NCBI Primer-BLAST

An online tool for designing target-specific PCR primer pairs (with internal probes)

https://www.ncbi.nlm.nih.gov/tools/primer-blast/

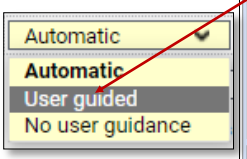
National Center for Biotechnology Information • National Library of Medicine • National Institutes of Health • Department of Health and Human Services

Scope and Access

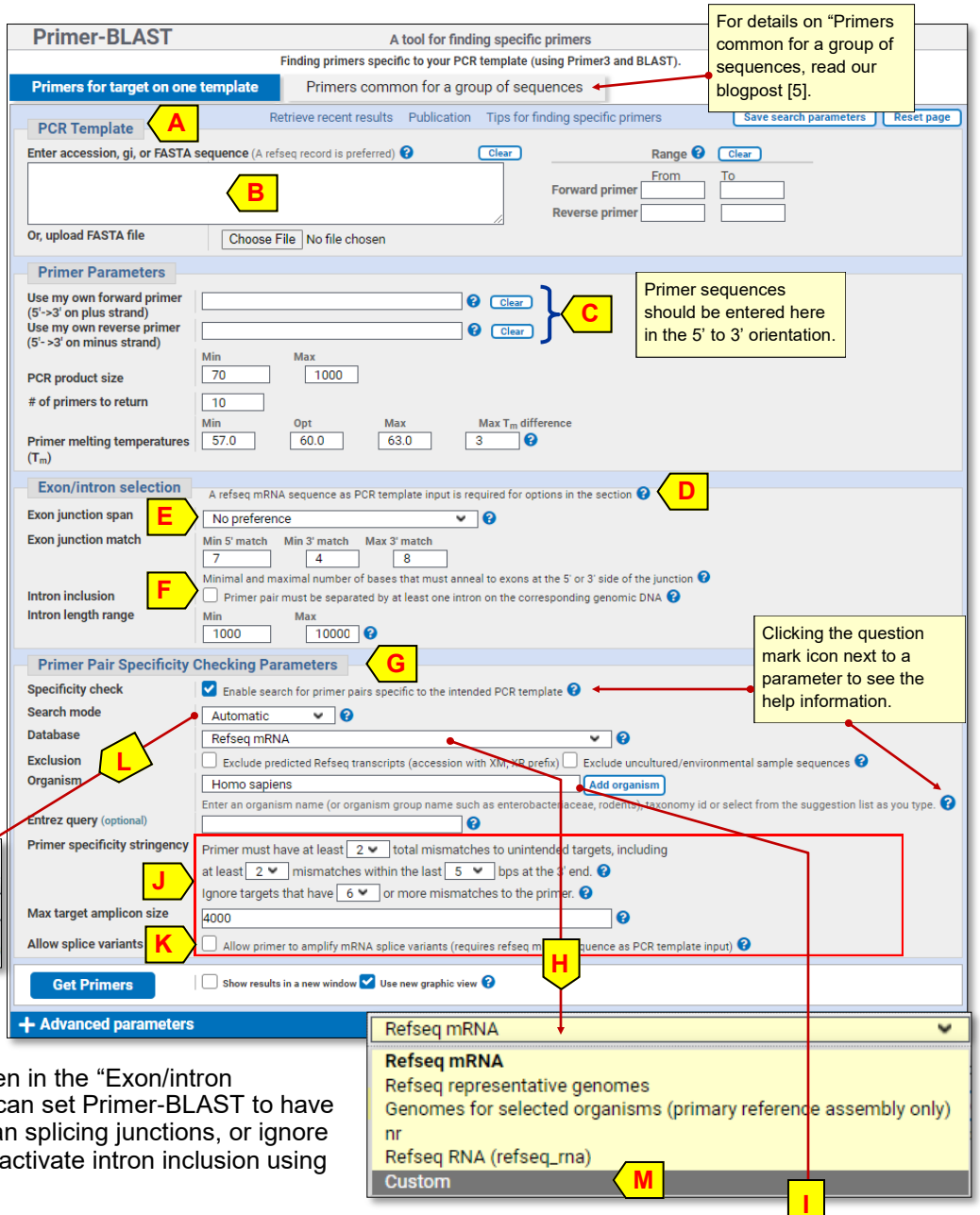
Primer-BLAST [1] is a PCR primer design and specificity checking tool from NCBI. It picks primers using the Primer3 algorithm [2] and then uses BLAST [3] to screen for primers specific to the input template. Similar to other BLAST searches, you can limit a Primer-BLAST search to specific taxa or a custom st of sequences specified by Entrez queries. It presents candidate primers along with their alignment to targets. Primer-BLAST is a web only application accessible through the "Specialized BLAST" section of the BLAST homepage (https://blast.ncbi.nlm.nih.gov/) or directly at https://www.ncbi.nlm.nih.gov/tools/primer-blast/.

