

SUPPLEMENTARY MATERIAL (FIGURES)

TBIO Method of Primer Design and PCR-Based Gene Synthesis

Figure 1S: Amino acid and DNA coding sequence of human protein kinase B-2 (PKB2). The synthetic DNA coding sequence for human PKB2 that was optimized for high-level protein expression in *E. coli* was generated by replacing the indicated amino acid codons.

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          ctg                               cgtccgcgc       tta
1  atgaatgaggtgtctgtcatcaaagaaggctggctccacaagcgtggtgaatacatcaagacctggaggccacgggtacttctgtgaag  90
1  M N E V S V I K E G W L H K R G E Y I K T W R P R Y F L L K  30
          cgtcca                               ctgccg
91  agcgacggctccttcattgggtacaaggagggcccgaggcccctgatcagactctaccccccttaaacacttctccgtagcagaatgc  180
31  S D G S F I G Y K E R P E A P D Q T L P P L N N F S V A E C  60
          cgt  cgccct                               attcgt                               cgt
181  cagctgatgaagaccgagggccgcgaccacaacacctttgtcatacgtgcctgcagtgaccacagtcacgagaggaccttccacgtg  270
61  Q L M K T E R P R P N T F V I R C L Q W T T V I E R T F H V  90
          cgc                               cgt                               ctg                               cgc                               ccg
271  gattctccagacgagagggagtgatgcgggccatccagatggtcgccaacagcctcaagcagcgggccccaggcgaggacccccatg  360
91  D S P D E R E E W M R A I Q M V A N S L K Q R A P G E D P M  120
          cca                               gtt                               cgc
361  gactacaagtgtggctccccagtgactcctccacgactgaggagatggaagtggcgggtcagcaaggcacgggctaaagtgaccatgaat  450
121  D Y K C G S P S D S S T T E E M E V A V S K A R A K V T M N  150
          ctg  ttgctg                               ggc                               cgt
451  gacttcgactatctcaaactccttggcaagggaaacctttggcaaagtcatcctggtgcgggagaaggccactggccgctactacgccatg  540
151  D F D Y L K L L G K G T F G K V I L V R E K A T G R Y Y A M  180
          cgt                               attatc                               cgc  ctg                               cgt
541  aagatcctgcgaaaggaagtcatcattgccaaaggatgaagtcgctcacacagtcaccgagagccgggtcctccagaacaccaggcaccgg  630
181  K I L R K E V I I A K D E V A H T V T E S R V L Q N T R H P  210
          tta                               ttt
631  ttctcactgcgctgaagtatgccttccagaccacgaccgcctgtgctttgtgatggagtatgccaacgggggtgagctgttcttccac  720
211  F L T A L K Y A F Q T H D R L C F V M E Y A N G G E L F F H  240
          cgt  cgc                               cgtgcgcgc                               ctg                               cgt
721  ctgtcccgggagcgtgtcttcacagaggagcgggcccgggttttatggtgcagagattgtctcggctcttgagtacttgactcgcgaggac  810
241  L S R E R V F T E E R A R F Y G A E I V S A L E Y L H S R D  270
          gag  ctg                               ttg                               gaa
811  gtggtataaccgacatcaagctggaaaacctcatgctggacaaagatggccacatcaagatcactgactttggcctctgcaaagagggc  900
271  V V Y R D I K L E N L M L D K D G H I K I T D F G L C K E G  300
          acg                               gcc                               cgt
901  atcagtgacggggccaccatgaaaaccttctgtgggaccccggagtacctggcgcctgaggtgctggaggacaatgactatggccggggc  990
301  I S D G A T M K T F C G T P E Y L A P E V L E D N D Y G R A  330
          ccg                               ctg
991  gtggactggtgggggctgggtgtggtcatgtacgagatgatgtgcgccgcctgcccttctacaaccaggaccacgagcgcctcttcgag  1080
331  V D W W G L G V V M Y E M M C G R L P F Y N Q D H E R L F E  360
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Figure 1S (continued)

	tta	ctg	gaggaa	ttt	tta	cca	ctg	tta	ccg	
1081	ctcatcctcatggaagagatccgcttcccgcgcacgctcagccccgaggccaagtccctgcttgctgggctgcttaagaaggaccccaag									1170
361	L	I	L	M	E	E	I	R	F	P
							R	T	L	S
							P	E	A	K
							S	L	L	A
							G	L	L	K
							K	D	P	K
	390									
	cggctg		cca		cgt		ctg			
1171	cagaggcttgggtggggggcccagcgcgatgccaaggaggtcatggagcacaggttcttcctcagcatcaactggcaggacgtgggtccagaag									1260
391	Q	R	L	G	G	G	P	S	D	A
							K	E	V	M
							E	H	R	F
							F	L	S	I
							N	W	Q	D
							V	V	Q	K
	420									
	tta		ccg		cgc		gac			
1261	aagctcctgccacccttcaaacctcaggtcacgtccgaggtcgacacaaggtacttcgatgatgaatttaccgcccagtcctatcacaatc									1350
421	K	L	L	P	P	F	K	P	Q	V
							T	S	E	V
							D	T	R	Y
							F	D	D	E
							F	T	A	Q
							S	I	T	I
	450									
	cct		gat		cgt		tttccg		gcg	
1351	acacccccctgaccgctatgacagcctgggcttactggagctggaccagcggaccacttccccagttctcctactcggccagcatccgc									1440
451	T	P	P	D	R	Y	D	S	L	G
							L	L	E	L
							D	Q	R	T
							H	F	P	Q
							F	S	Y	S
							A	S	I	R
	480									
1441	gagtga									1443
481	E									481

Figure 2S: Amino acid and DNA coding sequence of human p70 40S ribosomal protein S6 kinase-1 (S6K1). The synthetic DNA coding sequence for human S6K1 that was optimized for high-level protein expression in *E. coli* was generated by replacing the indicated amino acid codons.

