





This tool calculates the T_m of primers and estimates an appropriate annealing temperature when using different DNA polymerases. How to use this calculator

Quickly find the right annealing temperature for Platinum SuperFi DNA polymerase (also works for SuperScript IV One-Step RT-PCR Kit), Phusion and Phire DNA polymerases.

 $\textbf{Important note:} \ \text{If the PCR primer contains desired mismatches, e.g., for creating a mutation or a restriction site, make sure to calculate the T_m only for the T_m only fo$ correctly matched sequence

The T m calculator is not required for Platinum II Taq DNA Polymerase, Platinum SuperFi II DNA Polymerase, and Platinum Direct PCR Universal Master Mix, and Phusion Plus DNA Polymerase due to their buffers specially formulated for a universal annealing temperature of 60°C for primers.

1.	Select	vour	DNA	noly	merase

\circ	Platinum SuperFi DNA polymerase
	(Also select this option if using the SuperScript IV One-Step RT-PCR Kit)
	Phusion or Phire DNA polymerase

O DreamTaq DNA polymerase or other *Taq*-based DNA polymerase

2. Select input method

O Single pair

Batch

3. Paste your sequences

P3-3-3-3-3-3-3-3-3-3-3-3-3-3-3-3-3-3-3-
p94 AGTGGGATTCCCTACTCG; p194 CTCCCGCTCATAGCTTCC
p95 CCTCCGTTAGATAGCCCT; p195 TACGGTCTAGAAAAGTCT
p96 TACTAGTCTCGACCAGTA; p196 GGAAATTAATTGCTAACT
p97 AGGCCAATAAGCCTTAAT; p197 GTACAAGGCAAACCGGTG
p98 ATGCCGCGGTCCTGAACC; p198 TGGACCCCTGTCTCACGT
p99 TTGCACACCCTTGAGGCC; p199 ACAACTAAGACTCTAGGA
p100 GTGAGCTGACTTTTCAGT; p200 TAAACCATAATCACGACT

Clear

4. PCR conditions

Primer conc. 0.5 μΜ

Results

		Export table data into	Excel
ID Sequence #1	Molecular	Extinction Tm ID Sequence #2 Molecular Extinction Tm A	nnealing
#1	weight	coefficient °C #2 weight coefficient °C Tem	perature
	g/mol	l/(mol ⁻ cm) g/mol l/(mol ⁻ cm)	°C
`TCTCTTTTGGATG	5431.6	159500.0 56.2 p1 AATTAATTTATGTATAA 5495.7 188700.0 42.3 01 C	42.3

