

**Name:** Laura Das Neves, Krithika

**Date:** 10/02/2019

**Goal:**

1. Make Glycerol Stocks of pcb302 into *A. Tumefaciens* from 9/26/19
2. Create a large overnight culture of pcb302 into *A. Tumefaciens* from 9/26/19
3. Blank transformation on *S. microadriaticum* using yeast electroporation protocol

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2. Create a large overnight culture of pcb302 into A. Tumefaciens from 9/26/19

**Protocol:**

1. 1 mL of overnight culture + 1 mL of 50% glycerol was placed into 9 cryotubes.
2. Tubes were placed in -80°C freezer.

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1. Blank transformation on *S. microadriaticum* using yeast protocol

Protocol:

1. 90  $\mu$ L of symbiodinium at an OD of 0.077 was added to an ice cold electroporation cuvette
2. The symbiodinium was incubated on ice for 5 minutes
3. The cells were pulsed at 1.5V and given 1mL 10% glycerol recovery
4. The cells were transferred to a 15 mL tube and placed in a non-shaking water bath at 30 °C for 1 hour
5. The cells were then put in 2 mL of ASP8A media and placed in artificial light for rapid growth