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SNPtype Assay Protocol

** Thaw and spin down all new plates for 30 sec; pipette up and down several times to mix (do not vortex)

SNPtype Pool for PreAmp

- Use multichannel to pipette assays into a clean trough, add 5 uM tris water, and transfer to a 1.7 ul tube and vortex.

STA Assays (plate)	2 ul each (x 96)
LSP Assays (plate)	2 ul each (x 96)
Tris H2O	16 ul
	400 ul

PreAmp

- Pipette 1.3 ul/well of DNA into "PreAmp plate" (dilution factor depends on source)
  - 1:2 dilution for Chinook and Steelhead: 5 ul dH2O + 5 ul DNA
- Make the PreAmp MM as follows:

	96x	100x	105x
Qiagen Multiplex MM	250 ul	260.4 ul (130.2 x 2)	273.4 (136.8 x 2)
SNPtype Pool (above)	50 ul	52 ul	55
H2O	75 ul	78 ul	82
	375 ul	390.4 ul → 3.8 ul/well	

- Add 3.8 ul/well of PreAmp mix to DNA, seal with thick tape and spin down
- PCR Program: SNP > Pre60-15

PreAmp Dilution

- Dilute PreAmp products (1:100) the day of the chip run **Do NOT transfer any DNA to NTC wells, just add 2.5 ul Tris H2O instead!
  - 1 ul PreAmp into a dilution plate with 99 ul 2uM Tris H2O
  - 2.5 ul/well of dilution into "Sample Plate"

SNPtype Assay Stock Plates

- Use the multichannel to pipette the ASP Assays and LSP Assays into a stock plate, add 2 uM Tris H2O (** Assays are well specific!)

ASP Assays (plate)	3 ul	**well specific
LSP Assays (plate)	8 ul	**well specific
Tris H2O	29 ul	
	40 ul/well	

Assay Plate Aliquots

- Use the multichannel to carefully pipette 1 ul/well from the Assay Stock Plate into a new plate. Assays are well-specific – change tips each time! Make assay plate aliquots before the day of the run to save time (store covered in freezer and check for evaporation before use).

Preparing the Assay Plate

- Make up the Assay MM as follows and add 4 ul/well into the pre-prepared Assay Plate (above); cover with thin sealing tape and spin down; pipette 4 ul into left side of chip

	105x	108x
2x Assay Loading Reagent	262.6 ul (2 x 131.4 ul)	270 ul (2 x 135 ul)
H2O	157.6 ul	162
	420.4 ul → 4 ul/well	432

Preparing the Sample Plate

- Make up the Sample MM as follows and add 3.6 ul/well into the pre-prepared Sample Plate (above); cover with thin sealing tape and spin down; pipette 5 ul into right side of chip

	102x	105x
Biotium 2x MM	315 ul (2 x 157.6 ul)	324 ul (2 x 162 ul)
20x SNPtype Sample Loading Reagent	31.6 ul	32.4 ul
60x SNPtype Reagent *(purple)*	10.6 ul	11 ul
ROX (50x) *(pink)*	3.8 ul	4 ul
H2O	6.8 ul	7 ul
	368 ul → 3.6 ul/well	378.4

Fluidigm Chip Protocol

Priming a Chip

Materials:

- Chip plate (above Fluidigm machines)
- Syringes (above Fluidigm machines)

- Unwrap chip plate, save box (note barcode #), check for scratches
- Pull back on syringe and remove stopper carefully to avoid any oil from ejecting
- Insert syringe into holes on either side of the chip so that the inner black bulb is pressed down, begin to inject oil, repeat with new syringe on other side
- Remove blue sticker on underside
- Insert chip into controller aligning angled corner with 1A
- Prime ⇒ User ⇒ Run Script
    - This takes about **18 mins**

After a Chip is Primed

- Eject the chip plate and take to Post-PCR room for loading with Assays and Samples
- Interface Cleaning message: pull red part out and wipe with alcohol wipes
- System Cleaning message: load a blank plastic tray from drawer beneath machines, follow directions on screen

Loading a Chip

Materials:

- Assay Plate
- Sample Plate
- Black chip coaster

- *Load Assays* into *left* side of chip plate with "Chip Loading" P10 manual 8 channel pipet set to 3.9 or 4 ul
    - Stop dispensing at the first stop, you can touch the sides
    - When you get to 7A move one row down for the remaining columns to offset sample loading
- *Load Samples* into the *right* side of the chip plate with pipet set to 4.9 or 5 ul
- Check for bubbles, use universal P200 tips or LTS P20 tips to remove them by hand
- Load chip plate into a Fluidigm controller with angled corner aligned to 1A: Load Mix ⇒ Run Script
    - This will take about **1 hr 30 mins**

When Program Finishes Transfer Chip to Fluidigm Thermocycler

- For SNPtype use the color touch screen machine on the right
- Align angled corner of chip to 1A
- Start ⇒ Continue ⇒ use arrow to scroll to SNPtype96x96dep
    - This will take about **2 hours**

Reading the Chip

- Turn on EP1 Program to warm it up (blue icon) (this takes 5 minutes)
- Use scotch tape to clean Chip carefully
- Insert Chip into large camera machine with angled corner aligned to 1A
- EP1 Software protocol:
    - Start New Run
    - Use barcode, unclick to change to (PreAmp letters)_SNPtype_barcode#
    - Probe 1 = FAM-MGB, Probe 2 = VIC-MGB
    - Saved in Runs folder on Desktop
    - All the rest of the defaults
    - Start Run (this takes 3-4 minutes to read)
    - Eject
    - Label Chip plate (ie. TXXX SNPtype) and color in two dots
- Fluidigm SNP Software (green icon)
    - Sample Set-up: New, OK, double click on wells you want to change to NTC; Mapping, M96…
    - Assay Set-up: New, OK, Mapping, M96…
    - Details View: Analyze, Expand too see all SNP results to check
    - Save!
- Log all info in your notebook
- Turn off EP1 software to let lamp cool down
- Turn off computer over the weekend. This will turn off the camera. If computer is off wait at least 30 minutes before imaging a chip