	cgtcgccgctgccgt	cgc	cgt	att	
1	atgaggcgacgaaggaggcgggacggccttttaccagccccggacttccgagacaggggaagctgaggacatggcaggagtgtttgacata				90
1	M R R R R R R D G F Y P A P D F R D R E A E D M A G V F D I				30
		ctgaacgag	gat	ggc	
91	gacctggaccagccagaggacgcgggctctgaggatgagctggaggaggggggtcagttaaatgaaagcatggaccatgggggagttgga				180
31	D L D Q P E D A G S E D E L E E G G Q L N E S M D H G G V G				60
	tac	cgc	cgc		
181	ccatatgaacttggcatggaacattgtgagaaatgtgaaatctcagaaactagtgtgaacagagggccagaaaaaatcagaccagaatgt				270
61	P Y E L G M E H C E K F E I S E T S V N R G P E K I R P E C				90
	ctg	gtg	caggtgcgc	atcttc	
271	tttgagctacttcgggtacttggtaaagggggctatggaaaggtttttcaagtacgaaaagtaacaggagcaaatactgggaaaatattt				360
91	F E L L R V L G K G G Y G K V F Q V R K V T G A N T G K I F				120
	attgtgcaac				
361	gccatgaaggtgcttaaaaaggcaatgatagtaagaaatgctaaagatacagctcatacaaaaagcagaacggaatattctggaggaagta				450
121	A M K V L K K A M I V R N A K D T A H T K A E R N I L E E V				150
	ccg	ctgatctac	ctg	ctg	
451	aagcatcccttcatcgtggatttaattatgcctttcagactgggtggaaaactctacctcatccttgagtatctcagtgaggagaacta				540
151	K H P F I V D L I Y A F Q T G G K L Y L I L E Y L S G G E L				180
	ctggagcgt	att	ttc	ctggcc	agc
541	tttatgcagttagaaagagaggaatatttatggaagacactgcctgcttttacttggcagaaatctccatggctttggggcatttacat				630
181	F M Q L E R E G I F M E D T A C F Y L A E I S M A L G H L H				210
	cag	cgc	ctgaac	ctg	ggcctg
631	caaaaggggatcatctacagagacctgaagccggagaatatcatgcttaatcaccaaggtcatgtgaaactaacagactttggactatgc				720
211	Q K G I I Y R D L K P E N I M L N H Q G H V K L T D F G L C				240
	acc	att	cgc		
721	aaagaatctattcatgatggaacagtcacacacacattttgtggaacaatagaatacatggcccctgaaatcttgatgagaagtggccac				810
241	K E S I H D G T V T H T F C G T I E Y M A P E I L M R S G H				270
	ctg	ccg	cgcaaaaag		
811	aatcgtgctgtggatttgggtggagtttgggagcattaatgtatgacatgctgactggagcacccttactgaggagaatagaaagaaa				900
271	N R A V D W W S L G A L M Y D M L T G A P P F T G E N R K K				300
	aag	ctgaagtgc	ccg	cgt	cgc
901	acaattgacaaaatcctcaaagttaaactcaatttgcctccctacctcacacaagaagccagagatctgcttaaaaagctgctgaaaaga				990
301	T I D K I L K C K L N L P P Y L T Q E A R D L L K K L L K R				330
		cgc			
991	aatgctgcttctcgtctgggagctggctcctggggacgctggagaagttcaagctcatccattcttttagacacattaactgggaagaactt				1080
331	N A A S R L G A G P G D A G E V Q A H P F F R H I N W E E L				360

Figure 2S (continued)

	cgc	ccaccg		gaggaa		cgc	
1081	ctggctcgaaaggtggagccccctttaaacctctggtgcaatctgaagaggatgtaagtcagtttgattccaagtttacacgtcagaca						1170
361	L A R K V E P P F K P L L Q S E E D V S Q F D S K F T R Q T						390
	gatagt				tac		
1171	cctgtcgacagcccagatgactcaactctcagtgaaagtgccaatcaggtctttctgggttttacatatgtggctccatctgtacttgaa						1260
391	P V D S P D D S T L S E S A N Q V F L G F T Y V A P S V L E						420
		ttc ttcgag	cgc	cgtcgc	cg		
1261	agtgtgaaagaaaagttttcctttgaaccaaaaatccgatcacctcgaagatttattggcagcccacgaacacctgtcagcccagtcaaa						1350
421	S V K E K F S F E P K I R S P R R F I G S P R T P V S P V K						450
	agc	cg	gca				
1351	ttttctcctggggatttctggggaagaggtgcttcggccagcacagcaaattcctcagacacctgtggaataccaatggaaacaagtggc						1440
451	F S P G D F W G R G A S A S T A N P Q T P V E Y P M E T S G						480
	att			attcgc	aagaag gcc		
1441	atagagcagatggatgtgacaatgagtggggaagcatcggcaccacttccaatacgacagccgaactctgggccatacaaaaaacaagct						1530
481	I E Q M D V T M S G E A S A P L P I R Q P N S G P Y K K Q A						510
	ccg		ctg				
1531	tttcccatgatctccaaacggccagagcacctgcgtatgaatctatga						1578
511	F P M I S K R P E H L R M N L						526

Figure 3S: Amino acid and DNA coding sequence of human phosphoinositide-dependent kinase-1 (PDK1). The synthetic DNA coding sequence for human PDK1 that was optimized for high-level protein expression in *E. coli* was generated by replacing the indicated amino acid codons.

