Notes for next trip:

When I left I plated out most DNA to 384 well plates and zeroed in on a protocol for genotyping.

First step when returning (day 1) is to immediately set up a PCRs and run a full set, so that a test plate (panel 1) is ready for Dave to run on the ABI the next day (day 2).

From there, the tasks are:

* Install genemapper (day 1 + 2 + 3)
* Ask Cristin to open the file for me on her copy of genemapper to confirm PCR and ABI success (day 3), ? is this necessary or just look at some intermediate ABI output?
* Schedule/submit the rest of the panels for the plates (panels 2,3 and sex marker) for the plates 1-4 run (day 3)
* Once we have data at all panels, overview MCKR genotyping specifics with Dave (day 5 – can also schedule this early if we use old data)
* Plate DNA and run PCRs for plates 5-8 (day 4), submit to ABI queue
* Run PCRs for plates 5-8 (day 6), submit to ABI queue
* Run PCRs for plates 9-12 (day 8) submit to ABI queue
* Run PCRs for plates 13-16 (day 10) submit to ABI queue

**PCR**

*Make 20x Primer Stocks*

Make 20x working primer stocks from 100uM stocks according to spreadsheet

Notes:

* 100uM Primer stocks are in PCR room
* Make up my own 20X stocks for project
* Do not remove primers from PCR room
* Do not need to anneal

*Master Mixes and protocol*

1. Assemble MM, notes below
2. Thaw and spin down DNA plates (1uL per well)
3. Dispense 5uL per well using MM to 384 protocol on robot (save reservoirs)
4. Vortex and spin down
5. Seal with silicone septa and run

Notes:

* MM must be assembled in PCR room
* dNTPs, MgCl, “additive”, Promega and may buffers and Taq are all right most freezer on West side of room.
* Promega buffer is in “GoTaq G2” box. There will be extras in here like dye etc. These will accumulate.

*Running Machines*

Notes:

* There is a scheduling system for PCR machines accessible on the gel imaging computer using the google calendar for the computer user account
* Keep machine use consistent within panels/markers.

**ABI**

1. Vortex and spin down PCR products
2. Label 384 plate with run name
   1. Name according to paper entry sheet for Dave, standard format everyone is used to is MMDDYY\_initials\_R#, e.g. the second run for me would be 010922\_DID\_R2
   2. Just use the suffix here to label plate, note date (e.g. DID\_R2)
3. Add 1ul of PCR product to plate (from each PCR plate if coloading, e.g. if panel has 4 markers each amplified separately than add 1ul from each)
4. Add 15uL LIZ (400bp for main markers, 1200bp for sex marker + extra volume for 1200bp ladder) and 800uL HiDi-Formamide mix per 96-well plate. (volumes on note taped to bench)
5. Add 8uL of HiDi-formamide + ladder mix per well
6. Seal vortex and spin down plate
7. Run “denature” program on PCR machine (95C 5 min to rapid cool)
8. Set up ABI plate (boxes and grey septa in SW corner of ABI room)
9. Fill out paper form, send Dave txt version of ABI run sheet

Notes:

* Liz ladders in 4c
* Dave will make run folder and email to me, this will be added to path in the run sheet (dd\_genotyping)