Lab Notebook Management
Sample, Data, and File Organization

BGS Orientation

Wednesday, August 23, 2023

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Notebook organization is critical

- Find data faster
- Easily share protocols, reagents, data
- Minimizes mistakes

Adopt a system EARLY!

- Electronic, paper, or combo?
- If all electronic, ELN or your own system?
- Standardize your protocols

TG Quantification for Plasma

Reagents

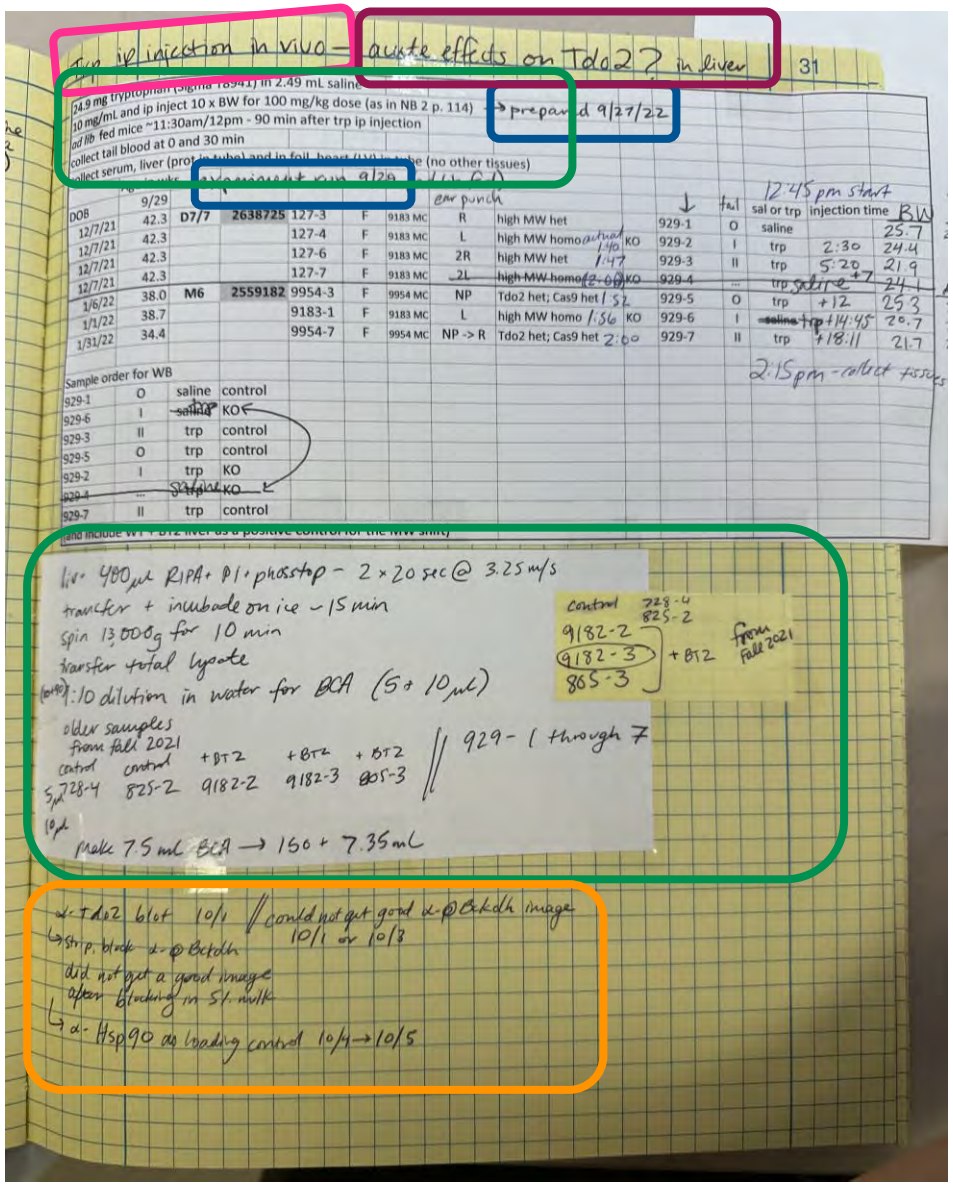
- Infinity Triglycerides Kit (Fisher Scientific TR22421)
- Triglycerides Standards (Pointe Scientific T7531-STD)
- DEPC Water
- 96-well clear flat-bottom plate
- Multichannel pipette
- Plasma (collected after blood was centrifuged at 10000rpm, 7 min, 4C)

Protocol

1. Make TG standards (serial dilutions with DEPC water from 200 mg/dl stock):
   a. 200 mg/dl
   b. 100 mg/dl
   c. 50 mg/dl
   d. 25 mg/dl
   e. 12.5 mg/dl
   f. 6.25 mg/dl
   g. 3.125 mg/dl
   h. 0 mg/dl
2. Dilute the plasma 1:1 with DPEC water.
3. Add 5uL of diluted plasma to 96-well plate.
4. Add 5uL of standards to 96-well plate in duplicate.
5. Add 200uL of TG infinity reagent to all wells.
6. Incubate at room temperature for 15 minutes in the dark (foil or drawer).
7. Measure absorbance at 500nm on the plate reader.
8. Graph standard curve to get equation to calculate triglycerides in samples.
9. Plug in absorbance readings for samples into the standard curve, and make sure to account for any dilutions.

