

Silent mutagenesis and fragmentation of the coding sequence of the VEGF homology domain of VEGF and VEGF-C

Numbering according to VEGF; **Bold black** = restriction sites/overlapping sequences used for annealing short double-stranded DNA fragments, **Bold dark blue** = non-coding sequence of oligos, **Bold grey** = vector sequence, **Red** = conserved cysteine residues, **Green** = identical amino acid residues

VEGF-A	BamHI																																								
	G	GAT CCT		GGG CAG AAT CAT CAC GAA GTG																																					
VEGF-C	C CTA G	GA		CCC GTC TTA GTA GTG CTT CAC																																					
	G	GAT CCT		GCA CAT TAT AAT ACC GAG ATC																																					
	C CTA G	GA		CGT GTA ATA TTA TGG CTC TAG																																					
VEGF-A		D	P	G	Q	N	H	H	E	V																															
VEGF-C		D	P	A	H	Y	N	T	E	I																															
		6	7	8	9	10	11	12	13	14																															
VEGF-A	GTG AAA TTC ATG GAT	.	GTC TAT CAG CGC AGC TAC TGC CAT CCG ATC GAG ACA CTG																																						
	CAC TTT AAG TAC CTA	.	CAG ATA GTC GCG TCG ATG ACG GTA GGC TAG CTC TGT GAC																																						
VEGF-C	CTG AAA TCT ATT GAT AAT GAG TGG AGA AAG ACT CAG TGC ATG CCG AGA GAG GTG TGT																																								
	GAC TTT AGA TAA CTA TTA CTC ACC TCT TTC TGA GTC ACG TAC GGC TCT CTC CAC ACA																																								
VEGF-A	V K F M D	.	V Y Q R S Y C H P I E T L																																						
VEGF-C	L K S I D N E W R K T Q C M P R E V C																																								
	15	16	17	18	19	19B	20	21	22	23	24	25	26	27	28	29	30	31	32																						
	SapI XhoI KpnI SapI																																								
VEGF-A	GTG GAC ATC TTC CAG G	AATAGAAGAGCTCGAG														GGTACCGCTCTTCG	AA TAC CCT GAT																								
	CAC CTG TAG AAG GTC CTT A	TCTTCTCGAGCTC														CCATGGCGAGAAGCTTA	TG GGA CTA																								
VEGF-C	ATC GAC GTG GGG AAG G	AATAGAAGAGCTCGAG														GGTACCGCTCTTCG	AA TTT GGA GTC																								
	TAG CTG CAC CCC TTC CTT A	TCTTCTCGAGCTC														CCATGGCGAGAAGCTTA	AA CCT CAG																								
VEGF-A	V D I F Q																																								
VEGF-C	I D V G K																																								
	33	34	35	36	37																							38	39	40	41										
	MscI NcoI																																								
VEGF-A	GAG ATC GAG TAC ATC TTC AAG CCA TCC TGC GTG CCC CTG ATG AGA TGT GG													C CAT GG																											
	CTC TAG CTC ATG TAG AAG TTC GGT AGG ACG CAC GGG GAC TAC TCT ACA CC													G GTA CC																											
VEGF-C	GCG ACA AAC ACC TTC TTC AAG CCA CCA TGT GTG TCC GTG TAC AGA TGT GG													C CAT GG																											
	CGC TGT TTG TGG AAG AAG TTC GGT GGT ACA CAC AGG CAC ATG TCT ACA CC													G GTA CC																											
VEGF-A	E I E Y I F K P S C V P L M R C G																																								
