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PCR Run Protocol for Bacterial 16S rRNA Gene Amplification

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We use this protocol and it's working

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Disclaimer

This protocol follows standard PCR guidelines and has been validated for bacterial 16S rRNA amplification. Users must follow biosafety regulations when handling microbial DNA and PCR reagents. The authors are not responsible for procedural errors or misuse.

Abstract

This protocol describes the polymerase chain reaction (PCR) setup and amplification of bacterial 16S rRNA genesusing 27F and 1492R primers. The method utilizes Promega GoTaq® Green Master Mix (2x) and follows a standard thermal cycling program optimized for bacterial DNA. Proper sterile techniques, contamination prevention, and accurate pipetting are crucial to obtaining high-quality amplification results. The protocol includes negative controls to ensure reaction validity.

Guidelines

- Work in a clean PCR setup area to avoid contamination.
- Always use filter pipette tips and change them between reagents.
- Keep primers, master mix, and DNA on ice during preparation.
- Prepare negative controls to detect contamination.
- Use a dedicated pipette for master mix handling to prevent DNA contamination.
- Ensure DNA is of good quality (measured by NanoDrop™ or gel electrophoresis).

Materials

Reagents & Solutions

- Promega GoTaq® Green Master Mix (2x) – Taq polymerase, dNTPs, buffer
- Forward Primer (27F) – Tm: 50°C / Length: 20 bp
- Reverse Primer (1492R) – Tm: 47°C / Length: 19 bp
- Template DNA (Bacterial Culture or Extracted DNA)
- Nuclease-Free Water (for negative controls and master mix preparation)

Consumables & Equipment

- PCR Tubes (0.2 mL) – For reaction setup
- 2 mL Microcentrifuge Tubes – For master mix preparation
- Pipettes & Filtered Tips – To prevent contamination
- Ice Bucket – For keeping reagents cold
- Vortex Mixer – For sample homogenization
- Centrifuge (for PCR tubes) – To collect contents at the bottom
- Thermal Cycler (PCR Machine) – For amplification

Safety warnings

- Use gloves and filter pipette tips to prevent contamination.
- Handle PCR products carefully to avoid cross-contamination.
- Ensure PCR tubes are tightly closed before centrifugation.
- Dispose of used tips and reagents according to lab biosafety guidelines.

Ethics statement

This protocol involves PCR amplification of bacterial DNA and does not involve animal or human subjects. However, researchers must adhere to institutional biosafety regulations when handling microbial cultures, DNA samples, and PCR reagents to ensure compliance with laboratory safety guidelines.
If this protocol is adapted for DNA extraction or amplification from animal or human samples, prior approval must be obtained from an Institutional Animal Care and Use Committee (IACUC) or an equivalent ethics committee. Researchers must comply with internationally accepted ethical standards, and any relevant permit numbers and committee approvals should be documented accordingly.
Proper biosafety precautions, contamination control, and ethical considerations must be followed throughout the experimental process to ensure safe and responsible research practices.

Before start

- Ensure all reagents and samples are properly labeled.
- Confirm all reagents are thawed and mixed properly.
- Clean the workspace and use sterile pipette tips.
- Prepare a sample sheet to track tube numbers and sample names.
- Set up the thermal cycler before loading the samples.

Procedures

1 1. Label all PCR tubes according to the sample chart (including negative controls, Contains all PCR reagents except DNA).
2. Place samples, primers, master mix, and controls on ice to thaw.

Primers Used:

- Forward Primer (27F): Tm = 50°C, 20 bp
- Reverse Primer (1492R): Tm = 47°C, 19 bp

Reagents Kept on Ice:

- Promega GoTaq® Green Master Mix (2x)
- Forward and Reverse Primers (27F and 1492R)
- Positive (A DNA sample known to amplify successfully with the selected primers) and Negative Controls (to validate the experiment and ensure the amplification is specific and free from contamination)

2 **Master Mix Preparation**

1. Calculate the total reaction volume per tube: 25 µL
2. Multiply by the number of reactions, including controls and extra blanks.

Master Mix Composition (Per Reaction):

- 12.5 µL Promega GoTaq® Green Master Mix (2x)
- 5 µL Forward Primer (1 µM, 27F)
- 5 µL Reverse Primer (1 µM, 1492R)

3. Mix all components in a 2 mL microcentrifuge tube to create the master mix.
4. Vortex briefly and centrifuge to collect liquid at the bottom.

3 **Distributing Master Mix into PCR Tubes**

1. Pipette 22.5 µL of master mix into each PCR tube (including positive and negative control).

4 **Adding DNA Samples**

1. Vortex bacterial culture (if using directly) before pipetting.
2. Add 2.5 µL of template DNA/culture to each PCR tube.
3. Mix by pipetting up and down gently.
4. For the negative control, add 2.5 µL of nuclease-free water instead of DNA.
5. For the positive control, add 2.5 µL of previously confirmed bacterial genomic DNA (e.g., a reference strain or validated sample)

Note: Avoid contamination by changing pipette tips between samples.

5 **Centrifugation & Loading PCR Machine**

1. Briefly centrifuge PCR tubes to collect liquid at the bottom.
2. Load tubes into the PCR machine, placing them near the center to ensure even heating.

6 **PCR Thermal Cycling Parameters**

Set up the thermal cycling conditions as follows: an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute and 30 seconds. After cycling, a final extension step at 72°C for 10 minutes ensures complete amplification. The reaction is then held at 10°C indefinitely (∞) to prevent DNA degradation until further processing.

7 **Running the PCR & Post-Run Handling**

1. Start the PCR machine with the volume set slightly higher than the actual reaction volume for accuracy.
2. Once the PCR cycle is completed, remove the tubes.
3. Store amplified PCR products at -20°C or proceed directly to gel electrophoresis for validation.

8 **Expected Results**

Successful 16S rRNA gene amplification produces a ~1,500 bp band on agarose gel electrophoresis.

Agarose Gel Verification:

- Load 5 µL of PCR product + 1 µL loading dye into a 1% agarose gel.
- Run at 120V for 30–40 minutes.

Expected results:

- Positive control: A clear ~1.5 kb band, confirming that the PCR conditions, primers, and polymerase are working correctly.
- Negative control: No bands should appear; any bands indicate contamination in the reagents or pipetting process.
- Samples: Presence of a ~1,500 bp band confirms successful amplification of bacterial 16S rRNA genes.

Key Takeaway: If the positive control fails (no band), check the PCR setup, reagents, and primers. If the negative control has bands, there may be contamination.

Protocol references

Lane, D.J. (1991) 16S/23S rRNA Sequencing. In: Stackebrandt, E. and Goodfellow, M., Eds., Nucleic Acid Techniques in Bacterial Systematic, John Wiley and Sons, New York, 115-175.

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