# Designing LAMP primers

## A) Select target sequence:

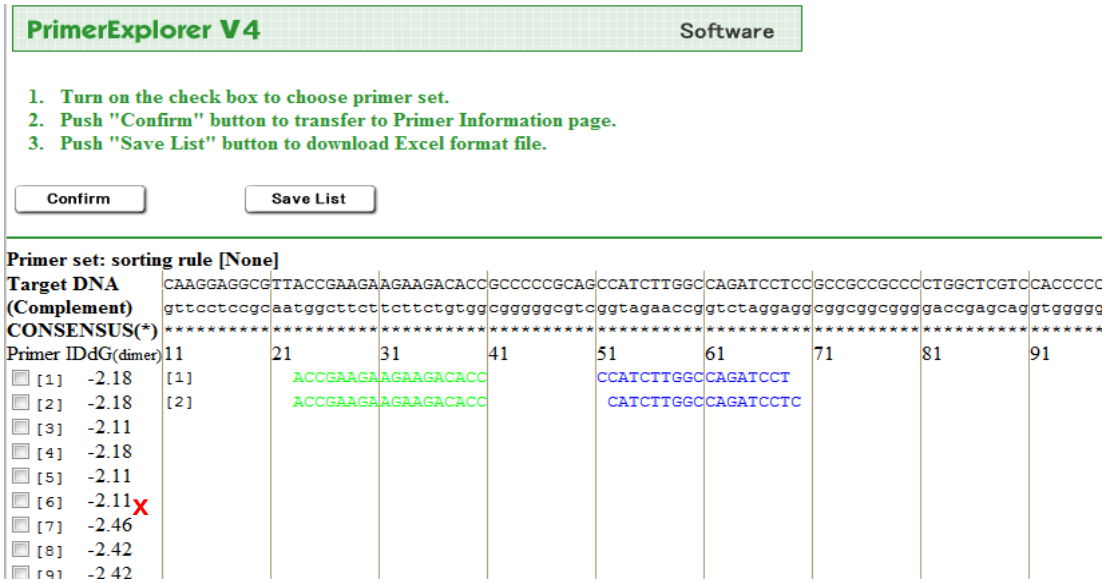
1. Select a highly conserved region of target gene.
2. Target region should be 200 - 2000 bp long.
3. Save the target sequence file in FASTA, GenBank, or text (.txt) format.

## B) LAMP Primer design:

1. Launch LAMP primer design software: http://primerexplorer.jp/elamp4.0.0/index.html
2. Upload the sequence file and click “Primer Design”.
3. (Optional) On the next screen, click on “Detail Settings”. If necessary, select AT-rich or GC-rich option. The Tm of primers may also be varied, but changing the length of primers is not recommended.
4. Click “Generate” to display number of primers designed.
5. Click on “Display” to display the list of primers in new window. This page lists 1-100 primer sets.
6. To look at additional primers, change the page number, and it will open a new window with list of primer sets. Each page lists maximum of 100 primers.

## C) Selecting the primers:

1. Select the desired primer set by checking the box on the left. Lower dG values are usually preferred. Click “Confirm” to select the primer set.

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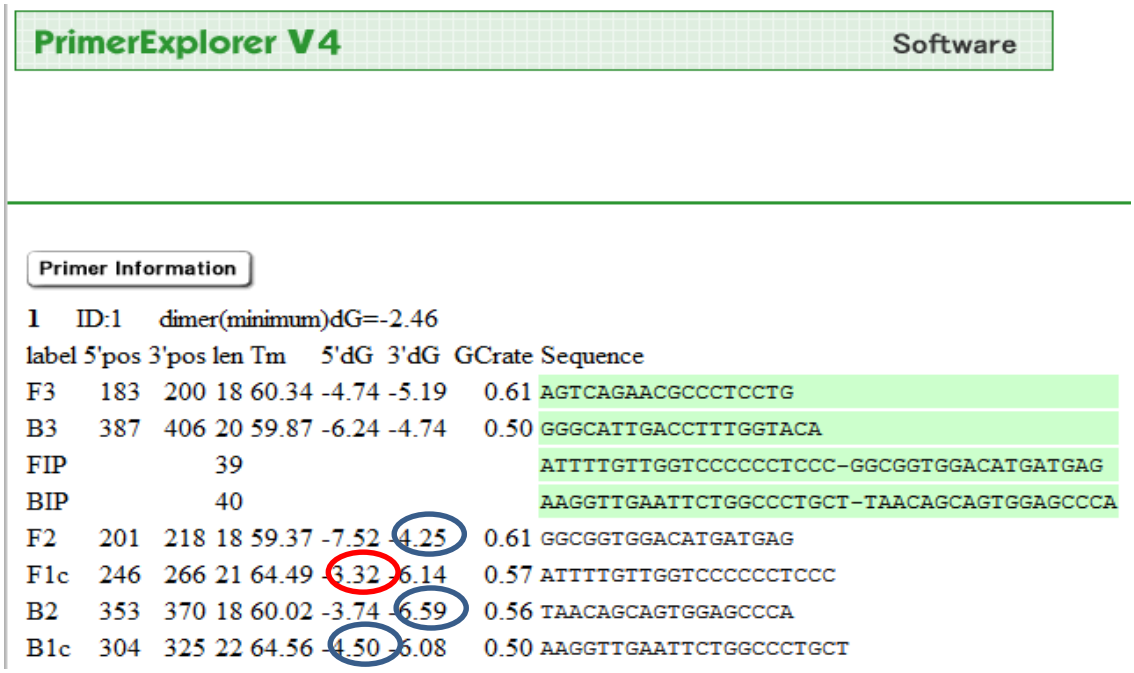
2. A new window will open to show the details of the selected primer set.



