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# **GenomeLab™ SNPstream® Genotyping System**

## **Autoprimer Tutorial**

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## Introduction

### 1.1 Autoprimer

Autoprimer (<http://www.autoprimer.com>) has guidelines and requirements for sequence content and format. The rule for minimum sequence length is mandatory, and it is highly recommended that other content guidelines be adhered to. The sequence format requirements are strict and must be followed exactly for successful primer design.

In addition to the sequence length, the content guidelines cover the sequence characteristics such as quality of the sequence data, and the GC content. There are also recommendations regarding BLASTing (Basic Local Alignment Sequence Tool) and masking of repeat/low complexity sequences.

If the user does not have the option of excluding a SNP with suboptimal characteristics, Autoprimer might still be able to design primers from the submitted sequence. However, certain deficiencies, such as insufficient number of bases surrounding the SNP locus, could result in failure to design the primers.

Once a sequence is qualified as a good candidate for the primer design, it must be formatted correctly for submission to Autoprimer.

This tutorial is a step-by-step guide to the primer design process. Italicized bullet points are from the SUPPORT section from <http://www.Autoprimer.com>. Some users may prefer a slightly different sequence of steps, i.e. performing repeat masking before BLASTing. It is important that all steps be performed, if possible.

#### Programming Tips

The Sections 6 and 7 provide tips on how to force Single Base Extension (SBE) primer design to one strand and how to use the *InSilico* PCR to check for PCR primer specificity.

#### URLs and Web References

The URLs and websites referenced in this tutorial are up-to-date at the time of print.

For updated information, send an email to [autoprimer-support@beckmancoulter.com](mailto:autoprimer-support@beckmancoulter.com), or contact your local Beckman Coulter Technical Support Representative.





## Autoprimer Design Process Tutorial

### 2.1 Sequence Content Guidelines

For this tutorial, panels will be designed from a test set of 70 human T/G SNPs obtained from the NCBI SNP database. The test sequences are shown in [Appendix A](#).

It is helpful to have the sequences in FASTA format for direct submission to BLAST and RepeatMasker. A description of the FASTA format is found in [www.ncbi.nlm.nih.gov/BLAST/fasta.shtml](http://www.ncbi.nlm.nih.gov/BLAST/fasta.shtml).

Briefly, the format requires:

- Single-line description preceded by ">"
- Lines of sequence data following the description line
- Standard IUPAC code
- Lower case letters
- A dash to indicate a gap of indeterminate length

**NOTE** Autoprimer does not accept dashes. Before submission to Autoprimer, dashes must be replaced by Ns.

#### Determine the Sequence Length

Verify that the sequence meets the minimum sequence length requirement. (Mandatory)

- *The sequence should have at least 150 bases flanking each side of the SNP for a minimum sequence length of 301 bases.*

**NOTE** The SNP locus (represented by the IUPAC code "K") has been highlighted to make inspection easier. Since one line of sequence comprises ~100 bases, a SNP should have at least one-and-a-half lines of sequence on either side to qualify.

#### Determine the Acceptable Sequence

Determine if the sequence has acceptable GC-content (Recommended).

- *GC-content should be between 40 and 65%.*

Check the region  $\pm 150$  bases surrounding the SNP locus. Highly GC-rich regions could yield primers with "sticky" 3'-ends that bind non-specifically to other regions of the genome, or form heterodimers with other primers, or self-dimerize. Conversely, highly AT-rich primers do not bind efficiently to the target sequence.

GC-content calculators are usually available in the web as part of oligonucleotide analysis software. An example of a website that does not restrict sequence length is <http://www.pitt.edu/~rsup/OligoCalc.html>. The analysis of ~300 bases surrounding the SNP in the first test sequence, rs36950, is shown in Figure 2.1.

Figure 2.1 GC Content Calculator

The screenshot shows a web browser window with the address bar containing <http://www.pitt.edu/~rsup/OligoCalc.html>. The page title is "Oligo Calculator". Below the title is a text input box labeled "Enter Oligo Sequence in Box" containing the following sequence: TCACCTCAGGACTAATTTTCTCCGCCTGCTTGTGTAAAAGA, TCTCTGCATTATTAATGGGTGTAACGCTAAAATAAAGCAT, CCTGGTGTGTTTACAGAGCAGCATCACAAATGAATGAAG, AGTGGAGCTTTAGGTTTACCACAAATGGGTTTTCTAGAG, TATCCAACCTGTTTCTGGGCCAT. Below the sequence box are several input fields: "Length" (296), "Melting Temperature (Tm)" (80 °C), "%GC content" (41), "Molecular Weight" (91350 daltons (g/M)), and "OD of 1 is equal to" (311 nanoMolar). A "Calculate" button is located below the input fields. The browser's status bar at the bottom indicates "Internet".

### Data Quality Score

Obtain a Sequence Data Quality Score (Recommended).

- *Sequence quality should have a score of PHRED 20 or higher (alternative quality measurements to PHRED can also be used).*

**NOTE** PHRED is a common DNA quality measurement. For more information, please visit the following website: <http://www.phrap.org>

The PHRED software assigns a quality score to each called base in a sequence, while PHRAP assigns a quality score to each position on sequence contigs based on the PHRED scores. The error probability is approximately  $10^{-Q/10}$ , where Q is the quality score. For example, a PHRAP score of 20 corresponds to 99% accuracy for a base in the assembled sequence. The sequence quality scores, as shown below, may be included in the Genbank reports.

Figure 2.2 Sequence Quality Score in Genbank Report

1 [AC025761](#). Reports Homo sapiens chro...[gi:12957685] Links

```

LOCUS          AC025761             112944 bp    DNA     linear   PRI 17-FEB-2001
DEFINITION    Homo sapiens chromosome 5 clone CTB-109C23, complete sequence.
ACCESSION     AC025761
VERSION       AC025761.5  GI:12957685
KEYWORDS      HTG.
SOURCE        Homo sapiens (human)
  ORGANISM    Homo sapiens
              Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
              Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
              Hominidae; Homo.
REFERENCE     1 (bases 1 to 112944)
  AUTHORS     DOE Joint Genome Institute and Stanford Human Genome Center.
  TITLE       Direct Submission
  JOURNAL     Unpublished
REFERENCE     2 (bases 1 to 112944)
  AUTHORS     DOE Joint Genome Institute.
  TITLE       Direct Submission
  JOURNAL     Submitted (14-MAR-2000) Production Sequencing Facility, DOE Joint
              Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA
REFERENCE     3 (bases 1 to 112944)
  AUTHORS     DOE Joint Genome Institute and Stanford Human Genome Center.
  TITLE       Direct Submission
  JOURNAL     Submitted (17-FEB-2001) DOE Joint Genome Institute, 2800 Mitchell
              Drive, Walnut Creek, CA 94598, USA
COMMENT       On Feb 17, 2001 this sequence version replaced gi:10305165.
              Draft Sequence Produced by DOE Joint Genome Institute
              www.jgi.doe.gov
              Finishing Completed at Stanford Human Genome Center
              www-shgc.stanford.edu
  1 → Quality: Phrap Quality >=40 99.8% of Sequence;
              Estimated Total Number of Errors is 0.2.
FEATURES             Location/Qualifiers
  source              1..112944
                    /organism="Homo sapiens"
                    /mol type="genomic DNA"

```

Legend

1 = Quality Score

## **BLAST Search**

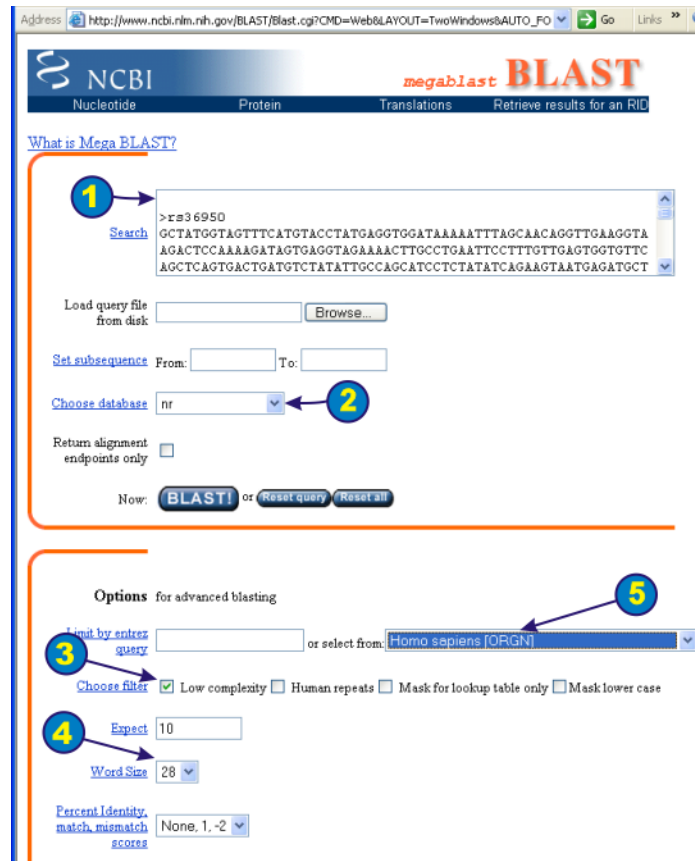
Perform a BLAST search. (Recommended)

- *You should BLAST your sequences to make sure they do not return High-Scoring Hits to more than one locus (e.g. pseudogenes).*

BLAST (Basic Local Alignment Sequence Tool; <http://www.ncbi.nlm.nih.gov/BLAST/>) compares nucleotide sequences to sequence databases and calculates the statistical significance of matches. There are several BLAST programs available. Megablast is suggested for performing quick searches for highly homologous sequences. Since the 70 test sequences are in a FASTA format, they may be submitted directly for BLASTing.

**NOTE** The Megablast accepts batch queries in a FASTA format.

Figure 2.3 Sequence Submission to BLAST



Legend:

1. Paste sequences in FASTA format in the **Search** field or browse to the query file.
2. Select **nr** (non-redundant) database. This database includes GenBank + EMBL + DDBJ + PDB sequences (but no EST, STS, GSS, or phase 0, 1, or 2 HTGS sequences). According to the NCBI, is no “no longer ‘non-redundant’ due to computational cost.”
3. Select **Low complexity filter** (default) to remove the low-complexity sequences that may cause artifactual hits.
4. Use default **Expect** and **Word Size** values.
5. Limit the search by specifying the organism.

Figure 2.4 Sequence Submission to BLAST, cont'd.

**Format**

Show  Graphical Overview  Linkout  Sequence Retrieval  NCBI.gov Alignment in HTML format

Masking Character: Default (X for protein, n for nucleotide) Masking Color: Black

1 Number of: Descriptions 100 Alignments 50

Alignment view: Pairwise

Start formatting from query #

Limit results by: [ ] or select from: Homo sapiens [ORGN]

Expect value range: [ ] [ ]

Layout: Two Windows Formatting options on page with results: None

Autoformat: Semi-auto

Results file

2 **BLAST!** or **Reset all**

Get the URL with preset values? **Get URL**

Legend:

1. Select **Pairwise** as the alignment view.
2. Click **BLAST!** to start the search.

Figure 2.5 Sequence Submission to BLAST, cont'd.

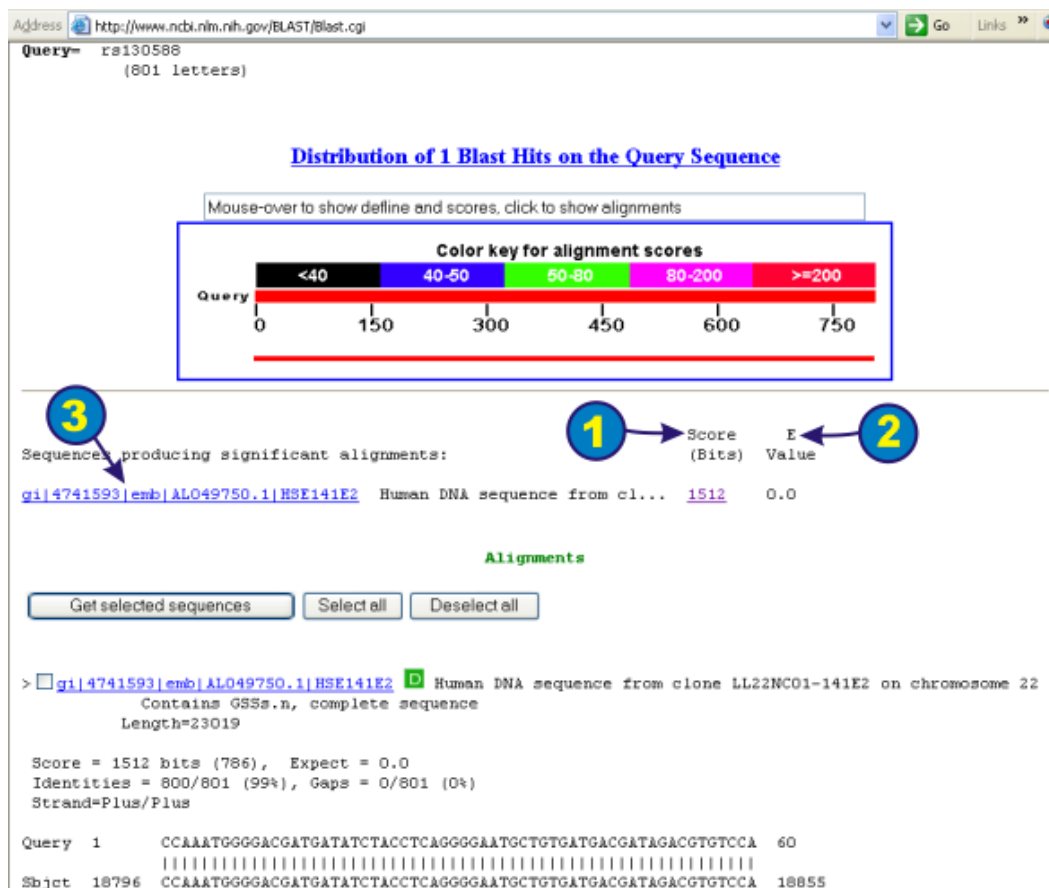


Legend:

1. A request ID is assigned to the query.
2. Click **Format!** to view the results.

Of the 70 test sequences submitted, 48 have single hits. The result for rs130588 is shown in the figure below.

