SOP: SP064

Designing PCR Primers using VectorNTI

Materials and Reagents:

1. Dell Dimension XPS T450 Rm C210 with VectorNTI installed
2. Electronic sequence chromatogram or text doc of target DNA template,

Protocol:

1. _____ Open the Vector NTI program designed by Invitrogen. Go to File and open the Create New Sequence drop down menu. Click on using sequence editor (DNA/RNA).

2. _____ In the General Tab, rename the sequence appropriately to the template sequence being inputted, where it says NEWMOL

3. _____ In the DNA/RNA Molecule tab, change the bullets to Linear and DNA.

4. _____ Under the Sequence and Maps tab is where the sequence will be inputted. Click on Edit Sequence… and copy and paste the acquired sequence of the target template.

5. _____ Click OK to open the sequence in the main VectorNTI interface.

6. _____ Highlight the entire sequence and go to Primer Design and click on Amplify selected.

7. _____ The Tm (C) should be >=65.0 and <=70.0 (Primer tab)

8. _____ The GC content should be between about 65-80%. (Primer tab)

9. _____ The primer length should be no less than 23bps and no more than 30bps in order to obtain the optimal Ta. (Primer tab)

10. _____ The Tm difference and %GC difference should be < 5 degrees C. (Pairs tab)

11. _____ Exclude T and A nucleotides at the first two or three base pairs of the 3' end of the primers. (3' tab)

12. _____ Once the parameters are set, select OK.

13. _____ In the upper left display a folder labeled "PCR Analysis" will appear. This contains the designed primers. The rating in parentheses indicates how "good" the program thinks the primers are. The higher the rating, the better the primers. At the very least, this rating needs to be over 100.

14. _____ The Tm (melting temperature) should be between 60-70 degrees C for both primers, otherwise redesign the primers, same as above, and only move the outlying boundary of what is highlighted 20bps.

15. _____ Run a BLAST analysis of the designed primers to confirm they align in the proper direction and coordinates of the template genome. (note 1)

16. _____ Pay attention to the information of the first Query only. There should be 100% identity for the size of the submitted primer. Use caution if the preceding Query show more than 50% identity, this could result in non-specific binding of the primer, re-design of the primer may be needed.

17. _____ The primers may also be aligned using VectorNTI to ensure confidence in primer binding location, especially if other non-genomic sequence elements are included in the template. Go to Align in the upper tool bar and select AlignX- open new alignment window.

18. _____ Copy and paste the template sequence into the upper top left display.

19. _____ Next copy and paste the Sense primer sequence into the window.

20. _____ Finally the Antisense primer sequence will not align correctly to the Sense strand because it is "facing" the opposite direction and binds to the antisense strand. Create a new sequence of the Antisense primer (step 1-5), then highlight and right click, then select reverse selection to complementary.

21. _____ Copy and paste the complementary into the alignment window.

22. _____ Select the template sequence and the sense primer and hit Align in the alignment window tool bar.

23. _____ Next, hold Ctrl and select the complementary of the antisense primer and hit Add to Alignment in the alignment window tool bar. Take note that the primers need to bind at opposite ends of the sequence.

24. _____ If the alignment looks satisfactory, then the primers can be ordered through http://www.idtdna.com/ColoradoState-PMF/login.aspx online resource.

Notes:
1. For example: If the template is of Mycobacterium tuberculosis origin, online resources such as Tuberculist database from Institut Pasteur http://genolist.pasteur.fr/TubercuList/, or TB Database http://www.tbdb.org/, may be used to BLAST the primers.

References:
Vector NTI product Manual