ID Sequence #1 Solutions to support your pre-clinical three Discover now > Molecular Extinction Tm Annealing coefficient °C #2 weight coefficient °C Temperature a/mol I/(mol⁻cm) a/mol l/(mol⁻cm) Tm differer or greater is not recommended. Annealing temperature lower than 45°C is not recommended. p2 AAACCCGGATAGCAC 5502.6 178200.0 65.4 p1 GCCGCATGCCCATTTT 5410.6 159500.0 62.8 62.8 GGC 02 AC p3 GGCATACCACTCTAGA 173000.0 59.4 p1 GGGGATTGCCCCTTA 5530.6 172400.0 61.2 5468.6 59.4 03 GTA GC p4 GACGCCCCCTACGG 5405.5 161300.0 68.6 p1 TCCAAATTAATTTAGA 5480.7 184400.0 46.7 46.7 CTA 04 AT Tm difference of more than 5°C or greater is not recommended. p5 GGAGAGCGAGCGCG 5574.7 176800.0 69.9 p1 CTACAGTAGATGTGAG 5563.7 181600.0 56.7 56.7 CTCA 05 GC Tm difference of more than 5°C or greater is not recommended. 163500.0 61.3 p1 CCCTGCCGGCAATAC 5413.6 165300.0 64.1 p6 GCGTGCAGCTTCTGT 5496.6 61.3 TTA 06 AAC 165100.0 66.6 p1 TTAAGGGCCGTCAGC 5490.6 165800.0 63.6 p7 CTCACCTGGAGGTCC 5516.6 63 6 p8 TCAGGCAATTGGCGT 5523.7 176000.0 63.0 p1 GTCCCTAACGTCCAAT 5418.6 171300.0 56.4 56.4 CAA 08 AT Tm difference of more than 5°C or greater is not recommended. 5467.6 176000.0 59.5 p1 CAGCACGGTTACTAG p9 AATGACGTAGCCCAA 5484.6 169800.0 62.8 59.5 CTT 09 GCC p1 TCATTATCATAAGGAG 5521.7 189200.0 49.2 p1 AAAATAGACTTTATAA 5529.7 193500.0 45.6 45.6 10 GT 0 TA p1TTCACAAGTCGATTTG 5529.7 173700.0 58.1 p1 GACCTATGAGATCCC 5484.6 171500.0 61.8 58.1 1 GG 11 GGC p1 TCTACCATGGGAGATA 5538 7 180200.0 55.1 p1 TAAATACACAAAGATA 5468 7 191100 0 49 2 49 2 12 CC Tm difference of more than 5°C or greater is not recommended. p1 TGGGGTACGGTTAAT 5585.7 175300.0 58.9 p1 GGCTTTTAGACAAAC 5522.7 181000.0 55.5 55.5 3 CTG 13 GTA p1 GCTTGCACCTGCGCA 5450.6 158900.0 65.2 p1 AAGACTAGACGTCTTA 5522.7 183000.0 53.3 53.3 4 ATT 14 TG Tm difference of more than 5°C or greater is not recommended. 5371.5 153800.0 69.6 p1 CTCTCAAGAACGCTG 5467.6 175500.0 56.6 p1 AGCCCCCAGTGCCC 56.6 5 TTT 15 ATA Tm difference of more than 5°C or greater is not recommended. p1 GCCTTACTCTTCAGG 5449.6 169200.0 55.7 p1 GTACGTATCTGCTCCA 5450.6 165400.0 59.9 55.7 16 GC 6 ATA p1 ACCTCTAACTCCGCC 5339.5 158700.0 60.6 p1 GCTTATACGATAGATC 5488.7 176600.0 50.9 50.9 7 TAC 17 TT Tm difference of more than 5°C or greater is not recommended. 154700.0 63.5 p1 GCTATGACAGGGGTT 5619.7 182000.0 59.4 p1CTCCTTGCCAACCAT 5355.5 59.4 8 CCG p1 GGAGCTGACGCTCCC 5509.6 172100.0 66.5 p1 GAATTTTGGTTTACCG 5495.6 166200.0 56.5 56.5

