User Guide: Primer Design

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1 About

This document will outline how to use the PrimerDesign application created for use by students of Molecular Methods to learn about Polymerase Chain Reactions (PCR) and primer design.

If you encounter any problems while using the application, feel free to ask your tutor, or email us at `TeamProjectQ@gmail.com`.

2 Getting Started

2.1 Downloading and Starting the Application

First, find the `PrimerDesign.jar` link on the Molecular Methods moodle site and run it. You can run it by clicking on the link, however this is dependant on your browser. Alternatively, you can save the program to your computer and double click on it. If you encounter problems here, ensure that you have Java 6 or newer installed on your computer.

2.2 Obtaining a DNA Sequence

You will need an internet connection to perform these steps.

Now that you have successfully launched the application, you should prepare a DNA sequence that you wish to manipulate. As an example, we will show you how to obtain the L1CAM sequence, which you should already be familiar with, from the NCBI (National Center for Biotechnology Information) website at `http://www.ncbi.nlm.nih.gov/`.

Use your web browser of choice to go to the NCBI website. You should see something similar to figure 1.

In order to search for a compatible sequence, change the search type to "Nucleotides" from the drop-down menu next to the search bar (highlighted in yellow in figure 2) and search for the sequence you want, for our example this is "`L1CAM`", using the search bar (highlighted in red in figure 2).

Now you will be presented with your search results, if you are following our example click on the link highlighted in yellow on figure 3.

Once clicked, you should be presented with something similar to figure 4.

Most of this information is irrelevant to this application, so scroll down until you see the DNA sequence, in our example it should look like figure 5. Now you can simply highlight the sequence (highlighted in yellow in figure 5) and press Ctrl-c (or equivalent) to copy the sequence.

3 The Application

Please note: the following information and screenshots are subject to change and may not necessarily reflect the current build of the system. Use your best judgement where differences appear.

3.1 Overview Screen

On starting the application (as described in section 2.1), you should see the overview screen (figure 6) with a button on the bottom right labelled "Start". Once you have finished reading the information on this page, you should press start.

3.2 Sequence Entry

Remember the DNA sequence you copied in section 2.2? Well now is the time to paste it! Simply paste (using Ctrl-V or using the EditPaste menu) the sequence into the large white area and press the next button. As you can see in figure 7, you do not need to worry about including the "ORIGIN" from the sequence as this will be removed when you press the 'Next' button.

3.3 Target Area Selection

Now we have to select what it is we want to produce from the reaction. To do this, you have to specify the first and the last base of the sequence you wish to copy, using it's position in the sequence. So if you wish to copy a sequence from position 100 to 500, as in the example on figure 8, you would enter these into the "From" and "To" text boxes. An easier way to do this is to highlight the sequence you wish to use and the numbers will be filled in for you.

Note that you can also view the complementary strand, by using the tabs just above where the sequence is.

3.4 Primer Design

You can now see your selected area more clearly and, since primers can include bases from before and after the target, the rest of the sequence is still available to you.

You should design your primers and insert them into the "Forward Primer" and "Reverse Primer" fields, as shown in figure 9, note however that the example data is not designed to be correct.

As you type in a primer, you will notice that what you type is highlighted in the sequence (as long as you are viewing the correct strand). A red highlight corresponds to a primer with at least one rule broken to an "unacceptable" degree, which means that you cannot proceed.

A yellow highlight corresponds to a primer with at least one rule broken to an "acceptable" degree, which will (after 3 attempts) allow you to proceed if you are absolutely sure you want to continue.

A blue highlight means that you have found a primer which meets all the requirements and may continue.

For the reverse primer, there is an additional text box which will reverse the order of the primer you enter, so you should enter the primer in the 3'—5' direction. The 5'—3' primer is the one which is checked against the rules.

So if you were to enter `aattccggt`, the additional text box would show `tggccttaa`.

You can also see the primer design rules again by pressing the "Show Primer Design Rules" button.

You can check your primers individually against the primer design rules by using the "Check Primer" buttons on the bottom of the window. These will give you information on where your primers pass and where they fail.

When you click next, both primers are checked against the rules described at the start of the application, and you are given a report of where your primers pass and where they fail, if at all. This will look something like 10.

