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7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008

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Library generation by point mutation

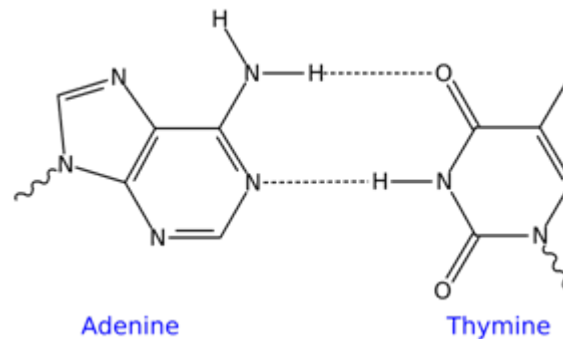
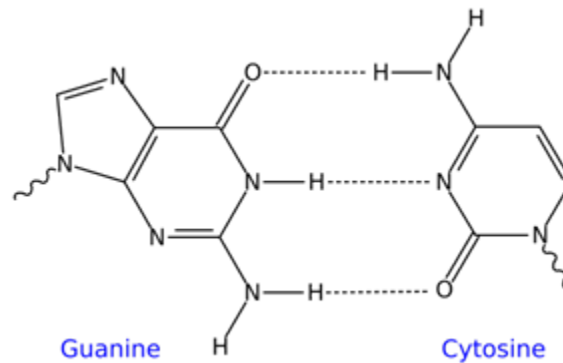
Cadwell, R.C.; Joyce, G.F. Randomization of genes by PCR mutagenesis. *PCR Methods Applic.* **1992**, 2, 28-33.

Kegler-Ebo, D.M.; Docktor, C.M.; DiMaio, D. Codon cassette mutagenesis: a general method to insert or replace individual codons by using universal cassettes. *Nuc. Acids Res.* **1994**, 22(9), 1593-1599.

PCR mutagenesis

- What is the method? What are the steps by which it is conducted?
- What are the results?
- What is mutational bias? Why is it a problem in library construction?
- What are the benefits to using this method?
- What are the pitfalls?

Watson-Crick base pairing



Guanosine tetraplex

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Please see http://www.imb-jena.de/images/groups/suehnel/suehnel_website_2b_big.gif.

H-DNA

Image of triple-stranded DNA removed due to copyright restrictions.

Table 1

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Please see Table 1 in Cadwell, R.C., and G. F.
Joyce. "Randomization of genes by PCR
mutagenesis." *PCR Methods Applic.* 2(1992): 28-
33.

Figure 3

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Please see Fig. 3 in Cadwell, R.C., and G. F. Joyce. "Randomization of genes by PCR mutagenesis." *PCR Methods Applic.* 2(1992): 28-33.

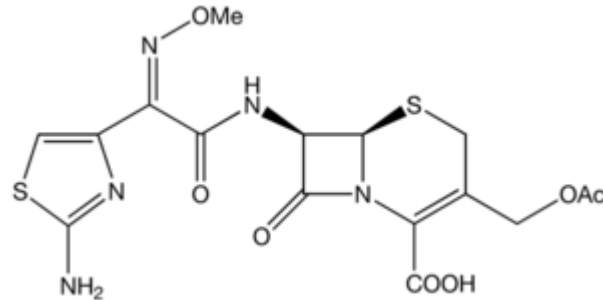
Codon cassette mutagenesis

- What is the method? What are the steps by which it is conducted? (Figure 1, Figure 2)
- What are the results?
- What are the benefits to using this method? When would you use this method?
- What are the pitfalls?

Figure 1 and 2

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Please see Figs. 1 and 2 in Kegler-Ebo, D. M., C. M. Dockett, and D. DiMaio. "Codon cassette mutagenesis: a general method to insert or replace individual codons by using universal mutagenic cassettes." *Nuc. Acids. Res.* 22(1994): 1593-1599.

For next week...



- TEM-1 β -lactamase – digests antibiotics like cefotaxime
- MIC (minimum inhibitory concentration) the amount of a small molecule that arrests bacterial growth
- Subtilisin E – a serine protease from *B. subtilis*