7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008

Enzyme evolution using *in vitro* compartmentalization

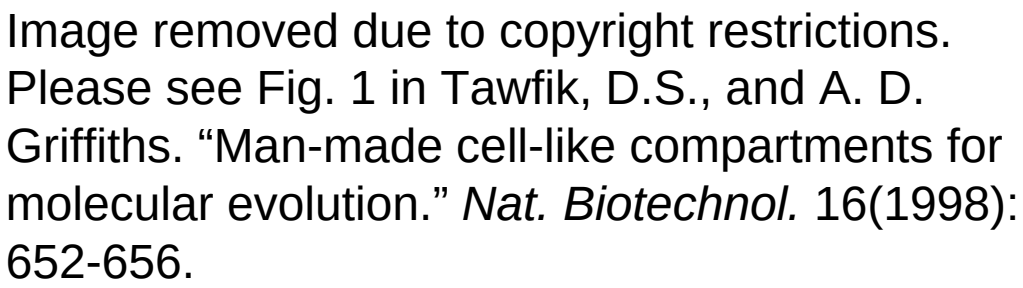
Tawfik, D.S.; Griffiths, A.D. Man-made cell-like compartments for molecular evolution. *Nat. Biotechnol.* **1998**, *16*, 652-656.

Griffiths, A.D.; Tawfik, D.S. Directed evolution of an extremely fast phosphotriesterase by in vitro compartmentalization. *EMBO J.* **2003**, *22*, 24-35.

In vitro compartmentalization

- What is the strategy presented by the authors for linking genotype and phenotype? (Figure 1, A)
- How are the compartments made? How big are they? What is the ratio of reaction volume to library size?
- What is the selection method?
- What sort of controls do the authors do to test their strategy?
- What are the results?
- What are the problems with this strategy?

IVC: The first generation



IVC controls (Figure 4, panel A)

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Results: Figure 4, panel B

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IVT: the second generation

- What enzyme is being examined? Why is this significant?
- What about the assay is different from the first paper? (Figure 1) Why do the authors make these changes?
- What are the controls used to test the strategy?
- What is the mutagenesis strategy?
- What are the results? Figure 5
- What are the pitfalls of this method?

Figure 1: Double emulsion-based IVC

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Figure 5: Controls

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Figure 8: Results

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