Accepted Inputs

The Primer-BLAST search page (right) defaults to single template input form. This contains multiple sections. The top one (**A**) takes your input and allows the adjustment to a basic set of parameters. Given a template alone (**B**), Primer-BLAST will find a set of primer pairs optimal for PCR amplification. Primer-BLAST also accepts existing primers (**C**) and supports other combinations of input: 1) a primer pair with its template, 2) a template with a single primer, and 3) a pair of primers alone. In the case 1), Primer-BLAST validates the primer pair for the template sequence and performs a specificity check if this option is selected. In the case 2), Primer-BLAST finds candidate primers that work with the input primer and reports their target-specificity. In case 3) with primer pairs alone, Primer-BLAST finds the amplification target and provides primer template alignments.



With a RefSeq mRNA accession as a template, Primer-BLAST can take exon junctions into consideration through options given in the "Exon/intron selection" section (**D**). There you can set Primer-BLAST to have candidate primers span or not span splicing junctions, or ignore those junctions (**E**). You can also activate intron inclusion using the checkbox (**F**).

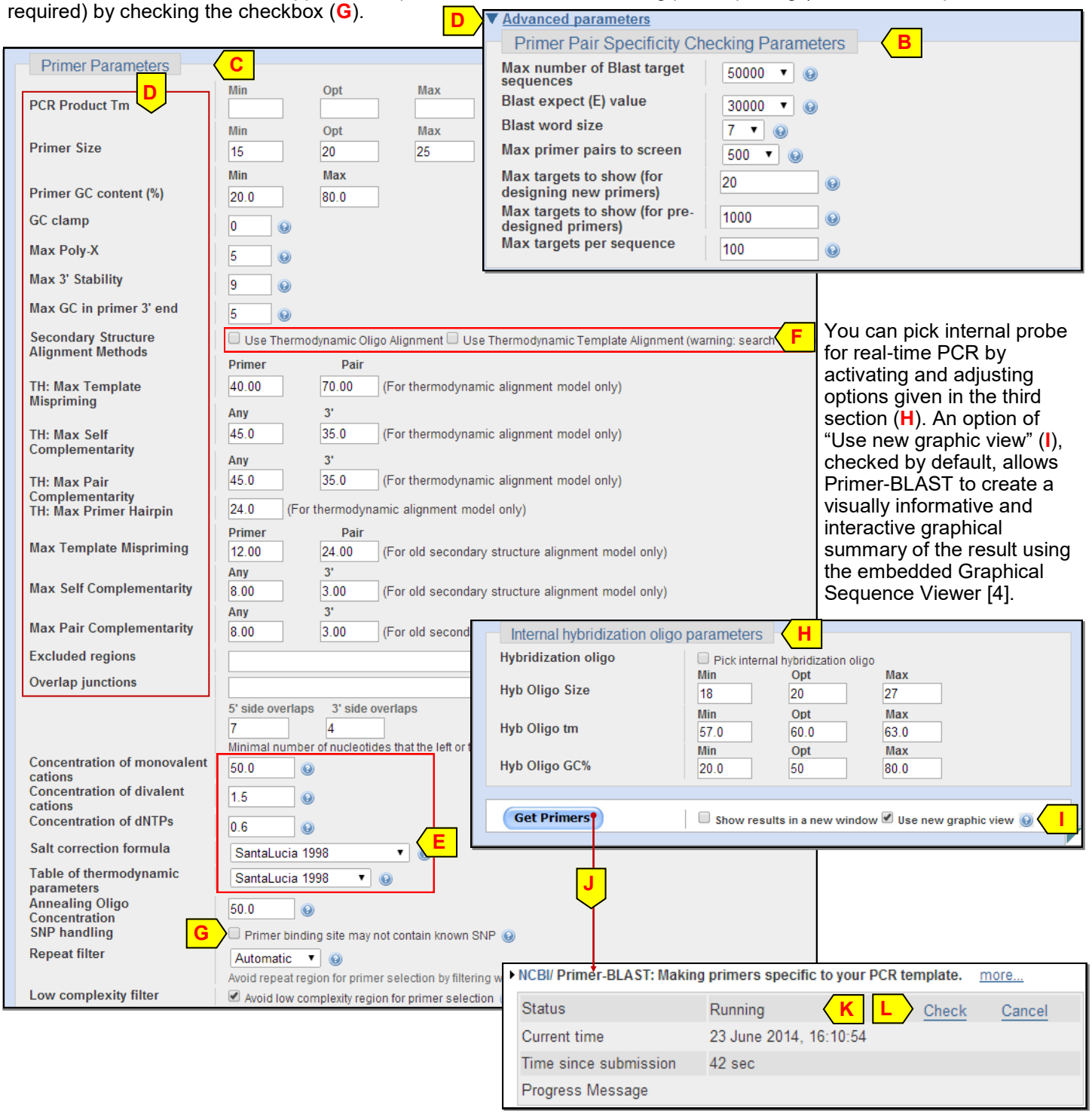


In the Primer Specificity Checking Parameters section (**G**), you can select different databases using the pull-down menu (**H**), restrict the search to a different organism by selecting from the suggested list upon typing (**I**), adjust the stringency of the specificity checking through parameters listed below the database (**J**), and check the box (**K**) to