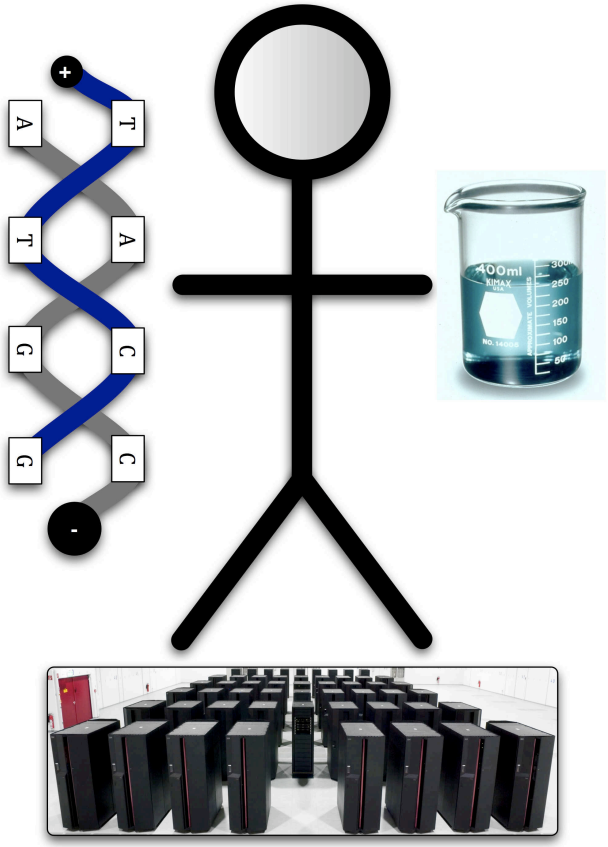
Research Progress Report IV

PCR Optimization Exercise or Perl Exercise, Gene Deletion & Verification by PCR, RNA-seq Experimentation & Library Preparation

Functional Genomics Research Stream • Freshman Research Initiative • by Dr. Patrick J. Killion and Dr. Travis B. White



EID:	
Assigned:	March 9, 2012
Due:	3 Dates (details inside)
Submission:	Results Central
Format:	Online

Evaluation Guidelines		
Section	**Possible**	**Earned**
Research Progress Report IV - PCR Optimization Exercise or Perl Exercise	200	
Research Progress Report IV - Update II	200	
Research Progress Report IV - Update III	200	
Late Penalty	-	
Final Grade	600	

Part 1 - Introduction

Now that you know the basics of growing yeast cultures, harvesting genomic DNA and RNA from these cultures, performing basic PCRs and running gels, we're going to advance to addressing a common problem in molecular biology research: a failed PCR result. Here you will change the PCR conditions of a non-productive reaction with the goal of forming the intended DNA product. Familiarity with productive PCR manipulations will help you in optimizing PCRs and RT-PCRs in your future research.

Alternatively, you may instead focus on the computational aspect of the Functional Genomics Research Agenda. To become familiar with a computer language often used in bioinformatic studies, you may do an exercise in the computer language Perl based on an excellent online tutorial.

Option 1 - PCR Optimization Exercise

If you wish to engage in the PCR Optimization Exercise for this Research Progress Report IV, please follow the instructions below.

There is a box in the freezer labeled RPR4 PCR Box.

In this you will find:
25 mM $MgCl_2$
Taq 10x buffer - (minus) $MgCl_2$
Act1L F 10 μM Tm = 51°C
Act1L R 10 μM Tm = 58°C
dNTPs 10 mM each
Taq 1U/μl
yeast genomic DNA (120 ng/μl)

These primers (Act1L F and Act1L R) amplify a 1.5 kb product from genomic DNA.

Each component has been aliquoted into several tubes (labeled 1-9 on the side). **Write in your lab notebook which tube number for each component you used.**

Please setup the following 20 μl reaction:

2 μl - 10x buffer
0.4 μl - dNTPs (10 mM each)
0.4 μl - Act1L F (10 μM)
0.4 μl - Act1L R (10 μM)
0.5 μl genomic DNA (120 ng/μl)
0.2 μL Taq (1U/μl)
0.5 μl $MgCl_2$ (25 mM)
15.6 μl dH_2O

Also setup a no template control (NTC) - identical except dH_20 instead of genomic DNA

Run it in the thermocycler with these conditions:

94°C 5:00

_____
25 cycles of:
94°C 0:30
58°C 0:30
72°C 1:30

_____
72°C 10:00
4°C hold

This thermocycler program is already in the machines under the name **TWPCR**.

Please make a **master mix** for your PCR and the NTC of your PCR when setting up these reactions. The master mix should include water and Taq polymerase.

Although this reaction **should not** give any product, please set it up.

Now, please set up three additional reactions - each with an alteration to a condition in the PCR specified above. At least one reaction should change a PCR reaction condition and at least one reaction should change a thermocycler setting. The third reaction may be a combination of the first two condition changes or a new condition change. These changes should have the goal making your 1.5 kb product. Please include no template controls for these reactions too. You may use the Fermentas PCR protocol as a guide, if you wish.

http://www.fermentas.com/templates/files/tiny_mce/coa_pdf/coa_ep0401.pdf

You may run the non-productive PCR specified above in the thermocycler along with your other reactions. (This is only applicable when you are changing PCR reaction conditions only. When you change thermocycler conditions you will need to place the reaction in a different thermocycler or at a different time from the non-productive PCR run specified above). Please analyze all reactions using gel electrophoresis.