Must-haves in notebook entries

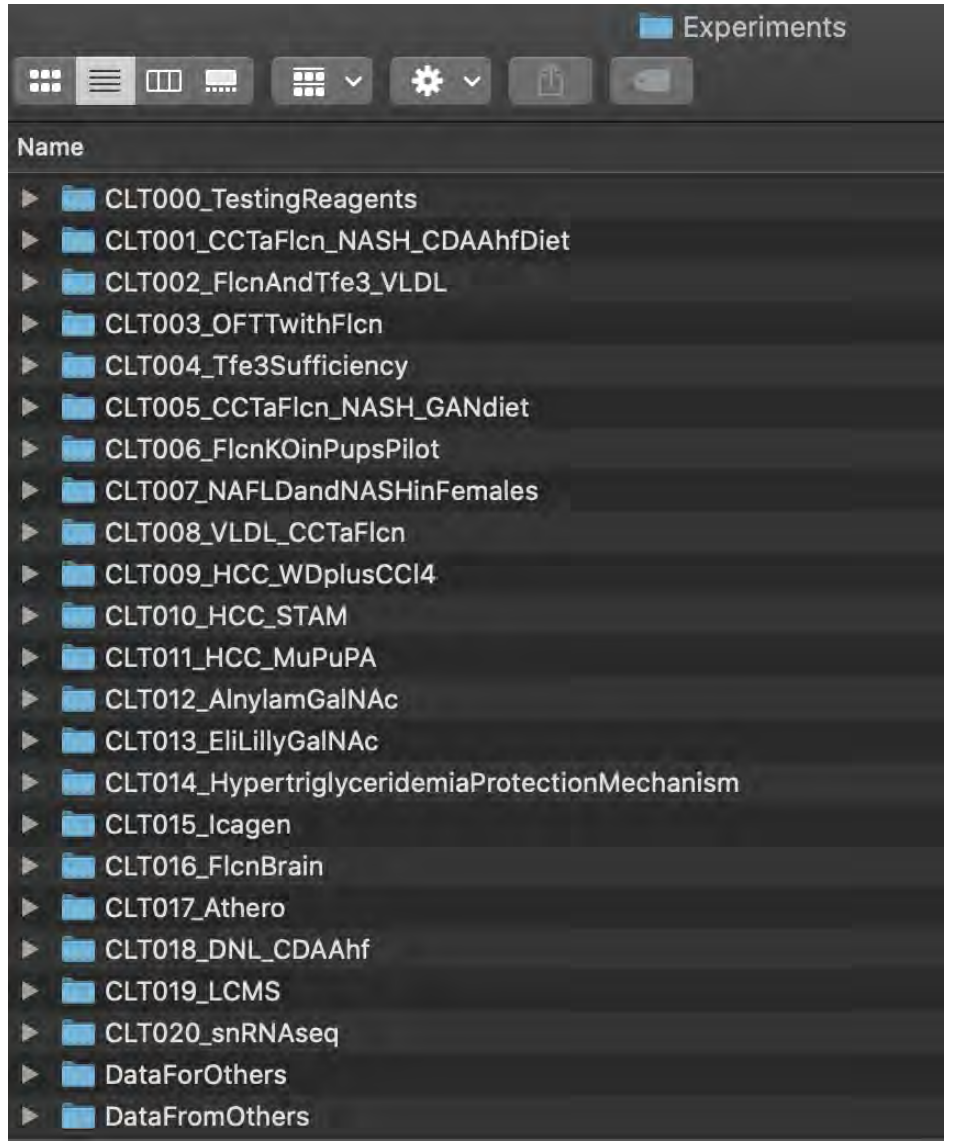
- Experimental Identifier
- Date
- Purpose
- Methods/Protocols
- Conclusion (if possible)



Utilize a number system

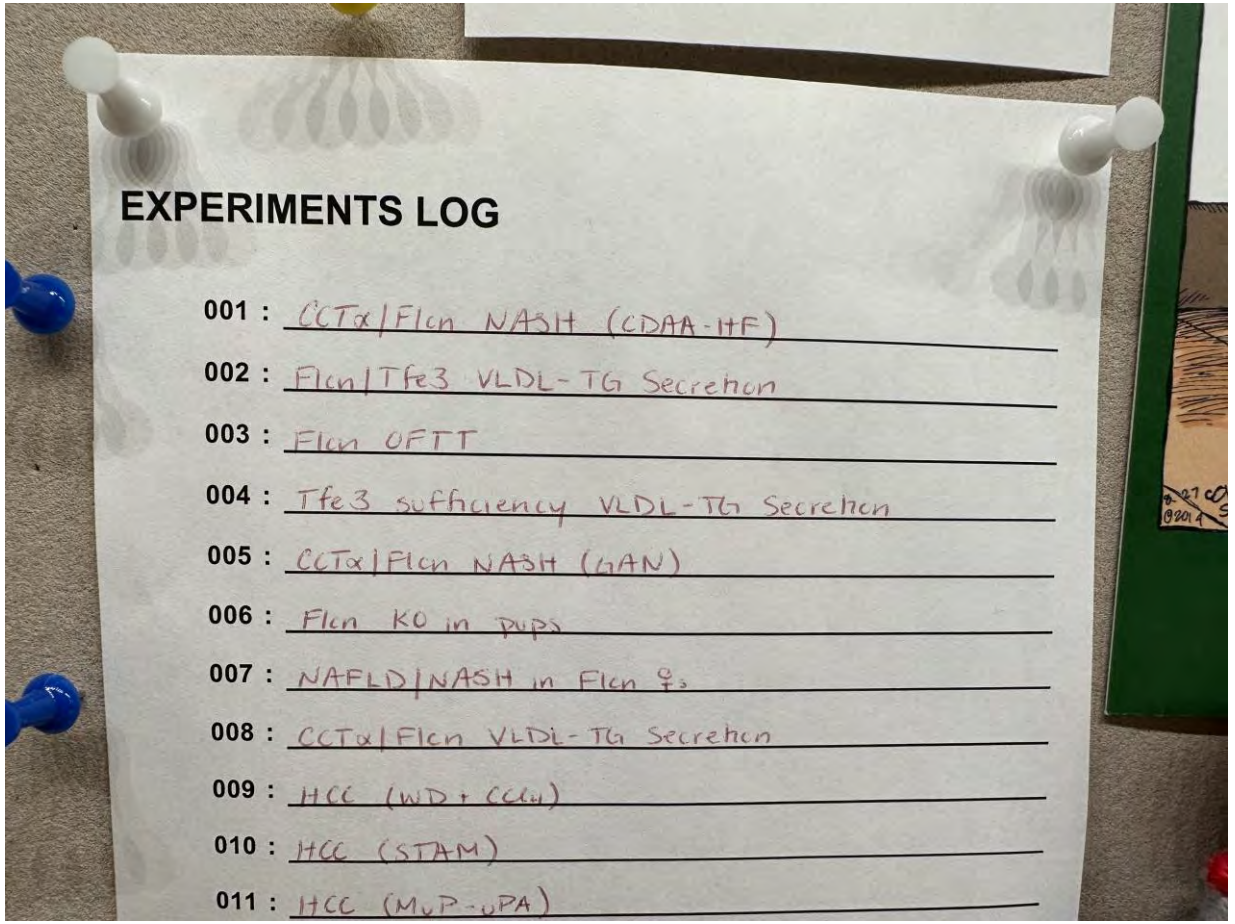
- Every experiment (or overarching experiment) gets a number
  - Include your initials
- Every mouse/animal gets a number

	A	B	C	D	E	F	G	H	I	J	K	L
	Cage #	Mouse #	sex	ear	origin	dob	8wks	Type of cohort	Notes/Injection Dates	Rough Experimental Planning	FLCN	TFE3
49	1754	6143	F	R	B308	6/4/23	7/30/23					Tfe3 +/+
50	1754	6144	F	LR	B308	6/4/23	7/30/23					Tfe3 +/+
51	1754	6145	F	LS	B308	6/4/23	7/30/23					Tfe3 +/-
52	1755	6146	M		B308	6/4/23	7/30/23	Fl/3	inject 8/1/23	CLT014 FPLC C9		Tfe3 +/Y
53	1755	6147	M	L	B308	6/4/23	7/30/23					Tfe3 -/Y
54	1755	6148	M	R	B308	6/4/23	7/30/23					Tfe3 +/Y
55	1755	6149	M	LR	B308	6/4/23	7/30/23					Tfe3 -/Y
56	1774	6216	M		B306	6/22/23	8/17/23	Fl/3	inject 8/18/23	CLT014 FPLC C10		Tfe3 -/Y
57	1774	6217	M	L	B306	6/22/23	8/17/23					Tfe3 -/Y
58	1774	6218	M	R	B306	6/22/23	8/17/23					Tfe3 -/Y