19 CT

9 AGA

rence of more than 5°C or greater is not recommended

#1 ThermoFisher SCIENTIFIC	Solutions to support you weight	ur pre-clinica tinfase coefficient l/(molˈcm)			weight	r Extinction coefficient l/(mol ⁻ cm)	°C	Annealing Temperature
p2ATTCGTAGATGTCCAG	5522.7	185500.0		p1 ACTAGGGGTATTTCCA 20 AC	5498.7	176700.0	56.3	55.6
p2TCATGGCTTCTCACG 1 GTG	5481.6	161700 0		p1.GATACTTTCGAGCATG 21.CC	5474 6	169400 0	58 7	58 7
p2 CGCGTGTCATGGCCG 2 GCG Tm difference of more than 5°C or greater is not re	5532.6	161800.0		p1 TGGCATACTGATACCC 22 TT	5449.6	166900.0	58.0	58.0
p2 CCTCCCCATATACCGC 3 CT	5315.5	154200.0		p1 GCAGCAAGTCGTCAC 23 GTG	5524.6	174100.0	64.2	63.3
p2GCGGATGCCCTAATC 4 GCT	5475.6	164100.0		p1 AACGGGCGATCTCTA 24 CAC	5468.6	172500.0	61.8	61.8
p2 ATGGGCAACCCCTAC 5 CCT	5404.6	163100.0		p1 CATGAAACTCTAAACA 25 AA	5468.7	187800.0	50.8	50.8
Tm difference of more than 5°C or greater is not re p2GTTTCAATTTTGTATTT 6 A Tm difference of more than 5°C or greater is not re	5484.7	173000.0		p1 CTAAGGGTGGTTCTT 26 CGC	5521.6	164800.0	60.5	46.5
p2CTGTAGTTAGTACGAG 7 GC	5554.7	178300.0		p1 CGGCTTACAGGCACG 27 CGT	5500.6	165600.0	68.2	56.6
Tm difference of more than 5°C or greater is not re p2 GACCCCGTACGGGG 8 GGTC	5541.6	169300.0		p1 GGGCGCGCAGTCGG 28 TTAA	5580.7	174600.0	68.3	68.3
p2TTCGGCCTGTTCATGT 9 CT	5447.6	155800.0		p1 CTGCGACCCGCATCC 29 AGC	5405.5	158200.0	68.7	61.8
Tm difference of more than 5°C or greater is not re p3 CCAGGGTTGCCTTGG	commended. 5515.6	166600.0	66.1	p1 GTGCTGTACGAGGGG	5556.6	168200.0	67.7	66.1
0 CAA p3GCCAGGCTCCGCGTG 1 CCG	5477.6	156900.0	73.8	30 CCC p1 TCGTAGTGCATACGC 31 GAA	5523.7	179200.0	61.7	61.7
Tm difference of more than 5°C or greater is not re	commended.			31 374				
p3TCATTCGACCTTCGTA 2 GC	5425.6	163200.0		p1 AGTGCATCCGGTCAG 32 TCG	5515.6	172500.0	64.7	59.5
Tm difference of more than 5°C or greater is not re								
p3 CTACTCCTCTGATCAG 3 GC	5410.6	159800.0		p1 GCAAACGTCTGCTCA 33 AGT	5483.6	171200.0	61.2	58.7
p3 CCCTCCGGTTTAATCT 4 AC	5385.6	159600.0		p1 ACAACTCCGTATTCTG 34 AG	5458.6	173500.0	56.6	56.6
p3CGCAACTGCGATTGC 5 TTT	5465.6	160100.0		p1 CTAGCATGTGACGTAA 35 TT	5513.7	176700.0	55.0	55.0
Tm difference of more than 5°C or greater is not re								
p3 GCGACATAAGAGCTC 6 AAG	5541.7	185300.0		p1 TGTACGCACGTATAGT 36 AC	5498.7	178800.0	56.3	56.3
p3 GATAACGGTCCTACTT 7 CG	5474.6	171300.0		p1 GCGTAGTGCTACCGT 37 ACT	5490.6	168800.0	61.5	57.0
CCACTGATTTTCCA	5409.6	163400.0		p1 GTTAAGAGACTATCGA 38 CA	5531.7	188500.0	53.3	53.3