VEGF-C	A T N T F F K P P C V S V Y R C G																																								
	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58																								
	KpnI SmaI																																								
VEGF-A	GG TAC CC	G GGT TGC TGC AAT		GAC GAA GGG CTG																																					
	CC ATG GG	C CCA ACG ACG TTA		CTG CTT CCC GAC																																					
VEGF-C	GG TAC CC	G GGT TGC TGC AAT		AGT GAG GGG CTG																																					
	CC ATG GG	C CCA ACG ACG TTA		TCA CTC CCC GAC																																					
VEGF-A		G	C	C	N	D	E	G	L																																
VEGF-C		G	C	C	N	S	E	G	L																																
		59	60	61	62	63	64	65	66																																
	Tsp509I																																								
VEGF-A	GAG TGC GTT CCC ACC GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG AGA ATT																																								
	CTC ACG CAA GGG TGG CTC CTC AGG TTG TAG TGG TAC GTC TAA TAC TCT TAA																																								
VEGF-C	CAG TGC ATG AAC ACG TCC ACG AGC TAC CTC AGC AAG ACG CTG TTT GAA ATT																																								
	GTC ACG TAC TTG TGC AGG TGC TCG ATG GAG TCG TTC TGC GAC AAA CTT TAA																																								
VEGF-A	E C V P T E E S N I T M Q I M R																																								
VEGF-C	Q C M N T S T S Y L S K T L F E																																								
	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82																									
	Tsp509I																																								
VEGF-A	A ATT AAA CCT CAC	.	.	GTT CCC GTC GTG TAG CCT CTC TAC TCG																																					
	T TAA TTT GGA GTG	.	.	GTT CCC GTC GTG TAG CCT CTC TAC TCG																																					
VEGF-C	A ATT ACA GTG CCT CTC TCT CAA GGG CCC AAA CCA GTG ACA ATC AGC																																								
	T TAA TGT CAC GGA GAG AGA GTT CCC GGG TTT GGT CAC TGT TAG TCG																																								
VEGF-A	I K P H	.	.	Q G Q H I G E M S																																					
VEGF-C	I T V P L S Q G P K P V T I S																																								
	83	84	85	86	86B	86C	87	88	89	90	91	92	93	94	95																										
	BbsI NotI																																								
VEGF-A	TTT CTC CAG CAT AAC AAA TGT GAA TGT AGA CCA AAG AAA GAT TGA GTC TTC GCG GCC GC																																								
	AAA GAG GTC GTA TTG TTT ACA CTT ACA TCT GGT TTC TTT CTA ACT CAG AAG CGC CGG CG																																								
VEGF-C	TTT GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAG CTG GAT TGA GTC TTC GCG GCC GC																																								
	AAA CGG TTA GTG TGA AGG ACG GCT ACG TAC AGA TTC GAC CTA ACT CAG AAG CGC CGG CG																																								
VEGF-A	F L Q H N K C E C R P K K D *																																								
VEGF-C	F A N H T S C R C M S K L D *																																								
	96	98	98	99	100	101	102	103	104	105	106	107	108	109																											

