GenRec Assembly Master Mix Kit

# CL08010	10 reactions	100ul
# CL08020	20 reactions	200ul
# CL08050	50 reactions	500ul

Kit Contents and Storage

The GenRec Assembly Master Mix Kit would be shipped on dry ice, Store the kit components at -20℃.

Components	CL08010	CL08020	CL08050
2×GenRec Assembly Master Mix	100 ul	200 ul	500 ul
Positive inserts	8 ul	8 ul	8 ul
Linear positive vector(25ng/ul)	2 ul	2 ul	2 ul
Primers for bacterial colony PCR	20 ul	20 ul	20 ul

Introduction :

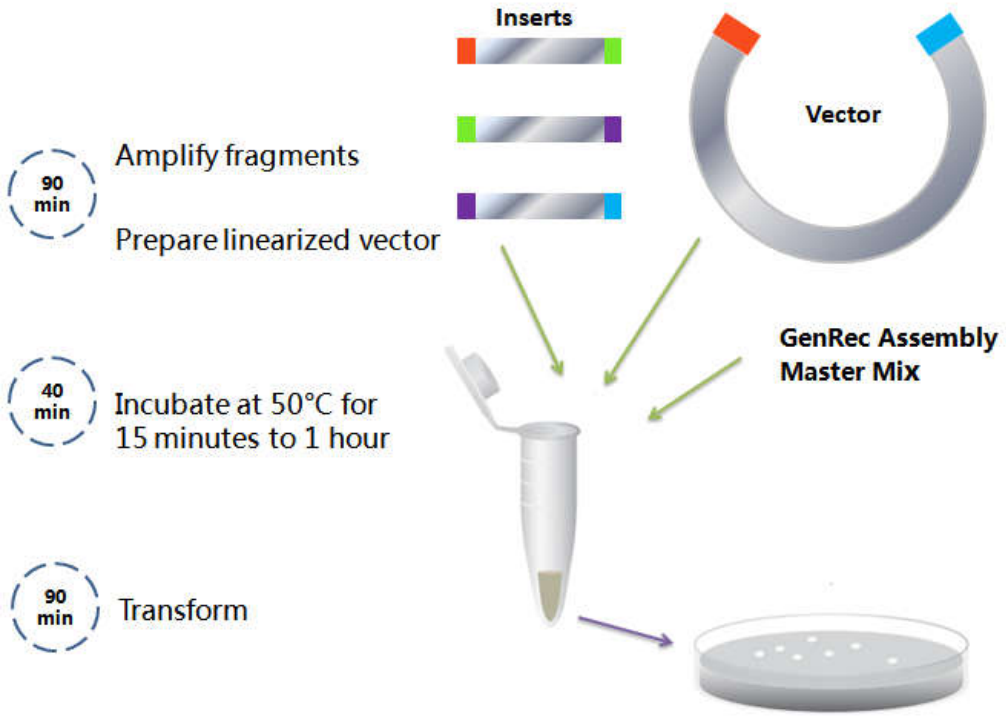
GenRec Assembly Master Mix Kit is a product for high-efficient, simultaneous, and seamless in vitro assembly of up to 5 DNA inserts plus any vector in a pre-determined order. This product could be used to assemble different sizes of DNA fragments with varied overlaps(15–80 bp) in about one hour even without the use of restriction enzymes.

Applications :

- Assembly of one insert with one vector
- Assembly of multiple Fragments with one vector

Detection of quality control:

Four inserts and one vector were assembled, and yield blue colonies on a Chloramphenicol plate when incubated overnight at 37°C after transformation. More than 87.5% colonies were assembled successfully, verified by colonies PCR or restriction enzyme digestion.



Protocols

1. Design and PCR of Fragments for DNA Assembly

PCR primers for use in DNA assembly must have two sequence components:

- Overlap sequence for the assembly of vector or of adjacent fragment(20-40 nt overlap are preferred);
- Gene-specific sequence, required for template priming during PCR.

2. Prepare linearized vector

Using a high-fidelity DNA polymerase or by restriction enzyme digestion.

3. GenRec assembly

Set up the following reaction on ice(Total Volume 20 ul):

2×GenRec Assembly Master Mix	10 µL
Linearized vector	A ng
Inserts	B ng
ddH_2O	Up to 20µL

- Optimized cloning efficiency is 50–100 ng of vector with 2-fold excess of inserts.
- Add inserts and vector first, then ddH_2O, and finish with Assembly Mix, mix gently and thoroughly.
- Do a short centrifugation after the setup, and go to step 4.
- A total of 0.03 pmols of DNA fragments are recommended when assembling fragments into a vector. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, the following formula are recommended :

  pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)

  Or: Weight in ng = 0.02×base pairs (0.03 pmol)

4. Incubate samples

Incubate samples in a thermocycler at 50°C for 15 minutes (assembling 2 or 3 fragments) or 60 minutes (assembling 4–6 fragments).
Following incubation, store samples on ice or at –20°C for subsequent transformation.

5. Transform

Transform E. coli Competent cells with 2-10 µl of the assembled product(less than 1/10 volume).

Notes

- To achieve the optimal efficiency, high quality DNA samples were recommended: A260/280>1.8, concentration>40ng/ul, without extra nonspecific amplification PCR fragments.
- The optimal length of the overlap region depends on the fragments number & length of the assembly reaction.
- Longer overlap regions will result in higher efficiency. 40 nt overlap were recommended to ensure directional assembly of multiple fragments Assembly. Avoid tandem repeats, homopolymers, high secondary structure, and extremely high or low GC content.
- PCR product purification is not necessary if the PCR products are not contaminated by non-specific amplification products, primer-dimers or large quantities of unused PCR primers.