

Primer Design with Primer 3

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11:59 AM

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<http://fokker.wi.mit.edu/primer3/input.htm>

[Edit 2013.03.12 by James: A new version of Primer 3 is at <http://primer3.wi.mit.edu/>]

I have had great success with Primer 3 and find it consistently picks good primers. If not stated in the protocol leave settings at their default.

1. Open Primer 3 webpage, <http://fokker.wi.mit.edu/primer3/input.htm>
2. Paste the sequence you want to find primers for in the top box
3. If you already have Left (forward) or Right (reverse) primers enter them into the box below the sequence box
4. Enter the product size range you wish to be amplified
5. Choose a primer size between 20 – 35 bp, with no optimum
6. Choose a primer Tm between 55 - 65°C with the optimum being 60°C
7. Choose a max Tm Difference between the primers of 5°C
8. Don't worry about product Tm, leave blank
9. Choose a primer %GC content between 40 and 60% with the optimum being 50%
10. Leave the rest of the setting at the default, except GC clamp
11. Set GC clamp at 1, 2, or 3 if you want, this requires the program to pick primers with 1, 2, or 3 G's or C's at the 3' end

** it is usually advised to have a G or a C at the 3' end but I leave this at 0 and my primers that do not have a G or C at the 3' end work great **

12. If you want to check the primers that Primer 3 chooses, use Integrated DNA technologies website Oligo Analyzer

<http://www.idtdna.com/SciTools/SciTools.aspx?cat=DesignAnalyze>

Sample of Important part of the Primer 3 webpage

Primer3 (v. 0.4.0) Pick primers from a DNA sequence.

Checks for mispriming in template, disclaimer, Primer3 Home, Primer3plus interface, cautions, FAQ/WIKI

Paste source sequence below (5'>3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINES, etc.) or use a [Mispriming Library](#) (repeat library): NONE

Pick left primer, or use left primer below: Pick hybridization probe (internal oligo), or use oligo below: Pick right primer, or use right primer below (5' to 3' on opposite strand):

Pick Primers Reset Form

Sequence Id: A string to identify your output.

Targets: E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the [source sequence](#) with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.

Excluded Regions: E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the [source sequence](#) with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.

Product Size Ranges 75-200

Number To Return 5 Max 3' Stability 9.0

Max Repeat Mispriming 12.00 Pair Max Repeat Mispriming 24.00

Max Template Mispriming 12.00 Pair Max Template Mispriming 24.00

Pick Primers Reset Form

General Primer Picking Conditions

Primer Size Min: 20 Opt: Max: 35

Primer Tm Min: 55 Opt: 60.0 Max: 65 Max Tm Difference: 5.0 [Table of thermodynamic parameters:](#) Breslauer et al. 1986

Product Tm Min: Opt: Max:

Primer GC% Min: 40.0 Opt: 50 Max: 60.0

Max Self Complementarity: 8.00 Max 3' Self Complementarity: 3.00

Max #Ns: 0 Max Poly-X: 5

Inside Target Penalty: Outside Target Penalty: 0 [Note: you can set Inside Target Penalty to allow primers inside a target.](#)

First Base Index: 1 CG Clamp: 0

Concentration of monovalent cations: 50.0 Salt correction formula: Schildkraut and Lifson 1965

Concentration of divalent cations: 0.0 Concentration of dNTPs: 0.0

[First Base Index:](#) [CG Clamp:](#)
[Concentration of monovalent cations:](#) [Salt correction formula:](#) Schildkraut and Lifson 1965 ▾
[Concentration of divalent cations:](#) [Concentration of dNTPs:](#)
[Annealing Oligo Concentration:](#) (Not the concentration of oligos in the reaction mix but of those annealing to template.)
 [Liberal Base](#) [Show Debuging Info](#) Do not treat ambiguity codes in libraries as consensus [Lowercase masking](#)

[Pick Primers](#) | [Reset Form](#)
frodo.wi.mit.edu/primer3/input-help.htm#PRIMER_DNTP_CONC