Oligonucleotides used for the assembly of the VEGF/VEGF-C mosaic molecules

Sequence of the VEGF (A) and VEGF-C (C) oligonucleotides used for assembly of the hybrid molecules and the corresponding amino acid sequences. Sequences that give rise to overhangs after annealing of the corresponding oligonucleotides (e.g. A1 fwd with A1 rev, A2 fwd with A2 rev, etc.) are shown in boldface.

A1 fwd **GAT** CCT GGG CAG AAT CAT CAC GAA GTG **GTG AAA T**
D P G Q N H H E V V K

A2 fwd TC ATG GAT GTC TAT CAG CGC AGC TAC TGC CAT
F M D V Y Q R S Y C H

A3 fwd **CCG** ATC GAG ACA CTG GTG GAC ATC TTC CAG GAATAGAAGAGC
P I E T L V D I F Q

A4 fwd CGCTCTTCGAA TAC CCT GAT GAG ATC GAG TAC A
E Y P D E I E Y

A5 fwd **TC TTC AAG CCA** TCC TGC GTG CCC CTG ATG AGA TGT GGC
I F K P S C V P L M R C G

A6 fwd CCG GGT TGC TGC AAT GAC GAA GGG CTG G
G C C N D E G L

A7 fwd **AG TGC** GTT CCC ACC GAG GAG TCC AAC ATC ACC ATG CAG ATT ATG AG
E C V P T E E S N I T M Q I M R

A8 fwd **A ATT** AAA CCT CAC CAA GGG CAG CAC ATC GGA GAG ATG **AGC TTT**
I K P H Q G Q H I G E M S F

A9 fwd CTC CAG CAT AAC AAA TGT GAA TGT AGA CCA AAG AAA GATTGAGTCTTCGC
L Q H N K C E C R P K K D

A1 rev CCACTTCGTGATGATTCTGCCAG

A2 rev **TCGGATGGCAGTAGCTGCGCTGATAGACATCCATGAATTTCA**

A3 rev **TCGAGCTCTTCTATTCTGGAAGATGTCCACAGTGTCTCGA**

A4 rev **TGGCTTGAAGAT**GTACTCGATCTCATCAGGGTATTCTGAAGAGCGGTAC

A5 rev **CATGGCCACATCTCATCAGGGGCACGCAGGA**

A6 rev **GCACTCCAGCCCTTCGTCAATTGCAGCAACCCGGGTAC**

A7 rev **AATTTCTCATAATCTGCATGGTGTGTTGGACTCCTCGGTGGGAAC**

A8 rev CATCTCTCCGATGTGCTGCCCTTGGTGAGGTTT

A9 rev **GGCCGCGAAGACTCAATCTTTCTTTGGTCTACATTACATTTGTTATGCTGGAGAAAAGCT**

C1 fwd **GAT** CCT GCA CAT TAT AAT ACC GAG ATC **CTG AAA T**
D P A H Y N T E I L K

C2 fwd CT ATT GAT AAT GAG TGG AGA AAG ACT CAG TGC ATG
S I D N E W R K T Q C M

C3 fwd **CCG** AGA GAG GTG TGT ATC GAC GTG GGG AAG GAATAGAAGAGC
P R E V C I D V G K

C4 fwd CGCTCTTCGAA TTT GGA GTC GCG ACA AAC ACC T
E F G V A T N T

C5 fwd **TC TTC AAG CCA** CCA TGT GTG TCC GTG TAC AGA TGT GGC
F F K P P C V S V Y R C G

C6 fwd CCG GGT TGC TGC AAT AGT GAG GGG CTG C
G C C N S E G L

C7 fwd **AG TGC** ATG AAC ACG TCC ACG AGC TAC CTC AGC AAG ACG CTG TTT GA
Q C M N T S T S Y L S K T L F E

C8 fwd **A ATT** ACA GTG CCT CTC TCT CAA GGG CCC AAA CCA GTG ACA ATC **AGC TTT**
I T V P L S Q G P K P V T I S F

C9 fwd GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAG CTG GATTGAGTCTTCGC
A N H T S C R C M S K L D

