

## ABI 3730 Instructions for Genotyping

1. Sign up for run at: <http://imbi.bio.indiana.edu/>  
Choose “genescan signup”. Click the “submit” button to schedule a time to run. Allow 1h per plate.
2. Set up PCR reactions (supported primer dyes = NED, 6FAM, PET, VIC; HEX may be o.k.,too)
  - use flexible 96-well PCR plates
  - seal with clear plastic plate adhesive
3. Dilute reactions with dH<sub>2</sub>O (1:20), into flexible 96-well PCR plates
  - may need to dilute more, given sensitivity of 3730, or not at all (try range 1<sup>st</sup> time)
  - if using HEX-labeled primers, may have to dilute less to overcome wavelength difference between HEX and VIC
  - seal with clear plastic plate adhesive
4. Set up fragment analysis plate
  - use non-flexible PCR plate (ABGene #AB-1100)
  - mix 890ul dH<sub>2</sub>O + 10ul LIZGS500 ladder (ABI #4322682) in Eppendorf tube
  - add 9ul ladder mix per well + 1ul diluted PCR reaction
  - seal with plastic or foil seal
5. Denature 95°C for 5 minutes, immediately place on ice.
6. At DNA facility, spin plate in Beckman fuge, then replace seal with rubber septum
7. Place plate in black base, snap white cover over top (make sure it snaps on!).
8. Open right drawer on 3730, open door on right, and place plate on stack (notch in back right corner)
  - plates are taken from the bottom first.
  - when the plate is done, it will be moved to the left stack.
9. On computer, click on Run Scheduler.
  - In the “Add Plate” box, enter a plate name, click enter, choose “yes”.
  - In the “New Plate Dialogue” window:
    1. Change application to “Genemapper generic”
    2. Choose “septa”
    3. Enter your name for “Owner” and “Operator”
    4. Click OK
10. In Plate Editor:
  - In Sample Name, can enter marker name, then fill down
  - In Results Group, enter CISAB\_LAB (fill down)
  - In Instrument Protocol, enter Genemapper (fill down)
  - Click OK
11. Click green arrow on top left of screen to initiate run
12. When run is complete:
  - retrieve plate from drawer, rinse septa for reuse
  - retrieve Data online at <http://imbidata.bio.indiana.edu/>
  - find results in “Data” folder, then in “CISAB” folder.
  - click on plate name, ftp to your computer.
  - analyze results on Genemapper