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Chesapeake Genomic Systems

# 2× ExSembly™ Cloning Master Mix

Cat. #: M0005 Size: 10 rxn

## Introduction

**2× ExSembly™ Cloning Master Mix** is a simple, fast, and high efficient product which enables directional insertion of any amplified DNA product into **CIRCULAR vector**. The ExSembly™ technology completely eliminates the time consuming step of preparing linear vector. The creative buffer system in our master mix allows restriction enzymes to efficiently digest the circular DNA, and maintain high exonuclease and polymerase activity to enable the assembly to occur. This technology takes advantage of the fact that once the vector DNA successfully assembles with insert DNA, the restriction sites in the vector disappear. It robustly removes background negative clones by completely digest unassembled vector DNA. One can conveniently use a relatively large amount of vector DNA, up to 500 ng, and obtain high number of positive clones, which consequently increase success rate.

Choose one (or more) restriction enzyme(s) that linearize the vector and **DO NOT** cut within the fragment(s) to be inserted. For single fragment insertion, using PCR, add 20 bp homology upstream of the unique restriction site in the vector to the 5' end of the fragment, and 20 bp of vector homology downstream of the restriction site to the 3' end.

## Package Information

| Component | M0005 |
|---|---|
| 2× ExSembly™ Master Mix | 100 µl |
| Positive control | 50 µl |

* An ampicillin resistant plasmid working as a transformation positive control.

## Storage

Store at -20°C

## Additional Materials Required

PCR templates, primers and circular vectors;

Restriction enzymes: High-Fidelity Restriction Enzymes (Hi-Fi®, NEB) or FastDigest™ Restriction Enzymes (Thermo-Fisher™) recommended. The following enzymes have been demonstrated to be active in 2× ExSembly Master Mix: ApaI, BamHI, EagI, EcoRI, EcoRV, HindIII, KpnI, SalI, SmaI, XmaI, NcoI, Not I, NdeI, NheI, PstI, SpeI, SacI, StuI, XbaI, XhoI;

High-fidelity polymerase: LiSpark™ Ultra SuFi DNA Polymerase (M0030) recommended;

Competent cells: DH5α competent *E.Coli* strain for conventional cloning, applicable to plasmids <15 kb ; XL10 competent *E.Coli* strain for long-fragment cloning, applicable to plasmids >10 kb;

Other materials: dd$H_2O$, PCR tubes, PCR instrument, *etc.*

## Protocol

1. Thaw 2× ExSembly™ master mix, vortex briefly. Upon first use, make 10× 10 µl aliquots and store at -20°C.

2. Set up reaction according to the following:

**Vector + 1 or 2 inserts**

| ExSembly™ Reaction Components & Recommended Ratios | |
|---|---|
| vector:insert[a] ratio | 1: ≥3 insert A + ≥3 insert B |
| 0.03-0.15 pmols circular vector[b] | X µl |
| Purified PCR fragment(s)[c] | Y µl |
| 2× ExSembly™ master mix | 10 µl |
| Vector-linearizing restriction enzyme(s)[d]† | 1 or 2 µl |
| Ultrapure water | 8 or 9 - (X + Y) µl |
| Total volume | 20 µl |

**Vector + 3 to 5 inserts**

| ExSembly™ Reaction Components & Recommended Ratios | |
|---|---|
| vector:insert[a] ratio | 1: 1 (each insert) |
| 0.03-0.15 pmols circular vector[b] | X µl |
| Purified PCR fragment(s)[c] | Y µl |
| 2× ExSembly™ master mix | 10 µl |
| Vector-linearizing restriction enzyme(s)[d]† | 1 or 2 µl |
| Ultrapure water | 8 or 9 - (X + Y) µl |
| Total volume | 20 µl |

a. For vector + 1 or 2 inserts, the molar ratio of vector to insert A is 1: ≥3, and the ratio of vector to insert B is also 1:≥3 (vector:total insert = 1:≥6). For 3 to 5 inserts, the molar ratio of vector:EACH insert is 1:1 (vector:total insert=1:3-5).

b. For a 5K bp vector, 0.03-0.15 pmols=100-500 ng. Higher input vector amount increases colony yield upon transformation. To convert ug DNA to pmols DNA use the following equation, where # base pairs is the length of linearized vector or PCR fragment:

$$\mu g\ DNA \times \frac{1\ pmol}{600\ pg\ DNA} \times \frac{10^6\ pg}{1\ \mu g} \times \frac{1}{\#\ base\ pairs} = pmols\ DNA$$

c. PCR products should be purified from oligos and primer-dimer, dissolved in $H_2O$ & accurately quantified (PEAKEclear™ PCR clean-up kit recommended).

d. High-Fidelity Restriction Enzymes (Hi-Fi®, New England Biolabs®) or FastDigest™ Restriction Enzymes (Thermo-Fisher™ Scientific) recommended.

3. Incubate at 37°C for 15 minutes and 50°C for 15-45 minutes.

4. Transformation to *E.coli* following standard protocols. Both chemical competent cells and electro competent cells can be used.