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BIO 343

9 May 2013

Identification of Drought Stress Genes in *Vaccinium corymbosum* and Selection of PCR Primers for Amplification of Nearby Simple Sequence Repeats

**Abstract**

The blueberry, *Vaccinium corymbosum*, is an important agricultural product in many areas of the United States, and like most crops it is susceptible to moderate and severe drought. In an effort to help researchers selectively breed for drought-resistant blueberries, I performed a literature search to identify genes in model organisms that may play a role in drought-resistance pathways. I identified orthologs of known drought-resistance genes. I utilized a web-based program to locate simple sequence repeats (SSRs) near the genes of interest, and I selected PCR primers that could be used to amplify these SSRs. The SSRs can be used to help researchers genotype individual plants and identify alleles, promoting selective breeding efforts.

**Introduction**

*Vaccinium corymbosum*, the blueberry, is the second-most harvested berry crop in the United States, with over 560 million pounds of blueberries harvested annually and worth over $850 million in 2012 (Blueberries, 2012). In North Carolina alone, over 28 million pounds are harvested, with a value of approximately (U.S., 2012). Blueberries contain many purportedly-healthful compounds, including phytonutrients, vitamins C and K, manganese, fiber, and more (Health, 2012). Compounds such as these may play significant roles in cardiovascular and brain health.

 Because of the nutritive and commercial value of blueberries, there is research interest in the biology of blueberries. We have partnered with researchers at North Carolina State University and the North Carolina Research Center in order to study the blueberry genome, with the goal of helping breeders produce better blueberries. I examined genetic pathways involved in resistance to drought stress. Soil water content of less than 50% can lead to blueberry plants producing fewer and/or smaller berries (Longstroth, 2012). The generation of a drought resistant crop could improve annual blueberry harvests, increasing profits for growers and making blueberries more available and less expensive to the public. I first studied universal drought resistance mechanisms in the model plant *Arabidopsis thaliana*, and then I searched the blueberry genome for orthologs to *Arabidopsis* drought resistance genes.

 Once I identified an ortholog of a drought resistance gene in the blueberry genome, I searched the genome for nearby simple sequence repeats, or SSRs. SSRs are frequently mutated by DNA polymerase, which has a tendency to make errors when synthesizing repeat units of DNA. These SSRs serve as molecular markers for the nearby gene. I produced PCR primers that could be used to clone and amplify these SSRs, so that blueberry growers and researchers could identify blueberry plants that contain an allele associated with a particular SSR. Hopefully, cloning these SSRs will give blueberry growers the ability to identify certain alleles of drought-resistance genes and ultimately selectively breed for a more drought-resistant crop.

As a part of this project, I also tested the recently developed Genome Sequence Annotation Server, or GenSAS. The goal of this program was to automatically annotate features of the blueberry genome using different computer programs. I tested a program that has been integrated into GenSAS called BLAT. BLAT is similar to BLAST, and it uses an index derived from assemblies of entire genomes to predict genes. The GenSAS program has been recently developed, and as one of its first users, I attempted to see if it could be used to produce viable annotations.
  **Methods**

I focused on the drought stress genes that I found to be most well-characterized in the literature, which were abscisic acid- (ABA) dependent genes in the model plant *Arabidopsis thaliana*. I took genes that I had identified as involved in drought-resistance in *Arabidopsis* and searched for them in NCBI’s protein database (REF). When I found a protein on NCBI, I saved its amino-acid FASTA sequence to a plain-text ile so that I could BLAST it against the blueberry genome to search for alignments.

In order to BLAST amino acid sequences of genes of interest against the blueberry genome, I first set up a local BLAST engine on my computer. I did this using a Mac OS X machine. I opened up the Terminal application and entered the following command – “cd Desktop/ncbi-blast-2.2.27+” - where ncbi-blast-2.2.27 was the name of the folder that contained the blueberry genome. The folder was located on the desktop, as indicated by the command. I entered the command – “./bin/makeblastdb -in Sorted\_454Scaffolds.txt -input\_type fasta -dbtype nucl -title blueberry\_Genome” – where Sorted\_454Scaffolds.txt was the text file containing the nucleotide sequences that composed the blueberry genome. This completed the setup of local BLAST.

