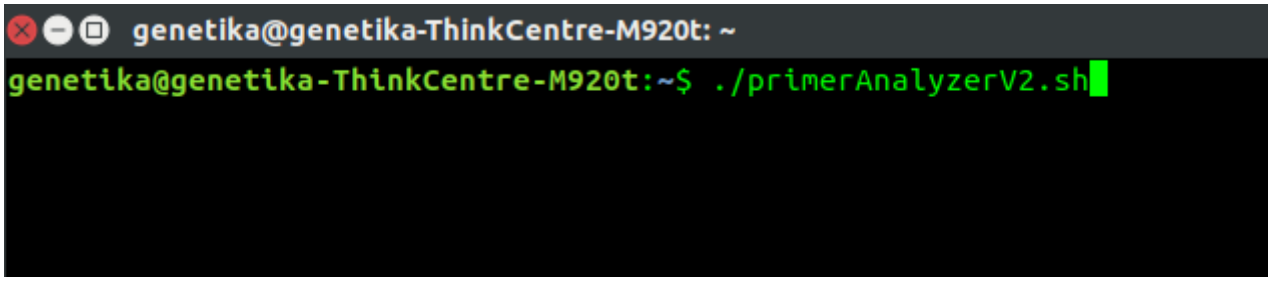
PrimerAnalyzerV2 manual

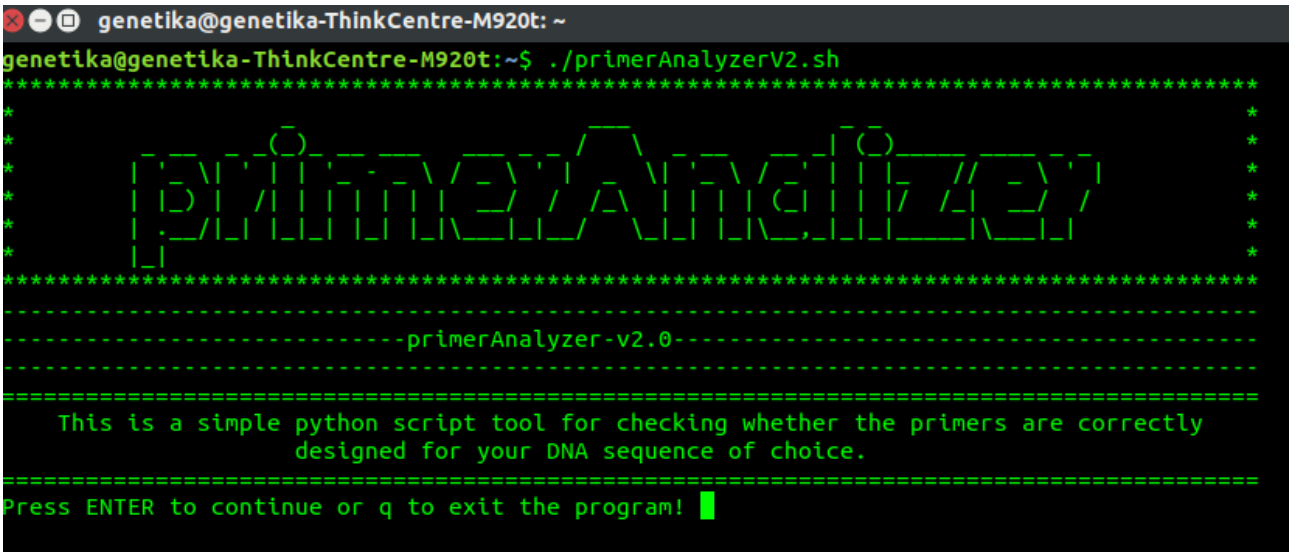
1. Open the Terminal window!