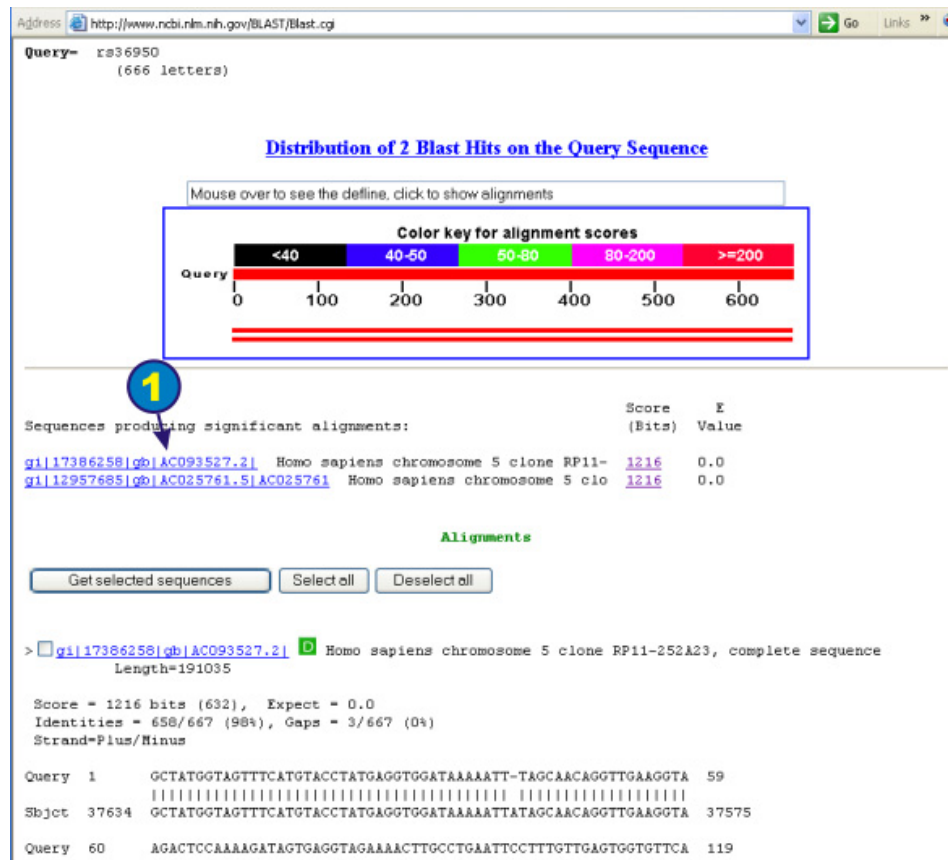
**Figure 2.6 BLAST Results: Single Hit**





Sequence rs36950 has two hits (a single hit on each of two distinct clones), both on chromosome 5, with the same score, as shown in the figure below.

**Figure 2.7 BLAST Results: Double Hit**



**Legend**

1. The Genbank accession number

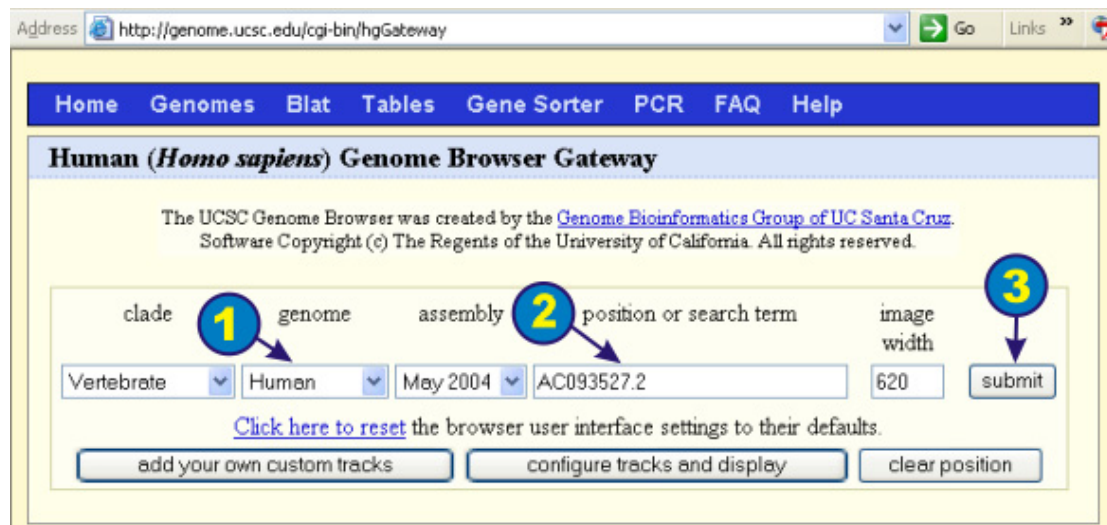
Are these hits overlapping and located in the same chromosomal position? In some cases, following the Genbank links and looking at the annotation for each clone does not give an unambiguous answer to this question.

It is possible to do a pairwise alignment of the two hits using Blast 2 Sequences (a program also available at the BLAST website) to determine how similar they are.

Another way to check the relationship between the two hits is to align them to the genome using the UCSC Genome Browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>).

In the case of rs36950, the two hits have Genbank accession numbers of AC093527.2 and AC025761.5. See the figure below.

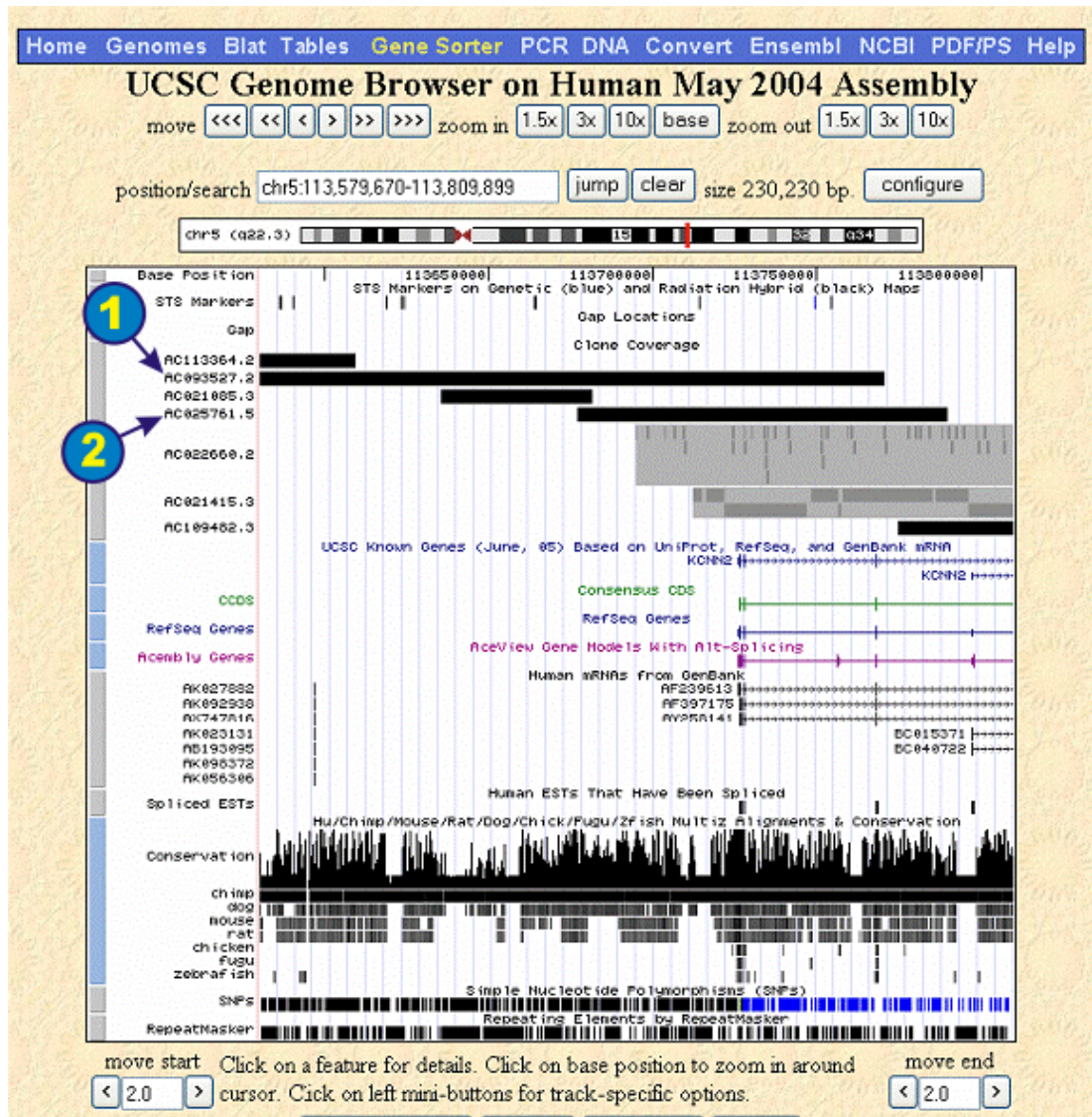
**Figure 2.8 UCSC Genome Browser Search**



Legend

1. Select the organism.
2. Enter the Genbank accession number of one of the two hits.
3. Click **Submit**.

Figure 2.9 Genome Browser Search Results



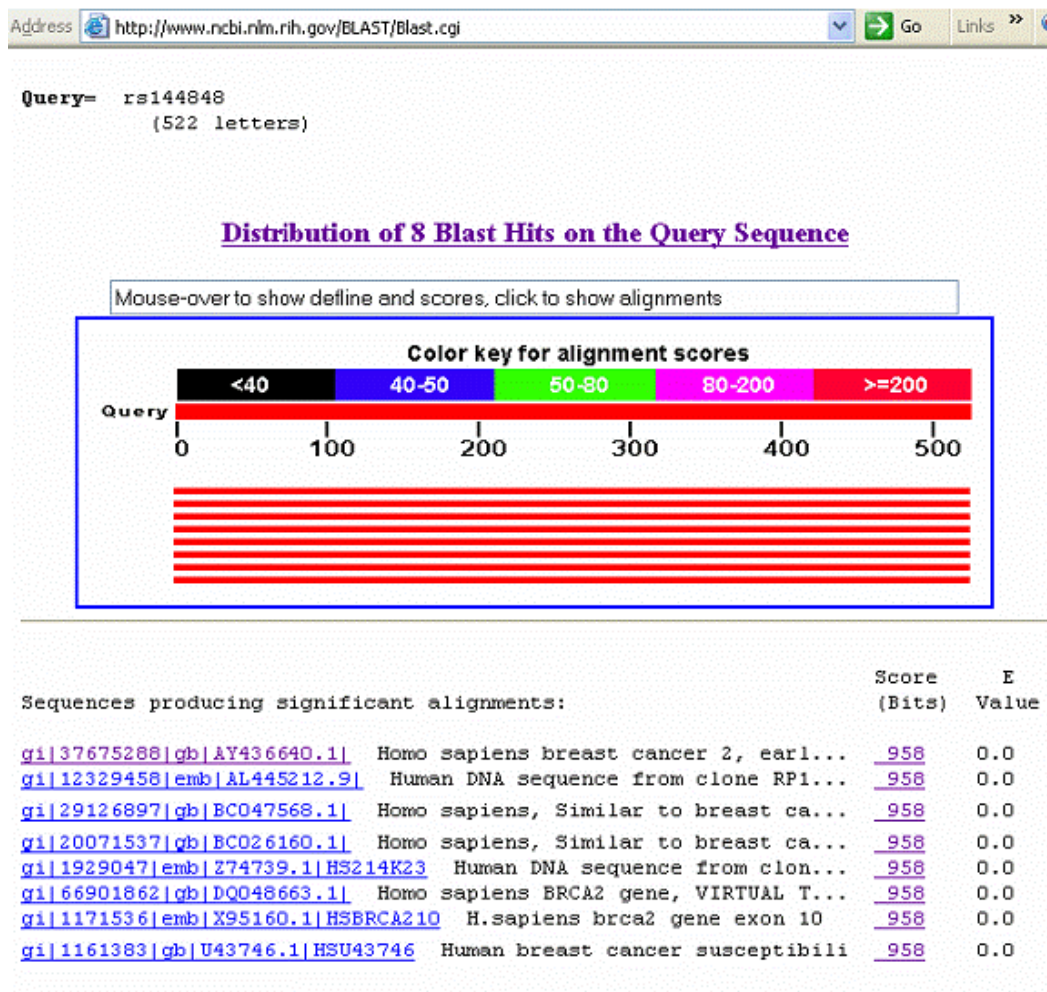
Legend

1. Hit #1, AC093527.2
2. Hit #2, AC025761.5

The results indicate that the two clones overlap, and suggest that there is in fact only one hit in chromosome 5.

**NOTE** This hypothesis may be checked by performing *InSilico* PCR with the Autoprimer-designed primers as shown in Section 7.

Figure 2.10 Blast Results: Multiple Hits



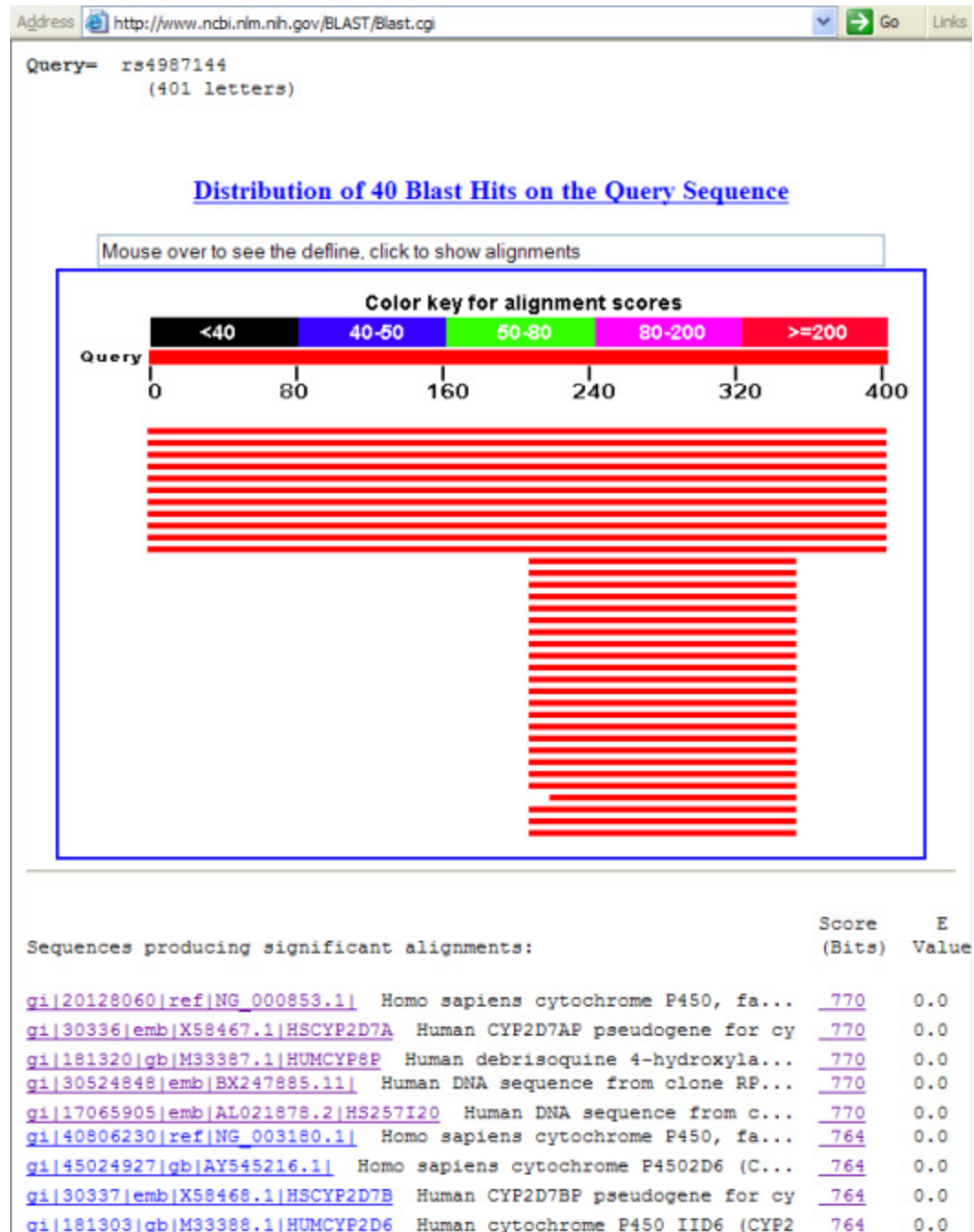
Most of the hits for rs144848 are clearly identified as the brca2 gene. Following the Genbank links on the other hits reveals that they are clones carrying the brca2 gene. Therefore, the multiple hits in BLAST are to the same locus. See Figure 2.10 above.