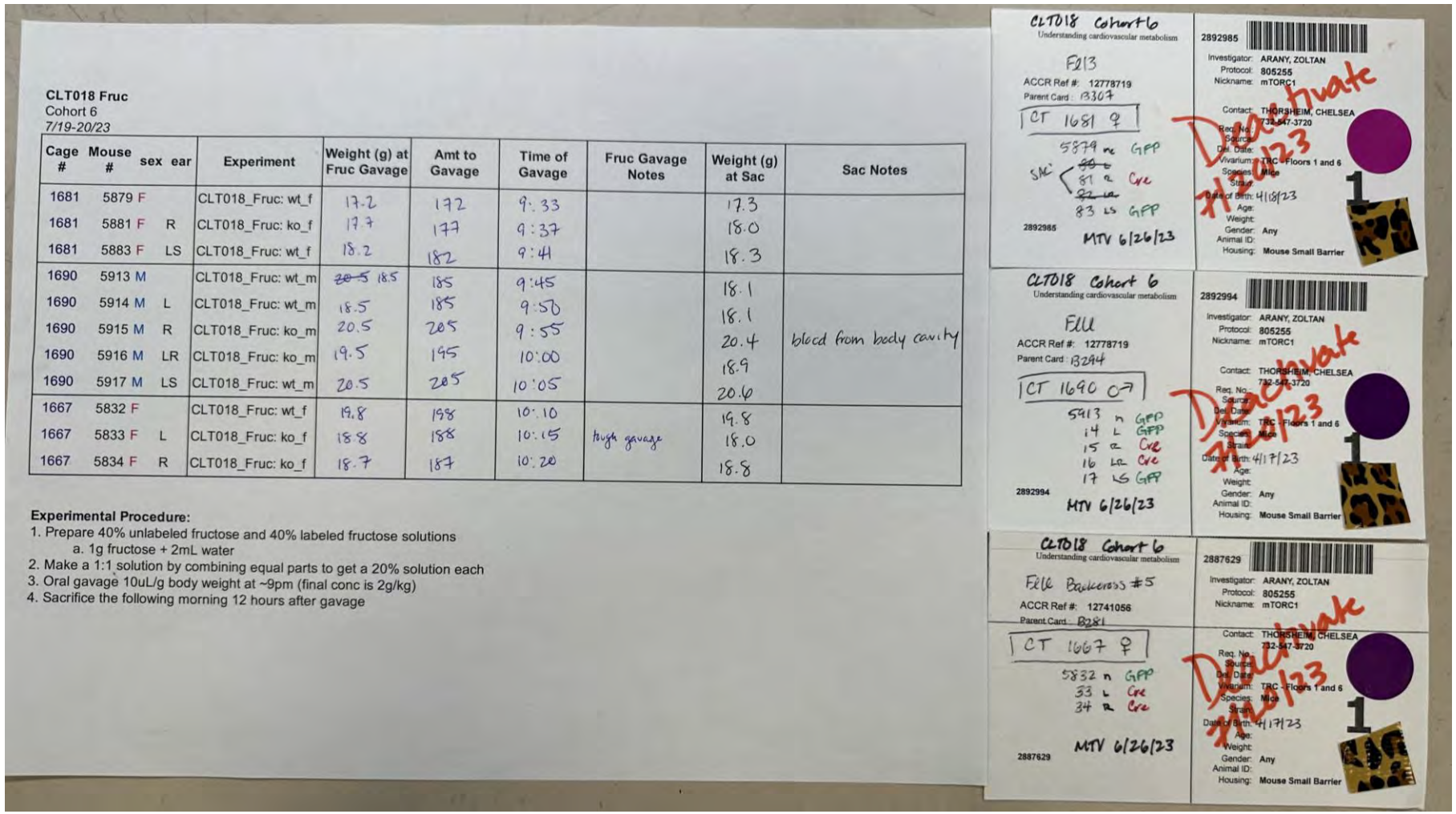
Utilize a number system: Table of Contents

- Keep a printout with blank spaces to label experiments
  - Put it somewhere easily accessible
  - Fill it out consistently



Utilize a number system: Sample organization

Utilize a number system: Notebook keeping



CLT018 Fruc
Cohort 6
7/19-20/23

Cage #	Mouse #	sex	ear	Experiment	Weight (g) at Fruc Gavage	Amt to Gavage	Time of Gavage	Fruc Gavage Notes	Weight (g) at Sac	Sac Notes
1681	5879	F		CLT018_Fruc: wt_f	17.2	172	9:33		17.3	
1681	5881	F	R	CLT018_Fruc: ko_f	17.7	177	9:37		18.0	
1681	5883	F	LS	CLT018_Fruc: wt_f	18.2	182	9:41		18.3	
1690	5913	M		CLT018_Fruc: wt_m	~~20.5~~ 18.5	185	9:45		18.1	
1690	5914	M	L	CLT018_Fruc: wt_m	18.5	185	9:50		18.1	
1690	5915	M	R	CLT018_Fruc: ko_m	20.5	205	9:55		20.4	bleed from body cavity
1690	5916	M	LR	CLT018_Fruc: ko_m	19.5	195	10:00		18.9	
1690	5917	M	LS	CLT018_Fruc: wt_m	20.5	205	10:05		20.6	
1667	5832	F		CLT018_Fruc: wt_f	19.8	198	10:10		19.8	
1667	5833	F	L	CLT018_Fruc: ko_f	18.8	188	10:15	tough gavage	18.0	
1667	5834	F	R	CLT018_Fruc: ko_f	18.7	187	10:20		18.8	

Experimental Procedure:
1. Prepare 40% unlabeled fructose and 40% labeled fructose solutions
   a. 1g fructose + 2mL water
2. Make a 1:1 solution by combining equal parts to get a 20% solution each
3. Oral gavage 10uL/g body weight at ~9pm (final conc is 2g/kg)
4. Sacrifice the following morning 12 hours after gavage

CLT018 Cohort 6
Understanding cardiovascular metabolism
F213
ACCR Ref #: 12778719
Parent Card: 13307
CT 1681 ♀
5879 n GFP
81 a Cre
83 LS GFP
2892985
MTV 6/26/23

2892985
Investigator: ARANY, ZOLTAN
Protocol: 805255
Nickname: mTORC1
Contact: THORSHEIM, CHELSEA 732-547-3720
Vivarium: TRC - Floors 1 and 6
Species: Mice
Date of Birth: 4/18/23
Gender: Any
Housing: Mouse Small Barrier
Deactivate 7/26/23