2. Once opened enter the following command and press ENTER:

```
$ ./primerAnalyzerV2.sh
```

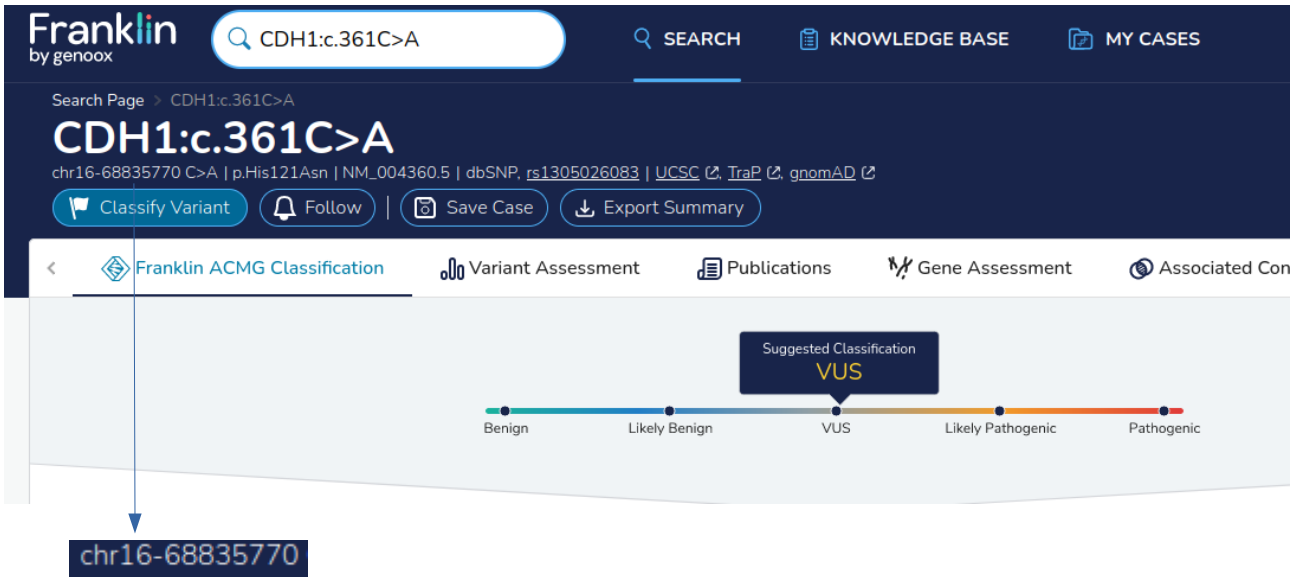


3. After running the command you should see this screeen:



4. Once you've entered the program you will be asked to go to the https://franklin.genoox.com/clinical-db/home and enter the transcript of your variant to the search bar (example for *CDH1:c.361C>A*):
Example one (search by gene): *CDH1:c.361C>A*
Example two (search by transcript): *NM_004360:c.361C>A*

5. Once you get the output of the variant of choice, you should copy the chromosome number and location to the terminal:




```
-----------------------------------IMPORTANT!-----------------------------------------
First part of the analysis is obtaining the DNA of choice!
You will be asked to provide the tool with the chromosome number and the location on the
chromosome where the variant is located as well as the length of the region where it occurs!
Go to https://franklin.genoox.com/clinical-db/home to get the location of the variant of
interest on the chromosome. Press ENTER to continue!
Enter the number of chromosome where the variant is located: 16
Enter the location on the chromosome, where the variant is located: 68835770
Enter the length of the region where the variant occurs: 1
```

Note: length of the region in this case is 1 (C>A); C is replaced by A.

6. Next step is getting the primer3file ready.

```
First step of the analysis done! Press ENTER to continue!
Name the sequence:
a - 150-200
b - 200-250
c - 250-300
d - 300-350
Select the letter for primer product size range: c
(✓) Data saved to primer3file.
```

Name your sequence and choose the product size range.

