

# PRIMER DESIGN USING CLC GENOMICS

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## ***Tutorial: a quick way to find the location of primers within a gene or the expected size of the resultant PCR product***

Get a sequence from NCBI nucleotide database. For this tutorial, we will use CO1 (cytochrome oxidase subunit 1 (KJ555477.1))

### **1. Using primer3**

Paste your sequence to the box provided

### **2. Using primer-blast**

- a. Go to the [Primer BLAST](#) submission form.
- b. Enter the target sequence in FASTA format or an accession number of an NCBI nucleotide sequence in the PCR Template section of the form. If the NCBI mRNA reference sequence accession number is used, the tool will automatically design primers that are specific to that splice variant.
- c. If one or both primer sequences are to be used in the search, enter these in the Primer Parameters section of the form. Primer BLAST performs only a specificity check when a target template and both primers are provided.
- d. In the Primer Pair Specificity Checking Parameters section, select the appropriate source Organism and the smallest Database that is likely to contain the target sequence. These settings give the most precise results. For broadest coverage, choose the nr database and do not specify an organism.
- e. Click the "Get Primers" button to submit the search and retrieve specific primer pairs.

## ***Validating specificity of your primers***

1. Concatenate the two primer sequences into one sequence separated by ~20 Ns and enter into BLAST sequence box.
2. Example: AATGATCCGCGACGCTAATGAGGANNNNNNNNNNNNNNNNNNNNNNNNNNNGG
3. TTTGCTCGTTGGCTTACTGCTT
4. Note: both primers can be in the same orientation as BLAST will search both strands for matches.
5. Before submitting, narrow the search by selecting the species, if known; otherwise, choose Nucleotide Collection (nr/nt). If you're looking for RT-PCR primers, select the reference mRNA sequences (refseq\_mRNA) database.
6. Under "Program Selection", select the "Somewhat similar sequences (blastn)" program.
7. Under Algorithm parameters, decrease word size to 16, increase expect threshold (or leave it as default), and turn off the low complexity filter.