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7.344 Directed Evolution: Engineering Biocatalysts
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Enzyme evolution using ribosome display

Amstutz, P.; Pelletier, J. N.; Guggisberg, A.; Jermutus, L.; Cesaro-Tadic, S.; Zahnd, C.; Pluckthun, A. *In Vitro* Selection for Catalytic Activity with Ribosome Display
J. Am. Chem. Soc. **2002**, *124*(32), 9396-9403.

Takahashi, F.; Funabashi, H.; Mie, M.; Endo, Y.; Sawasaki, T.; Aizawa, M.; Kobatake, E. Activity-based *in vitro* selection of T4 DNA ligase. *Biochem. Biophys. Res. Commun.* **2005**, *336*, 987-993.

Selection of β -lactamases using catalytic activity

- What limitations do the authors identify with in vivo or “partially” in vitro type selections?
- What is the strategy used by the authors? Does everyone understand IVT?
- What is the library size limitation?
- What sort of controls do the authors do to test their strategy?
- What are the results?
- What are the problems with this strategy?

Ribosome display

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Please see Fig. 1 in Amstutz, P., J. N. Pelletier, A. Guggisberg, L. Jermutus, S. Cesaro-Tadic, C. Zahnd, and A. Pluckthun. “*In Vitro* Selection for Catalytic Activity with Ribosome Display.” *JACS*. 124(2002): 9396-9403.

Mechanism of lactamase inhibition by suicide inhibitors

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Please see Fig. 2 in Amstutz, P., J. N. Pelletier, A. Guggisberg, L. Jermutus, S. Cesaro-Tadic, C. Zahnd, and A. Pluckthun. “*In Vitro* Selection for Catalytic Activity with Ribosome Display.” *JACS*. 124(2002): 9396-9403.

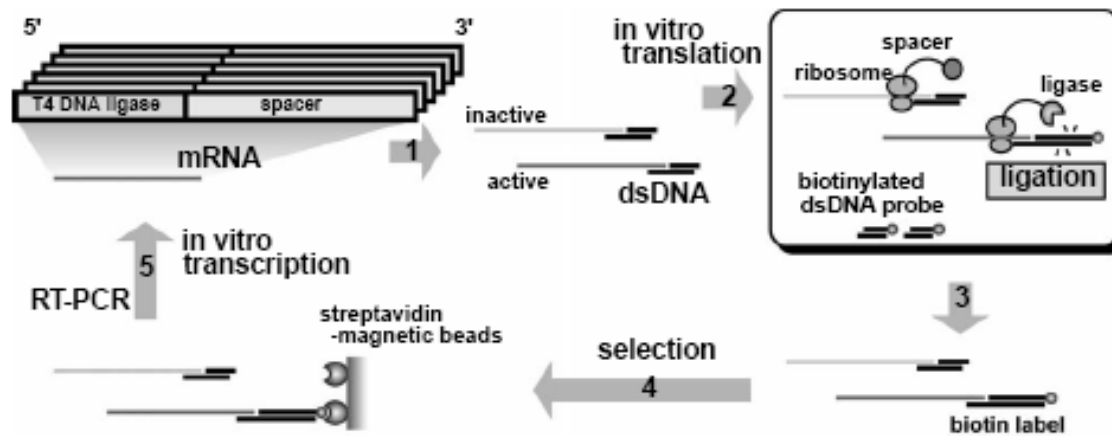
Figure 6: Enrichment and controls

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Please see Fig. 6 in Amstutz, P., J. N. Pelletier, A. Guggisberg, L. Jermutus, S. Cesaro-Tadic, C. Zahnd, and A. Pluckthun. “*In Vitro* Selection for Catalytic Activity with Ribosome Display.” *JACS*. 124(2002): 9396-9403.

T4 DNA ligase selection

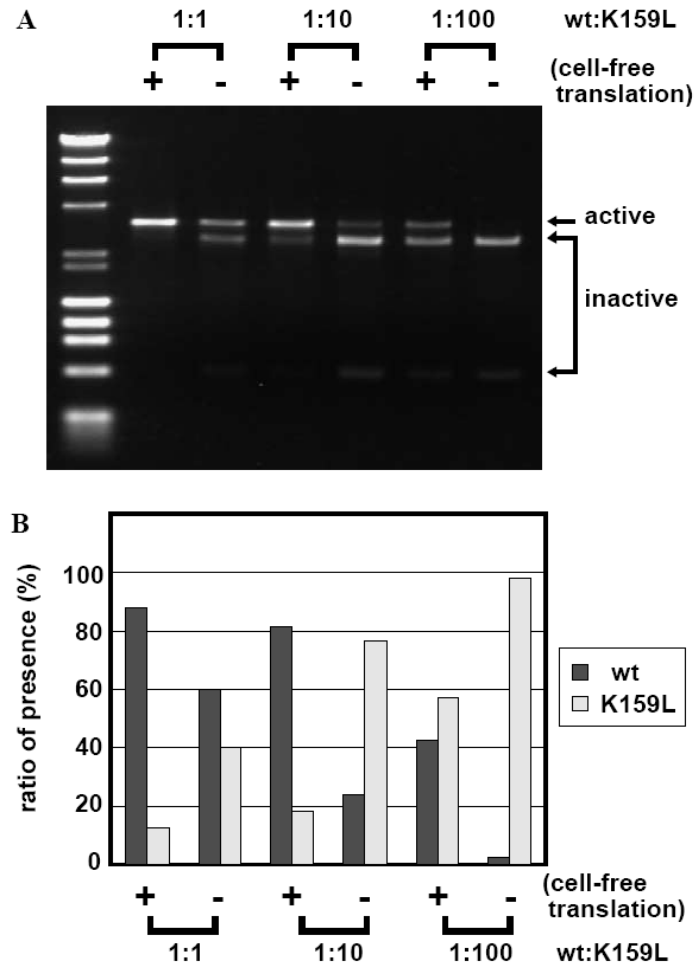
- What is the authors' strategy for selection using ribosome display? (Figure 1)
- What are the controls used to test the strategy? (Figure 3 and 4)
- What are the results? Figure 5
- What are the pitfalls of this method?
- Has ribosome display been used for enzyme evolution?

Figure 1: T4 display and selection



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Figure 5: Catalytic enrichment



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