ID Sequence #1	Solutions to support weight	your pre-clinicar phase coefficient				Extinction coefficient		Annealing Temperature
Thermo Fisher SCIENTIFIC	g/mol	l/(mol ⁻ cm)			g/mol	l/(mol ⁻ cm)		0 🗁
p3 GTGGGTTTTATTCGG	5591.7	172900.0	57.6	p1 CGGTATGTATTGCTGC	5520.7	170800.0	57.1	57.1
9 GAT				39 TA				
p4 GTGGTCCTCGGATTA 0 ACA	5514 7	175100.0	59 2	p1 CGCCAGAAGCAAAGC 40 TCA	5486 6	178700 0	63 6	59 2
p4TACGCCTTCTCAGCA 1 AGG	5459.6	166800.0	62.1	p1 GATAAAGCCTTAACCT 41 TA	5466.6	180400.0	51.6	51.6
Tm difference of more than 5°C or greater is not re-	commended.							
p4 CTCCATGGACATCCA	5443.6	173500.0	58.4	p1 AATACAGTCTGCTACT	5457.6	177800.0	52.5	52.5
2 GTA				42 TA				
Tm difference of more than 5°C or greater is not re-	commended.			1				
p4 CCAGCTGACTCTAAA	5468.6	169800.0	60.5	p1 GGGCAGGAGGGTGG	5645.7	180700.0	69.1	60.5
3 GCG	oommondod			43 TCCA				
Tm difference of more than 5°C or greater is not re-								
p4 TAATTTTTACCCCTATG 4 A	5423.6	171200.0	50.4	p1 ATGGGACAATAACGC 44 AGC	5541.7	184700.0	60.9	50.4
Tm difference of more than 5°C or greater is not re-	commended.							
p4 TATCGATTATCTCTAAG	5488.7	177200.0	49.9	p1 TGCTACACGTCGCCG	5491.6	163000.0	67.6	49.9
5 G				45 GTG				
Tm difference of more than 5°C or greater is not re-	commended.							
p4 GACCTAAAGTAGAGA	5556.7	188000.0	53.6	p1 GGTTCAGCCACGTATA	5459.6	170900.0	60.2	53.6
6 CTG Tm difference of more than 5°C or greater is not re-	commended			46 CC				
_		470000		1.071.00700771010	55547	470000 0	00.0	
p4 CAGTCATACTTCCGAA 7 AC	5427.6	1/3200.0	55.6	p1 AGTAGGTGCTTAGAC 47 CGT	5554.7	178800.0	60.2	55.6
p4ATGCATGGCCGAGGT	5564.7	176800.0	67.9	p1 GTGAATAGGGGGTATT	5634.7	184900.0	57.1	57.1
8 GCA				48 CG				
Tm difference of more than 5°C or greater is not re	commended.							
p4 CGAGATGTTTATTCTT	5519.7	175000.0	52.3	p1 GATTGATCCATGAATG	5553.7	180300.0	55.0	52.3
9 GA				49 GT				
p5 CATCGTCTCTGAAGA 0 ACG	5483.6	173500.0	57.9	p1 TAACGTGTGGCGCTAT	5490.6	166900.0	62.4	57.9
	5378.5	145700.0	70.0		EE40.7	100000 0	E47	54.7
p5 TCCTCGGCCCGTGCC 1 TCT	3376.3	143700.0	70.9	p1 GCTAGCAGATGAATAC 51 AA	5540.7	190000.0	34.7	54.7
Tm difference of more than 5°C or greater is not re-	commended.							
p5 TTATAGTGAGCCGAG	5579.7	178800.0	61.4	p1 ACCTTCCCGACTAACT	5363.5	165700.0	60.2	60.2
2 GGC				52 CA				
p5 TGATGTTCGAGGGCG	5635.7	179200.0	66.2	p1 CCGCCTATCCCTGAC	5404.6	163300.0	62.3	62.3
3 GGA				53 AAG				
p5TCAACTCTTGACAGC 4 GAT	5458.6	170000.0	59.1	p1 TTTATGTTACAACGAG 54 AC	5497.7	179400.0	52.6	52.6
Tm difference of more than 5°C or greater is not re-	commended.							
p5 TGGTGGACCGTACAA	5529.7	173700.0	59.4	p1 GAATGGCTTCCCAGA	5539.7	174400.0	60.5	59.4
5 TTT				55 GTG				
p5 AGATTAGAATGAAGAC	5564.7	197700.0	52.8	p1 CTATGGCAAATAGCTC	5467.6	175700.0	57.1	52.8
6 ^4				56 CA				