7. The next step is selecting the primer sequences that the tool provided you with:

```
PRIMER_PAIR_EXPLAIN=considered 25, unacceptable product size 20, ok 5
PRIMER_LEFT_NUM_RETURNED=5
PRIMER_RIGHT_NUM_RETURNED=5
PRIMER_INTERNAL_NUM_RETURNED=0
PRIMER_PAIR_NUM_RETURNED=5
PRIMER_PAIR_0_PENALTY=0.068535
PRIMER_LEFT_0_PENALTY=0.034198
PRIMER_RIGHT_0_PENALTY=0.034337
PRIMER_LEFT_0_SEQUENCE=ttgaagattgcaccggtcga
PRIMER_RIGHT_0_SEQUENCE=gtcaacggtaccaaggctga
PRIMER_LEFT_0=59,20
PRIMER_RIGHT_0=358,20
PRIMER_LEFT_0_TM=59.966
PRIMER_RIGHT_0_TM=59.966
PRIMER_LEFT_0_GC_PERCENT=50.000
PRIMER_RIGHT_0_GC_PERCENT=55.000
```

8. Once you've selected the primer sequences that fit you the most, you'll be asked to copy them to the terminal.

```
Next step is selecting the left primer and right primer sequences!
Press ENTER to continue!
Copy the selected left primer sequence! ttgaagattgcaccggtcga
Copy the selected right primer sequence! gtcaacggtaccaaggctga
(✓) Both primers saved to their respective files.
```

9. Once you've entered both of the primer sequences to the terminal, tool will start checking whether the primers are compatible with your DNA of choice, if everything is OK you should be able to start the analysis.

```
This is the last step of the primer analysis. Press ENTER to continue!
(✓) The length of your raw DNA is OK.
(✓) The nucleotide(s) in your raw DNA sequence of choice is/are highlighted.
(✓) Your raw DNA sequence of choice does not include any illegal characters.
(✓) Length of your both primer sequences is OK.
(✓) None of your primers include any illegal characters.
(✓) Combined length of both of your primers is not longer than the DNA sequence.


OK to start the primer analysis!
Press ENTER to start the analysis!
```

If analysis is successfull you will see this!

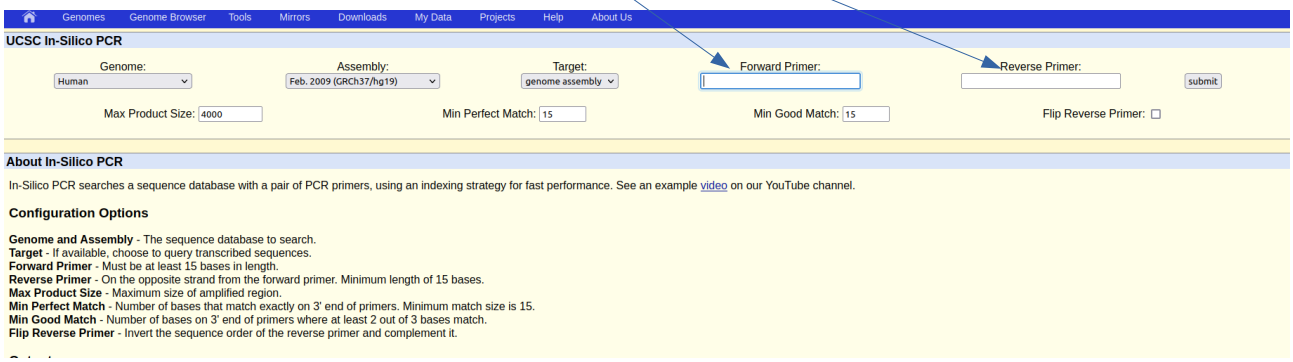
```
Loading |                              | 100%
(✓) DONE


Your analysis of the designed primers is now complete! Press ENTER to save the data.

================================================================================
     ANALYSIS DONE! The results of the analysis have been saved to 'analysisFile.txt'.
================================================================================
```

