Program of Genomic and Nutrition Laboratory
UCLA Department of Epidemiology
Prepared by Yuko You (yuko@ucla.edu)
10/30/06

The PCR-RFLP Assay for SNP genotyping

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. Primer 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 PCR assay mix
   - Prepare a clean sterilized 50 ml centrifuge tube
   - Prepare the PCR assay mix for 10 uL reaction volume each sample (make 5% more overall)

	1X
Master Mix	5
H2O	4.850
Primer (each)	0.075
Total	10

4. Aliquot 10ul PCR 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

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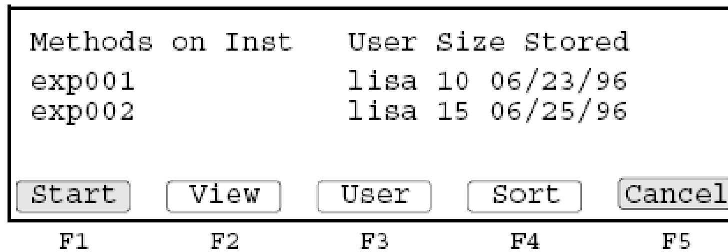
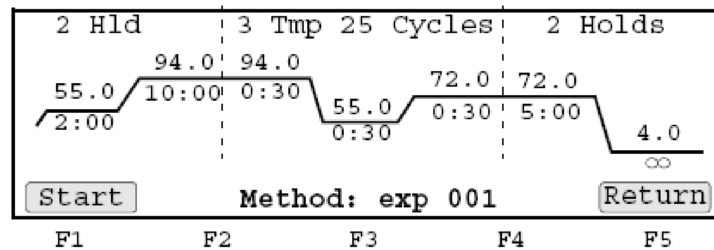
(See Appendix for PCR 9700 quick manual)

- Place cover on each sample plates then perform PCR amplification run
- Select method from menu or create program manually

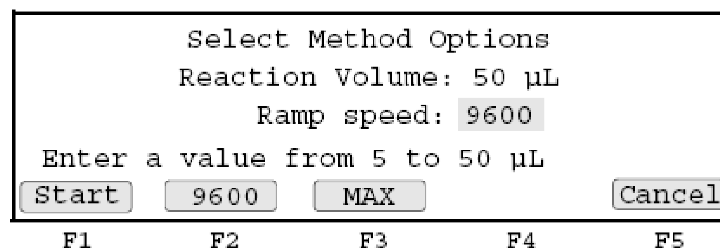
1. Select from menu
    - From the **Main** menu, press **Run**.



- From the **Stored Methods** screen, press **View**.
- The **View Method** screen appears. The screen shows all the parameters of the method you selected.

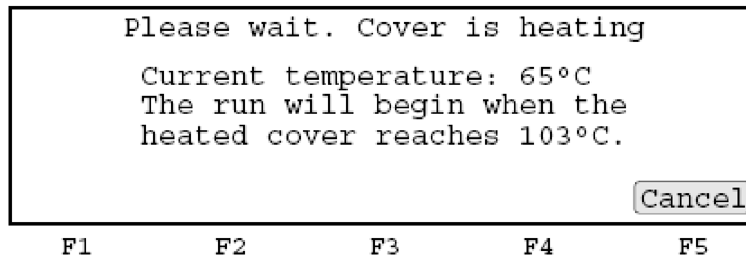


- After reviewing PCR and post-PCR parameters of a stored method, make sure it is the right program, and you can press **Start** to start the method.(If not, Press **Return** and return to the **Stored Methods** screen.)
- The **Select Method Options** screen appears.

