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7.344 Directed Evolution: Engineering Biocatalysts  
Spring 2008

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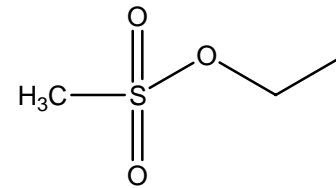
# Alternative methods for library generation

Lai, Y.-P.; Huang, J.; Wang, L.-F.; Li, J.; Wu, Z.-J. A new approach to random mutagenesis *in vitro*. *Biotech. Bioeng.* **2004**, *86*, 622-627.

Coia, G.; Ayres, A.; Lilley, G.G.; Hudson, P.J.; Irving, R.A. Use of mutator cells as a means for increasing production levels of a recombinant antibody directed against Hepatitis B. *Gene* **1997**, *201*, 203-209.

# Chemical mutagenesis

- How does mutagenesis occur?  
What do the authors use this method to study?
- What are the results?
- What are the benefits to using this method?
- Are there any pitfalls to this method?

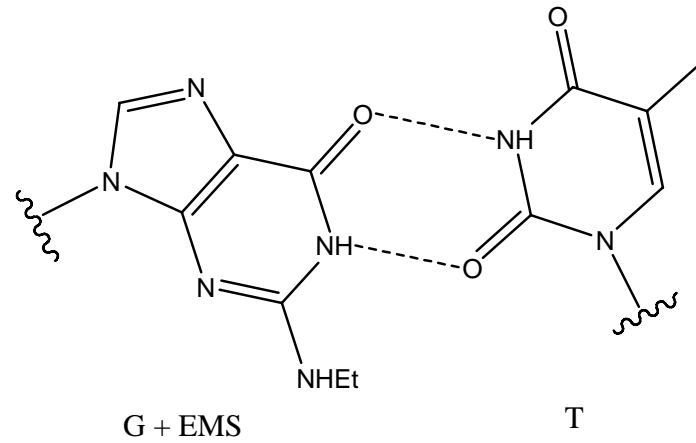
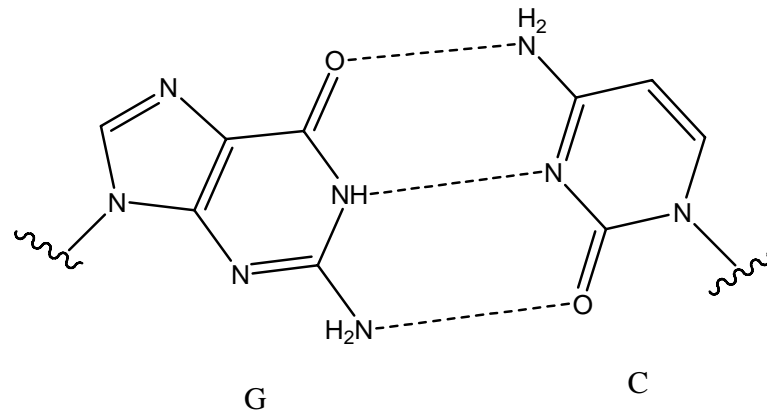


EMS

# Figure 1

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Please see Fig. 1 in Lai, Y.-P, J. Huang, L.-F.  
Wang, J. Li, and Z.-J. Wu. "A new approach to  
random mutagenesis *in vitro*." *Biotech. Bioeng.*  
86(2004): 622-627.

