Gene Fragment Cloning Guidelines

For Research Use Only (RUO). Not for use in diagnostic procedures.

FEATURES:

- High Quality Gene Fragments with industry-leading error rates
- Linear dsDNA from 0.3 to 3.2 kb and 3.2 to 5.0 kb
- Twist Gene Fragments are delivered with 100 ng to 1 µg
- Compatible with all downstream cloning strategies
- Available with and without Twist Adapters
  - Twist Adapters may be advantageous for your application as they enable amplification of multiple Gene Fragments with a common set of primers.

GENE FRAGMENTS

To help ensure success, please see the information below for design and cloning considerations specific to your desired cloning strategy.

Gene Fragments without Adapters

Gene Fragments without adapters are blunt-ended, double-stranded DNA sequences. The sequence you submit is the sequence you receive.

Gene Fragments with Adapters

Gene Fragments with adapters are blunt-ended, double-stranded DNA sequences that have adapter sequences on the 5' and 3'ends of your submitted sequence. Gene fragments without these adapters are also available during project configuration on eCommerce.

The current Twist adapter sequences are provided on eCommerce when you configure your Gene Fragments with Adapters project. For the current and previous adapter sequences, please see the Twist FAQ, *What are the adapter sequences used on the Adapter-On Gene Fragments?*, at the link below.

https://www.twistbioscience.com/faq/gene-synthesis/what-are-adapter-sequences-used-adapter-gene-fragments

Removing Twist Adapters

Some cloning methods, such as Restriction Enzyme, Golden Gate, and Gateway cloning (Thermo Fisher Scientific), will naturally result in the removal of the Twist Adapters. For TA, blunt-end, and homology-based cloning, it is advantageous to remove the Twist Adapters from your Gene Fragments. The simplest method to remove the Twist Adapters is through PCR amplification using internal primers as indicated in the diagram below.

Pink arrows highlight PCR priming sites:

CLONING GUIDELINES

Blunt-End Cloning

Gene Fragments are compatible with blunt-end cloning and can be easily cloned using this method. Gene Fragments without Adapters will result in a construct free from adapter sequence while Gene Fragments with Adapters will result in a construct containing the Twist Adapters. If the Twist Adapters are not desired, we recommend ordering Gene Fragments without Adapters or simply removing the the Twist Adapters using PCR prior to cloning.

The 5' ends of all Twist Bioscience Gene Fragments are not phosphorylated. Some traditional blunt-end cloning methods require a 5' phosphate, however most methods do not require a 5' phosphate. For specific guidance, please refer to your preferred cloning method's protocol.

If 5' phosphates are required, please use a T4 PNK or equivalent method. For specifics, we recommend referring to your cloning method protocol for more details.

TA-Cloning

Gene Fragments are compatible with TA cloning methods. To prepare the fragment for cloning, please add an adenine to the 3' end using Taq polymerase.

PROTOCOL OVERVIEW:

1. Set-up the reaction by adding the following components:
    a. Twist Gene Fragment: ~50–100 ng
    b. Taq DNA Polymerase
    c. Taq Buffer
    d. dATP
    e. Nuclease Free Water: up to desired reaction volume
    f. Note: for specific volumes, please refer to the instructions that accompany your Taq Polymerase.
2. Incubate the reaction at 72°C for 10–20 minutes.
3. After incubation, the Gene Fragment is now ready for TA-Cloning. Please consult your specific TA-Cloning kit for the next steps.

Please note that if you are performing TA-cloning using Gene Fragments with Adapters, the Adapters will be integrated into the construct. If desired, the Twist Adapters can be removed prior to cloning.

Restriction Enzyme-Based Cloning

Gene Fragments are compatible with restriction enzyme-based cloning methods. To add restriction sites, please include the sequences for the desired restriction sites when configuring your project.

Please note, some restriction enzymes do not cleave efficiently close to the ends of DNA fragments and require extra bases to be added for efficient cleavage. If ordering Gene Fragments without Adapters, 6 base pairs should be added on either side of the restriction enzyme recognition site, however, we recommend reviewing your specific restriction enzyme's requirements.

Golden Gate Cloning

Gene Fragments are made compatible with Golden Gate cloning methods through the inclusion of the Type IIS restriction sites on the ends of the submitted fragment sequences as indicated in the diagram below.

Pink: Restriction Sites (BsaI, a Type IIS enzyme in this example)

Up to 10 DNA fragments can be assembled in a single cloning reaction. Please consult your cloning kit's protocol for specific use instructions.

Gateway Cloning

Gene Fragments are compatible with Gateway cloning. When configuring your project, please include the flanking attB1 and attB2 sites (or appropriate att site) in your fragment design.

AttB1

5' GGGG-ACA-AGT-TTG-TAC-AAA-AAA-GCA-GGC-TNN--(template-specific sequence) 3'

AttB2

5' GGGG-AC-CAC-TTT-GTA-CAA-GAA-AGC-TGG-GTN--(template-specific sequence) 3'

Gene Fragments with and without Adapters are compatible with Gateway cloning.

GENE FRAGMENT WITHOUT ADAPTERS

GENE FRAGMENT WITH ADAPTERS

Homology-Based Cloning

Gene Fragments are compatible with homology-based cloning methods such as Gibson Assembly and many other homology-based cloning methods. The optimal length of homology at the ends of the DNA fragments vary based on the number of inserts and cloning method used, please review the design guidelines for your specific homology-based cloning method prior to ordering your Gene Fragments.

Gene Fragments without Adapters can be designed and ordered with homologous sequences or homology can be added using PCR primers.

For Gene Fragments with Adapters, we recommend adding homology ends and removing the adapters prior to your homology-based cloning reaction if necessary.

A. INTERNAL PRIMERS TO ADD HOMOLOGY

Add homology tails to PCR primers for downstream seamless cloning