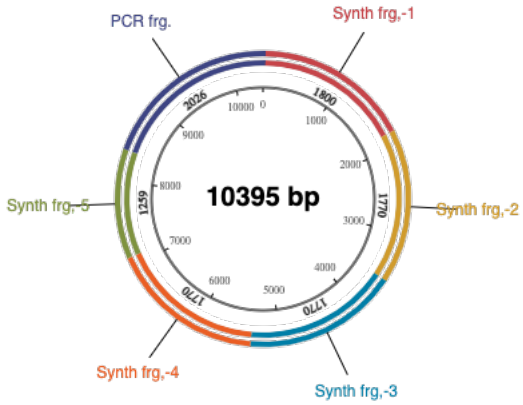
New Assembly

Created: 12/30/2021, 3:01:59 PM
Saved: not saved

Component Fragments

Name	Length	Produced by	5' End	3' End
Synth frg,-1	1800	Synthetic	---	---
Synth frg,-2	1800	Synthetic	---	---
Synth frg,-3	1800	Synthetic	---	---
Synth frg,-4	1800	Synthetic	---	---
Synth frg,-5	1289	Synthetic	---	---
PCR frg.	2086	PCR	Fwd Primer (auto)	Rev Primer (auto)



Notes

- For assemblies of 4 or more fragments, we recommend using overlaps of at least 25 bp when using NEBuilder.
- A 60 minute reaction is recommended for the assembly of more than 3 fragments.

Required oligos

Name	Primer 5' (overlap/spacer/ANNEAL) 3'	Len	%GC	3' %GC	3' Tm	3' Ta
PCR frg._fwd	**AGCAGACAAGCCCGTCAG**	18	61	61	67.9	64.4
PCR frg._rev	**TTGGTTTGTGCCAGGTTTC**	19	47	47	63.4	64.4

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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#ACCESSION   .
#VERSION     .
#KEYWORDS    NEBuilder
#SOURCE      synthetic DNA construct
#  ORGANISM  synthetic DNA construct
#REFERENCE   1  (bases 1 to 10395)
#  AUTHORS   .
#  TITLE     NEBuilder-generated Construct
#  JOURNAL   Exported  30-DEC-2021 from NEBuilder https://nebuilder.neb.com
#COMMENT     NEBuilder-generated oligos (UPPERCASE = gene-specific, lowercase = overlap)
#COMMENT     PCR frg._fwd: AGCAGACAAGCCCGTCAG
#COMMENT     PCR frg._fwd 3'Tm: 67.9  3'Ta: 64.4
#COMMENT     PCR frg._rev: TTGGTTTGTGCCAGGTTTC
#COMMENT     PCR frg._rev 3'Tm: 63.4  3'Ta: 64.4
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