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/]

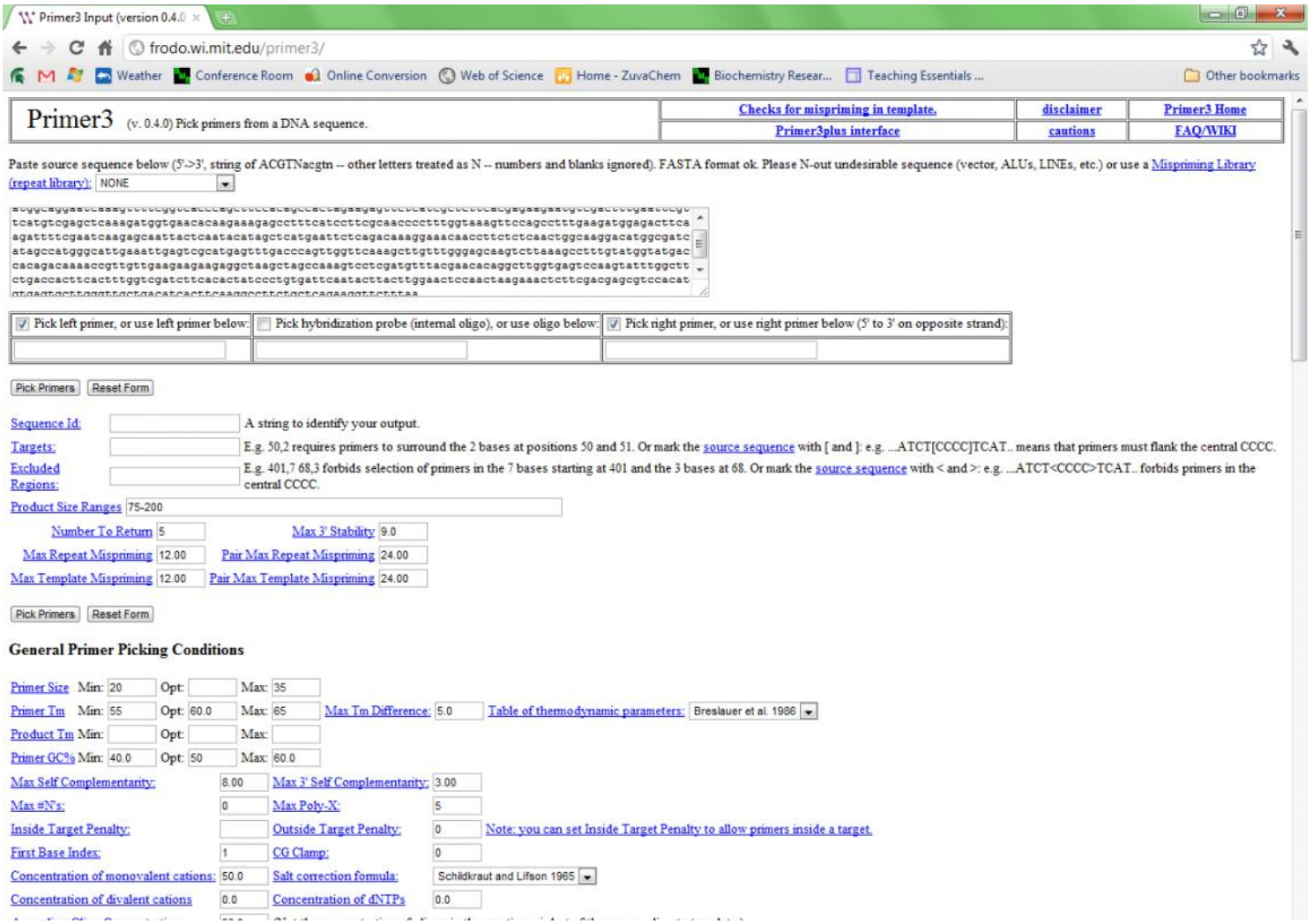
I have had great success with Primer 3 and find it consistently picks good primers. If not stated in the protocol leave settings at their default.

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. Chose 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




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
Annealing Oligo Concentration: 50.0
(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
12:23 PM
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