

Supplementary Data

A Protocol for Seamless and Simultaneous Fusion of Multiple DNA Fragments via Homology-Annealing Extension Mediated Fusion PCR (HAEMF-PCR)

by

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1. Fragment sequences

Human TERT gene Promoter Sequence from (NCBI reference sequence NG_009265.1):

**TTTCCTACTTGGCAGTACATCTACGTAACGGCATTTCGTGGTGCCCGGAGCCCGACGCCCCGCGT
CCGGACCTGGAGGCAGCCCTGGGTCTCCGGATCAGGCCAGCGGCCAAAGGGTCGCCGCACGCAC
CTGTTCCAGGGCCTCCACATCATGGCCCTCCCTCGGGTTACCCACAGCCTAGGCCGATTTCG
ACCTCTCTCCGCTGGGGCCCTCGCTGGCGTCCCTGCACCCTGGGAGCGCGAGCGGGCGCGGGG
GGGAAGCGCGGCCAGACCCCCGGGTCCGCCCGGAGCAGCTGCGCTGTCGGGGCCAGGCCGGG
CTCCAGTGGATTTCGCGGGCACAGACGCCAGGACCGCGCTTCCACGTGGCGGAGGGACTGGG
GACCCGGGCACCCGTCTGCCCTTACCTTCCAGCTCCGCTCCTCCGCGCGGACCCCGCCCC
GTCCCGAC**

NSP4 (nonstructural protein 4, partial, synthetic sequence)

**AGAGAGTTGCCCGTACTTGACAGCGCAGCGTTCAATGTTGAGTGTTTCAAGAAGTTCGCTTGTA
ATCAGGAATACTGGAAGACATTC AAGGAGAATCCATTAGACTGACCACTGAGAACGTGACGAA
CTACATCACGAAGTTGAAAGGGCCTGAAGCAGCCGCATTCTTTGCG**

SGP promoter+51 nt cis element

**AGAGGACCTGTTATACACCTCTACGGCGGTCTAGATTGGTGCGTTAATACACAGAATTCTGAT
TATAGCGCACTATTATAGCACC**

miniCMV:

GGTAGGCGTGACGGTGGGAGGCCTATATAAGCAGAGCT

Synthetic PLA2: UniProtKB/Swiss-Prot: Q9PVF1.1

**ATGGCACGAACCCTGTGGATACTGGCCGTGCTGCTGGTGGGCGTAGAAGGATCTCTGGTGCAAT
TTGAGACCATGATCATGAAACTCGCCAAAAGGTCTGGCTTCTTCTGGTACTCTTTCTATGGTTG
TTATTGTGGTTGGGGCGGGCATGGACTGCCACAGGATCCTACAGATCGGTGTTGCTTCGTGCAC
GATTGTTGCTACGGCAAGGTCACCAACTGTAACCCTAAAACAGCTACATACTCCTACACAGAGG
AGAATGACGGGATTGTGTGTGGGGGTGATGATCCATGCAAAAACAGGTCTGCGAATGTGATCG
AGTGGCAGCCATGTGTTTCCGAGACAACAAGACACTTACGACGGCGATAAGTATTGGAAACTG
CCTCCTCAGAAGTGTGAGGAAGATCCTGAGCCATGTAGACCCCCCTCCTCCACCCAAT**

Synthetic SVMP: UniProtKB/Swiss-Prot: [U5PZ28.1](#)

**ATGATCCAGGTACTGCTGGTGACCATCTGCCTCGCCGCACTGCCATAACCAGGGGAGCAGCATCA
TTCTGGAGAGCGGCAACGTGAACGACTACGAGGTGGTGTACCCCCGGAAGGTGACGGCTCTGCC
CAAGGGCGCCGGCCAGCCCAAGTACGAGGACGCCATGCAGTACGAGTTC AAGTGAACGGCGAG
CCTGTGGTGTGCACCTGGAGAAGAACAAGGGACTGTTTAGTAAGGACTACTCCGAAACTCACT
ACTCCAGCGACGGGCGGAAAATCACAATAACCCCCCTGTGGAGGATCACTGCTACTACCACGG**

GAGGATCGAGAATGACGCCGACTCTACTGGGTCCATCTCTGCCTGTAATGGCCTAAAGGGACAC
TTCAAGCTGCAGGGGGAGATGTATCTGATCGAGCCACTGAAACTGAGTGACAGCGAGGCCCATG
CCATCTACAAGTACGAGAACGTGGAAAAAGAAGACGAGGCCCTAAAATGTGTGGCGTGACCGA
GACCAACTGGGAAAGCTACGAGCCTATTA AAAAGGCTAGCCAGAGCAACCTGACCCCCGAACAG
CAGAGGTTCAACCCCTTTAAGTACGTGGAGCTGGTGTATCGTGGCCGATCACAGGATGTTCACTA
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ACAACAGGTGTACAGGACAGAGCGCCGATTGCCCTTCAAATGGAAGACCCCCCTTCTCCACC
CAAT