	cgt		ccg	agctccggt		ccg		cgt		
1	atggccaggaccaccagccagctgtatgacgccgtgcccatccagtcagcgtgggtgttatgttcctgcccatccccatcaatggtgagg								90	
1	M A R T T S Q L Y D A V P I Q S S V V L C S C P S P S M V R								30	
		agctcc	ccg		cgt	ccg		cgccccg		
91	accagactgagtccagcagccccctggcattcctgggtggcagcaggcagggccccgccatggacggcactgcagccgagcctcggccc								180	
31	T Q T E S S T P P G I P G G S R Q G P A M D G T A A E P R P								60	
			cca		aaa	cgc		ttt	ggt	ggt
181	ggcgccggctccctgcagcatgccagcctccgcccagcctcggaagaagcggcctgaggacttcaagtttgggaaaatccttggggaa									270
61	G A G S L Q H A Q P P P Q P R K K R P E D F K F G K I L G E									90
			cgt		cgt		atc		cgt	att
271	ggctctttttccacggttgtcctggctcgagaactggcaacctccagagaatatgcgattaaaattctggagaagcgacatatcataaaa									360
91	G S F S T V V L A R E L A T S R E Y A I K I L E K R H I I K									120
	gaa	ccg	cgt		ccg					
361	gagaacaaggtcccctatgtaaccagagagcgggatgtcatgtcgcgcctggatcacccttctttgttaagctttacttcacatttcag									450
121	E N K V P Y V T R E R D V M S R L D H P F F V K L Y F T F Q									150
			ggt	ctg						
451	gacgacgagaagctgtatttcggccttagttatgccaaaaatggagaactacttaaatatattcgcaaaatcggttcattcgatgagacc									540
151	D D E K L Y F G L S Y A K N G E L L K Y I R K I G S F D E T									180
		cgt				cgt				
541	tgtaccgcattttacacggctgagatcgtgtctgcttttagagtacttgcacggcaagggcatcattcacaggaccttaaacggaaaac									630
181	C T R F Y T A E I V S A L E Y L H G K G I I H R D L K P E N									210
			att		ggt			cgt		
631	attttgtaaataagatatgcacatccagatcacagattttggaacagcaaaagtcttatccccagagagcaacaagccagggccaac									720
211	I L L N E D M H I Q I T D F G T A K V L S P E S K Q A R A N									240
		ggt						ggt		
721	tcattcgtgggaacagcgcagtacgtttctccagagctgctcacggagaagtccgcctgtaagagttcagacctttgggctcttggtatgc									810
241	S F V G T A Q Y V S P E L L T E K S A C K S S D L W A L G C									270
	attatt		ggt	cct	cgt	ggt		att		
811	ataatataccagcttgtggcaggactcccaccattccgagctggaaacgagtatcttatatttcagaagatcattaagttggaatatgac									900
271	I I Y Q L V A G L P P F R A G N E Y L I F Q K I I K L E Y D									300
		tttttt	cgt		cgc					
901	ttccagaaaaattcttccctaaggcaagagacctcgtggagaaacttttggtttttagatgccacaaagcggttaggctgtgaggaaatg									990
301	F P E K F F P K A R D L V E K L L V L D A T K R L G C E E M									330
		ggt	ggt		ttt		caa			
991	gaaggatacggacctcttaaagcacaccggttcttcgagtcggtcacgtgggagaaacctgcaccagcagacgcctccgaagctcaccgct									1080
331	E G Y G P L K A H P F F E S V T W E N L H Q Q T P P K L T A									360

Figure 3S (continued)

		gat	
1081	tacctgccggctatgtc	ggaagacgacgaggactgctatggcaattatgacaatctcctgagccagtttggctgcatgcaggtgtcttcg	1170
361	Y L P A M S E D D E D C Y G N Y D N L L S Q F G C M Q V S S		390
	agc	ccg cgt	atc
1171	tcctcctcctcacactccctgtcagcctccgacacgggcctgccccagaggtcaggcagcaacatagagcagtacattcacgatctggac		1260
391	S S S S H S L S A S D T G L P Q R S G S N I E Q Y I H D L D	cgtctg ctg	420
		ggt	
1261	tcgaactcctttgaactggacttacagttttccgaagatgagaagaggttggttggtggagaagcaggctggcggaaacccttggcaccag		1350
421	S N S F E L D L Q F S E D E K R L L L E K Q A G G N P W H Q		450
	aac attgtg	cgtcgc	
1351	tttgtagaaaataatttaataactaaagatgggcccagtgataagcgggaagggtttatttgcaagacgacgacagctggtgctcacagaa		1440
451	F V E N N L I L K M G P V D K R K G L F A R R R Q L L L T E		480
	ggt tac gac	cgt	
1441	ggaccacatttatattatgtggatcctgtcaacaaagttctgaaaggtgaaattccttggtcacaagaacttcgaccagaggccaagaat		1530
481	G P H L Y Y V D P V N K V L K G E I P W S Q E L R P E A K N	cgt tac	510
		ccg ggt	cgt
1531	tttaaaactttctttgtccacacgcctaacaggacgtattatctgatggaccccagcgggaacgcacacaagtgggtgcaggaagatccag		1620
511	F K T F F V H T P N R T Y Y L M D P S G N A H K W C R K I Q		540
	cgtcaacgt		
1621	gaggtttggaggcagcgataaccagagccacccggacgccgctgtgcagtga		1668
541	E V W R Q R Y Q S H P D A A V Q		556

SUPPLEMENTARY MATERIAL (TABLES)

Tables 1S-3S: Oligonucleotide primers designed for the thermodynamically-balanced conventional (TBC) (11,12) and thermodynamically-balanced inside-out (TBIO) methods of PCR-based synthesis of the codon-optimized PKB2 (Table 1S), S6K1 (Table 2S), and PDK1 (Table 3S) gene sequences. In each table, the coding- or "sense"-strand oligonucleotide primers (5'→3') and the noncoding- or "antisense"-strand oligonucleotide primers (3'←5') designed for both the TBC and TBIO strategies are listed for (A) the amino-terminal half and (B) the carboxy-terminal half of the gene sequence. For each primer, the overall length (nt), the coding region of the gene sequence, the melting temperature, T_m , and the number of base pairs (bp) for each overlapping region are given. For each primer, the 5'-terminal overlapping nucleotides are indicated in blue color, and the 3'-terminal nucleotides are indicated in red color. Green colored regions of primers indicate nucleotides that are common to both the 5'- and 3'-overlapping regions. The start and stop codons are capitalized in the primer sequences.

Table 1AS: Oligonucleotide primers designed for the thermodynamically-balanced conventional (TBC) (11,12) and thermodynamically-balanced inside-out (TBIO) methods of PCR-based synthesis of the amino-terminal half of the codon-optimized protein kinase B-2 (PKB2) gene sequence.