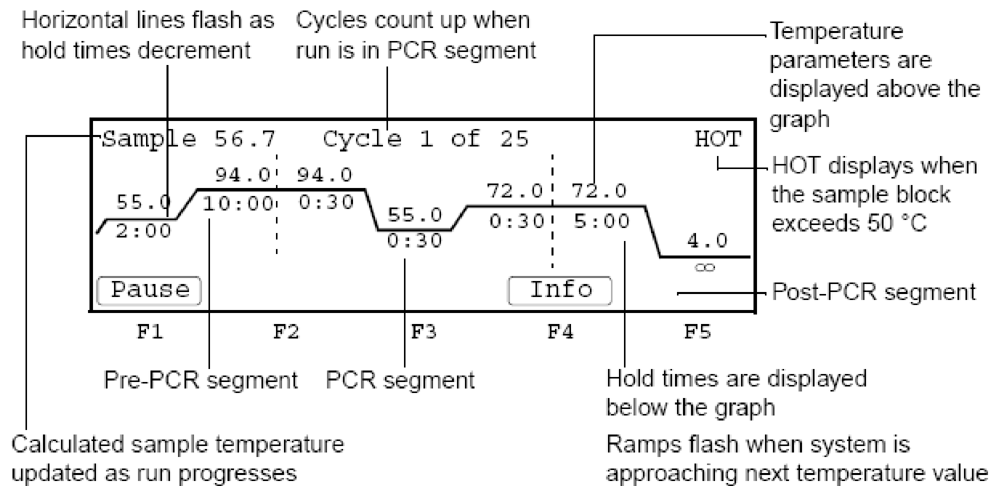


Adjust reaction volume to 10ul, then press **Start** to start a run

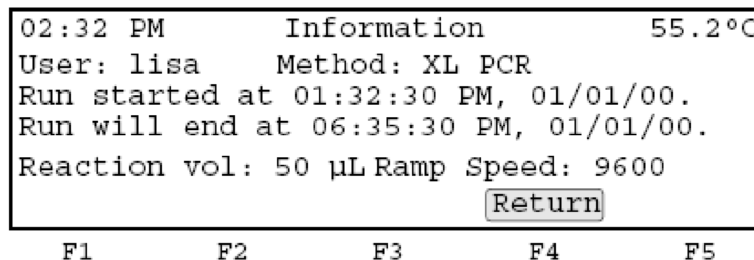
- If the temperature of the heated cover is less than 103 °C, this message, “Cover is heating” appears.



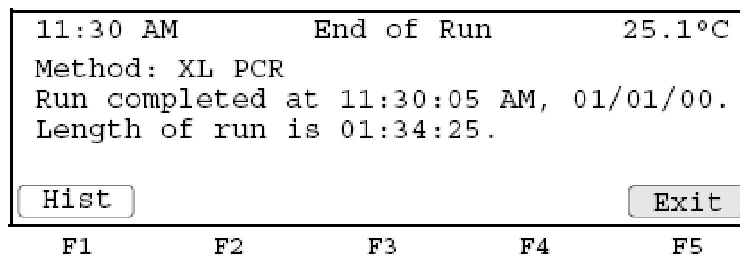
- When the heated cover reaches 103 °C, the **Run Time** screen displays and the method you selected starts running.



- You can view the Method Information screen during a run by pressing Info. Press **Return** to return to the Run Time screen.

