7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008
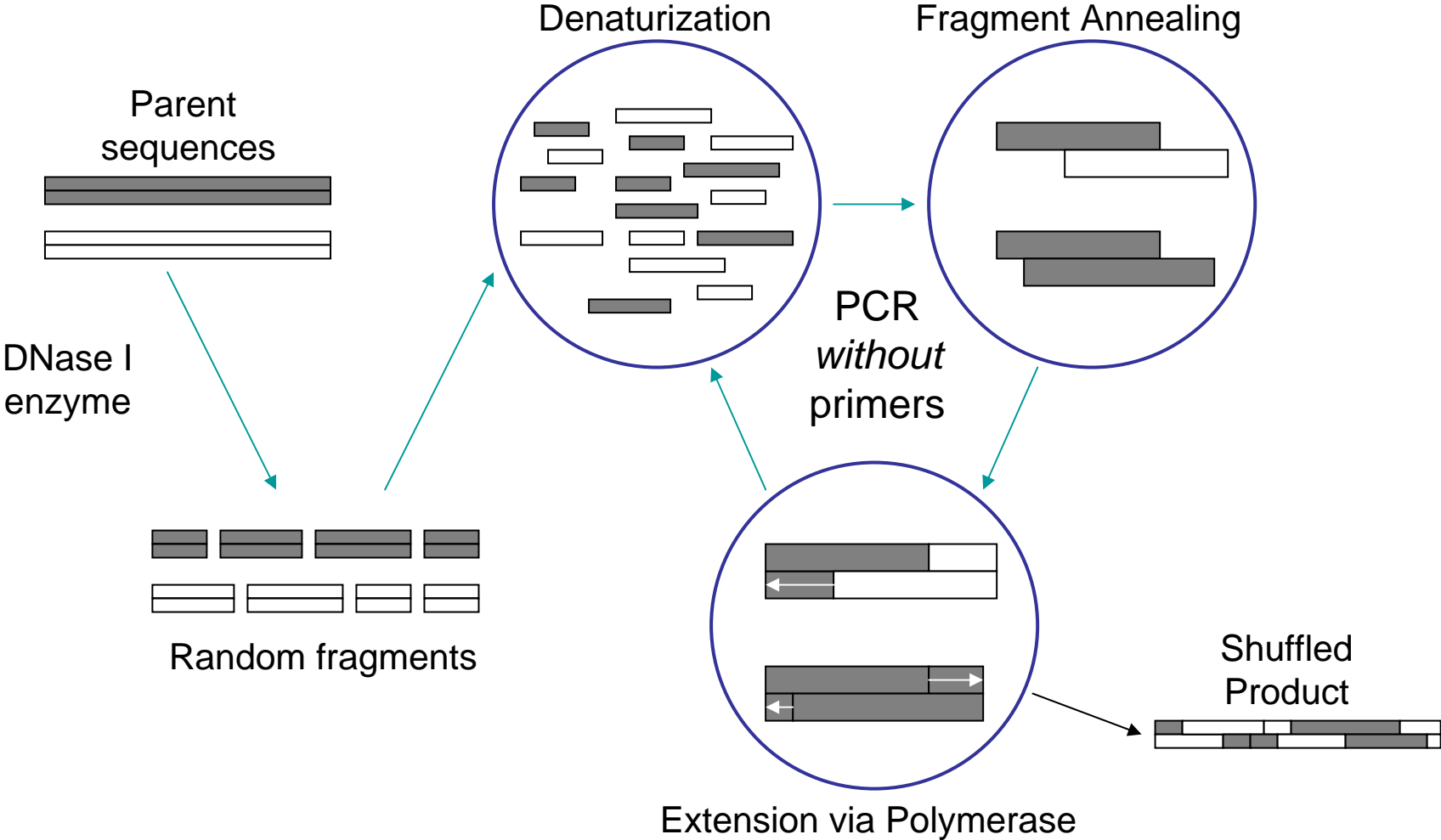
Library generation by recombination

Stemmer, W.P.C. Rapid evolution of a protein in vitro by DNA shuffling. *Nature* **1994**, *370*, 389-391.

Zhao, H.; Giver, L.; Shao, Z.; Affholter, J. A.; Arnold, F. A. Molecular evolution by staggered extension process (StEP) in vitro recombination. *Nature Biotechnol.* **1998**, *16*, 258-261.

DNA shuffling

- What is the method? What are the steps by which it is conducted?

- What are the results?

- How does it compare with other methods?

- What are the benefits to using this method?

- What are the pitfalls?

DNA shuffling

Denaturization

Fragment Annealing

Parent sequences

DNase I enzyme

Random fragments

PCR *without* primers

Extension via Polymerase

Shuffled Product

StEP (Staggered extension process)

- What is the method? What are the steps by which it is conducted?