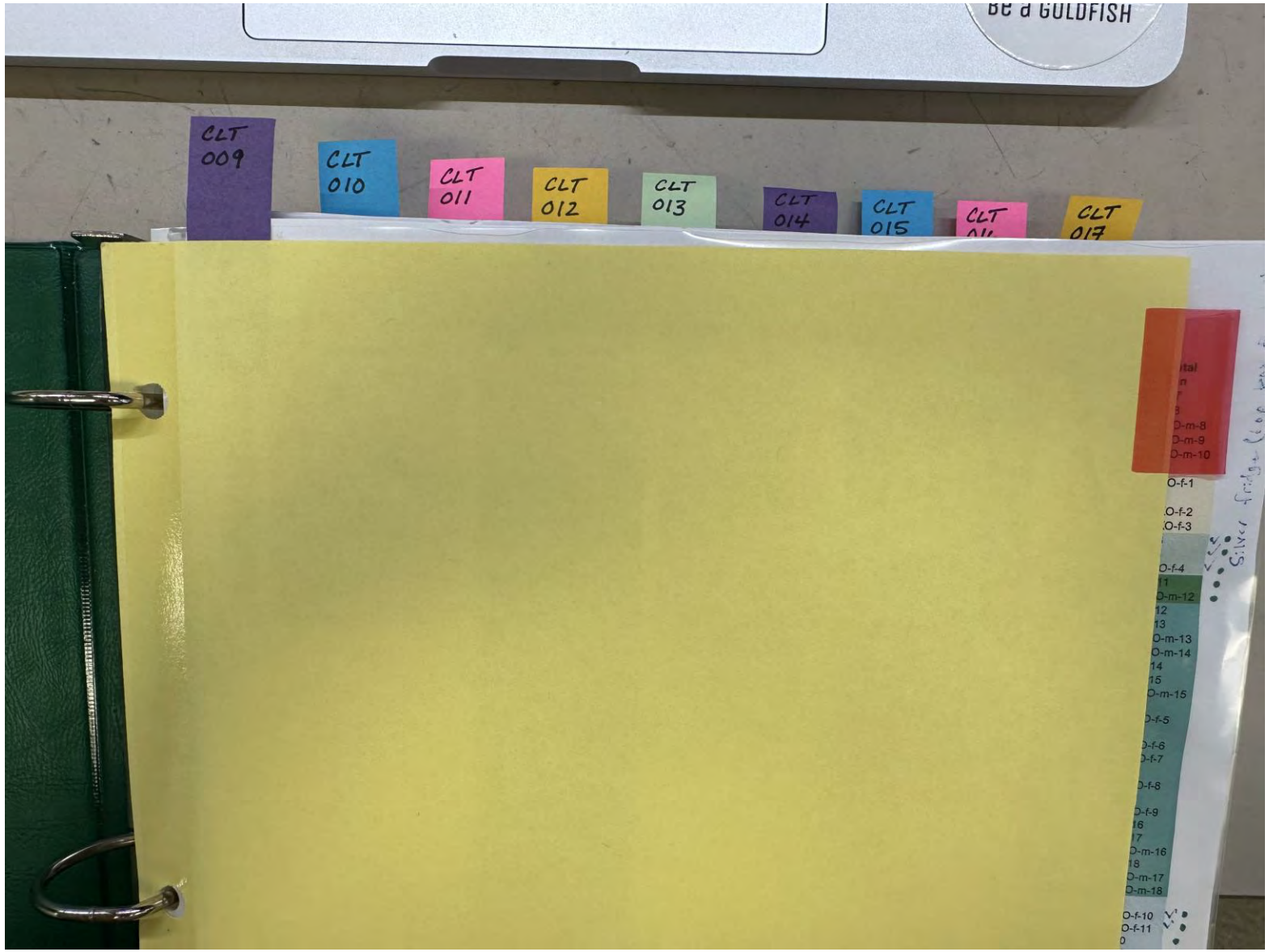
CLT018 Cohort 6
Understanding cardiovascular metabolism
F111
ACCR Ref #: 12778719
Parent Card: 13294
CT 1690 ♂
5913 n GFP
14 L GFP
15 a Cre
16 LR Cre
17 LS GFP
2892994
MTV 6/26/23

2892994
Investigator: ARANY, ZOLTAN
Protocol: 805255
Nickname: mTORC1
Contact: THORSHEIM, CHELSEA 732-547-3720
Vivarium: TRC - Floors 1 and 6
Species: Mice
Date of Birth: 4/17/23
Gender: Any
Housing: Mouse Small Barrier
Deactivate 7/26/23

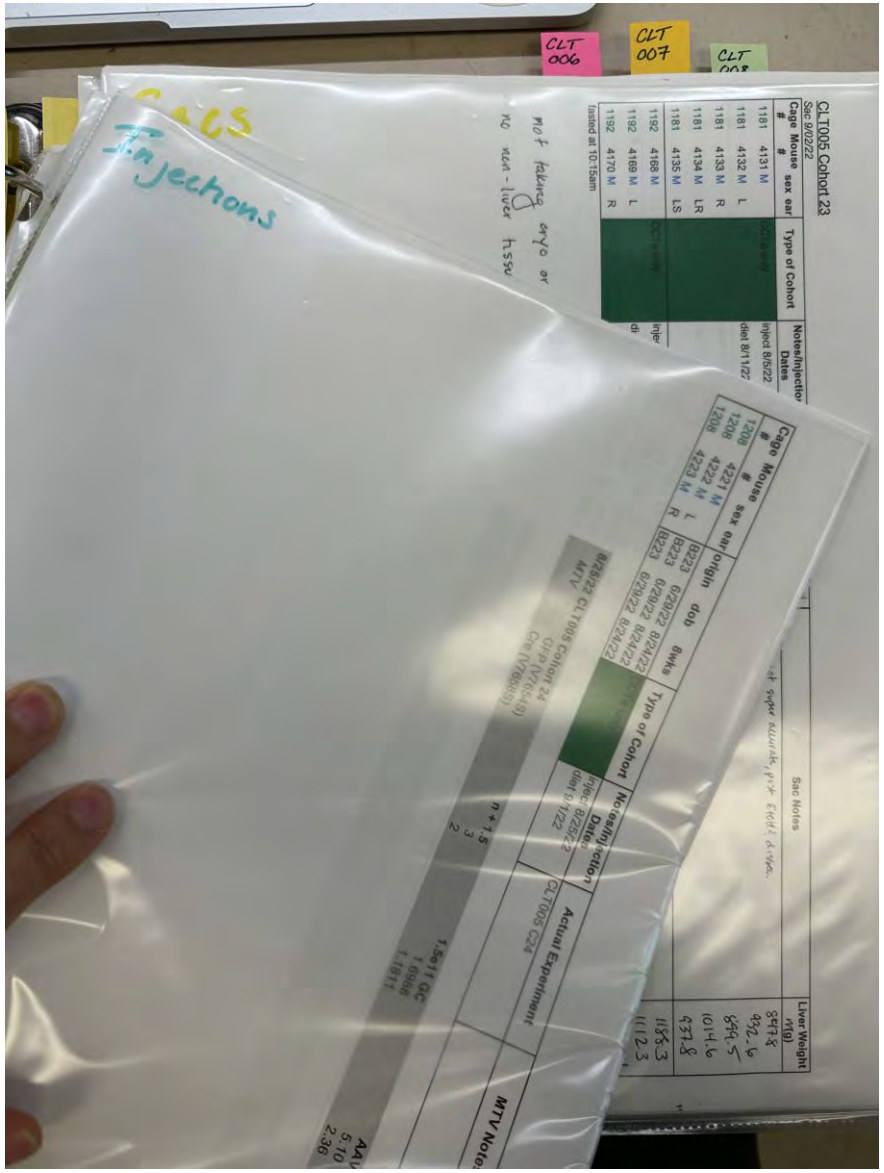
CLT018 Cohort 6
Understanding cardiovascular metabolism
F111 Backcross #5
ACCR Ref #: 12741056
Parent Card: B281
CT 1667 ♀
5832 n GFP
33 L Cre
34 R Cre
2887629
MTV 6/26/23

