New Assembly

Created: 5/30/2024, 11:51:39 AM
Saved: not saved

Component Fragments

Name	Length	Produced by	5' End	3' End
Linear_Fragment_Hifi	7812	PCR	Fwd Primer (auto)	Rev Primer (auto)
Synthetic_fragment	94	Synthetic	---	---
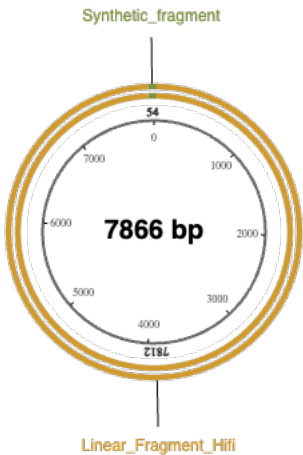
Notes

- Synthetic_fragment is 94 bp long. Fragments smaller than 100bp may be at risk of disintegration during exonuclease digestion.
- For optimal results, use Synthetic_fragment in ≥ 5-fold excess over longer fragments.

Required oligos

Name	Primer 5' (overlap/spacer/ANNEAL) 3'	Len	%GC	3' %GC	3' Tm	3' Ta
Linear_Fragment_Hifi_fwd	GGGAAATTTGGACAGCAG	18	50	50	60.9	59.8
Linear_Fragment_Hifi_rev	CATGTTTGATTGTGTTGAGG	20	40	40	58.8	59.8

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 7866)
#  AUTHORS   .
#  TITLE     NEBuilder-generated Construct
#  JOURNAL   Exported  30-MAY-2024 from NEBuilder https://nebuilder.neb.com
#COMMENT     NEBuilder-generated oligos (UPPERCASE = gene-specific, lowercase = overlap)
#COMMENT     Linear_Fragment_Hifi_fwd: GGGAAATTTGGACAGCAG
#COMMENT     Linear_Fragment_Hifi_fwd 3'Tm: 60.9  3'Ta: 59.8
#COMMENT     Linear_Fragment_Hifi_rev: CATGTTTGATTGTGTTGAGG
#COMMENT     Linear_Fragment_Hifi_rev 3'Tm: 58.8  3'Ta: 59.8
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#     gene            7813..19
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