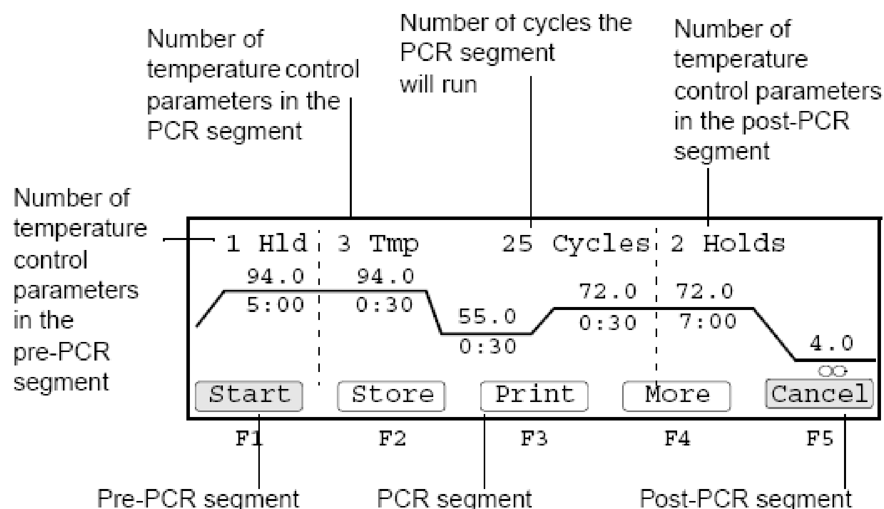


- At completion of a run, the instrument beeps and the End of Run screen appears. From the End of Run screen you can perform the same functions as you can from the Stop Run screen.



2. Create program manually

- From the **Main** menu, press **Create**. The create screen appears.
- The GeneAmp PCR System 9700 comes with a default PCR thermal profile called a method. The create screen displays this default method. You can run the default method shown above, or use it as a template to create a customized method.



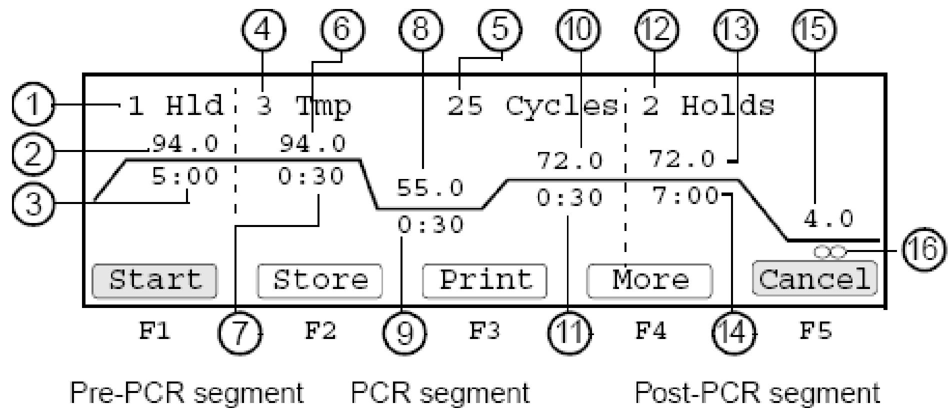
- From the **Create** screen, you can take the following action:

If you want to...	Then...
start running the default method	press **Start**.
store the method under a user's name	press **Store**.
print a record of the method parameters	press **Print**.
enter temperature control parameters	enter the information on the create screen and create a new method.
display more functions for modifying methods	press **More**. **Note** The More function only displays when you select a time or temperature parameter. For detailed information on using the More function to modify cycles, see "Modifying Cycling" on page 5-11.
return to the previous screen	press **Cancel**.

- When you enter temperature control parameters, you define values for parameters in each of the three segments of a method: pre-PCR, PCR, and post-PCR.

- To enter temperature control parameters:

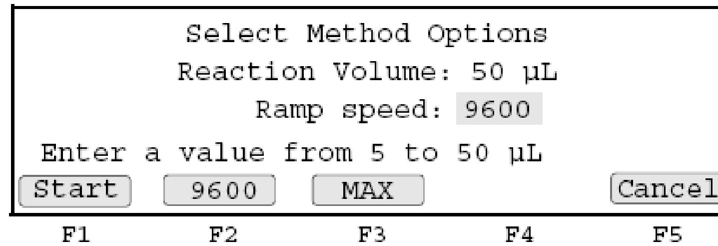
Step	Action
1	On the **Create** screen, select a field. When you first display the Create screen, the **Hld** field is highlighted.
2	Use the numeric keys to enter values.
3	Press **Enter** to accept a value. The next field is then selected in the order shown in Figure 5-1.



