

Primer BLAST Activity

A frequent use of nucleotide-nucleotide BLAST is to check oligonucleotides for hybridization or PCR. The goal most people have when doing this is to make sure that the primer will give a unique product from the target genome or cDNA population.

Check the following pair of candidate PCR primers in a search against the RefSeq mRNA database. Identify the gene that is amplified. In your opinion are these good primers?

F1 GTCAAGTGGCAACTCCGTCAG

R1 TTGAGAGATGGATTGTTGCGC

Now try these modified primers. There is one mismatch in each near the middle.

F2_mod GTCAAGTGGCTACTCCGTCAG

R1_mod TTGAGAGATGTATTGTTGCGC

Do you still see the previous hits?

Click on the link to the first hit [NM_001190942.1](#). This should take you directly to the Entrez Nucleotide record. From here click the link on the side 'Pick Primers'. Using default settings run Primer BLAST. Compare the primer pairs to those above. Why might the primer pairs found using the target sequence be better to use?