7.02 Fall 2001

RDM

Recombinant DNA methods

Agenda

DAY 6

- differences between AG1111 and BL21 strains
- another case discussion about ligation results

Differences between AG1111 and BL21 strains

• Why are we doing another transformation today? Why didn't we just transform into BL21 in the first place instead of using AG1111? Let's review the differences between these two strains of *E. coli.*

AG1111	BL21
high transformation efficiency	low transformation efficiency
does not express T7 RNA Polymerase	does express T7 RNA polymerase
normal complement of proteinases	proteinase-deficient

• Transformation efficiency is the main reason we didn't use BL21 to start with. When we did our first transformation, we were using plasmid DNA in the form of a ligation mixture. This mixture contained all kinds of fragments of DNA in various conditions: some of it was still the linear DNA that we'd added. It's probably safe to say that only a small fraction of it was intact circular plasmid. To ensure that we get a good number of transformants from such a low concentration of plasmid DNA, it was crucial to use a bacterial strain with a high transformation efficiency.
• The DNA we are using in today's transformation is the result of a miniprep; it is a pure preparation of plasmid DNA in high concentration. Because of this, we can get away with using a bacterial strain with a lower transformation efficiency and still expect to see a good number of colonies.

• Remember that the reason we require T7 RNA Polymerase for expression of GFP is that the GFP gene was inserted into pET ahead of a T7 promoter.
• Using a proteinase-deficient strain increases the chances successfully and stably expressing GFP. It allows it to stick around longer for us to watch it glow green under UV light the next morning.

Another strange case to explain

• Many of the Tues/Thurs groups saw a strange result on their analytical plasmid miniprep redigestions. In their control minipreps (just pET), they used XbaI and EcoRI, and they saw two bands: a 4000-bp band representing the vector backbone, and a 600-bp band representing the default "insert" that pET has between its XbaI and EcoRI sites. So far, this is what we expect.
• In all six of their "v+i" minipreps, they also the 4000-bp backbone band. But instead of a 1000-bp band representing the GFP insert, these groups all saw a 2700-bp band.

• What could this represent? I believe the simplest explanation is that the 2700-bp fragment is the backbone of pUGFP. How did this get into the ligation? I think it happened on Day 1 and resulted from cutting out the incorrect band from the pUGFP digest they ran out on their low-melt gels. As you'll recall, there were two bands in that digest, one from the GFP insert (1 kb) and one from the rest of the plasmid (2700 bp), essentially just pUC19.
• How could we validate this theory? It is a useful exercise in understanding restriction mapping to draw out this chimeric plasmid and demonstrate what bands you'd get from digesting it with SspI and XbaI. This digestion would yield a particular set of bands that none of the other constructs we discussed would yield.

• Why should it be surprising that this chimeric plasmid can be propagated in bacteria? Presumably it has two replication origins, one deriving from pET, and the other from pUGFP/pUC19. This might have been expected to destabilize the plasmid in bacteria. Apparently it doesn't.