TaqMan® Protocol for Copy Number Variation (CNV):
Custom Protocol for *Hph* gene copy

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Materials Needed:		Source	Catalog#	Price
TaqMan® Genotyping Master Mix		Life Technologies	4371355	$690.00
Primers and Probes:				
Hpt F3	5' – ATACGAGGTCGCCAACATCT			
Hpt R3	5' – CGTCTGCTGCTCCATACAAG			
Hpt Probe3	5' – CCAACCACGGCCTCCAGAAGA	5'FAM 3' QSY	TaqMan Probe	$200.00
SvPCK F3	5' – ACTGTGGACGGCATAAAGG			
SvPCK R3	5' – CGTACTTGGTCGGATGGAG			
SvPCK Probe3	5' – ACGTTCTCGGCCTGCTTCGG	5'VIC 3' TAMRA	TaqMan Probe	$255.00
MicroAmp® Fast Optical 96-well Reaction Plate		Life Technologies	4346907	$ 35.00
MicroAmp® Optical Adhesive Film Kit		Life Technologies	4313663	$ 65.50
ABI real-time PCR machine		Life Technologies	Various	Variable
CopyCaller™ Software		Life Technologies		Free Download

Plate Layout Design:

1. Plan your plate keeping in mind the following:
    a. Samples will be run in triplicate
    b. Controls (NTC, WT+, Vector, Calibrator gDNA) will take up 12 wells
    c. Triplicates should be loaded horizontally (ie. A1, A2, A3)
    d. One plate will hold 28 samples and 4 Controls
2. Use this layout to

Reaction Assembly:

1. Assemble the master mix according to the following recipe: (Try to keep probes out of the light)

	Rxn Vol	10.0
	# of Rxns	1.0
	mqH2O	0.3
	Genotype MM Buffer	5.0
F-primer1	HptF3	0.9
R-primer1	HptR3	0.9
Probe1	Hpt3	0.025
F-primer2	SvPCKF3	0.9
R-primer2	SvPCKR3	0.9
Probe2	SvPCK3	0.025
	Total:	9.0

2. Mix well and aliquot 30ul of MM into the appropriate # of 0.5mL microtubes. You should have the same number of tubes/aliquots as the number of samples + controls.
3. Add 3ul of the appropriate sample or control to each tube. *Make sure you add 3ul of mqH20 to the NTC.

4. Mix well and load 10ul from each tube into the corresponding 3 wells in your plate layout. Keep plate in a rack to avoid exterior contamination that would affect the optical quality of the plate.
5. Apply Optical Adhesive Film using the tool in the film kit to ensure a good seal. Spin down plate briefly to collect samples.

Run Setup:

1. Turn on ABI Step One Plus real-time PCR machine. (Rocker switch on back left bottom, to the left of the power cord).
2. Open ABI Step One Plus Software. (Continue with software even if it can't find machine, it will eventually)
3. In the Experiment Menu on the left side of the screen, in Experiment Properties select: Advanced Setup.
    a. In Experiment ID section: Experiment name, etc.
    b. In Instrument section: Step One Plus Instrument (96 wells)
    c. In Experiment Type section: Quantification-Standard Curve
    d. In Reagents section: TaqMan Reagents
    e. Select Standard ramp speed (2 hr run)
4. In the Experiment Menu on the left side of the screen, in Plate Setup select
    a. Define Targets: click Add Saved Target, scroll to and select

Target	Reporter	Quencher	Date
Pck	VIC	TAMRA	Aug 22, 2014
Hyg	FAM	None	Aug 22, 2014

    b. Define Samples: click Add New Sample for each sample on the plate and type in the sample names
    c. Click Assign Targets and Samples. Under View Plate Layout, click Show in Wells-> Sample Color. Highlight all samples and assign both targets to every sample well, then highlight all replicates of each sample and assign samples to wells.
5. Save the run in the appropriate folder.
6. Put plate in PCR machine.
7. In the Experiment Menu on the left side of the screen, in RUN:
    a. Highlight the entire plate and click Start Run
8. When run is complete, remove plate and turn off PCR machine

Analysis: (When run is complete)

1. In the Experiment Menu on the left side of the screen, in Analysis: highlight all wells and click Analyze.
2. Look at Amplification Plot to confirm expected amplification, especially in controls. Adjust thresholds for each target if necessary in Log Graph Type View. Reanalyze and save.
3. Click Export:
    a. Export Results as a .txt file to your "public" folder. This is the data and format that CopyCaller analyzes. You can export many other forms of data and formats as well.
4. Open CopyCaller on your desk computer, and click Import. Browse to saved .txt file.
5. Highlight the imported run and click Analyze. Use your Calibrator gDNA control and enter appropriate copy number.
6. In right-hand table, omit outliers (one per sample, max). In lower table adjust samples that appear in bar chart.
7. Save analysis
8. Bar chart can be copy/pasted into Powerpoint, Word, etc.