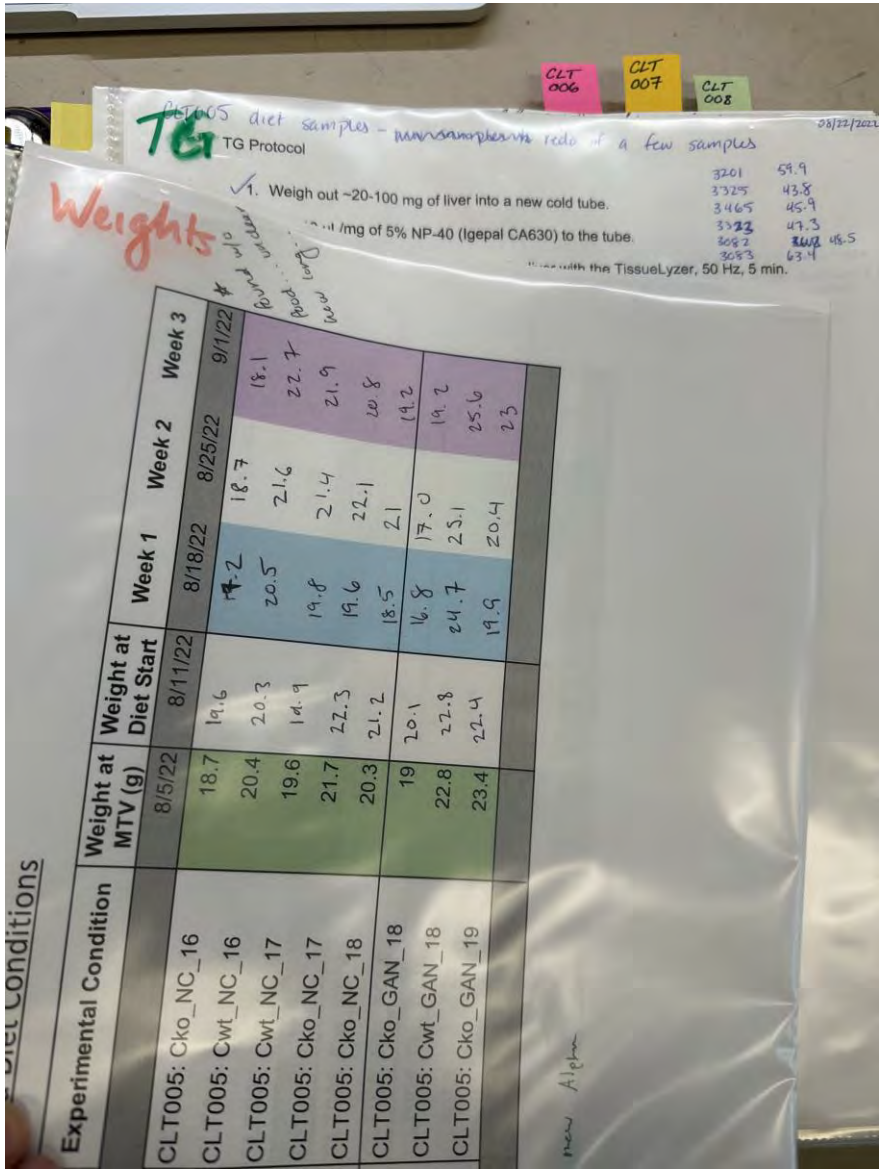
2887629
Investigator: ARANY, ZOLTAN
Protocol: 805255
Nickname: mTORC1
Contact: THORSHEIM, CHELSEA 732-547-3720
Vivarium: TRC - Floors 1 and 6
Species: Mice
Date of Birth: 4/17/23
Gender: Any
Housing: Mouse Small Barrier
Deactivate 7/26/23

