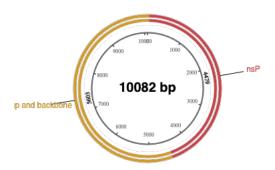
Component Fragments

Name	Length	Produced by	5' End	3' End
nsP	4479	PCR	Fwd Primer (custom)	Rev Primer (custom)
sp and backbone	5687	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_fwd	TGTGTGTCAGAATAGTGAATG	21	38	38	59.2	60.2
nsP_rev	GTTAGCTCACTCATTAGGC	19	47	47	59.6	60.2
sp and backbone_fwd	AGCATAAAGTGTAAAGCCTG	20	40	40	59.6	60.6
sp and backbone_rev	AAAGCCAACTGGTTGGTG	18	50	50	63.1	60.6

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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                                                          SYN 13-DEC-2021
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#ACCESSION
#VERSION
#KEYWORDS
             NEBuilder
#SOURCE
             synthetic DNA construct
# 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_fwd 3'Tm: 59.2 3'Ta: 60.2
#COMMENT
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#COMMENT
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#COMMENT
             sp and backbone_fwd: AGCATAAAGTGTAAAGCCTG
#COMMENT
             sp and backbone_fwd 3'Tm: 59.6 3'Ta: 60.6
#COMMENT
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#COMMENT
             sp and backbone_rev 3'Tm: 63.1 3'Ta: 60.6
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