ID Sequence #1	Solutions to support you	ur pre-clinica phase	™B	iBcoverce#2v >	Molecula	r Extinction	Tm	Annealing
#1	weight	coefficient			_			Temperature
Thermo Fisher SCIENTIFIC	g/mol	l/(mol ⁻ cm)			g/mol	l/(mol ⁻ cm)		Q H
p5 TAAGGGGCCCGTCTT 7 AAA	5523.7	177000.0	61.0	p1 GTGTGATCGTCTGCC 57 ACA	5490.6	169100.0	62.6	61.0
1 AVA								
p5 CCTTAGGGCTCCACA 8 CCG	5420 6	160600 0	64 8	p1 GGGACAGACTGCATT 58 CCA	5508 6	176100 0	62 2	62.2
				JUCCA				
p5 CTCAGTTCCCCTCGA 9 AAG	5419.6	165000.0	59.8	p1 AAACTCAGGATATTCG 59 TG	5522.7	182200.0	54.6	54.6
Tm difference of more than 5°C or greater is not re	commended.							
p6 GGACCTCCCCCTGCA	5380.5	156000.0	66.7	p1 AATGCTATACTTCGCC	5409.6	163900.0	58.7	58.7
0 ACT				60 CT				
Tm difference of more than 5°C or greater is not re	commended.							
p6 TACGTTTATGAAAATG	5497.7	177600.0	53.6	p1 ACACAACATTGCTGTT	5466.6	179500.0	55.6	53.6
1 CC				61 AA				
p6 CAGTTTAAGCCTCGG	5475.6	164500.0	63.4	p1 GCAGAACACCGATGC	5468.6	170500.0	63.4	63.4
2 GCC				62 CTT				
p6 ATAGCCCTGTCGTAG	5530.6	174000.0	61.0	p1 ATTAGAATGATTCCAA	5496.7	183800.0	49.1	49.1
3 GTG				63 TT				
Tm difference of more than 5°C or greater is not re	commended.							
p6 ATTGTATACTGAAGCG	5562.7	186200.0	56.0	p1 ACCTAAGGGAAGGGC	5558.7	178200.0	67.0	56.0
4 GA				64 GCC				
Tm difference of more than 5°C or greater is not re	commended.							
p6 GGTGATTGCTTCCTTT	5502.6	159000.0	57.1	p1 CGCCTACTCTGAACG	5450.6	162000.0	60.7	57.1
5 TG				65 TTG				
p6 CCGATTACGTAAATCT	5458.6	171800.0	56.1	p1 GTGTTTTGAGTTGTAT	5606.7	174600.0	54.2	54.2
6 GC				66 GG				
p6 AAGACCTTCAAGGGA	5531.7	186600.0	55.5	p1 ATTCCTCGCTATGTCG 67 GC	5441.6	160600.0	62.3	55.5
7 TTA Tm difference of more than 5°C or greater is not re	aammandad			67 GC				
Till dillerence of more than 5 C or greater is not re	commended.							
p6 ACAATTCAAATAGCAG 8 TT	5490.7	185600.0	52.8	p1 GAAGTTAACACCGCT 68 TAG	5507.7	179600.0	55.9	52.8
p6 TAACTCATGCGCCGA 9 ATA	5467.6	176400.0	59.3	p1 AAAGGGTTACCCGAT 69 GGC	5548.7	178900.0	62.6	59.3
p7GTTTTTATGTCGTTCT 0 TA	5476.6	166100.0	50.6	p1 AGGAGGAACACTTAT 70 GTT	5562.7	184500.0	55.8	50.6
Tm difference of more than 5°C or greater is not re	commended.							
p7 AAAATAACGGCACATA	5533.7	195200.0	53.6	p1 CAACGTAACGCCACA	5430.6	177700 0	60.1	53.6
1 AG	3335.1	200200.0	00.0	71 AAC	0.00.0	211100.0	00.2	55.5
Tm difference of more than 5°C or greater is not re	commended.							
p7 TCAGGCATTGCATCA	5539.7	173800.0	63.0	p1 TCTCGACAGGACTGA	5459.6	168500.0	60.6	60.6
2 GGG				72 TCC				
p7 ACGTGATACACCGGC	5508.6	177900.0	61.5	p1 TACTAAACAGATAACT	5490.7	185100.0	50.1	50.1
3 TAG				73 GT				
Tm difference of more than 5°C or greater is not re	commended.							
p7 CTTGGAACTGCGGCC	5515.6	165200.0	65.3	p1 CTTATGTCTGAGAACG	5489.6	171200.0	55.0	55.0
4 TGA				74 TC				
Transference of more than 5°C or greater is not re	commended.							

