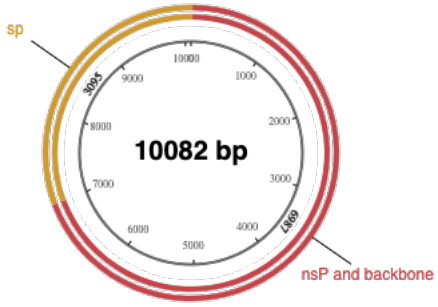
New_Assembly

Created: 12/13/2021, 5:52:38 PM
Saved: not saved

Component Fragments

Name	Length	Produced by	5' End	3' End
nsP and backbone	6987	PCR	Fwd Primer (custom)	Rev Primer (custom)
sp	3135	PCR	Fwd Primer (custom)	Rev Primer (custom)



Notes

- Everything looks OK. No major issues detected.

Required oligos

Name	Primer 5' (overlap/spacer/ANNEAL) 3'	Len	%GC	3' %GC	3' Tm	3' Ta
nsP and backbone_fwd	**GGGATTCAATGGATCGAGC**	19	53	53	63.0	62.5
nsP and backbone_rev	**TTGTTCATGTCTAGCGTCTC**	20	45	45	61.5	62.5
sp_fwd	**GAGACGCTAGACATGAACAAG**	21	48	48	62.6	62.9
sp_rev	**TGCTCGATCCATTGAATCC**	19	47	47	61.9	62.9

Build Settings

Property	Value
Product/Kit	#E5520 NEBuilder HiFi DNA Assembly Cloning Kit
Minimum Overlap	20 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

Assembled Sequence

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#VERSION     .
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#  ORGANISM  synthetic DNA construct
#REFERENCE   1  (bases 1 to 10082)
#  AUTHORS   .
#  TITLE     NEBuilder-generated Construct
#  JOURNAL   Exported  13-DEC-2021 from NEBuilder https://nebuilder.neb.com
#COMMENT     NEBuilder-generated oligos (UPPERCASE = gene-specific, lowercase = overlap)
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#COMMENT     nsP and backbone_rev: TTGTTCATGTCTAGCGTCTC
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#COMMENT     sp_fwd: GAGACGCTAGACATGAACAAG
#COMMENT     sp_fwd 3'Tm: 62.6  3'Ta: 62.9
#COMMENT     sp_rev: TGCTCGATCCATTGAATCC
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