RNAi Day 2 and Molecular toolbox

Before class, please watch the following videos and participate in the forum:

1. GFP tagging: https://www.youtube.com/watch?v=wVuA-EpV64c
2. Sequencing (watch at least the first 12 minutes): https://www.youtube.com/watch?v=8n2LvJ-m0n0
3. Antibodies: https://www.youtube.com/watch?v=68T-QUIEp_o&

Today's missions:

1. Continue with your RNAi experiment. For many of you, this involves induction of your dsRNA producing bacterial strains.
2. Finalize your experimental plan for next week. Give Lil a list of materials you will need (THIS IS VITAL!).
3. Complete the molecular toolbox flow chart (instructions below).

Instructions for the Molecular toolbox Flowchart:

I have designed this activity to give you an idea of how molecular biologists mix and match approaches to execute the experiments that answer specific biological questions. One way to think about this is based on three categories:

1. Starting material.
There are many possibilities here, but today we will limit ourselves to three:

a. Whole, intact cells
b. Purified DNA
c. Purified protein

2. Analytical methods.
These can be used singly, or in combination (think restriction digest, then gel electrophoresis). Again, there are many options, but today we will focus on the following:

a. PCR
b. Restriction digest
c. GFP tagging
d. Gel electrophoresis
e. Incubation with antibodies (e.g. a Western blot OR immunofluorescence).

3. Detection methods.
As above, we will only consider a subset of possibilities:

a. Staining
b. Sequencing
c. Fluorescence microscopy

Under this framework, experimental design simply requires choosing a starting material, choosing one or more analytical methods, and a method for detecting the final output.

When designing such experiments, it's important to keep in mind what the requirements are for each analytical method, and what they actually can tell you. Begin your flow chart by completing the following table (I have completed the first row to give you a concrete example:

Analytical method	**Required materials/knowledge specific to your experiment (input)**	**What does the method give out (output)**
PCR	Starting DNA template Sequence specific primers	Lots of copies of the DNA template sequence from between where the primers bind
Restriction digest		
GFP tagging		
Gel electrophoresis		
Antibody incubation		

Now comes the fun part: mixing and matching analytical and detection methods to answer specific scientific questions! The next step of your flowchart is diagram ***two different*** approaches to answering each of the following questions. Each diagram should include what the starting material is, what will be the input to each analytical method used (again, some approaches use a single analytical method, others use several), what the output from each analytical method is, and at the end, how you will observe the results (detection).

1. I *think* I successfully cloned my favorite gene into an expression plasmid. Did I?
2. How much protein are my cells expressing from my favorite gene?
3. Where does the protein encoded by my favorite gene localize in a cell?