Number	Description
1	Number of pre- PCR holds
2	Pre-PCR temperature parameter
3	Pre-PCR hold time parameter
4	Number of PCR segment temperatures
5	Number of cycles for the PCR segment
6	PCR segment temperature parameter
7	PCR segment time parameter
8	PCR segment temperature parameter
9	PCR segment time parameter
10	PCR segment temperature parameter
11	PCR segment time parameter
12	Number of post-PCR holds
13	Post-PCR temperature parameter
14	Post-PCR hold time parameter
15	Post-PCR temperature parameter
16	Post-PCR hold time parameter

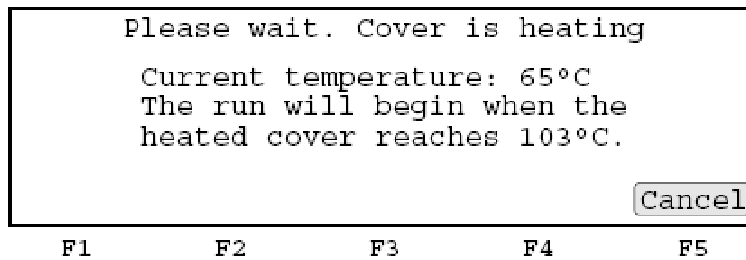
(For details of programming, see appendix)

- After reviewing PCR and post-PCR parameters of a stored method, make sure it is the right program, and you can press **Start** to start the method.(If not, Press **Return** and return to the **Stored Methods** screen.)
- The **Select Method Options** screen appears.



Adjust reaction volume to 10ul, then press **Start** to start a run

- If the temperature of the heated cover is less than 103 °C, this message, "Cover is heating" appears.



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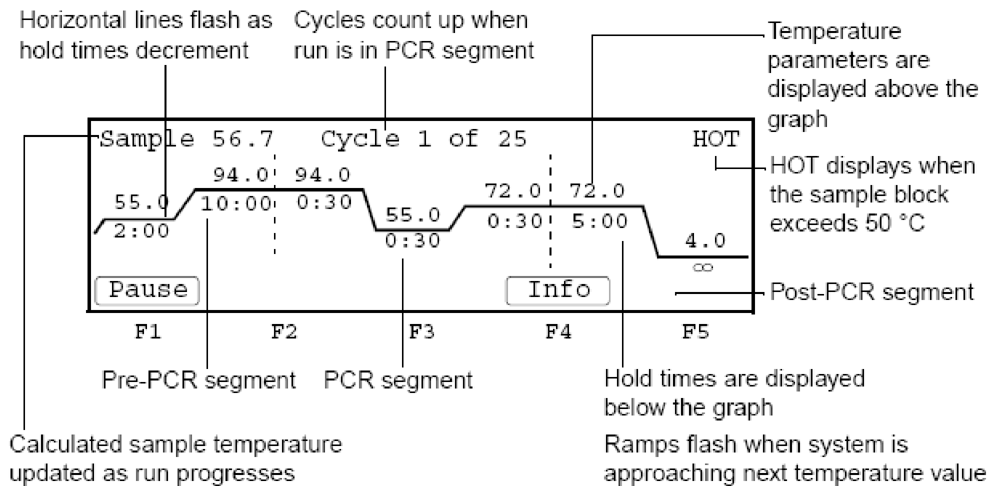
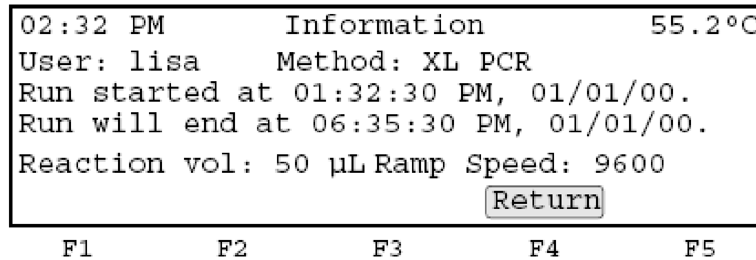


- You can view the Method Information screen during a run by pressing Info. Press **Return** to return to the Run Time screen.



