Farber Lab Notebook

**5/24**: Intro to Fish husbandry, self-cross 5 nluc(+/-). Crosses 3 and 4 had two females

**5/25**: Picked eggs from my crosses. 30,30,100,100,52 eggs. Refreshed media for some of McKenna’s fish. Removed shells. Literature search for PRKD2 drug targets.

**5/26**: Made microinjection needles and introduced fish to nursery. Designed CRISPR gRNA primers

**5/27**: designed verification and CRISPR primers. See prkd2\_CRISPR primers for more info.

**5/28**: nluc assay. See protocol in protocols folder. Extracted DNA on nluc crosses.

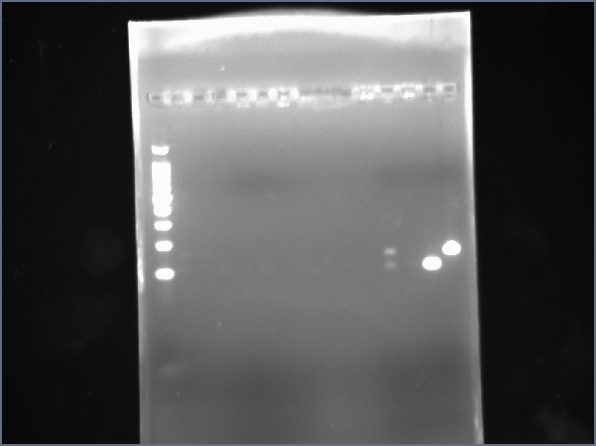
**6/1**: PCR for nluc assay. See protocol in protocols folder. For low used A1, H6, D4. For medium (putative hets) used E7, C3, B7. For homos used F3, B9,D2.

-Practiced injections with Darby. Calibration was really hard.

-Set up a cross tomorrow with AB 12109 tank F08-2-04. Made 5 crosses for plenty of eggs.

**6/2**: Practiced injections again. Someone removed the gates from my crosses so most of the eggs were already 2 cell stage. Very annoying! Getting better at injection, but I need to practice more. Probably will do a cross on Thursday 6/4 to practice again.

Ran nluc genotype gel: Very few bands. Got one heterozygote, which I thought was a homozygote, but was one of the lower values for that phenotype.



Lane 1: 100 bp ladder, lane 2 sample 1, lane 3 empty, lane 4 sample 2, lane 5 sample 3, lane 6 sample 4, lane 7 sample 5, lane 8 sample 6 lane 11 sample 7 lane 12 sample 8, lane 13 sample 9 lane 14 nluc(+/+) lane 15 nluc(-/-)

Finished drug plan: see document.

Plan for the rest of this week/next week:

Thursday: Set up crosses for drug screen. Start reverse transcription? If not read papers about ApoB and the different CRISPR priming method.

Friday: Practice injections and set up eggs for drug screen

Saturday/Sunday. Come in to check on eggs.

Monday: Set up

**6/3**: Self-cross of F17-3-08 #12716 FUS9ApoBb.1 nluc (-\-). Will be using these both for the drug screen and for injection practice.

**6/4**: Injection practice went well, but only got one good clutch of eggs after injections (3 crosses bred, and one was bad and one I used for injections). Also lost one fish down the sink (it was a dude though so probably okay). Set up PCR for verification primers to find the optimal melting temperature. Recipe for 20 ul solution below:

dNTP 0.5

FWD primer 0.5 uL

REV primer 0.5 uL

MgCl2 1.5uL

5x MM 4.0 uL

0.25 Taq uL

11.75 H20 uL

1.0 gDNA uL

Ran for 34 PCR cycles on a gradient from 55 C to 65 C. Tube 1 is the highest, tube 8 is the lowest

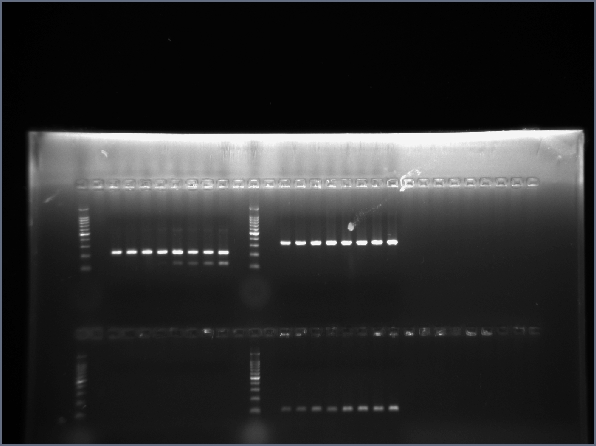
Expected bands:

E1: ~290 bp

PH: ~180 bp

K1: ~63 bp

K2: ~586 bp



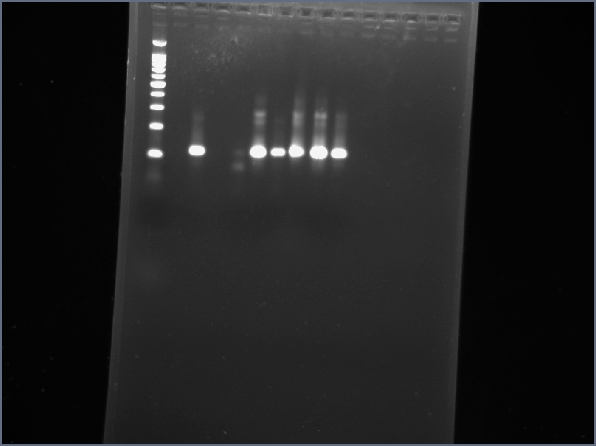
First set of 8 is PH which matches our expected size. 65 C looks like it has the best performance. Second is E1 which matches our expectations. The second row has the two kinase primers, one of which should be 583 bp, the other should be ~63. Add primers and this could be the 100 bp band we see in the fourth one, with the best temperature being 55 deg. No priming for the other, possibly because the product is too long.

**6/7**: Started drug experiment. Only had enough embryos for a single dose, went with the IC50 of 0.6 uM. Thought I saw an early dark yolk phenotype, but upon reflection was probably just the angle of light. Took 7 samples from the vehicle, and 7 samples from the CID 2011756 0.6 uM treated plate at 3 hours and 6 hours. Removed as much liquid as possible and pre-froze on dry ice before placing in the -20 C. Also ran the PCR for the sgRNA templates.

**6/8**: Lots of meetings today, but learned how to use the Nikon camera to image fish. Pipet onto a glass slide covered with 4% methyl cellulose on ice. Remove as much liquid with possible by tilting the slide. Microscope had weird yellow tint today- will have to get that checked out. Confirmed no dark yolk phenotype- maybe have to up the dose.

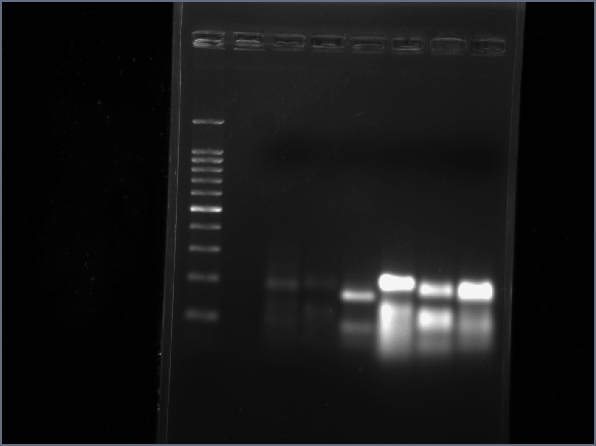
Harvested 25 hr time point. Same procedure as yesterday.

Ran gel of the RT template. Good bands except for the second guide for E1. We will have to see if RT works tomorrow. Nanodrop concentrations are in the experiment\_2 folder.

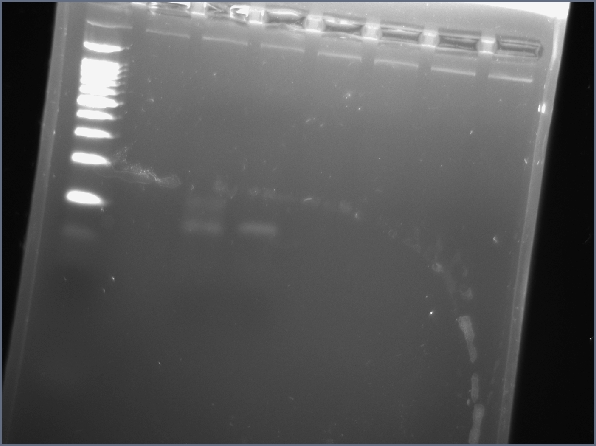


**6/9** 48 hr timepoint, started RT-PCR reaction. Meetings, meetings, meetings.

**6/10** Crosses. Prepared 6 crosses for tomorrows injection. Also ran RNA product on gel and purified. Nanodrop concentrations were extremely high (in the 2000s).