10. After the analysis is done, you will see this screen:

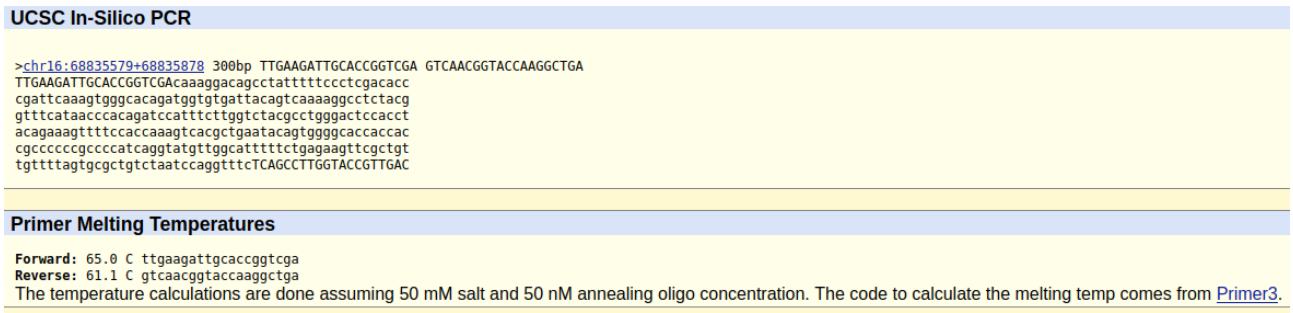
```
================================================================================================
     ANALYSIS DONE! The results of the analysis have been saved to 'analysisFile.txt'.
================================================================================================

Opening the UCSC In-Silico PCR site...

Left primer (FORWARD):
TTCCCGCTGCTTTCTTCCTC

Right primer (REVERSE):
TTCTGGTCCACGTGAGCTTC
```

In a few seconds browser will open and take you to the UCSC In-Silico PCR site, where you will copy the left and right primer sequence respecitvely to perform the In-Silico PCR.

