5.36 Biochemistry Laboratory
Spring 2009

5.36 Lecture Summary #5 (shortened) Thursday, March 5, 2009

CI-M assignments: The first draft of your mini-review is due today.

Prior to the March 10th CI-M lecture please read: Gorre, M. E. et al. Clinical Resistance to STI-571 *(STI-761 = Gleevec)* Cancer Therapy Caused by BCR-ABL Gene Mutation or Amplification. *Science* **293**, 876-880 (2001). The article is posted on steller.

Next Laboratory Session: #9

Topic: Site-Directed Mutagenesis and Transformation

Overview of what you've completed in Module 4 and what will come in Module 5:

Module 4 (Sessions 1-8)

- Expressed H396P Abl(229-511) in BL21-DE3 expression cells
- Purified, dialyzed, concentrated, and quantified your expressed protein
- Visualized the purified H396P Abl(229-511) by SDS-PAGE gel analysis
- Isolated wt Abl kinase domain vector DNA for mutagenesis in Module 5 (and checked for the expected insert by restriction digestion)
- Designed mutagenesis primers.

Module 5 (Sessions 9-15)

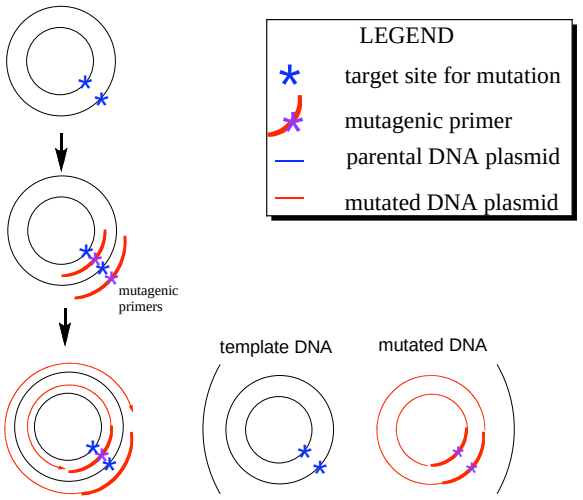
- Use Quikchange mutagenesis to create vector DNA of your selected mutant
- Isolate your mutant Abl(229-511) DNA and prepare it for DNA sequencing
- Perform kinase activity assays on the wt and H396P Abl kinase domains in the absence and presence of kinase inhibitors (Gleevec and Dasatinib)
- Analyze crystal structures of wt and mutant Abl kinase domains complexed with inhibitors

I. QUIKCHANGE MUTAGENESIS (Sessions 9-11)

Modified from the QuikChange handbook (http://www.stratagene.com/manuals/200518.pdf).

Step 1: plasmid preparation
(session 2)

Step 2: thermal cycling (_____)
(session 9)



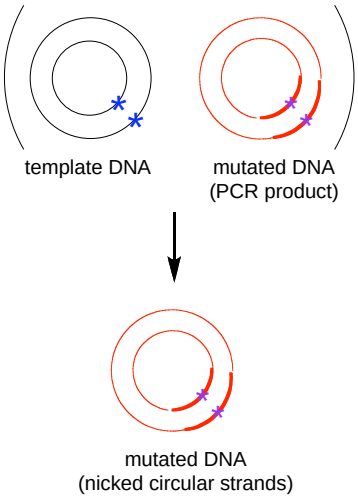
Note: For PCR using the Quikchange method (versus standard PCR replication of a DNA fragment)

- Instead of replicating small (____-____ kb) fragments, replicate entire plasmid
- Need a much more powerful polymerase that has higher fidelity than Taq
- Use Pfu Turbo (isolated from *pyrococcus furiosus*)
- 3′ to 5′ _____ gives Pfu Turbo higher fidelity.

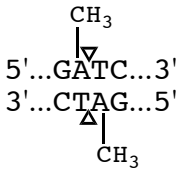
A standard PCR Mutagenesis program is as follows:

95 °C for 30 sec
16-20 cycles of
    95 °C for 30 sec (DNA denaturation)
    55 °C for 1 min (_____)
    65 °C for 2 min per kb of plasmid (_____)
Hold at 10°C

Step 3: Digest the methylated, non-mutant DNA template with Dpn1. (*session 10*)



Dpn1 is a restriction enzyme that cleaves within a _____ recognition sequence.



In many types of bacteria (including DH5-α *E. coli*) methylase enzymes add CH_3 groups to specific sequences of DNA. This originated as a primitive bacterial immune system to differentiate native from foreign DNA.

In the Quikchange mutagenesis procedure, this means that the template DNA that was amplified in DH5a cells is methylated, but the PCR product is not. Dpn1 digests away the _____ (methylated) DNA.

Step 4: Transformation *(session 10)*

Bacterial transformation is the uptake of _____ DNA by bacteria. Naked DNA refers to DNA that is not associated with cells.

In is rare for bacteria to display natural *competence* (the ability to uptake DNA) in a laboratory setting. To facilitate transformation, scientists have engineered artificially competent bacteria that are passively permeable following heat shock or electroporation.

We will be using heat-shock (or _____)- competent cells in Session 10.

Mutant plasmid (with nicks)

Chemi-competent *E. Coli* (DH5-α cells)

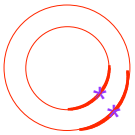
E. Coli transformed with plasmid (nicks repaired)

Heat-shock competent cells are prepared by incubating chilled cells with divalent metal ions to increase cell permeability. We will use commercially available chemi-competent cells, but many labs prepare their own cells.

After incubation with plasmid DNA, briefly heating the cells (ie. At ____ °C for 45 s) results in uptake of the foreign DNA plasmid.

It is essential for transformation success that the cells are heated for the optimized time depending on the cell preparation, since the goal is to maximize DNA incorporation while minimizing cell destruction.

Step 5: Isolation of the mutant DNA *(session 11)*



In Session 11 you will isolate the mutant DNA by miniprep and prepare samples for DNA sequencing.

*Note that Quikchange can be hit or miss; both skill **and** luck are required for it to work on the first try. If you don't get any colonies following Session 10, select a colony from another group to isolate in Session 11. This will increase the chances that the class isolates a perfect plasmid.*