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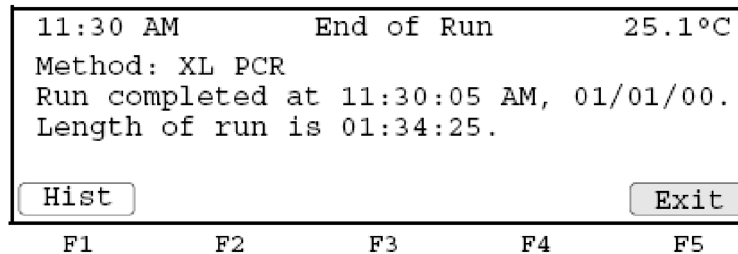
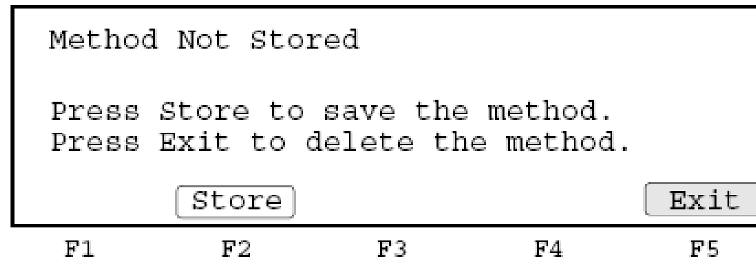


- If you attempt to exit the End of Run screen before storing the method, the Method Not Stored screen displays.



- The following table lists the actions you can take.

If you...	Then press...
want to store the method	**Store**.
do not want to store the method	**Exit**. **Note** The **Method Not Stored** screen appears for a few seconds before the Exit key is recognized. This prevents you from losing a newly created method. You return to the top level screen and the method you created is not saved.

Step 3: Post-Run Electrophoresis

By pre-cast gel or agarose gel

1. pre-cast gel

- Cut the pouch open on one end and remove the gel. **Exercise caution when removing the gel as the pouch contains a small amount of buffer that may contain ethidium bromide.**
- Leave the gel in the tray and place directly on the chamber platform.
- Cover the gel with the appropriate buffer (1X TAE or 1X TBE) to a ***depth of 2 mm-3 mm*** over the gelsurface.
- Place the appropriate size Cambrex TruBandAnchor over the tray so that the white gaskets fit snugly on the flanges on the short ends of the tray. Position the anchor so that both the top and middle tiers of wells are exposed.

