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7.344 Directed Evolution: Engineering Biocatalysts
Spring 2008

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Enzyme evolution using *in vitro* compartmentalization


**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

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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Please see Fig. 8 in Griffiths, A. D., and D. S. Tawfik. “Directed evolution of an extremely fast phosphotriesterase by in vitro compartmentalization.” EMBO J. 22(2003): 24-35.