Quick10™ Cloning Kit

Manual

Catalog #	**4122**
Package Size	6 reactions

Important!

-80°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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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 fragments (<6kb total) through PCR into linearized cloning-ready IG® AmpR/KanR-pCR vector (4kb, 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 fragments or PCR products need to fuse. The unpurified PCR products are then diluted 5-10 times by the dilution buffer included in this kit. No purification step is required. The diluted PCR products and linearized IG® AmpR/KanR-pCR vector can then be assembled in just 7 minutes at 37 ºC, followed by a 3 minute 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 products 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.**

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 (<6kb total) DNA fragments
- Single PCR product cloning
- Site directed mutagenesis
- High throughput cloning

Product Components:

- 5x Quick10-Assemble™ Premix
- IG® AmpR/KanR-pCR vector
- Dilution buffer
- 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 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 to save a significant amount of 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	50.0 µl

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 gene.

Assembly to Plate in 10 Minutes!

PCR

Set up PCR for assembling DNA fragment.

single fragment <6kb

12-16bp homologous arm

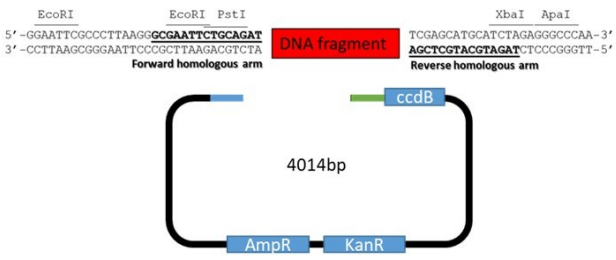
multi fragments <3kb Each

2-3 fragments

We recommend 15bp homologous 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/µl)	1µl
DNA fragment (100ng/µl)	Xµl
5x Quick10-Assemble™ premix	4µl
Deionized water	20µl

Incubate the reaction for 7 min at 37ºC in.02ml PCR tube. Then place on ice to stop reaction.

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

DirectPlate

Set up the transformation.

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

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

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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