TBC name	coding or "sense" (S) oligonucleotide primers (5'→3') noncoding or "antisense" (A) primers (3'←5')	T_m (°C)	overlap (bp)	length (nt)	coding region	TBIO name
C-S01	ggggggggcatATGaatgaggtgtctgtcatcaaagaaggctggctgcacaagcgtggt	64.8	19	60	-12-48	IO-S19
C-A01	gaccgacgtgttcgcaccacttatgtagttctggaccgcaggcgcgatgaagaatgactt	63.4	22	60	29-89	IO-S18rc
C-A01rc	ctggctgcacaagcgtggtgaatacatcaagacctggcgtccgcgctacttcttactgaa					IO-S18
C-S02	gtccgcgctacttcttactgaagagcgcagcggctccttcattgggtacaaggagcgtccag	65.2	22	60	68-127	IO-S17
C-A02	taaccatgttcctcgcaggtctccggggactagtctgagacggcgggaatttgttgaag	65.4	24	60	106-165	IO-S16rc
C-A02rc	attgggtacaaggagcgtccagaggcccctgatcagactctgcccccttaaacaacttc					IO-S16
C-S03	actctgccgcccttaaacaacttcctcgtagcagaatgacagctgatgaagaccgagcgt	65.3	21	60	142-201	IO-S15
C-A03	gtcgactacttctggctcgcaggcgcgggattgtggaaacagtaagcaacggacgtcacc	65.3	21	60	181-240	IO-S14rc
C-A03rc	cagctgatgaagaccgagcgtccgcgccctaacaccttctgctattcgttgccctgcagtgg					IO-S14
C-S04	gtcattcgttgccctgcagtggaaccacagtcatcgagcgtaccttccacgtggattctcca	63.4	22	60	220-279	IO-S13
C-A04	atggaaggtgcacctaaagaggtctgctcgcgctcctcacctacgcacggtaggtctacca	65.3	21	60	258-317	IO-S12rc
C-A04rc	taccttccacgtggattctccagacgagcgcgaggagtgatgctgctgccatccagatggt					IO-S12
C-S05	gatgctgcccacccagcgcgcccagggcagggaccgatggacta	64.8	19	69	297-365	IO-S11
C-A05	cgctcctgggctacctgatgttcacaccgagggggttcaactgaggaggtgc	64.8	19	50	347-396	IO-S10rc
C-A05rc	gcgaggaccgcgatggactacaagtgtggctccccaagtgactcctccacg					IO-S10
C-S06	cccaagtgactcctccacgactgaggagatggaagttgctggtcagcaaggcagcgcgctaa	64.5	20	60	378-437	IO-S09
C-A06	cagtcggtcctcgtgctgctgatttcaactggtacttactgaagctgatagactttaacgacccg	64.8	26	60	418-477	IO-S08rc
C-A06rc	gtcagcaaggcagcgcgctaaagtgaccatgaatgacttcgactatctgaaattgctgggc					IO-S08
C-S07	acttcgactatctgaaattgctgggcaagggcaccttggcaagtcatcctggtgctg	65.3	21	60	452-511	IO-S07
C-A07	cgtttcagtaggaccacgcactcttccgggtgaccggcgatgatgctggtacttctaggacg	63.4	22	60	491-550	IO-S06rc
C-A07rc	gcaaagtcatcctggtgctgctgagaaggccactggccgctactacgccatgaagatcctgc					IO-S06
C-S08	tactacgccatgaagatcctgctgtaaggaagtcattatgcccaaggatgaagtcgctcac	65.3	21	60	529-588	IO-S05
C-A08	cggttctacttccagcagtggtgtcagtggtctctcggcgcaggacgtcttgtgggcagtg	64.8	19	60	568-627	IO-S04rc
C-A08rc	gccaaggatgaagtcgctcacacagtcaccgagagccgcgctcctgcagaacacccgtcac					IO-S04
C-S09	cctgcagaacaccgctcacccgttcttaactgcgctgaagtatgcctttcagaccacga	63.5	23	60	609-668	IO-S03
C-A09	ttcatacggaaagtctgggtgctggcggacacgaaacactacctatacgggtgccccca	64.5	20	60	646-705	IO-S02rc
C-A09rc	aagtatgcctttcagaccacgaccgcctgtgcttctgtgatggagtatgcccaacgggggt					IO-S02
C-S10	tggagtatgcccaacgggggtgagctgttcttccacctgtccgctgagcgcgctcttcacag	64.8	19	60	686-745	IO-S01

Table 1BS: Oligonucleotide primers designed for the thermodynamically-balanced conventional (TBC) (11,12) and thermodynamically-balanced inside-out (TBIO) methods of PCR-based synthesis of the carboxy-terminal half of the codon-optimized protein kinase B-2 (PKB2) gene sequence.

TBC name	coding region	length (nt)	overlap (bp)	T_m (°C)	coding or "sense" (S) oligonucleotide primers (5'→3')	noncoding or "antisense" (A) primers (3'←5')	TBIO name
C-A10	727-786	60	19	64.8	gcactcgcgcagagaagtgtc	tcctcgcacgcgcgaaataccacgtctctaacagagccga	IO-A01
C-S11					tttatgggtgcagagattgtctcggct	ctggagtacttgcactcgcgtgacgtgggtataacc	IO-A02rc
C-S11rc	761-820	60	26	64.8	aaataccacgtctctaacagagccga	gacctcatgaacgtgagcgcactgcaccatatgg	IO-A02
C-A11	801-860	60	20	64.5	gagcgcactgcaccatatgg	cgctgtagtctcgacctcttggactacgacctgtttctacc	IO-A03
C-S12					acctgatgctggacaaaagatgg	ccacatcaaatgcactgacttttggcttgtgcaaagaag	IO-04rc
C-S12rc	839-898	60	22	63.4	tggactacgacctgtttctacc	gggtgtagtcttagtgactgaaaccgaacacgtttcttc	IO-A04
C-A12	875-934	60	24	63.7	gactgaaaccgaacacgtttcttc	cgtagtcaactgccccgggtggtacttttggagacac	IO-A05
C-S13					ggccaccatgaaaaccttctgtg	ggacgcggagtagcctggccctctgaggtgctggagga	IO-A06rc
C-S13rc	912-971	60	23	65.3	ccgggtggtacttttggagacac	cctcgcggcctcatggaccgggactccacgacctcct	IO-A06
C-A13	954-1013	60	18	65.3	gggactccacgacctcct	gttactgataccggcacggcaccctgaccacccccgaccaca	IO-A07
C-S14					actggtgggggctgggtgt	gggtcatgtacagatgatgtgctgcccgcctgcccgttctaca	IO-A08rc
C-S14rc	995-1054	60	19	66.9	tgaccacccccgaccaca	ccagtacatgctctactacacgcggcggacggcaagatgt	IO-A08
C-A14	1037-1096	60	18	65.2	cgccggacggcaagatgt	tggctcctggtgctcgcggacaagatcaattaggactacctcc	IO-A09
C-S15					ctgttctagttaatcctgatggagg	aaatccgctttccgcgcacgttaagcccagaggcc	IO-A10rc
C-S15rc	1072-1131	60	25	64.6	gacaagatcaattaggactacctcc	tttaggcgaaaggcgcgtgcaattcgggtctccgg	IO-A10
C-A15	1113-1172	60	19	64.8	gtgcaattcgggtctccgg	ttcagggacgaccgaccccgacaatttcttctcctgggettctgt	IO-A11
C-S16					ctgttaaagaaggacccgaagca	gcgcctgggtggggggccaagcgtgccaaggagggtc	IO-A12rc
C-S16rc	1150-1209	60	23	63.5	gacaatttcttctcctgggettctgt	cgccgacccacccccggttcgtctacggttctcctccag	IO-A12
C-A16	1190-1249	60	20	64.5	gttcgctacggttctcctccag	tacctcgtggcaagaaggactcctcgtagttgacctcctgc	IO-A13
C-S17					gagcatcaactggcaggacgt	tggtccagaagaagtactgccaccggttcaaacctcaggt	IO-A14rc
C-S17rc	1230-1289	60	20	64.5	ctcgtagttgacctcctgc	accaggctcttcttcaatgacgggtggcaagtttggagtcca	IO-A14
C-A17	1269-1328	60	21	65.3	cggtggcaagtttggagtcca	gtgcaggctccagctgtgtgctgatgaagctgctacttaa	IO-A15
C-S18					gacacacgctacttcgacgatgaatt	taccgcccagtcctacacaaatcacacctcctgac	IO-A16rc
C-S18rc	1303-1362	60	26	64.8	ctgtgtgctgatgaagctgctacttaa	atggcgggtcaggtagtgttagtgtggaggactg	IO-A16
C-A18	1341-1400	60	22	63.4	gtagtgttagtgtggaggactg	gcgatactatcggacccgaatgacctcgacctggctgc	IO-A17
C-S19					ttactggagctggaccagcgt	taccacactttccgcagttctcttactcggcgagcatccgc	IO-A18rc
C-S19rc	1381-1440	60	20	64.5	aatgacctcgacctggctgc	atgggtgaaaggcgtcaagaggatgagccgctcgtagggc	IO-A18
C-A19	1423-1482	60	18	65.2	atgagccgctcgtaggcg	ctcACTattactgagctcctaggccgcgactcgcgcgcgcgc	IO-A19