### Part of the control of the contr	54.9
Transitifierence of more than 5°C or greater is not recommended.	54.9
p7 AAGATTTGCCTATCCG 5474.6 169800.0 60.6 p1 GCCGGAAGCCTATAG 5532.7 184100.0 58.2 p7 TCGTGAGGTTCAACG 5538.7 180500.0 59.0 p1 TTCGGGCACAACCGA 5509.6 169700.0 68.1 7 TAA 7 GGC 77 GGC 163900.0 71.2 p1 GACTGGAGAGCACCGA 5509.6 177300.0 61.5 7 GTTAGCGCCCACCGG 5501.6 163900.0 71.2 p1 GACTGGAGAGACAGC 5533.6 177300.0 61.5 8 GCG Tm difference of more than 5°C or greater is not recommended. PTGGACTCAATCATGG 5579.7 177700.0 60.3 p1 TAACGAATTTCGTTTC 5454.6 167300.0 52.8 9 TGG Tm difference of more than 5°C or greater is not recommended. PBTTACCTGATCGTATCG 5480.6 169300.0 56.4 p1 GTTCAATTACACCCTT 5417.6 174000.0 52.5 0 TG 80 AA PTAGAGACTGACATC 5492.6 177600.0 58.9 1 CT 81 CAC 81 CAC 81 CAC 81 CAC 177600.0 59.7 PB TGCGAAGGATATTCCA 5547.7 184200.0 58.3 p1 AGCTTATATCAGCGC	
FOR TIT CONTROL STATE AND	
Trind difference of more than 5°C or greater is not recommended. P7GTTAGCGCCCACCGG 5501.6 163900.0 71.2 p1 GACTGGAGAGACAGC 5533.6 177300.0 61.5 78 TCC	58.2
p7 GTTAGCGCCCACCGG 5501.6 163900.0 71.2 p1 GACTGGAGAGACAGC 5533.6 177300.0 61.5 8 GCG 78 TCC 78 TCC 177300.0 61.5	59.0
## RECG Tm difference of more than 5°C or greater is not recommended. pr GGGACTCAATCATGG	
p7 GGGACTCAATCATGG 5579.7 177700.0 60.3 p1 TAACGAATTTCGTTTC 545.6 167300.0 52.8 79 TT 545.6 167300.0 52.8 79 TT 552.8 79 TT 545.6 167300.0 52.8 79 TT 552.8 79 TT	61.5
9 TGG Tm difference of more than 5°C or greater is not recommended. p8 TTACCTGATCGTATCG TG 5480.6 169300.0 56.4 p1 GTTCAATTACACCCTT 5417.6 174000.0 52.5 80 AA p8 TCAACTCTCTGTGCTT 5391.6 152600.0 58.4 p1 TAAGGAGCTGACATC 5492.6 177600.0 58.9 1 CT 81 CAC p8 TGCGAGGAATATTCCA 5547.7 184200.0 58.3 p1 AGCTTATATCAGCGCT 5474.6 170800.0 59.7 2 GA p8 TACGGAAGGGCACAT 5573.7 180500.0 64.5 p1 ACGAGACGCGCGACA 5487.6 177300.0 67.9 83 CAC p8 TACGGTAGATTGTTTA 5552.7 185600.0 51.1 p1 TGACTGAATATTGGGA 5553.7 177700.0 55.6	
p8 TTACCTGATCGTATCG 5480.6 169300.0 56.4 p1 GTTCAATTACACCCTT 5417.6 174000.0 52.5 0 TG 80 AA 80 AA 58.4 p1 TAAGGAGCTGACATC 5492.6 177600.0 58.9 1 CT 81 CAC 5492.6 177600.0 58.9 2 GA 184200.0 58.3 p1 AGCTTATATCAGCGCT 5474.6 170800.0 59.7 2 GA 82 CG 82 CG 177300.0 67.9 3 CGG 83 CAC 83 CAC 177700.0 55.6	52.8
0 TG 80 AA p8 TCAACTCTCTGTGCTT 5391.6 152600.0 58.4 p1 TAAGGAGCTGACATC 5492.6 177600.0 58.9 1 CT 81 CAC 81 CAC 5492.6 177600.0 58.9 p8 TGCGAGGAATATTCCA 5547.7 184200.0 58.3 p1 AGCTTATATCAGCGCT 5474.6 170800.0 59.7 2 GA 82 CG 82 CG 82 CG 5474.6 177300.0 67.9 3 CGG 83 CAC 83 CAC 5552.7 185600.0 51.1 p1 TGACTGAATATTGGGA 5553.7 177700.0 55.6	
1 CT 81 CAC p8 TGCGAGGAATATTCCA 5547.7 184200.0 58.3 p1 AGCTTATATCAGCGCT 5474.6 170800.0 59.7 2 GA 82 CG p8 TACGGAAGGGCACAT 5573.7 180500.0 64.5 p1 ACGAGACGCGCGACA 5487.6 177300.0 67.9 3 CGG 83 CAC p8 TACGGTAGATTGTTTA 5552.7 185600.0 51.1 p1 TGACTGAATATTGGGA 5553.7 177700.0 55.6	52.5
2 GA 82 CG p8 TACGGAAGGGCACAT 5573.7 180500.0 64.5 p1 ACGAGACGCGCGACA 5487.6 177300.0 67.9 3 CGG 83 CAC p8 TACGGTAGATTGTTTA 5552.7 185600.0 51.1 p1 TGACTGAATATTGGGA 5553.7 177700.0 55.6	58.4
3 CGG 83 CAC 83 CAC 98 TACGGTAGATTGTTTA 5552.7 185600.0 51.1 p1 TGACTGAATATTGGGA 5553.7 177700.0 55.6	58.3
	64.5
	51.1
p8 TCAGGATAGATGCCC 5474.6 170100.0 60.2 p1 TGTCCATTCAAACCAG 5443.6 169400.0 60.8 5 CTT	60.2
p8 CCATTGCATGGGGCC 5515.6 167500.0 63.4 p1 AACCGACCTGCCCTC 5380.5 156900.0 66.9 6 TAG	63.4
p8 GCCTCGTTTATGAAAA 5538.7 177200.0 56.6 p1 ATGACGGCCAGCATC 5499.6 172000.0 65.1 87 GTT	56.6
Tm difference of more than 5°C or greater is not recommended.	
p8TCCCCGGGTCTGGTC 5451.6 162200.0 68.0 p1TTGTGCAGCGGATCG 5521.6 163400.0 65.6 8 ACA 88 CTT	65.6
p8 TGAACACTTATACGCT 5473.6 171900.0 56.5 p1 CGTGCAATCCAGCAA 5492.6 176800.0 61.1 9 GT	56.5
p9 TTAAAGCCAAGTCTCC 5451.6 178100.0 56.7 p1 GTGATCGGAATTCCCT 5514.7 174800.0 57.1 90 AG	56.7
p9 AGCGCCATTTGGGTG 5595.7 175100.0 65.8 p1 TAGAAGGGTTCGTGG 5619.7 181600.0 61.8 91 CAG	61.8
p9 TAGCTCAATGTGACAG 5498.7 173500.0 58.9 p1 TAGCCCGACAACCAA 5461.6 177900.0 60.5 2 CT	58.9
p9 GGTCAGGCTAGGAGC 5540.6 171300.0 64.0 p1 AGGTATTGTTTCCCAC 5465.6 168100.0 59.9 93 CG	59.9
p9 AGTGGGATTCCCTACT 5490.6 169300.0 60.7 p1 CTCCCGCTCATAGCTT 5346.5 151300.0 62.3 94 CC	60.7

