Program of Genomic and Nutrition Laboratory
UCLA Department of Epidemiology
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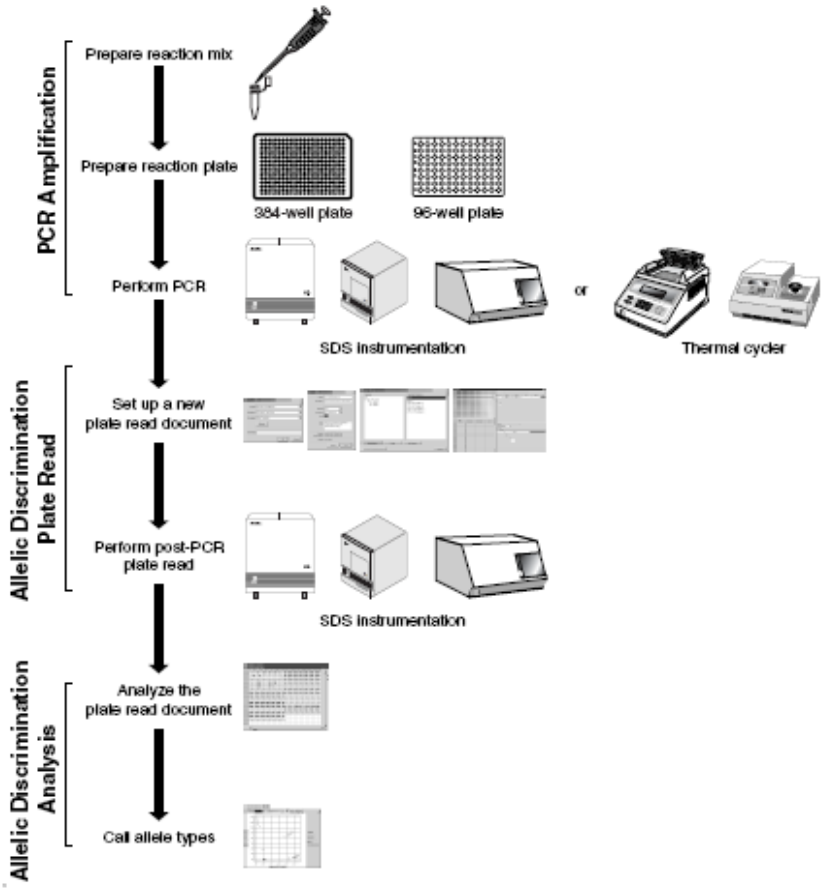
10/30/06

The Taqman Assay for SNP genotyping

Procedure flowchart



Step1: Prepare the samples

1. Prepare DNA plates
   - Label plates including the following information: SNP ID(rs #), gene name, and SNP#
   - Scan plates into the log file include the following information:

GENE	CRP	CRP	CRP
no#	1	2	3
date	2006/7/26	2006/7/26	2006/7/27
rs#	1470515	2369146	2808629
abi#	C_7479355_10	C_26627365_10	C_177485_10
P_1	A302LNR4	A302LNR5	A302LNOX
P_2	A302LNAI	A302LNAJ	A302LSA7
P_3	A302LPNJ	A302LO33	A302LPMH
Total rx(ul)	5	5	5
Anneal Temp	60	60	62 1min20sec

2. Prob preparation
   - Make sure the SNP ID and Assay ID on the tube is right for the SNP
   - Take assay probs out of the freezer

3. Prepare for Taqman assay mix
   - Prepare a clean sterilized 50 ml centrifuge tube
   - Prepare the Taqman assay mix for 5 uL reaction volume each sample (make 5% more overall)

	1X	4248X (for 4032 samples in WHI)
Master Mix	2.5	10620
H2O	2.375	10089
Probe (each)	0.125	531
Total	5	21240

4. Aliquot 5ul Taqman Assay mix for each sample

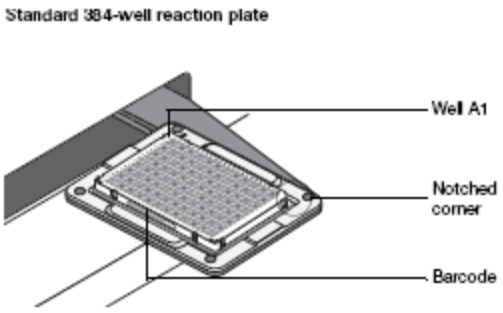
5. Seal plate with the Optical Adhesive Cover

