F1-X™ Next-Generation 1-Step Gibson Assembly® Master Mix
Quick Start Guide

F1-X™ Guidelines

Parameter	Range
Fragment size	100 bp – 32 kb per fragment
Fragment number	2-12 fragments per reaction
Assembly size	Up to 100 kb total
Overlap length	• 20-40 bp (for 2-3 fragment assemblies), • 40+ bp (4+ frags)
Reaction conditions	• 2-3 fragments: 50°C for 15 minutes • 4-12 fragments: 50°C for 60 minutes
Reaction temp.	50°C (tolerance: 50-56°C)
Reaction volume	20 µL (tolerance: 2.5-20 µL)
Compatibility	• Mismatches in overlaps • Crude PCR products (≤20% v/v)
Storage temp.	-20 °C

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)

✓ **Designed overlaps:** Ensure homologous overlaps of ~40 bp are present on all DNA fragments

2. Calculate DNA Amounts and Prepare Fragment Mixes

Prepare DNA mixtures at >2× final target concentration.

Simple Assemblies 2-3 Fragments	Complex Assemblies 4-12 Fragments
Total DNA*: 0.03-0.2 pmols	Total DNA*: 0.2-0.8 pmols
Vector: 50-100 ng per reaction	Per fragment: 0.02-0.1 pmol per reaction (0.05 target)
Ratio (Vector:Insert): 1:1-1:3, with 1:3 preferred*	Ratio: Equimolar for all

**Final amounts in standard 20 µL reaction. For DNA fragments ≤100 bp, use 5× molar excess.*

3. F1-X™ Assembly Procedure

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

Assembly Protocol

1. Thaw F1-X™ Master Mix (2×) on ice

2. Vortex Master Mix vigorously for 15 seconds before use

3. Combine on ice: (see table below)

4. Mix thoroughly and spin down briefly

5. Incubate at 50°C for 15 mins for 3 frags or less, 60 mins for 4+ frags. If elevating incubation temp for complex assemblies (tolerance: 50-56°C), incubate for no less than 60 mins regardless of fragment number.

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

Component	Volume
F1-X™ Master Mix (2×)	**10 µL**
DNA fragment mix*	**X µL**
Nuclease-free water	**10-X µL**
Total reaction volume	**20 µL**

**if using F1-X™ Positive Control (2×), add 10 µL. No water needed.*

Controls

• Positive Assembly, Vector Only, No-assembly

4. Quick Tips For Best Performance

If **scaling reactions down**, scale DNA amount linearly with reaction volume.

Keep reactions on ice until they are transferred to a pre-heated heat block.

For transformation, start with a ratio of **~1 µL Assembly to 20 µL of chemically competent cells**. Dilution series may be needed to find optimum.

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