Simply Seamless DNA Assembly Protocol

1. Set up the assembly reaction in a microtube on ice: (keep all components on ice and transfer stocks back to freezers promptly after use)

	2-3 Fragment Assembly	4-6 Fragment Assembly	(+) control
Amount of DNA fragments	1:1 molar ratio of fragments, 50-200 ng DNA per reaction, volume 8 µl or less	1:1 molar ratio of fragments, 50-200 ng DNA per reaction, volume 8 µl or less	8 µl (S95)
Simply Seamless Enzyme Mix (S92)	1 µl	1 µl	1 µl
10X Reaction Buffer (S94)	1 µl	1 µl	1 µl
H2O	Bring volume up to 10 µl	Bring volume up to 10 µl	-
Total Volume	10 µl	10 µl	10 µl

2. Incubate the samples in a thermocycler, water bath or a hot block at 37°C for 15-30 minutes. (incubation time may be extended to 60 minutes when more than 4 fragments are being assembled)

3. Transfer the completed reaction to ice and use immediately or store at -20°C for subsequent transformation.

4. Transform Competent *E. coli* cells (DH5α provided in the cloning kit or purchased separately, S96) with 3 µl of the assembled product, following the transformation protocol.