In order to BLAST a particular amino acid sequence against the blueberry genome, I entered the following command – “bin/tblastn -query XXXXXXXX.txt -db Sorted\_454Scaffolds.txt.” XXXXXXXX.txt should be replaced by the name of the .txt file that contains your query amino acid sequence. The results of an example BLAST for are shown in Figure 1. Higher bit scores and lower E-values correspond to better alignments.

**Figure 1.** Example results of a tBLASTn search. Circled in red is the best alignment the computer identified, its raw bit score, and E-value. Underlined below are details about that alignment, including the scaffold that it is on and the number of the nucleotide on that scaffold where the alignment began.

 I performed tBLASTn searches, as indicated in the search command, to search the genome (a nucleotide sequence) using an amino acid sequence query. Amino acid queries produced better alignments than nucleotide queries. If the search produced any significant (E-value < 1x10-3) alignment between the query sequence and the genome, I identified the scaffold which contained the lowest E-value hit, and noted the base pair position of the alignment on that scaffold. If I had already identified a gene of interest on the scaffold with the lowest E-value alignment, I selected the alignment of the nest-lowest E-value.

I searched the blueberry genome for the scaffold I had identified. I did this by opening up the file containing the blueberry genome, and using the Cmd+F “find” feature to find the right scaffold within the genome. I copied and pasted the entire scaffold into a separate document, and saved it as a plain-text file. I submitted that file to the SSR identification tool (REF). This tool can be accessed by mousing over the “Tools” header, and selecting “SSR.” Into the SSR server, I entered my email address and attached the .txt file containing the scaffold with the alignment to the gene of interest. When submitting the file, I entered “0” next to tetra- and pentanucleotide repeats so that they would be ignored. SSRs with repeat units ≥4 bases do not produce as much variation as the shorter repeat units (REF). The SSR server returned excel documents to my email that included the position of all di- and trinucleotide SSRs along the query scaffold. The excel file also generated PCR primers that could be used to amplify those SSRs. A sample excel file with relevant data highlighted is included in Figure 2.



**Figure 2.** Example of the Excel file returned by vaccinium.org SSR tool. Important information is highlighted in red, including the repeat motif, the number of times that motif repeats, the number of the scaffold nucleotide where the repeat motif begins, sequences for PCR primers that can amplify the repeats, and the size of the PCR product that would be produced by those primers.

 I selected SSRs and their accompanying PCR primers based on the number of repeats a SSR contained and its distance on the scaffold from the gene of interest. Higher-repeat SSRs and close proximity to the gene of interest are more desirable. I generally selected PCR primers for the highest-repeat SSR on the scaffold, for the SSR nearest to the gene of interest, and for an SSR that struck a balance between number of repeats and distance from the gene of interest. I recorded the PCR primers for each SSR, noting the repeat unit, the number of repeats, the length of the PCR product, and the base pair on the scaffold where the primer began. I also recorded the name of the gene of interest and the number of the scaffold the gene was found on. A sample of my finished report on a gene is shown in figure 3.



**Figure 3**. Example report for gene of interest ABF2. Includes PCR primers for three different SSRs. Repeat unit, number of repeats, size of PCR product, and location along on the scaffold are included for each.

I also submitted several of my scaffolds to the GenSAS automatic annotation website (REF). I uploaded the scaffold a particular gene of interest was on onto GenSAS, and then selected the BLAT tool as the program I wanted to use to analyze that sequence. I attempted to run the BLAT tool on multiple scaffolds.

**Results**

I generated useful SSR primers for 39 genes. I generated three pairs of primers for 35 of the 39 genes, but 4 of the genes presented only one nearby SSR that was suitable for amplification (Appendix A). The genes I generated primers for are listed in table 1. Some genes, which I have not included in this paper, did [NOT?] produce any significant alignments to the blueberry genome.

**Table 1**. Genes for which SSR primers were generated.



In addition, I found literature regarding the potential of select genes to generate drought resistant phenotypes. I found literature examining the biology of these “high-importance” genes, which included MYB96, IPT, and CKX. I identified these genes to examine in detail because the literature showed that they confer significant and well-characterized drought resistance.

 The GenSAS BLAT tool did not produce any results. After I submitted my scaffolds for annotation, I received an email informing me that there had been an error and that the annotation could not be completed. I tried this with several different scaffolds, but the GenSAS BLAT tool did not work properly on any of them.