Utilize a number system: Notebook keeping

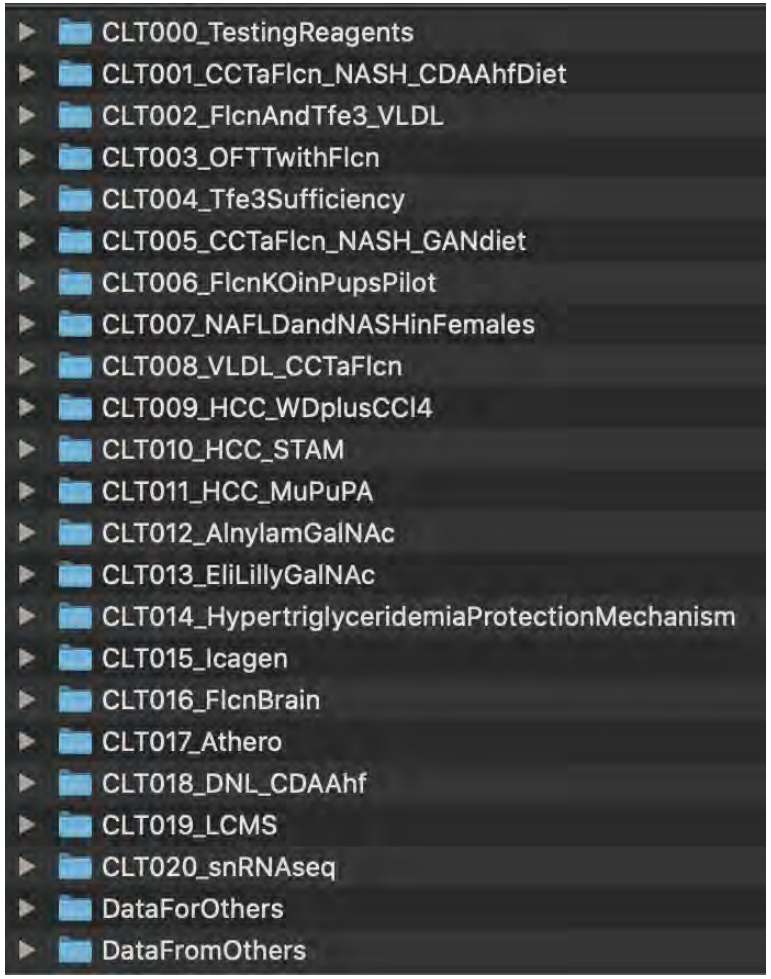


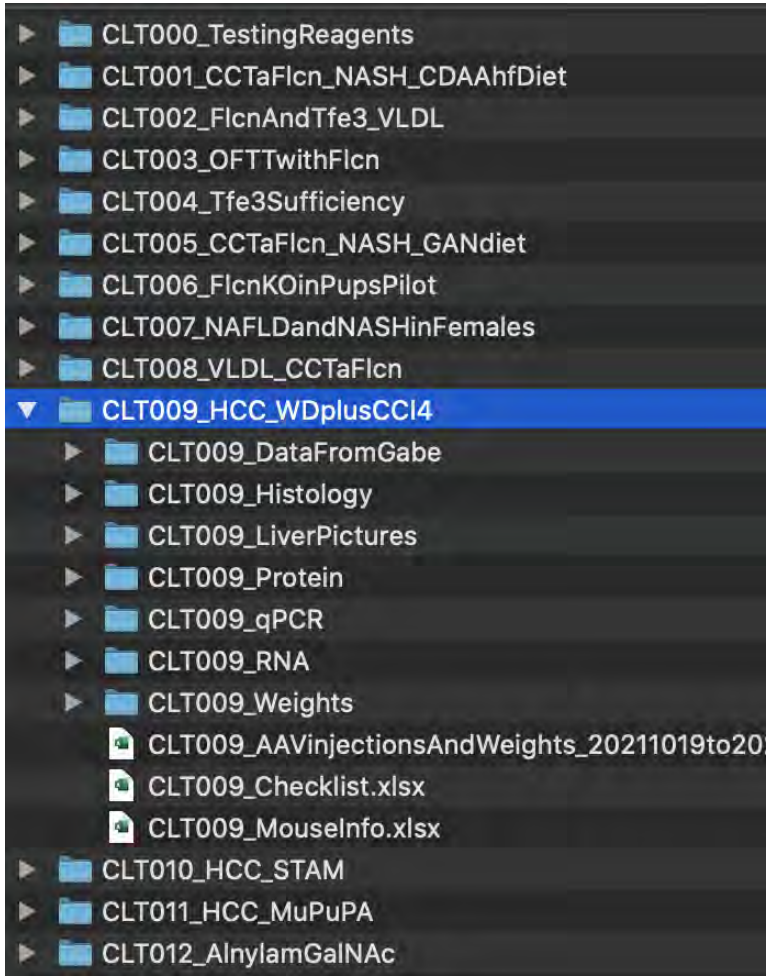
Utilize a number system: Notebook keeping

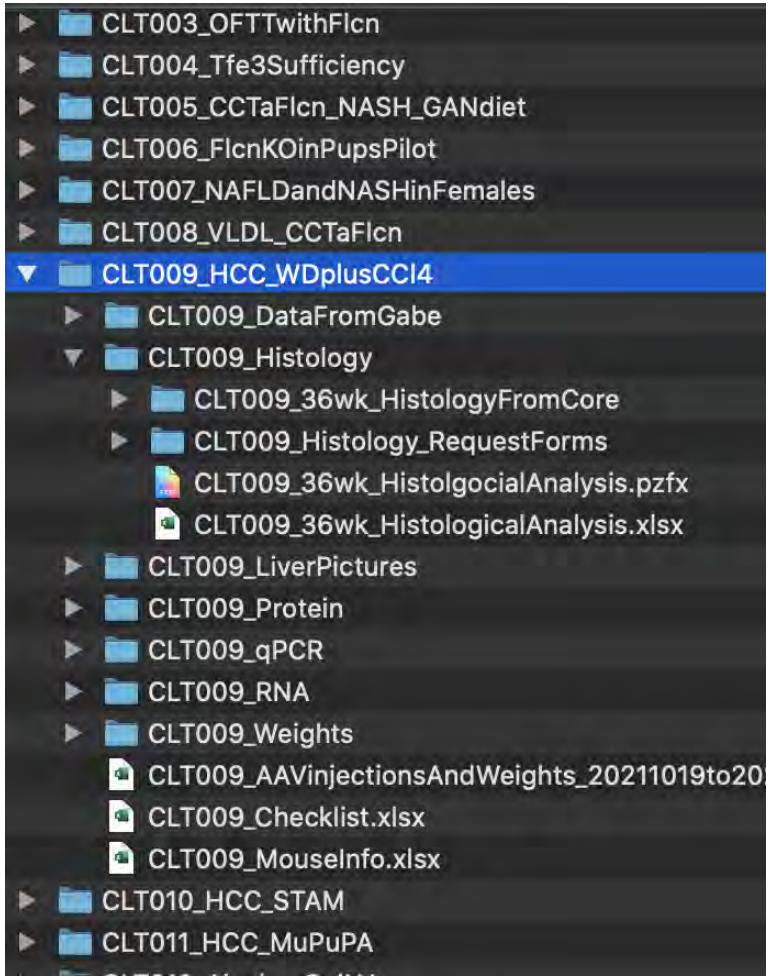




Utilize a number system: File organization







Example #1 of utilizing the number system

- Western Blots for CLT014
  - Experimental Identifier
  - Date
  - Purpose
  - Methods/Protocols
  - Conclusion (if possible)

Example #1 of utilizing the number system

- Western Blots for CLT014

CLT014 WB: Liver lysate

- To better characterize some VLDL components
- Order: (wt = green, ko = red)
  1. ladder
  2. 3331
  3. 3333
  4. 3704
  5. 3843
  6. 4071
  7. 3332
  8. 3705
  9. 3844
  10. 4072
  11. 4073
  12. 4094
  13. ladder

  Repeat to get 26 lanes total

CLT014 WB
4/05/23

Example #1 of utilizing the number system

- Western Blots for CLT014

CLT014: Fourth WB Round

Western Blot
TGX Gels for broad MW proteins

Reagents and Prep
- 4-20% TGX gels (Bio-Rad 5671095)
- 10x Tris/Glycine/SDS Running Buffer (Bio-Rad 1610772)
- 10x Tris/Glycine Transfer Buffer (Bio-Rad 1610771)
- Rader Lab Sample Buffer (see below for reagents needed)
- Spectra Multicolor Broad Range Protein Ladder (ThermoScientific 26634)
- SuperSignal West Femto Maximum Sensitivity Substrate (ThermoScientific 34095)
- Immobilon-P PVDF Transfer Membrane 0.45um (Millipore IPVH00010)
- TBS-T
- Dehydrated milk
- Antibodies (primary and secondary)
- Samples

Recipes:

Running Buffer
- 100mL 10x Tris/Glycine/SDS Running Buffer
- 900mL ddH2O

Transfer Buffer
- 100mL 10x Tris/Glycine Transfer Buffer
- 200mL methanol
- 700mL ddH2O

Sample Buffer (please scale accordingly)
- 2.5mL of 0.5M Tris-HCl, pH6.8
- 4mL of 10% SDS
- 2mL of glycerol
- 1mL of BME
- 0.5mL of ddH2O

Sample Prep
- For plasma: Add 1.5uL of plasma to 8.5uL of Sample Buffer (for 10uL total); scale up as necessary to load 10uL per well per sample
- For lysate: Add 50 uL of protein (at conc. 2 ug/uL) to 16.66uL of Sample Buffer (for 66.66uL total); load 11.11uL per well
- DON'T FORGET TO BOIL SAMPLES (95C for 5min)