Pressing the "Ok" button will close this window and allow you to continue only if you have passed each rule. If you have any "Close Fail" items in the report, you will only be allowed to proceed when you have clicked the "Next" button another two times, which will give you the option to "Accept and Continue".

3.5 Melting Temperature

This screen, which should be similar to figure 11, lets you review your design, showing the melting temperatures of both primers and the primers themselves.

Note that for our example we should not have been allowed to get here due to the feedback we received in figure 10.

4 Known Bugs

There are a few known issues with the current build of the system. These are constantly changing with the development of the application so are not listed here. If you suspect you have found something which should not have, please feel free to e-mail us at `TeamProjectQ@gmail.com`.

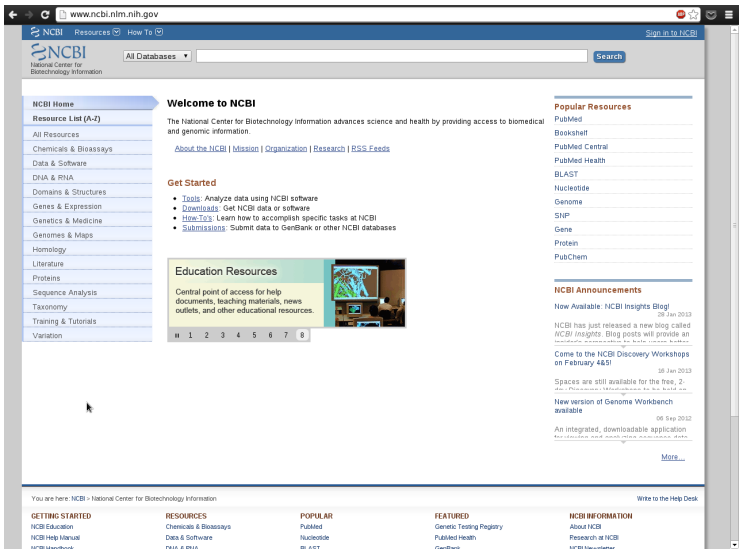


Figure 1: NCBI Home Page

Figure 2: Searching for a sequence

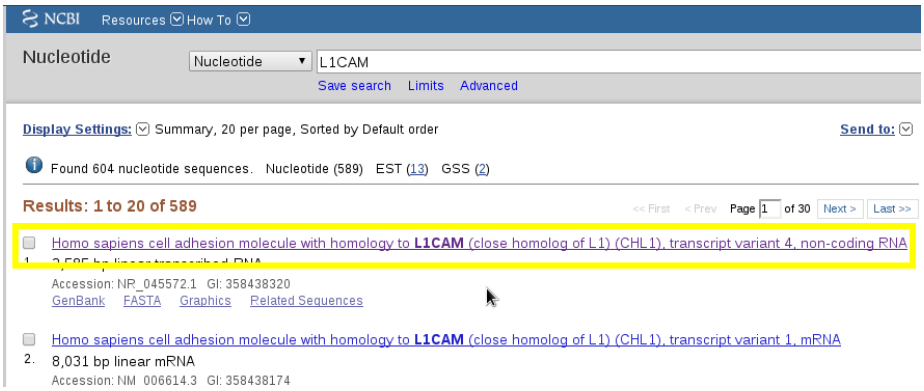
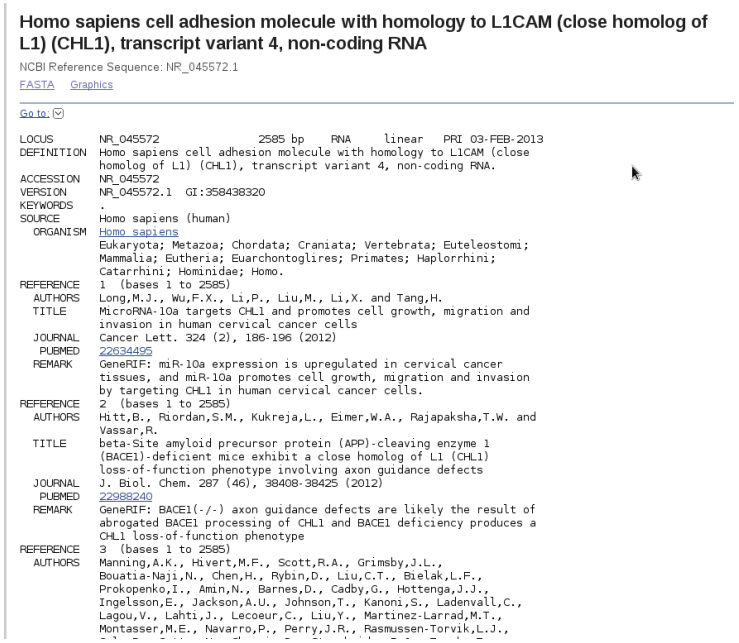


