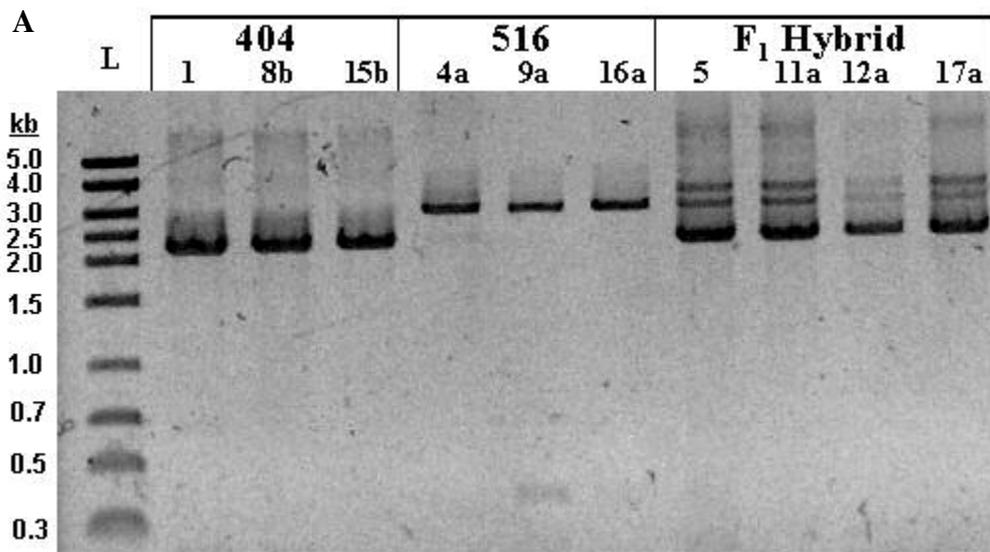
Figure 2. RpL7a Anchor Locus
3μL of Midranger (L) and PCR Products run on 1% agarose gel at 100V for 30 minutes.
A, 2/17/06. B, 1/24/06. C, 1/26/06. D, 1/31/06. E, 1/31/06.
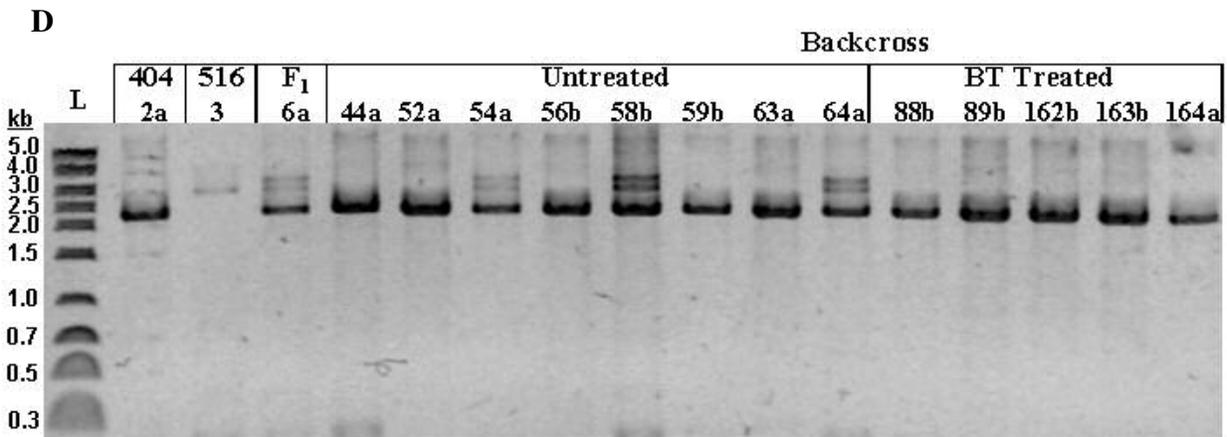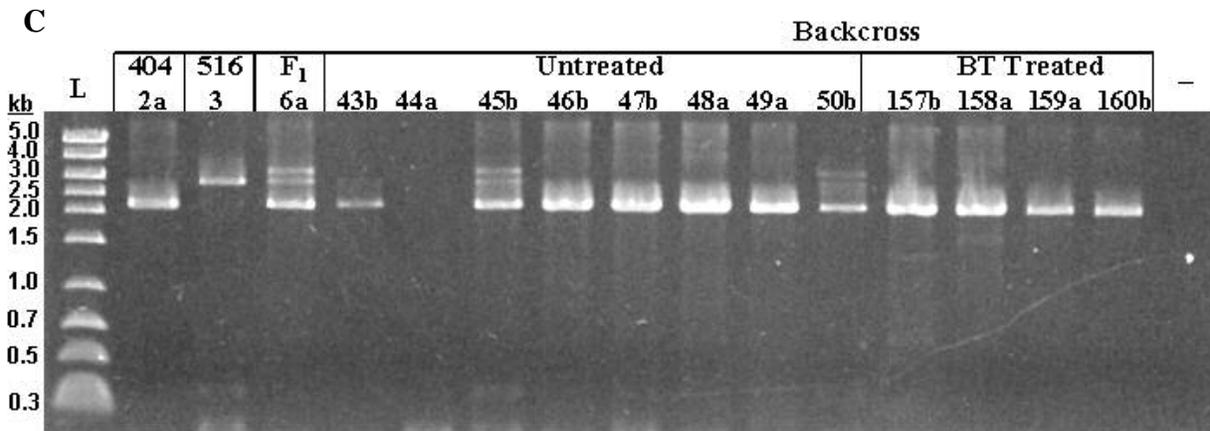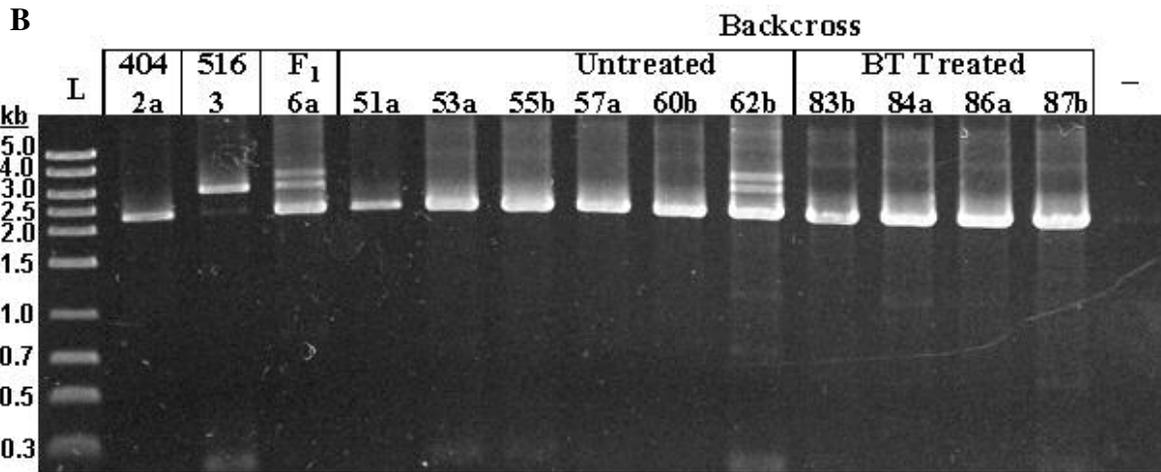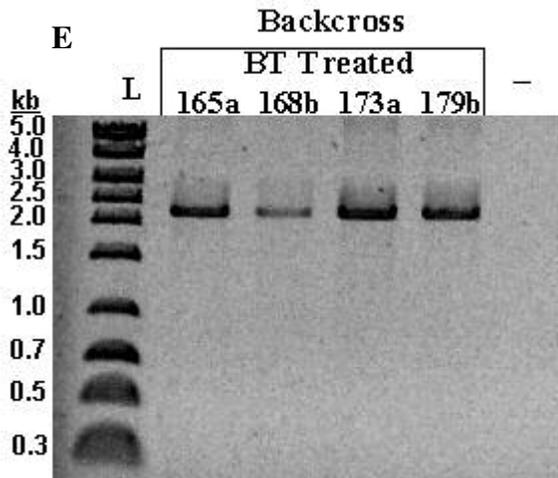
Figure 2 Continued