Synthetic SVSP: UniProtKB/Swiss-Prot: [Q9PTU8.1](#)

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CACTGCGACATGAGAAATATGAGAATCTACCTGGGCGTGCACAATGAGGGCGTGCAGCACGCAG
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CACCGTCTGCAGAGGCGCTCATGCAGGGCTGCCTGCCACCAGCAGGACCCTGTGTGCCGGCGTG
CTGCAAGGCGGGATCGACACTTGTGGCGGCGACTCTGGCGGGCCCCTGATCTGCAATGGGACCT
TCCAGGGCATCGTGTCTTGGGGGGGCCACCCCTGCGCCCAGCCTGGCGAGCCCGCTCTGTATAC
CAAAGTGTTTGATTACCTGCCTTGGATCCAGTCCATCATCGCTGGCAACACAACAGCCACCTGC
CCCCCGGACCTCTTGGGCTGGCT

2. Primers used in the Paper

Table 1. Primers Used in the Paper			
Name	Sequence (5'-3')	Overlap Length	Description
F01f	TTGGCAGTACATCTACGTAACGGCATTCTGGTGCCCGGAGCCCG CA202		Forward primer for F1 fragment with Sna BI
F01r	ACAGGGTTCGTGCCATGGTGCGGGTGCTATAATAG	20 bp	Reverse primer for F1 fragment
F01r-25	GCACGGCCAGTATCCACAGGGTTCGTGCCATGGTGCGGGTGCTATAATAG	25 bp	Reverse primer for F1 fragment (adjusted)
F02f	CACCATGGCACGAACCCTGTGGATACTGGCCGTGC	25 bp	Forward primer for F2 fragment
F02r-25	AGGTCAGCAGGCTTCCCCTTCTTCATTGGGTGGAGGAAGGGGGGGTCTAC	25 bp	Reverse primer for F2 fragment
F02r	CCCTTCTTATTGGGTGGAGGAAGGGGGGGTCTACATGGCTCAGGATCTTCTGACAC	20 bp	Reverse primer for F2 fragment
F03f-25	GAAGGAAGGGGAAGCCTGCTGACCT	25 bp	Forward primer for F3 fragment
F03f	TCCACCAATGAAGGAAGGGGAAGCCTGCTGACCT	20 bp	Forward primer for F3 fragment
F03r-25	GCTTGAGCAGGGAGAAGTTGGTGGCATTGGTGGAGG GGGGGTCTTCC	25 bp	Reverse primer for F3 fragment
F04f-25	GCCACCAACTTCCCTGCTCAAGC	25 bp	Forward primer for fragment 4 amplification
F04r	TTAAGCCAGCCCAAGAGGTCCGGGG		Reverse Primer for For fragment 4 amplification
F04r-2	GAAAAAAGCTTGAATTGTTTTT AAGCCAGCCCAAGAGGTCCGGGGGGGCAGGTGGCTGTT GTGTT		Reverse Primer for For fragment 4 amplification
F1f-003-N	CTACGTAACGCGTCGGCATTCTGGTGCCCGGAGCCCGACGCCCGCTCCGGACC		Forward Primer for CA401 fragment1 with MluI site
F1r-003	CCATGGTGGCAGCTCTGCTTATATAGGCCTCCAC	20 bp	Reverse Primer for CA401 F1 fragment
F2f(Tai)-003	AAGCAGAGCTGCCACCATGGCTTCTTGCT	20 bp	Forward Primer for CA401 fragment2
F2r-004-3	AGCAGGCTTCCCCTTCTCGCTGCCCGCCGCCAGACCCGCCCGCC	25 bp	Reverse Primer for CA401 F2 fragment
F3f-004	GCAGCGAAGGAAGGGGAAGCCTGCTGACCTGC	25 bp	Forward Primer for CA401 F3 fragment
F3r-003	GAAAAAAGCTTGAATTGTTTTAACTTAAGCCAGCCCAAGAGG		Reverse Primer for CA401 plasmid with PmeI site

3. Efficacy Predication for HAEMF-PCR efficiency

Numer of fusion fragments	10 HAEMF-PCR cycles	15 HAEMF-PCR cycles
2	65.13%	79.50%
3	12.80%	18.40%
4	2%	3.00%
5	0.32%	0.48%
6	0.05%	0.08%

Number of Cycles	2 fragments	3 fragments	4 fragments	5 fragments	6 fragments
5	41.00%	6.50%	1.02%	0.80%	0.03%
10	65.10%	12.80%	2.06%	1.60%	0.05%
15	79.50%	18.40%	3.11%	2.30%	0.08%

4. Plasmid Sequences used in the paper:

Ep-CA201

aaacaattcaagcttttttcaattctcgacctcgagacaaatggcagatattcatccacaatttttaaagaaaagggg
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