

INSERT PREPARATION

Starting materials



**Traditional Cloning
(RE Digestion & Ligation)**

**PCR Cloning
(TA & Blunt-End)**

**Seamless Cloning
(Gene Assembly)**

**Ligation Independent
Cloning (LIC)**

**Recombinational
(Gateway/Creator/Univector)**

DNA preparation

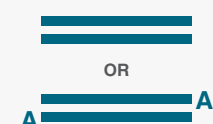
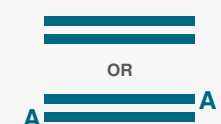
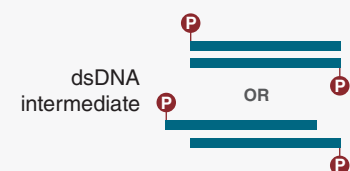
RE digestion
60 min. (standard)
5–15 min. (Time-Saver)

PCR
90 min.

PCR
90 min.

PCR
90 min.

PCR
90 min.



DNA end modification

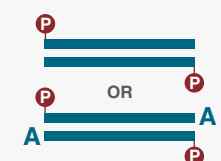
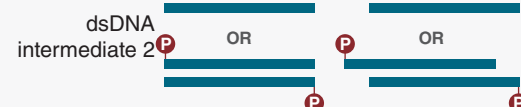
Dephosphorylation/
blunting (optional)
10–30 min.

Clean up
15 min.

Clean up
15 min.

Clean up
15 min.

Clean up
15 min.



Cohesive-end formation
by 5'→3' exo
30 min.

Cohesive-end formation
by 3'→5' exo
30 min.

RE digestion
60 min. (standard)
5–15 min. (Time-Saver)

Vector & insert joining

Gel & column purification
75 min.

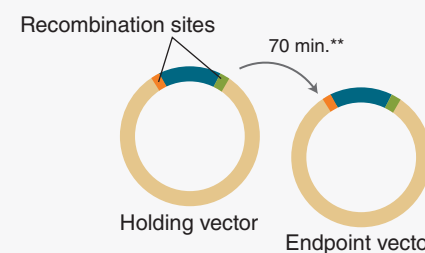
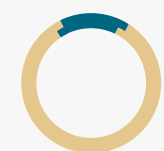
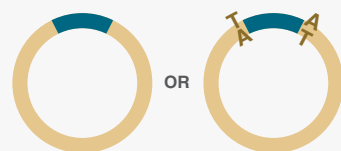
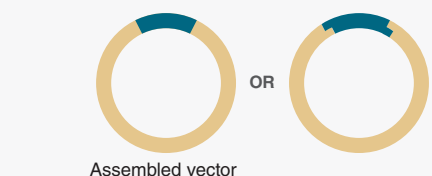
Ligation
15 min.

Ligation
Simultaneous with
previous step

Annealing
30 min.

Clean up
15 min.
Site-specific recombination
60 min.

Proteinase K treatment
10 min.



Estimated total time* 1 hr., 20 min. – 3 hr.

2 hr. – 2 hr., 30 min.

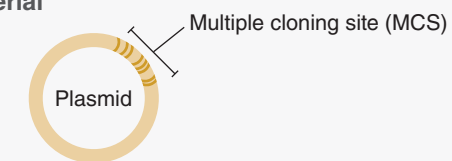
2 hr., 15 min.

2 hr., 45 min.

3 hr., 15 min. – 5 hr., 20 min.

VECTOR PREPARATION

Starting material



**Restriction Enzyme (RE)
Digestion**

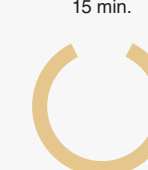
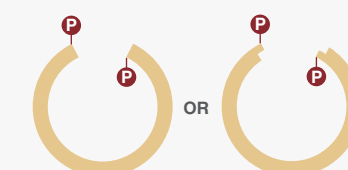
PCR

DNA preparation

RE digestion
60 min. (standard)
5–15 min. (Time-Saver)

PCR
2 hr.

Clean up
15 min.



Linear vector

DNA end modification

Dephosphorylation (optional)
10–30 min.

T-addition (optional)
1.5 hr.

Clean up
15 min.

Gel & column
purification
75 min.



Linear vector,
ready for joining

Estimated total time

20 min. – 2 hr., 25 min.

2 hr., 15 min –
3 hr., 45 min.