Figure 3: Search Results



Homo sapiens cell adhesion molecule with homology to L1CAM (close homolog of L1) (CHL1), transcript variant 4, non-coding RNA

NCBI Reference Sequence: NR_045572.1

FASTA Graphics

Go to:

```
LOCUS       NR_045572              2585 bp    RNA     linear   PRI 03-FEB-2013
DEFINITION  Homo sapiens cell adhesion molecule with homology to L1CAM (close
            homolog of L1) (CHL1), transcript variant 4, non-coding RNA.
ACCESSION   NR_045572
VERSION     NR_045572.1  GI:358438320
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 2585)
  AUTHORS   Long,M.J., Wu,F.X., Li,P., Liu,M., Li,X. and Tang,H.
  TITLE     MicroRNA-10a targets CHL1 and promotes cell growth, migration and
            invasion in human cervical cancer cells
  JOURNAL   Cancer Lett. 324 (2), 186-196 (2012)
   PUBMED   22634495
  REMARK    GeneRIF: miR-10a expression is upregulated in cervical cancer
            tissues, and miR-10a promotes cell growth, migration and invasion
            by targeting CHL1 in human cervical cancer cells.
REFERENCE   2  (bases 1 to 2585)
  AUTHORS   Hitt,B., Riordan,S.M., Kukreja,L., Eimer,W.A., Rajapaksha,T.W. and
            Vassar,R.
  TITLE     beta-Site amyloid precursor protein (APP)-cleaving enzyme 1
            (BACE1)-deficient mice exhibit a close homolog of L1 (CHL1)
            loss-of-function phenotype involving axon guidance defects
  JOURNAL   J. Biol. Chem. 287 (46), 38408-38425 (2012)
   PUBMED   22988240
  REMARK    GeneRIF: BACE1(-/-) axon guidance defects are likely the result of
            abrogated BACE1 processing of CHL1 and BACE1 deficiency produces a
            CHL1 loss-of-function phenotype
REFERENCE   3  (bases 1 to 2585)
  AUTHORS   Manning,A.K., Hivert,M.F., Scott,R.A., Grimsby,J.L.,
            Bouatia-Naji,N., Chen,H., Rybin,D., Liu,C.T., Bielak,L.F.,
            Prokopenko,I., Amin,N., Barnes,D., Cadby,G., Hottenga,J.J.,
            Ingelsson,E., Jackson,A.U., Johnson,T., Kanoni,S., Ladenvall,C.,
            Lagou,V., Lahti,J., Lecoeur,C., Liu,Y., Martinez-Larrad,M.T.,
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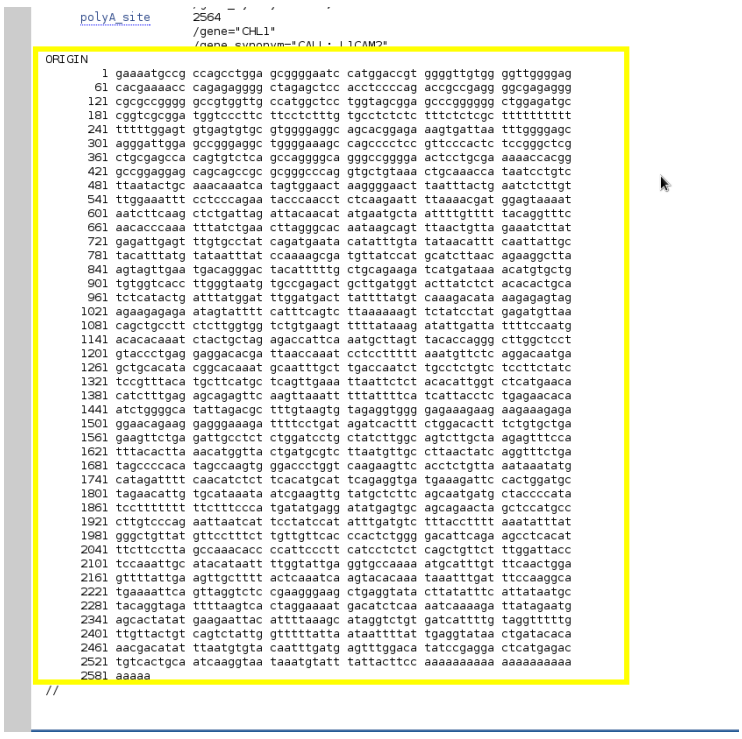
Figure 4: Sequence Page