Table 2AS: Oligonucleotide primers designed for the thermodynamically-balanced conventional (TBC) (11,12) and thermodynamically-balanced inside-out (TBIO) methods of PCR-based synthesis of the amino-terminal half of the codon-optimized p70 40S ribosomal protein S6 kinase-1 (S6K1) gene sequence.

TBC name	coding or "sense" (S) oligonucleotide primers (5'→3') noncoding or "antisense" (A) primers (3'←5')	T_m (°C)	overlap (bp)	length (nt)	coding region	TBIO name
C-S01	gaaggtcgtcatATGcgtcgccgtcgccgtcgggacggcttttaccagccccggacttc	64.5	20	60	-12-48	IO-S22
C-A01	aatgggtcggggcctgaagggcgtggcacttcgactcctgtaccgtcctcaciaactgt	63.4	22	60	29-88	IO-S21rc
C-A01rc	tttaccagccccggacttcgcgaccgtgaagctgaggacatggcaggagtgtttgaca					IO-S21
C-S02	gacatggcaggagtgtttgacattgacctggaccagccagaggacgcgggctctgaggat gagctggag	64.5	20	69	67-135	IO-S20
C-A02	cgagactcctactcgacctcctccccagtcgacttgctctcgtacctagtacc	63.4	21	55	116-170	IO-S19rc
C-A02rc	gctctgaggatgagctggaggaggggggtcagctgaacgagagcatggatcatgg					IO-S19
C-S03	gaacgagagcatggatcatggcggagtggaccatacgaacttggtatggaacattgtga	63.6	27	60	150-209	IO-S18
C-A03	tatgcttgaaccgtaccttgaactcctttaaacttttagagtctttgatcacacttggc	63.2	26	60	183-242	IO-S17rc
C-A03rc	atacgaacttggcatggaacattgtgagaaatttgaatctcagaaactagtgtgaaccg					IO-S17
C-S04	gaaatctcagaaactagtgtgaaccgcgggccagaaaaatccgccagaatgttttgag	63.6	27	60	217-276	IO-S16
C-A04	cttttttagggcgggtcttataaaaactcgacgaagcccatgaaccatttccccgatacct	63.3	21	60	250-309	IO-S15rc
C-A04rc	gaaaaaatccgccagaatgttttgagctgcttcgggtaacttggtaaagggggctatgga					IO-S15
C-S05	cttggtaaagggggctatggaagggtgtttcaggctgcgcaaagtaacaggagcaaatact	63.2	26	60	289-348	IO-S14
C-A05	acgcgtttcattgtcctcgtttatgacccttttagaagcggctacttccacgaatttttcc	63.5	23	60	323-382	IO-S13rc
C-A05rc	tgcgcaaagtaacaggagcaaatactgggaaaatcctcgccatgaaggtgcttaaaaagg					IO-S13
C-S06	cgccatgaaggtgcttaaaaaggcaatgattgtgcgcaacgctaaagatacagctcatac	64.8	26	60	360-419	IO-S12
C-A06	gcggttgcgattttctatgtcaggtatgttttcgtcttgcttataagacctccttcatttc	63.6	27	60	394-453	IO-S11rc
C-A06rc	cgcaacgctaaagatacagctcatacaaaagcagaacggaatattctggaggaagtaaaag					IO-S11
C-S07	gaacggaatattctggaggaagtaaacatccgttcatcgtggatctgatctacgccttt	63.5	23	60	427-486	IO-S10
C-A07	agcacctagactagatgcggaaggtctgaccaccttttgagatggagttaggacctcatag	63.5	23	60	464-523	IO-S09rc
C-A07rc	tcgtggatctgatctacgcctttcagactggtggaaaactctacctcatcctggagtatc					IO-S09
C-S08	actctacctcatcctggagtatctcagtgaggagaaactgtttatgcagctggagcgtga	63.4	22	60	501-560	IO-S08
C-A08	acaaatacgtcgacctcgacctccttataaaaataccttctgtgacggacgaagatggacc	64.5	20	60	539-598	IO-S07rc
C-A08rc	tgtttatgcagctggagcgtgagggaaatttttatggaagaactgctgctgcttctacctgg					IO-S07
C-S09	caactgctgcttctacctggccgaaatcagcatggctttggggcattttacatcagaaggg	63.5	23	60	579-638	IO-S06
C-A09	aaccccgtaaatgtagtcttcccctagtagatggcgtggacttcggcctcttatagtac	63.4	22	60	616-675	IO-S05rc
C-A09rc	ttggggcattttacatcagaaggggatcatctaccgcaactgaagccggagaatatcatg					IO-S05
C-S10	cctgaagccggagaatatcatgctgaaccaccaaggtcatgtgaaactgacagactttgg	64.8	26	60	654-713	IO-S04
C-A10	ccagtacactttgactgtctgaaaccggacacgcttcttagataagtaactacctgtcag	63.5	29	60	688-747	IO-S03rc
C-A10rc	ggtcatgtgaaactgacagactttggcctgtgcaagaatctattcatgatggaacagtc					IO-S03
C-S11	gcaaagaatctattcatgatggaacagtcacacacaccttttggtggaacaattgaataca	63.9	30	60	719-778	IO-S02
C-A11	gtgtgtggaaaacaccttgttaacttatgtaccggggactttagaactacgcgtcaccgg	63.4	21	60	749-808	IO-S01rc
C-A11rc	cacacaccttttggtggaacaattgaatacatggcccctgaaatcttgatgcgccagtgccc					IO-S01