**Discussion**

The PCR primers I have generated could be used to amplify an SSR, which could assist blueberry growers in identifying plants of a particular allele of the gene in question. Particular alleles of a drought-resistance gene are likely associated with a certain form of the SSR because those regions are so prone to mutation. By using the PCR primers I generated to amplify and sequence an SSR near a gene of interest, breeders will be able to determine what allele an individual plant has. Accurate genotyping of drought-resistance genes in blueberries could accelerate the selective breeding efforts of growers by allowing them the ability to breed only homozygous individuals. This would allow growers to produce cultivars that are true-breeding for a drought resistance gene. Enhanced selective breeding could lead to wide-spread growing of drought resistance blueberries and thus increased crop yields.

 To further the usefulness of amplifying SSRs for a gene, it would be ideal if the blueberry genome could be more thoroughly sequenced. If there were fewer scaffolds and fewer ambiguous nucleotides in the genome, better SSRs could be identified. In addition, it would be beneficial if more drought-resistance genes could be identified in the blueberry genome. A careful review of the current literature on drought-resistance pathways could help identify key drought-resistance genes that are of greatest importance to identify in blueberry. I focused only on abscisic acid- (ABA) mediated pathways, and so there are likely many ABA-independent pathways that are as yet un-annotated in the blueberry genome.

 I also examined the phenotypic impacts of several drought resistance genes that I identified in the blueberry genome. These genes - MYB96, and IPT/CKXs - were shown to confer significant drought resistance. Increased expression of IPT and CKX leads to higher levels of intracellular cytokinins (Nishiyama *et al*., 2011). Higher cytokinin levels confer significantly increased drought resistance, yet also stunt the growth of plants (Nishiyama *et al*., 2011). Plants that express high levels of IPT and CKX would thus be desirable under drought conditions when survival of the plant is more important than plant size. Under non-drought conditions, however, such plants would likely be smaller and undesirable. Perhaps there is potential for breeders to selectively breed for a number of cultivars with different expression levels of these genes, and they can then select for the one that strike the optimal balance between size and drought resistance based on the weather predictions for a growing season.

Increased expression of MYB96 leads to increased cuticular wax deposition and reduced water loss via transpiration (Seo *et al*., 2011). Unlike IPT/CKXs, increased MYB96 expression does not seem to stunt the growth of plants. Therefore, if a MYB96 allele that produces increased expression could be identified, it could be a good candidate gene to selectively breed for. Increased wax deposition could potentially alter the taste and texture of blueberries, however, which might make them undesirable.

 The BLAT tool I ran through GenSAS did not produce any results, despite several trials on different scaffolds. If GenSAS were fixed so that it produced automatic annotations, it could potentially be a useful tool for analyzing genomes.

**Acknowledgements**

I would like to thank Dr. Allan Brown of North Carolina State University, Dr. Jeannie Rowland of the U.S. Department of Agriculture, and Dr. Doreen Main of Washington State University for helping me gather this data. I would also like to thank Dr. Malcolm Campbell for his guidance and support throughout this project.

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1. ABF2 found in scaffold 00546 (query sequence starts at base 66768 on scaffold)

1)

For Primer CAACAGATGATAGCAGGACGAG

Rev Primer ATTAAAAGGAGTGAGAAGGCCC
repeats (ga) x12 PCR product = 277 bp & start at base 46931

2)
For Primer CGAACCCTAACCTTACCTTCCT
Rev Primer GGAAAACCAGTAAACCCATGAA
repeats (ct) x16 PCR product = 197 bp & start at base 49815

3)
For Primer ACCTAGGGTGATGCAGTGATTT
Rev Primer GTTCTACCAATACTGGCTTCGG
repeats (ct) x 10 PCR product = 130 bp & start at base 98319

2. ABF3 found in scaffold 00375 (query sequence starts at base 185340 on scaffold)

1)

For Primer AAATGGGACCTAATGTGGTACG

Rev Primer TCACTCCATAAATCATTGCAGC
repeats (ag) x23 PCR product = 285 bp & start at base 172031

2)
For Primer AGCTGGCCTAGGCTTGACTC
Rev Primer CTTAACTCGGCTCGATAAGGCT
repeats (ca) x13 PCR product = 288 bp & start at base 183449

3)
For Primer CACAGAGAGAGAGAGAGAGAGAGAG
Rev Primer TGCCAAGAGTAGTCATAAGCCA
repeats (ga) x 18 PCR product = 192 bp & start at base 192091

