Dec 08, 2020

TaqMan SNP genotyping protocol

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.bpapmidn

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ABSTRACT

The protocol is based on TaqMan SNP genotyping protocol

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243509

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Jin J, Robeson H, Fagan P, Orloff MS (2020) Association of *PARP1*-specific polymorphisms and haplotypes with non-small cell lung cancer subtypes. PLoS ONE 15(12): e0243509. doi: 10.1371/journal.pone.0243509

DOI

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PROTOCOL CITATION

Heather Robeson, Jing Jin, Mohammed S. Orloff 2020. TaqMan SNP genotyping protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bpapmidn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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CREATED

Nov 01, 2020

LAST MODIFIED

Dec 08, 2020

PROTOCOL INTEGER ID

44079

MATERIALS TEXT

TaqMan genotyping master mix
TaqMan genotyping SNP genotyping assays
Adhesive film

ABSTRACT

The protocol is based on TaqMan SNP genotyping protocol

BEFORE STARTING

-Extract and purify genomic DNA (gDNA)
-Quantify sample gDNA
-Prepare assays, DNA samples, and master mix
Dilute the predesigned and custom TaqMan SNP genotyping assays to 20X working stock solution

PCR reactions

1 384-well Fast (5-µL reaction)
Prepare the reaction mix:
Combine the following components for the number of reactions required, plus 10% overage.
2X TaqMan Master Mix: **2.5 µl**
20X Assay Working Stock: **0.25 µl**
Total volume per well: **2.75 µl**

2 Vortex to mix

3 Centrifuge to bring the reaction mix to the bottom of the tube and remove the air bubbles

4 Prepare the reaction plate:
Dilute each DNA sample, positive and negative controls in Nuclease-free water. The final concentration should be at least 0.2 ng/µL

5 Add reaction mix to each well of the reaction plate

6 Seal the plate with adhesive film, then centrifuge to bring the reaction mix to the bottom of the well and eliminate air bubbles.

7 Remove film from the plate, then add the appropriate volume of sample or control to the wells

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8 Seal the plate with adhesive film, then centrifuge to bring the reaction mix to the bottom of the well and eliminate air bubbles.

Perform PCR 10m

9 Place the plate in a Real-Time PCR instrument ABI 7900 HT. Use the thermal cycling conditions specified.
Polymerase activation:

Temp. **95 °C** , Time 10mins , Cycles HOLD

Denaturation:

Temp. **95 °C** , Time 15 seconds, Cycles 40

Annealing extension:

Temp. **60 °C** , Time 1 min, Cycles 40

Post-PCR plate read and analyses

10

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