**NOTE** The alignments of the different hits may also be checked using the Gene Browser, as discussed in the previous example.

Other sequences in the test set with more than 2 high-scoring BLAST hits were analyzed using the Gene Browser. In most cases, the "multiple" hits turn out to be to the same locus. In a few cases, the accession numbers for one of the hits could not be found in the Gene Browser database. Again, primers designed for these latter sequences may be checked by *InSilico* PCR as shown in Section 7.



Figure 2.11 BLAST Results: True Multiple Hits



The first five hits have identical high scores. At least two hits map to distinct chromosomal regions, namely the CYP2D8P2 and CYP2D7AP genes. In this situation, additional sequence information is needed in order to design PCR primers that target less homologous regions, so that only the target SNP locus will be amplified. (The query sequence used in this example is not a member of the test set.)

## Mask Repeat Sequences

(Recommended)

- Repeat masking of the target sequence during primer design is highly recommended. If the repetitive elements are located outside 25 bases upstream or downstream of the SNP of interest, remove all repetitive elements by inserting N bases in their place. If the repetitive elements are located inside 25 bases upstream or downstream of the SNP of interest, **DO NOT** replace them with N bases.
- The Autoprimer needs both masked and unmasked sequences. The mask sequence indicates the positions to avoid when designing primers. The unmasked sequence gives the bases to use when calculating the energetics for primer design and when considering cross-hybridization.

## Repeat Masker

RepeatMasker screens DNA sequences for interspersed repeats and low complexity sequences. See Figure 2.12. The following information is from the RepeatMasker website (<http://www.RepeatMasker.org/>):

- Interspersed repeats:
  - Processed Pseudogenes, Retrotranscripts, SINES - Non-functional copies of RNA genes which have been reintegrated into the genome with the assistance of a reverse transcriptase
  - DNA Transposons
  - Retrovirus Retrotransposons
  - Non-Retrovirus Retrotransposons (LINES)
- Low complexity sequences:
  - Simple repeats (micro-satellites)
  - Poly-purine/poly-pyrimidine stretches
  - Regions of extremely high AT or GC

"The settings are very stringent, and we think that few if any sequences informative in database searches are masked as low-complexity DNA."

"A 100 bp stretch of DNA is masked when it is >87% AT or >89% GC, a 30 bp stretch has to contain 29 A/T (or GC) nucleotides."

The output is:

- a. A detailed annotation of the repeats that are present in the query sequence, and
- b. A modified version of the query sequence in which all the annotated repeats have been masked (default: replaced by Ns).

Figure 2.12 Sequence Submission to RepeatMasker

Legend

1. Paste the sequences in FASTA format; batch submissions are allowed.
2. Select **Links**, for the **Return Format**.

3. Select **Repetitive sequences replaced by strings of N**, for the **Masking Options**.
4. Select **Mask interspersed and simple repeats**, for the **Repeat Option**.

Figure 2.13 RepeatMasker Results



The screenshot shows a web browser window with the address bar displaying `http://www.repeatmasker.org/tmp/18633c761b69ec4d5bef76ae816ab300.html`. The main content area has a large heading: **RepeatMasker completed 27-Sep-2005 10:55:05 PDT**. Below this, it provides version information: `RepeatMasker version development-$Id: RepeatMasker,v 1.165 2005/05/04 19:55:18 rhubley Exp $` and the search engine used: `Crossmatch`. The file being analyzed is `/usr/local/rmsrver/tmp/RM2sequpload_1127843671`. The process log includes: `Checking for E. coli insertion elements`, a list of identification steps (simple repeats, full-length ALUs, interspersed repeats, etc.), `processing output:`, `cycle 1` through `cycle 4`, and `done`. System statistics are shown at the bottom: `66.09user 2.58system 1:08.72elapsed 99%CPU (0avgtext+0avgdata 0maxresident)k` and `0inputs+0outputs (10407major+115296minor)pagefaults 0swaps`. Below the log, there are sections for **Repeat Annotations:** (with a link to view annotations) and **Masked Sequence:** (with a link to view the masked sequence). A blue circle with the number '1' and an arrow points to the masked file link: `Masked file: RM2sequpload\_1127843671\_masked`. The **Summary:** section is partially visible at the bottom.

Legend:

1. Click on the link to open the results file.



Save the results as a .txt file. A portion of the file is shown below. The entire file is shown in [Appendix B](#).

**Figure 2.14 RepeatMasker Results (.txt)**

```
rs36950
GCTATGGTAGTTTCATGTACCTATGAGGTGGATAAAAAATTTAGCAACAGG
TTGAAGGTAAGACTCCAAAAGATAGTGAGGTAGAAAACTTGCCGTGAATTC
CTTTGTTGAGTGGTGTTCAGCTCAGTGACTGATGTCTATATTGCCAGCAT
CCTCTATATCAGAAAGTAATGAGATGCTCAGTCCCCTTTGACTTTGTGTATA
CACTGTAGTAACGAAATCACTCAGGACTAATTTTCTCCGCCCTGTGTGT
AAAGATCTCTGCATTTAATGGGTGTAACGCTAAAATAAAGCKATCCTGG
TGTGTTTACAGAGCAGCATCACAAATGAATGAAGAGTGGAGCTTTAGGTT
TACCACAAATGGGTTTTCTAGAGTATCCAACCTGTTTTCTGGGCCATGTTT
TGTACAGTTGAGAGATAAGGGCACTGTGGGCTGTACCCCAAACCACGTT
TGGCAGGGAGCACCAAGGCAGCCTGCGTTGATTTAACTTCAGAGAAGTTA
TATTCAATTANGATAGGAAGTGGTTTTGGGGGGTGTTTTAAAGAGAAAT
TTCTACTCATTAAAGAAATAATAATAATTTTGGCTACTTTGGGAGTACATTT
TCAACCTGAAACTCCTTAATAATTCTATTTATAAAAAC TAGAAAAACACATG
GNAGGGATCTTAATTA
>rs130588
CCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCC
CAGCACCAAGGGGAAATAAGAAAAAGCAAAGAAAGGGAAAGTGTCTGGTATAA
AGGGAAAGGAAGGAAACC AAGGGCATCAGTGTGGATGGGGAAAGGACAAAA
TGCTCTCCTGGGGAATCACAACTTTGTATTATCAAGTGTCTTTTTTTGAA
TTCAAGCCATGCTATTTCTTGCAGCTCTGACTATGTTTTCAAGTTGGAATA
TCAAGCATCTCTGAGCCTTCACTACAGCAATTAAGGGCTCATAAAGCTAT
TCTTAGGAGTACAAAAATAAACTGAGATATATTTGAGGTTGCCAGAAAGTTT
KCCATGTGGAGAACTGAGTGTCTAACCCTGGAAGGTGCACATTAAGAC
AGCAGGTGTGCAGAAATGCTGTGGCCATAACTCTCTCCAGCTGAGGGCAGA
GGCCACAAAAAGGACCTGCTGAGGGGAGGCTGCCAAGGAGCTCCACACT
CTGCCAAGAGGGGCTGAGTTCTGGCGCCATCAGCAACTGTCTTGNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATCAACTAA
AGCCTGCAGATTTCTTCCACTGCCCCCATGGTGGCCATCTGTTGAGGTGGG
AGGGAAACATGCTGGCCCTCCCGTCTCTACCCTAAAGAAGACACTGTG
C
>rs144848
GAATTCCTGAAGTAAGAAAATCTTTCTTTCTTTTGTCTCTGTGTCTAA
TAGGTCTTTTCTGAAATATTTTGGTCACATGAAGAAATATGCAATAGGG
GTATTTTCTCCATCTGGGCTCCATTTAGACCTGAAAGGGTTAGTTGAGAC
CATTACAGGCCAAAGACGGTACAACCTTCTGGAGATTTTGTCACTTCC
ACTCTCAAAGGGCTTCTGATKTGCTACATTTGAATCTAATGGATCAGTAT
CATTTGGTTCCACTTCAGATACAAATGAGTATTTTTCTTTCACTTTGGTTT
TTAGATTTTTTACATTCATCAGCGTTTTGCTTCATGGAAAATTTTTTTTCT
AGTCTTGCTAGTTCTTACTTTTTGTAGATTTTTTGTCTACATTTAGAAA
AACATAATGAAAACTATCTTCTTCCAGAGGTATCTACAACGTTTTCATAG
ACTTCATCTTCTAGGACATTTGGCATTGACTTTCCACGTGGTCTTTGCA
GCTATTTACTTTAAATGAATTC
>rs187031
```

Next, delete the sequences that were not masked by the RepeatMasker. The resulting file is shown in [Appendix C](#).

Inspect the masked sequences. Make sure that there are at least 25 unmasked bases upstream and 25 unmasked bases downstream of the SNP. An easy way to do this is by **Edit | Find | K** (the SNP type for the test sequences). The following test sequences fail.



Figure 2.17 Example 3

```
>rs727023
GGATCTCAAAAAATGTGGAGGTTGCATTCAATTACATTTGTGAATGTTAG
GAAACTGTCAACTGCCTTTGGAGTCCAAGGAGAGAGCCAGCAGACACCCT
TCTCACCACCCTGGGCTTTTCATGAGAGTGGCACTGCAATATCATCATCC
TAGCTTAGGGGAATTKGTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNCACACTGCTTCCCTTCTCTCCGACAGGTTCTCTGGAGA
CAAAGGAAAAGGGATATCTGGTGAGGCAGTAGAGGTCTAGTTCATCCACT
TAGAGATCATAGAGTCCTCCAGGTCGCAGGACTGGATGCTGAGATCC
```

- Example 3: In Figure 2.17, there are less than 25 unmasked bases downstream of the SNP. If this is a must-have SNP, modify the masked sequence before submission to the Autoprimer by restoring the original 25 bases downstream of the SNP. This is demonstrated on Section 3 on page 12.



## Sequence Formatting Requirements

### 3.1 Transfer Requirements

The following are steps for transferring the sequence information, creating a .TXT file and the punctuation exceptions for use in the sequence.

- *Transfer the sequence information, one sequence per line, into the Notepad file. Be sure that no carriage returns are present within the sequence. Enter the SNP identifier/name at the beginning of the line and place one space between the identifier/name and the sequence. Sequence names should be restricted to the use of Alphanumeric characters with the following punctuation exceptions: Periods(.), dashes(-) and underscores(\_). The sequence name should be 20 characters or less.*

**NOTE** Save the file with the .txt extension.

- *Masking*
  - *The format for a sequence to be masked is:*  
*[SNP Name][whitespace][SNP sequence][whitespace][Mask sequence]*
  - *Entries that aren't to be masked only contain:*  
*[SNP Name][whitespace][SNP sequence]*
  - *Whitespace is any contiguous series of spaces and/or tab characters.*
  - *[Mask sequence] is the same length as the corresponding [SNP sequence].*
- *Sequence annotation*
  - *The SNP to be designed for should be represented by the two base types separated by a forward slash and encased in brackets (i.e. a T to C SNP would be represented as [T/C] in the sequence for ...ATGGATCTTTATT[T/C]TCTTATCTA...*
  - *Other SNPs located in the sequence are represented with the following single-letter annotation:*
    - *R = G or A*
    - *K = G or T*
    - *S = G or C*
    - *W = A or T*
    - *M = A or C*
    - *Y = T or C*
    - *D = G or A or T*
    - *V = G or A or C*
    - *B = G or T or C*
    - *H = A or T or C*
    - *N = G or A or T or C*
  - *Other sequence variations (e.g. insertion/deletions, repeat elements, etc.) should be annotated by an uppercase n: N.*

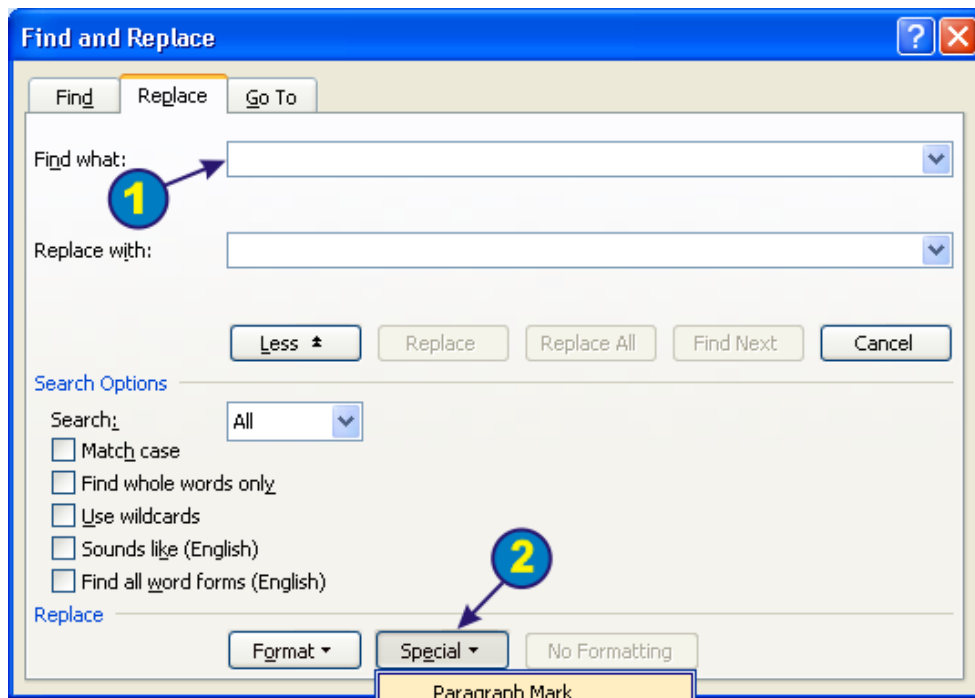


- Go to **Edit | Replace** and perform the following operations:

Function:	Find:	Replace with:	Effect:
Operation 1:	^p (paragraph mark)	(leave blank)	This removes all line breaks in the document.
Operation 2:	(initial character of the sequence name)	^p>	This will start each sequence, beginning with the sequence name, on a new line.

Use the **Find and Replace (CTL+F)** function box to perform operations 1 and 2 as shown below.

**Figure 3.2 Find and Replace Dialog Box**



**Legend**

- Place the cursor on the **Find what** drop-down field.
- Click **Special** and select ¶ **Paragraph Mark** from the drop-down menu. The ¶ symbol displays in the **Find what** drop-down combo box.

**NOTE** For operation 2, substitute the input as described above.

A portion of the reformatted file is shown in Figure 3.3. The entire file is shown in [Appendix E](#).