3. ABF4 found in scaffold 00054 (query sequence starts at base 66789 on scaffold)

1)

For Primer CGAACCCTAACCTTACCTTCCT

Rev Primer GGAAAACCAGTAAACCCATGAA
repeats (ct) x16 PCR product = 197 bp & start at base 49815

2)
For Primer ACCTAGGGTGATGCAGTGATTT
Rev Primer GTTCTACCAATACTGGCTTCGG
repeats (ct) x16 PCR product = 130 bp & start at base 98319

3)
For Primer TAGATTGGTGAGATCAGGGAGG
Rev Primer TGTCATCTGCAACTCTATTCGC
repeats (ct) x 10 PCR product = 156 bp & start at base 112538

4. ANAC019 found in scaffold 00116 (query sequence starts at base 390369 on scaffold)

1)

For Primer TAATTTGTCCTAAATGCTCCCG

Rev Primer TCCACATTGTACTGAAACTGCC
repeats (ct) x10 PCR product = 247 bp & start at base 327537

2)
For Primer CAAGAGAAGAGGAGAGCCAAAA
Rev Primer TCTCGTTCATGGTTATTGATGC
repeats (tc) x11 PCR product = 247 bp & start at base 338965

3)
For Primer CAAGAGAAGAGGAGAGCCAAAA
Rev Primer TCTCGTTCATGGTTATTGATGC
repeats (tc) x 17 PCR product = 247 bp & start at base 338783

5. ANAC055 found in scaffold 01837 (query sequence starts at base 43877 on scaffold)

1)

For Primer TTTATAGCGCTTTGATCAGGGT
Rev Primer CTCACACTTTGAGACAGGCATC
repeats (ag) x10 PCR product = 265 bp and start at base 23482

2)
For Primer AGAACTTCGAGGCTTTGACTTG
Rev Primer CTCCATCTGCCATCCCTATAAA
repeats (ga) x11 PCR product = 181 bp and start at base 34820

 3)

