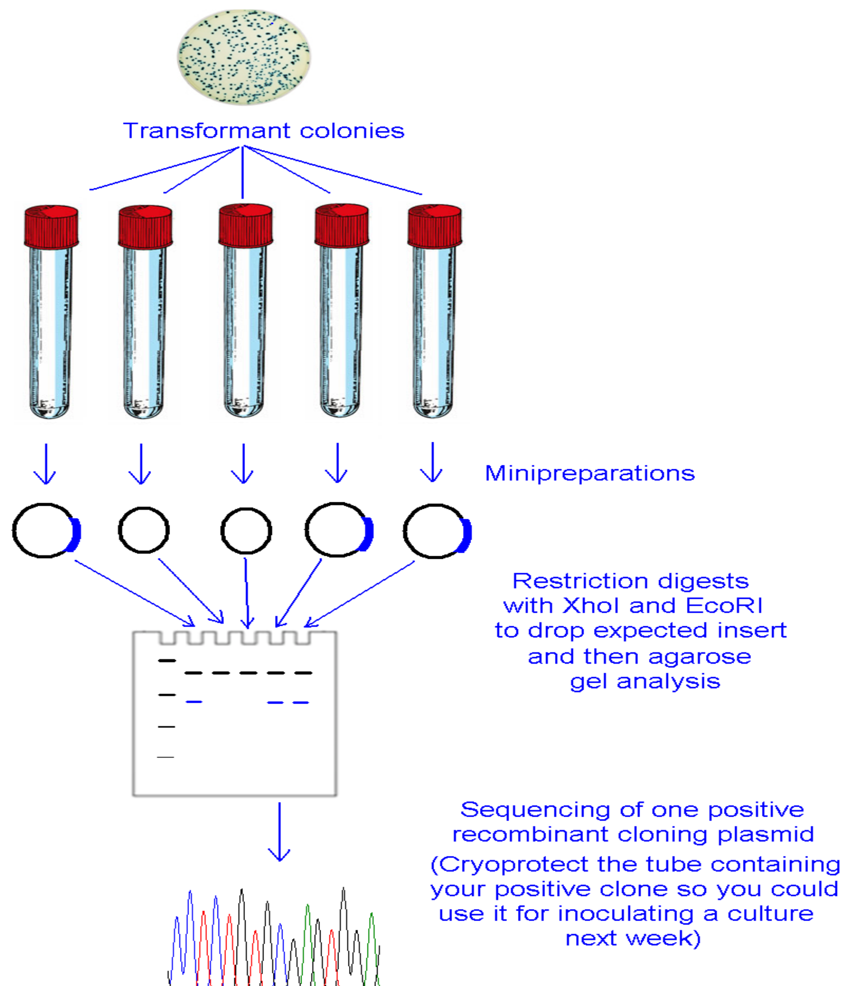
Laboratory Class 4

SCREENING FOR RECOMBINANT PLASMID AND DNA SEQUENCING

Your challenge for this week is to finalize the sub-cloning procedure of the T7 insert in pTrcHisB. You will first inoculate and screen five transformant colonies to confirm the presence of the expected recombinant plasmid, pTrcHisB/T7 – you will hopefully obtain at least one positive transformant colony out of your five inoculates. You will then sequence the T7 insert from your positive clone to identify the point mutation that had been introduced in the parent plasmid you received at the beginning of the semester.

The positive transformant cell line you will identify this week, i.e., the one for which the expected insert will be confirmed, will be put aside and used for the second part of the course that will relate with the expression, purification and functional assessment of your T7 RNA polymerase mutant.



LEARNING OBJECTIVES

Underlying molecular principles

- Identify and explain the main procedural steps for the (mini)preparation of plasmid DNA by alkaline lysis
- Identify and explain the main procedural steps for DNA sequencing

Hands-On Skills

- Inoculate a liquid culture with a transformant colony
- Isolate plasmid DNA (miniprep)
- Digest recombinant plasmid DNA to screen for a given DNA insert
- Sequence DNA
- Cryopreserve a cell culture in glycerol-supplemented liquid medium

Analytical Skills

- Analyze and discuss transformation results
- Refer to the positive and negative transformation controls to discuss the efficacy of the ampicillin screening system
- Discuss the efficacy of ligation based on the number of colonies obtained for the experimental treatment (transformation treatment I) and the positive control for transformation (Transformation treatment VI)
- Estimate the transformation yield in # colonies/ug plasmid DNA
- Select appropriate restriction enzymes to screen for the presence of a DNA insert in recombinant plasmids
- Use Nebcutter to simulate restriction digests and predict the size of the expected DNA fragments
- Use nucleotide BLAST to analyze DNA sequencing results
- Assemble the individual sequencing results to reconstruct the whole sequence of your DNA insert coding for T7 RNA polymerase

