DNA Engineering: M1D3 Lab Talk

20.109 (F10)
09.21.10
Why run this gel?
Your objective is a 1:4 vector:insert ratio – Why?

What if it was 1:100?
What if it was 100:1?
How do you figure out how to get a 1:4 molar ratio?
Agarose Gel Electrophoresis

SAFETY NOTES:
• Use nitrile gloves
• Need face shield when excising DNA bands from gel

NEXT STEPS:
1. Q-kit to melt agarose, isolate DNA
2. Remove aliquot to check recovery on gel:

   Bkb: _______________________

   Insert: ______  ______  ______
Figure + legend FNT assignments…

“Litmus test”: Figure must be able to stand alone, away from results text, and be interpretable

Descriptive Legends or Captions: To pass the "acid test" above, a clear and complete legend (sometimes called a caption) is essential. Like the title of the paper itself, each legend should convey as much information as possible about what the Table or Figure tells the reader:

- what results are being shown in the graph(s) including the summary statistics plotted
  - the organism studied in the experiment (if applicable),
- context for the results: the treatment applied or the relationship displayed, etc.
  - location (ONLY if a field experiment),
- specific explanatory information needed to interpret the results shown (in tables, this is frequently done as footnotes)
  - culture parameters or conditions if applicable (temperature, media, etc) as applicable, and,
  - sample sizes and statistical test summaries as they apply.
- Do not simply restate the axis labels with a "versus" written in between.

http://abacus.bates.edu/~ganderso/biology/resources/writing/HTWtablefigs.html
Figure 1. Cumulative germination of *Chenopodium* seeds after pregermination treatment of 2 day soak in NaCl solutions. 

$n = 1$ trial per treatment group (100 seeds/trial.)
Agarose gel electrophoresis of DNA isolated from yeast cell lysates (pre-IP). High quality DNA can be efficiently recovered from *S. cerevisiae* cell lysates using the ChIP DNA Clean & Concentrator™. Duplicate purifications were performed with 5, 15, and 30 μL cell lysate and an equal volume of eluted DNA was loaded into each lane. The size markers M1 and M2 are 100 bp and 1 kb ladders, respectively (Zymo Research).
Figure, example 2

(a) gel electrophoresis images showing the expression levels of centromeric repeats in different strains. 

(b) Bar graph showing the expression of centromeric repeats. 

- WT
- spt6-HhHAΔ
- nmt1-spt6-HhHAΔ

Fold increase over WT:
- WT (Spt6-TAP) 
- spt6-HhHAΔ 
- nmt1-spt6-HhHAΔ 
- ago1Δ 

Sample sizes: n = 2 for spt6-HhHAΔ, n = 4 for nmt1-spt6-HhHAΔ. 

Ponceau staining for protein loading control.
Figure 6. Derepression of centromeric repeats in HhHΔ is not due to decreased expression levels. (a) Western blot analysis of spt6+ levels demonstrates that Spt6-HhHΔ is expressed at a lower steady state level than wild-type Spt6, but is overexpressed by the nmt1 promoter in the absence of thiamine. Two exposures are shown: 1 m and 10 s, as well as a Ponceau stain loading control. (b) Real-time qPCR analysis of the dg repeats in these strains showed that they are derepressed comparably in spt6-HhHΔ strains when the protein is under- and overexpressed. This indicates that the silencing defect is not due to the reduced levels of Spt6-HhHΔ protein, and may be due to a specific role of the HhH domain.