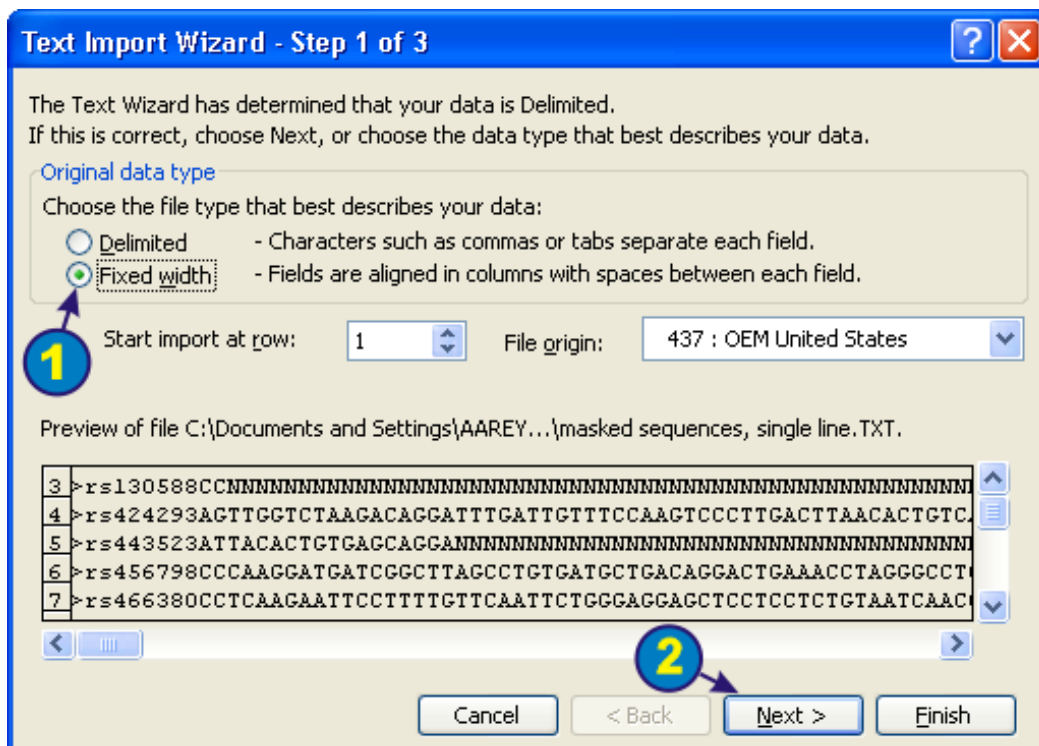




Next, insert a tab between the sequence name and the sequence. Do this manually for files containing a few sequences. For bigger files, open Microsoft Excel.

On the main menu, select **File | Open** and browse to the .txt file. The Text Import Wizard dialog box displays, as shown in the figures below.

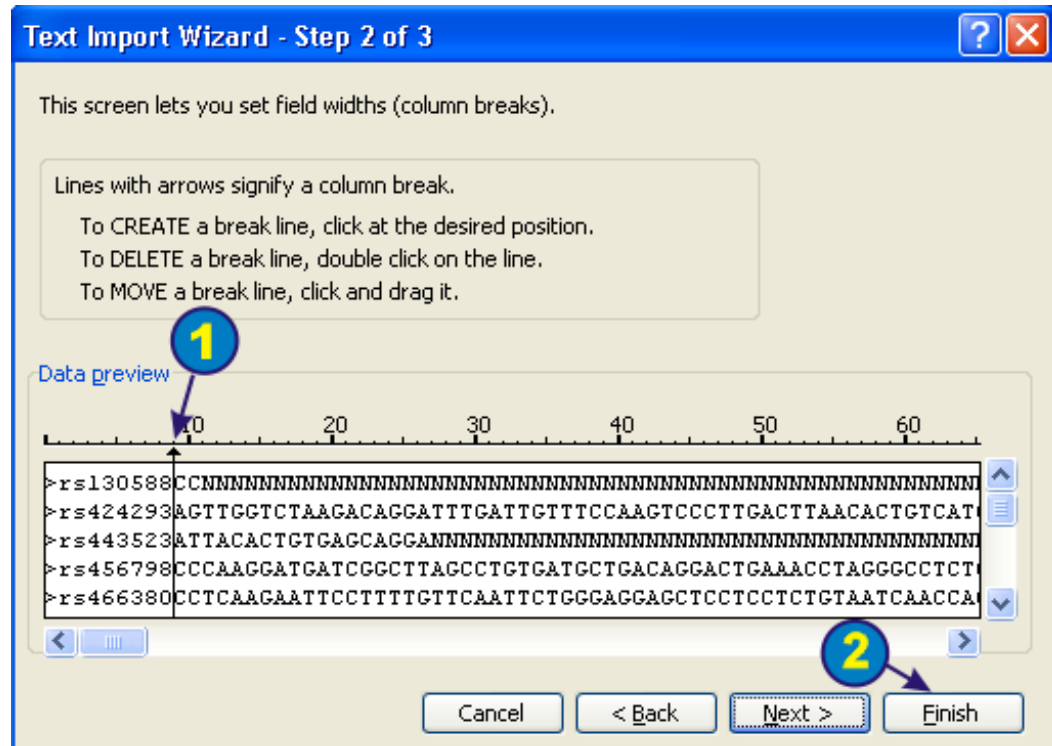
**Figure 3.5 Text Import Wizard Box: Step 1**



Legend:

1. Select **Fixed width**.
2. Click **Next**.

Figure 3.6 Text Import Wizard Box: Step 2



**Legend:**

1. Create a break line after the SNP name. If the SNP names vary in length, some sequence characters (bases) will end up in the name column, or vice-versa. Make corrections as necessary after saving the .xls file.
2. Click **Finish**.



A sample of the original sequence file saved as an .xls file, is shown in Figure 3.8 below. The entire file is shown in [Appendix H](#).

**Figure 3.8 Original Sequences, Single Line (.xls)**

```
>rs36950 GCTATGGTAGTTTCATGTACCTATGAGGTGGATA,AAAATTTAGCAACAGGTTGAAGGTAAGACTCC,
>rs130588 CCAAATGGGGACGATGATATCTACCTCAGGGGAATGCTGTGATGACGATAGACGTGTCCAGCAC/
>rs144848 GAATTCCTGAAAGT,AGAAAATCTTTCTTTCTTTTGTCTCTGTGTCTAATAGGCTTTTTCTGAAATAT
>rs187031 AGAAAGAAAGAAAA,AAACCCCTGCTGATTATGTATGCAAAAGTCGCAGCACGGTGCTAGAGTGCT
>rs277939 AGAGAAGTTGGCTCTTGC TTA,AAATCCAACACTTATCTTATGGGGAGAAAGCCATGGTTTACC TCT
>rs288422 GAATTCACCTTTTATT TTAGATAGAATGGCAGANAGTCTA,AGTAGAAGCAGGGCTTTGTGTATTCTGT/
>rs309133 CATAACATCCTGATGTCCCTGGCTTACTTCTATTTGGGAGAGGGGCCCACTCGTTCTCATTTTATG/
>rs309773 TCTTCCTTCAGGCTAGGTGACTGCA,AAATCCTCTAGAACTGCTATAGCCACATGTTTATA,ATAAAC,
>rs380607 AAGAGTGACACAGTCTCCATCAAGCTCTGGAGCCATGTAGTCATGGCAGAGCAAAGAGTATTTAG
>rs382515 AGCTAGAA,TACCCACAAGTGGCTTCACTCTGAGCTTCAGTTTATCTACTGTTTATGGCCCTTAGAA/
>rs383885 AGAAGAAA,ACTGATTCTTTGCAGGACATTGTGGGTGAGTTTCTGCCAAAA,ATAAATATCAATATTT'
>rs424293 AGTTGGTCTAAGACAGGATTTGATTGTTTCC AAGTCCCTTGACTTAACACTGTCATCTTATAACGCTT
>rs443523 ATTACACTGTGAGCAGGACA,AGATAAAGACCTTTACCTTCATTTATAACTGGGGAAATTGAGGCTC
>rs456798 CCCAAGGATGATCGGCTTAGCC TGTGATGCTGACAGGACTGAAACCTAGGGCCTCTGCCCTAAA
>rs466380 CCTCA,AGAATTCCTTTTGTTC,AAATCTGGGAGGAGCTCCTCCTCTGTAA,TCACCAGATTAGCATGG
>rs470490 TCGCTTTGATA,TGTAAGCTTGTGATCCCCTTTTACTTTTTTTTTTTTTTTTAGATGGAGTCTCGCTCG
>rs554653 GGAATTTCCATTACAGCATATAGCA,TTCTCCTATGAAGAAACCCCTCGCTCAGTGACTTACACG/
>rs615474 CTGGAGAGCA,AGCCACTCCAGCCCCACCCAGCCATCCTGTGGTTCTGAGGGCCTCCTCAAGGC'
>rs638250 GGATCTCTTGT,TTGCAATTGCTCTAATCTCTCAAGA,ATTATGAAGTGAGGTA,CTTCA,TTCTGCAGGGC
>rs713075 TCGTCGGAGGA,ATGCATAATATAAGAGTCGGTGGGCTAGTCTTTAGCCAGATGCCCTTCTGTAGAT
>rs713478 GGGGATCTCA,AGATCACATC,AAAATTA,AAACAGCCTCCA,ACTATTGCCACAATCTTAGAAGAA,ATC
>rs713538 AAATA,TTTTGTGTAGTTA,ATGTAAGGATACTCAGACAGGAAA,ATAAACACAGTTATTTTTAGAAAG/
>rs713718 CTTATA,AAAAATATTGTGCATTTAATACCTTTTGGTTTCTTTTATACTTGTCAAAAAGTCATTTATACAT/
>rs715755 TCTCCCACCAGGACCATC,TTCCCCGTTGTCTCTCTCTCTCCTTACCAGGACTGCAGGTC,ACTGCT
```

- Combine the original and masked sequences into one file.  
Copy the masked sequences from the single line .xls file (Figure 3.7 on page 8 and [Appendix G](#)), and append to the end of the original sequence .xls file. See Figure 3.8 on this page and [Appendix H](#).





Next, convert the .xls file into a .txt file for better alignment viewing. In Notepad, select **Format** and uncheck **Wordwrap**, to remove the wordwrap formatting. A portion of the file is shown below. The entire file is shown in [Appendix J](#).

**Figure 3.10 Original and Masked Sequences, Each in Single Line (.txt)**

```

>rs130588      CCAATGGGGACGATGATATCTACCTCAGGGGAATGCTGTGATGACGATAGACGTGTCCAGCACACAGTAAC
>rs130588      CCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>rs144848      GAATTCCTGAAAGTAAAGAAAATCTTTCTTTCTTTTGTCTCTGTGCTAATAGGCTTTTTCTGAAATATTT
>rs187031      AGAAAGAAAGAAAAAACCCTGCTGATTATGTATGCAAAAGTCGCAGCACGGTGTAGAGTGTAGGCACA
>rs277939      AGAGAAGTTGGCTCTTGCTTAAATCCAACACTTATCTTATGGGGAGAAAGCCATGGTTTACCTCTTTGGT
>rs288422      GAATTCACCTTTTTATTTGATAGAATGGCAGANAGTCTAAGTAGAAGCAGGGCTTTGTGTATTCTGTAAAGT
>rs309133      CATAACATCCTGATGTCCTTGGCTTACTTCTATTTGGGAGAGGGGGCCCACTCGTTCTCATTTTATGAGACT
>rs309773      TCTTCTTCAGGCTAGGTGACTGCAAACTCCCTAGAACTGCTATAGCCACATGTTTATAATAAACATTCA
>rs36950       GCTATGGTAGTTTCATGTACCATGAGGTGGATAAAAATTTAGCAACAGGTTGAAGGTAAGACTCCAAAAGA
>rs380607      AAGAGTGACACAGTCTCCATCAAGCTCTGGAGCCATGTAGTATGGCAGAGCAAAAGAGTATTTAGTCACTG
>rs382515      AGCTAGAATACCCACAAGTGGCTTCACTCTGAGCTTCAGTTTATTCTACTGTTTATGGCCCTTAGAACACTG
>rs383885      AGAAGAAAACGATTCTTTCAGGACATTTGTGGGTGAGTTTCTGCCAAAATAAATATCAATATTTTCTT
>rs424293      AGTTGGTCTAAGACAGGATTTGATTGTTTCCAAGTCCCTTGACTTAACTGTCACTTTATAACGCCTTCCA
>rs424293      AGTTGGTCTAAGACAGGATTTGATTGTTTCCAAGTCCCTTGACTTAACTGTCACTTTATAACGCCTTCCA
>rs443523      ATTACACTGTGAGCAGGACAAGATAAAGACCTTTACCTTCATTTTATAACTGGGGAAATTGAGGCTCTGAGG
>rs443523      ATTACACTGTGAGCAGGANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>rs456798      CCCAAGGATGATCGGCTTAGCCTGTGATGCTGACAGGACTGAAACCTAGGGCCCTCGCCTCAAATCTCAGT
>rs456798      CCCAAGGATGATCGGCTTAGCCTGTGATGCTGACAGGACTGAAACCTAGGGCCCTCGCCTCAAATCTCAGT
>rs466380      CCTCAAGAATTCCTTTGTTCAATTCTGGGAGGAGCTCCTCCTCTGTAATCAACCAGATTAGCATGGACCTC
>rs466380      CCTCAAGAATTCCTTTGTTCAATTCTGGGAGGAGCTCCTCCTCTGTAATCAACCAGATTAGCATGGACCTC
>rs470490      TCGCTTTGATATGTAAGCTTGTGATCCCTTTTACTTTTTTTTTTTTTTTAGATGGAGTCTCGCTCTGT
>rs470490      TCGCTTTGATATGTAAGCTTGTGATCCCTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
GGAATTTCCATTACAGCATATAGCATTCTGCTCATGAAGAAAACCCCTCGCTCAGTGACTTACACGAAAAAC
>rs554653      CTGGAGAGCAAGCCACTCCAGCCCCACCCAGCCATCTGTGGTCTGAGGGCTCCTCAAGGCTATAGGGA
GGATCTCTTGTGTTGCAATTGCTCAATCTCAAGAATTATGAAGTGAAGTACTTCACTTGCAGGGGGAAA
>rs615474      TCGTCGGAGGAATGCATAAATAAAGAGTCGGTGGGCTAGTCTTTAGCCAGATGCCCTTCTGTAGATCCTGAG
GGGATCTCAAGATCACATCAAATTAAGCAGCCCAACTATTGCCAATCTTAGAAGAAATCCCACTT
>rs638250      AAATATTTTGTGTAGTTAATGAAGGATCACTCAGACAGGAAAAAACAACAGTATTTTTAGAAAGATTTCT
CTTAAAAAATTTGTGCATTTAATACCTTTTTGGTTTCTTTTATACTTGTCAAAAGTCAATTTATACATAA
>rs713075      TCTCCACCCAGGACCATCTCCCCGGTGTGCTCCTCTCTCCTTACCAGGACTGCAGGTCAGCTGCAGAA
TCTAGATAGAAGTTAAAACACCTCACTGGCTCGAGGAGGAGGAGAGGAGGAGGTTTCAATTTCAAG
>rs713478      CCTGGTTGTTGATATTTATAGTCTCTTTCCCTGGGCTGGTCAAATTTCCCAAGAAAGATATCTTCAATCTC
>rs713538      CCTGGTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>rs713718
>rs715755
>rs717207
>rs717292
>rs717292

```

Make modifications to the masked sequences as necessary.

