TECHNICAL BULLETIN

hPSC GENETIC ANALYSIS KIT TECHNICAL TIPS & TRICKS

This document contains tips and tricks for optimal use of the hPSC Genetic Analysis Kit. This document should be used in conjunction with the Product Information Sheet (PIS; Document #DX22330).

Key Tips

- When using the hPSC Genetic Analysis Kit for the first time, run the Genomic DNA Control and only 1 - 2 samples to become familiar with the protocol before screening large numbers of samples.
- Vortex the mixtures thoroughly (5 seconds per sample) when indicated in the PIS.
- It is important to be precise and careful when pipetting. A digital single-channel repeating (multi-dispenser) pipette is recommended when analyzing multiple samples.

General Tips

When using the kit for the first time

1. Read the PIS thoroughly before resuspending the Genetic Assays or preparing the qPCR Master Mix + Dye.
2. The first run should be carried out on the Genomic DNA Control plus 1 - 2 samples at most. Once you are familiar with the technique and have low replicate variability, the sample number can be increased.
3. Plan your plate layout in advance and have it in front of you for reference when pipetting the reactions.

Vortex, Vortex, Vortex!

1. When working with DNA there is often a hesitation about vortexing, since it may lead to shearing. Although we do not recommend vortexing the stock genomic DNA samples, it is important to thoroughly vortex the DNA and Master Mix + Dye solution (prepared in step C5) for 5 seconds. This will help to reduce the variability between technical replicates.
2. Likewise, the Genetic Assay and water solution prepared in step D3 should be sufficiently mixed by vortexing (5 seconds per sample). This will help to reduce the variability between technical replicates.

Genomic DNA Sample Preparation

1. The amount of genomic DNA per reaction can be increased; in some cases this can improve reproducibility between replicates.
   e.g. Instead of using 300 ng in 90 µL (step C.4), use 600 ng or 900 ng in 90 µL.
2. Mix the genomic DNA and Master Mix + Dye, prepared by the end of C5, by vortexing thoroughly (5 seconds per sample) immediately before pipetting into the plate.
3. Determine the concentration and quality of genomic DNA samples using an appropriate method. Genomic DNA samples should have absorbance ratios in the range of A260/280 ~1.8 - 2.0 and A260/230 ~1.9 - 2.2.
4. If the concentration of the sample DNA is low (< 5 ng/µL), it is beneficial to concentrate using ethanol precipitation or other suitable method prior to analysis with the hPSC Genetic Analysis Kit. Running very low concentrations of DNA may introduce high variability between replicates and lead to difficult-to-interpret results.

Genetic Assays

1. The lyophilized primer-probe pellet may have become dislodged during shipping, so it is important to centrifuge the tube prior to reconstitution. If possible, try to locate the pellet and pipette the TE Resuspension Buffer directly onto the pellet for resuspension.
2. It is important to mix the Genetic Assay thoroughly before use, particularly if the suspension is going to be aliquoted. This can be done by flicking the tube followed by brief centrifugation.
3. Take care when pipetting the Genetic Assays; always use a clean pipette tip when moving between stocks or diluted assays to avoid cross-contamination of primer-probes.

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Troubleshooting

qPCR Reaction Fails/No Amplification

All reactions fail to amplify:

- Incorrect amount or no ROX Reference Dye added to qPCR Master Mix (2X).
  Note: Check that the correct amount of ROX Reference Dye was added to the qPCR Master Mix (2X) for the machine being used.
- Incorrect cycling conditions used.
  Note: If you are unsure which cycling conditions are required for your machine, use the standard cycling times provided in the PIS.

Individual reactions fail to amplify:

- One or more components are missing from the reaction including genomic DNA, qPCR Master Mix (2X), ROX Reference Dye, or Genetic Assay.
  Note: Take care when pipetting the reactions together; marking sample borders can help to keep track of wells that have been filled.
- Incorrect wells selected in the qPCR software.
  Note: Some qPCR machines use software that require the pre-selection of wells and their contents; check that the correct wells are selected prior to performing qPCR.

High Variability Between Technical Replicates

- Inaccurate pipetting of samples/reagents.
  Note: Properly calibrated repeating pipettes can be used to reduce variability.
- Insufficient mixing/vortexing of reagents.
  Note: Where indicated in the PIS, mixtures should be sufficiently mixed by vortexing.
- Insufficient volume of mixture.
  Note: If prepared correctly, the genomic DNA + qPCR Master Mix solution created in step C and the Genetic Assay mixes created in step D should have sufficient excess material to allow for loss during pipetting.
- Inaccurate loading of reagents into qPCR plate.
  Note: Take care when pipetting the reagents into the qPCR plate; loading variable amounts of DNA between wells will affect amplification dynamics.

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