

Primer Design for RT-PCR

1. Go to www.pubmed.gov
2. Select “Nucleotide” in the black menu or drop down “Search” box
3. Search for the gene sequence of interest
 - a. Example: “Peripherin Rat”
 - b. Search the records for the one that most appropriate fits the gene you are looking for.
 - c. If possible, do not use postulated gene sequences. You can also search the literature to see if other papers have done PCR for the same gene you are looking for. They should provide the PMID (pubmed ID) for the gene they used (i.e. AF031878)
4. Copy the gene sequence of interest
5. Open up Primer Express 3.0
6. Click “Continue with Primer Express” button
7. Go to “File -> New...”
 - a. Type :Taqman MGB Quantification
 - b. Parameters: Default
8. Paste sequence in sequence box
9. Click on Green Arrow button to “Find primers/probe”
10. The program will list out various types of primer sequences. The best bet to use is the one that has the lowest penalty score (meaning fewest problems in terms of primer-dimmers, hairpins, etc.)
11. Double check to make sure there are no hairpins or dimmers from the bottom right corner of the program.
12. Keep a record of all the primers and probes. Include information such as gene name, species, primer name, sequence, product size, annealing temperature. Also make sure that you keep a copy of the gene sequence that you used to design the primers. Keep the pubmed gene sequences in another file.
13. Order your primer/probe from a company that offers custom oligonucleotides (Invitrogen, Operon for example). Make sure that you label them in a way that you will know which one is which. Cut and paste the sequence from your record when you can to avoid typo. In general 50nmole is sufficient. You want it to be salt-free.
14. When you receive the primers from the company make a 0.5mM stock solution in PBS or DNase/RNase free water (50nmol in 0.1ml). Keep it in the freezer.
15. Dilute the stock solution 10 fold to make a 50µM working solution (10µl stock plus 90 µl PBS/water).

Note: When you run these primers for the first time, make sure you initially run a negative control to make sure the signal that is amplified is from the cDNA and not from the primers themselves.