|  |
| --- |
| For Primer GACAAAAGGTTGTTTTCCTTGCRev Primer ACCAGGTGGAAGTTGAATGTCTrepeats (at) x5 PCR product = 148 bp and start at base 435586. ANAC072 found in scaffold 00651 (query sequence starts at base 120766 on scaffold)1)For Primer GTACCGGTGAGTGTGTTGAAGARev Primer AATAGTGGATTCTGGGGTGAGArepeats (ag) x14 PCR product = 231 bp and start at base 1204472)For Primer AGAATAGCATGTCCCGACTCTCRev Primer CAACTAATAGCTCGCTCCCTTTrepeats (ct) x14 PCR product = 300 bp and start at base 1338433)For Primer CGACACAGGTCCTCTCTCTCTCRev Primer ACCTGCAACTAATAGCTCGCTCrepeats (ct) x24 PCR product = 277 bp and start at base 1338897. Apx1 found in scaffold 00868 (query sequence starts at base 63665 on scaffold)1)For Primer TTTCCTTTTCATCGTCAGAGGTRev Primer ACCTACCATGTGTGAGAATCCCrepeats (tc) x14 PCR product = 230 bp and start at base 155752)For Primer CAAGCTGGATGAGACTGTTGAGRev Primer CTTTTTCCCTTCCATGTTTCACrepeats (ac) x7 PCR product = 294 bp and start at base 409733)For Primer GCTGTAGAAAAAGCCAAGAGGARev Primer TTGTACTTCATTGTCCCGAATGrepeats (tc) x6 PCR product = 299 bp and start at base 637908. CDPK1 found in scaffold 00154 (query sequence starts at base 315099 on scaffold)1)For Primer TCCTCCACAACTACGATGTCTGRev Primer CAGCGAAAATACCCTATTCCACrepeats (ag) x25 PCR product = 246 bp and start at base 484042)For Primer TGTATCTTTCGAATCGCGTAGARev Primer CAGGGAAAGGAACATTTTCAAGrepeats (att) x17 PCR product = 219 bp and start at base 1943783)For Primer GTCTTTCTTTGACAACGGAACCRev Primer CCACGTATATAGTTGTTCGGCArepeats (ct) x9 PCR product = 283 bp and start at base 3058519. CDPK2 found in scaffold 01799 (query sequence starts at base 51713 on scaffold)1)For Primer ACGTTTAATTACGTACGTCCCARev Primer ACCGATAAGGACACGAGATTGTrepeats (ct) x23 PCR product = 231 bp and start at base 231152)For Primer ATGTTCTTTCAGAATCCGTCGTRev Primer TGCTGAACTATCTTTCAGGGGTrepeats (tc) x10 PCR product = 272 bp and start at base 519113)For Primer GTTTAAACCGTTACCTTTCCCCRev Primer GCTAATTATCCTCTCGTGGTGGrepeats (ct) x18 PCR product = 282 bp and start at base 5204810. CKX found in scaffold 00024 (query sequence starts at base 447697 on scaffold)1)For Primer GCCTTTGAAGAACAAAACTGCTRev Primer CGTAACGTAACGAAACGAAGAArepeats (ct) x16 PCR product = 215 bp and start at base 3240642)For Primer TTTTGTTTGACAGCTCGAAGACRev Primer ACGTAAACCGTAACCTTCGTTGrepeats (ga) x16 PCR product = 213 bp and start at base 4735093)For Primer GTAAAGAGAAAGGGCCAAATCCRev Primer ACCTACCACTACTCCCTCCCTCrepeats (ga) x18 PCR product = 140 bp and start at base 48076011. 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MBM1 found in scaffold 00028 (query sequence starts at base 413851 on scaffold)1)For Primer GCTCTTATCTGGTTGTGGCTCTRev Primer ATGAACATCTGTGAGCAGCAGTrepeats (ag) x13 PCR product = 288 bp and start at base 4101532)For Primer GCTCTTATCTGGTTGTGGCTCTRev Primer ATGAACATCTGTGAGCAGCAGTrepeats (ag) x13 PCR product = 288 bp and start at base 4101993)For Primer CTCTACCGTAATGACGCTACCCRev Primer TACTACCGTCCGTCGTCTTCTTrepeats (ga) x17 PCR product = 235 bp and start at base 46994322. MYB96 found in scaffold 00457 (query sequence starts at base 75842 on scaffold)1)For Primer TCTCTCTCTCACAAACACACGC Rev Primer GAGCAACCCCAACAATAGGTTArepeats (ac) x16 PCR product = 223 bp & start at base 465192)For Primer TTGCGGTCATTTCTTCTTCTTCRev Primer TGACATTGCATAGAAGCACACArepeats (tc) x15 PCR product = 174 bp & start at base 590213)For Primer CACCTACTCCCTTCGTTCAAAARev Primer CCCTAAAGTGCCTCAAGTCATTArepeats (ct) x 16 PCR product = 300 bp & start at base 7919123. 