## How to prepare a plate with microsatellites for the sequencer

Steffi Kautz - Field Museum Moreau Lab www.moreaulab.org

(August 2011)

After your PCRs with the microsatellite primers is complete, you will prepare a plate for the sequencer using the PCR products. Ideally, you will combine/multiplex 4 different primers (labeled 6-Fam, Ned, Pet, Vic) and add the size standard. Here are the instructions of how to prepare and load a plate of microsatellites.

**Before you begin:** Generate a file for fragment analysis. Get the template and save it under your name. Add the samples names (including primers).

## **Get started**

- 1. Get a grey septa specifically for loading onto the 3730 sequencer from the DNA Disco Lab, clean with ddH<sub>2</sub>O, dry really well with paper towels.
- 2. Get a sequencing plate from the 2nd drawer on the Moreau lab space, take off the old label with Ethanol and a kimwipe, and relabel with your plate's name (remember you can reuse these 96 well plates).
- 3. Place both the plate and the septa under the UV.
- 4. Get your 4 PCR reaction plates out of the PCR machines or the fridge, quickly spin all of them down in the centrifuge that holds plates, and keep on lab bench until needed.
- 5. Get two 500  $\mu$ l tubes filled with HiDi (from the freezer with the lab stocks, HiDi is free in our lab, so no need to sign out). Let sit in a rack (one that hold 80 samples) at room temperature to defrost.
- 6. Get two clean 500 μl tubes and label them "HiDi + LIZ"
- 7. Once the HiDi is defrosted pipet 450  $\mu$ l into each of the tubes that you labelled "HiDi + LIZ". Place these tubes in the rack and throw away the two tubes that the HiDi was in if they are empty.
- 8. Add 11  $\mu$ l LIZ to each tube (so it will be **450 \mul HiDi** and **11 \mul LIZ** in each of the 2 tubes), mix by vortexing very briefly and spin down quickly in the little benchtop centrifuge (these hold 6 samples).
- 9. Get your plate (labeled) and septa from the UV.
- 10. Add **9.2**  $\mu$ I of the HiDi + LIZ to each of well of the empty plate using the electronic pipet, spin down again quickly in the plate centrifuge.
- 11. Now it's time to load your samples into the plate which has the HiDi + LIZ mixture in each well. Be sure that you place all plates in the same orientation the plate that you will load onto the sequencer (well A1 should be on the upper left corner). You have to pipet  $0.5~\mu l$  of each sample into plate. Use the 8-channel multipipet for this. It can be difficult to get  $0.5~\mu l$  into the pipet tips. Check each tip carefully. I always pipet the very small amount of the PCR sample directly into the HiDi solution. So I go down all

the way to the bottom of the well with the pipet tips. If you do this and still see a tiny amount left in the pipet tips when you take them out of the well, this will be fine.

- 12. After you are done with the first PCR plate, seal the PCR plate back up, and get the second PCR plate.
- 13. Continue pipetting the PCR products from the PCR plate into the sequencing plate (labelled). Proceed exactly as in point 11 above, then continue with the third and fourth plate of PCR products.
- 14. Once you are done loading the PCR samples into the sequencing plate, quickly centrifuge the plate.
- 15. Get a pretty big piece of aluminum foil, close the PCR plates with the white silica mats, stack the four PCR plates on top of each other, wrap in aluminum and place in the fridge. Only keep these until you know your PCRs worked in the sequencing machine.
- 16. Get a box with ice. Then, to denature your sequencing plate: Find a PCR machine (an empty Biorad machine that will hold 96 samples, not the eppendorf machines), hit "instant", select 95°C, deselect heated lid. Wait for the PCR machine to heat up to 95°C, then place your sequencing plate in the machine, leave the lid open, wait 5 minutes, then place your plate directly on ice for 5 minutes.
- 17. Now load onto the sequencer: Sign up for a whole plate.
- 18. Find an empty 3730 sequencing gasket (black and white thing), place your plate in that in the correct orientation (corner lined up).
- 19. Load your plate into the sequencer, make sure you place it in the "In" stack in the correct orientation (watch for the corner).
- 20. Upload your file. Go to "Plate manager" on the left, "Import", then navigate to the USB device that the file is on. Hit "Open". Then, go to the "Run scheduler", "Search a...", select "Advanced", select "Plat name" and "contains", type something that was included in your file's name. Hit "Search". Then, your plate should come up. Select it, hit "Add" and "Done". If this is the first run of the day, you will need to hit the green play button on the top left corner of the window (after you have changed the buffer). If this is not the first run of the day, your plate will be run automatically.
- 21. If the buffer has not been changed, change the buffer. If you do not feel comfortable in doing this, always ask the lab manager or someone who knows.

## Master Mix for microatellites

Reagent	For 1 sample	For 50 samples (prepare twice)
HiDi	9 μΙ	450 μl
Liz	0.22 μΙ	11 μΙ
PCR sample 1	0.5 μl	-
PCR sample 2	0.5 μl	-
PCR sample 3	0.5 μΙ	-
PCR sample 4	0.5 μΙ	-
In each well	9.2 μl Master Mix	