**6/11**: Prepared injection and attempted to inject fish. However, all eggs were bad. Going to set up crosses again (with Darby’s fish) for injection on Tuesday. Also tried to run exon 1 R pcr for the template again, but something is wrong with the primer concentration or sequence or something. Analyzed nluc data from the drug screen. Results are in Jupyter Notebook.



6/14: Set-up crosses for tomorrow using Darby’s Fus(ApoBb.1-nluc (-/-)) 12716 from tank F21-03-06.

PCR with the Kinase verification primers again. Didn’t have much this afternoon

dNTP 0.5

FWD primer 0.5 uL

REV primer 0.5 uL

MgCl2 1.5uL

5x MM 4.0 uL

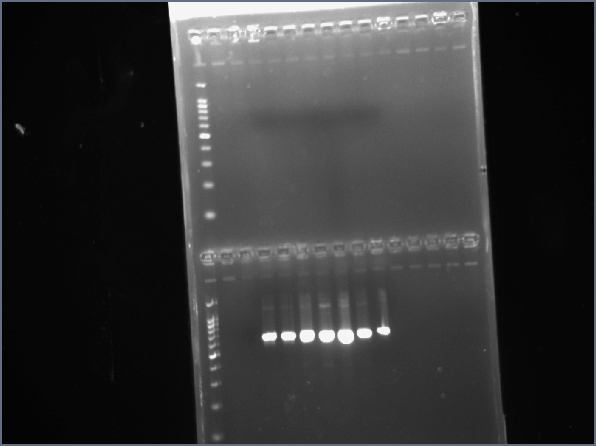
0.25 Taq uL

11.75 H20 uL

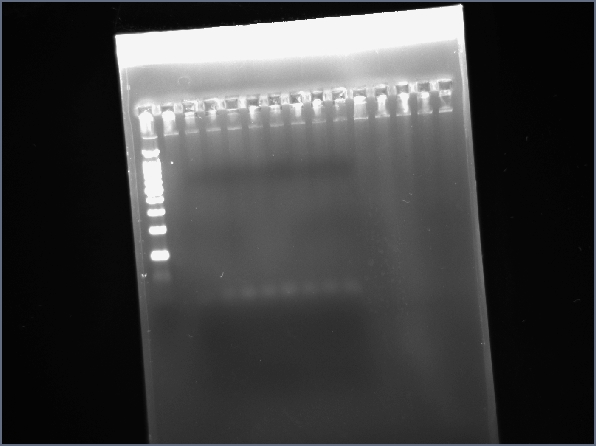
1.0 gDNA uL

Ran for 34 PCR cycles on a gradient from 55 C to 65 C. Tube 1 is the highest, tube 8 is the lowest 1 minute elongation time.

**6/15**: Crosses had all bad eggs… AGAIN! Ran PCR on the gel and got a band a little over 600bp for the second kinase. 65C seems to be the optimal temperature for this reaction again as well. No band for K1, but it may have run off the gel. Will try to rerun this afternoon. Rerun gel has no bands…. Weird. Rerun produced the same results.



K1 on top, K2 on bottom



Rerun

**6/16**: Setup crosses between AB 12657 F05-4-07 females and F17-3-08 #12716 FUS9ApoBb.1 nluc (-\-). Will be using these for a drug screen on Monday and injections tomorrow. Worked on the puzzle of ApoA4 for a bit as well. Summary of my findings is under exp3.

**6/17**: CRISPRed the cross from yesterday with the E1 guide RNA for PRKD2. Got some good dyeing and survivors on 6/18. Worked more on the ApoA4 puzzle. Setup a cross with Darby’s fish: Darby’s Fus(ApoBb.1-nluc (-/-)) 12716 from tank F21-03-06. And AB 11854 from tank F21-03-02. Also setup a self with Maggie’s fish from 12125.1  apoa4a; apobb.1-nluc  F26-3-20 and a cross between females of that fish and male: 11664 apoa4a  F06-3-19.

**6/18**: No laying from Darby’s Fish. Injected the PH domain guides into Maggie’s selfed fish. 2125.1 apoa4a (+/-); apobb.1-nluc(+/-)  F26-3-20. Injected about 30 embryos and used the rest for the drug screen that I started on 6/21.