TO BE INDIVIDUALLY HANDED IN TO YOUR TA WHEN ENTERING THE LAB
(/10 marks)

1. Predict the expected DNA bands for the XhoI/EcoRI digest of (1) pTrcHisB and (2) pTrcHisB/T7 (your recombinant plasmid vector with the T7 RNA polymerase insert) using NebCutter. You should first generate the exact sequence of your recombinant plasmid vector, and then paste this sequence into NebCutter. Don`t forget to take into consideration the ligation details. Explain your reasoning and insert a copy of your NebCutter results. (5 MARKS)

2. Figure out how to prepare the Master mix to be used for the digest of your five minipreps knowing that one reaction requires the followings:
   - 19ul of H2O
   - 5ul of minipreparation
   - 3ul of 10X **REACT 2**
   - 1.5ul of XhoI at 10 U/ul
   - 1.5ul of EcoRI at 10U/ul

   Show your calculations and justify your reasoning. (1 MARK)

3. Plasmid copy number
   - What is a plasmid copy number? What is a reasonable range for the copy number value to expect for the pTrcHisB/T7 plasmid you will purify in this lab? (1MARK)
   - Estimate the copy number for someone obtaining a concentration of 100ng/ul for his purified plasmid (see step 26). You can assume that there were 10^9 cells/ml of the liquid cell culture used for the miniprep. Your calculations should take into consideration all the dilution steps to be performed during lab 4. (3MARKS)

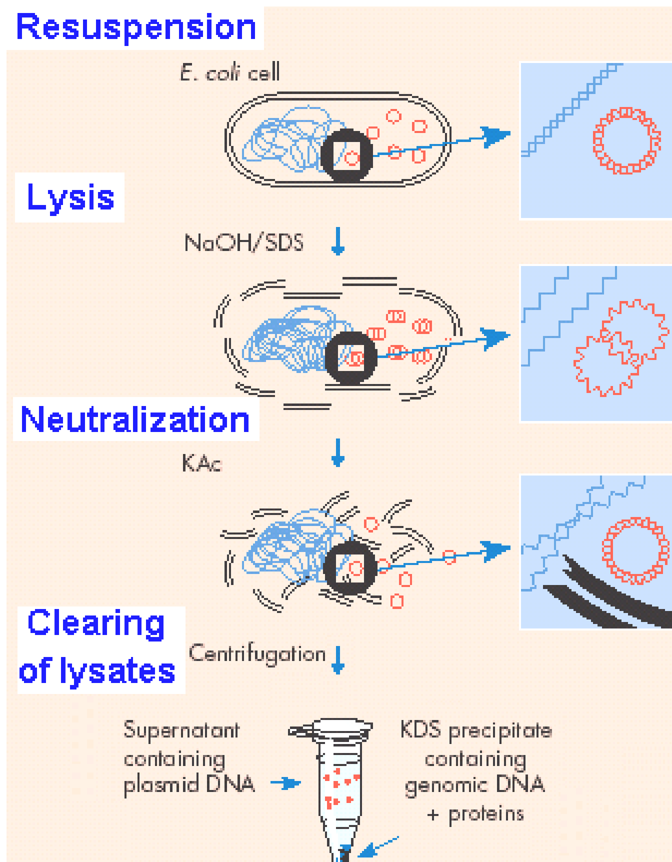
Don`t forget that you are expected to submit a formal lab report for the sub-cloning part that ends up with this laboratory. Your report can be submitted either on an individual basis or in groups, and it should contain the following sections: title, abstract (0.5 page maximum), introduction (limit of 2 pages, double-spaced), materials and methods (1-2 pages, double-spaced), results (no page limits, half a page per figure or table), discussion (2-3 pages, double-spaced), and references. Lab reports are to be organized as described in *A Guide to Writing in the Sciences.* Your lab report should be double-spaced using arial 12 text format. A template file with standard cover page and text format is available from the course website.

Your lab report is to be submitted in person to the coordinator at your arrival in the lab for the practical exam during the week of Nov 1-4.

