Precipio, Inc.

TaqMan SNP Genotyping Protocol

ICE COLD-PCR Product Analysis with TaqMan SNP Genotyping Assays on the ABI 7500 Fast System

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TaqMan SNP Genotyping Guidelines (ABI 7500 Fast System)

IMPORTANT! To avoid cross contamination, set up TaqMan SNP Genotyping reactions in an area or room that is separated from DNA extraction and the designated pre-PCR areas. Good laboratory practices, especially cleaning the bench and pipettes after reaction setup, are also required to avoid contamination.
IMPORTANT! The following procedures are optional but highly recommended prior to PCR setup:

- Turn on UV light inside hood or a UV crosslinker.
- Prior to preparing Master Mixes, UV crosslink all empty Master Mix tubes. Also UV crosslink 1.7 mL tubes containing appropriate volume of Molecular Biology Grade Water needed for Master Mix preparation. These tubes should be UV irradiated for 10 min.

Make sure all work areas are prepared for analysis of low level mutations. This includes correct use of the PCR Workstation, dedicated pipettes, tips, 10% bleach solution and/or DNA Away™ solutions.
IMPORTANT! TaqMan SNP Genotyping assays need to be diluted to 20X in Low TE prior to performing the real-time PCR assay.

1. Preparation of Template DNA for Real-time PCR Analysis
   a. Make a 1:200 dilution by adding 199 µL water into the wells of a clean, labelled 96-well plate. Add 1 µL of ICE COLD-PCR product from the ICE COLD-PCR plate to the water in the corresponding wells. Maintain the original plate layout. Mix the wells by pipetting or by vortexing for ~10 sec. Spin down briefly.
2. Real-time PCR after ICE COLD-PCR
   a. Remove the TaqMan SNP Genotyping assay from freezer, thaw on ice, vortex each for 10 sec, and spin the tubes briefly.
   b. Remove the 2XTaqMan Master Mix from fridge, mix by inverting the tube and briefly spin it down. Keep it on ice.
      i. Thermo Fisher GTXpress 2X Master Mix (Catalog number 4401892) has been shown to work well in TaqMan analysis following ICE COLD-PCR amplification.

      NOTE: Keep TaqMan reagents in the dark until ready to use. Add them last to reaction mixtures. Once added, keep the plate in the dark until the plate is disposed of following run on the ABI 7500 Fast. Minimize freeze-thaw cycles.

   c. Prepare reaction mix for each target, using **Table 1** as a guide.

      Table 1: Reaction Mix calculations for each TaqMan assay.

Components	1X Reaction*
Molecular Biology Grade Water (µL)	6.25
TaqMan Master Mix, 2X (µL)	12.50
TaqMan SNP Genotyping Assay Mix, 20X (µL)	1.25
Total Volume Reaction Mix (µL)	20.00
Volume DNA added to reaction	5.00

**Multiply volumes in this table by the number of samples being tested.*

NOTE: A Master Mix volume slightly greater than this calculation will be required to allow for losses during pipetting.

d. Mix the reaction mix tube by pulse vortexing on low and spin the tube briefly.
e. Aliquot 20 µL of the reaction mix to the relevant wells of a MicroAmp Optical 96-Well Reaction plate. See **Table 2** for an example plate layout for 24 samples with controls (real plate layout dependent on original ICE COLD-PCR layout).

Table 2: Example template for running **24** samples with controls.

	1	2	3	4
A	SMP01	SMP09	SMP17	WT
B	SMP02	SMP10	SMP18	CTRL1_1per
C	SMP03	SMP11	SMP19	CTRL2_1per[a]
D	SMP04	SMP12	SMP20	CTRL3_1per[a]
E	SMP05	SMP13	SMP21	
F	SMP06	SMP14	SMP22	
G	SMP07	SMP15	SMP23	NTC1
H	SMP08	SMP16	SMP24	NTC2*

[a] As supplied in the specific ICE COLD-PCR assay
* NTC2 is a reagent control for the HRM assay. NTC1 is carried over from the ICE COLD-PCR amplification.

f. To appropriate wells, add 5 µL of diluted ICE COLD-PCR product from Step 1a or water (NTC2). Pipette-mix to ensure sample is well mixed into reaction mixture.
g. Place MicroAmp Optical Film over plate and seal. Spin the plate briefly to eliminate any air bubbles from the solution.

3. Set up of the ABI 7500 Fast to perform TaqMan SNP Genotyping assay

IMPORTANT! TaqMan_Template file is available for download at www.precipiodx.com to facilitate experiment file setup on ABI 7500 Fast System. It is recommended to use the files and track the user sample names with relevant setup table file sample names.

a. System Set Up
   i. Power on the 7500 Fast instrument.
   ii. Power on the computer.
   iii. Load the reaction plate into the 7500 Fast instrument.
   iv. Launch the 7500 software from the icon on the desktop
b. If TaqMan_Template file is to be used:
   i. Make a copy of the downloaded file. Open the copied file in Microsoft Excel. On the “Plate Layout” sheet, fill in or copy/paste the sample names in the 96-well plate area. Check the resulted plate layout to make sure it is as expected. Save the "7500 Template" sheet separately as Text (Tab delimited, .txt) file. Use this .txt file as TaqMan_template later.
c. In the 7500 Software v2.3, create a new run file for the TaqMan genotyping reaction by clicking **New Experiment**. In the Experiment Properties Window, input the desired experiment name and select