- What are the results?

- What are the benefits to using this method?

- What are the pitfalls?

StEP

Image removed due to copyright restrictions. Please see Fig. 1 in Zhao, H., L. Giver, A. Shao, J. A. Affholter, and F. A. Arnold. "Molecular evolution by staggered extension process (StEP) in vitro recombination." *Nature Biotechnol.* 16(1998): 258-261.
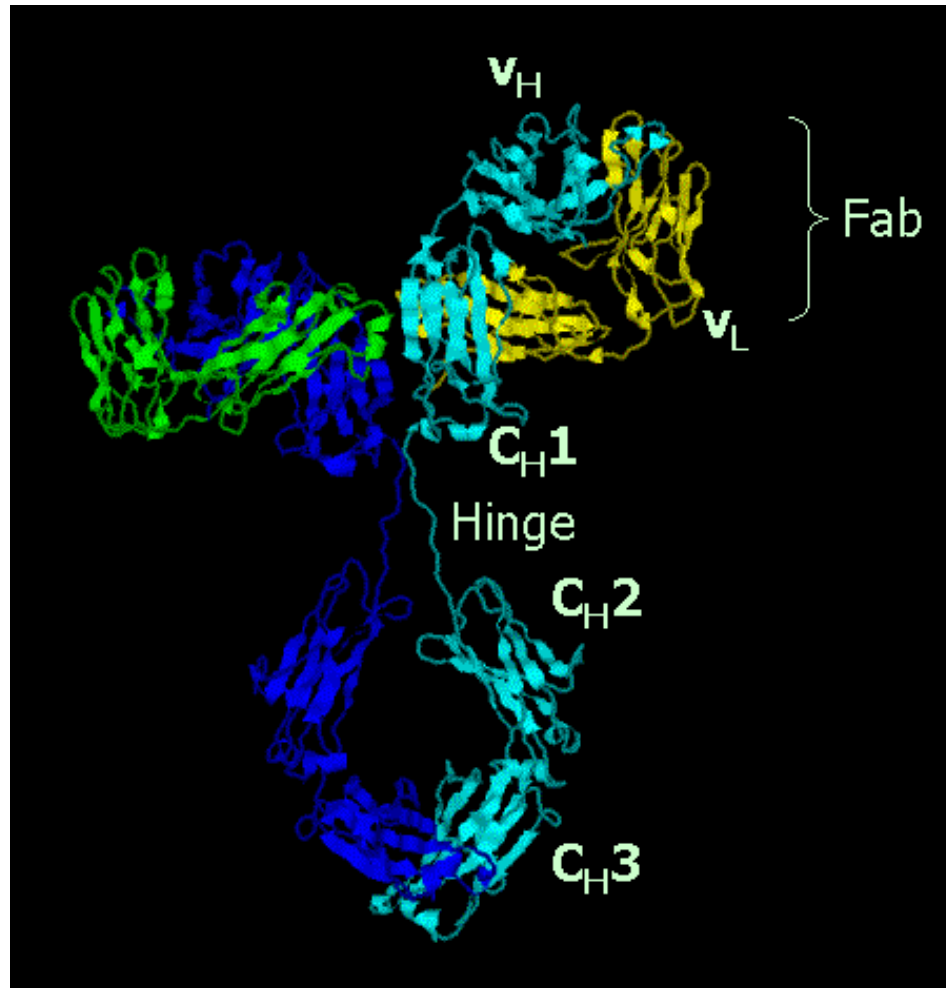
For next week…

- Zone of clearing assay
- Western blot
- Antibody structure/production
- Intro to phage display selection
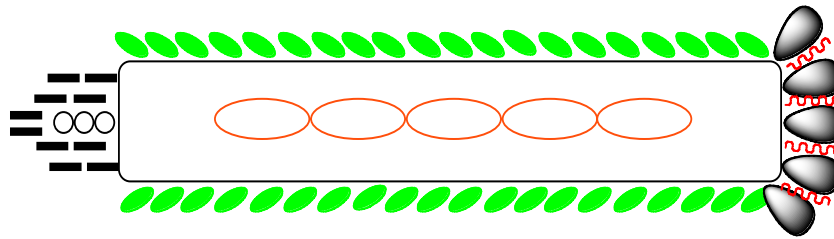
Antibody structure and function

Diagram of antibody removed due to copyright restrictions.

scFv

Intro to phage display

M13 phage particle

Phagemid

pVIII

pVII
+
pIX

ssDNA

pIII

pVI

Image removed due to copyright restrictions. Please see Fig. 2 in <http://www.biology-medicine.com/Technical-Articles/Molecular-Biology/2008-01-11/90.html>