Genetics 284 Laboratory Schedule (Tentative)

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	1/24	Intro to Flies		**Monday night clear vials of wildtype Canton-S, put at 18C for virgins in the morning.** 12 vials of wildtype Canton-S 12 vials of mutant flies, mapping stocks
T	1/31	Intro to Worms		12 plates of N2 normal worms 1 plate of each mutation Alcohol lamps (12) Picks (12 at least - ask Dave)

Day	Date	Lab Day	Outside class	Lab Prep Needs
M	2/6		Start overnight of RNAi plasmid containing bacteria pL4440 empty pL4440 + lsy-2 pL4440 + choice **37C incubator**	**Plates of bacteria with:** pL4440 empty pL4440 + lsy-2 (chemo assay) Groups will choose one of the following for phenotypic analysis: pL4440 + bli-1 pL4440 + dpy-5 pL4440 + unc-22 (from Chase Lab) **10ml per overnight of sterile LB + 12.5ug/ml tetracycline** **Adding 10ul of 50mg/ml ampilicillin stock** **15ml conical tubes for overnight, 3 per group, so 36 tubes = 360ml of broth minimum** **Sterile toothpicks**

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	2/7	Set up **PCR** to verify plasmid inserts Isolate plasmid DNA from bacteria	Begin collecting virgin females from the P-element lines this week	**PCR reagents:** **Invitrogen T7 Promoter Primer** **Catalog Number N560-02** (forward primer, 20uM) 50 ul of ultrapure sterile water PCR buffer 50mM MgCl2 10mM dNTP mix Taq Polymerase (5U/microliter) **PCR engines** **Parameters:** 94C 5min 94C 30sec 55C 30sec 72C 3min Repeat 30 72C 6min 4C HOLD **Solutions for DNA isolation:** **Solution 1 (~ 7.2 mls min)** 50 mM glucose 10 mM EDTA 25 mM Tris, pH 8 **Solution 2: (~14.4 mls min)** 0.2 M NaOH 1% SDS (sodium dodecyl sulfate) **Solution 3: (~10.8mls min)** 2.7M Potassium Acetate 6.6M Acetic Acid --- **100% ethanol** **80% ethanol** **sterile water** **Speed Vac??**

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	2/14	Gel analysis of PCR products, verification of inserts Transformation of HTT115DE3 feeding lines, plates to 37C ---------- Set up Cross 1 p-element virgins x males for mapping stock (18C incubator)		2% agarose 1 x TAE mini gels to check PCR products (EtBR) loading dye for samples? **Need competent HTT115DE3 E.coli made by this date (protocol by Inoue, see at end of this document)** **LB broth** **LB + amp + tet plates** ---------- Dissecting Scopes out, fly morgue, anethesitizers, Fly Nap, brushes, clean vials with food Multiple marker chromosome mapping stocks to get males from. 18C incubator
W	2/15		Check transformation and control plates Save transformation plates, notify TA/instructor if control plate is positive also!	
M	2/20		Overnight cultures of transformed bacteria	LB + tet broth amp stock sterile toothpicks

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	2/21	Induce HTT115DE3 bacteria to produce RNAi by IPTG Seed plates with induced bacteria for feeding plates, allow plates to dry ---------- Clear parent flies from Cross 1 vials put back in 18C incubator		Overnight cultures will need to be subcultured **by Prep Staff** in the morning in order to be in log phase by lab time for induction Diluting cultures 1:10 (500ul culture into 4.5ml of LB+tet+amp) put in 37C to grow 3-4 hours until lab time. **0.5M IPTG stock** **NGM plates with 0.4mM IPTG, 50ug/ml ampicillin, and 12.5ug/mL tetracycline.**
Fri	2/24	Put N2 and rrf-3 worms on the feeding plates to feed them RNAi		**L4s of** **N2 worms** **rrf-3 worms** 23C incubator

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	2/28	Collect phenotypic data on gene of interest Wash worms for chemotaxis assay from feeding plates, resuspend and put on chemotaxis plates Chemotaxis assay		Day before chemotaxis assay plates set up. (Prep staff or students??) **Experimental:** NGM plates no food, 10ul of 2.5M NaCl on one side of plate, 10ul of double deionized water for control, mark bottom of plate with location of each **Control:** have water on both sides marked ice cold sterile water **0.25M sodium azide** **2.5M NaCl**

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	3/6	Isolate Fly DNA from P-element line Digest DNA to cut out the P-element Ligate o/n at 4C		**Isolating DNA from flies:** Need bottles of P-element lines by here, 30 flies per group **Solution A: (~6 ml)** 100mM Tris-HCL pH 7.5 100mM EDTA **LiCL/KAc solution: (~12ml)** 1 part 5M KAc stock 2.5 parts 6M LiCl stock 65C heat block/water bath 37C heat block/water bath isopropanol 70% ethanol TE for resuspension **Digest:** 10X RE buffer 100um/ml RNase ddH20 Sau3A I, HinP1 I or Msp I (Kenny? which one for P[LacW]) **Ligation:** 10X Ligation buffer (+ATP) ddH20 Ligase (2 Weiss units)
W	3/7		Precipitate DNA and resuspend in 150ul TE	Ethanol ice Speed Vac TE for resuspension (Students do?? TA does??)

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	3/13	Run PCR to amplify flanking sequence from P-element primers Mini gel check for amplification, put in 4C Send out sequencing here?		**PCR on P-element DNA:** 2mM each dNTP 10uM forward primer 10uM reverse primer 10X Pharmacia Taq buffer ddH2O 2 U Taq PCR parameters: 95C 5min 95C 30sec 60 or 55C 1 min 68C 2min Repeat 35X 72C 10 min 4C HOLD **Gel Check PCR products:** 1.5% agarose mini gel with EtBr markers?? Who sets up sequencing reaction to go to GeneWiz?? Students or TA??
T	3/20	SPRING BREAK	SPRING BREAK	SPRING BREAK
T	3/27	Bioinformatics Session, use sequence to locate insertion site Sequence back here?		

Day	Date	Lab Day	Outside class	Lab Prep Needs
T	4/3	Score flies from the mapping cross, do three point cross and determine recombinant map position		fly sorting dishes! morgue
T	4/10	LaZ staining of P-element lines??		
T	4/17	HOLIDAY	HOLIDAY	HOLIDAY
T	4/24	Presentations		
T	5/1	Presentations		