ENFINIA™ Linear DNA

Recommendations for Amplification 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) and storing the DNA at -20°C for long-term use. 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 be 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 your sequence contains the required homologous sequences, ENFINIA Linear DNA can be used directly in Gibson or NEB HiFi assembly (see this Application Note). If your application is of lower efficiency, removing the adapters can help. As outlined above, our adapters can be removed by encoding restriction endonuclease sites in the target sequence, or using PCR with specific primers to amplify the target sequence.

COLONY PICKING

95% of the full-length molecules in ENFINIA Linear DNA are expected to be a perfect match to the ordered sequence. With Elegen's quality control specifications and average sequencing accuracy of 99.999%, picking two colonies should be sufficient to find a perfect clone in most cases.

IN VITRO TRANSCRIPTION (IVT)

Elegen offers ENFINIA IVT Ready DNA, templates with an encoded poly(A) tail that are immediately ready for in vitro transcription. Customers who elect this option should submit their sequence with an appropriate promoter for RNA polymerase (e.g., T7, T3, SP6), 3′ and 5′ UTRs and potential 5′ cap and select from one of multiple poly(A) tail options (more information here).

ENFINIA Linear DNA can also serve as a template for in vitro transcription (IVT) to generate mRNA. In this case, the same regulatory elements as described above should be included

A poly(A) tail can be added post-transcriptionally using poly(A) polymerase. Alternatively, a tail can be added directly to the ENFINIA Linear DNA template using a polyadenylated PCR primer. For the latter option, optimizing the PCR reaction conditions is recommended to ensure consistent results.

ELEGEN

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