ID Sequence #1 #1	Solutions to Support you weight	r pre-clinical phase coefficient		scover now >		r Extinction coefficient		Annealing Temperature
Thermo Fisher SCIENTIFIC	g/mol	l/(mol ⁻ cm)			g/mol	l/(mol ⁻ cm)		QE
p9 CCTCCGTTAGATAGCC 5 CT	5410.6	162300.0		p1 TACGGTCTAGAAAAGT 95 CT	5522.7	181100.0	54.8	54.8
Tm difference of more than 5°C or greater is n	ot recommended.							
p9 TACTAGTCTCGACCAG	5458.6	176500.0		p1 GGAAATTAATTGCTAA	5521.7	181500.0	50.6	50.6
6 TA Tm difference of more than 5°C or greater is n	ot recommended			96 CT				
The difference of more than 5 °C of greater is n	ot recommended.							
9 AGGCCAATAAGCCTTA	5491.7	181200.0	56.7	p1 GTACAAGGCAAACCG	5557.7	182400.0	61.6	56.7
7 AT				97 GTG				
9 ATGCCGCGGTCCTGA	5460.6	163800.0	68.0	p1 TGGACCCCTGTCTCA	5426.6	158300.0	65.5	65.5
3 ACC				98 CGT				
9 TTGCACACCCTTGAG	5435.6	159700.0	65.7	p1 ACAACTAAGACTCTAG	5500.7	187600.0	54.1	54.1
9 GCC				99 GA				
m difference of more than 5°C or greater is n	ot recommended.							
o1 GTGAGCTGACTTTTC	5520.7	168800.0	57.7	p2 TAAACCATAATCACGA	5435.6	180700.0	53.5	53.5
00 AGT				00 CT				

