illustra™ TempliPhi™ Sequence Resolver Kit

Codes:

28-9035-29 28-9035-30 28-9035-31

Description

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. illustra TempliPhi Sequence Resolver Kit uses the highly processive, strand-displacing Phi29 DNA polymerase and modified nucleotides to amplify circular templates for subsequent sequencing. The formulation is designed to help resolve sequencing problems associated with repeats, sequencing stops, and compressions due to high GC content. Product amplified using the TempliPhi Sequence resolver kit does not form the same secondary structure artifacts and provide greatly improved results with Phred20 scores greater than 800bp.

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Short Protocol

The steps outlined below describe a general protocol to amplify sequencing templates. This protocol is a starting point for optimizing the reaction in your laboratory.

NOTE: The short protocol is appropriate for researchers who have optimized their TempliPhi Sequence Resolver Kit reactions. First-time users must review the Full Protocol, which can be downloaded from <u>http://www.gelifesciences.com</u> The full protocol can also be obtained by e-mail or fax by

contacting your local GE Technical Support Group.

1. Heat denaturation of template in sample buffer

- **a.** Mix 1 μ l of template with 4 μ l of sample buffer
- **b.** Heat to 95°C for 3 minutes.
- c. Cool to 4°C on ice.

2. Preparation of amplification reaction

- **a.** For each amplification reaction, combine 4.5 µl of reaction buffer with 0.5 µl of enzyme
- **b.** Mix on ice.
- **c.** Add this to the cooled sample.

3. Incubation

Incubate the sample at 10°C for 16–18 hours.

4. Post-amplification heat inactivation Heat the sample to 65°C for 10 minutes.

Cool to 4°C.

5. Sequencing

Use 2 μ l of amplification product per 20 μ l sequencing reaction.