**6/21**: Following modified experiment exp1\_prkd2\_drug\_screen. Gave fish 6um, 3um,1.5um and 0.6um as well as DMSO control of CID 2011756. CRT0066101 hasn’t come yet. Also screwed up by diluting my stocks 1:100 instead of 1:1000 as was planned. Also took pictures of CRISPR fish and drugged fish at 4.5 hours. Those picture are saved in the Phenotyping\_images in the respective experiments folders. Looks like a dark yolk and cardiac edema, but could just be from sick fish. Also forgot to close the freezer today! Big oops!

**6/22**: Homogenized samples and nluc assayed them. Results do not look promising because of freezer issue. Harvested new CRISPR fish for genotyping (both the 2125.1 line and the 12657 crossed to nluc). Quick genotyping protocol is as follows:

30 ul 50mM NaOh

20 min @95C

+3ul 1 M Tris and pH 8 to neutralize

**6/23**: Ran a genotyping PCR on the fish from yesterday. Used the nluc PCR as a control (as at least the ApoBb.1 background fish should all be hets) and ran the E1 and PH primers with an annealing temp of 61 degrees. Full protocol below. Setup an incross with Darby’s Fus(ApoBb.1-nluc (-/-)) 12716 from tank F21-03-06.

PCR protocol for E1 and PH

3:00 @ 95 C

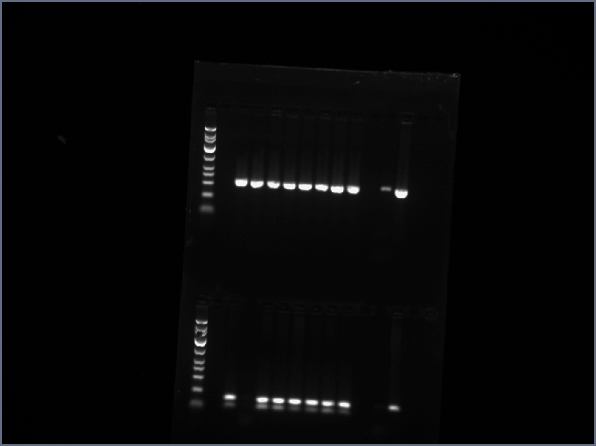
0:30 @ 95 C

0:30 @ 61.1 C

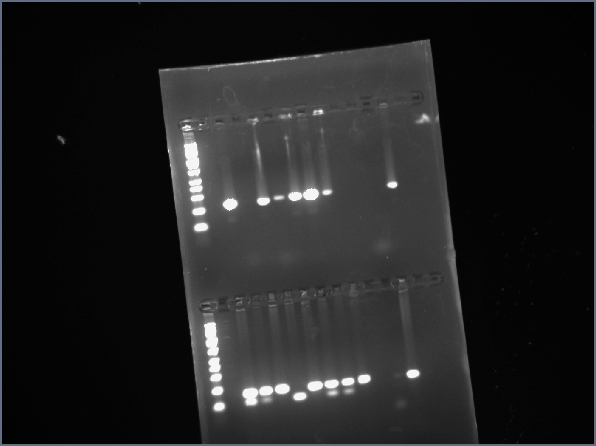
0:30 @ 72 C

34x to step 2

5:00 elongation.



Exon 1 CRISPR no hits.



PH domain no hits. Lots of nluc hets.

Will redo PCR tomorrow because of water contamination.

**6/24**: Ran a genotyping on the CRISPR fish from last week. Ran the E1 and PH primers with an annealing temp of 63 degrees. Full protocol below. Harvested about 30 good eggs from an incross with Darby’s Fus(ApoBb.1-nluc (-/-)) 12716 from tank F21-03-06.

PCR protocol for E1 and PH

3:00 @ 95 C

0:30 @ 95 C

0:30 @ 63 C

0:30 @ 72 C

34x to step 2

5:00 elongation.

Ran for 40 mins and took a pic but trying to run for longer so I can potentially get more separated bands.

Bands did not separate clearly even after another 40 minutes of running. Don’t think we got any CRISPRs, but I’ve saved the PCR products to run on two separate gels tomorrow morning.