BACKGROUND

A. Minipreparation of plasmid DNA

You will isolate plasmid DNA using a small scale or minipreparation protocol that was first designed by Dr. Birnboim, a cross-appointed member of the Biochemistry, Microbiology and Immunology department. The research article in which the miniprep procedure was first described (Nucleic Acids Res 1979, 7:1513) still constitutes one of the most commonly referred article nowadays in science. The miniprep protocol permits the rapid isolation of small amounts of plasmid DNA (1-10ug). Plasmid isolation procedures make use of the covalently closed circular nature of bacterial plasmids and their small size in relation to the bacterial chromosome. In the alkaline lysis method that you will be using, bacterial cells are lysed in a solution containing NaOH and sodium dodecyl sulphate (SDS). Effective lysis of bacterial cells is a key step in plasmid isolation and it directly affects DNA yield and quality. The alkaline conditions (pH 12-12.5) denature both the chromosomal DNA and the plasmid DNA and SDS denatures proteins. The solution is then neutralized with potassium acetate. Under these renaturing conditions, the plasmid DNA, whose two strands remained intertwined during the alkaline lysis, rapidly reanneals. The chromosomal DNA cannot renature as quickly and is therefore trapped along with proteins in an insoluble complex. The precipitate is removed by centrifugation and the plasmid is precipitated from the supernatant through the addition of ethanol. Typical yield for the alkaline lysis protocol is about 1-2ug of plasmid DNA per ml of liquid culture.



Alkaline lysis protocol for plasmid purification. Figure modified from Qiagen.

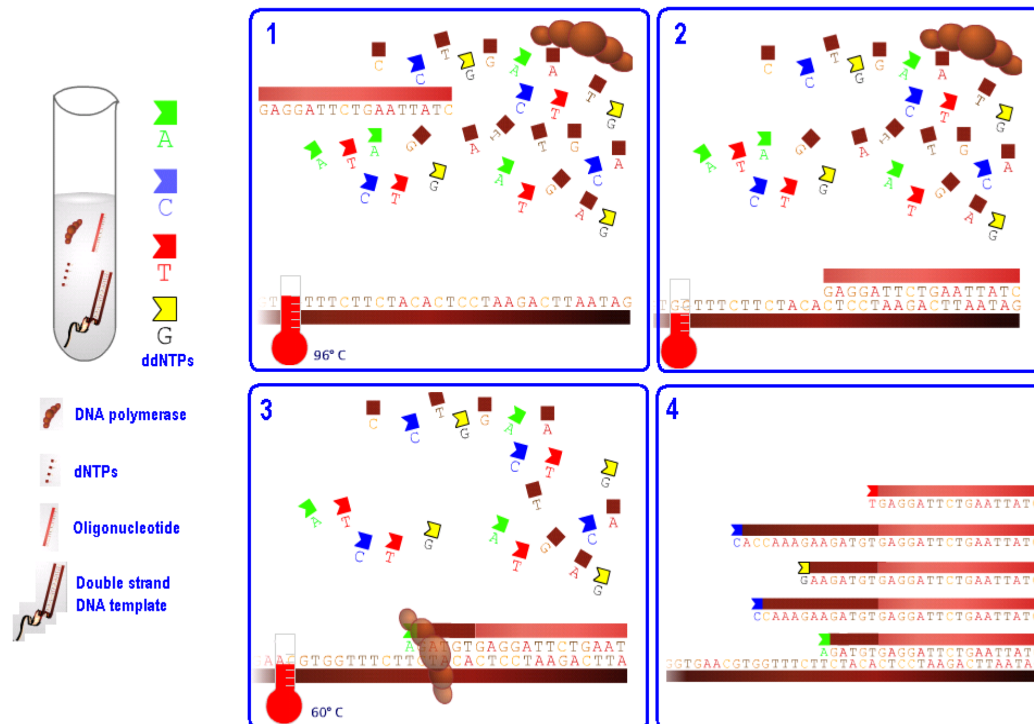
B. DNA Sequencing

Different methods can be used for sequencing DNA. In this lab, you will be using the enzymatic base-specific chain termination procedure, which is essentially a 'special' PCR amplification. The PCR and the sequencing reactions will be performed at the McGill University`s Sequencing Center and the sequencing results will be posted, usually within one week, through the Nanuq server.

Step 1: PCR amplification

In the enzymatic sequencing procedure, the DNA to be sequenced is denatured and annealed with a single primer that delineates the origin of sequencing. One peculiar feature of PCR-based sequencing is the use of a mixture of regular deoxynucleotides (dNTPs) and altered dideoxynucleotides (ddNTPs) whose hydroxyl groups on 2'C and 3'C of the deoxyribose sugar have been substituted with H`s. These ddNTPs, which are found in much lower proportions than the dNTPs, can bind to DNA polymerases and be transferred at the 3' end of the elongating strand, but they strictly prevent the addition of any further nucleotides upon their incorporation. All PCR amplicons are therefore characterized by a single terminator ddN at their 3' ends, and the identity of the chain-terminating nucleotide can be determined by tagging each ddNTP with a distinctive dye. In the next figure, for instance, a blue dye was specifically attached to the ddCTPs.

