Primer design for DNA sepuencing.v1

1. Obtain the target DNA sequence from your source or online, and save it in a word file. Print a hard copy of the sequence.

2. Use T7 and Sp6 primers if these sequences flank your sequence in the plasmid.

3. Pick up a 20-bp sequence at about 500 bp into your full sequence and paste to the query box of "Nucleotide base codes"at: www.basic.northwestern.edu/biotools/oligocalc.html.

4. Click the "Calculate" button to obtain the resulting calculation information. You should move your oligonucleotide sequence upstream and downstream this site to make sure that the temperature of "Salt Adjusted" is about 60°C and the GC content is about 50%.

5. Click the button of "Check self-complementarity" to make sure no potential hairpin formation and self-complementarity.

6. Repeat step 4 and 5 around the sites of 1kb, 1.5kb and so on for additional primers to cover all the whole sequence.

7. Login at www.idtdna.com/Home/Home.aspx using the username "zhenyan" and the password to order the primers. Order your primers as instructed. You should use the PO number to pay the charge.

8. Print the receipt. File everything in two places in Zhen Yan's office: Oligo folder and Gene folder.

9. Spin down the primers for 30 seconds at full speed and then resuspend the oligoes in TE buffer at 200 μM as a stock when the oligoes are received,. Dilute 20 μl in 380 μl of TE buffer (final concentration10 μM) as working stock and store in X oligo-box at 4°C. Store the 200 μM stock at -20°C. Log the primer information in the database (Yan'Office\Yan data\Database\YanLabDatabase). Please fill in the complete information.