Table 2BS: Oligonucleotide primers designed for the thermodynamically-balanced conventional (TBC) (11,12) and thermodynamically-balanced inside-out (TBIO) methods of PCR-based synthesis of the carboxy-terminal half of the p70 40S ribosomal protein S6 kinase-1 (S6K1) gene sequence.

TBC name	coding region	length (nt)	overlap (bp)	T_m (°C)	coding or "sense" (S) oligonucleotide primers (5'→3')	noncoding or "antisense" (A) primers (3'←5')	TBIO name
C-S12	788-847	60	21	63.4	aaatcttgatg	gattggtggagtctgggagcattaa	IO-A01rc
C-S12rc					tttagaactacgcgtcaccggtg	gttagcacgacacctaaccacctcagaccctcgtaatt	IO-A01
C-A12	822-882	60	25	64.6	ctaaccacctcagaccctcgtaatt	acatactgtacgactgacctcgtggcggtgaagtga	IO-A02
C-S13	863-922	60	20	64.5	ctggagcaccgccattcactggggaga	atcgcaaaaagacaattgacaagatcctgaagt	IO-A03rc
C-S13rc					gacctcgtggcggtgaagtga	cccctcttagcgtttttctgttaactgttctaggacttca	IO-A03
C-A13	890-949	60	33	64.8	tagcgtttttctgttaactgttctaggacttca	cgtttgagttaaacggagggcatggagt	IO-A04
C-S14	924-983	60	26	64.8	caaactcaatttgcctccgtacctca	cacaagaagcccgatctgcttaaaaagctgct	IO-A05rc
C-S14rc					gtttgagttaaacggagggcatggagt	gtgttcttcgggcactagacgaatttttcgacga	IO-A05
C-A14	956-1018	60	25	64.6	gggcactagacgaatttttcgacga	ctttgcttacgacgaagagcagaccctcgaccag	IO-A06
C-S15	999-1058	60	20	64.5	ttctcgtctgggagctggctc	ctggggacgctggaagttaacagctcatccattctttcg	IO-A07rc
C-S15rc					aagagcagaccctcgaccag	gaccctgacgacctcttcaagttcgagttaggtaagaaagc	IO-A07
C-A15	1032-1091	60	27	63.6	tcttcaagttcgagttaggtaagaaagc	gggtgaattgaccttcttgaagaccgagcgtt	IO-A08
C-S16	1071-1130	60	21	63.4	ggaagaacttctggctcgcaagg	gtggagccaccgtttaaacctctgttgcaatctgagga	IO-A09rc
C-S16rc					ccttcttgaagaccgagcgtt	ccacctcgggtggcaaatgggagacaacgtagactcct	IO-A09
C-A16	1104-1163	60	27	63.6	caaatgggagacaacgtagactcct	tctacattcagtcaaaactaagggttcaaatgtgc	IO-A10
C-S17	1135-1194	60	29	63.5	gtaagtcagtttgattccaagtttacag	ccagacacctgtcgatagttccagatgactca	IO-A11rc
C-S17rc					cattcagtcaaaactaagggttcaaatgtgc	ggctcgtggacagctatcaggtctactgagt	IO-A11
C-A17	1172-1231	60	23	63.5	gacagctatcaggtctactgagt	tgagagtcactttcaggttagtccagaaaagacccaa	IO-A12
C-S18	1210-1269	60	22	63.4	gccaatcaggtctttctgggtt	ttacatacgtggctccatctgtacttgaaagtgtgaaa	IO-A13rc
C-S18rc					cggttagtccagaaaagacccaa	aatgtatgcaccgaggttagacatgaactttcacacttt	IO-A13
C-A18	1243-1302	60	27	63.6	cgaggtagacatgaactttcacacttt	cttttcaagaggagagctcgggttttagggcaggt	IO-A14
C-S19	1281-1340	60	22	63.4	cttcgagccaaaaatccgctca	cctcgtcgtttatttggcagcccacgtacacctgtcag	IO-A15rc
C-S19rc					gaagctcgggttttagggcaggt	ggagcagcgaataaccgtcgggtgcatgtggacagtc	IO-A15
C-A19	1321-1380	60	20	64.5	tcgggtgcatgtggacagtc	gggtcagtttaaatcgggaccctaaagaccctgcgcca	IO-A16
C-S20	1362-1421	60	19	64.8	ggatttctggggacgcggt	gcttcggcaagcacaagcaaatcctcagacacctgtggaata	IO-A17rc
C-S20rc					cctaaagaccctgcgcca	cgaagccgttcgtgctggttaggagctctgtggacaccttat	IO-A17
C-A20	1396-1455	60	26	64.8	cgtttaggagctctgtggacaccttat	gggttacctttgttcaccgtaactcgtctacctta	IO-A18
C-S21	1433-1492	60	23	63.5	caagtggcattgagcagatggat	gtgacaatgagtggggaagcatcggcaccacttccaa	IO-A19rc
C-S21rc					gttcaccgtaactcgtctacctta	cactgttactcacccttcgtagccgtggtaaggtt	IO-A19
C-A21	1472-1531	60	21	63.4	ttcgtagccgtggtagaggtt	aagcggtcggcttgagaccgggtatgttcttcgttcgga	IO-A20
C-S22	1511-1570	60	21	63.4	ggccatacaagaagcaagcct	ttccgatgatctccaaacgcccagagcaccctgcgtatga	IO-A21rc
C-S22rc					cgggtatgttcttcgttcgga	aaggctactagaggtttgcccgtctcgtggacgcatact	IO-A21
C-A22	1551-1610	60	20	64.5	cggctcgtggacgcatact	tagacACTattatcccagcagcgtggcgactcgttattg	IO-A22

Table 3AS: Oligonucleotide primers designed for the thermodynamically-balanced conventional (TBC) (11,12) and thermodynamically-balanced inside-out (TBIO) methods of PCR-based synthesis of the amino-terminal half of the codon-optimized phosphoinositide-dependent protein kinase-1 (PKD1) gene sequence.

