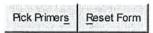
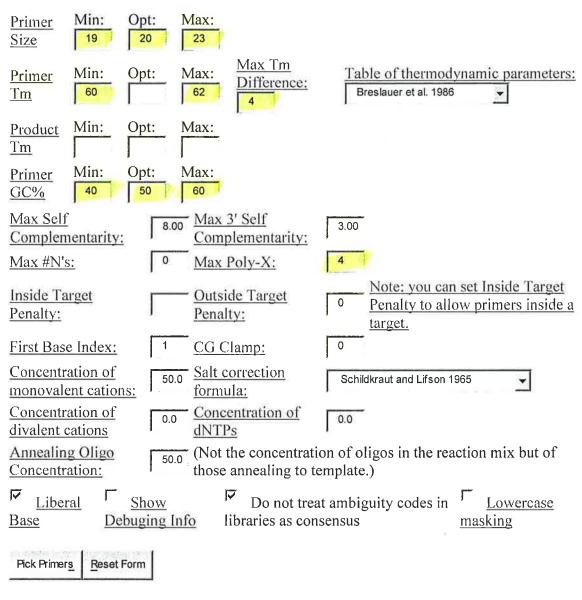
# Change when designing gPCR primers

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):					
			<u> </u>		
41-1			¥ •		
Pick left primer, or use left primer below:		r use oligo below:	se right prim	primer, or er below posite strand)	
Pick Primers Reset Form  Sequence Id:  A s	string to ident	ify your output.			
E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [ and ]: e.gATCT[CCCC]TCAT means that primers must flank the central CCCC.					
Excluded Regions: at 4	101 and the 3	forbids selection of print bases at 68. Or mark the CT <ccc>TCAT for</ccc>	e source sequ	ence with <	
Product Size Ranges	75-150				
Number To Return	20	Max 3' Stability	9.0		
Max Repeat Mispriming 12.00 Pair Max Repeat Mispriming 24.00					
Max Template Mispriming	12.00 <u>Pair Ma</u>	x Template Mispriming	24.00		



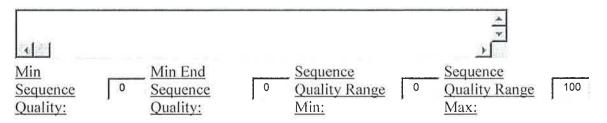
#### **General Primer Picking Conditions**



#### **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 <u>source sequence</u> to mark the beginning and end of the included region: e.g. in ATC{TTCTCT}AT the included region is TTCTCT.
Start Codon Position:	

### **Sequence Quality**



## **Objective Function Penalty Weights for Primers**

<u>Tm</u> Lt: .5 Gt: .5	Ĭ.
Size Lt: 0 Gt: 0	Ì
GC% Lt: 0.0 Gt: 0.0	
Self Complementarity	0.5
3' Self Complementarity	1
<u>#N's</u>	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	
<u>Tm Difference</u>	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 18  Hyb Oligo Tm: Min 57.0	Opt 20 Opt 60.0	Max 63.0		
Hyb Oligo GC% Min: 20.0	Opt:	Max: 80.0	4	
Hyb Oligo Self Complementarity:	12.00		Hyb Oligo Max 3' Self Complementarity:	12.00
Max #Ns:	0		Hyb Oligo Max Poly-X:	5
Hyb Oligo Mishyb Library:	NONE	<u> </u>	Hyb Oligo Max Mishyb:	12.00
Hyb Oligo Min Sequence Quality:	0			
Hyb Oligo Conc of monovalent cations:	50.0		Hyb Oligo DNA Concentration:	50.0
Hyb Oligo conc of divalent cations:	0.0		Hyb Oligo [dNTP]	0.0

# Objective Function Penalty Weights for Hyb Oligos (Internal Oligos)

Hyb Oligo Tm Lt: 1.0 Gt:	1.0
Hyb Oligo Size Lt: 1.0 Gt:	1.0
Hyb Oligo GC% Lt: 0.0 Gt:	0.0
Hyb Oligo Self Complementarity	0.0
Hyb Oligo #N's	0.0
Hyb Oligo Mishybing	0.0
Hyb Oligo Sequence Quality	0.0
Pick Primers Reset Form	