***Naegleria* 101**

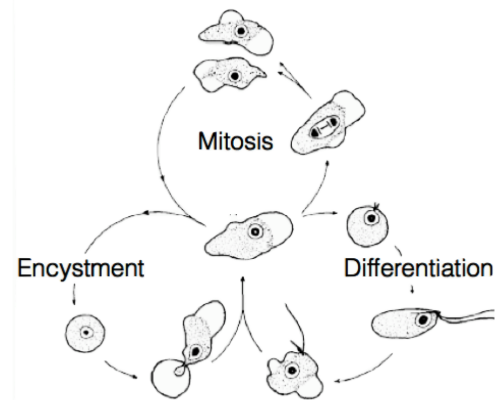
Today's missions:

1.) Analyse the results of your cloning. Make liquid cultures for bulking your dsRNA expression plasmids.
2.) Practice culturing and differentiating *Naegleria*
3.) Collect images for your comparative project

Part 2: Cloning bootcamp part 1B

1. Examine the plates from your transformation. Did your cloning work? How do you know? When deciding, be sure to examine and include the results from your control plates.
2. If you think your cloning worked, pick up to 4 colonies to grow cultures for plasmid extraction. Sterilize your forceps by dipping them into alcohol and shaking off the excess. Using these sterile forceps, pick up a sterile tip or toothpick, touch a colony (this is called "picking" a colony). Drop the colony into 5mL of LB broth containing antibiotic (Carb or Amp). When deciding how many colonies to pick, think about the ratio of colonies on your control plates to your experimental plate.

Part 1: Naegleria 101



Today we will practice culturing, observing, and handling *Naegleria*. *Naegleria* grows in the leaft litter at the bottom of ponds, where it hunts and eats bacteria. In the lab, we can grow *Naegleria* by feeding it bacterial strains of our choice. Dr. Fritz-Laylin will discuss and demonstrate the 3 main methods of culturing *Naegleria*: 1) co-spreading *Naegleria* cells with bacteria, 2) Plating *Naegleria* in a drop at the edge of preformed bacterial lawns, and 3) Plating *Naegleria* on "triple stripe" plates.

As shown above, *Naegleria* can adopt three distinct forms: actively dividing ameobae that eat bacteria, temporary swimming flagellates, and dormant cysts. Today, we will observe the 3 different *Naegleria* life stages, and collect time lapse movies and still images of each. Finally, we will stain differentiating cells with Lugol's Iodine solution, and measure rates of differentiation. *Be sure to save your data as you may want to use it for the presentation at the end of the module, and may also be used for your model systems projects*.

1.) Transfer *Naegleria* amoebae onto pre-cultured triple stripe plates of RNAi-production bacteria to transfer *Naegleria* from growing on Aerobacter to growing with our RNAi bacterial strain:
    a.) Use a sterile loop to transfer *Naegleria* cysts (from the center of the cleared region of the edge plate) to the end of one of the outer stripes on the prepared Triple Stripe plate.
    b.) Check on the microscope to make sure you've transferred cysts.
    c.) Label your plate, and put at 30C to grow.
    d.) If all goes well, what do you expect to see next class?

2.) Differentiate cells:
    a.) Use a 2mM tris filled loop to put amoebae into 1mL of 2mM Tris.
        i.) Dip a clean loop into the Tris. The circle should be full of buffer
        ii.) Touch the drop within the loop to the surface of the plate at the edge of the cleared zone. Do not drag the loop.

iii.) When you lift the loop, the droplet should remain inside, and the liquid should appear slightly cloudy.

iv.) Dip the loop into the tube of Tris buffer and swirl. This should release the cells into the liquid, and the tris should appear slightly cloudy

b.) Check cell densities: 10ul on a slide should show lots of amoebae. If you don't see any, try again.

c.) Shake your tube gently at 30C, and note your start time

d.) At time 0, 30 min, 60 min, 90 min, 120 min, collect a 50ul sample of your differentiating cells.

e.) At each time point:

i.) put a 20ul drop on a slide, and look at it on your scope. Note what you see. Try taking timelapse movies.

ii.) Also add 20 ul of cells to a tube with 20 ul of Lugol's iodine to fix and stain the cells. Make a wetmount and count the percent cells with flagella at each time point.

3.) Make lawns of the bacteria for cultivation next class.

a.) Add 5-10 beads to two Naegleria growth medium plate.

b.) Pipette 150 ul of HT115 culture onto each plate.

c.) Shake to spread the culture. Return beads to the dirty bead jar.

d.) Put the plates at 30C to grow overnight.