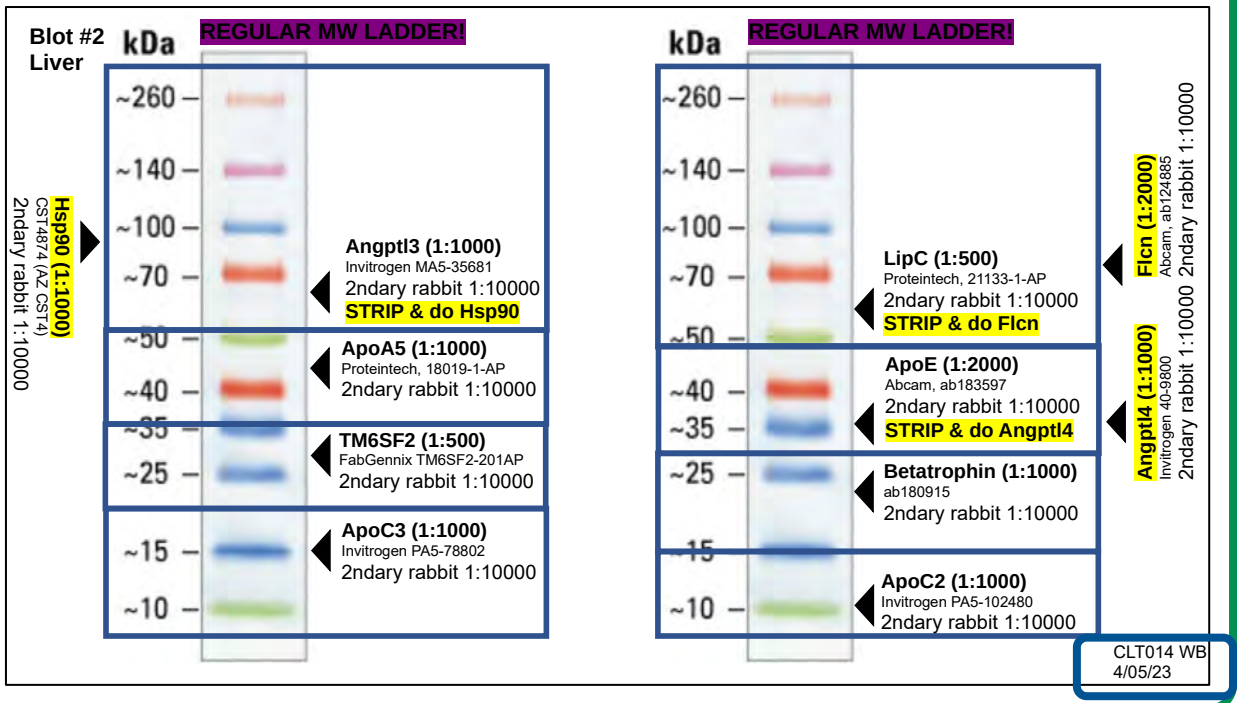
Running
1. Prep the 4-20% TGX gel by removing tape and rinsing the wells with Running Buffer.
2. Add the samples and ladders to the wells.
   a. Make sure to use the normal MW ladder!!
3. Run at 100 V (constant voltage) for 60 minutes.

Transfer
1. Make sure to chill the transfer buffer while the samples are running.
2. Put entire transfer chamber in a tray filled with ice and add some Transfer Buffer and the ice pack.
3. Activate PVDF membrane with methanol before transfer by shaking in methanol for 5 minutes. Rinse with water and then add Transfer Buffer until ready to sandwich. Make sure it stays wet!!
4. Rinse gel before transfer with regular water.
5. Equilibrate the gel in transfer buffer as you make the sandwich.
6. Sandwich as follows: red, foam, filter, membrane, gel, filter, foam, black
7. Add sandwich to the chamber and top with Transfer Buffer as needed.
8. Run at 500 mAmp (constant Amps) for 80 minutes.
9. Ponceau stain for 10 minutes. Image against a white background, not in the container.
10. Wash with ddH2O 3x 1 minute or until stain seems gone.

Blotting
1. After transfer, block for 30-60 min in 5% milk in TBS-T.
2. Wash 3 x 5 min with TBS-T.
3. Incubate with primary overnight.
4. Wash 3 x 5 min with TBS-T.
5. Incubate with secondary for 30-60 min in 5% milk in TBS-T.
6. Wash 3 x 5 min with TBS-T.
7. Image using Femto on the 10th floor imager.

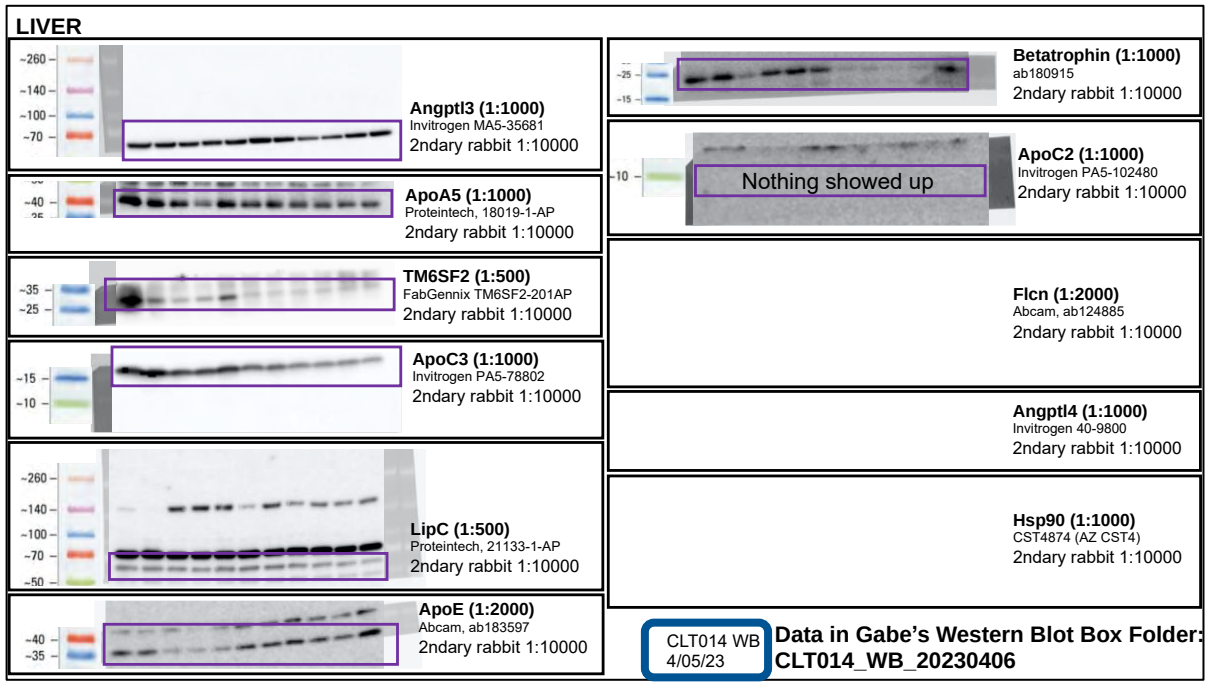
Notes:
Blot #1 is plasma, Blot #2 is liver