Ready to order primers? >

How to use the $T_{\rm m}$ calculator

The calculator calculates recommended T_m (melting temperature) of primers and PCR annealing temperature based on the primer pair sequence, primer concentration, and DNA polymerase used in PCR. The calculator also calculates the primer length, percentage of GC content, molecular weight, and extinction coefficient.

The modified Allawi & SantaLucia's thermodynamics method [1] is used for T_m and annealing temperature calculation of reactions with Platinum SuperFi, Phusion and Phire DNA Polymerases. The parameters were adjusted on a set of primers seeking to maximize specificity and retain high yields.

To use this calculator select your DNA polymerase, type in or paste your primer sequences, and provide your final primer concentration. T_m values, annealing temperature, and other data are automatically generated.

If necessary, use a temperature gradient to further optimize and empirically determine the ideal annealing temperature for each template-primer pair combination. The annealing temperature gradient should start with temperature 6–10 °C lower than annealing temperature generated by the calculator and increased up to the extension temperature (**two-step PCR**).

1. Allawi, H. T., and SantaLucia, J. (1997). Thermodynamics and NMR of internal G-T mismatches in DNA. Biochemistry, 36(34), 10581-10594.

Additional technical resources

Optimizing T_m and primer annealing

Oligos tools and utilities

Molecular biology web tools

Molecular biology resource library

Invitrogen School of Molecular Biology

Related products

DreamTaq DNA polymerase

Phusion Plus DNA Polymerase

Platinum SuperFi II DNA Polymerase