Setup Crosses for AB 12657 F05-4-07 females and F17-3-08 #12716 FUS9ApoBb.1 nluc (-\-)

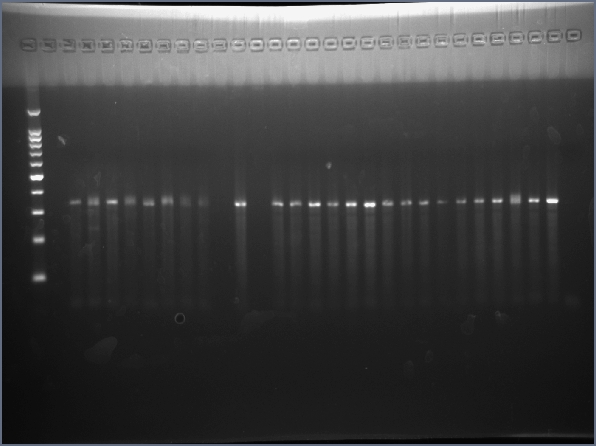
**6/25**:

Injected E1 sgRNAs with McKenna. We injected from laying pair 3 and used laying pair 4,5 and 8 for the drug screen. I presorted each family into 4 round plates for each of the drug conditions, aiming for 40 fish in each condition.

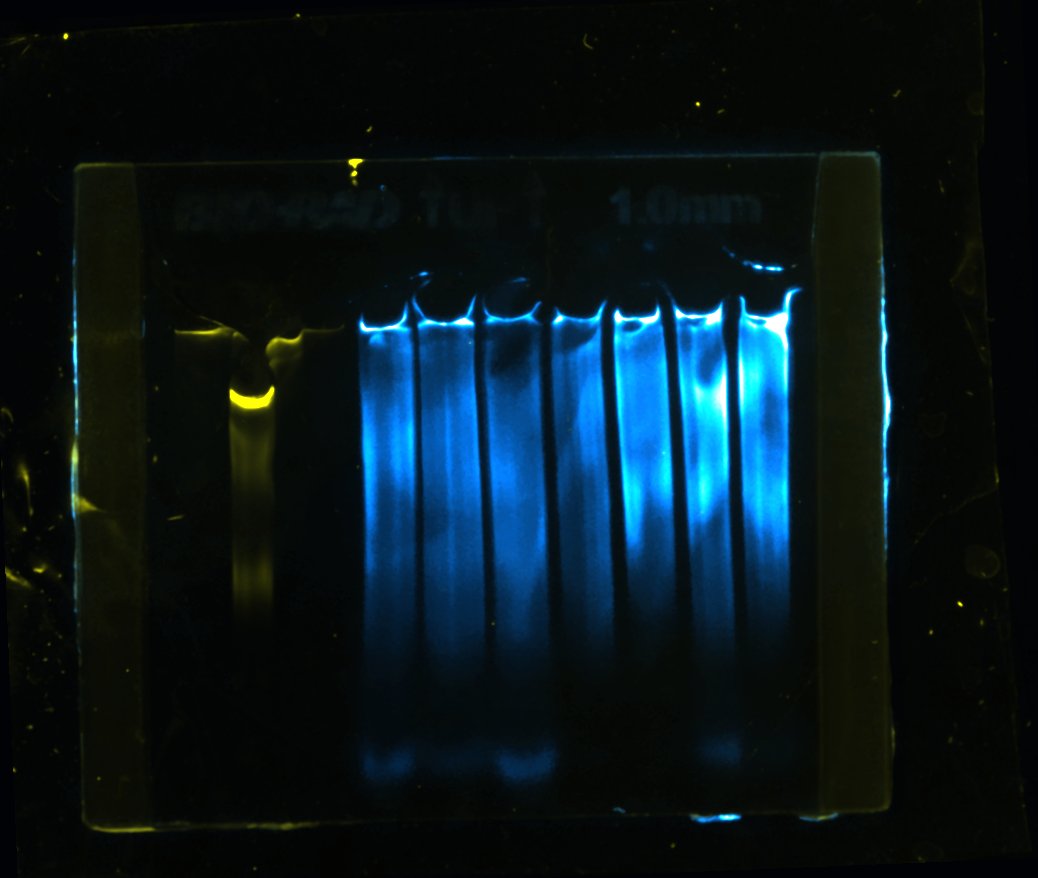
**6/28**:

Drug experiment with 0,0.6,1.5,6 um of CID2011756. Harvested 8 fish from each family at each timepoint (although ran out of fish from family 4 for some reason!!!). Homogenized and ran nluc immediately. Results analyzed in Jupyter Notebook screen\_analysis28). Also homogenized CRISPR samples and prepped PAGE gel for tomorrow.

**6/29**: Made DNA and ran genotyping PCR with CRISPR samples. No definitive cuts, but we see some potential heteroduplexes. Ran Native PAGE with McKenna, but my gels were shite, so no result. Images are in the drug treatment and CRISPR folders respectively. But I’ll also upload them to this document.



Gel



Nglo assay from today.

Also made new PAGE gels with Monica.