- Example 1. As shown in Figure 2.17 on page 19, rs727023 after repeat masking does not have the minimum 25 bases downstream of the SNP required for primer design. Assuming this is a must-have SNP, copy 25 bases from the unmasked sequence and replace the corresponding Ns in the masked sequence.

In the figures below, note that the top sequence is changed to the bottom sequence.

```

ATCCTAGCTTAGGGGAATTKGTGTGCAATAAGCAAGAGGCAGGACTTGGGAGTCAGGAAGGTTTGAATCCT
ATCCTAGCTTAGGGGAATTKGTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

```

```

ATCCTAGCTTAGGGGAATTKGTGTGCAATAAGCAAGAGGCAGGACTTGGGAGTCAGGAAGGTTTGAATCCT
ATCCTAGCTTAGGGGAATTKGTGTGCAATAAGCAAGAGGCAGGACNNNNNNNNNNNNNNNNNNNNNNNNNN

```









Finally, convert the .xls file into a .txt file for submission to the Autoprimer. A portion of the file is shown in Figure 3.13 below. The entire file is shown in [Appendix N](#).

**Figure 3.13 Original and Masked Sequences, Single Line (.txt)**

```

>r130588      CCAATGGGGACGATGATATCTACCTCAGGGGAATGCTGTGATGACGATAGACGTGCCAGCACAGTAACTATTCAGT
TGGGGAATCAACCTTTGATTATCAAGTGC TTTTGAATCAAGCCATGCTATTTCTTGCAGCTC TGACTATGTTCAAGTTGGAATCA/

>r144848      GAATTCCTGAAGTAAGAAAATCTTCTTCTTTCTTTGTTCTCTGTGTC TAATAGGTC TTTTCTGAAATATTTTGGTCAC/
AGAAAGAAAGAAAAAACCCCTGCTGATTATGTATGCAAAAAGTCGCAGCACGGTGC TAGAGTGTAGGCACATCATCAGT
>r187031      AGAGAAGTTGGCTCTTGC TAAATTC AACACTTATTCCTATGGGGAGAAAGCCATGGTTTACCTCTTTGGTTTGTAC/
>r277939      GAATTCACCTTTTATTTTGTAGAATGGCAGANAGTCTAAGTAGAAGCAGGGCTTTGTGATTCGTAAAAGTTAACCTAC/
>r288422      CATAACATCCTGATGTCCCTGGCTTACTTCTATTTGGGAGAGGGGGCCCACTCGTTCATATTTATGAGACTATAACTTI
>r309133      TCTTCCCTCAGGCTAGGTGACTGCAAACTCCCTAGAAAATGCTATAGCCACATGTTTATAAATAAACATTCAAGGCCAGT
>r309773      GC TATGGTAGTTTATGTACC TATGAGTGGATAAAAAATTTAGCAACAGTTGAAGGTAAAGACTCCAAAAGATAGTGAGC
>r36950       AAGAGTGACACAGTCTCCATCAAGCTCTGGAGCCATGTAGTCATGGCAGAGCAAGAGATTTAGCTC ACTGGGATTC/
>r380607      AGCTAGAATACCCAC AAGTGGCTTCACTCTGAGCTTCAGTTTATCTACTGTTTATGGCCCTTAGAACACTGGCATAAGC
>r382515      AGAAGAAAACCTGATCTTTG CAGGACATTGTGGGTGAGTTTCTGCCAAAAATAAATTATCAATATTTTCC TTGCACAAT/
>r383885      AGTTGGTCTAAGACAGGATTTGATTGTTTCCAAGTCCCTTGACTTAACAC TGTCATCTTATAACGC TTTCCAATTCCCA/
>r424293      AGGTTGTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTAGAAACATATGAGAGATGATT

>r443523      ATTACACTGTGAGCAGGACAAGATAAAGACC TTTACCTCATTTTATAAC TGGGGAAATTGAGGCTCTGAGGGGTTAAGT
TCTGAAAATAATCTAGAATTC

>r456798      CCCAAGGATGATCGGCTTAGCCTGTGATGCTGACAGGACTGAAACCTAGGGCTCTGCCTCTAAATCTCAGTTCAATTTG/
>r466380      CCTCAAGAATTC TTTTGTCAATCTGGGAGGAGCTCCTCTCTGTAATCAACAGATTAGCATGGACTCTATGTTTC
GCCCTGCAAAAGGAATCCATAGCCTTTGAAATCACATGTGTTACTACTAGGATCCAAGGCTCCTGCAAAATTC AAGACAAAACCAACCCCTAG/

>r470490      TC GCTTTGATATGTAAGCTTGTGATCCCC TTTTACTTTTTTTTTTTTTTTTATAGTGGAGTCTCGCTCTGTTGCCCGGC
GTTAAGAATATTTTCTAGAAGAAAACACAGTAAGGAAATGGGGAAAGTTGAAGATGCCAAACAAAGGTAATCTTCAGGTAATGTGTAGCCTCTC

>r554653      GGAATTTCCATTACAGCATATAGCATTCTGCTTATGAAGAAACCCCTCGCTCAGTGACTTACACGAAAAAC TGGGGTAT
>r615474      CTGGAGAGCAAGCCACTCCAGCCCCACCCAGCCATCCTGTGGTTCTGAGGGCCCTCTCAAGGCTATAGGGATACCAGAC
>r638250      GGATCTTTGTTTGC AATTGCTCTAATCTCAAGAATTATGAAGTGAAGTACTTCAATCTCAGGGGGAAAAAAGAAAGC
>r713075      TCGTCGGAGGAATGCATAATAAAGAGTGGTGGGCTAGTCTTTAGCCAGATGCCCTTCTGTAGACTCTGAGTCCCAAA1
>r713478      GGGGATCTCAAGATCACATCAAAATTAACAGCCCTCAACTATTGCCACAATCTTAGAAGAAATCCCACTTACTACTC/
>r713538      AAAATATTTTGTGTAGTTAATGTAAGGATACTCAGACAGGAAAAATAAACACAGTTATTTTTTAGAAAAGATTTCTCCACA1
>r713718      CTTATAAAAAATTTGTGATTTAATACCTTTTGGTTTCTTTTATACTTTGCAAAAAGTCAATTTATACATAAAATGACA1
>r715755      TCTCCACCAGGACCATCTCCCCCGTTGCTCCCTCTCTCCCTCACAGGACTGCAGGTCACTGCTCAGAACCATCATC
>r717207      TCTAGATAGAAGTTAAAACCCCTCACTCTGGCTC GAGGAGGAGGGAGAAGGAGGCAGGGTTTCATTTCAAGATCAGTC/

```

**Sequence Formatting Requirements**  
*Transfer Requirements*

## Sequence Submission

### 4.1 Sequence Submission to the Autoprimer

Figure 4.1 Autoprimer File Confirm Page

The screenshot shows the 'File Confirm Page' on the Autoprimer website. The page is divided into five numbered steps:

- Step 1:** A dropdown menu is set to 'SNPstream 12plex'. Below it is the text '- Product to Design Primers'.
- Step 2:** A text box contains 'C:\Documents and S...', followed by 'Browse...' and 'Submit File' buttons. Above the text box is the instruction: 'Step 2: Select file to process and submit. For help in creating a file, click here. To "cut and paste" sequences, click here.'
- Step 3:** A text area titled 'Confirm Data:' contains a list of sequence identifiers and their corresponding DNA sequences, such as '>rs130588 CCAAATGGGGACGATGATATCTACCTCAGGGGAATGCTG'. A blue arrow points to the first line of the list.
- Step 4:** A text box contains the word 'tutorial', followed by '- Optional comment about the Primers or file (30 characters max)'.
- Step 5:** A 'Design Primers' button.

Legend:

1. Select SNPstream 12plex or 48plex.
2. Browse to the sequence file then click **Submit File**. If no platform is selected in Step 1, the following error message displays: "You entered too many sequences. Only the first will be processed."
3. Confirm the sequence data.
4. Add any optional comments.
5. Click **Design Primers**.

**Figure 4.2 Autoprimer Results**

Address: [https://www.autoprimer.com/primer\\_app/process.asp](https://www.autoprimer.com/primer_app/process.asp)

home support design password

**Results**

**Application Options**

[Print Page](#)   [Run New Design](#)   [Download Matching Text File](#)   [Back to Home](#)

**Primers Designed For:**  
 SNPstream Customer  
 Senior Scientist  
 BCI  
 D25B  
 4300 N. Harbor Blvd  
 Fullerton, CA 92834  
 Telephone: 7149923669  
 Facsimile: 7149923669

**General Info:**  
 Submission Date: 10/13/2005  
 Primers Designed for SNPstream 12plex Kits  
 Comments: tutorial

**References:**

- Oligo's direction: 5' to 3'

SNP Name- Probe Name	SNP Type	Primer Type- Mod.	Amplicon Length	Sequence
>rs763342				No Primers could be found for this sequence...
>rs754257				No Primers could be found for this sequence...
>rs727678				No Primers could be found for this sequence...
>rs727023				No Primers could be found for this sequence...
>rs717292				No Primers could be found for this sequence...
>rs721580				No Primers could be found for this sequence...
Panel # 1				
>rs723651- U4	CA	PCR PCR SNPL	152	TCATGAGACTCCCAGAAAG ATTACTTCCCTTCAGATAGAGGTATCT AGCGATCTGCGAGACCGTATGTGGCATTCTTGACCTGGTTGAAC
>rs380607- U9	CA	PCR PCR	141	GGATTGGAATTCAGCATCG AGAAAATGTAGAGAGGCAGGATC

Legend:

1. The sequences for which the Autoprimer could not design primers.

The Autoprimer designed a total of six panels:

- four 12plex panels with extension type G/T (Panels #2, 3, 4, 5),
- one 10plex G/T (Panel #6)
- one 6plex C/A (Panel #1)

A portion of the results file is shown below.

**Figure 4.3 Autoprimer Results, cont'd.**

Address					http://www.autoprimer.com/primer_app/process.asp				
Panel # 1									
>rs723651-U4	CA	PCR SNPL	152	TCATGAGACTCCCCAGAAAG ATTATACTTCCCTTCAGATAGAGGTATCT AGCGATCTGCGAGACCGTATGTGGCATTCTTGACCTTGTTGAAC					
>rs380607-U9	CA	PCR SNPL	141	GGATTGGAATTCAGCATCG AGAAAATGTAGAAGAGGCAGAATC GACCTGGGTGTCGATACCTATATCAGCTATGAGGGCAAAAAGAA					
>rs187031-U5	CA	PCR SNPL	152	ATAATAAAGGTGTGCTTACTCTCCC TTTGAAATTTGGTTGAGCAAC GCGGTAGGTTCCCGACATATAAAACCATCCTTGAATTTGTGCTT					
>rs554653-U3	CA	PCR SNPL	100	ACCATTACATCACTCTCTCCG CTGTGGACTGTGCTCGAG CGTGCCGCTCGTGATAGAATACAGCGCCATCTGGTGGTTATGATC					
>rs748253-U2	CA	PCR SNPL	91	ACCGGAAATGCAGATACG GGCACCCACATGCCTTGG GGATGGCGTTCGCTCTATTTCAGCCCTGTTTCTCCAGTATG					
>rs724903-U1	CA	PCR SNPL	100	ATAGATATAGAGGTTAAACCCAAACACA TTCTCATGGCATCCAAT ACGCACGTCCACGGTGATTTAGTTATTCATCTGGCCCAGCTGTTC					
Panel # 2									
>rs456798-U12	GT	PCR SNPU	90	GAAATGAGCACTCCATTGG TCCCCAACTCTCATGCC CGACTGTAGGTGCGTAACTCGCTCGAACCCAGGACCACCTGGATA					
>rs383885-U10	GT	PCR SNPU	131	TGGAATGCTGGTTTCTTTAAGT TCTAAAGCTCTAAAGTGTGAAGGTC AGATAGAGTCGATGCCAGCTCCACACTCACACCCAGTAATGACAT					
>rs720460-U9	GT	PCR SNPU	94	CATGGCTCTGTCATGAGTATTC AATGAGCCCCGCTGGCAC GACCTGGGTGTCGATACCTAGGTATGGGGTGGGTGTCCTGTGA					
>rs720513-U7	GT	PCR SNPU	130	ACTGGCTCAAGGTAACCAAA TTATTGCTGTGGACAGTTGG AGGGTCTCTACGCTGACGATTTTGATCTTGACCTGCACCTCTCC					
>rs745458-U8	GT	PCR SNPU	142	TAAATGCAGTTCAACTACTATGGG TATTCCCTTATTTCTGGTGAGG GTGATTCTGTACGTGTCGCCCTCCTGTTTTCTGTTAAGGTGATT					
>rs726482-U5	GT	PCR SNPU	102	ACCACTCGTGCCCTCACT TTTTGCTGTGGGTCTGAAG GCGGTAGGTTCCCGACATATCCAGTGGATGTTTGCTGTCTCTG					
>rs726122-U4	GT	PCR SNPU	153	ATCAGCTCCAATCCCAAAC TAACTTTCCTTAGTTTATGACCAGG AGCGATCTGCGAGACCGTATACTGGACTGCCATCCCATTAACTGT					

Download the matching .txt file as shown below. The entire file is shown in [Appendix O](#).

**Figure 4.4 Autoprimer Results (.txt)**

```

Primers Designed For:
SNPstream Customer
Senior Scientist
BCI
D25B
4300 N. Harbor Blvd
Fullerton, CA 92834
Telephone: 7149923669
Facsimile: 7149923669

General Info:
Submission Date: 10/13/2005
Primers Designed for SNPstream 12plex Kits
Comments: tutorial

Powered By: Autoprimer v2.3 (Build 48)

SNP Name      SNP      oligo      Amplicon
>rs763342     Type    Type-Mod.  Length    Sequence
                No Primers could be found for this sequence...
>rs754257     No Primers could be found for this sequence...
>rs727678     No Primers could be found for this sequence...
>rs727023     No Primers could be found for this sequence...
>rs717292     No Primers could be found for this sequence...
>rs721580     No Primers could be found for this sequence...

Panel # 1
>rs723651-U4  CA      PCR/      152      TCATGAGACTCCCCAGAAAG
                PCRL
                SNPL      ATTATACTCCCTTCAGATAGAGGTATCT
                AGCGATCTGCGAGACCGTATGTGGCATTCTTGACCTTGGTTGAAC
>rs380607-U9  CA      PCR/      141      GGATTGGAATTCAGCATCG
                PCRL
                SNPL      AGAAAATGTAGAAGAGGCAGAATC
                GACCTGGGTGTCGATACCTATATCAGCTATGAGGGCAAAAAGAA
>rs187031-U5  CA      PCR/      152      ATAATAAAGGTGTGCTTACTCTCCC
                PCRL
                SNPL      TTTGAAATTTGGTTGAGCAAC
                GCGGTAGGTTCCCGACATATAAAACCATCCTTGTAAATTTGTGCTT
>rs554653-U3  CA      PCR/      100      ACCATTACATCACTCTCTCCG
                PCRL
                SNPL      CTGTGGACTGTGCTCGAG
                CGTGCCGCTCGTGATAGAATACAGCCCATCTGGTGGTTATGATC
>rs748253-U2  CA      PCR/      91       ACCGAAATGCAGATACG
                PCRL
                SNPL      GGCACCCACATGCCTTGG
                GGATGGCGTTCGGTCTATTTCCAGCCCTGTTTCTCCAGTATG
>rs724903-U1  CA      PCR/      100      ATAGATATAGAGGTTAAACCCAAACACA
                PCRL
                SNPL      TTCTCATGGCATCCAAT
                ACCGACGTCCACGGTGATTTAGTTATTCATCTGGCCAGCTGTTG

Panel # 2
>rs456798-U12 GT      PCR/      90       GAAATGAGCACTCCATTGG
                PCRL
                SNPU      TCCCCAACTCTCATGCC
                CGACTGTAGGTGCGTAACCTCGTCTCGAACCCAGGACCACCTGGATA
    
```



## Completing Partial Panels

### 5.1 Reverse Complementing G/A:T/C or C/A:T/G SNPs

Partial panels are formed when not enough SNPs of one extension type are submitted to the Autoprimer. Submission size for a given extension type may be increased by taking the reverse complement of sequences that have the complementary SNP type. For example, the following panel, as shown below, was submitted for 12plex design as 8 C/T and 5 G/A SNPs. With one exception, the extension type selected by Autoprimer was the same as the input extension type; thus, the panel consists of 7 C/T and 6 G/A extension types.

