UCL Laboratory Schedule

Monday 3rd September

Session 1 **11:00-13:00**	
	Set up reactions for PCR- **Assembling PCR mix** *PCR of marine genes-antifreeze (ANF), mercury reductase (MR), CMP plasmid backbone* Primer dilution
13:00-14:00	**Lunch**
Session 2 **14:00-17:20**	
	Genomic DNA extraction
	Put DNA template in reaction mix and start PCR

Tuesday 4th September

Session 1 **10:00-12:00**	
	Gel electrophoresis Making 1% agarose gel (20mins); while gel is running mix loading buffer with sample
	Running gel
12:00-13:00	**Lunch (iGEMers Nanodrop)**
Session 2 **13:00-15:30**	
	Gel Visualisation
	PCR clean up of ANF, MR,CMP, (40mins)
	Set Up Digest for Ligations (15mins), incubation (35mins) _____- Cut CMP backbone with E &P Cut marine gene with E & P Expression vector: Appropriate digest of ANF and expression vector
	Perform ligation (20mins) _____ Ligate marine gene with CMP backbone (2 ligation, one for ANF one for MR) Ligate CMP backbone (control) Ligate marine gene with expression vector -for characterisation
Session 3 **15:30-18:00**	***Transformation***
	Transform ligation into *E. coli* (3hrs)
	Spread plate

Wednesday 5th September

11:00-11:30	Pick colony from plate into 2ml of LB Put liquid culture into 37C (approx. 2hr to reach ~0.5 OD)
11:30-12:00	Miniprep something inoculated beforehand not the public biobrick
12:00-13:30	**Lunch**
13:30-3:00	**Characterisation** • 10 fold serial dilutions of culture containing ANF construct plated onto LB agar + CMP • Store plates at 37C-data for before freezing • 10 fold serial dilutions of culture containing CMP plasmid (control) + CMP • Store plates at 37C-control data for before freezing • Freeze original ANF tubes at -20C

Next morning, we make serial dilutions of ANF and plate; incubate at 37C

Take pictures of plates, for biohackers to calculate relative freezing tolerance