

Change when designing qPCR primers

Primer3 (v. 0.4.0) Pick primers from a DNA sequence.	<u>Checks for mispriming in template.</u>	<u>disclaimer</u>	<u>Primer3 Home</u>
	<u>Primer3plus interface</u>	<u>cautions</u>	<u>FAQ/WIKI</u>

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

<input checked="" type="checkbox"/> Pick left primer, or use left primer below:	<input type="checkbox"/> Pick hybridization probe (internal oligo), or use oligo below:	<input checked="" type="checkbox"/> Pick right primer, or use right primer below (5' to 3' on opposite strand):
<div></div>	<div></div>	<div></div>

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**75-150**

<u>Number To Return</u>	20	<u>Max 3' Stability</u>	9.0
<u>Max Repeat Mispriming</u>	12.00	<u>Pair Max Repeat Mispriming</u>	24.00
<u>Max Template Mispriming</u>	12.00	<u>Pair Max Template Mispriming</u>	24.00

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General Primer Picking Conditions

Primer Size Min: Opt: Max:

Primer Tm Min: Opt: Max: Max Tm Difference:

Table of thermodynamic parameters:

Breslauer et al. 1986

Product Tm Min: Opt: Max:

Primer GC% Min: Opt: Max:

Max Self Complementarity: Max 3' Self Complementarity:

Max #N's: Max Poly-X:

Inside Target Penalty: Outside Target Penalty:

Note: you can set Inside Target Penalty to allow primers inside a target.

First Base Index: CG Clamp:

Concentration of monovalent cations: Salt correction formula:

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 Debugging Info ☒ Do not treat ambiguity codes in libraries as consensus ☐ Lowercase masking

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Other Per-Sequence Inputs

Included Region:

E.g. 20,400: only pick primers in the 400 base region starting at position 20. Or use { and } in the source sequence to mark the beginning and end of the included region: e.g. in ATC{TTC...TCT}AT the included region is TTC...TCT.

Start Codon Position:

Sequence Quality

Min Sequence Quality:	0	Min End Sequence Quality:	0	Sequence Quality Range Min:	0	Sequence Quality Range Max:	100
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Objective Function Penalty Weights for Primers

Tm	Lt:	.5	Gt:	.5
Size	Lt:	0	Gt:	0
GC%	Lt:	0.0	Gt:	0.0
Self Complementarity				0.5
3' Self Complementarity				1
#N's				0.0
Mispriming				0.0
Sequence Quality				0.0
End Sequence Quality				0.0
Position Penalty				0.0
End Stability				0.0
Template Mispriming				0.0

Objective Function Penalty Weights for Primer Pairs

Product Size	Lt:	0.0	Gt:	0.0
Product Tm	Lt:	0.0	Gt:	0.0
Tm Difference				0.5
Any Complementarity				0.5
3' Complementarity				1
Pair Mispriming				0.0
Primer Penalty Weight				0
Hyb Oligo Penalty Weight				0.0
Primer Pair Template Mispriming Weight				0.0

Hyb Oligo (Internal Oligo) Per-Sequence Inputs

Hyb Oligo Excluded Region:

Hyb Oligo (Internal Oligo) General Conditions

Hyb Oligo Size:	Min <input type="text" value="18"/>	Opt <input type="text" value="20"/>	Max <input type="text" value="27"/>	
Hyb Oligo Tm:	Min <input type="text" value="57.0"/>	Opt <input type="text" value="60.0"/>	Max <input type="text" value="63.0"/>	
Hyb Oligo GC%:	Min <input type="text" value="20.0"/>	Opt <input type="text"/>	Max <input type="text" value="80.0"/>	
Hyb Oligo Self Complementarity:	<input type="text" value="12.00"/>		Hyb Oligo Max 3' Self Complementarity:	<input type="text" value="12.00"/>
Max #Ns:	<input type="text" value="0"/>		Hyb Oligo Max Poly-X:	<input type="text" value="5"/>
Hyb Oligo Mishyb Library:	<input type="text" value="NONE"/>	<input type="text"/>	Hyb Oligo Max Mishyb:	<input type="text" value="12.00"/>
Hyb Oligo Min Sequence Quality:	<input type="text" value="0"/>			
Hyb Oligo Conc of monovalent cations:	<input type="text" value="50.0"/>		Hyb Oligo DNA Concentration:	<input type="text" value="50.0"/>
Hyb Oligo conc of divalent cations:	<input type="text" value="0.0"/>		Hyb Oligo [dNTP]	<input type="text" value="0.0"/>

Objective Function Penalty Weights for Hyb Oligos (Internal Oligos)

Hyb Oligo Tm	Lt: <input type="text" value="1.0"/>	Gt: <input type="text" value="1.0"/>
Hyb Oligo Size	Lt: <input type="text" value="1.0"/>	Gt: <input type="text" value="1.0"/>
Hyb Oligo GC%	Lt: <input type="text" value="0.0"/>	Gt: <input type="text" value="0.0"/>
Hyb Oligo Self Complementarity	<input type="text" value="0.0"/>	
Hyb Oligo #N's	<input type="text" value="0.0"/>	
Hyb Oligo Mishybing	<input type="text" value="0.0"/>	
Hyb Oligo Sequence Quality	<input type="text" value="0.0"/>	
<input type="button" value="Pick Primers"/>	<input type="button" value="Reset Form"/>	