Applied Biosystems introduced BigDyes^{TM}, which consist of a fluorescein energy donor dye directly linked to an energy acceptor dichlororhodamine dye. Together the dyes make up an energy-transfer system that provides significantly greater sensitivity compared to single dyes. BigDyes also exhibit improved color resolution, a property that allows better base-calling. The additional sensitivity conferred by energy transfer dyes means that less template is needed for a sequencing reaction, a feature that is valuable for sequencing very large templates and even for direct sequencing of genomic DNA.



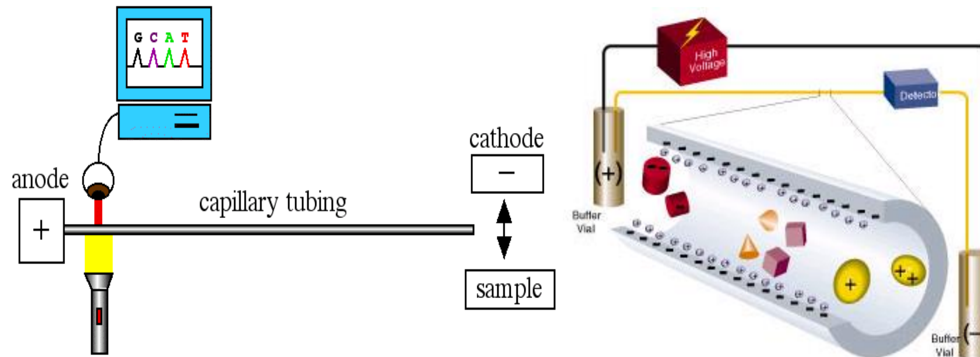
The reagents that are needed for a sequencing reaction are shown at the left. The double strand DNA template is first denatured by heat (1). Then the temperature is lowered so that the oligonucleotide primer could hybridize to its complementary sequence (2). The free 3' hydroxylated group is recognized by DNA polymerase and elongation occurs until the addition of a dideoxynucleotide stops the reaction (3). Several fragments are generated with each one having a ddNTP at its 3' end (4). Figures modified from the animation at http://www.dnai.org/b/index.

Step 2: Analysis of PCR amplicons by capillary electrophoresis

A mixture of DNA fragments with different lengths is obtained upon PCR amplification, but another step is required to resolve the fragments and identify their terminal ddNs. Capillary electrophoresis (CE) chromatography is commonly used for resolving the mixture of amplicons generated by PCR-based sequencing. In CE, DNA separation is achieved in a fused-silica capillary with a 25-100 um diameter. The high ratio of surface area to volume of the small capillary tube serves to efficiently dissipate heat produced during electrophoresis, allowing the use of higher electric fields that can decrease the run time and improve DNA resolution. High voltages (up to 30 KV) are used to separate molecules based on differences in charge, size and hydrophobicity.

Step 3: Automated base-calling

The time profile of the fluorescence signal (color and intensity) is recorded at the output of the capillary tube throughout the analysis run of the PCR sequencing product. The fluorescence signal is then analyzed with a computer to determine the DNA sequence (this procedure is usually referred as base-calling analysis: a nucleotide is assigned to each fluorescence peak based on its fluorescence spectrum).



Basics of Capillary Electrophoresis (CE) DNA Samples enter the tube from the right and travel to the left to the detection system that records the chromatogram output on a computer. The magnified view at the right depicts the capillary interior with molecules of different size and charge separating. For DNA separation, a solution of polymer, which serves as a molecular sieve, is added into the capillary (capillary gel electrophoresis). This allows solutes having similar charge-to-mass ratios to be resolved by size. Figure on the right obtained from Beckman Coulter.

PROCEDURES

EXPERIMENT A: Inoculation of Transformant Colonies into Liquid Culture Medium

Experiment A shall be completed between 8-10:30am on the day preceding your regular laboratory class, except for Monday sections that are to return to the lab on the preceding Thursday morning. You should plan about 30 min to complete the work. You should therefore arrive in the lab by 10am at the latest to make certain you can exit the lab by 10:30am.

You will inoculate five transformant colonies from your transformation treatment I into five culture tubes. Pick colonies that are well delineated to avoid cross contamination among neighboring colonies. Inoculated transformant cells will grow and divide rapidly (up to ~10^9 cells/ml of liquid broth) and each cell will contain several copies of the recombinant plasmid vector.