```
     polyA_site      2564
                     /gene="CHL1"
                     /gene_synonym="CALL; L1CAM2"
ORIGIN
        1 gaaaatgccg ccagcctgga gcggggaatc catggaccgt ggggttgtgg ggttggggag
       61 cacgaaaacc cagagagggg ctagagctcc acctccccag accgccgagg ggcgagaggg
      121 cgcgccgggg gccgtggttg ccatggctcc tggtagcgga gcccgggggg ctggagatgc
      181 cggtcgcgga tggtcccttc ttcctctttg tgcctctctc tttctctcgc ttttttttct
      241 tttttggagt gtgagtgtgc gtggggaggc agcacggaga aagtgattaa tttggggagc
      301 agggattgga gccgggaggc tggggaaagc cagcccctcc gttcccactc tccgggctcg
      361 ctgcgagcca cagtgtctca gccaggggca gggccgggga actcctgcga aaaaccacgg
      421 gccggaggag cagcagccgc gcgggcccag gtgctgtaaa ctgcaaacca taatcctgtc
      481 ttaatactgc aaacaaatca tagtggaact aaggggaact taatttactg aatctcttgt
      541 ttggaaattt cctcccagaa tacccaacct ctcaagaatt ttaaaacgat ggagtaaaat
      601 aatcttcaag ctctgattag attacaacat atgaatgcta attttgtttt tacaggtttc
      661 aacacccaaa tttatctgaa cttagggcac aataagcagt ttaactgtta gaaatcttat
      721 gagattgagt ttgtgcctat cagatgaata catatttgta tataacattt caattattgc
      781 tacatttatg tataatttat ccaaaagcga tgttatccat gcatcttaac agaaggctta
