

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.



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 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 amplification¹.

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)

ENFINIA Linear DNA can be easily modified to serve as a template for in vitro transcription (IVT) to generate RNA. To ensure RNA can be transcribed, an appropriate promoter (e.g. that for T7 RNA polymerase) should be either encoded in your ENFINIA Linear DNA or added by PCR. mRNA production is a common application of IVT. To ensure mRNA stability and enhance translation efficiency, both a 5' cap and a poly(A) tail must be added.²

- **5' Capping:** Cap structures can be formed on IVT transcribed RNA either during or after transcription using a variety of cap analogs and enzymatic methods. Users should select an approach based on their application.
- **Polyadenylation:** The poly(A) tail can be added enzymatically to the transcribed RNA using a poly(A) polymerase or added directly on to the ENFINIA DNA template using a polyadenylated PCR primer. For direct addition of poly(A) tails to ENFINIA DNA templates, optimizing the PCR reaction conditions is recommended to ensure consistent results.

Various commercially available kits support IVT, capping, and polyadenylation of IVT RNA.³ We highly recommend consulting the information associated with these kits when considering the design elements to incorporate into your ENFINIA Linear DNA.

1. Kalva et al., *Biological Procedures Online* (2018) 20:2
2. New England Biolabs. *Minding your caps and Poly A tails – Strategies for synthesizing in vitro transcribed (IVT) mRNA*
3. Example: *MEGAscript™ T7 Transcription Kit*



3565 Haven Ave, Suite 3, Menlo Park, CA 94025

© 2025 Elegen Corp. All rights reserved. All trademarks are the property of Elegen Corp. or their respective owners. ENFINIA DNA is intended for research purposes only, not for use in diagnostic procedures. 26.03.06

Order Now
ecommerce.elegen.com

