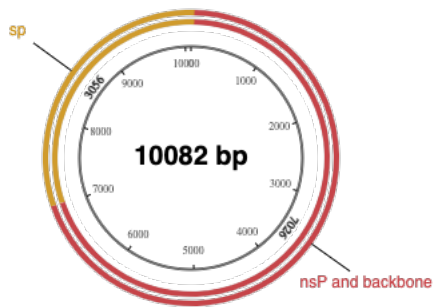


## New\_Assembly

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

### Component Fragments

Name	Length	Produced by	5' End	3' End
nsP and backbone	7026	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	ATTGCTTTGAAGGCC	17	47	47	60.2	60.7
nsP and backbone_rev	GCTCAATAGACTCTTCACAC	20	45	45	59.7	60.7
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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#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)
#COMMENT     nsP and backbone_fwd: ATTTGCTTTGAAGGCC
#COMMENT     nsP and backbone_fwd 3'Tm: 60.2 3'Ta: 60.7
#COMMENT     nsP and backbone_rev: GCTCAATAGACTCTTCACAC
#COMMENT     nsP and backbone_rev 3'Tm: 59.7 3'Ta: 60.7
#COMMENT     sp_fwd: GAGACGCTAGACATGAACAAG
#COMMENT     sp_fwd 3'Tm: 62.6 3'Ta: 62.9
#COMMENT     sp_rev: TGCTCGATCCATTGAATCC
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#

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