      841 agtagttgaa tgacagggac tacatttttg ctgcagaaga tcatgataaa acatgtgctg
      901 tgtggtcacc ttgggtaatg tgccgagact gcttgatggt acttatctct acacactgca
      961 tctcatactg atttatggat ttggatgact tattttatgt caaagacata aagagagtag
     1021 agaagagaga atagtatttt catttcagtc ttaaaaaagt tctatcctat gagatgttaa
     1081 cagctgcctt ctcttggtgg tctgtgaagt ttttataaag atattgatta ttttccaatg
     1141 acacacaaat ctactgctag agaccattca aatgcttagt tacaccaggg cttggctcct
     1201 gtaccctgag gaggacacga ttaaccaaat cctccttttt aaatgttctc aggacaatga
     1261 gctgcacata cggcacaaat gcaatttgct tgaccaatct tgcctctgtc tccttctatc
     1321 tccgtttaca tgcttcatgc tcagttgaaa ttaattctct acacattggt ctcatgaaca
     1381 catctttgag agcagagttc aagttaaatt tttattttca tcattacctc tgagaacaca
     1441 atctggggca tattagacgc tttgtaagtg tagaggtggg gagaaagaag aagaaagaga
     1501 ggaacagaag gagggaaaga ttttcctgat agatcacttt ctggacactt tctgtgctga
     1561 gaagttctga gattgcctct ctggatcctg ctatcttggc agtcttgcta agagtttcca
     1621 tttacactta aacatggtta ctgatgcgtc ttaatgttgc cttaactatc aggtttctga
     1681 tagccccaca tagccaagtg ggaccctggt caagaagttc acctctgtta aataaatatg
     1741 catagatttt caacatctct tcacatgcat tcagaggtga tgaaagattc cactggatgc