C1 rev GGATCTCGGTATTATAATGTGCAG

C2 rev **TCGGCATGCATGAGTCTTTCTCCACTCATTATCAATAGATTTCA**

C3 rev **TCGAGCTCTTCTATTCTTCCCCACGTCGATACACACCTCTC**

C4 rev **TGGCTTGAAGA**AGGTGTTTGTGCGGACTCCAAATTCTGAAGAGCGGTAC

C5 rev **CATGGCCACATCTGTACACGGACACACATGG**

C6 rev **GCACTGCAGCCCTCACTATTGCAGCAACCCGGGTAC**

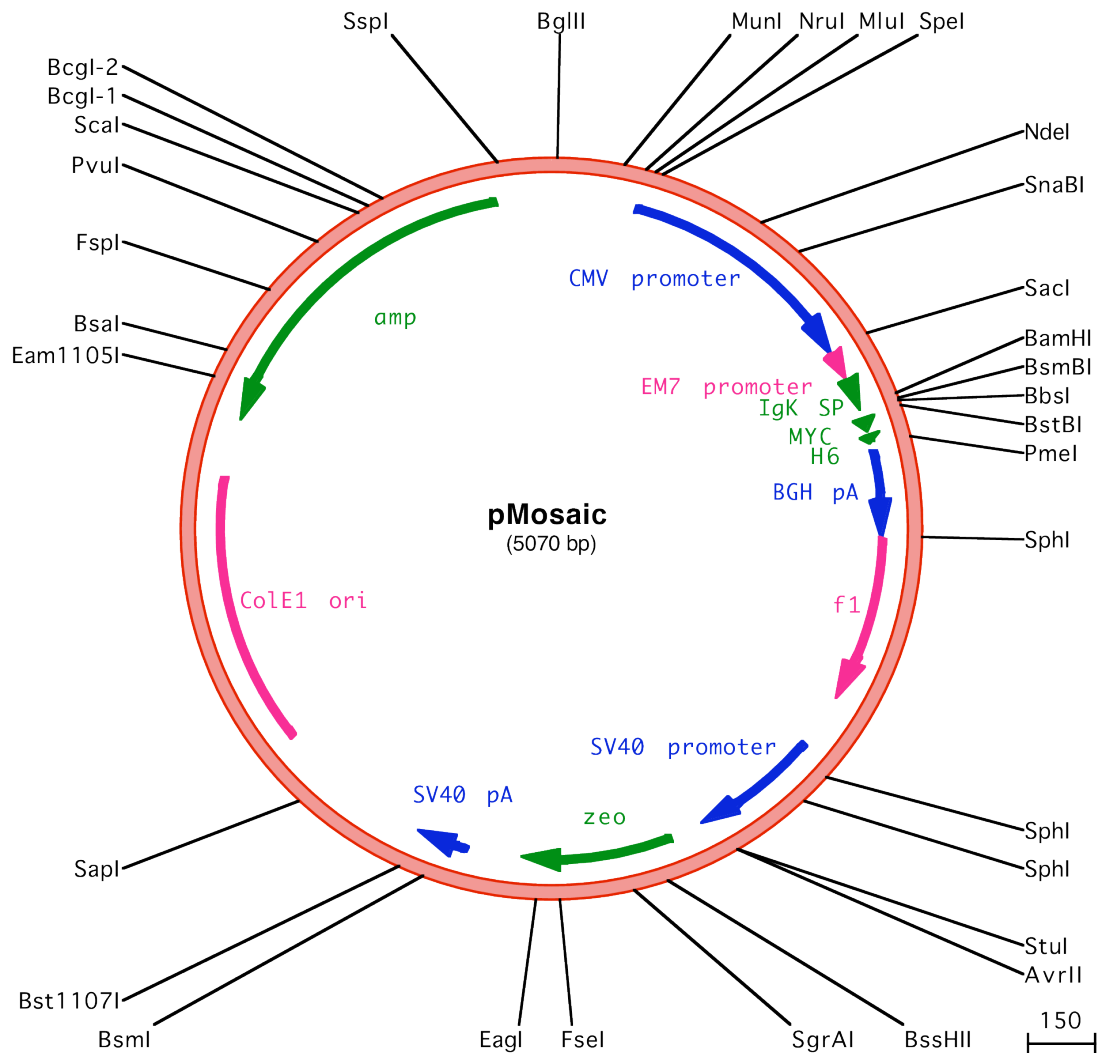
C7 rev **AATTTCAAACAGCGTCTTGCTGAGGTAGCTCGTGGACGTGTTTCAT**

C8 rev GATTGTCACTGGTTTGGGCCCTTGAGAGAGAGGCACTGT

C9 rev **GGCCGCGAAGACTCAATCCAGCTTAGACATGCATCGGCAGGAAGTGTGATTGGCAAAGCT**

Map of pMosaic

pMosaic is based on pSacTagA (Invitrogen). The latter was modified to eliminate the restriction sites for MscI (in the zeocin coding sequence) and the BbsI site in the bovine growth hormone polyadenylation sequence. The EM7 promoter (from pTRACER-CMV, Invitrogen) was inserted between the CMV promoter and the IgK leader sequence. The polylinker was modified eliminating most restriction sites and introducing a BsmBI site, that creates a NcoI-compatible overhang upon cleavage.