1. First count your transformant colonies.

Treatment	Transformation	DH5α cells (μl)	Plasmid DNA source (μl)	Number of colonies
I	Doubly digested pTrcHisB and doubly digested PCR amplicon (ligation treatment I)	100	40	
II	pTrcHisB without any insert DNA (negative control for ligation: treatment II)	100	40	
III	pTrcHisB digested with XhoI (positive control for ligation: treatment III)	100	40	
IV	1st negative transformation treatment without DNA (40ul of H_2O is added)	100	0	
V	2nd negative transformation treatment with doubly digested pTrcHisB (aliquot of 40ul put aside at step 5 of Laboratory Class 2)	100	40	
VI	Positive transformation treatment with undigested pTrcHisB (aliquot of 40ng in 40ul put aside at step 1 of Laboratory Class 2)	100	40	

2. You need five polystyrene snap cap tubes (17mm X 100mm) each containing 3ml of LB broth with 100ug/ml ampicillin.

3. Examine your agar plate for transformation treatment 1 and identify five well delineated colonies. Use a marker to identify the five colonies to be inoculated.

4. Use a sterile plastic tip to slightly touch the external surface of a colony, and then simply drop the tip into a culture tube containing 5ml of LB broth with ampicillin (200ug/ml). Repeat the inoculation procedure for your four other colonies.

5. Quickly vortex your culture tubes and put them back on a rack. The Support Staff will transfer all tubes in a shaking incubator (200 rpm) at 37°C.

EXPERIMENT B: Minipreparation of plasmid DNA (miniprep)

In this experiment, you will purify plasmid DNA from five different transformant colonies. Two important procedural details are:

- **Before you begin with the miniprep, it is important that you put aside 0.5ml of each of your five liquid cultures (step 7) because one of them is to be cryopreserved (step 23) and used for protein expression next week. In Experiment C, you will perform a screening analysis using restriction enzymes. The results of this screening analysis will allow you to determine which one(s) among your five transformant colonies contain(s) the expected pTrcHisB/T7 recombinant plasmid.**
- **Do not discard any of your five plasmid preparations; make sure you keep them on ice until the end of the laboratory class. Not only you will have to digest part of your five minipreps in Experiment C, but you`ll also have to retrieve one miniprep to be used as template for DNA sequencing in Experiment D (the screening step to be done with restriction enzymes in Experiment C will allow you to define which miniprep is suitable for sequencing).**

6. Recover and vortex the five culture tubes, which you inoculated yesterday, to resuspend the cells.

7. Transfer 0.5ml of each of your five culture tubes into a distinct 1.5ml microcentrifuge tube.

Those five microfuge tubes are to be kept on ice until you could identify, by restriction digest (Experiment C), which one(s) contain(s) the expected pTrcHisB/T7 recombinant plasmid. Once you have identified at least one positive clone, you will retrieve the appropriate tube and proceed to its cryopreservation as described at step 23.

8. Centrifuge your five polystyrene culture tubes at 3,000g for 30 sec using the centrifuge with a swinging bucket rotor. Discard the supernatant.

9. Add 150ul of Resuspension Buffer into each of your tubes, and then vortex at high speed to resuspend the pellets. Once the pellets have been **completely resuspended**, transfer the content of each polystyrene culture tube into a pre-labeled 1.5 microcentrifuge tube.

10. Add 150ul of Lysis Buffer into each of your 1.5 tubes and mix the content by inverting the tubes five times. Describe how the properties of the samples have changed.

Do not vortex, shake or incubate for more than 5 min to minimize shearing of genomic DNA. Breakdown of genomic DNA into small fragments is not desirable as the smaller fragments might be extracted along with plasmid DNA.

11. Immediately add 150 ul of K-acetate buffer into each of you tubes. Mix thoroughly by inverting your tubes 3 times.

12. Centrifuge your tubes at maximum speed for 5 min to pellet cell debris and chromosomal DNA.

13. Use a 1ml pipettor to transfer about 75-80% of the supernatants into clean 1.5 microcentrifuge tubes. Avoid transferring any of the white precipitate. If some of the precipitate gets transferred, it can be removed with a pipet tip.

14. Add 900ul ethanol 95-99% pre-cooled to -20°C. Mix well by shaking.

15. Centrifuge at maximum speed for 5 min.

16. Discard the supernatants. Add 1ml of 75% ethanol in each tube and rinse the pellets by vortexing for 5-10 sec (pellets do NOT have to be completely resuspended). Centrifuge again at maximum speed for 5 min then invert the tubes over absorbent paper for two minutes to drain out the remaining ethanol.

After the 75% ethanol wash, the pellets tend to weakly adhere to the bottom of the tubes: be careful not to lose your plasmid DNA pellets!

