Quick10™ Cloning Kit

Catalog #	Package Size
4122	6 reactions

Description

Intact Genomics (IG®) propriety Quick10™ Cloning kit and DirectPlate® technologies enable simple, rapid and highly efficient cloning. This cloning technology utilizes homologous assembly and ccdB selection which allows for nearly 100% cloning accuracy. This kit enables the direct cloning of one or two DNA fragment(s) (<6kb total) through PCR into linearized cloning-ready Ig AmpR/ KanR-pCR vector(4 kb, provided in this kit)*. The PCR fragments can be generated by Intact Genomics high fidelity i7® DNA polymerase or other high fidelity DNA polymerases. Primers need to have 12 to 15 bases of homology at Ig AmpR/KanR-pCR vector linear ends where the DNA fragment(s) or PCR product(s) needs to fuse. The unpurified PCR product (s) is then diluted 5-10 times by the dilution buffer included in this kit. No purification step is required. The diluted PCR product (s) and linearized IG AmpR/ KanR-pCR vector can then be assembled in just 7 minutes at 37 ºC, followed by a 3 minutes transformation step on ice utilizing our Directplate® XL DH10B competent cells (included in this kit). DirectPlate® cells eliminate heat shock, lengthy incubations, and time-consuming outgrowth procedures. Utilizing this kit, DNA/PCR product(s) can be assembled/transformed in **10 minutes** vs. other seamless/fusion cloning, conventional T/A, or restriction ligation cloning methods that can take up to 2 hours or more.

***Single selection vector or custom vector available upon request**

Key Benefits

- Combination of high fidelity PCR, assembly, and DirectPlate® transformation cloning technologies.
- Homologous assembly (vs. T/A or restriction ligation methods) displays <1% false-positive clones or nearly 100% cloning accuracy.
- Assembled/transformed in 10 minutes. Less time and less effort spent on cloning, transformation, and positive clone screening/ identification.

Applications

- Streamlined cloning of one or two (<6 kb total) DNA fragments
- Single PCR product cloning
- Site directed mutagenesis
- High throughput cloning

Product Includes

1) 5x Quick10-Assemble™ Premix
2) IG AmpR/KanR-pCR vector
3) Dilution buffer
4) Directplate® XL DH10B competent cells (6x50 µl)

Storage Temperature

-20 ºC for premix, vector and buffer
-80 ºC for Directplate® XL DH10B competent cells

Protocol

1. Design PCR primers for the DNA of interest with 12 to 15 bp at 5'-extensions to the ends of the linearized Ig AmpR/ KanR-pCR vector sequence (e.g. 5'-extension forward primer: 15bp homology 5'-GCGAATTCTGCAGAT-3' and 3'-extensions reverse primer: 15bp-homology complementary strand 5'-TAGATGCATGCTCGA-3')
2. Amplify the DNA of interest with Intact Genomics i7® High-Fidelity DNA Polymerase 2x Master mix (cat. #3257) or any other high fidelity DNA polymerase. Run the PCR product on an agarose gel to determine the integrity of the PCR product.
3. Dilute the unpurified PCR product 5-10 times utilizing the provided dilution buffer.
   ***Note**: Increasing PCR product purity correlates to increasing cloning efficiency.*
4. Set up the assembly reaction as follows. Insert (s) and vector molar ratio should be 2:1 to produce the highest number of colonies.

IG AmpR/KanR-pCR vector 50ng	1.0 µl
Diluted PCR product(s) 100ng	x µl
5x Quick10-Assemble™ premix	4.0 µl
H_2O up to	20.0 µl

5. Mix the reaction mixture thoroughly in 0.2 ml PCR tube.
6. Incubate the reaction mixture at 37 °C for 7-15 minutes, then place on ice.
   ***Caution:** The assembly reaction maximum incubation is 30 minutes. Longer incubation times decrease cloning efficiency.*
7. Use 1.0 to 5.0 µl of the reaction mixture and transform into DirectPlate® XL DH10B competent cells (included in this kit).
   ***Note:** DirectPlate® competent cells are optimized for high transformation efficiency and save significant time.*
8. Remove competent cells from the -80 °C freezer and thaw completely on ice and set up the transformation reaction as follows:

Assembly reaction	1.0 to 5.0 µl
Directplate® XL DH10B competent cells	50.0ul

9. Mix by gently pipetting up and down a few times then place on ice or room temperature for 3-10 minutes.
   ***Caution**: Longer incubation times decrease cloning efficiency.*
10. Spread 25 to 50 µl from each transformation directly onto LB plate containing 100 µg/ml ampicillin. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies. Use a sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
    ***Caution**: Drying the plate too much will decrease cloning efficiency*
11. Incubate the plates overnight at 37 °C.

Note: The procedures above are for IG® AmpR/KanR-pCR vector containing Ampicillin and Kanamycin resistant markers. To prevent self ligation, IG® AmpR/KanR-pCR vector has the *ccdB*

QUICK10™ Cloning Kit — Assembly to Plate in 10 Minutes!

Additional Information and Process Overview

PCR

Set up PCR for assembling DNA fragment

single fragment <6kb

12-16bp homologous arm

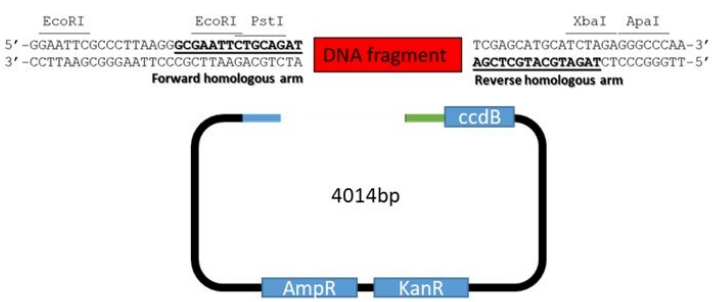
multi fragments <3kb Each

2-3 fragments

We recommend 15bp homologous arm in IG AmpR/KanR-pCR Vector(forward primer: 5'-GCGAATTCTGCAGAT-3' and reverse primer: 5'-TAGATGCATGCTCGA-3'). This Kit can clone multiple fragments, although efficiency will decrease with larger fragments.

Vector

IG AmpR/KanR-pCR Vector



IG AmpR/KanR-pCR Vector is a linearized vector that has ampicillin, kanamycin-resistant genes and ccdB gene for removing self-ligated clones.

Assembly

Set up the assembly cloning reaction

IG AmpR/KanR-pCR Vector (50ng/ul)	1ul
DNA fragment (100ng/ul)	Xul
5x Quick10-Assemble™ premix	4ul
Deionized water	20ul

Incubate the reaction for 7min at 37°C in 0.2ml PCR tube.
Then place on ice to stop reaction.

NOTE: The assembly reaction is completed within a 30min incubation. Longer incubation times decrease cloning efficiency.

DirectPlate

Set up the transformation

IG assembly reaction mixture	1-5ul
DirectPlate competent cell	50ul

Mix the IG assembling reaction mixture and DirectPlate™ competent cell gently.
Place the tube on 3min in ice.
Immediately spread the mixture on YT plate containing Ampicillin.
The plate then incubates overnight at 37°C.

1 12-16bp Homology

IG AmpR-PCR Vector

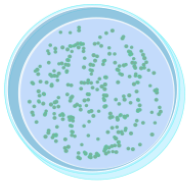
2

Assembly
7min in 37°C

3

DirectPlate
3min in Ice

4



Growth Time
Overnight
in 37°C