Figure 5.1 Partial SNP Panels

SNP Name- Probe Name	SNP Type	Primer Type- Mod.	Amplicon Length	Sequence
Panel # 1				
>rs22124-U6	CT	PCR SNPU	146	TTCTCATGTGGGGTTCTT TTATGGTGCTAAACACTGTACCAC GGCTATGATTCGCAATGCTTTTCTTCACTGCAAAACAGTTTAGAG
>rs52911-U5	CT	PCR SNPU	152	TATACTCTACTCAACAACATTCCCATC ATTTTAGCAGCTCTGCTTGATG GCGGTAGGTTCCCGACATATTCTCTTTGCTCCTCTTAATCAAA
>rs2624-U3	CT	PCR SNPU	112	AATGTCCAGAAATCATTATTGTC AATGTCCCAATATCAAGGACAA CGTGCCGCTCGTGATAGAATCATAGACCGTGTAGTCTTGATCTAA
>rs55778-U4	CT	PCR SNPU	131	AGAGGACATATGCTCTTCTATCAAAT AAGAATACATTAAATTTTCTTTGGAGG AGCGATCTGCGAGACCGTATCCACTGGGAAAGTATTTATTTTCAT
>rs49698-U1	CT	PCR SNPU	96	TAAAACACTATATTACCCAGCACCT AGGGCCAATTAGGATAGGAG ACGCACGCTCCACGGTGATTTTGTGGTTCCTCTAGCTCACACA
>rs31226-U10	CT	PCR SNPU	153	ATTCTACAAAAAACAAATCCAA ATGATCTGTGTCATGGGAA AGATAGATCGATGCCAGCTTTGTCTTTGTCTAAAAATAAT
>rs13180-U2	CT	PCR SNPU	99	TGATTAGAGGGAATAAATGCAC CCAATTCCAATCAAATGATCTT GGATGGCGTCCGCTCTATTTACAGGGTGTGAAAGCTGTTTTGGC
Panel # 2				
>rs2246-U7	GA	PCR SNPU	152	AGTTGAAGTATGGCCAGC TTTCTTGTGGATTGTCATGC AGGGTCTCTACGCTGACGATACAAACCTAAGAAACACAGAAATG
>rs19417-U5	GA	PCR SNPU	152	TATATTGGTCAGAGACTGAGTATGTC AAGTCAATCAGATGAAATAAAAAATTC GCGGTAGGTTCCCGACATATGAACAAAGGAAGATTCAAACTT
>rs923-U4	GA	PCR SNPU	90	TCCCAAGTCAGGCCTCC ATCTCCCCACTCCACTACT AGCGATCTGCGAGACCGTATACACTGAATTAGAGAGAAAGGACAA
>rs17422-U3	GA	PCR SNPL	116	ACAAGCTGAGTCTGAGACC TTTAGGACCTGGACATCCAC CGTGCCGCTCGTGATAGAATGGACCTAGAACACTCTGTACCTGAT
>rs17504-U1	GA	PCR SNPU	89	CTTCCAGTGCTCCAGAAA AGGAATTTATGTTTACCTTTCC ACGCACGCTCCACGGTGATTTTGAACCTTCTAGTGACGTCTGCTAC
>rs9239-U2	GA	PCR SNPU	107	ATTTGAAGTCTCAGAGCTTGC CTGTAAGAGGATTATCTTAGTTTATACTTTG GGATGGCGTCCGCTCTATTAGTTAAACACCGCCTTCAGTACCGA

The two partial panels may be combined into one full panel, for example, C/T.

1. Reverse complement all the G/A SNP sequences.

Use the website below to reverse complements degenerate positions (e.g. SNPs) and accepts batch files, as shown in the figure below.

[http://www.bioinformatics.vg/bioinformatics\\_tools/batch\\_reverse\\_complement.shtml](http://www.bioinformatics.vg/bioinformatics_tools/batch_reverse_complement.shtml).

**Figure 5.2 Batch Reverse Complement**

Address [http://www.bioinformatics.vg/bioinformatics\\_tools/batch\\_reverse\\_complement.shtml](http://www.bioinformatics.vg/bioinformatics_tools/batch_reverse_complement.shtml) Go Links

Links Forum BioDirectory Search News Jobs Books

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Bioinformatics.Net

SeqWright: DNA Sequencing  
Genotyping, Mutagenesis, Synthesis,  
Shotgun Lib, SNPs, Contigs, GLP.  
[www.seqwright.com](http://www.seqwright.com)

Bioinformatics s  
Anova, clustering, F  
800 citations. Free  
[www.agilent.com/ch](http://www.agilent.com/ch)

### Batch Reverse Complement Tool (fasta format)

```
>rs923
TTAAGCCCCAAGTTACCCAGCAAAGAGCTACACTCCAGTTAGCTATTAGCTTTTATTT
ACTCAAAAACATCCACAGGTGAATTCAGCAGTTAAAGTCCCCCTTAAACTCAGTTTATT
TTTTCTGTTCCCAACATCTCCTAGGCATCCTCCGAGAGCAGGTTCTCTGCTCAATTCCA
CAATGGTATGAAATGAGTCACCCACACACATGTTTCAGCCAACTGGAGCCTTTTGT
CAGTAAAGTCAACTAGGCACCCCTTCAGGCTCCATGACTTATTCTTCTATTGGGAAGAAG
AAAACAGACAATAATATCAATAATACGTTGTCCCAAGTCAGGCCTCCAAAACACTGAA
```

Reverse complement Example Clear  Include input sequence in output

Please enter a sequence or sequences in the box above consisting of the letters A,C,G,T,U or N or the *degenerate* code as listed below. Sequence should be in FASTA format (click example). Press the button marked **reverse complement**, and the result will appear in the box below. Reverse complements degenerate nucleotide sequence. This tool is convenient for batch reverse complementation of primer sequences.

### Results of DNA Reverse Complement Calculator

```
>rs923 (rev comp)
GTTATCCATCAGAGATGACTCTGTCTCATTGACCTTCAGATTCCCAATCAACAAACACTTGAGGGCCTG
TATGCTGGGCCAAGGGATTTTACATCCTTGATTACTTCCACTGAGGTGGGAGCATCTCCAGTGTCTCC
CAAATTATATCTCCCCCACTCCACTACTCTCTTCTCTCCACTTCATTTTTCCYTTGTCTTTCTCTCTAA
TTCAGTGTTTTGGAGGCCCTGACTTGGGGACAACGTATTATTGATATTATTGTCTGTTTTCTCTTCC
AATAGAAGAATAAGTCATGGAGCCTGAAGGGTGCCTAGTTGACTTACTGACAAAAGGCTCCAGTTGGGG
TGAACATGTGTGGTGGTGACTCATTTCCATACCATTGTGGAATTGAGCAGAGAACCCTGCTCTCGGGAG
GATGCCATAGGAGATGTTGGGAACAGAAAAATAAACTGAGTTTTAAGGGGGACTTAAACTGCTGAATTCA
CCTGTGGATGTTTTGAGTAAATAAAAAGCTAATAGCTAACTGGAGTGTAGCTCTTTGCTGGGGTAACTT
GGGCTTAA
```

Legend:

1. Paste the sequence in FASTA format.
2. Click **Reverse complement**
3. View the results.

- Assemble a new Autoprimer submission file.

Retain the original C/T SNPs. Replace the G/A SNP sequences with the corresponding C/T sequences.

- Submit to the Autoprimer.

The resulting Autoprimer design, as shown below, shows a full 12plex C/T panel.

**NOTE** The "refractory" G/A SNP, rs17422, may be switched to C/T using the method described in Section 6.

**Figure 5.3 Full SNP Panel After Reverse Complementation**

SNP Name- Probe Name	SNP Type	Primer Type- Mod.	Amplicon Length	Sequence
Panel # 1				
>rs2246rev-U12	CT	PCR SNPU	142	ATTTGCATGCTATGGGGA AGTTGAACTGATGGCCAGC CGACTGTAGGTGCGTAACTCGAAAGCATCTTCATGGGCAGGAATT
>rs22124-U11	CT	PCR SNPU	146	TTCTCATGTGGGGTTCTT TTATGGTGCTAAACACTGTACCAC AGAGCGAGTGACGCATACTATTCTTCACTGCAAACAGTTTAGAG
>rs19417rev-U9	CT	PCR SNPU	152	AAGTCAATCAGATGAAATAAAATTCC TATATTGGTCAGAGACTGAGTATGTCC GACCTGGGTGTCGATACCTAGTGCCACTCAATCTTTAAGCAAAA
>rs2624-U7	CT	PCR SNPU	112	AATGTCCAGAAATCACATTATTGC AATGTCCCAATATCAAGGACAA AGGGTCTCTACGCTGACGATCATAGACCGTGTAGTCTTGATCTAA
>rs17504rev-U6	CT	PCR SNPU	113	GAGGTCTTAGATATATCCTGAGTTCAG TTCCAGTGCTCCAGAACT GGCTATGATTCGCAATGCTTTTCCATTAGGTTTCCAAAACAAC
>rs923rev-U8	CT	PCR SNPU	93	AATTATATCTCCCCACTCCAC CCAAGTCAGGCCTCCTCAA GTGATTCTGTACGTGTGCGCCCTCTCTTCTCCACTTCATTTTCC <a href="#">Possible PCR Primer Cross Hybridization...</a> <a href="#">Possible SBE Primer vs Amplicon Cross Hybridization...</a>
>rs52911-U5	CT	PCR SNPU	152	TATACTCTACTCAACAACATTCCCATAC ATTTTAGCAGCTCTGCTTGATG GCGGTAGGTTCCCGACATATTTCTTTGCTCCTCTTAATCAAA
>rs49698-U3	CT	PCR SNPU	96	TAAAACACTATATTACCCAGCACCT AGGGCCAATTAGGATAGGAG CGTGCCGCTCGTGATAGAATTGTTGGGTTCTCCTAGCTCACACA
>rs31226-U10	CT	PCR SNPU	153	ATTCTACAAAAAACAATTCCAA ATGATCTGTGCATGGGGAA AGATAGAGTCGATGCCAGCTTTGTCTTTGTCTAAAAATAAT <a href="#">Possible SBE Primer Cross Hybridization...</a>
>rs55778-U4	CT	PCR SNPU	131	AGAGGACATATGCTTCTATCAAAT AAGAATACATTAATTTCTTTGGAGG AGCGATCTGCGAGACCGTATCCACTGGGGAAAGTATTTATTTAT <a href="#">Possible PCR Primer Cross Hybridization...</a> <a href="#">Possible SBE Primer vs Amplicon Cross Hybridization...</a>
>rs9239rev-U1	CT	PCR SNPU	124	TTTTAATGCACTAATCTGAATCTGTA AAGTCTCAGAGCTTGCCAACT ACGCACGTCCACGGTGATTTATTTATAATGTTCTTGATAGCAG
>rs13180-U2	CT	PCR SNPU	99	TGATTAGAGGGAATAAAATGCAC CCAATTCGAATCAATGATCTT GGATGGCGTTCCGTCTATTACAGGGGTGAAAGCTGTTTGGC
Panel # 2				
>rs17422-U1	GA	PCR SNPL	116	ACAAGCTGAGTCTGAGACC TTTAGGACCTGGACATCCAC ACGCACGTCCACGGTGATTTGGACCTAGAACACTCTGTACCTGAT

## Completing Partial Panels

*Reverse Complementing G/A:T/C or C/A:T/G SNPs*

## Completing Partial Panels

### 6.1 Forcing SBE Primer Design to One Strand

As seen in the panels designed for the 70 G/T SNPs (Section 4 and [Appendix O](#)), the Autoprimer switches the extension type of a submitted SNP if it calculates more favorable energetics for the opposite strand. This could result in partial panels. For example, Panel #6 comprises 10 G/T SNPs. If a full 12plex G/T panel is preferred, two SNPs that were designed as C/A (Panel #1) may be "forced" to the G/T extension type. To do this, follow the steps below:

1. Obtain the sequence of the strand that, as written, shows the desired SNP extension type. This could be the sequence that was originally submitted. If not, get the reverse complement of the original sequence.

Suppose that C/A SNPs rs723651 and rs380607 from Panel #1 are selected for inclusion in the new G/T panel. Locate the sequences in the Autoprimer submission file. Only the regions surrounding the SNP loci are shown below:

```
rs723651    GGGTCTAGGCCCTCTTGCTGTTTGGGT[G/T]GTTCAACCAAGGTCAAGAATGCCACATTC
rs380607    TCTCCTTGGATGTCTGGATAATCTCCAC[G/T]TCTTTTTTGGCCCTCATAGCTGATATGGT
```

2. Replace the base immediately upstream of the SNP locus with N. Repeat with the corresponding masked sequence, if available.

```
rs723651    GGGTCTAGGCCCTCTTGCTGTTTGGGN[G/T]GTTCAACCAAGGTCAAGAATGCCACATTC
rs380607    TCTCCTTGGATGTCTGGATAATCTCCAN[G/T]TCTTTTTTGGCCCTCATAGCTGATATGGT
```

3. Create a new submission file.

Create a new .txt file for the new panel. It consists of the original 10 sequences from Panel #6, plus the two modified sequences from Panel #1. A portion of the file is shown below. The entire file is shown in [Appendix P](#).