Nucleotide sequence of pMosaic

GACGGATCGGGAGATCTCCCGATCCCCATGGTGCAGTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTG
TGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTT
AGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT
TAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTGACGTC
AATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTA
CATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTACATGACCTTATGGG
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTT
TGACTACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAAAC
AACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGGCGGTGTTGACAATTAATCATCGGCA
TAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTCCAG
GTTCCACTGGTGACGCGGCCAGGATCCGTCTCCCATGCCGATTTGGTCTTCGAACAAAACTCATCTCAGAAGAGGATCTGAATAGCGCCGTC
GACCATCATCATCATCATATTGAGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCG
TGCTTCTCTGACCTGGAAGGTGCCACTCCCACTGTCTTTCTAATAAAATGAGGAAATGTCATCGCATTGTCTGAGTAGGTGTCATTCTAT
TCTGGGGGGTGGGGTGGGCGAGGACAGCAAGGGGAGGATTGGGAAGATAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAG
GCGGAAAGAACAGCTGGGGCTCTAGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGA
CCGCTACACTTGGCAGCGCCCTAGCGCCCGCTCCTTTGCTTTCTTCCCTTCTTCTCGCCACGTTTCGCGGCTTTCCCGCTCAAGCTCTAAA
TCGGGGCATCCCTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTACAGTAGTGGGCCATCG
CCCTGATAGACGGTTTTTCGCCCCTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCATCT
CGGTCTATTCTTTGATTATAAAGGATTTTGGGGATTTTCGGCTATTGGTTAAAAATGAGCTGATTAAACAAAAATTAACGCGAATTAATT
CTGTGGAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAATATGCAAAGCATGCATCTCAATTAGTCAGCAACCA
GGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAATATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACCTCCGCC
CATCCCGCCCCTAACCTCCGCCAGTTCCGCCCATTTCTCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAAGGCGGAGGCCGCTCTGCC
TCTGAGCTATTCAGAAGTAGTGAGGAGGCTTTTTGGAGGCTTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCGGATCTG
ATCAGCACGTGTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAACCATGGCTAAGTTGACCACT
GCCGTTCCGGTGCTCACC CGCGCGACGTCGCGGAGCGGTGAGTTCTGGACCGACCGGCTCGGTTCTCCCGGACTTCGTGGAGGACGACT
TCGCGGTGTGGTCCGGGACGACGTGACCCGTTCATCAGCGCGGTCCAGGACAGGTGGTGCCGACAACACCTGGCTGGGTGTGGGTGCG
CGGCTGGACGAGCTGTACGCCGAGTGGTTCGAGGTGCTGTCCAGAACTTCGGGACGCTCCGGGCGGGCCATGACCGAGATCGGCGAGCAG
CCGTGGGGGCGGAGTTTCGCCCTGCGCGACCCGCGCGCAACTGCGTGCACTTCGTGGCCGAGGAGCAGGACTGACAGTGTACGAGATTTTCG
ATTCACCGCGCCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGA
GTTCTTCGCCCACCCCACTTGTATTATGCACTTATAATGGTTACAAATAAAGCAATAGCATCACAATTTACAAATAAAGCATTTTTTTCA
CTGCATTCTAGTTGTGGTTGTCCAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGGT
CATAGCTGTTTCTGTGTGAAATGTTATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCCTAATG
AGTGAGCTAACTACATTAATTGCGTTGCGCTCACTGCCCGCTTTCAGTCGGGAAACCTGTCTGTCCAGCTGCATTAATGAATCGGCCAACGC
GCGGGAGAGGCGGTTTTCGCTATTGGGCGCTCTTCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTCTCGCTGCGGCGAGCGGTATCAG
CTCACTCAAAGGCGGTAATACGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCG
TAAAAAGGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCTCAAGTCAGAGGTGGCGAAACCCG
ACAGGACTATAAAGATACAGGCGTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCGACCTGCGGCTTACCGGATACCTGTCCGCT
TTCTCCCTTCGGGAAGCGTGCGCTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCTGCTCCTCAAGCTGGGCTGTGTGCA
CGAACCCCGCTTCAGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCA
GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAG
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TTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAAAC
GAAACTCACGTAAAGGATTTTGGTTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAGGTTTAAATCAATCT
AAAGTATATATAGTAACCTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTATCCATAGT
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GCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTGCACTTTATCCGCTCCATCCAGTCTATTAATT
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CCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCATCCGTAAGAT
GCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAAATAGTGTATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAA
TACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTTGGAACCGTTCTTCGGGGCGAAACTCTCAAGGATCTTACCCTGTTGAGATCC
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