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PKABA1 found in scaffold 00012 (query sequence starts at base 630459 on scaffold)1)For Primer AGATGCGAAAGCTGTAAAAGGARev Primer AGTATCTCCGAGCCACTTTCTGrepeats (ag) x15 PCR product = 291 bp and start at base 5442222)For Primer TGGTGGTCTACTAGATGCGAAARev Primer CATGTTTATGGTCCGAGTGCTArepeats (ag) x15 PCR product = 194 bp and start at base 5443723)For Primer ATAAAAACCCTGCCTTGACTGARev Primer TGATCAGAAGCATCTTCTCCAArepeats (ga) x18 PCR product = 233 bp and start at base 65083126. PLC1 found in scaffold 00254 (query sequence starts at base 241728 on scaffold)1)For Primer NCTCTCCTACCTTACTACACACACARev Primer AAGGCACTCCGATGGTTAAGTrepeats (tc) x14 PCR product = 110 bp and start at base 1921352)For Primer TGACCATCCCTTCTGAATTTCTRev Primer TTTCGACAATGCCAACAGATACrepeats (ct) x40 PCR product = 288 bp and start at base 2317573)For Primer TACACCTCTTCTCTCTCCTGCCRev Primer GACTGTTACAACTCCGCTTCCTrepeats (ca) x12 PCR product = 216 bp and start at base 23379427. PPC1 found in scaffold 00939 (query sequence starts at base 17909 on scaffold)1)For Primer CTCAACGATTGACCAAATAACGRev Primer ATTCACACTCATGGCTTACCCTrepeats (ag) x12 PCR product = 267 bp and start at base 40522)For Primer CATAGTTACCGATGTCCGATCARev Primer ACTCTGTACCTCTCCTTCTGCCrepeats (aga) x10 PCR product = 225 bp and start at base 468093)For Primer GCTGTATCAGTCGTTGCTGTTCRev Primer GATACCTTTGGCCACGTAAAAGrepeats (tc) x10 PCR product = 259 bp and start at base 6457028. PUB18 found in scaffold 00746 (query sequence starts at base 16200 on scaffold)1)For Primer CTCTAAACTTTCCGCCTCCTTT Rev Primer GCTAATCAACCGAAACACCATTrepeats (tc) x14 PCR product = 119 bp & start at base 87592)For Primer AAAGAGAGGAACGAAACAAACGRev Primer TTTGTGCTATTAGGGTTTGGCTrepeats (tg) x14 PCR product = 286 bp & start at base 268713)For Primer GTGTGTGTGTGTGTGTGTGTGARev Primer TTTGTGCTATTAGGGTTTGGCTrepeats (ga) x 16 PCR product = 201 bp & start at base 2690229. RD19 found in scaffold 00009 (query sequence starts at base 735401 on scaffold)1)For Primer CACACAAGACGTGTATGCAGAARev Primer CCCTCATTATGAAATGGCTATGrepeats (ag) x24 PCR product = 298 bp and start at base 6253762)For Primer TAGAGAATGCGATGGGAAATCTRev Primer GAACGGTAAATCTGACTCCGTCrepeats (ag) x12 PCR product = 168 bp and start at base 6818893)For Primer GAAACGAACAAGGAATAATCGCRev Primer TCTTCCTCCTTATTTAGCACGCrepeats (ct) x10 PCR product = 256 bp and start at base 69916230. RD21 found in scaffold 11065 (query sequence starts at base 489 on scaffold)1)For Primer TGAAATTGTGACAGCTTGTGTGRev Primer CATCACAAAGCCTATTGATTGCrepeats (at) x5 PCR product = 283 bp and start at base 172031. RD22 found in scaffold 10193 (query sequence starts at base 2435 on scaffold)1)For Primer TCCGTCAGTTCGTTTTATTGTG Rev Primer ATAACATCTGTTCTTGCAGCGArepeats (ac) x5 PCR product = 243 bp & start at base 300332. RD28 found in scaffold 00872 (query sequence starts at base 72552 on scaffold)1)For Primer AGAGGACACGAGAAATTTGGAARev Primer GAGGATCACCACTAGATCGGACrepeats (tc) x15 PCR product = 300 bp and start at base 824342)For Primer AGTTTTTGTTTGGAGCGAAGAGRev Primer ATTCGTACCCAAGTTTTTCTCGrepeats (ag) x13 PCR product = 266 bp and start at base 919703)For Primer TCTCGTTAACGTTTGCTCAATGRev Primer AAAGAGGGGAAAAGAAAGAAGGrepeats (ct) x12 PCR product = 203 bp and start at base 9303033. SAM1 found in scaffold 00742 (query sequence starts at base 28366 on scaffold)1)For Primer TGGGATCTATCGATCTAGGCTGRev Primer TGAAAACGAACCTGTAGACGAArepeats (tc) x8 PCR product = 238 bp and start at base 267202)For Primer AAATATTGCCGGCTTGTGATACRev Primer TTTTAAGTTCAACTAGGGTGGCrepeats (ct) x8 PCR product = 230 bp and start at base 424793)For Primer GCCTGGTAAAAGAAACTGCAAARev Primer