F1-X™ Next-Gen Gibson Assembly® Quick Start Guide

RACER BIOSCIENCES
accelerate innovation

This guide is designed for users experienced with Gibson Assembly. F1-X™ uses a nearly identical protocol to legacy Gibson Assembly kits, making setup fast & straightforward.

F1-X™ Guidelines at a Glance

Table 1. Guidelines for using F1-X™

Parameter	Range
Reaction volume	10 µL (tolerance: 2.5 – 20 µL)
Fragment size	100 bp – 32 kb per fragment
Fragment number	2-12 fragments per reaction
Assembly size	Up to 100 kb total
Homologous overlap lengths	• 20-40 bp (2-3 fragments) • 40+ bp (4+ fragments)
Reaction conditions	• 50°C for 15-50 mins (time depends on No. of fragments)
Temperature	50°C (tolerance: 50-56°C)
Compatibility	• Mismatches in overlaps • Crude PCR products

1. DNA Fragment Preparation and Quality Control

✓ **Concentration:** Quantify by fluorimetry or absorbance

✓ **Integrity:** Verify full-length products by electrophoresis. If <80% full-length product, consider gel extraction

✓ **Purity:** A260/280 ≥1.8, A260/230 ≥2.0. Crude PCR products may be used (≤20% v/v of reaction)

✓ **Homologous overlaps:** Ensure proper designs

2. Reaction Planning (10 µL Assembly)

Table 2. Assembly setup and reaction parameters

Number of fragments	Simple Assembly (2-3 fragments)	Complex Assembly (4-12 fragments)
Total DNA*	0.03-0.2 pmols	0.2-0.4 pmols
How to set up	Add 25-50 ng vector per reaction, then add 1 to 3-fold molar excess of insert*	Add 0.02-0.04 pmol of each DNA fragment per 10 µL reaction
Molar ratio	(Vector:Insert): 1:1-1:3	Equimolar amounts for all fragments*
Rxn conditions	50°C for 15 minutes	50°C for 60 minutes

**Final amounts in standard 10 µL reaction. For DNA fragments ≤100 bp, use 5× molar excess.*

3. F1-X™ Assembly Procedure

Materials Required: DNA fragments, F1-X™ Master Mix (2×), Positive Control DNA Mix (2×), Nuclease-free water, Thermocycler (50°C), Ice

Assembly Protocol

1. Prepare DNA mixtures on ice. Target >2× final target DNA concentrations determined via Table 2

2. Thaw F1-X Master Mix (2×) on ice

3. Vortex Master Mix vigorously for 15 seconds before use

4. Combine reaction on ice according to Table 3

5. Mix thoroughly and spin down briefly

6. Incubate at 50°C for 15-60 minutes (depending on number of fragments)

7. Store at -20°C or use immediately for transformation

Table 3. Reaction assembly

Component	Volume
F1-X™ Master Mix (2×)	5 µL
DNA fragment mix	X µL
Nuclease-free water	5-X µL
Total reaction volume	10 µL

Note: for best performance, keep reactions on ice until they are transferred to 50°C.

Essential Controls

• **Positive Assembly Control:** Use 5 µL of F1-X™Positive Control (2×) in the reaction. After transformation, select on LB agar plus Ampicillin or Carbenicillin

• **Vector Only Control:** Add vector only (no inserts) to F1-X Master Mix to gauge vector preparation background

• **No-assembly Control:** DNA fragments without F1-X Master Mix as a control for unintended recombination and gel electrophoresis

Need more information? Scan below for detailed, comprehensive User Guide

For technical support: help@racerbio.com | For purchasing: sales@racerbio.com