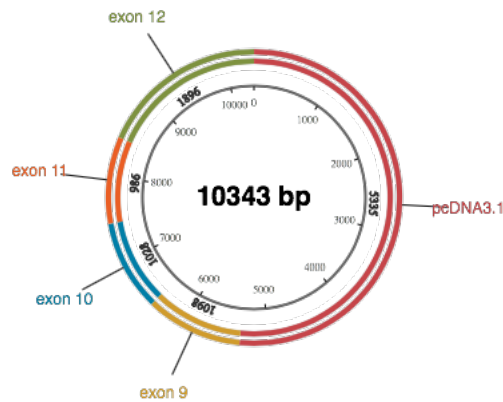


New Assembly

Created: 1/15/2019, 2:37:35 PM
Saved: not saved

Component Fragments

Name	Length	Produced by	5' End	3' End
pcDNA3.1	5360	PCR	Fwd Primer (auto)	Rev Primer (auto)
exon 9	1124	PCR	Fwd Primer (auto)	Rev Primer (auto)
exon 10	1053	PCR	Fwd Primer (auto)	Rev Primer (auto)
exon 11	1011	PCR	Fwd Primer (auto)	Rev Primer (auto)
exon 12	1920	PCR	Fwd Primer (auto)	Rev Primer (auto)



Notes

- A 60 minute reaction is recommended for the assembly of more than 3 fragments.
- Primer pcDNA3.1_fwd has %GC outside of desired range (35-65%) in the annealing segment.
- Primer exon 9_fwd has %GC outside of desired range (35-65%) in the annealing segment.
- Primer exon 9_rev contains a run of 4+ repeats of a mono/di/trinucleotide.
- Primer exon 9_rev has %GC outside of desired range (35-65%) in the annealing segment.
- Primer exon 10_fwd contains a run of 4+ repeats of a mono/di/trinucleotide.
- Primer exon 10_rev contains a run of 4+ repeats of a mono/di/trinucleotide.
- Primer exon 10_rev has %GC outside of desired range (35-65%) in the annealing segment.
- Primer exon 11_fwd contains a run of 4+ repeats of a mono/di/trinucleotide.
- Primer exon 11_rev contains a run of 4+ repeats of a mono/di/trinucleotide.
- Primer exon 12_fwd contains a run of 4+ repeats of a mono/di/trinucleotide.
- Primers for fragment pcDNA3.1 have internal complementarity.

Required oligos

Name	Primer 5' (overlap/spacer/ANNEAL) 3'	Len	%GC	3' %GC	3' Tm	3' Ta
pcDNA3.1_fwd	gggcccgtttaaaCCCCTGATCAGCCTCGA	31	61	67	70.3	68.4
pcDNA3.1_rev	tgcgccagcgtAAGCTTAAGTTTAAACGCTAGCCAGC	38	53	42	67.4	68.4
exon 9_fwd	taacttaagcttACGCTGGCCGCAGGGATT	31	48	67	73.8	72.0
exon 9_rev	cctgtgatttttGGCCACGAGTGGAGATGC	32	56	68	71.6	72.0
exon 10_fwd	cactcgtgggccAAAAATCCACAGGTGATTCTGATGC	37	51	40	65.4	66.4
exon 10_rev	tgctggaaggcaGAGTGGGGTATCTGCGGC	31	61	67	68.6	66.4
exon 11_fwd	gatacccccactcTGCCTTTCCAGCAAGATTTTTC	34	47	41	63.6	63.6
exon 11_rev	accatgacccacAGTAGCTGGGACTACAGG	31	58	56	62.6	63.6
exon 12_fwd	gtcccagctactGTGGGGTCATGGTTTACAG	31	55	53	62.9	62.0

exon 12_rev	ctgatcagcgggTTTAAACGGGCCCTCTAG	30	57	50	61.0	62.0
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Build Settings

Property	Value
Product/Kit	#E5520 NEBuilder HiFi DNA Assembly Cloning Kit
Minimum Overlap	25 nt
Minimum Overlap Tm	48 °C
Circularize	Yes
PCR Polymerase/Kit	Q5 High-Fidelity DNA Polymerase
PCR Primer Conc.	500 nM
Min. Primer Length	18 nt