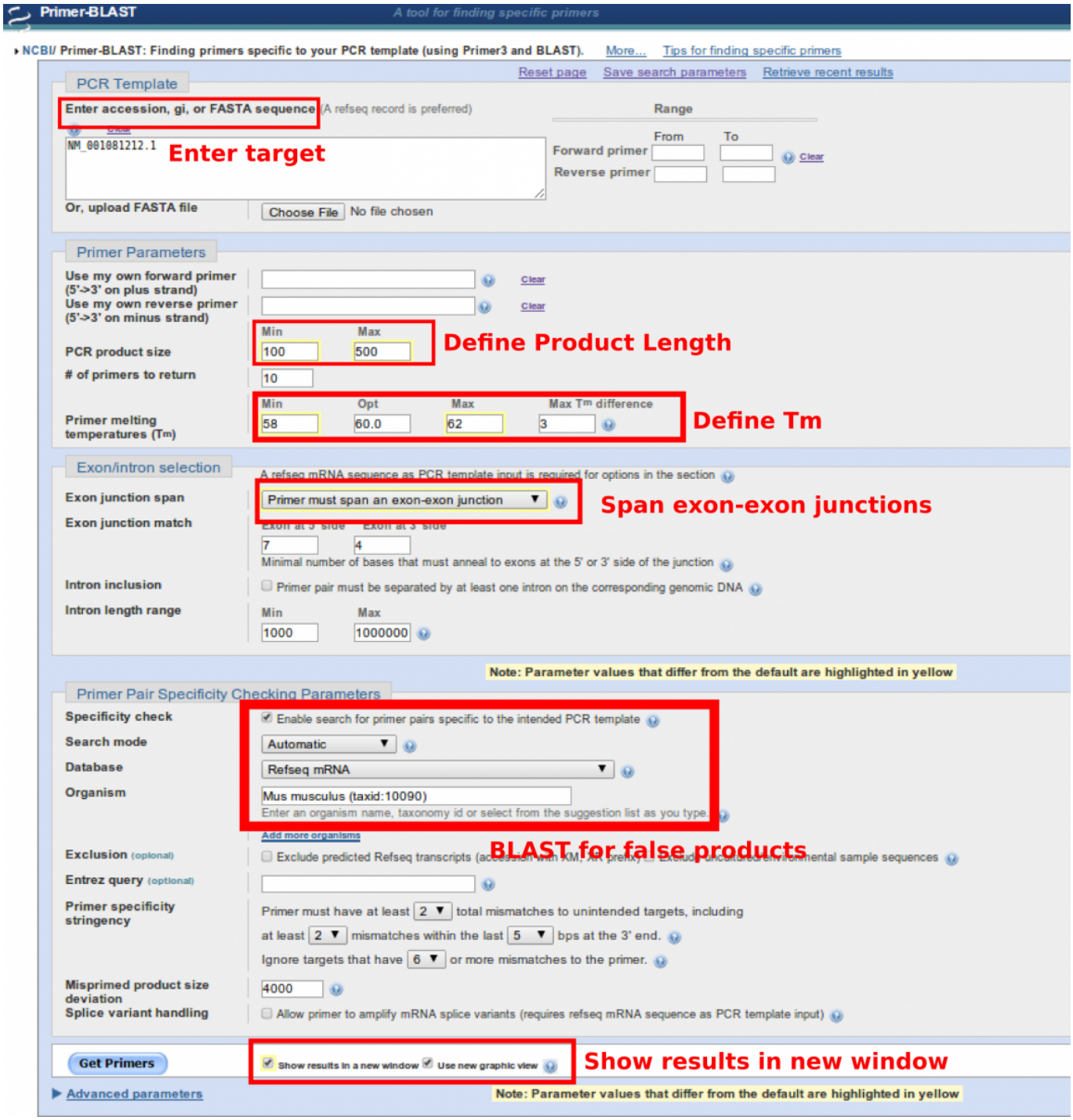
Primer-BLAST

Primer-BLAST is a combination of a program called **Primer3** that aids in the design of primers with specific properties and BLAST. Primer-BLAST allows for the construction of primers for qPCR where the user can specify the melting temperature, reduce the amount of self-priming and to span exon-exon junctions in order to avoid amplification of contaminating genomic DNA. After the design of primers, each primer pair is sent into BLAST to identify if similar products within the genome of the model organism will also be primed and amplified. This process ensures that the primers designed fall within your design parameters and most likely only amplify your gene of interest.

1. Enter the sequence OR the NCBI accession number for the gene of interest
2. Define the PCR product length
    1. limiting the product between 100-500 permits for good efficiency in thqPCR
    2. longer products may not be efficiently replicated depending on your cycling protocol
3. Define the desired melting temperature (T_m) of the primers (minimum, optimal, maximum, difference between the set)
    1. 60ºC is fairly high and will aid in the enhanced specificity of the primer with the target during amplification to avoid false priming
    2. Try to have the T_m as close as possible so that they are annealing about equally
4. Choose the option “Primer must span an exon-exon junction”
    1. This aids in amplifying cDNA and not genomic DNA that may be contaminating
    2. do not select this if it a single exon gene as this will fail
5. Select Refseq mRNA as the database to search against.
    1. **Refseq** provides sequences to naturally occurring sequences.
    2. Things like plasmid sequences or vector constructs do not show up in Refseq
6. Select the organism you are BLASTing against
    1. there are options for model organisms as well as cell lines
    2. If you are using something like PC12 cells, you may use *Rattus norvegicus* or PC12 genome since that is also an option in the database
7. Evaluate the location of the primers and the other parameters. We generally choose primers at the 3? end of the RNA since RT reactions often have a 3? bias in eukaryotes by using oligo-dT priming in the reverse transcription


Primer-BLAST
A tool for finding specific primers
NCBI/ Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST).
More...
Tips for finding specific primers
Reset page
Save search parameters
Retrieve recent results
PCR Template
Enter accession, gi, or FASTA sequence
(A refseq record is preferred)
NM_001081212.1
Enter target
Range
From
To
Forward primer
Reverse primer
Clear
Or, upload FASTA file
Choose File
No file chosen
Primer Parameters
Use my own forward primer (5'->3' on plus strand)
Use my own reverse primer (5'->3' on minus strand)
PCR product size
Min
Max
100
500
Define Product Length
# of primers to return
10
Primer melting temperatures (Tm)
Min
Opt
Max
Max Tm difference
58
60.0
62
3
Define Tm
Exon/intron selection
A refseq mRNA sequence as PCR template input is required for options in the section
Exon junction span
Primer must span an exon-exon junction
Span exon-exon junctions
Exon junction match
Exon at 5' side
Exon at 3' side
7
4
Minimal number of bases that must anneal to exons at the 5' or 3' side of the junction
Intron inclusion
Primer pair must be separated by at least one intron on the corresponding genomic DNA
Intron length range
Min
Max
1000
1000000
Note: Parameter values that differ from the default are highlighted in yellow
Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq mRNA
Organism
Mus musculus (taxid:10090)
Enter an organism name, taxonomy id or select from the suggestion list as you type.
Add more organisms
BLAST for false products
Exclusion (optional)
Exclude predicted Refseq transcripts
Entrez query (optional)
Primer specificity stringency
Primer must have at least 2 total mismatches to unintended targets, including
at least 2 mismatches within the last 5 bps at the 3' end.
Ignore targets that have 6 or more mismatches to the primer.
Misprimed product size deviation
4000
Splice variant handling
Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)
Get Primers
Show results in a new window
Use new graphic view
Show results in new window
Advanced parameters
Note: Parameter values that differ from the default are highlighted in yellow