RC Cloning Kit

Catalog No.: N-RC-20

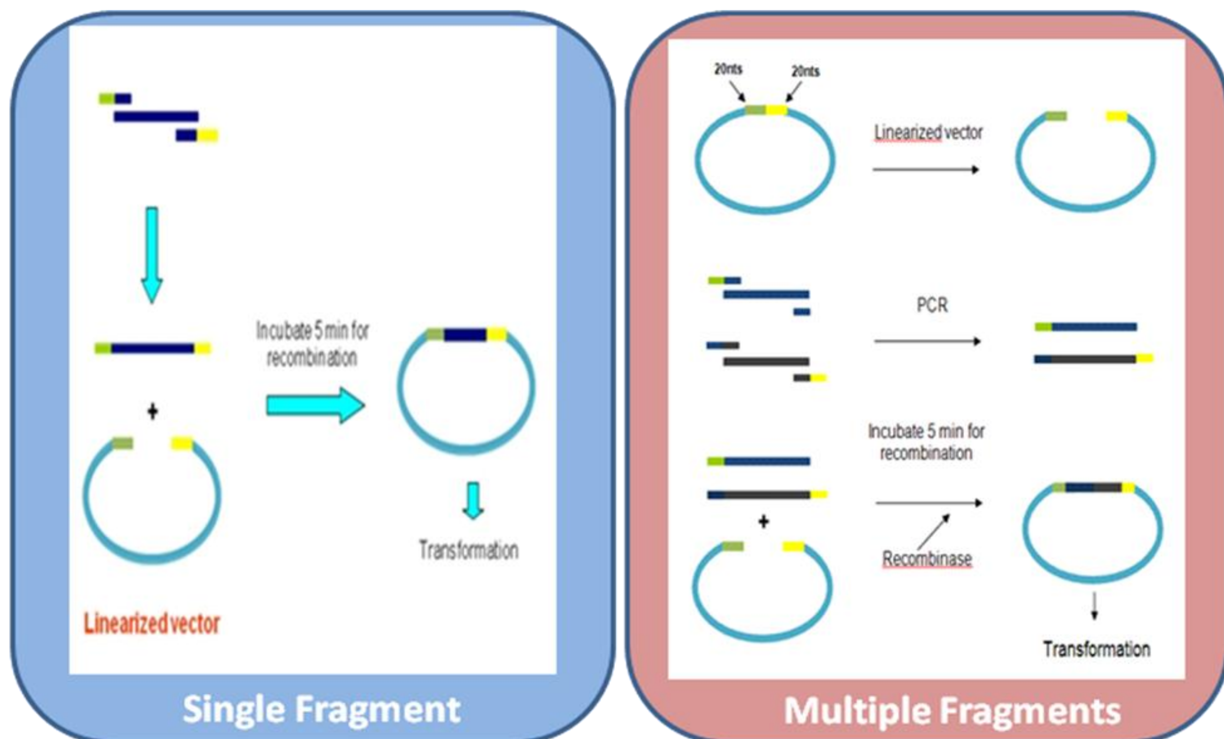
List of components:

RC enzymes, 5x RC buffer, Control vector, Control Insert, DNAase-free ddH2O

The RC Cloning Kit provides an easy and robust method to clone one or multiple PCR fragments into the desired vector seamlessly. **There is no need to either design compatible cohesive restriction ends as in traditional cloning methods or employ a cloning shuttle.**

Overview:

As shown in following figure, the desired vector is linearized by using the restrict enzymes and the interested DNA sequence is amplified with PCR by using the primer carrying extra twenty nucleotides homologous to the two open ends of the vector backbone. Incubation of the linearized vector and PCR fragments with the recombinase cocktail results in circulated plasmid bearing interested PCR product for transformation.



Pre-Protocol:

PCR Primer Design:

The PCR primers require a piece of sequence homologous to each end area of the linearized vector (***figure 1***). We recommend adding 20nt homology to each end of the vector, although as few as 12nt homology at each end is enough for the recombination.

PCR and the Product:

The template could be Genomic DNA, plasmid or cDNA, but treatment with DpnI is highly recommended in removing the template when plasmid is used as the template.

The PCR can be performed by using DNA polymerase such as, Taq, Pfu and Fusion DNA polymerase. Please follow the manufacturer's instructions.

The PCR product can be used directly without purification, but the purification of the PCR product will result in much higher yield of positive clones.

The Vector:

Linearization of circular vector can be prepared by either digesting of restrict enzyme or PCRing. Treatment with Dpn I is necessary for linearized vector prepared by PCR.

Summary

Table 1 shows the cloning efficiency of different length of PCR products by using the RC Cloning Kit. The results demonstrate that the RC Cloning Kit is capable of cloning the PCR product up to 8kb.

Table 1:

Vector(pRDcmv) (30ng)*	PCR product (40ng with 20bp homologous sequence at each end)	Positive Colonies	Total Colonies
+	500	6 out of 6	>200
+	1000	6 out of 6	>200
+	2000	5 out of 6	>200
+	4000	4 out of 6	>200
+	6000	6 out of 6	~100
+	8000	5 out of 6	~40
-	500	0 out of 3	3

Protocol:

1. Generate the linearized vector with restriction enzymes; or generate the linearized vector by PCR followed by treatment with Dpn**I.**

2. Design the gene-specific primers with a tag of 20nt homology to each end of the linearized vector.

3. Amplify desired DNA fragment by PCR with DNA polymerase (Taq, pfu, phusion etc.) following the manufacturer's instructions.

4. Purify the PCR product (unpurified PCR product may be used directly, but purification is highly recommended).

5. Set up a 5 µl of recombination reaction:

x µl (30-100ng) linearized vector.
x µl (30-100ng) inserts.
0.25 µl of RC enzymes
1 µl of 5x RC buffer
Add dH2O up to 5 µl

Mix and incubate at RT for 5 min and then keep on ice.

6. Use 3-5 µl of the reaction mixture for transformation.

Comparing of PCR cloning methods:

	Topo	**TA cloning**	**RC clong Kit**	**Restrict Enzyme cloning**
Restrict enzyme	Need for next step	Need for next step	-	Need for cloning
Vector	Specific	Specific	Interesting	Interesting
Ligase	-	+	-	+
Reaction time	5min	10min-1hr	5 min	10min-1hr
Insert	1	1	multiple	multiple
Yield (1kb)	High	Medium-Low	High	Up to digestion
Background	Low	Medium-high	Low	Up to digestion