GCGTAACATGTTTAGGTCGGTArepeats (ag) x25 PCR product = 299 bp and start at base 12139734. SAM3 found in scaffold 00014 (query sequence starts at base 424166 on scaffold)1)For Primer TTTGAGGAGAAGTCCAAAGGAGRev Primer GTTAAGTTTGGGCGACTGAGAGrepeats (tc) x15 PCR product = 297 bp and start at base 4276512)For Primer TTTTCAGTCTCGGACCCTTTTARev Primer TGCTAACCTCCACTCCAAAAATrepeats (taa) x22 PCR product = 259 bp and start at base 5202803)For Primer TGGGGACTCTCTTAGATGCAATRev Primer CCAAATACTTGGCCACCTTAGArepeats (ca) x20 PCR product = 282 bp and start at base 59097235. SC514found in scaffold 00065 (query sequence starts at base 272119 on scaffold)1)For Primer AAGTTATCTCTTCATGAGGGCGRev Primer ACCATCTTCAGATCCAGAAAGCrepeats (tc) x16 PCR product = 245 bp and start at base 2603032)For Primer TTGGGTAAAGAATGGAGATGCTRev Primer TTAAAGGTCATTTTCTCCCCCTrepeats (att) x18 PCR product = 161 bp and start at base 2693653)For Primer CCATACCCCATGATGATTTCTTRev Primer ACCTCTCTCTCCCATTTCATCArepeats (ga) x14 PCR product = 173 bp and start at base 27482236. SEH found in scaffold 00629 (query sequence starts at base 131051 on scaffold)1)For Primer CCAAGTAGTGGAACGAAACACARev Primer CGGGTTATGACTTATGAGGGAGrepeats (tc) x9 PCR product = 222 bp and start at base 1110632)For Primer GCGAAATCAACAAACTCAGTCARev Primer GCCGTGTAGGAATAGTACAGCCrepeats (ag) x7 PCR product = 187 bp and start at base 1244263)For Primer TCTCTTGTTCTTGGGTCGAACTRev Primer ATTTGTTGTGTGTTTGTCCTCGrepeats (tc) x17 PCR product = 147 bp and start at base 14459137. SOD2 found in scaffold 01664 (query sequence starts at base 5569 on scaffold)1)For Primer GGCAAAAGACAAAATTCGAGACRev Primer CTCTCTCTCTGCACCATTCCTTrepeats (ga) x9 PCR product = 187 bp and start at base 26532)For Primer CACTCAAATGTAAGTCGGTCCARev Primer TCTCCTCACTCCCAACTCTCTCrepeats (tc) x10 PCR product = 259 bp and start at base 250183)For Primer CGCTTTCTTACGATTTTTCTGGRev Primer CACCGCAACTACACTCATCAATrepeats (ag) x11 PCR product = 103 bp and start at base 2965838. SS1 found in scaffold 01481 (query sequence starts at base 27541 on scaffold)1)For Primer CCCACGAGAAGTCTCTCTCTCTRev Primer ACGAATCACAAGAAGGAGAAGGrepeats (tc) x10 PCR product = 239 bp and start at base 86592)For Primer TGCTCTTCCATTCTCTCCTCTCRev Primer CAACATGCTAAATGCCTAACCArepeats (tct) x14 PCR product = 275 bp and start at base 224233)For Primer GCTCCAAGCTTCTTGGTGTATTRev Primer GGGACTTTCACTCTTTGCATTCrepeats (ga) x12 PCR product = 261 bp and start at base 2659639. STZ found in scaffold 02547 (query sequence starts at base 19296 on scaffold)1)For Primer CCGTAGCGTTACTACCCAAAACRev Primer TTCGTGAGATGGTAGATGTTGCrepeats (ct) x5 PCR product = 149 bp and start at base 89792)For Primer GGTCTTCAGCAAAGTCTCCACTRev Primer AGACGTGGAGCACGTTTAATTTrepeats (ct) x5 PCR product = 157 bp and start at base 182033)For Primer ATTTTTGTCACCGCTCGTACTTRev Primer GAGCTGAAACAGCAGAGTAGCArepeats (ct) x5 PCR product = 263 bp and start at base 1914740. UBQ1 found in scaffold 12687(query sequence starts at base 335 on scaffold)1)For Primer TCGTTTGCTCTGGTACTCTCTGRev Primer CATATCCCCATCACTCCATTCTrepeats (taa) x4 PCR product = 173 bp and start at base 70541. ZFHD1 found in scaffold 00085 (query sequence starts at base 50788 on scaffold)1)For Primer TTTTTCGATTCTCCTCTCGTTGRev Primer TAGCCAAACTAGGCCTTAGCACrepeats (aat) x20 PCR product = 219 bp and start at base 432992)For Primer TATTCCTTAGGGGAACATGTGGRev Primer GCGAACCCATCTTCTACAAAACrepeats (ag) x7 PCR product = 136 bp and start at base 537903)For Primer TTCTGTGCACACCTGTTCTTTTRev Primer AGCGTATGAGAGAAATGCAAGArepeats (ttc) x13 PCR product = 247 bp and start at base 76563 |