ENFINIA™ Linear DNA

Recommendations for ampification and cloning

RESUSPENSION

ENFINIA™ Linear DNA is dried down in 100% molecular grade water before shipment instead of buffer to avoid excess salts. We recommend resuspending ENFINIA Linear DNA in molecular grade water or TE buffer (10mM Tris, 0.1mM EDTA, pH 7.5). TE buffer is typically preferred for downstream quantification including gel or capillary electrophoresis.

ADAPTORS INCLUDED

ENFINIA Linear DNA is provided with a 46 bp adaptor on the 5′ end and a 42 bp adaptor on the 3′ end of the ordered sequence. The innermost 22 bp sequence on the 5′ end and the 42 bp sequence on the 3′ end are constant sequences listed below.

24 bp Variable Sequence | 22 bp Constant Sequence A | Your Ordered Sequence | 42 bp Constant Sequence B

Constant Sequence A: 5′— GCGAGTCTTAGCCTGCGACGCT — 3′
Constant Sequence B: 5′— AGACGCGCAGACGACGACACACGCTAGTACGGACGTACGCCT — 3′

AMPLIFICATION

If it is desired to amplify ENFINIA Linear DNA using the 22 bp inner adaptors, the following primer sequences can used:

Forward primer (5′-3′): GCGAGTCTTAGCCTGCGACGCT
Reverse primer (5′-3′): GTGTGTCGTCGTCTGCGCGTCT

TRADITIONAL CLONING AND GOLDEN GATE ASSEMBLY

ENFINIA Linear DNA can be used directly in a restriction digest reaction without further purification; followed by ligation for traditional cloning. Additionally, ENFINIA Linear DNA can be added directly to a Golden Gate Assembly reaction without purification. Desired restriction sites must be included in the target sequence to facilitate digestion and assembly.

GIBSON ASSEMBLY

If using ENFINIA Linear DNA in a downstream Gibson or NEB HiFi assembly, amplify the target sequence using sequence specific primers or the ~20 bp innermost constant adaptor sequences. A polymerase with 3′→5′ exonuclease activity should be included in the reaction mix if adaptor sequences are used for fragment amplification[1]. Assembly can be attempted post cleanup of the amplification reaction.

COLONY PICKING

95% of the molecules in each ENFINIA Linear DNA sequence are a perfect match to the ordered sequence. Picking two colonies should be sufficient to find a perfect clone.

[1] Kalva et al., Biological Procedures Online (2018) 20:2

24.10.05