PrimerExplorer V4 Software

Primer Information

1 ID:1 dimer(minimum)dG=-2.46

| label | 5'pos | 3'pos | len | Tm | 5'dG | 3'dG | GCrate | Sequence |
|---|---|---|---|---|---|---|---|---|
| F3 | 183 | 200 | 18 | 60.34 | -4.74 | -5.19 | 0.61 | AGTCAGAACGCCCTCCTG |
| B3 | 387 | 406 | 20 | 59.87 | -6.24 | -4.74 | 0.50 | GGGCATTGACCTTTGGTACA |
| FIP | | | 39 | | | | | ATTTTGTTGGTCCCCCCTCCC-GGCGGTGGACATGATGAG |
| BIP | | | 40 | | | | | AAGGTTGAATTCTGGCCCTGCT-TAACAGCAGTGGAGCCCA |
| F2 | 201 | 218 | 18 | 59.37 | -7.52 | -4.25 | 0.61 | GGCGGTGGACATGATGAG |
| F1c | 246 | 266 | 21 | 64.49 | -3.32 | -6.14 | 0.57 | ATTTTGTTGGTCCCCCCTCCC |
| B2 | 353 | 370 | 18 | 60.02 | -3.74 | -6.59 | 0.56 | TAACAGCAGTGGAGCCCA |
| B1c | 304 | 325 | 22 | 64.56 | -4.50 | -6.08 | 0.50 | AAGGTTGAATTCTGGCCCTGCT |

Check the stability of the following regions to confirm that the dG is $\leq$ -4.0 kcal/mol:

- the 3' end at the region F2
- the 5' end at the region F1c
- the 3'end at the region B2
- the 5' end at the region B1c

In the above example, the 3' end of Primer B2 (dG = -6.59 kcal/mol) has the highest stability. The dG of 5' end of Primer F1c (-3.32 kcal/mol) is above -4.0, indicating it is unstable. Therefore, this entire primer set should not be used. Another primer set should be selected from the list.

3. Once a primer set with required stability is found, use the BLAST program (www.ncbi.nlm.nih.gov) to test the specificity of each primer (F3, B3, F2, B2, F1c, and B1c). An ideal primer set should have high specificity for the targeted pathogen.
4. Select the primer set with highest specificity for the target.
5. Save the file by clicking on "Primer information". This file is required to design loop primers. DO NOT CLOSE THE WINDOW until you have copied the primers (F3, B3, FIP, and BIP; highlighted in green) into a separate file (Word or Excel) to save the sequences of the primers. If you close this window (step C.2) you will not be able to recover the primer sequences.

**Troubleshooting:** If no primers are designed or too few primer pairs are generated, try changing parameters:

6. Use a broader range of Tm for primers, but make sure to keep the Tm of F1c/B1c about 3-4 degrees higher than that of F2/B2 and F3/B3 primers.
7. Change the minimum length of F2/B2 and F3/B3 primers to 15 rather than 18.
8. Change the GC rate to 20 or 25 rather than 40.

## D) Designing Loop primers: Including loop primers speeds up the LAMP reaction significantly. Steps for designing loop primers are similar to LAMP primer design.

1. Launch LAMP primer design software: http://primerexplorer.jp/elamp4.0.0/index.html
2. Upload the sequence file saved in step C.5 and click “Primer Design”.
3. Under parameter conditions, you can change Tm of primers and GC rate.
4. Click “Generate” and it will display number of primers designed. If too few primers are generated, try using a broader range of Tm.

   Make sure the result window says “X **sets** were generated” and not “X **pieces** were generated”. If the result screen says primer sets, then both forward and backward loop primers (FL and BL) were generated; if it says primer pieces, then either an FL or BL primer were generated. For a good LAMP reaction, both FL and BL are required.

5. Click on “Display” to display the list of primers in new window. This page lists a maximum of 100 primer sets. Repeat the steps as described in section C to check the specificity of loop primers by BLAST.