Pro Ligation-Free Cloning Kit: Quick-Start Protocol

To download the full manual, visit Cat. No. E086 or E087 on *www.abmgood.com*.

Design and Amplify Overlapping PCR Products

1. Design primers to amplify insert fragments with 15-25 bp of overlapping homology to each other and to the linearized vector.
2. PCR amplify the fragments and visualize on an agarose gel.

Critical Step: For more details on primer design, see page 4 of the manual.

Purification Steps (Recommended)

3. Column purify your products, or gel extract if the PCR amplification contained non-specific bands.
- Alternatively, add 0.5 µl of Assembly Enhancer and 1.0 µl Cloning Optimizer to 50 µl of PCR product. Incubate at 37°C for 5 minutes, then at 80°C for 20 minutes.

Note: Gel extraction will help remove any residual PCR template plasmid, reducing background colony growth.

Assembly Reaction

4. Quantify the insert and vector DNA by a UV spectrophotometer or by comparing the target band against a known molecular weight marker run on the same gel.

5. Set up the following reaction on ice:

Reagent	Cloning Reaction	Example Reaction
2X Pro Ligation-Free MasterMix	10 µl	10 µl
Linearized Vector	100 ng	100 ng (5 kb vector)
Each Insert	3:1 insert-vector molar ratio	60 ng (1 kb insert)
Nuclease Free H_2O	Up to 20 µl	Up to 20 µl

To calculate amount of insert to add:

$$\text{insert (ng)} = 3 \times \frac{\text{insert (bp)}}{\text{vector (bp)}} \times \text{vector (ng)}$$

6. For 1 insert, incubate at 50°C for 15 minutes. For multiple inserts or difficult assemblies, incubate at 50°C for 1 hour.
7. Transform immediately, or store samples at -20°C until transformation.

ProClone™ Chemical Transformation

8. Add reaction mix to 60 µl of ProClone™ competent cells (Cat. No. E003).
9. Incubate on ice for 30 minutes.
10. Heat-shock for 45 seconds at 42°C, then chill on ice for 2 minutes.
11. Add 150 µl of LB Medium without antibiotics.
12. Shake at 37°C for 1 hour for recovery.
13. Spread cells onto pre-warmed LB agar plates with an appropriate antibiotic.
14. Incubate plates overnight at 37°C.

Troubleshooting: If no colonies are present, check: primer design, purity and concentration of DNA inserts, vector-insert ratio, antibiotic used, and transformation efficiency.

Screening and Analysis

15. Screen by colony PCR, or grow up colonies overnight in LB medium containing the appropriate antibiotic.
16. Isolate plasmid DNA. We recommend using our Plasmid Mini-Prep Kit (D504).
17. Confirm correct assembly by restriction analysis or sequencing.

Troubleshooting: If too many colonies are present or clones do not pass screening, consider the following possibilities: vector was not completely digested, presence of residual PCR template plasmid, antibiotics are old, or amplification was not specific.