Intact Genomics® ig-Fusion™ Cloning Kit

Catalog #	4111	4115
Package Size	10 reactions	50 reactions
Concentration	N/A	

Description

Intact Genomics propriety ig-Fusion™ cloning technology is a simple, rapid and highly efficient cloning kit which allows to directly clone any PCR product(s) to any linearized expression vector at any site. The PCR fragments can be generated by Intact Genomics high fidelity Pfu DNA polymerase or other high fidelity DNA polymerases, with primers having 15 to 18 bases of homology at their linear ends to where the product need to fuse. The linearized vector can be generated by PCR or restriction enzymes. The kit is so robust that multiple DNA fragments can be assembled simultaneously and cloned into one construct in a single reaction step within short times (usually 10-30 min) with more than 95% cloning efficiency.

Applications

- Clone any insert at any site within any vector
- Restriction enzyme and phosphatase free system
- Joining multiple large fragments at once
- Precise insertion at a desired orientation
- Rapid and high efficiency with > 95% positive clones

Product Includes

1) 5x ig-Fusion™ enzyme premix
2) 2x PCR premix
3) High efficiency competent cells
4) Recovery medium

Storage Temperature

5x ig-Fusion™ enzyme premix:	-20 ºC
2x PCR premix:	-20 ºC
High efficiency chemical competent cells:	-80 ºC
Recovery medium	4 ºC or -20 ºC

Protocol

1. Linearize the vector by restriction enzyme digestion or inverse PCR and purify the product with spin column.
2. Design PCR primers for the gene of interest with 15 to 20 bp at 5'-extensions that are complementary to the ends of the linearized vector.
3. Amplify the gene of interest with Intact Genomics 2x PCR premix or any other high fidelity DNA polymerase. Run the PCR product on an agarose gel to determine the integrity of the PCR product.
4. Purify the PCR product with spin column.
5. Set up the ig-Fusion™ cloning reaction as follows: Insert and vector molar ratio 3:1 produce the highest number of colonies.

Linearized vector	x µl (50-100 ng)
Insert	x µl (50-100 ng)
5x ig-Fusion™ enzyme premix	2.0 µl
H_2O up to	10.0 µl

6. Mix the reaction mixture thoroughly.
7. Incubate the reaction mixture at 50 °C for 10-30 min, then place on ice. Number of colonies depend on the incubation time, insert size and number of inserts need to clone.
8. Use 2.0 µl of the reaction mixture and transform into high efficiency ig™ 10B chemical or electroporation competent cells (included). To get the maximum number of colonies, we recommend to use ig™ 10B electrocompetent cells (Cat # 1212).