Datasheet for ABIN491483

PCR Cloning Kit (CloneEZ)

34 Publications

Overview

Quantity:	24 reactions
Application:	Cloning (Clon)

Product Details

Purpose:	CloneEZ® PCR Cloning Kit is designed for quick and convenient PCR cloning.
Brand:	CloneEZ®
Characteristics:	CloneEZ® PCR Cloning Kit is designed for the quick PCR cloning , it is especially powerful in high-throughput cloning of PCR products into any destination vector effectively by sidestepping the tedious and limiting tasks such as selecting proper restriction enzymes, phosphatases, or ligases. Using our proprietary CloneEZ® Enzyme, this kit is able to rapidly generate precise and directional constructs by carrying out 30-minute incubation at room temperature (20 °C-25 °C).
Components:	CloneEZ® Enzyme (5 U/µL): 50 µL 10X CloneEZ® Buffer: 100 µL pUC57 Linearized with EcoRI/HindIII, positive control: 10 µL 1-kb Control Insert, positive control: 10 µL

Application Details

Comment:	The CloneEZ® PCR Cloning Kit offers flexible and quick directional cloning and enables users to: Clone into any vector without any need for restriction enzymes, phosphatase treatments, or ligases Provide up to 10 kb PCR cloning at any restriction site Rapidly and precisely generate correctly oriented constructs and inserts Work with PCR that employ any thermostable polymerases

Application Details

Assay Time:	< 1 h
Reagent Preparation:	To clone any DNA fragment into a linearized vector using this kit, the insert fragment should be obtained by PCR using primers with an add-on of 15 base sequences homologous to either side of the restriction site that is used to linearize the vector. Therefore, a primer should cover a 15-base sequence add-on at the 5'-end, an optional restriction site in the middle, and the insert-specific sequence at the 3'-end. PCR amplification can be performed using any thermostable DNA polymerase. However, primers and primer dimers produced in PCR reactions are inhibitory to the CloneEZ® PCR cloning reaction. If the PCR produces a single specific band (from an agarose gel), PCR DNA can be purified by simply using a PCR purification kit.
Sample Preparation:	To achieve a successful CloneEZ® PCR cloning reaction, complete linearization of the vector is critical. Incomplete linearization of the vector will result in high background. The linearized vector should be purified using a gel or PCR purification kit, such as QuickClean I PCR Purification Kit
Assay Procedure:	CloneEZ® Recombination Procedure 1. Set up the following reaction in a 0.5 mL Eppendorf tube by mixing the following reagents gently and then spin down briefly to collect the reagents at the bottom of the tube. Linearized vector (100-200 ng/μL): 6 μL Purified PCR products (100-200 ng/μL): n μL 10X CloneEZ® Buffer: 2 μL CloneEZ® Enzyme: 2 μL Deionized water: up to 20 μL In general, add more than 10 μL of PCR DNA (n = 10) to the reaction can produce nearly 95% positive clones. In addition, less amount of DNA is appropriate for short PCR DNA fragments. For different sizes of PCR DNA, different amount of DNA is recommended below: PCR DNA of 1 kb: 4 μL PCR DNA of 2 kb: 6 μL PCR DNA of 3 kb: 8 μL PCR DNA of >3 kb: 10 μL 2. Incubate the reactions at 22°C for 30 minutes, and then transfer tubes to ice and incubate on ice for five minutes. 3. Proceed with transformation (Section D). The reaction can also be stored at -20°C for later transformation. Transformation Materials needed but not provided along with the kit: Water bath or heating block (42°C)

Application Details

	SOC liquid mediumDH5α competent cells (>1×108 cfu/μg) 1. Thaw one vial of frozen 50 μL competent cells on ice. Tap the tube gently to ensure that the cells are suspended. 2. Add 5–8 μL of reaction mixture to the competent cells. Tap the tube gently and incubate the tube on ice for 30 minutes. 3. Heat shock the cells by placing them in 42oC water bath for 45–90 seconds and then place the tube on ice for 2–3 minutes. 4. Add 600 μL of SOC medium to the cells and then incubate the cells on a shaker set at 250 rpm at 37 °C for 60 minutes. 5. Centrifuge the cell down at 4000 rpm for five minutes and then remove and discard about 500 μL of medium. Gently suspend the cells by tapping the tube. 6. Transfer 10 μL and 100 μL of the suspension to two different plates containing appropriate antibiotics, respectively. Spread the cells evenly on the plates. 7. Incubate the plates overnight at 37 °C.
Restrictions:	For Research Use only

Handling

Storage:	-20 °C
Storage Comment:	The kit should be stored at -20°C. It will remain stable for at least one year.
Expiry Date:	12 months

Publications

Product cited in:	Johnson, Drugan, Miller, Evans: "38" in: , Vol. 1363, Issue Nucleic acids research, pp. 28-39, (1991)