generates primer pairs that amplify all known transcript variants for the same gene. You can also adjust the search mode (**L**) to increase the chance of finding specific primers when the input template is highly similar to other targets in the database, and use the "Custom" database (**M**) option to upload a custom set of sequences (accessions or FASTA) for use as the specificity checking database.

Contact: info@ncbi.nlm.nih.gov

Advanced Parameters for Primer-BLAST

Clicking the "Advanced Parameters" link (**A**) toggles open the section with infrequently adjusted parameters. The first section (**B**) contains parameters for BLAST that specify the exhaustiveness of specificity checking. The second section (**C**) contains parameters specific to the selected primers and their PCR products (**D**): such as, the Tm of the PCR product, the primer length, the primer GC content, and GC clamps at the 3'-end of the primer. It also contains settings on PCR buffer conditions (**E**) since they can greatly affect the primer Tm calculation. Note that, in favor of search speed, Primer-BLAST does not use thermodynamic alignment features by default (**F**). This section also allows you to instruct Primer-BLAST to take SNPs mapped to template into consideration during primer picking (Human RefSeq accession required) by checking the checkbox (**G**).



You can pick internal probe for real-time PCR by activating and adjusting options given in the third section (**H**). An option of "Use new graphic view" (**I**), checked by default, allows Primer-BLAST to create a visually informative and interactive graphical summary of the result using the embedded Graphical Sequence Viewer [4].