Here's a checklist for the 8 reactions you will set up:

PCR	✔	No Template Control (NTC) PCR	✔
PCR specified above		NTC for PCR specified above	
PCR with change to reaction condition		NTC for PCR with change to reaction condition	
PCR with change to thermocycler setting		NTC for PCR with change to thermocycler setting	
PCR with combination of both changes above, or different change		NTC for PCR with combination of both changes above, or different change	
		Post to **Results Central**	

Post to Results Central

Please post your experimental design, rationale for the condition/thermocycler changes you chose, and results (gel picture and description of what's in each lane) on Results Central. **In your report, make note of the**

findings from other students that have preformed this exercise. State general conclusions from your results **and others' results** as to what condition changes contribute to making a non-productive PCR become productive. The due date is April 1, but please post earlier than that if you have the data. This will help the students posting after you by contributing to their results discussion.

- **DUE DATE**
  Sunday, April 1, 10:00 PM
  Use the title: *Research Progress Report IV - PCR Optimization Exercise*
  Will be evaluated on Monday, April 2.

Option 2- Perl Programming Exercise

If you wish to engage in the Perl Programming Exercise **instead of** the PCR Optimization Exercise, please follow the instructions below.

Please read Chapters 12-15 of the **course website** textbook.

Then, read the following online tutorial:

http://www.perl.com/pub/2000/10/begperl1.html

Be sure to do each example and run each Perl program on the interpreter (Cygwin for PCs, Terminal for Macs).

At the end of the online tutorial there is a program calculating compound interest.

Your assignment is to use this compound interest program as a foundation for writing a program that calculates the number of DNA molecules made for each cycle of a PCR. You may use the following guidelines, but are welcome to change these variables and explain your changes, if you'd like.

Starting molecules of DNA: 100
Amplification efficiency: 100% (each molecule of DNA gets doubled with each cycle)
Number of cycles: 30

Post to Results Central

Please post your experiences learning Perl. Explain in your own voice how the compound interest calculator works and how you approached taking this framework to come up with your PCR program.

- **DUE DATE**
  Sunday, April 1, 10:00 PM
  Use the title: *Research Progress Report IV - Perl Programming Exercise*
  Will be evaluated on Monday, April 2.

Part 2 - Introduction

Given our transition from training to research, gone are the enormous packets, questions, long written procedures and sign-off requirements. You are now expected to manage your research progress on a day-to-day, week-to-week basis. We will continue to use *Results Central* as a tool by which you as researchers share your experiences and overall progress.

Your research updates must contain the following sections (clearly **bolded** in your post):

- **Projects Engaged**
- **Results Achieved**
- **Open Questions**

Projects Engaged

A summation of the various projects you have engaged. No details here - just a list of problems you have addressed.

Results Achieved

This will be by far the longest section. This section includes all the details of your work. In this section you should order your work temporally and separate experiments that ask different questions. Do not just go through your day to day procedures. I can read protocols. I know how PCR, RT-PCR, transformations, library preparation, etc work and I do not need details of protocols here. Sentences like "*I added 1 mL of Lysis Buffer to ....*" are not the least bit interesting to me. I want to know which experiments you are performing, why, and the results achieved. I require specifics: details of the work you engaged, how the experiments deviated from normal and most importantly - the results. **I expect to see all important data from your experiments.**

I want you to present this data in the form most understandable by anyone who might read your post (tables, graphs, etc). All reactions should clearly include details such as primers used, the parameters of the PCR program utilized and any deviations from normal. All gel images should have a very clear summary of what is loaded into each well of the gel and any other specifics that might be of interest. I should clearly understand which strain with which you are working. I should clearly understand experimental decisions you may make (the use of pre-clearing, modifications to the normal PCR program, etc). **I should clearly understand your work and should not have to ask you questions relative to important details.**

Open Questions

You should always have open and unaddressed questions with respect to your work. Be critical. Think hard. Ask questions that might guide your next step or at least interest the audience.

Progress Reports

Starting in mid-March you will be fully trained and focused upon research progress in the lab. You will be making individual transcription factor deletion strains in several species of yeast. With these strains you will engage RNA-seq experimentation. During this time you will be accounting for your work and productivity by posting to *Results Central*. I expect you to **make THREE independent *Blog Postings*** to *Results Central* during this *Research Progress Report*.

When do I want you to make these ***Blog Posts***? Specific dates.

- **FIRST UPDATE DUE DATE**
  Sunday, April 15, 10:00 PM
  Use the title: *Research Progress Report IV - Update I*
  Will be evaluated on Monday, April 16.
- **SECOND UPDATE DUE DATE**
  Sunday, April 29, 10:00 PM
  Use the title: *Research Progress Report IV - Update II*
  Will be evaluated on Monday, April 30.

What do I wish for you to Blog Post? See the text earlier in this section for complete details on the **required contents** of a good progress report. Please note that fulfilling expectations is extremely important in the reporting of scientific results to an advisor. I will be looking closely at **both the content of your reports and whether you have clearly followed the instructions provided**.

Evaluation of Reports

It is important to note that the total point value for *Research Progress Report IV* is 600 points (**200 points per posted update and 200 points for your research proposal**). I expect these updates to be clear, complete, readable, **scientifically voiced**, proof-read and truly account for **two weeks of your work** at a time. Given that your laboratory hours are 6 hours per week I will evaluate each report asking myself "*Does this look like 12 productive, focused hours of preparation work, results and analysis?*". A single update will likely take **at least two hours** of your time to produce. **You can use lab time.** Do not simply throw up the first thing that comes to mind, a few scattered details and hope that it will be enough for decent credit. It will not. Finally, please also note that these updates will be significantly easier (even somewhat trivial) to produce if you keep a **clear, complete, readable and accurate laboratory notebook**.

Example Report

There is an example research progress report posted on *Results Central*. Search for **"Example Progress Update"** (include the quotes in your search).