17. Resuspend each pellet in 50ul of water.

EXPERIMENT C: Analysis of the Minipreparations by Restriction Digest and Cryopreservation of One Positive Clone

18. Prepare a Master mix to digest your five minipreps knowing that one single reaction requires 3.0ul of a miniprep and the followings:
    - 21ul of H_2O
    - 3.0ul of 10X **REACT 2**
    - 1.5ul of XhoI at 10 U/ul
    - 1.5ul of EcoRI at 10U/ul

19. Mix well each tube and incubate for at least one hour at 37°C.

20. Cast a 1% agarose gel with a 20-well comb. Two groups can share one gel as follows:
    a. Lanes 1-10: 5 digested and 5 undigested samples from 1st group
    b. Lane 11: 5ul of the Alpha Quant DNA ladder
    c. Lanes 12-20: 5 digested and only 4 undigested samples from 2nd group.

Each TA also determines one volunteer to cast an extra 1% agarose gel to be used in step 25.

21. Prepare your samples to be loaded on the agarose gel as follows:
    a. Digested samples (5X): Mix 13.5ul of each digest with 1.5ul of 10X loading buffer
    b. Undigested controls (4-5X see note below): Mix 1.5ul of each miniprep with 12ul of H_2O and 1.5ul of 10X loading buffer (1.5ul of an undigested miniprep represents approximately the same DNA amount as 13.5ul of its digested preparation: band intensities should be similar).

One of the two groups sharing a gel will only be able to load 4 undigested control (see step 20).

22. Load 15ul of each digested or undigested miniprep sample along with 5ul of the DNA ladder, and then carry out electrophoresis at 100V for about 40 minutes. Take a picture of your results.

23. Refer to your gel picture to identify one positive colony whose band profile corresponds to the expected bands for pTrcHisB/T7. This positive miniprep is to be used as a template for DNA sequencing (Experiment D). You should also retrieve the transformant cell line corresponding to your positive miniprep among the five cell lines that were put on ice at step 7. You will require this cell line next week for the protein expression procedure. Cell cultures can be cryopreserved for long periods of time if frozen at -70ºC in presence of 25% glycerol. Add the appropriate volume of glycerol to your positive cell culture (0.5ml) to reach a final concentration of 25% glycerol, invert a few times to homogenize the content, and then hand in your tube to your TA who will store it at -70ºC.

EXPERIMENT D: DNA Sequencing

Each sequencing reaction can accurately sequence about 600 to 800 bp. In this experiment, you will proceed to five sequencing reactions to ensure the full coverage of your T7 RNA polymerase insert containing about 2,655bp. Once the five sequencing results will be returned to you, your challenge will be to examine the overlaps among the five sequencing results to deduce the complete sequence of your insert. The assembly process of different sequencing results is commonly referred to as gene assembly.

The five sequencing primers you will use for priming DNA sequencing are:

- Seq F1: 5'CACTCGACCGGAATTATCG3'
- Seq F2: 5'GGTCTTCGTGGCATAAGGAAG3'
- Seq F3: 5'GTTCAGGACATCTACGGGATTG3'
- Seq R1: 5'GAACATCAGACCCTTGCC3'
- Seq R2: 5'CTGCCGCCAGGCAAATTC3'

24. Retrieve the miniprep corresponding to your positive transformant and purify it using the QIAQuick Spin column as explained in Appendix I, except that only 35ul of water should be used for the final elution volume instead of 50ul (see step 6 in Appendix I). This modification will ensure a higher concentration of the purified plasmid DNA to meet the minimum concentration requirement for DNA sequencing.

The procedure for DNA sequencing is relatively straightforward, but very sensitive. The amount of DNA template is critical: either a too low or a too high concentration of DNA can significantly reduce the number of nucleotides that can be read or sequenced. To ensure a good estimate of the DNA concentration of your purified miniprep product to be sequenced, an aliquot will be electrophoresed along with a quantitative DNA ladder (AlphaQuant1). Your miniprep DNA could also be

quantitated by absorbance reading at 260nm, but that approach would require too much of your purified 50ul sample.

25. Combine 2ul of your purified plasmid to be sequenced with 8ul of water and 1ul of the 10X loading buffer in a microcentrifuge tube. Mix, and then load the whole volume on a 1% agarose gel. Electrophorese at 100V for about 40 min.

26. Take a picture of your gel and accurately estimate the concentration of your purified recombinant plasmid DNA.

DNA sequencing will be performed at the McGill and Genome Quebec Innovation Centre in Montreal. Samples are to be labeled as Day_lab#_Group#_Mutant#_Primer. You will be asked to enter your sample names in an electronic file to be directly sent to the sequencing centre along with your samples. The sample names you enter are the ones to be used for posting the sequencing results.

27. Samples are to be loaded on a 96 well plate with 1 row for each group. Load 5ul of your purified DNA plasmid in each of the first five wells of your lane, and 10ul of the appropriate primer in each of the next five wells.

The minimal concentration required for proceeding to DNA sequencing is 85ng/ul. If your concentration is below that minimum threshold, you will have to borrow the sequencing results of another group having worked with the same mutant number.