6. Spin plate for 1000rpm, 1 minute. (Important!!)

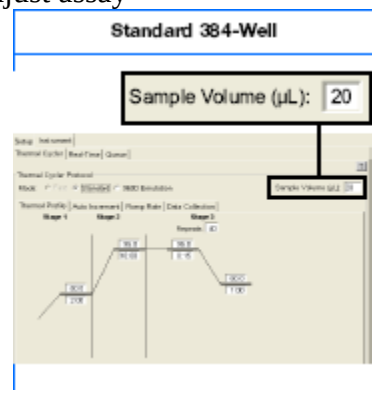
Step 2: Perform amplification run

Two ways to perform PCR amplification run:

1. Absolute Quantification by using Taqman 7900 HT
   1. In SDS software, select "Absolute Quantification"
      - Use "384 well SNP genotyping" as template
      - Scan plate barcode
      - Place plate as the figure shows



- Adjust assay



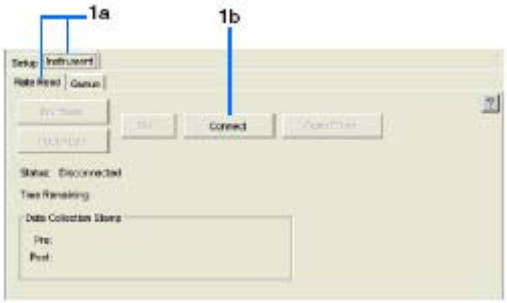
  - For assays require 60°C annealing temperature 1 minute
  - For assays require 62°C annealing temperature 1 minute 20 sec

2. PCR
   - Place cover on each sample plates then perform PCR amplification run
     i. Taqman60- For assays require 60°C annealing temperature 1 minute
     ii. Taqman62- For assays require 62°C annealing temperature 1 minute 20 sec

STEP3: Allelic Discrimination

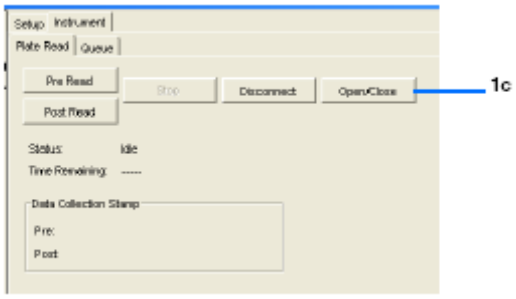
In SDS software

1. Open a new document
2. Choose “Allelic Discrimination”
3. Use “SNP score” as template
4. Scan plate barcode, then hit OK
5. In the Allelic Discrimination (AD) plate document, select the **Instrument** > **Plate Read** tabs.(1a)

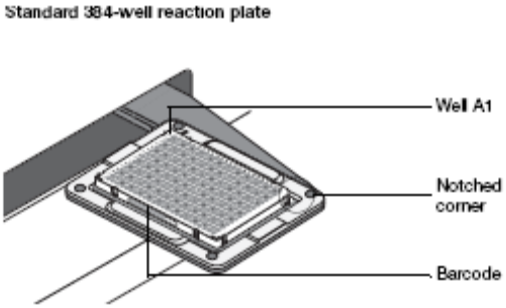


6. Click **Connect** to connect the plate document to the instrument. (1b)

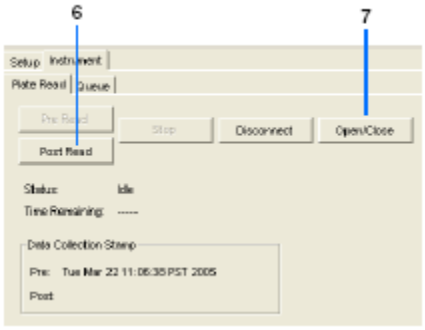
7. Click **Open/Close**. The instrument tray rotates to the OUT position.



8. Place the prepared reaction plate into the instrument tray as shown.



9. Click **Post Read** (6). The instrument tray rotates to the IN position and the instrument performs the run. Save file.



10. As the instrument performs the run, it displays status information in the Plate Read tab. After the run, the status values and buttons are grayed-out, the Analysis button is enabled ( ), and a message indicates whether or not the run is successful.

11. When the run is complete, click **Open/Close** (7) to eject the reaction plate.