

## GenPart Fragments Usage Instructions

### Background

GenPart fragments are double-stranded DNA fragments which are ready for cloning with a variety of methods, including restriction cloning, homologous recombination, and blunt end ligation. GenPart fragments are a fast, cost effective, and flexible alternative to get your synthetic DNA.

### Resuspending and storing your GenPart fragments

GenPart fragments are delivered dried down. Upon receipt of your GenPart fragments, the following steps are recommended:

- ✓ Briefly centrifuge the tube to pellet the material to the bottom prior to opening.
- ✓ Add TE to the tube for your desired final concentration, briefly vortex and centrifuge.
- ✓ For long term storage, freeze GenPart fragments at  $-20^{\circ}\text{C}$  and avoid freeze-thaw cycles.

### PCR amplification of GenPart fragments

At least 250 ng GenPart fragments are delivered for each order, which should provide sufficient material for various cloning applications. However, in some cases it may be necessary to amplify your GenPart fragments to get more starting material. When amplifying your GenPart fragments using PCR, we recommend:

- ✓ Using high fidelity DNA polymerase to avoid introducing mutations during amplification
- ✓ Using 0.5-5 ng GenPart fragments as template in 50  $\mu\text{l}$  PCR reactions
- ✓ Using 15~25 thermo cycles. Fewer cycles would provide better fidelity.

### Cloning of GenPart fragments

GenPart fragments can be easily cloned into the vector of your choice using a wide variety of cloning methods. The following table lists several commonly used methods and relevant recommendations for better use of GenPart fragments.

Cloning Method	Description	Note
<b>Restriction Cloning</b>	Restriction digestion/ligation is a simple and easy way of cloning your GenPart fragments into your desired vector.	<ul style="list-style-type: none"> <li>✓ Require adding 6–8 nucleotides on each end of your GenPart fragments</li> <li>✓ Use <math>\sim 0.05</math> pmol of insert fragment (25 ng of 750 bp fragment is about 0.05 pmol) and <math>\sim 0.01</math> pmol of linearized vector in each ligation reaction</li> </ul>
<b>Gibson Assembly</b>	Gibson assembly, also known as isothermal assembly, is a homologous recombination based method which is able to simultaneously assemble several fragments into the target vector.	<ul style="list-style-type: none"> <li>✓ Require adding, on each end of the fragments, 20–80 bp sequence overlap with the adjacent insert or vector</li> <li>✓ Use <math>\sim 0.1</math> pmol of each fragment (50 ng of 750 bp fragment is about 0.1 pmol) and <math>\sim 0.05</math> pmol of linearized vector in each reaction</li> </ul>
<b>Golden Gate Cloning</b>	Golden Gate cloning uses Type IIs restriction enzyme and DNA ligase to efficiently assemble up to ten fragments in one reaction. It is not based on homologous recombination and thus can be used to clone highly repetitive sequences.	<ul style="list-style-type: none"> <li>✓ Require adding type IIS sites and additional 6–8 nucleotides on each end of your GenPart fragments</li> <li>✓ Use <math>\sim 0.05</math> pmol of each fragment and vector (25 ng of 750 bp fragment is about 0.05 pmol) in each reaction</li> </ul>
<b>Blunt-end Cloning</b>	GenPart fragments are blunt-ended and therefore can be directly used for blunt-end cloning	<ul style="list-style-type: none"> <li>✓ Require adding 6–8 nucleotides on each end of your GenPart fragments</li> <li>✓ Use <math>\sim 0.05</math> pmol of insert fragment (25 ng of 750 bp fragment is about 0.05 pmol) and <math>\sim 0.01</math> pmol of linearized vector in each ligation reaction</li> </ul>
<b>T/A Cloning</b>	T/A cloning relies on the hybridization of adenine (A) and thymine (T) on different DNA fragments.	<ul style="list-style-type: none"> <li>✓ GenPart fragments are blunt-ended, and therefore further manipulation to add end-terminal A-overhangs is required prior to cloning</li> <li>✓ Use Taq DNA polymerase with ATP in reaction buffer to add 3' A-overhangs to GenPart fragments</li> </ul>

### Screening for the correct clone

To have at least 90% chance of obtaining your desired clone, we recommend sequencing 4-6 clones for each GenPart fragments.

Caution:

For laboratory or further manufacturing use only. If you have any questions, please contact our customer service representative at 1-877-436-7274 (Toll-Free), or email: [gene@genscript.com](mailto:gene@genscript.com).