     1801 tagaacattg tgcataaata atcgaagttg tatgctcttc agcaatgatg ctaccccata
     1861 tccttttttt ttctttccca tgatatgagg atatgagtgc agcagaacta gctccatgcc
     1921 cttgtcccag aattaatcat tcctatccat atttgatgtc tttacctttt aaatatttat
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     2281 tacaggtaga ttttaagtca ctaggaaaat gacatctcaa aatcaaaaga ttatagaatg
     2341 agcactatat gaagaattac attttaaagc ataggtctgt gatcattttg taggtttttg
     2401 ttgttactgt cagtctattg gtttttatta ataattttat tgaggtataa ctgatacaca
     2461 aacgacatat ttaatgtgta caatttgatg agtttggaca tatccgagga ctcatgagac
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     2581 aaaaa
//
```

Figure 5: The Sequence

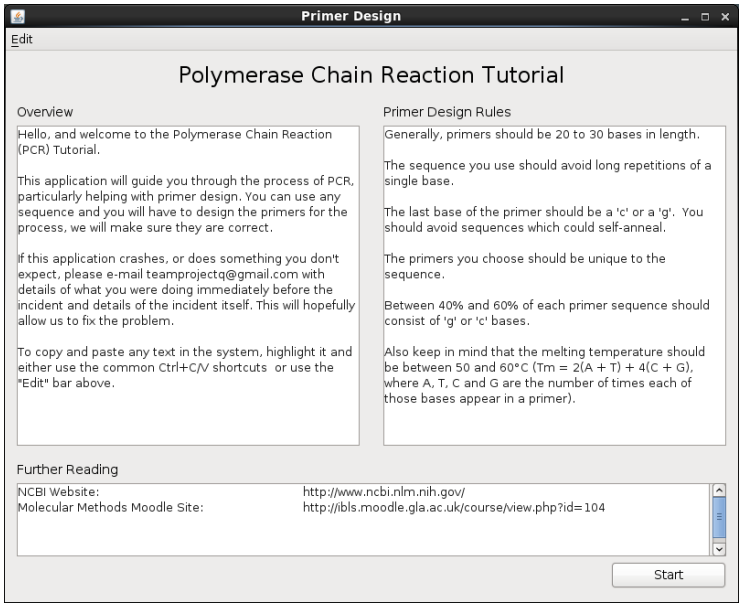

Figure 6: Overview Screen

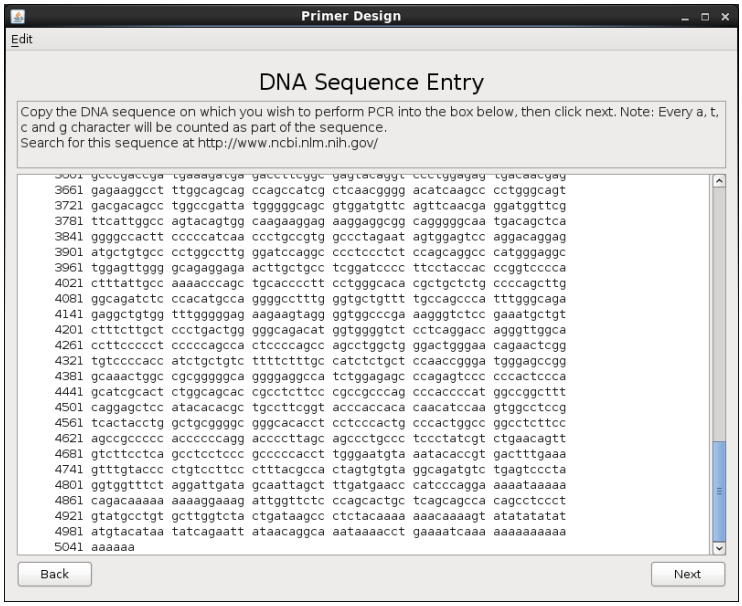

Figure 7: DNA Sequence Entry Example

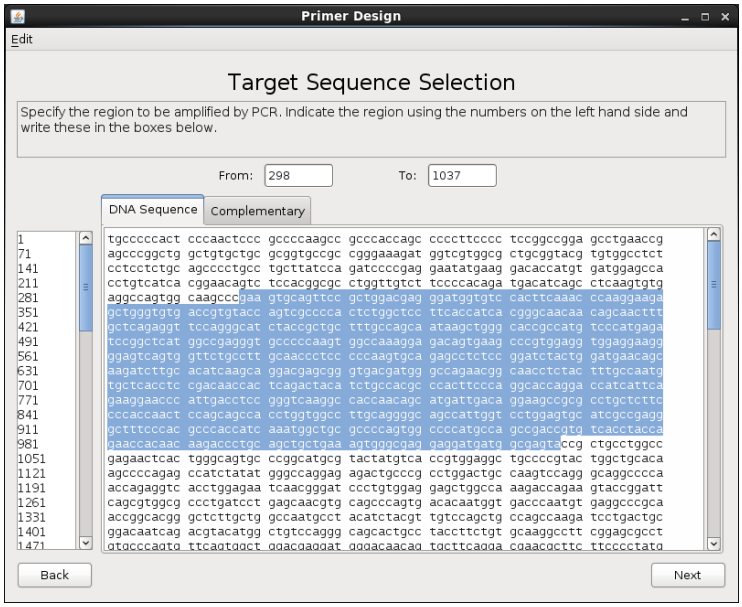

Figure 8: Target Area Selection Example

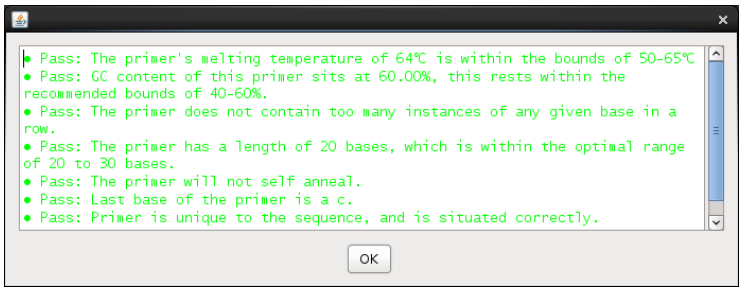

Figure 9: Primer Selection Example

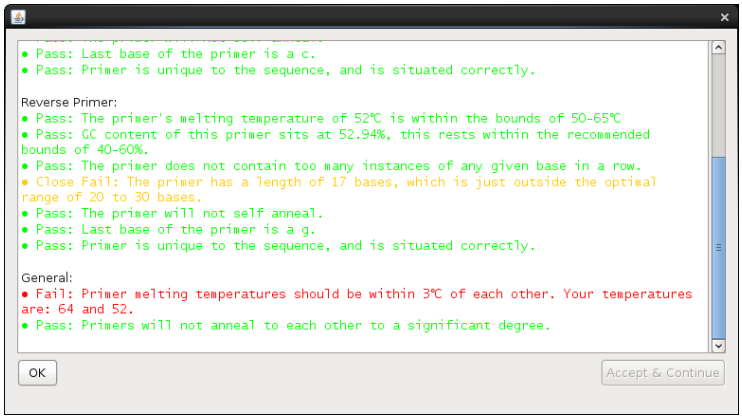

Figure 10: Primer Design Feedback Example

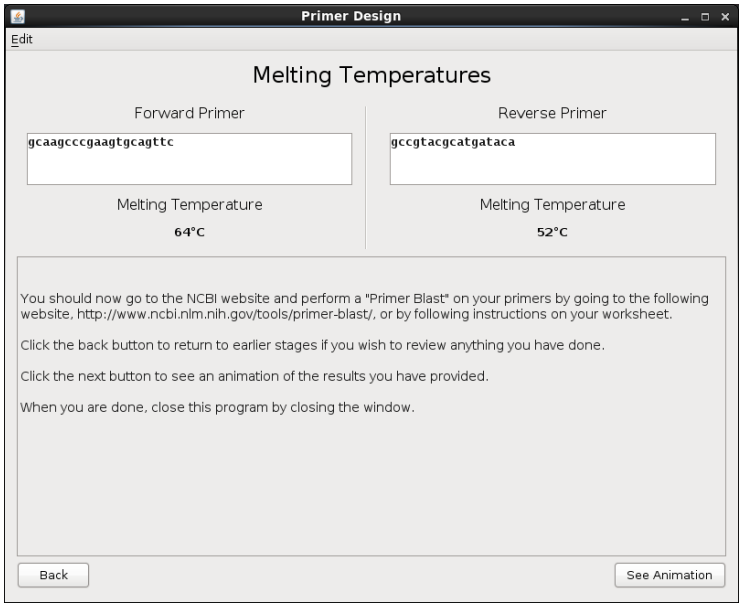

Figure 11: Melting Temperature Screen Example