Well	Sample name (5ul @ 85-500ug/ul)	Primer (10ul @ 5uM)	Project name
A01	Monday_202_Group8_Mutant3_PrimerSeqF1	SeqF1	
A02	Monday_202_Group8_Mutant3_PrimerSeqF2	SeqF2	
A03	Monday_202_Group8_Mutant3_PrimerSeqF3	SeqF3	
A04	Monday_202_Group8_Mutant3_PrimerSeqR1	SeqR1	
A05	Monday_202_Group8_Mutant3_PrimerSeqR2	SeqR2	
A06	SeqF1		
A07	SeqF2		
A08	SeqF3		
A09	SeqR1		
A10	SeqR2		
A11			
A12			

TIMELINE

OVERVIEW WITH TA

- Pre-lab questions are handed in to the TA
- Pre-lab quiz
  - **20 min (20 min)**

EXPERIMENT A: Inoculation of Liquid Cultures with Transformant Colonies

- **To be done the day before your regular laboratory class**
  - Experiment A shall be completed between 8am and 10:30am on the day preceding your regular laboratory class (Monday sections should get back on the previous Thursday morning to do their inoculation). You should plan about 30 min to complete the work. You should therefore arrive in the lab by **10am at the latest** to make certain you can exit the lab by 10:30am.

EXPERIMENT B: Minipreparations

- **90 min (110 min)**
  - Agarose gel to be cast
  - Preparation of Master mix for restriction digest

EXPERIMENT C: Analysis by Restriction Digests of the Minipreparations

- **130 min (240 min)**
  - Digest
    - Pre-assigned teaching
  - QIAQuick Spin column to purify one positive miniprep
  - Electrophoresis
    - Pre-assigned teaching

EXPERIMENT D: DNA Sequencing

- **60 min (300 min)**
  - DNA quantitation by agarose gel electrophoresis
  - Set up sequencing reactions

IN LAB PERFORMANCE

- **To be discussed with your TA before leaving the lab**
  - Transformation results
    - Positive and negative ligation controls
    - Positive and negative transformation controls
    - Ratio of numbers of colonies for the two dilutions on agar plates without ampicillin should be consistent with the ratio of the two dilutions performed
    - Experimental treatment
  - Technical quality of agarose gels (resolution of markers)
  - Quality of minipreps : the supercoiled conformation should predominate
  - Quality of restriction digests : the supercoiled conformation should be negligible if complete digest
  - Quality of the purified miniprep : only one visible band on the gel and reasonable yield

QUESTIONS/PROBLEMS

Underlying molecular principles

1. **DNA sequencing** Briefly explain how and where sequencing is initiated. Which DNA strand is to be sequenced with (1) a positive and (2) a negative primer (the sense or the antisense strand)? Will the primer sequence be included in the sequencing results? Are the amplified products generated during sequencing single- or double-stranded?

2. **DNA sequencing** The PCR amplification of a sequencing reaction results in linear amplification, though conventional PCR amplification with two delineating primers gives exponential amplification. What does linear amplification mean? How many strands of DNA are amplified from a one single double-stranded template after 20 cycles of (1) a conventional PCR amplification and (2) a sequencing PCR amplification?

Analysis of results

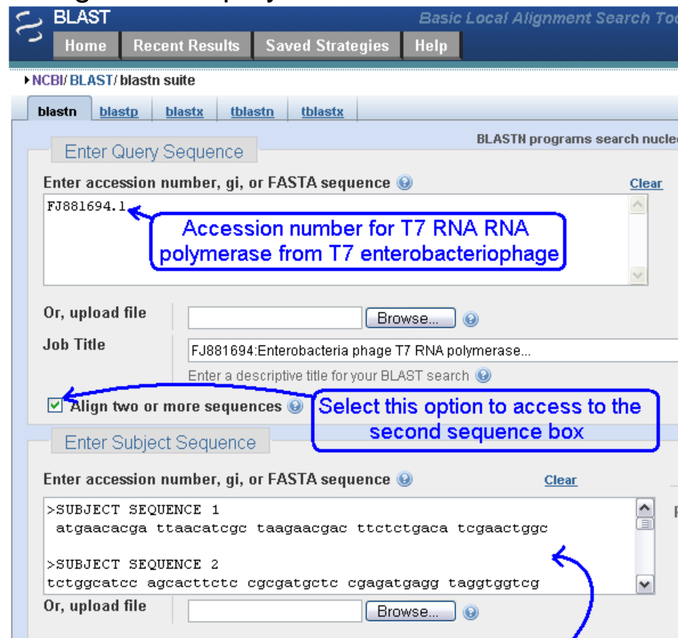
For next week, you do not have to hand in a hard copy of your answers to the questions listed in this section. Don't forget, however, that your first formal lab report is due by Nov 1-4.