# EMS induced mutation



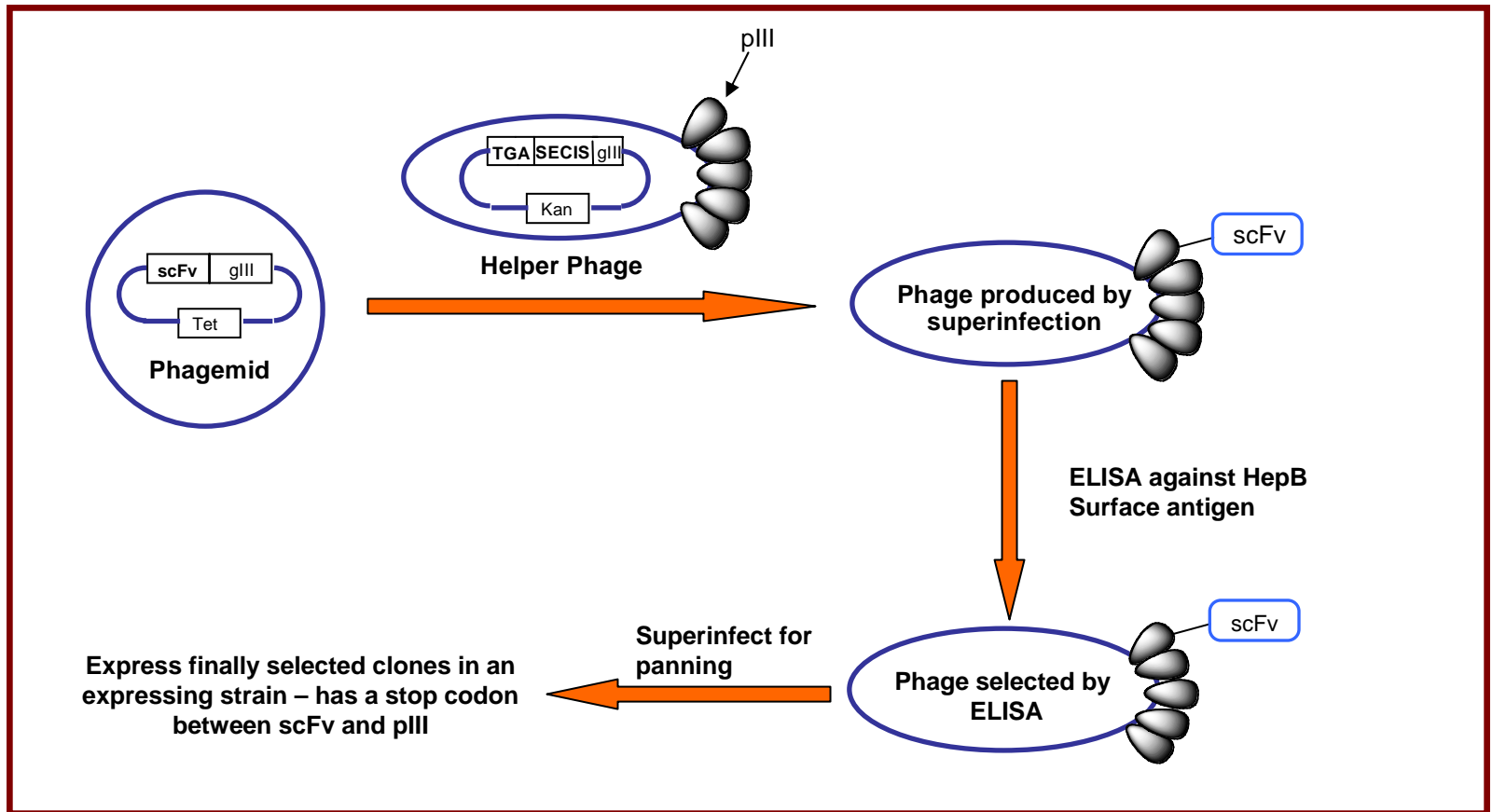
# Figure 2

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Please see Fig. 2 in Lai, Y.-P, J. Huang, L.-F.  
Wang, J. Li, and Z.-J. Wu. "A new approach to  
random mutagenesis *in vitro*." *Biotech. Bioeng.*  
86(2004): 622-627.

# Use of *E. coli* mutator strains

- What is the technique and how does it work?
- What are the authors trying to use this technique for? What are the results? (Figs 4-6)
- How could you calculate the mutational frequency for this method? (Hint: use Figure 4)
- What are the benefits to this method?
- What are the pitfalls?

# Scheme of technique



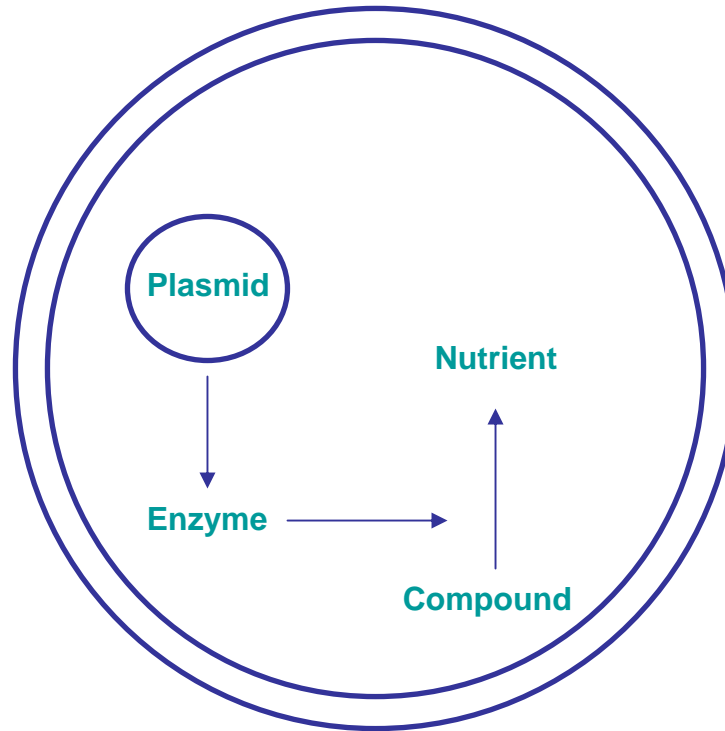


# Mutator strain results

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Please see Figs. 4 and 5 in Coia, G, A. Ayres, G. G. Lilley, P. J. Hudson, and R. A. Irving. "Use of mutator cells as a means for increasing production levels of a recombinant antibody directed against Hepatitis B." *Gene* 201(1997): 203-209.

# For next week...genetic complementation



# Aspartate 2-oxoglutarate aminotransferase



# Cephalosporin acylase

