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PCR Protocol for TaqMan® Genotyping Assays

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Eyleen Nabyla Alvarenga Niitsuma[1], Gabriel da Rocha Fernandes[2], Francisco Carlos Félix Lana[3]

[1]Instituto Federal de Educação, Ciência e Tecnologia do Norte de Minas Gerais;
[2]Instituto René Rachou - Fiocruz Minas;
[3]Departamento de Enfermagem Materno-infantil e Saúde Pública, Escola de Enfermagem, Universidade Federal de Minas Gerais - UFMG

Eyleen Nabyla Alvarenga Niitsuma
Instituto Federal de Educação, Ciência e Tecnologia do Norte...

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Protocol status: Working
We use this protocol and it's working

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Guidelines

1 Keep all reagents protected from light until you are ready to use them. Excessive exposure to light may affect the fluorescent probes.

2 Minimize freeze-thaw cycles.

3 Prior to use:
– Mix the TaqMan Genotyping Master Mix thoroughly by swirling the bottle.
– Thaw any frozen TaqMan reagents by placing them on ice. When thawed, resuspend the samples by vortexing, then centrifuge the tubes briefly.
– Resuspend the TaqMan reagents by vortexing, then centrifuge the tube briefly.
– Thaw any frozen genomic DNA samples by placing them on ice. When thawed, resuspend the samples by vortexing, then centrifuge the tubes briefly.

Materials

MATERIALS

- TaqMan® Genotyping Master Mix **Applied Biosystems (ThermoFisher Scientific) Catalog #**4371355
- TaqMan® SNP Genotyping Assays **Applied Biosystems (ThermoFisher Scientific) Catalog #**4351376
- MicroAmp® Optical Adhesive Film **Applied Biosystems (ThermoFisher Scientific) Catalog #**4360954

STEP MATERIALS

- TaqMan® Genotyping Master Mix **Applied Biosystems (ThermoFisher Scientific) Catalog #**4371355
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Protocol materials

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Before start

1. Extract and purify genomic DNA.

2. Quantitate the gDNA in samples by spectophotometry.

1 Normalize the samples of gDNA.

Note

The DNA concentration per sample is 20ng / µL.

2 Calculate the number of reactions to be performed for each assay, including recommended controls.

Note

Use negative and positive control. Prepare excess volume to account for pipetting errors.

Prepare the reaction mix - volume per well is 10.5 µL for a 96-well plate.

3 Swirl the bottle of TaqMan® Genotyping Master Mix gently to mix the contents. Vortex and centrifuge the Genotyping Assay Working Stock, then mix briefly. Pipette the required volumes of TaqMan® Genotyping Master Mix and Genotyping Assay mix into a sterile tube. Cap the tube.

0.5 µL Genotyping Assay Working Stock (40x)

10 µL TaqMan® Genotyping Master Mix (2x)

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4 Vortex the tube briefly to mix the components. Centrifuge the tube briefly to spin down the contents and to eliminate air bubbles from the solution.

Prepare the reaction plate

5 Pipette 10.5 of the reaction mix (Master mix genotyping and TaqMan Assay) into each well of the reaction plate.

6 Into each well of the plate, pipette 2ul of normalized gDNA sample (concentration of 20ng/ul) and 7.5ul of ultrapure water.
Be sure to include wells for use as no template controls (no gDNA and 9.5ul of ultrapure water).

Note

Use a calibrated, positive-displacement pipettor to minimize contamination and error. Be sure that no cross-contamination occurs from well to well.

7 Cover the plate with MicroAmp® Optical Adhesive Film and seal the plate.

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8 Centrifuge the plate briefly to spin down the contents and eliminate air bubbles from the solutions.

Perform the PCR

9 Place the plate in a Real-Time PCR instrument. Use the thermal cycling conditions specified.

Enzyme activation
Temp. 95°C - Duration 10 minutes - Cycles HOLD

Denaturation
Temp. 95°C - Duration 15 seconds - Cycles 40

Annealing/Extension
Temp. 60°C - Duration 1 minute - Cycles 40

PCR plate read and analysis

10