User Instruction

U-Clone Master Mix for

DNA Cloning and Mutagenesis

(Cat#: UC20 or UC100)

Important Note:

Store the U-Clone master mix at –20°C.

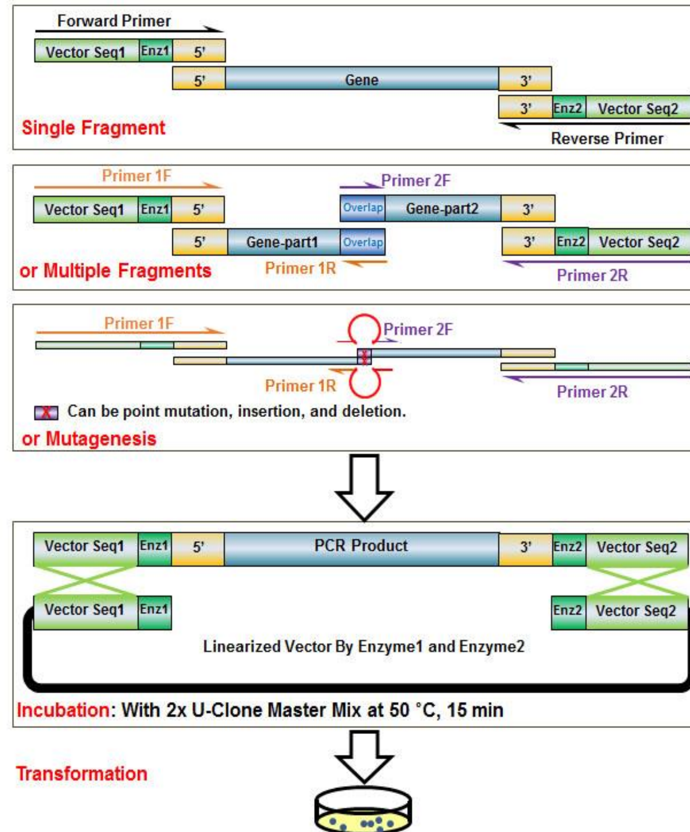
Key Features of U-Clone Master Kit:

- Seamless cloning within 15 min;
- Clone any insert at any site of any vector;
- Clone single, or multiple fragments, or mutagenesis at once;
- Add any adaptor, linker and tag in insert;
- No ligase and dephosphatase;
- No more than five colonies pick-up.

How U-Clone Works

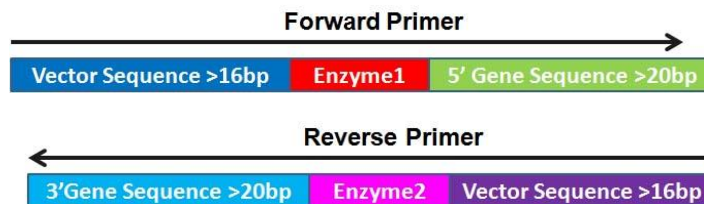
The 2x U-Clone master mix contains a proprietary blend of enzymes that direct the assembly of DNA fragments with homology ends and further extend with the highest accuracy so far under cozy condition. A brief procedure was outline in chart below.

U-Clone Master kit is also suitable for DNA mutagenesis, including point mutation, insertion and deletion mutation, through multiple pair primers and multiple fragment assembly.



Preparation of insert and vector

1. **Linearize vector**: vector would be digested with your desired restrict enzymes and then the linearized vector would be purified.
2. **PCR the target gene:** The key part for successful cloning your target DNA into linearized vector is to design appropriate PCR primers. Each primer contains three parts: at least 16 bp of homology with the end of the linearized vector; plus restrict enzyme sites; and at least 20 bp of the gene-specific sequence. The guidelines for primer designs are shown in the diagram below.



3. **Multiple DNA fragment cloning**: If multiple DNA fragments will be assembled, the joining sequence should be at least 20 bp of homology in each end. More U-Clone master mix and longer incubate time will need.
4. **Other:** The PCR fragments should be generated by high fidelity DNA polymerase. Clean PCR product ensures the successful cloning. The melting temperature (Tm) would be based on the gene-specific end of the primer, NOT the entire primer. Enzyme 1 and 2 can be the same or different one in the vector. Additional sequences can be included between vector and gene sequences.

Kit Components

- U-Clone Master Mix (2x) suitable for 20 or 100 reactions
- **Required Materials but Not Included:**
  - High-Fidelity DNA Polymerase and relative PCR materials (for generating PCR products).
  - 20 or 100 tubes of *E. coli* DH5α Super Efficiency Competent Cells
  - 5 or 25 ml of SOC medium
  - LB (Luria-Bertani) plates with appropriate antibiotic.

Brief Protocol for U-Clone Reaction

1. A typical U-Clone reaction for vector with one insert fragment was described below. Generally, 50–100 ng of vectors with 2~4 fold of excess inserts will give a good transformation rate.

   - Optimized cloning efficiency will be obtained at 3~5x molar ratio of insert to vector. DNA insert and vector molar concentration is dependent on insert and vector's length and weight, respectively. The following formula: DNA pmols = 1.54x ng / (PCR product or vector bps). e.g.
     - 100 ng of 3000 bp dsDNA is about 0.05 pmols.
     - 100 ng of 300 bp dsDNA is about 0.5 pmols.
   - If insert is less than 200 bps, more of inserts may need.
   - If more than two fragments will be assembled, additional U-Clone Master Mix may be required.

2. Linearize and purify vector DNA (20~100 ng in 2 µl). Usually, if 2 µg of vector DNA is digested, and eluted by 50 µl of EB buffer at final step when using Qiagen Gel Extraction kit or other kits, the DNA concentration would be 20~30 ng/µl. Use 2 µl of this product per reaction. Note: Do not use the linearized vector after two months of storage, even though stored in -20˚C.

3. Purify PCR product(s). Mostly, the purification step of PCR products is not necessary for DNA assembly. Adding PCR reaction directly to assembly system will give a similar result.

4. Mix the linearized vector (2 µl) and PCR product (1~8 µl) in a clean PCR tube, bring to 10 µl with water. All reaction should be set up on ice.

5. Add the above mixture to 10 µl of 2x U-Clone master mix, flick to mix it and then spin down briefly.

6. Set a program on Thermal Cycler (PCR machine) with step1= 50˚C for 15 min (one fragment) to 60min (more than one fragment)→ step 2=4 ˚C/∞. Product can be frozen at - 20˚C if desired.

U-Clone Transformation Protocol

1. Thaw chemically Super Efficiency Competent Cells on ice, aliquot 50 µl to 1.5ml micro-centrifuge tube.
2. Add 5-10 µl of the chilled U-Clone Reaction product to the competent cells. Mix gently by flicking the tube 4–5 times.
3. Place the mixture on ice for 30 minutes.
4. Heat shock on a 42°C heat block for 60 seconds.
5. Transfer tubes to ice for 2 minutes.
6. Add 250 µl of room-temperature SOC media to the tube.
7. Incubate the tube at 37°C for 60 minutes with shaking (250 rpm).
8. Spread 60 µl of the cells onto the antibiotic-selection plates.
   - Option: Spin at 3000 rpm for 1 min, remain 50-100 µl of supernatants to resuspend pellet and spread all on the plate.
9. Incubate overnight at 37°C.

Question and Troubleshooting

Please contact: info@evomicscience.com

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