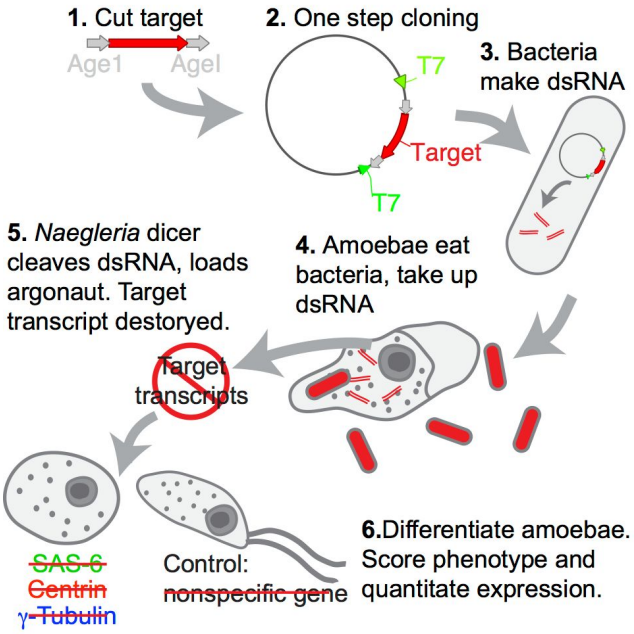
Naegleria RNAi Day 1

Diagram of overall plan for *Naegleria* module as we discussed in class. This diagram may help you design a flow diagram to represent your experiment. Note: this diagram does not include every step of of the process, but is intended to give you a visual picture of the entire *Naegleria* module. We have already completed step 2, and last class we introduced our plasmids into dsRNA bacteria, the first part of step 3. Today we will be making concrete plans for executing the rest of step 3 and steps 4-6 which will be done over four days (including today).



Today's mission: Plan your RNAi experiment. To do this, begin by reading over two different methods used for other model systems. Compare the two different methods: what seems to be important (hint: what is the same between the protocols, what is different?) Decide how you want to proceed for your first attempts at RNAi in *Naegleria*. Make a flow diagram overview of your planned RNAi experiment(s). Set up bacterial and/or *Naegleria* cultures you will need for Friday.

Part 1: Background Videos and Readings

When using one model system to manipulate another model system, it's important to keep in mind the biology of both organisms. Here is a youtube video for you to watch that 1.) uses some elements you might want to try out for your own video projects 2.) Discusses the biology of bacterial growth that influences how we use it to induce RNAi: https://www.youtube.com/watch?v=s3JpVZjhEYM

Reading 1: Excerpts from paper describing RNAi by feeding in C. elegans
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5396541/

RNAi can be induced by feeding worms bacteria expressing dsRNA (Timmons et al., 2001). First, a cDNA corresponding to the gene of interest is cloned into a bacterial expression vector between opposing phage T7 polymerase promoter sites. The feeding vector is then transformed into the E. coli HT115 strain carrying the DE3 lysogen (unit 1.8), providing IPTG inducible expression of the phage T7 RNA polymerase. The HT115(DE3) strain also lacks the Rnc gene, which encodes RNase III and is therefore deficient in degrading

dsRNA. The RNAi food is prepared and seeded onto NGM plates containing IPTG. Animals are placed onto the RNAi food and both the hermaphrodite and progeny are examined and scored for phenotypes.

1. Prepare the RNAi feeding strain

Clone a cDNA for the gene of interest into L4440 double T7 RNAi feeding vector. Transform the RNAi feeding construct into the E. coli feeding strain HT115(DE3).

2. Grow bacteria

Pick a colony and inoculate 2 ml terrific broth (TB) containing 50 µg/ml each ampicillin and tetracycline, and grow overnight at 37°C. Seed the starter culture into 1 liter TB containing 50 µg/ml ampicillin and incubate with shaking at 37°C for 8 to 16 hr. Tetracycline in this culture is unnecessary and, in fact, may reduce the efficiency of RNAi (Kamath et al., 2001).

3. Harvest the bacteria

Centrifuge bacteria 10 min at 800 × g, 4°C. Resuspend the bacterial pellets in M9 buffer (use 25 ml for each liter of culture) and transfer to a 50-ml conical tube. Remove broth by aspiration. These pellets tend to be soft and pouring off the medium could result in loss of the bacterial pellet. Wash the bacteria in 5 pellet volumes M9 buffer by vortexing until the clumps are dissolved. Pellet the bacteria in a clinical centrifuge 10 min at 800 × g (4500 rpm), 20°C. Resuspend washed bacteria in 5 pellet volumes of M9 buffer. Store up to 1 month at 4°C.

4. Seed plates

Seed an appropriate number of NGM/amp/IPTG plates with one drop (~100 µl) each RNAi food. Allow plates to dry overnight at room temperature. This also allows induction of dsRNA production and consistently produces the most penetrant RNAi phenotypes by feeding. Seeded plates can be stored in a sealed container up to several weeks at 4°C. Warm the RNAi plates to room temperature prior to use.

5. Feed the worms

Cultivate worms on RNAi food and analyze phenotypes

A second method for inducing RNAi by feeding in C. elegans

(abbreviated from https://www.ncbi.nlm.nih.gov/pubmed/12828945)

Bacterial preparation and induction. For feeding plates, NGM agar [26] was prepared including 25 µg/ml carbenicillin (carb) and 1 mM IPTG; 2.5 ml of agar was dispensed into each plate. Plates were allowed to dry inverted for 4–8 days at room temperature before use. Large HT115 bacterial colonies were picked, inoculated into LB with 50 µg/ml Amp, and grown for 6–8 h with shaking at 37 °C; 1 drop of this culture was seeded into the above plates in triplicate, and the plates were dried thoroughly before being incubated overnight (~12–24 h) at room temperature to allow the bacteria to grow and to begin induction. The L4440 vector without insert in HT115(DE3) is used as a negative control.

Part 2: Flow chart

Based on the above video and readings, build a flow chart of your proposed RNAi experiments by doing the following:

1. Write out the important steps for each of the two above methods. Draw lines between the equivalent steps of the two methods.
2. Decide which steps you think are necessary. Write down why each is needed (what is happening inside the bacteria and/or *Naegleria*). Discuss these with the instructor before proceeding.

3. For each group member: draw a flow diagram of the experiment, being sure to answer the following questions:
    a. How will you assess whether Naegleria “listens” to your dsRNA?
    b. How can you quantitate your results (often phenotypes are only partially penetrant, *i.e.* only some of the population will show an effect).
    c. What control(s) do you want to use. Why this one(s)? How will you interpret the results of the control(s)? What does success look like? Failure?
    d. What is going to be done on each day? Remember, after today we have 3 more lab days scheduled for this project (Full 3 hour lab period on Feb. 23 and Feb. 28, and two hours on March 2).
    e. What are you going to need each day? If you need to grow *Naegleria* and/or bacterial cultures, when do those need to be set up?
    f. When phenotyping Naegleria, what might make things easier (hint: how many cells did you start with last time? Was this enough to be efficient?).
4. Switch flow diagrams with someone from another group. Give honest/thoughtful feedback. Did the other person answer the above questions?
5. Based on your flow diagram, write out a protocol (one per group), including date(s) for each step, what you will need for each step, and what volumes (and/or numbers of plates) you want to use (you may need help deciding on this, but please take a stab at it first).

Part 3: Start your engines (errr… cultures)

Depending on your proposed protocol, you might need to start cultures before next class. Now might be a great time to do this!