3. **Ligation controls**
   a. Did some of the pTrcHisB plasmid doubly digested with XhoI and EcoRI recircularize for the negative control performed without any insert DNA? If yes, explain what might have happened?
   b. Out of 100 digested plasmid molecules, would you expect more successful ligations for your positive control than your experimental treatment? Why? Are the experimental results in agreement with that?
   c. What can you conclude from the results of your positive ligation control?

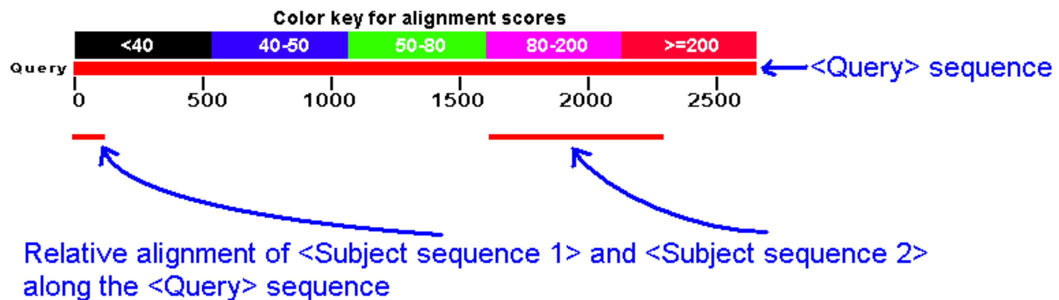
4. **Transformation controls**
   a. What was the purpose for having a negative transformation control with the XhoI digested pTrcHisB? Are the results similar for your two negative transformation controls? If not, why? What can you conclude from your transformation controls?
   b. Your positive transformation control was plated on LB-agar with and without ampicillin. Why these two conditions were both assessed? What's the information you can derive from each treatment?
   c. Refer to your positive transformation control to estimate the percentage of competent cells that were successfully transformed. For the purposes of this question, you can assume that a cell culture contains about 10^9 cells per ml. Explain your reasoning.
   d. Refer to your positive transformation control to estimate the transformation yield in colonies per ug of plasmid vector DNA. How does your transformation yield value compare with the expected transformation yield, which is 10^6-10^7 colonies per ug of plasmid DNA?

5. **Ligation success rate**
   a. How many of your five colonies you analyzed contain the expected product, pTrcHisB/T7?

6. **Assembly of overlapping DNA sequences**
   a. NCBI`s BLAST multiple alignments. The top and bottom figures respectively illustrate the alignment options and a set of returned alignment results. In this example, the two DNA stretches do not overlap at all as they align at two distinct regions along T7 RNA polymerase



Descriptive is introduced after a > sign and the DNA sequence pasted on the next line(s). It is possible to insert as many sequences as possible, but each one should be delineated from the previous one by a text descriptive (> blablab...)



Relative alignment of <Subject sequence 1> and <Subject sequence 2> along the <Query> sequence

   b. Did you successfully sequence the whole length of your DNA insert coding for T7 RNA polymerase? If not which part(s) were not sequenced? How would you proceed to sequence the missing part(s)?

7. **DNA sequencing**
   a. **BLAST alignment** Access to nucleotide-nucleotide alignment to align one of your assembled sequencing result. Proceed first by keeping the default reference database, i.e., <Human genomic + transcript>, and then repeat the same alignment using <Others>. Do you get the same results for both alignment options? How can you be sure that the alignment hit is from the proper organism which, in your case, should be the T7 bacteriophage?
   b. Can you identify the point mutation that had been inserted in your DNA insert coding for T7 RNA polymerase?

Reading/viewing materials for teaching topics

8. **Groups 5 and 13**: Specificity of the DNA sequencer, Applied Biosystems model 3730. This is the sequencer model to be used at McGill for sequencing your DNA.

9. **Groups 6 and 14:** Emerging DNA sequencing technologies for human genomic medicine (Drug Discov Today 2008, 13-14:569). Discuss the differences between Sanger versus pyrosequencing technologies.

REFERENCES

- Miniprep: Nucleic Acids Res 1979, 7:1513
  - Resuspension buffer
    - Tris HCl (50mM, pH8.0)
    - EDTA (10mM) 5.0ml
  - Lysis buffer
    - SDS (1%)
    - NaOH (0.2N)
  - Neutralization buffer
    - Potasium Acetate (3M)
    - Acetic Acid: 11.5ml of glacial acetic acid in a final volume of 100ml

- McGill`s sequencing facilities: https://genomequebec.mcgill.ca/nanuq