

Name: Rehmat Babar, Chiara Brust, Krithika Karunakaran

Date: 7/5/19

Goals:

1. PCR on pcb302 in E. Coli straight from papers 1 & 2
2. Overnights of pcb302 in Agrobacterium Tumefaciens from papers 1 & 2

Name: Krithika Karunakaran

Date: 7/5/19

Goal:

1. Overnights of pcb302 in Agrobacterium Tumefaciens

Protocol:

**Overnights of pcb302 Protocol**

1. 15mL of YM (media) was added to each 50mL Falcon tube (3 tubes total)
2. 15 $\mu$ L of kanamycin was added to each tube
3. A p20 was dipped into each of the 3 colonies and dropped into the corresponding tubes.
4. The tubes were placed in the shaker (next to the incubator on the back counter) at 28°C and shaking at 220rpm. They should be removed from the incubator first thing Monday (7/8/19)
  - a. The pcb302 plates are in the fridge.

Name: Rehmat Babar

Date: 7/5/19

Goals:

1. PCR on pCB302-gfp-MBD plasmid in E. Coli that came off of the filter paper that was sent to us.

Materials:

DreamTaq Green PCR Master Mix (2x) Lot 00603571

Protocol:

### **PCR Protocol**

#### **20 $\mu$ L Reaction**

1. Prepared a PCR concentration cocktail with the following proportions: 7  $\mu$ L of diH<sub>2</sub>O, 10  $\mu$ L Dream Taq PCR Mastermix (2x), 1  $\mu$ L of the forward primer, and 1  $\mu$ L of the reverse primer.

- 1 reaction with primers 1 & 2
- 1 reaction with primers 1 & 4

2. Placed PCR tube in the thermocycler at the following settings:

1. 95° C for 3:00 minutes
2. 95° C for 1:00 minute
3. **48° C for 1:00 minute**
4. 72° C for 1:00 minute
5. 30X (Go to Step 2)
6. 72° C for 5:00 minutes

Lid Temperature: 105° C

The PCR was analyzed on a gel electrophoresis gel

## Results:

