PCR Protocol for TaqMan® Genotyping Assays

Eyleen Nabyla Alvarenga Niitsuma,Gabriel da Rocha Fernandes,Francisco Carlos Félix Lana

Abstract

Citation: Eyleen Nabyla Alvarenga Niitsuma,Gabriel da Rocha Fernandes,Francisco Carlos Félix Lana PCR Protocol for TaqMan® Genotyping Assays. **protocols.io**
dx.doi.org/10.17504/protocols.io.sfzebp6
Published: 28 Aug 2018

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.

Before start

1. Extract and purify genomic DNA.

2. Quantitate the gDNA in samples by spectophotometry.

Materials

TaqMan® Genotyping Master Mix 4371355 by Applied Biosystems
TaqMan® SNP Genotyping Assays 4351376 by Applied Biosystems
MicroAmp® Optical Adhesive Film 4360954 by Applied Biosystems

Protocol

Step 1.

Normalize the samples of gDNA.

NOTES

The DNA concentration per sample is 20ng / µL.

Step 2.

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

NOTES

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.

Step 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.

AMOUNT
0.5 µl : Genotyping Assay Working Stock (40x)
AMOUNT
10 µl : TaqMan® Genotyping Master Mix (2x)
REAGENTS
TaqMan® Genotyping Master Mix 4371355 by Applied Biosystems
TaqMan® SNP Genotyping Assays 4351376 by Applied Biosystems

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

Step 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

Step 5.

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

Prepare the reaction plate

Step 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).

NOTES

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

Prepare the reaction plate

Step 7.

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

READINGS

MicroAmp® Optical Adhesive Film 4360954 by Applied Biosystems

Prepare the reaction plate

Step 8.

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

Perform the PCR

Step 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

Step 10.