**Figure 6.1 Forced panel (.txt)**

```
>rs722979    GGATCTCTGCTGAGGTGTTGAAGCTTTGAGGAAGAGATAGCATTATACATGCATTGGAGATCAAGTTGTAAACAAATGGAATTTTAACTACA
>rs309773    TCTTCCCTTCAGGCTAGGTGACTGCAAAATCTCCCTAGAACTGCTATAGCCACATGTTTATAAATCAACATCAAGGCCAGTAGAATACTGAGA
>rs721757    TCTAACCATGTCATATTCAGAGTTTCTGCTTTTGGCCCTTTTGGCCGATCTTATCATCATCCCAAGCAATTAATGCATTCCATAAATACA
>rs5424293    AGTTGGTCTAAGACAGGATTGATTGTTTCCAAAGTCCCTTGACTTAAACACTGTCTATTAACGCCTTCCAATTCOCCAAATACCTTTAAGG
AGGTTGTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>rs727432    TTATCTCTTACCCECAAAGCCACCAGCGGTTTCTCCATCCTTACGCTCACTTACCTGGATGCTGTGCACACGGACCCATCCTG
>rs36950     GCTATGGTAGTTTCATGTACCTATGAGGTGGATAAAAATTTAGCAACAGGTTGAAGGTAAAGACTCCAAAAGATAGTGAAGTAGAAAACCTGC
>rs720596    TCTAGACATCTTCAAGTGTGCCACACTGCGCAGACCCAGGACGTCGTTACGTTGAAGTCCAAGCATTCAAGGGCTGCTGTTCACGTGGAATAT
>rs721623    TTTGGTTTCATGTAGAAGTCAAAATTCGCTACAATGAAAGTAGTTCCCAATCCAGTGTGAAATAAAAGCAGGGCTGTCACAGTCATTA
>rs470490    TCGCTTGATATGTAAGCTTGTGATCCCTTTTACTTTTTTTTTTTTTTTTTTTTATAGATGGAGTCTCGCTGTGTCOCCGGCTAGAGTCAAGT
GTTAAGAATATTTCTAGAAGAAAACAGTAAAGAAATGGGAAAAGTTGAAGATGCCAAACAAAGGTAATCTTCAAGTAAATGTGTAGCCCTGCATAATTCATA
>rs756384    TCCTGCTTTAGCAAACTTGAAGTACCTACTGAGATGTTAGAGAGTCTGTAAGTCTGGGTACCTAGAGTGACTACATTGAGTACCCCTGTGGA
>rs723651    GGTGGCAAATGGGGTTCGCTGAATATTCTGTTTGTCAATCTTGAGGCCCTTACTATCTGGAGAGCAAAAAGAGGTGATGAACCTGCTGT
```

4. Submit the file to the Autoprimer.

The resulting design of 12-SNP T/G panel is shown in Figure 6.2 below. The entire results file is shown in [Appendix Q](#).

**Figure 6.2 Full SNP Panel After Strand Forcing**

SNP Name- Probe Name	SNP Type	Primer Type- Mod.	Amplicon Length	Sequence
Panel # 1				
>rs380607- U11	GT	PCRU PCRL SNPU	141	GGATTGGAATTCAGCATCG AGAAAATGTAGAAGAGGCAGAATC AGAGCGAGTGACGCATACTACCTTGGATGTCTGGATAATCTCCAN
>rs722979- U10	GT	PCRU PCRL SNPU	90	TGAAGGCCAAAAAATCCAA AGGAGGTAAAAAGGTATCTACAGACTTAT AGATAGAGTCGATGCCAGCTGTCCAAAGCGTGCCTAAGAGTCGTT
>rs309773- U12	GT	PCRU PCRL SNPU	141	AGTCAAATGTTGCATCCTAAAAG ATCTAATAGCATATATACTAGGTCTTCTGAGA CGACTGTAGGTGCGTAACTCGGCCACAGATGATCATACACTTGCT
>rs727432- U9	GT	PCRU PCRL SNPU	108	TTTACCCAAACCATCGTCTG TCAATTGCTAATTCAAAATGAATG GACCTGGGTGTCGATACCTAGGTATGAGGTCTTACCCCCATGCCT <a href="#">Possible PCR Primer Cross Hybridization...</a>
>rs36950- U7	GT	PCRU PCRL SNPU	97	CTGCTTGTGTAAGATCTCTGC ACTCTTCATTCAATTTGTGATGCT AGGGTCTCTACGCTGACGATTAATGGGTGTAACGCTAAATAAAGC <a href="#">Possible PCR Primer Cross Hybridization...</a>
>rs424293- U8	GT	PCRU PCRL SNPU	101	AATCAGAAATGGAATGAAGCATAT CATAAAATACATATTTATATTGCTCACTGG GTGATTCTGTACGTGTCGCCGAGAAGTAAAAGAAATTTCCCA <a href="#">Possible SBE Primer vs Amplicon Cross Hybridization...</a>
>rs721757- U5	GT	PCRU PCRL SNPU	132	AACATGGAAAAATTCATCCTAACT AACAAATTTGGGGATGTTATTAGG GCGGTAGGTTCCCGACATATTCTATCAACACTTGAATAACAGAT
>rs720596- U6	GT	PCRU PCRL SNPU	151	AGGGTTGGGATAAAACAACAG ATTATATTTTCAGTACAACATCTACTCTTCAA GGCTATGATTGCAATGCTTATGAATAGAAAAGGATTAGAGCAGG
>rs756384- U4	GT	PCRU PCRL SNPU	153	AAAGGTAATATCTGACCAATGTGA ATTTATAGCTCCAGGATTACA AGCGATCTGCGAGACCGTATCTGCTCAGATTTTATATCCAGGAA
>rs721623- U2	GT	PCRU PCRL SNPU	107	AGTATTTGATATGAAAATAATTGTGCTG AATTCTATTTGGCACAGGTGG GGATGGCGTTCCGTCCTATTCTTCATAAATGTAACATCAAGCAC
>rs470490- U3	GT	PCRU PCRL SNPU	102	AATTCAAGGCGAGATGACAA AAATGTATGTCTGCTTTCTTCCC CGTGCCGCTCGTATAGAATCTCACAGCAGTTATTGGCCAAGGAC <a href="#">Possible SBE Primer vs Amplicon Cross Hybridization...</a>
>rs723651- U1	GT	PCRU PCRL SNPU	90	ATGAGACTCCCCAGAAAGG TTTAAGTGATGATTTGAATGTGG ACGCACGTCCACGGTGATTTCTAGGCCCTCTTGCTGTTTGN

**NOTE** For the forced SNPs, the SBE primer has an N at the 3'-end.

- Replace the 3' N in the SBE primer with the original base prior to ordering the oligonucleotide.

Although this procedure has been shown to be a reliable method for forcing AutoPrimer to pick a specific strand, there exist conditions where the AutoPrimer algorithm will select only one primer orientation no matter what is done to the opposite strand sequence. In these cases, replacing the SNP may be necessary to complete the panel.



## InSilico PCR

### 7.1 InSilico PCR

InSilico PCR, available at <http://genome.ucsc.edu/cgi-bin/hgPcr>, predicts the number of distinct amplicons that may be generated from a given primer pair. This method is useful for checking a sequence with multiple BLAST hits that can not be unambiguously determined to overlap and align to the same chromosomal position.

For example, rs36950 has two BLAST hits (Figure 2.3 on page 5). PCR primer sequences are given in the Autoprimer results file, as shown below.

**Figure 7.1 Autoprimer Results**

SNP Name Panel #	SNP Type	Oligo Type-Mod.	Amplicon Length	Sequence
>rs380607-U11	GT	PCR PCR SNPU	141	GGATTGGAATTCAGCATCG AGAAAATGTAGAAGAGGCAGAATC AGAGCGAGTGACGCATACTACCTTGGATGCTCGATAATCTCCAN
>rs722979-U10	GT	PCR PCR SNPU	90	TGAAGGCCAAAAAATCCAA AGGAGGTAAAAAGGTATCTACAGACTTAT AGATAGAGTGCATGCCAGCTGTCCAAAGCGTGCCTAAGAGTCGT
>rs309773-U12	GT	PCR PCR SNPU	141	AGTCAAATGTTGCATCCTAAAAG ATCTAATAGCATATATACTAGGCTCTCTGAGA CGACTGTAGGTGCGTAACTCGGCCACAGATGATACACTTGTCT
>rs727432-U9	GT	PCR PCR SNPU	108	TTTACCCAAACCATCGTCTG TCAATTGCTAATTCAAAATGAATG GACCTGGGTGTCGATACCTAGGTATGAGGTCTTACCCCCATGCCT Possible PCR Primer Cross Hybridization...
>rs36950-U7	GT	PCR PCR SNPU	97	CTGCTTGTTGTAAGATCTCTGC ACTCTTCATTCATTGTGATGCT AGGGTCTCTACGCTGACGATTAATGGGTGAACGCTAAATAAAGC Possible PCR Primer Cross Hybridization...
>rs424293-U8	GT	PCR PCR SNPU	101	AATCAGAAATGGAATGAAGCATAT CATAAAAACATATTTATTTGCTCACTGG GTGATTCGTACGTGTCGCGGAGAACTAAAGAAATTTCCCA Possible SBE Primer vs Amplicon Cross Hybridization...
>rs721757-U5	GT	PCR PCR SNPU	132	AACATGGAAAAATTCCTCAACT AACAAATTTGGGGATGTTATTAGG GCGGTAGGTTCCGACATATCTATCAACTTGGAAATACAGAT
>rs720596-U6	GT	PCR PCR SNPU	151	AGGGTTGGGATAAACAACAG ATTATATTCAGTACAACATCTACTTCAA GGCTATGATTCCGAATGCTTATGAATGAAAAAGGATTAGAGCAGG
>rs756384-U4	GT	PCR PCR SNPU	153	AAAGGTAATATCTGACCAATGTGA ATTTCATAGCTCCAGGATTACA AGCGATCTGCGAGACCGTATCTGCTCAGATTTTATATCCAGGAA
>rs721623-U2	GT	PCR PCR SNPU	107	AGTATTTGATATGAAAATAATTGTGCTG AATTCTATTTGGCACAGGTGG GGATGGCGTTCGGTCTATTCTCATAAATGTAACATCAAGCAC
>rs470490-U3	GT	PCR PCR SNPU	102	AATTCAAGGCGAGATGACAA AAATGTATGCTGCTTTCTTCCC CGTGCCGCTCGTGATAGAATCTCACAGCAGTTATTGGCCAAGSAC Possible SBE Primer vs Amplicon Cross Hybridization...
>rs723651-U1	GT	PCR PCR SNPU	90	ATGAGACTCCCCAGAAAGG TTTAAGTGATGATTGAATGTGG ACGCACGTCCACGGTGATTTTCTAGGCCCTCTTGTCTTTGGGN

**Figure 7.2 Primer Submission for *InSilico* PCR**

Legend

1. Select **Genome**.
2. Click in the field and paste the forward primer.
3. Click in the field and paste the reverse primer.
4. Click **Submit**.

**Figure 7.3 *InSilico* PCR Results**

The results show the chromosomal location and size of a single amplicon generated from the submitted primers. See Figure 7.3 above.

**NOTE** The predicted size agrees with that calculated by the Autoprimer from the submitted sequence. The web-based tool does not accept batch queries.



## Original Sequences

## A.1 Original Sequences (.doc)

&gt;rs36950

GCTATGGTAGTTTCATGTACCTATGAGGTGGATAAAAATTTAGCAACAGGTTGAAGGTAA  
GACTCCAAAAGATAGTGAGGTAGAAAACCTGCCTGAATTCCTTTGTTGAGTGGTGTTC  
GCTCAGTGACTGATGTCTATATTGCCAGCATCCTCTATATCAGAAGTAATGAGATGCTCA  
GTCCCTTTGACTTTGTGTATACTGTAGTAAACGAAATCACTCAGGACTAATTTTCTCCG  
CCTGCTTGTGTAAAGATCTCTGCATTATTAATGGGTGTAACGCTAAATAAAGKATCCTG  
GTGTGTTTACAGAGCAGCATCACAATGAATGAAGAGTGGAGCTTTAGGTTTACCACAA  
ATGGGTTTTCTAGAGTATCCAACCTGTTTCTGGGCCATGTTTTGTACAGTTGAGAGATA  
AGGGCACTGTGGGCTGTCATCCCAAACCACGTTTGGCAGGGAGCACCAAGGCAGCCT  
GCGTTGATTTAACTTCAGAGAAGTTATATTCAATTANGATAGGAAGTGGTTTTGGGGG  
GTTGTTTTAAAGAGAATTTCTACTCATTAAGAAATAATATAATTTGCTACTTTGGGAGTA  
CATTTTCAACCTGAAACTCCTTAATAATTCTATTTATAAAACTAGAAAACACATGGNAGG  
GATCTTAATTA

&gt;rs130588

CCAAATGGGGACGATGATATCTACCTCAGGGGAATGCTGTGATGACGATAGACGTGTCC  
AGCACACAGTAACTATTCAGTAGATATTATCTTTGTTCCCTCAGCACCAGGGGAAATAAG  
AAAAGCAAAGAAAGGGGAAGTGCTGGTATAAAGGGAAGGAAGGAAACCAAGGGCATCA  
GTGTGGATGGGGAAGGCAGAAAATGCTCTCCTGGGGAATCACAACCTTTGTATTATCAA  
GTGCTTTTTTTGAATTCAGCCATGCTATTTCTTGCAGCTCTGACTATGTTTCAAGTTGG  
AATATCAAGCATCTCTGAGCCTTCACTACAGCAATTAAGGGCTCATAAAGCTATTCTTAG  
GAGTACAAAATAAACTGAGATATATTTGAGGTTGCCAGAAGGTTKCCATGTGGAGAACT  
GAGTGCTAACCCTGGAAAGGTGCACATTAAGACAGCAGGTGTGCAGAATGCTGTGG  
CCATAACTCTCTCCAGCTGAGGGCAGAGGCCACAAAAAGGACCCTGCTGAGGGGAGG  
CTGCCAAGGAGCTCCACACTCTGCCAAGAGGGGCCTGAGTTCTGGCGCCATCAGCAA  
CTGTCTTGTCTCTGTGACTCACATCTTCTCACATAAAATGAGTAGTTGAATGAGTTGATT  
TTGAATGTTCAATTTCTGTTCTAATGTTGTGTGATCCAATATGAATCAACTAAAGCCTGC  
AGATTCTTCCACTGCCCCCATGGTGGCCATCTGTTGAGGTGGGAGGGAAACATGCTGG  
CCCTCCCCGTCTCCTACCCTAAAGAAGACACTGTGC

&gt;rs144848

GAATTCTCTGAAGTAAGAAAATCTTTCTTTCTTTGTTCTCTGTGTCTAATAGGTCTTTT  
TCTGAAATATTTTGGTCCATGAAGAAATATGCAATAGGGGTATTTTCTCCATCTGGGCT  
CCATTTAGACCTGAAAGGGTTAGTTGAGACCATTACAGGCCAAAGACGGTACAACCTT  
CCTTGGAGATTTTGTCACTTCCACTCTCAAAGGGCTTCTGATKTGCTACATTTGAATCT  
AATGGATCAGTATCATTTGGTTCCACTTCAGATACAAATGAGTATTTTTCTTTCACTTGG  
TTTTTAGATTTTTCACATTCATCAGCGTTTGCTTCATGGAAAATTTTTTCTTAGTCTTG  
CTAGTTCTTACTTTTTGTAGATTTTTGTTCTACATTTAGAAAAACATAATGAAAACTAT  
CTTCTCAGAGGTATCTACAACCTGTTTCATAGACTTCATCTTCTAGGACATTTGGCATTG

