TECHNICAL NOTE

Azure Cielo: SNP Genotyping/Allelic Discrimination

Introduction

Genotyping is the process by which genetic variations can be identified among individuals within a population. SNPs (single nucleotide polymorphisms) are the most frequently occurring type of variation, defined as a single point base difference in the genomic DNA that can be found in more than 1% of the population. To date, there have been over 9 million SNPs reported in public databases. Although not always located in coding regions of the genome, SNPs can result in altered gene and protein expression or altered protein structure or function and can be associated with various disease states. Thus, SNPs can be used as molecular biomarkers in genetic studies looking closely at disease and drug responses.

Genotyping typically requires the amplification of allele-specific products containing SNPs of interest, followed by SNP detection for genotype determination. In this technical note, we will detail SNP genotyping using the Azure Cielo qPCR instrument. We will detect 2 SNPs using a commercially available kit. Using a 5'-nuclease allelic discrimination assay, we will amplify the target from a DNA sample using a standard primer pair, followed by detection of each SNP with two differently labeled probes (FAM or VIC reporter molecule). Detection of each SNP will be determined based on increases in either FAM or VIC dye fluorescence and the genotype assigned based on which SNP is present. Increases in only FAM or VIC will indicate homozygosity for either the FAM- or VIC-detected alleles; while increases in both FAM and VIC will indicate heterozygosity at this SNP location.

Materials and Methods

- High quality DNA extracted from samples of interest
- Positive and negative control (NTC) samples
- Taq Man genotyping master mix
- 40X Taq Man SNP genotyping assay, diluted to 20X
- Molecular grade water
- qPCR, white, opaque, low-profile 96-well plates
- Optical sealing sheets
- Benchtop plate spinner
- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- Instrument: Azure Cielo 3 or 6 Real Time PCR System
- Data Analysis: Azure Cielo Manager Software (v. 1.0.6.4 or newer)

1. Count the number of samples that will be included in the run. Be sure to include the positive control, negative control, and non-template control in the count.

   # of samples + 3 (controls) = **Total # of samples** x 3 (triplicate) = **# of reactions**

2. Prepare the master mix for all reactions in a microcentrifuge tube, excluding the template. Prepare a volume 10% greater than calculated to allow for pipetting loss. Spin contents of tube down in benchtop microcentrifuge.

	X 1	X # of reactions + 10%
Taqman Master Mix (2X)	12.5 µL	
Taqman Assay Mix (20X)	1.25 µL	
mH2O	10.25 µL	
Template	1 µL	—
	= 25 µL	

3. Add 24 µL of the prepared master mix to each well.
4. Add 1 µL of the desired template to each well.

   See example plate map below:

   - 3 wells each of each positive control (PC 1/1, PC 1/2, PC 2/2)
   - 3 wells of non-template control
   - 3 wells each of each sample
5. Cover plate with optical sealing film and briefly spin plate in benchtop microplate spinner.
6. Load plate into Azure Cielo qPCR instrument.
7. Program the following protocol into the instrument:

Step 1	95°C for 10 minutes	
Step 2	95°C for 15 seconds	Repeat steps 2 and 3, 39 times
Step 3	60°C for 1 minute	

8. Run the protocol.
9. Following the completion of the run protocol, extract the AZD file and open it in the Azure Cielo Manager software.
10. Ensure that the Experiment Mode is specified as Genotyping.
11. Label the plate map according to the controls and number of samples included (see plate map below for reference). Each well should have a Well Type, Sample, and both FAM and VIC dye labels.
12. Navigate to Analysis > Graphical Displays. Click on the Allelic Discrimination Plot tab.
13. Check that samples are labeled correctly per positive controls (PC 1/1, PC 1/2, and PC 2/2) and non-template control.
14. Check that assigned genotypes cluster nicely together; otherwise, reassign genotypes by right clicking on dot on allelic discrimination plot. Genotypes may need to be reassigned if genotype call does not align with clustering pattern.
15. Navigate to Analysis Data tab to see genotype calls in table format.

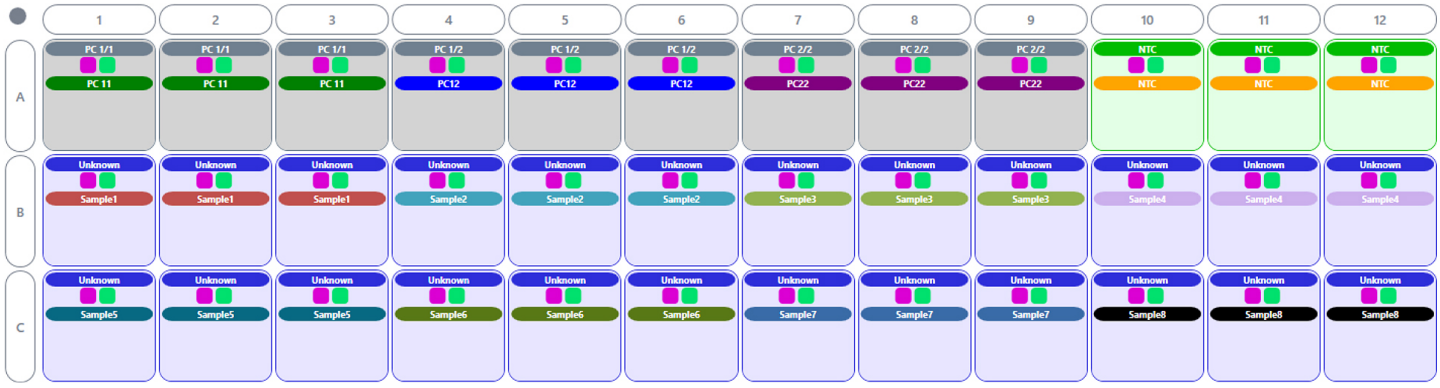

Figure 1. Sample Plate Map with all controls.