TBC name	coding or "sense" (S) oligonucleotide primers (5'→3')	noncoding or "antisense" (A) primers (3'←5')	T _m (°C)	overlap (bp)	length (nt)	coding region	TBIO name
C-S01	actgactcatATGgcccgtaccaccagccagctgtatgacgccgtg		66.5	20	46	-10-36	IO-S24
C-A01	cggtcgacatactgcgccacggctaggtctcgaggcaaca	caatacaaggacgggcaggg	64.5	20	60	17-76	IO-S23rc
C-A01rc	gccagctgtatgacgccgtg	ccgatccagagctccgttgtgttatgttcctgcccgtccc	68.6	20	60	57-116	IO-S23
C-S02	gttatgttcctgcccgtccc	catcaatgggtgctgaccagactgagagctccacgccc	68.6	20	60	57-116	IO-S22
C-A02	tgactctcgaggtgcccgggaccgtaaggaccaccgtcggcagt	cccgggcccgtacctg	67.2	16	60	97-156	IO-S21rc
C-A02rc	actgagagctccacgcccgtg	ggcattcctgggtggcagccgtcagggcccggccatggac	67.2	17	60	141-200	IO-S21
C-S03	gggcccggccatggac	ggcactgcagccgagcctcgcccgggcgcccgtccctgcagca	67.2	17	60	141-200	IO-S20
C-A03	gggcccgggacgtcgt	acgggtcggaggcgggtgctcggagcgttttttcgcccgtacctg	63.6	26	60	184-243	IO-S19rc
C-A03rc	gccgggtccctgcagcat	gccagcctccgccacagcctcgcaaaaagcgcctgaggac	63.6	27	60	218-277	IO-S19
C-S04	agcctcgcaaaaagcgcctgaggac	tttaagtgttggtaaaatccttgggtgaaggctctt	63.6	27	60	218-277	IO-S18
C-A04	aaccatttttaggaaccacttccgagaaa	aaagggtgccaacaggaccgagcacttgaccgtt	65.3	21	60	251-310	IO-S17rc
C-A04rc	ttggtaaaaatccttgggtgaaggctctt	tttccacgggtgtcctggctcgtgaactggcaa	64.8	26	60	290-349	IO-S17
C-S05	tcctgggtcgtgaactggcaac	cctcccgtgaataatgcatcaaaaattctggagaagcgtc	64.8	26	60	290-349	IO-S16
C-A05	acgctagtttttaagacctcttcgag	tatagtaatttctttgttcagggcatacattg	64.0	32	60	324-383	IO-S15rc
C-A05rc	tgcatcaaaaattctggagaagcgtc	atcatttaagaaaacaagggtcccgtatgtaac	66.5	20	60	352-411	IO-S15
C-S06	atcatttaagaaaacaagggtcccgtatgtaac	ccgtgagcgcgatgtcatgtcgcgcctg	66.5	20	60	352-411	IO-S14
C-A06	cgctacagtagcagcgcggac	ctagtgggcaagaaa	65.3	25	60	392-451	IO-S13rc
C-A06rc	gcgatgtcatgtcgcgcctg	gatcaccggttctttgttaagctttacttcacatttcagg	65.3	25	60	392-451	IO-S13
C-S07	gttaagctttacttcacatttcagg	acgacgagaagctgtatttcggccttagttatgcc	63.7	24	60	427-486	IO-S12
C-A07	gacataaagccggaatcaatacgggtttttaccactgacgaat	ttatataagcgttttag	63.3	36	60	463-522	IO-S11rc
C-A07rc	ctgtatttcggccttagttatgccc	aaaaatgggtgaactgcttaaatatattcgcaaaatc	65.4	24	60	487-546	IO-S11
C-S08	aaaaatgggtgaactgcttaaatatattcgcaaaatc	gggttcattcgatgagacctgtacc	65.4	24	60	523-582	IO-S10
C-A08	ccaagtaagctactctggacatggg	caaaaatgtgcccgactctagcacagacgaaatctc	65.4	24	60	523-582	IO-S09rc
C-A08rc	gggttcattcgatgagacctgtacc	cgtttttacacggctgagatcgtgtctgcttttagag	65.4	24	60	559-618	IO-S09
C-S09	gctgagatcgtgtctgcttttagagt	acttgcacggcaagggcatcattcaccgtgacctt	65.4	24	60	559-618	IO-S08
C-A09	ttcccgtagtaagtgccactggaa	ttggccttttgtaaaacaatttacttctatacgtg	63.8	35	60	595-654	IO-S07rc
C-A09rc	aagggcatcattcaccgtgacctta	aacccggaaaacattttgttaaatgaagatatgcac	64.0	28	60	620-679	IO-S07
C-S10	aaccggaaaacattttgttaaatgaagatatg	cacattcagatcacagatttttggtacag	64.0	28	60	620-679	IO-S06
C-A10	gtgtaagtctagtgctaaaaccatgtc	ggttttcagaataggggtctctcgtttgttcgg	65.3	27	60	652-711	IO-S05rc
C-A10rc	cacattcagatcacagatttttggtacag	caaaaagctttatcccagagagcaacaagcc	65.3	21	60	685-744	IO-S05
C-S11	gtcttatcccagagagcaaaacagcc	cgtgccaaactcattcgtgggtacagcgcagtac	65.3	21	60	685-744	IO-S04
C-A11	aagcaccatgtcgcgtcatg	caaagaggtctcgacgagtgccctctcagggcagcattc	65.3	21	60	724-783	IO-S03rc
C-A11rc	ttcgtgggtacagcgcagtac	gtttctccagagctgctcaccggagaagtcgcgctgtgtaag	63.0	26	60	763-822	IO-S03
C-S12	acggagaagtcgcctgtaag	agttcagacctttgggctcttgggtgcattatttaccag	63.0	26	60	763-822	IO-S02
C-A12	cccgagaaccaacgtaataaatggtc	gaacaccgtccagaggggtggaaagccagccat	64.5	20	60	797-856	IO-S01rc
C-A12rc	gggctcttgggtgcattatttaccag	cttgggtggcaggtctcccaccttccgtgctggtgta					IO-S01

Table 3BS: Oligonucleotide primers designed for the thermodynamically-balanced conventional (TBC) (11,12) and thermodynamically-balanced inside-out (TBIO) methods of PCR-based synthesis of the carboxy-terminal half of the codon-optimized phosphoinositide-dependent protein kinase-1 (PDK1) gene sequence.