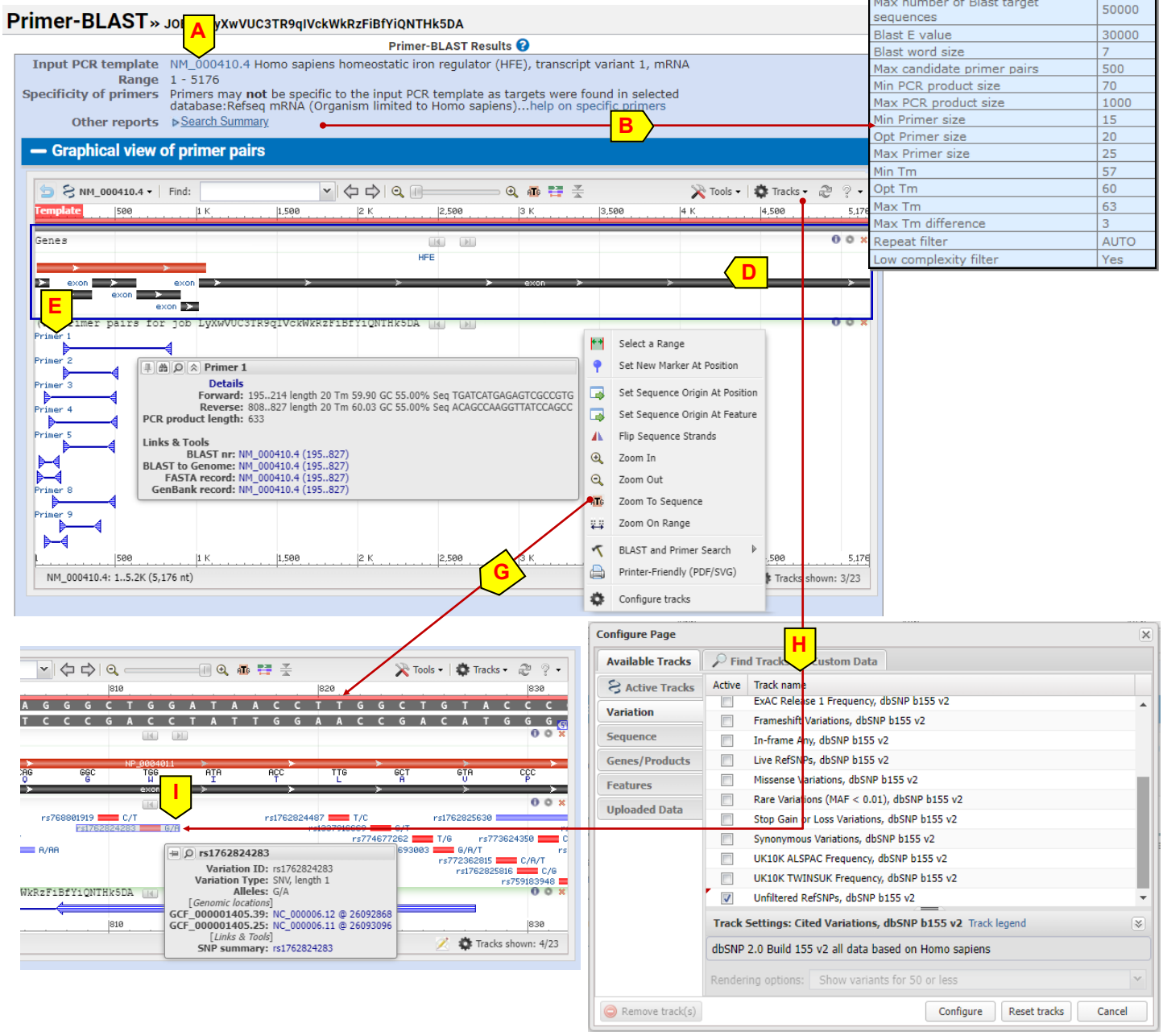
Submitting a Search

Click the "Get Primers" button (**J**) to submit the search. The browser tracks the progress of the submitted job via an intermediate polling page (**K**) and displays the result when it becomes available. You can manually check it by using the "Check" link (**L**).

Primer-BLAST Results: the Graphical Summary

The Primer-BLAST displays results by breaking them into several sections: the search summary, the graphical overview, and a tabular list of primer pairs with their properties plus alignments to the annealing sites on different targets. The summary section (**A**) reiterates the template, an informational message with additional details on the primers returned, and a "Search Summary" link (**B**) with detailed search statistics.