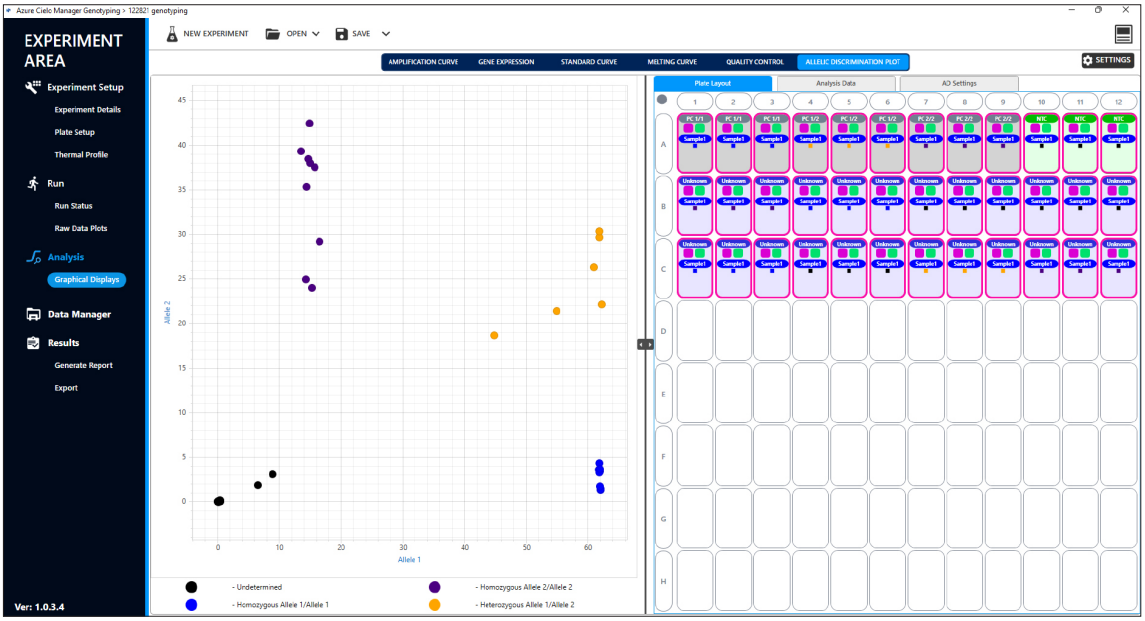

Figure 2. Allelic Discrimination Plot with automatic calls.

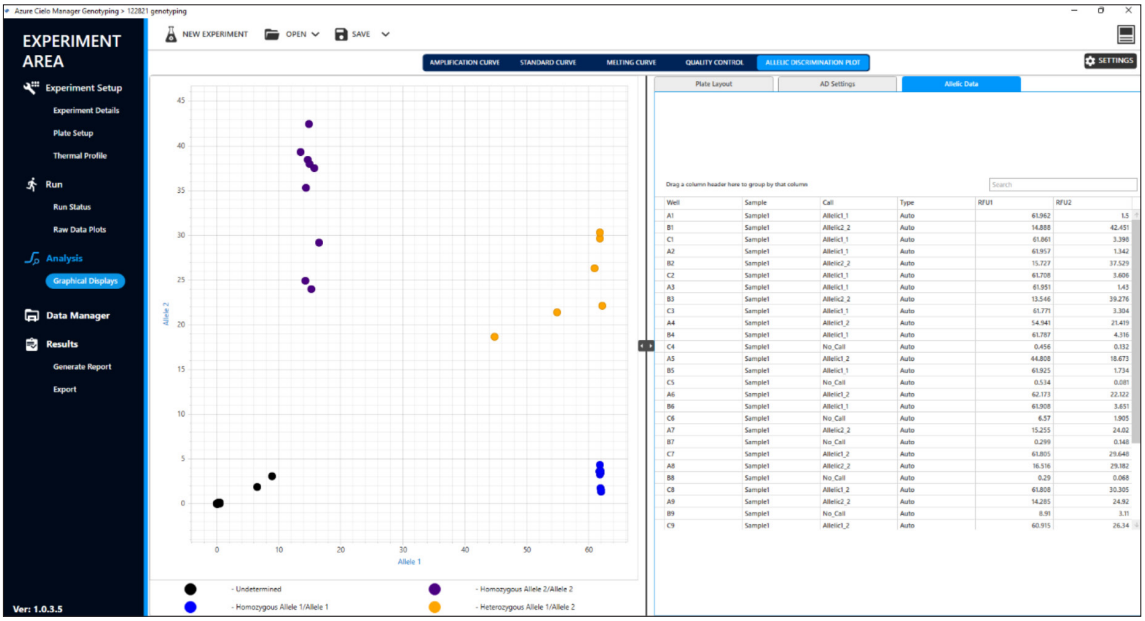

Figure 3. Allelic Discrimination Plot with Analysis Data table.

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