TBC name	coding region	length (nt)	overlap (bp)	T_m (°C)	coding or "sense" (S) oligonucleotide primers (5'→3') noncoding or "antisense" (A) primers (3'←5')	TBIO name
C-S13	837–896	60	20	64.5	cccacctttccgtgctggtaacgagtatccttatttttcagaagatcattaagttggaata	IO-A01rc
C-S13rc					gggtggaaaggcagcaccattgctcatagaataaaaaagtcttctagtaattcaaccttat	IO-A01
C-A13	859–918	60	38	62.7	ctcatagaataaaaaagtcttctaataattcaaccttataactgaaaggtccttttaagaag	IO-A02
C-S14	882–941	60	37	64.1	cattaagttggaatatgactttccagaaaaattcttccttaaggcacgtgacctcgtgga	IO-A03rc
C-S14rc					gtaattcaaccttataactgaaaggtccttttaagaagggattccgtgactggagcacct	IO-A03
C-A14	922–981	60	20	64.5	ttccgtgactggagcacctccttgaaaaccaaactctacgggtgttccggaatccgaca	IO-A04
C-S15	960–1019	60	22	65.3	tgccacaaagcgttaggctgtgaggaaatggaaggttacggctcctcttaagcacacccc	IO-A05rc
C-S15rc					acgggtgttccggaatccgacactcctttaccttccaatggcaggagaatttcgtgtggg	IO-A05
C-A15	998–1057	60	22	65.3	tgccaggagaatttcgtgtgggcaagaaactcaggcagtgcaacctcttggacgtggctg	IO-A06
C-S16	1040–1099	60	18	65.2	gggagaacctgcaccagcaaacgcctccgaagctcaccgcttacctgcccggctatgtcgg	IO-A07rc
C-S16rc					ccctcttggacgtgtgcttctgagggcttcgagtgccgaaatggacggccgatacagcc	IO-A07
C-A16	1082–1141	60	18	65.2	tgacggccgatacagccttctgctactcctgacgataccgttaatactgttagaggact	IO-A08
C-S17	1113–1172	60	29	64.9	ctgctatggcaattatgacaatctcctgaagcagtttggctgtatgcaggtgtcttcgag	IO-A09rc
C-S17rc					gacgataccgttaataactgttagaggactcggtaaacacgacatacgtccacagaagctc	IO-A09
C-A17	1152–1211	60	21	63.4	gacatacgtccacagaagctcgaggaggagtgtagggacagtcggaggctgtgcccgga	IO-A10
C-S18	1196–1255	60	16	64.7	cctccgacacgggctgccgcagcgttcaggcagcaacatcagcagcagcattcacgac	IO-A11rc
C-S18rc					ggaggctgtgcccggacggcgtcgcaagtcctgctgttagctcgtcatgtaagtgttag	IO-A11
C-A18	1233–1292	60	23	63.5	gtagctcgtcatgtaagtgtcagacctgagcttgaggaaacttgacctgaatgtcaaaag	IO-A12
C-S19	1266–1325	60	27	63.6	ctcctttgaaactggactttacagttttccgaagatgagaagcgtcgttggctggagaagca	IO-A13rc
C-S19rc					gaggaaacttgacctgaatgtcaaaagccttctactcttcgagacaacgacctcttcgt	IO-A13
C-A19	1304–1363	60	22	65.3	tcgagacaacgacctcttcgctccgaccgcattgggaacctgtgtcaaacatcttttat	IO-A14
C-S20	1334–1393	60	30	65.3	gtaacccttggcaccagttttagaaaaataacttaattctgaagatgggcccagtgata	IO-A15rc
C-S20rc					cattgggaacctgtgtcaaacatcttttatgtaattaaacttctaccgggtcacctat	IO-A15
C-A20	1372–1431	60	22	65.3	gacttctaccgggtcacctatctcgttcccaaataaacgtgcagcggctgtcgacaac	IO-A16
C-S21	1413–1472	60	19	64.8	acgtcgcgacagctgtgtctcacagaaggtccacatttataattacgtggacctgtcaa	IO-A17rc
C-S21rc					tgacggcgtgtcgacaacgagtgcttccaggtgtaaatataatgcacctgggacagtt	IO-A17
C-A21	1445–1504	60	28	64.0	gtgtaaatataatgcacctgggacagttgtttcaaactttccactttaaggaaccagtg	IO-A18
C-S22	1480–1539	60	25	62.9	ctgaaaggtgaaattccttggcacaagaactctgctccagaggccaagaattttaaaact	IO-A19rc
C-S22rc					gactttccactttaaggaaccagtggttcttgaagcaggtctccggttcttaaaattttga	IO-A19
C-A22	1512–1571	60	28	64.0	agcaggtctccggttcttaaaaattttgaagaacaggtgtgcggattggcatgcatgat	IO-A20
C-S23	1551–1610	60	21	63.3	cacgcctaaccgtactactatctgtagccggcaggtaacgcacacaagtggtgccc	IO-A21rc
C-S23rc					gtgcccattggcatgcatgatagactacctggcctcgcattgctgtgttccaccaggg	IO-A21
C-A23	1592–1651	60	19	64.8	tgctgtgttccaccagggcattctagctcctcaaaccgcagttgcaatggctcgggtgg	IO-A22
C-S24	1631–1690	60	21	65.3	gtcaacgttaccagagccaccggagcggctgtgcagTAAtgatagctcagggatccgc	IO-A23rc
C-S24rc					cagttgcaatggctcgggtggcctgcccgcacacgtcATTactatcgagctcctagggc	IO-A23
C-A24	1673–1702	60	18	63.0	ctatcgagctcctagggcagtcgaatttat	IO-A24