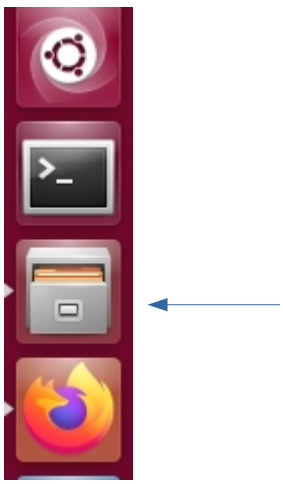
Once on the page

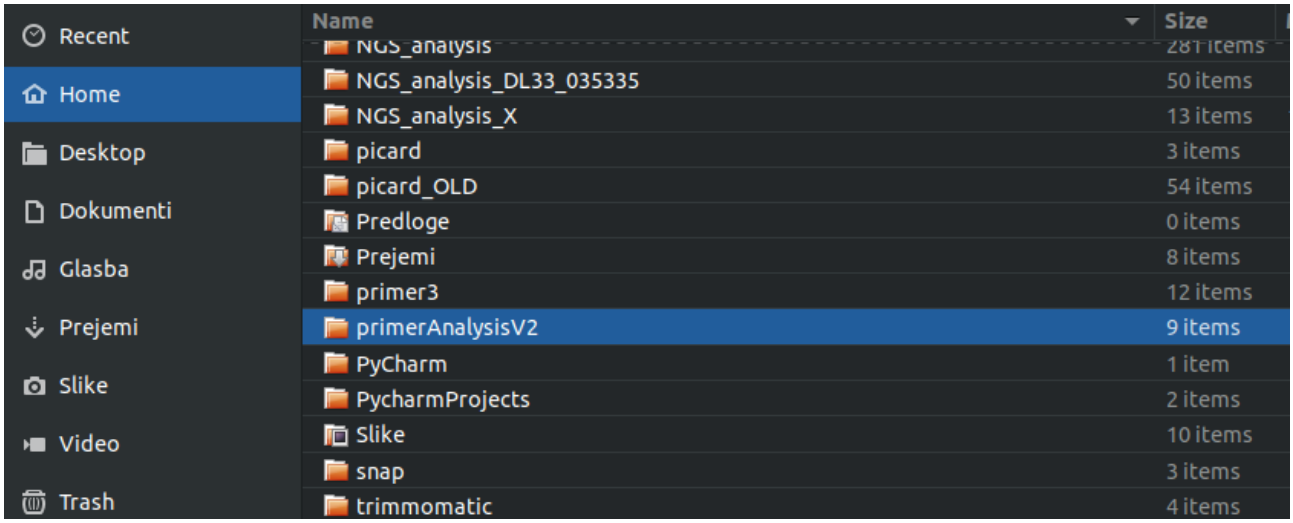


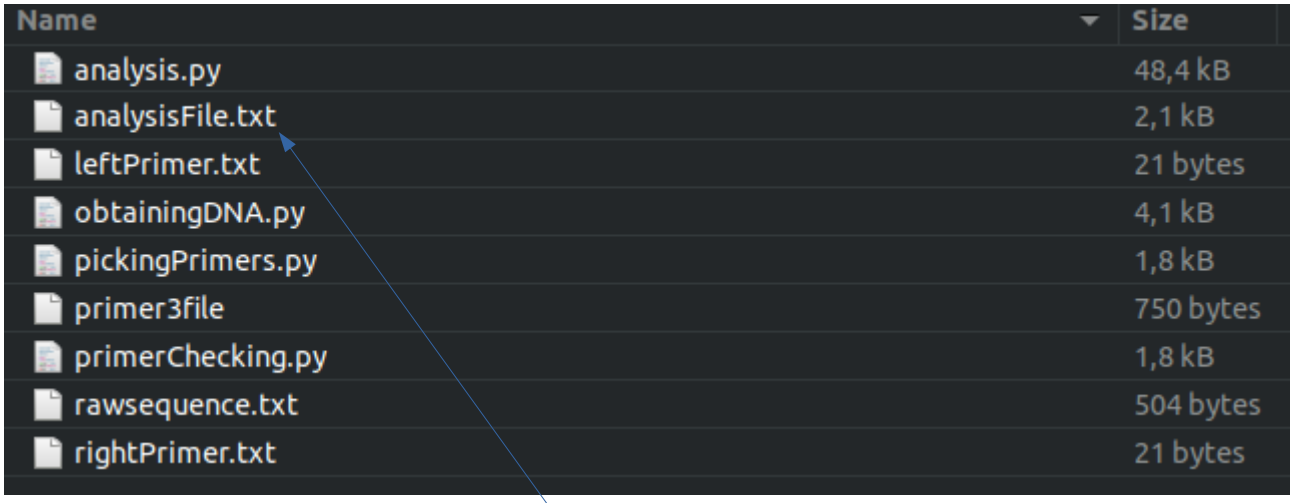
Enter both primers; Forward (Left) and Reverse (Right) and click »submit«.



11. Once the analysis is done the tool creates a report which is located in files Home/primerAnalysisV2 directory under the name »analysisFile.txt«.

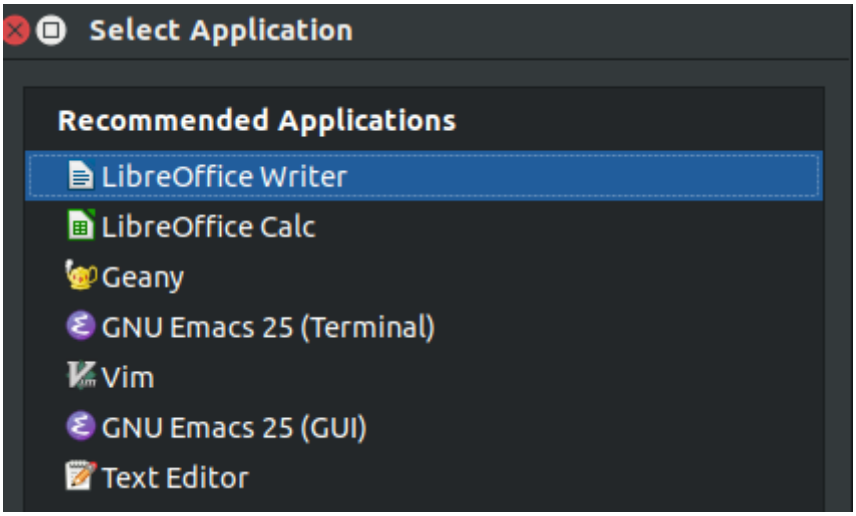






Your report file.

Select open the file with other application and select LibreOffice Writer



12. Once you have the file opened in LibereOffice paste the In-Silico PCR content to the report.

```
{Go to the https://genome.ucsc.edu/cgi-bin/hgPcr and perform the In-Silico PCR
and paste it here!}
```

Choose save as to rename and save the file under the name chosen »GENE_DNAcode«!

And DONE!