LOAD
with TruBand Anchor

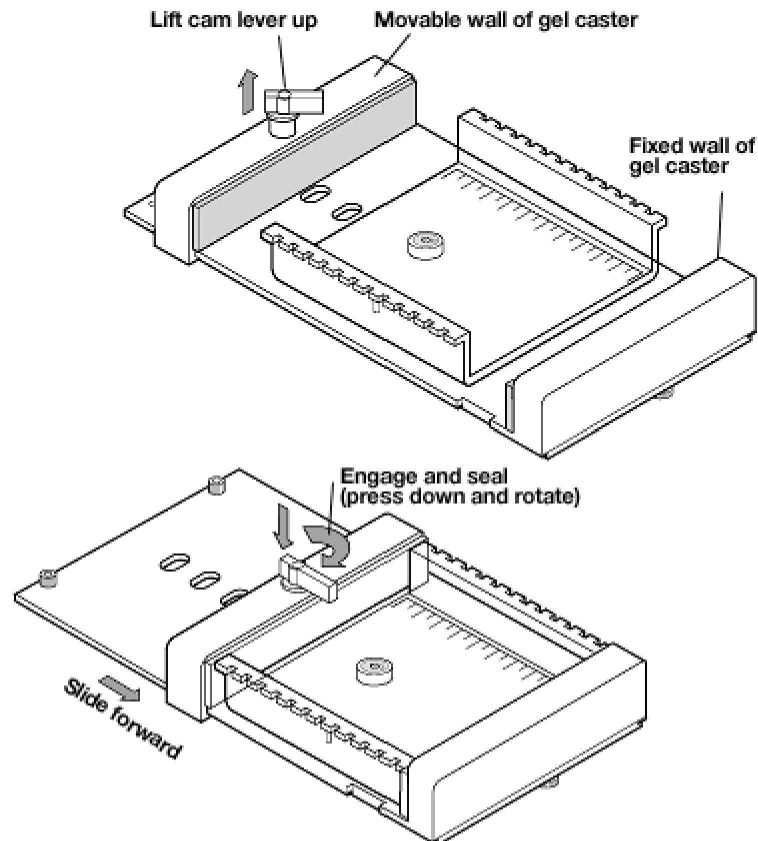
- Add buffer until the level is even with the top of the anchor. **Very Important! Do not flood buffer over the top of the anchor.** It is better to have the buffer level slightly below the top of the anchor than even with the top of the anchor.
- Load DNA samples following standard procedures. The gels are configured for alternate or consecutive-well loading using an 8 or 12 tip multichannel pipette.
- **Shift the TruBand Anchor so that it is centered over the gel and all wells are completely covered before beginning electrophoresis.**

RUN
With Truband Anchor

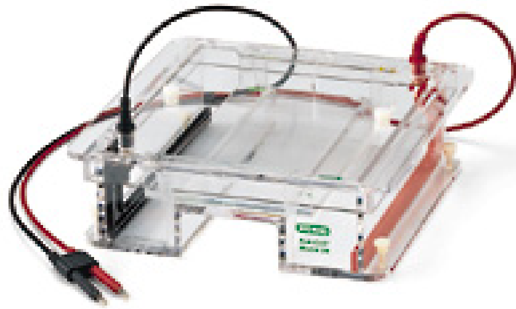
- Electrophorese the gel at 5-10 V/cm interelectrode distance, until the bromophenol blue is about 1 cm from the bottom of the gel or from any row of wells.
- Remove the anchor, and remove the gel tray from the chamber. Carefully remove the gel from the tray and place on the UV light box. Photograph as usual. If the smaller DNA fragments are not detected, briefly stain the gel (10 to 15 minutes) in 0.5 µg/ml ethidium bromide.

2. Agarose gel

- Prepare the gel, using 200 ml electrophoresis buffer (TBE or TAE, 1 X) and electrophoresis-grade agarose (1.0%=2g, 2%=4g, 4%=8g) by melting in a microwave oven until consistent.
- Cooling at 50ºC, mixing with ethidium bromide (0.5 µg/ml= 10ul) and then pouring into a sealed gel casting platform, and inserting the gel comb.



- After the gel has hardened, remove the seal from the gel casting platform and withdraw the gel comb. Place into an electrophoresis tank containing sufficient electrophoresis buffer (TBE or TAE; 1 X) to cover the gel (~ 1 mm).
- Prepare DNA samples with an appropriate amount of loading buffer (10 µl PCR product, 3 µl loading buffer) and load samples into wells with a pipettor. Be sure to include appropriate DNA molecular weight markers.
- Attach the leads so that the DNA migrates to the anode or positive lead and electrophorese at 100 V/cm gel for 40 min. Red to red, black to black. You can confirm that current is flowing by observing bubbles coming off the electrodes. **DNA will migrate towards the positive electrode, which is usually colored red.**



- Turn off the power supply after 40 minutes or when the bromophenol blue dye from The loading buffer has migrated a distance judged sufficient for separation of the DNA fragments.
- Photograph the gel on a UV translluminator.

Step 4. Scoring

Sample scoring for the results from CAPN10 19 RFLP