

Running 12 inch well-to-read gel on ABI 377

After preparing gel (see gel prep protocol)...carry it down to the 377 room.

- 1) Make sure sequencer is on and REBOOT computer.
- 2) Make sure glass around the laser is as clean as it can get. Use kimwipes and H₂O to clean it to the greatest degree possible.
- 3) Go ahead and remove comb from gel. Carry gel from prep area over to 377.
- 4) Check to see if bottom tray is clean, if not, empty and rinse it out and replace.
- 5) Choose FILE-> NEW
- 6) Choose Pre-run module GS PR 12D-2400
- 7) Choose Run module GS Run 12D-2400
- 8) Choose **matrix file Quail D, Well to read distance=12, Collect Time=2.0**
- 9) Now, run plate check. To see what is going on choose WINDOW->STATUS.
If you see large spikes in the viewing area of the window...you need to re-clean glass.
- 10) To re-clean glass, choose INSTRUMENT->CANCEL RUN. Re-clean plates.
- 11) Run plate check again and check for spikes in output.
- 12) If no spikes, go ahead and proceed. If spikes, clean plates again, plate check again.
- 13) Prepare 1500 ml 1X TBE (150 ml 10X TBE, 1350 dH₂O).
- 14) Carry TBE and top tray over to machine. Add TBE to top tray so that it just flows over the lip of the comb area. Add TBE to bottom tray so that it just spills into catchment device on the front of the tray.
- 15) Grab syringe and dye, fill syringe w/ 5 ml or so of dye and run across lip of area where comb was. Check to see if all of dye moves between plates. Run pipet tip across area to free it of any acrylamide that may be in the wells. Once this is done, go ahead and insert sharks-tooth comb into well area until teeth reach the bottom of the well (do not insert into gel).
- 16) Go to PAPER beside RUN MODULE, click it, and make sure VOLTAGE=750, CURRENT=60, WATTS=200.

- 17) Now, choose PRE-RUN, click on WINDOW->STATUS to see what is going on.
- 18) While gel is heating up, choose NEW->GENE SCAN SAMPLE SHEET, and fill out information for each well as it will be entered.
- 19) Once Gel temperature, as indicated by STATUS window, reaches 45 C, spin 96-well plate down and put it on the heating block for 5 minutes at 95 C. Remove samples after 5 minutes, put immediately on ice, and get ready to load.
- 20) Hit PAUSE to pause pre-run.
- 21) Load odd-numbered samples in odd-numbered wells. Flush multi-channel loader with warm dH₂O. Make sure loader is not in open position and draw 2 μ l of sample into loader. Hold loader over paper towel and push out enough sample to see it on the tips of the loader. Blot on paper towel. . Open loader to load lanes. Close after loading lane. Flush again. Continue loading lanes until all odd-numbered lanes are loaded.
- 22) Run gel for 2 minutes. After 2 minutes, PAUSE run and load even-numbered samples.
- 23) TERMINATE PRE-RUN and begin RUN.