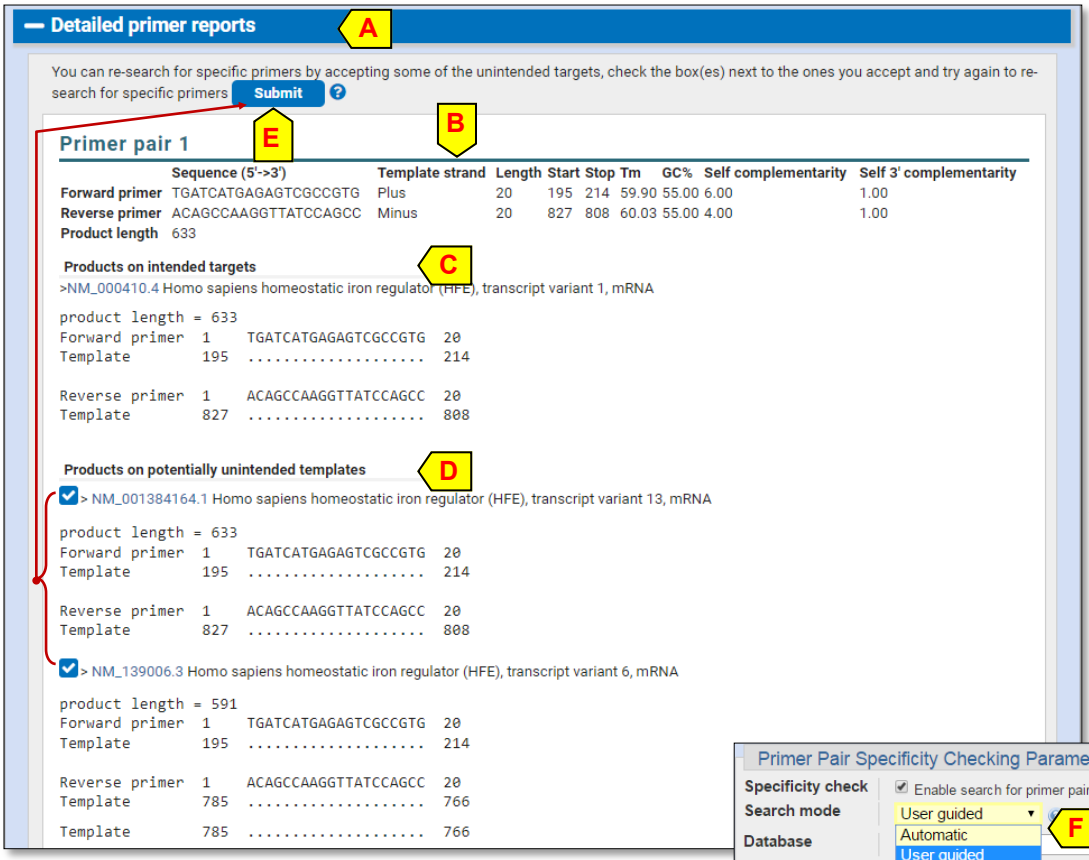
Search parameters and other details	
Number of Blast hits analyzed	432066
Entrez query	
Min total mismatches	2
Min 3' end mismatches	2
Defined 3' end region length	5
Mismatch threshold to ignore targets	6
Misprimed product size deviation	4000
Max number of Blast target sequences	50000
Blast E value	30000
Blast word size	7
Max candidate primer pairs	500
Min PCR product size	70
Max PCR product size	1000
Min Primer size	15
Opt Primer size	20
Max Primer size	25
Min Tm	57
Opt Tm	60
Max Tm	63
Max Tm difference	3
Repeat filter	AUTO
Low complexity filter	Yes



For the template sequence submitted in RefSeq accession format, NM_000410 in this case, the Graphical Sequence Viewer provides much more information. Specifically, it displays:

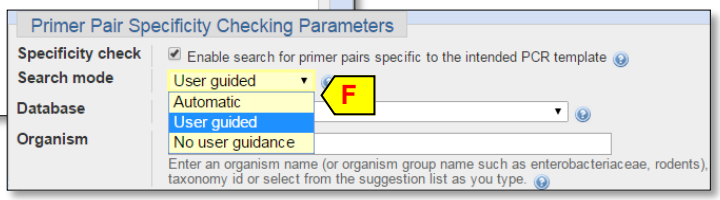
- A clear overview of the results in the context of the target sequence, by showing the exon boundaries of the template plus its annotated protein product, pulled out from the feature table of this record (**D**),
- The candidate primer pairs, their predicted products, and exact locations on the template (**E**),
- The properties of a specific primer pair, viewable in the hovering activated popup (**F**),
- The sequence-level details of the annealing site through the "Zoom to Sequence" option (**G**) in the right-click menu
- The highlighted relationship of suggested primers with other features through the "Configure page" dialog box (**H**) activated by clicking the "Tracks" button, with the example shown being one of the known SNPs (**I**) mapped to one of the primer's annealing site on this human mRNA template.

Primer-BLAST Results: Primer Pairs and Their Alignment to Targets



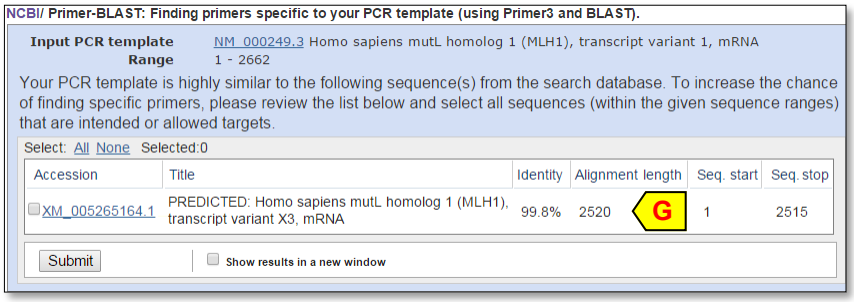
The "Detailed Primer Reports" section (**A**) contains the details for returned primer pairs. Each primer pair is in its own subsection (**B**), with a summary of basic properties along with alignments to their intended target (**C**) and to potentially unintended targets (**D**).

In the example pair of primers for human HFE gene transcript variant 1 (NM_000410) also amplify variants 13 and 6 (under **D**). Alignments, which are considered unintended in automatic mode. Checking them will mark them as intended targets in re-search through Submit button (**E**).

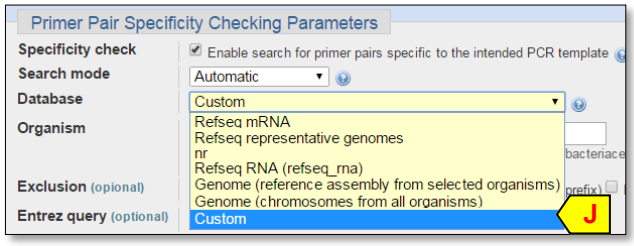


More on "User guided" Mode and "Custom" Database

The "User guided" (**F**) search mode allows you to instruct Primer-BLAST whether certain targets that are highly similar to the input template should be considered as intended target upon job submission (**G**).



The Custom database option (**H**) allows you to provide your own input dataset for specificity checking. System constraints limit the size of sequence files to 300 MB. For sequences from the NCBI Nucleotide database, you can use their accessions or GI's to specify a larger custom dataset.



References

1. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. (2012) Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 13:134.
2. Rozen, S and Skaletsky, HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) *Bioinformatics Methods and Protocols: Methods in Molecular Biology.* Humana Press, Totowa, NJ, pp 365-386.
3. Altschul, SF, Madden, TL, Schäffer, AA, Zhang, J, Zhang, Z, Miller, W and Lipman, DJ (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res. 25:3389-3402.
4. The Graphical Sequence Viewer Factsheet: https://ftp.ncbi.nih.gov/pub/factsheets/Factsheet_Graphical_SV.pdf.
5. NCBI Insight Blogpost: Primer-BLAST now designs primers for a group of related sequences. https://go.usa.gov/xuJcg

Technical Assistance

Please send you feedback, questions and bug reports to blast-help@ncbi.nlm.nih.gov