ACTTTCCCACGTGGTCTTTGCAGCTATTTACTTTAAATGAATTC

>rs187031

AGAAAGAAAGAAAAAACCCCTGCTGATTATGTATGCAAAAGTCGCAGCACGGTGCTA  
GAGTGCTAGGCACATCATCAGTGCATAATAAAGGTGTGCTTACTCTCCCCGGGGATGAG  
ATTAAATCCTGGGTGCAAAGCAAGTCTTGATTTCCATGCTC**K**AAGCACAAATTACAAGG  
ATGGTTTTTCAGGATTGTTTTGGGGATTGCCCCAAATGTTGCTCAACCAAATTTCAAAC  
GAGGGTGGTCTCTTTGACACAGGCTACTTTTCAGATTTCTGCTCCCCTGCACTCCAG  
CACCTGAGTCAGCAGTCACTTTTTGACAGGAGGCCAGCAGGTGCTTTCTGAGCTG  
TCATGATTACCTTCCAGTGAAAACACACTACGCTTCTTGCTGCCAGTTTGAAGTGCAG  
GCAACCTCC

>rs277939

AGAGAAGTTGGCTCTTGCTTAAATTCCAACACTTATTCTTATGGGGAGAAAGCCATGGT  
TTACCTCTTTGGTTTGTCAAGGGAGAAAAATGACACATTATTTGGGACTTCAGTAAA  
ACAACCTCTGATGTCAGCATCCATACATA**K**GTGCATTAGTAGCTACTGAACATGGTAGAT  
TATGTAATGATCAAATAACCATGGATAATCTAAAAAATAAGTGTAACAAATATTCTAAAG  
ATTTTCTATCTAAAAAGTTTTAGCACTTAAGCTCTTATTGTACAGAATGTGTATCTGCAA  
TATTACTGTATTTCTTTGGCTGAAACTTTTTCTTTACACTGATGGAGGAATATGTTT  
ATTAAACACCATCCTTAGTACTGGATCTAATCTGTTCTGTATAGGTTATATTATTAAGACA  
GAGAGCAAAACACTTATTAAGATTTGTTTCATGATACCTGGATAGTGAGGCTTTAGAAGA  
CGATGTTCTGCTATCCATTTGCTGAGACTTAGGAGCAGCATCAGTTCTTTCTTCTTATC  
AGACACAATCATTTTCATCCTGAGGTAACCTGAGGCAC

>rs288422

GAATTCACCTTTTATTTTGATAGAATGGCAGANAGTCTAAGTAGAAGCAGGGCTTTGTG  
TATTCGTAAAGTTAACCTACCTCCATCTGCAGCTGACTTTGCTAGGAGGAATACACAG  
AGAAGTCTGGCATCCTTAGAAATGTGCTGTTGTGACACAGTTACCTGAATAACTAAACA  
CACTGGAATCTCCCCTAAACACAGCTCAGGTGGCTAATGCTAACAATTATAGGGTTACC  
TATAGGGAGAACTAATGACCATTTCAACCAAATAATGAATACTGATAATAACAAGAATA  
ACACATCAGCTGGCATTAAAGAGTGTGTGTCCCTACTGTGCTCCTTCTTGAGGATGTTG  
ATTATCATGCCATTAACCTAGAAGAACTCAAATCTCTGGTTGTAGTGGTCACGCTCCCT  
AGATTTACAT**K**TATCTAATTTACATTATCTCATTTTCATGATCTTTAACCATTCTATTGCCTA  
GAAAGAAAACTCCTGAGATCAAATAATTTGACTTTGTTACAATTGTTAAGAACAACAA  
ACAACAAAAGAATTGTCCCTCACTTGTCTTTGTTGCAGGCAATGGGTATAACACATGCC  
TTCTTTTCCAGCTNTTTAGAATTC

>rs309133

CATAACATCCTGATGTCCTGGCTTACTTCTATTTGGGAGAGGGGCCCCACTCGTTCTC  
ATTTTATGAGACTATAACTTTTTAGTATTCATTTTACTGTTTGTCAATTTATTGAGTTGTTTC  
TACCCAGTGTGTTTACAACCTCACATAAAATACTAAAGACAATCAAATATACAGAAACAAG  
GCTGGGAAATGCAACTTGCCAGTGAAAATGTCATTAGTATCTAGTGGCAGCTGCGTAC  
TGTAACCACTTGGCATCGCCTCAGATAAAAGGCAGATGAAAAACAAGGAAATGACTA  
TCTAGATGCCTTGATGGAAAACCTGTTTTAATAGTAGGTGGTTTTAAATAGGCTGTCAGA  
GAAAGAGGATAAGTTGTCCAAGTG**K**AAACTAGACAATCCATCAAGAACAGAATGCAAA  
GAGGAGAAAAAGGTAAAGTTCTCATGGGAACAGGATATAAAGTTGATGACAAAAGGCCT  
TCACAGGGAATGAAGAGCAACATAACAATGTGTGCCATGTATTATTTGCATAAACTCCAA

AGAACTTGGCACAATTTATATTTCCACATCACAATCATAAACAGGAAGTAAGAAAAA  
GAGTGTCAAGCCGCCAAAACAGGTGGAGTATTCAACTTCACTCAAGGGAGTTAAGGGA  
GTTCTCAGGTCCTCACTTTAACAAT

>rs309773

TCTTCCTTCAGGCTAGGTGACTGCAAATCTCCCTAGAACTGCTATAGCCACATGTTTAT  
AATAAACATTCAAGGCCAGTAGAATACTGAGAGAGTGTACTTAGGATACAAGTTCTTGAT  
TCCAAGCCCCTGATCAAAGTAAAGGAAAACTTGGAGGCCAAGAATTTGCTTTCTTTTA  
CTAGTTTAAATGTAAGACTTGACCCCTAATTTTTGATCATCCAGGGGAGGAGGTGGCA  
ATTCTCAATCATGCAGCTTAAAAGTCTTGAAAGCCAAAGGTTAGGATGCTTCTCTGAG  
TCAAATGTTGCATCCTAAAAGGTCCATCTGCATTGTTCTAGAATTAAGGGCCACAGAT  
GATCATACTTGCTKTTGTCTCTGTGCATTTCTCCAATAAATGCCGTCTCAGAAGACC  
TAGTATATATGCTATTAGATCCTTTGCCCTTACTTGTTGACAGTGGTTTACCCAATACGT  
GCTTTTTACTAATCCCTGAGATACAGAACTCCAGTGATTACAGGTCTAACATGAGTGGA  
GTTAATGAG

>rs380607

AAGAGTGACACAGTCTCCATCAAGCTCTGGAGCCATGTAGTCATGGCAGAGCAAAGAG  
TATTTAGTCACTGGGGATTGGAATTCAGCATCGTTACAAATGGACACAGTTCACACTG  
GGACACGATATCTCCTTGGATGTCTGGATAATCTCCACKTTCTTTTTTGCCTCATAGCT  
GATATGGTCCAGGGATTCTGCCTCTTCTACATTTCTCCCCTGTTAGAATGGAGGTCT  
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## RepeatMasker Results

### B.1 RepeatMasker Results (.txt)

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*

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>rs288422

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*

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>rs713478

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>rs713538

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>rs713718



## RepeatMasker Results

*RepeatMasker Results (.txt)*

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>rs718896

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>rs719025

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>rs719113

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>rs719931

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>rs720033

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>rs720394

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>rs720460

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>rs720513

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*

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>rs720596

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>rs720659

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>rs720710

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>rs721368

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>rs721580

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>rs721757

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>rs721992

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*

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>rs722525

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>rs722979

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>rs723081

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>rs723651



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>rs723886

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>rs724263

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>rs724691

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*

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>rs724903

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>rs725046

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>rs725301

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*

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**RepeatMasker Results**  
*RepeatMasker Results (.txt)*



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## Masked Sequences

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## Masked Sequences

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**Masked Sequences**

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## Masked Sequences

*Masked Sequences (.doc)*



## Masked Sequences, Single Line

### E.1 Masked Sequences, Single Line (.doc)

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*Masked Sequences, Single Line (.txt)*

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*Masked Sequences, Single Line (.txt)*



**Masked Sequences, Single Line**  
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## Original Sequences

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## Original Sequences

*Original Sequences, Single Line (.xls)*

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## Original and Masked Sequences

### I.1 Original and Masked Sequences, Single Line Each (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.xls)

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Original and Masked Sequences, Single Line Each (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.xls)

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## Original and Masked Sequences

*Original and Masked Sequences, Single Line Each (.xls)*

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## Original and Masked Sequences

### J.1 Original and Masked Sequences, Single Line Each (.txt)

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Original and Masked Sequences, Single Line Each (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.txt)

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Original and Masked Sequences, Single Line Each (.txt)

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Original and Masked Sequences, Single Line Each (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each (.txt)

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>rs754257

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>rs754257

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## Original and Masked Sequences

*Original and Masked Sequences, Single Line Each (.txt)*

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## Original and Masked Sequences

### K.1 Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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CACCTGAGTCAGCAGTCACTTTTTGACAGGAGGCCAGCAGGTGCTTTCTGAGCTG  
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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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## Original and Masked Sequences

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## Original and Masked Sequences

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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**Original and Masked Sequences**

*Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)*

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)

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## Original and Masked Sequences

*Original and Masked Sequences, Single Line Each, Masking Adjusted (.txt)*

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## Original and Masked Sequences

### L.1 Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)

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## Original and Masked Sequences

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## Original and Masked Sequences

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)

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**Original and Masked Sequences**

*Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)*

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)

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**Original and Masked Sequences**

*Original and Masked Sequences, Single Line Each, Masking Adjusted (.xls)*

## Original and Masked Sequences

### M.1 Original and Masked Sequences, Single Line (.xls)

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>rs144848

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>rs187031

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.xls)

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Original and Masked Sequences, Single Line (.xls)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.xls)

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## Original and Masked Sequences

*Original and Masked Sequences, Single Line (.xls)*

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## Original and Masked Sequences

### N.1 Original and Masked Sequences, Single Line (.txt)

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## Original and Masked Sequences

*Original and Masked Sequences, Single Line (.txt)*

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.txt)

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## Original and Masked Sequences

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.txt)

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## Original and Masked Sequences

*Original and Masked Sequences, Single Line (.txt)*

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.txt)

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**Original and Masked Sequences**

*Original and Masked Sequences, Single Line (.txt)*

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.txt)

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## Original and Masked Sequences

Original and Masked Sequences, Single Line (.txt)

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## **Original and Masked Sequences**

*Original and Masked Sequences, Single Line (.txt)*

## Autoprimer Results

### 0.1 Autoprimer Results (.txt)

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## Autoprimer Results

Autoprimer Results (.txt)

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**Autoprimer Results**

*Autoprimer Results (.txt)*

## Forced Panel

### P.1 Forced Panel (.txt)

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>rs720596

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>rs721623

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**Forced Panel***Forced Panel (.txt)*

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>rs723651

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>rs380607

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## Autoprimer Results

### Q.1 Autoprimer Results, Forced Panel (.txt)

Primers Designed For:  
 SNPstream Customer  
 Senior Scientist  
 BCI  
 D25B  
 4300 N. Harbor Blvd  
 Fullerton, CA 92834

Telephone: 7149923669  
 Facsimile: 7149923669

General Info:  
 Submission Date: 9/7/2005  
 Primers Designed for SNPstream 12plex Kits  
 Comments: tutorial new panel 12plex

Powered By: Autoprimer v2.3 (Build 48)

SNP Name	SNP Type	Oligo Type-Mod.	Amplicon Length	Sequence
Panel # 1				
>rs380607-U11	GT	PCR	141	GGATTGGAATTCAGCATCG
	PCRL			AGAAAATGTAGAAGAGGCAGAATC
	SNPU			
				AGAGCGAGTGACGCATACTACCTTGGATGTCTGGATAATCTCCAN
>rs722979-U10	GT	PCR	90	TGAAGGCAAAAAAATCCAA
	PCRL			AGGAGGTAAAAAGGTATCTACAGACTTAT
	SNPU			
				AGATAGAGTCGATGCCAGCTGTCCAAAGCGTGCGTAAGAGTCGTT
>rs309773-U12	GT	PCR	141	AGTCAAATGTTGCATCCTAAAAG
	PCRL			ATCTAATAGCATATATACTAGGTCTTCTGAGA
	SNPU			
				CGACTGTAGGTGCGTAACTCGGCCACAGATGATCATACTTGCT
>rs727432-U9	GT	PCR	108	TTTACCCAAACCATCGTCTG
	PCRL			TCAATTGCTAATTCAAATGAATG
	SNPU			
				GACCTGGGTGTCGATACCTAGGTATGAGGTCTTCACCCCATGCCT

Possible PCR Primer Cross Hybridization...

## Autoprimer Results

Autoprimer Results, Forced Panel (.txt)

```
>rs36950-U7      GT   PCRU    97  CTGCTTGTGTAAGATCTCTGC
                PCRL          ACTCTTCATTCATTTGTGATGCT
                SNPU
AGGGTCTCTACGCTGACGATTAATGGGTGTAACGCTAAATAAAGC
                Possible PCR Primer Cross Hybridization...

>rs424293-U8    GT   PCRU    101 AATCAGAAATGGAATGAAGCATAT
                PCRL          CATAAAATACATATTTATATTGCTCACTGG
                SNPU
GTGATTCTGTACGTGTCGCCGAGAAGTAAAAGAAATTTCCCA
                Possible SBE Primer vs Amplicon Cross Hybridization...

>rs721757-U5    GT   PCRU    132 AACATGGAAAAATTCATCCTAACT
                PCRL          AACAATATTGGGGATGTTATTAGG
                SNPU
GCCGGTAGGTTCCCGACATATTCTATCAACACTTGAATAACAGAT

>rs720596-U6    GT   PCRU    151 AGGGTTGGGATAAAACAACAG
                PCRL          ATTATATTTCAGTACAACATCTACTCTTCAA
                SNPU
GGCTATGATTTCGCAATGCTTATGAATAGAAAAGGATTAGAGCAGG

>rs756384-U4    GT   PCRU    153 AAAGGTAATATCTGACCAATGTGA
                PCRL          ATTCATAGCTCCCAGGATTACA
                SNPU
AGCGATCTGCGAGACCGTATCTGCTCAGATTTTATATCCCAGGAA

>rs721623-U2    GT   PCRU    107 AGTATTTGATATGAAAATAATTGTGCTG
                PCRL          AATTCTATTTGGCACAGGTGG
                SNPU
GGATGGCGTTCCGTCCTATTCTTCATAAATGTAACATCAAGCAC

>rs470490-U3    GT   PCRU    102 AATTCAAGGCGAGATGACAA
                PCRL          AAATGTATGTCTGCTTTCTTCCC
                SNPU
CGTGCCGCTCGTGATAGAATCTCACAGCAGTTATTGGCCAAGGAC
                Possible SBE Primer vs Amplicon Cross Hybridization...

>rs723651-U1    GT   PCRU    90  ATGAGACTCCCCAGAAAGG
                PCRL          TTTAAGTGATGATTTGAATGTGG
                SNPU
ACGCACGTCCACGGTGATTTTCTAGGCCCTCTTGCTGTTTGGGN

>===== UHT Probe Panel Info =====<
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>rs309773_GTGT
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>rs727432_GTGT
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2 1 CONTROL_YY 1 4
2 1 NEGATIVE 4 4
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**Autoprimer Results**

*Autoprimer Results, Forced Panel (.txt)*