Universal16s PCR Barcoding Kit

Amplification of the 16s rRNA gene region

Catalog Number: NA2029

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

The 16S ribosomal RNA (rRNA) plays a crucial role in bacterial and archaeal ribosomes. This sequence is Highly conserved across bacteria and archaea, it contains variable regions useful for species differentiation and is part of the 30S subunit of prokaryotic ribosomes. 16s rRNA Binds to the Shine-Dalgarno sequence and contributes to the subunit's structure. Acts as a scaffold for ribosomal proteins.

2. Test Principle

Attogene's 16s PCR kit is designed for barcoding the ribosomal RNA gene region. In cyanobacteria. A sample of algae is obtained and washed to extract a clean algal gDNA sample. A reaction mixture is assembled from primers, master mix, and gDNA samples as required. The qPCR machine of choice is set up and loaded as needed and the mixture undergoes PCR amplification. The Primer mix provided exploits the Taq polymerase to amplify the gene region of interest.

3. Applications

This kit can be used for specific amplification of the 16s gene region in liquid gDNA samples such as water, wastewater, and algal cultures.

4. Equipment and Reagents Needed (not provided)

- Real-time PCR Instrument
- qPCR 2X Master Mix
- DNA extraction kit/ gDNA sample
- PCR reaction tubes/plate
- Vortex and centrifuge
- PCR clean 1mL tube
- Micropipettes & Tips

5. Components Provided in This Kit

- 170ul 16s region specific primer mixture (150 reactions)
- 1.5ml PCR clean water

6. Reagents Preparation

Master Mix (not Provided)

-Caution this reagent is sensitive to contamination and should only be handled in a clean area away from positive control template.

-Store at -20C. Master Mix is stable when stored at -20C. Freeze and thaw cycles should be minimized to increase shelf life.

-If required aliquots of Master Mix should be made and stored at -20C to minimize freeze thaw and contamination risk.

Primer Mixture

-Caution these reagents are sensitive to contamination and should only be handled in a clean area away from positive control template.

-Store at -20C. Primer is stable when stored at -20C. Freeze and thaw cycles should be minimized to increase shelf life.

-If required, aliquots of Primer should be made and stored at -20C in the dark to minimize freeze thaw and contamination risk.

7. Control Preparation

Negative extraction control (NEC)

-If necessary, prepare one NEC each time extracting DNA from your sample.

-RNase/DNase free water is used in place of a sample in the extraction system to create a negative for the DNA isolation method.

- The NEC will serve as a contamination control method for the isolation.

No Template control (NTC)

-If necessary, a NTC can made by replacing gDNA in the PCR reaction with RNase/DNase free water

-The NTC is used to check for contamination during PCR plate set up

8. Assay Set Up

-gDNA isolation will need to be done before starting an experiment. For optimal results use >10ng/uL of gDNA with a ratio of >1.80 in your experiment. IEC multiplexing can also be done to ensure proper DNA extraction.

-Plate set up will vary with the quantity of samples you need to run on your plate. A NEC is preferably included in each plate set up. NTCs should be included in each plate set up.

-Determine the number of reactions to set up in your assay (including NEC and any NTCs for your plate). It is necessary to make extra reaction mixture to allow for pipetting error.

-For convenience a large solution of PCR components will be mixed shortly before starting a reaction and subsequently aliquoted into your plate or tubes. Each PCR run will use 19uL of this reaction mixture and 1ul of isolated gDNA/NTC/NEC based on the experiment set up.

9. qPCR detection protocol

1. For each DNA sample prepare a reaction mix according to the table below:

(Include sufficient reactions for positive and negative controls)

Reagent	Quantity
2X Master Mix	10uL
PCR Water	8uL
Dual 16s Primer set	1uL
Final Volume	19uL

2. Pipette 19uL of this mixture into each well according to your qPCR experimental plate set up.

3. Prepare your gDNA templates for each reaction

4. Pipette 1uL of sample gDNA into each well, according to your experiment. For negative controls replace the gDNA sample with 1uL of RNase/DNase free water to bring the total volume to 20uL.

10. qPCR Amplification Protocol

Amplification conditions using 2x qPCR Master Mix:

Steps	Time	Temperature	Cycles
Initial Denaturation (Taq Activation)	2 Minutes	94C	1
Denaturation	20 sec.	94C	35
Annealing*	30 sec.	50C	
Extension	45 sec.	74C	

*Fluorogenic data should be read during this step through the FAM channel

11. Expected Performance

Before Interpreting results, it is necessary to verify the integrity of the reaction. If the criteria are not satisfied, then testing needs to be repeated.

13. General Instructions

13.1 Shaking of Reagents

- Shake each reagent gently before use.

13.2 Out of Date Kits

- Don't use kits that are expired. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

14. Storage

- Storage condition: -20°C
- Storage period: 12 months

Customer Notes:

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