CLT014 WB
4/05/23



Example #1 of utilizing the number system

- Western Blots for CLT014



CLT014 WB: Fourth WB Round Summary

- ApoC2 Ab not great with these experimental conditions?
  - Try low MW gel?
- Maybe increased ApoE in Flcn KO
- Decreased betatrophin, ApoC3
- Maybe decreased ApoA5, TM6SF2, Angptl3, LipC
- **TO DO:** densitometry, redo ApoC2 blot with different conditions

CLT014 WB 4/05/23

Example #2 of utilizing the number system

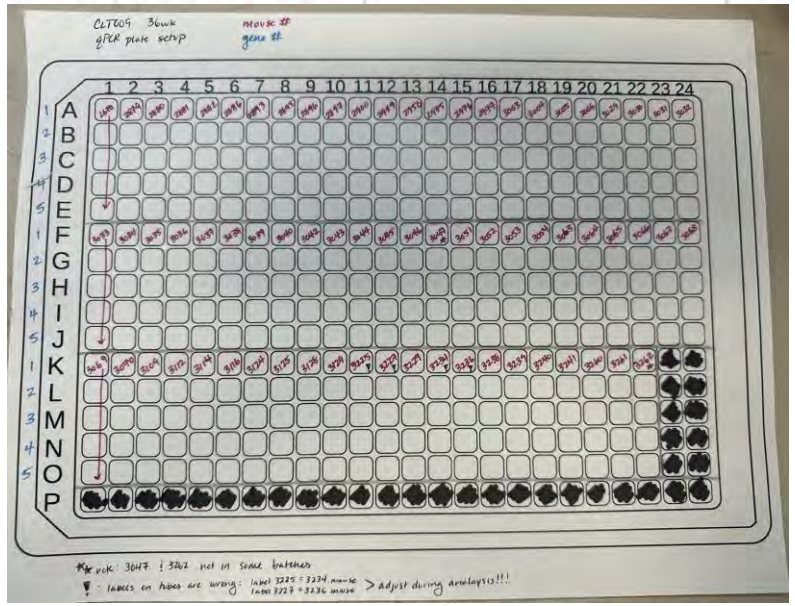
- qPCRs for CLT009

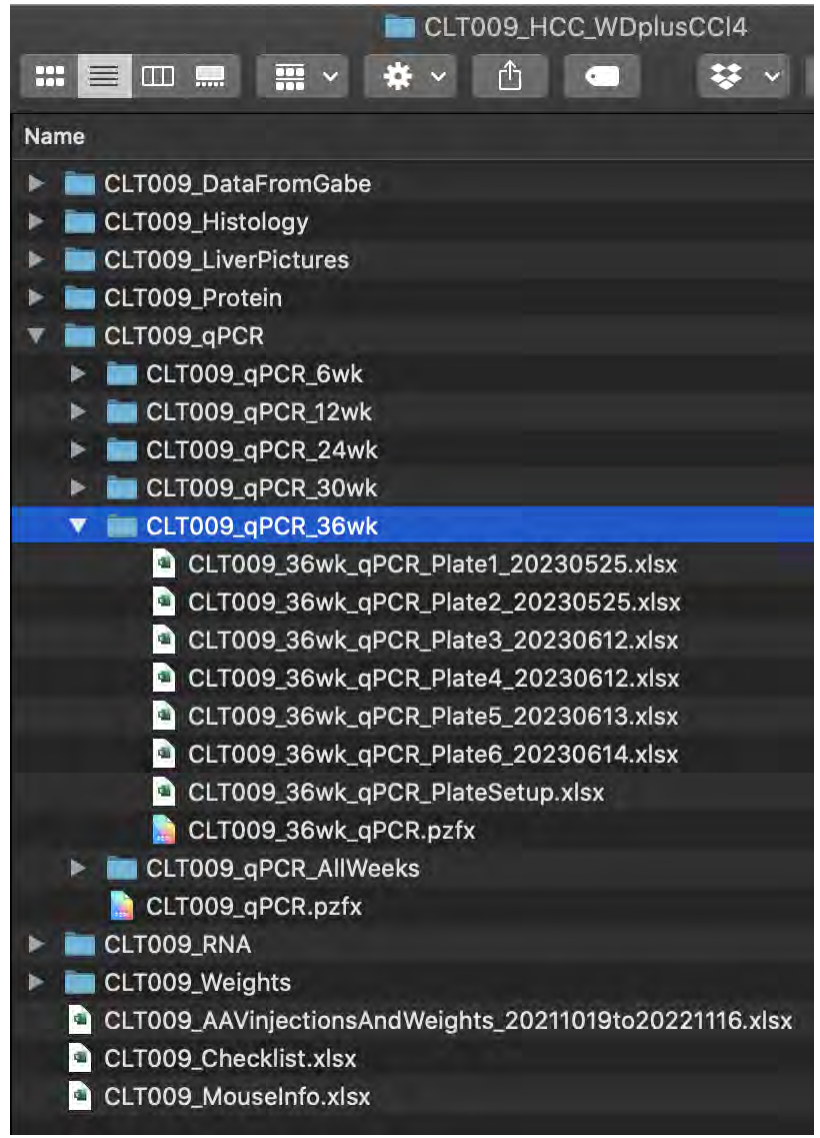
CLT009 36wk

Mouse #	Experiment	Sort
2878	CLT009: wt-m	1
2879	CLT009: wt-m	1
2880	CLT009: FlcnKO-m	2
2881	CLT009: FlcnKO-m	2
2882	CLT009: FlcnKO-m	2
2886	CLT009: wt-f	3
2893	CLT009: wt-f	3
2895	CLT009: FlcnKO-f	4
2896	CLT009: wt-f	3
2897	CLT009: FlcnKO-f	4
2900	CLT009: FlcnKO-f	4
2949	CLT009: wt-f	3

CLT009
36 week

Plate 1	Plate 2	Plate 3
36b4; 483/4	36b4; 483/4	36b4; 483/4
HPRT; 768/9	HPRT; 768/9	HPRT; 768/9
FLCN; 5391/2	GPNMB; 3833/4	Acly; 4865/6
Cre; 5507/8	Srebp1c; 336/7	ACSS2; 5655/6
aFetoprotein; 6472/3	Tnfa;	scd1; 503/4





Advice

- Try out different methods during rotations if you have flexibility
  - Also ask if there is a particular method that is required of the lab

- Don’t make too rigid of a system
  - Too much work to maintain the notebook will make you less likely to do it

Key Takeaways

- Adopt a system that works best for you and your lab
  - All electronic
  - All paper
  - Combo
- Assign numbers to your (overarching) experiments and consistently use those numbers
- Someone should be able to go look at your notebook and find all of the information they need to reproduce or find data

Questions?

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