Figure 2 Continued

LDH

LDH introns were amplified using two different primer sets on both the 404/516 and C108/p50 mapping panels. The LDH2 amplicon of the C/P panel has an extra wide band, possibly a doublet, in some individuals. If LDH is sex-linked, P/- females and P/C males are expected in the F1 and C/- females and P/C males are anticipated in the backcross. If it is autosomal, one expects all the F1 to be P/C and a 1:1 ratio of P/C to C/C in the F1. Of the 1 F1 and 3 backcross individuals amplified, each had a banding pattern consistent with sex-linked inheritance.

Using the C/P mapping panel, digestion of LDH1 amplicons with MseI and digestion of the LDH2 amplicons with DdeI reveals a p50-specific band.

Discussion

This mapping panel consists of a 404/516 hybrid backcrossed to a 404 parent, so half of the backcross population should be 404 homozygous and the other half heterozygous under normal circumstances. However, half of the backcross population was treated with the BT toxin, and since any individual with a 516 allele should be sensitive to the insecticide, only 404 homozygotes should survive. The control population size (21) is rather small, and the variation from the expected 1:1 ratio in the control (p=.05) fails to reject the null hypothesis. However, the fact that all of the survivors in the backcross treated population are 404 homozygous strongly

suggests that the trait maps to LG15.   It is possible that the 516-specific band of the RpL7a anchor locus is due to a repeated element or transposon present in the intron.

Although the RpS8 anchor locus could not be used in this study, it would be useful with a mapping panel where the F_1 was backcrossed to the 516 parent.

The fact that the digests of 404 and 516 introns generally led to similar banding patterns (which corresponded to the *in silico* digestions of the p50 genomic sequences) indicates that there is less polymorphism in introns than expected, despite the fact that 404 and 516 are of different geographic origins.

References Cited

Lawlor, C. (2004). A Determination of the Genetic Basis For BT Resistance Using RAPD Markers in the Silkworm, *Bombyx mori*. M.S. Thesis, University of Rhode Island.

Maekawa, M., Taniguchi, T., Ishikawa, J., Toyoda, S., and N. Takahata, 2004 Problem with Detection of an Insertion-Type Mutation in the BCHE Gene in a Patient with Butyrylcholinesterase Deficiency. Clinical Chemistry **50**: 2410-2411

Shi, J., D. G. Heckel, and M. R. Goldsmith, 1995 A genetic linkage map for the domesticated silkworm, *Bombyx mori*, based on restriction fragment length polymorphisms. Genet. Res. **66**:109-126.

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