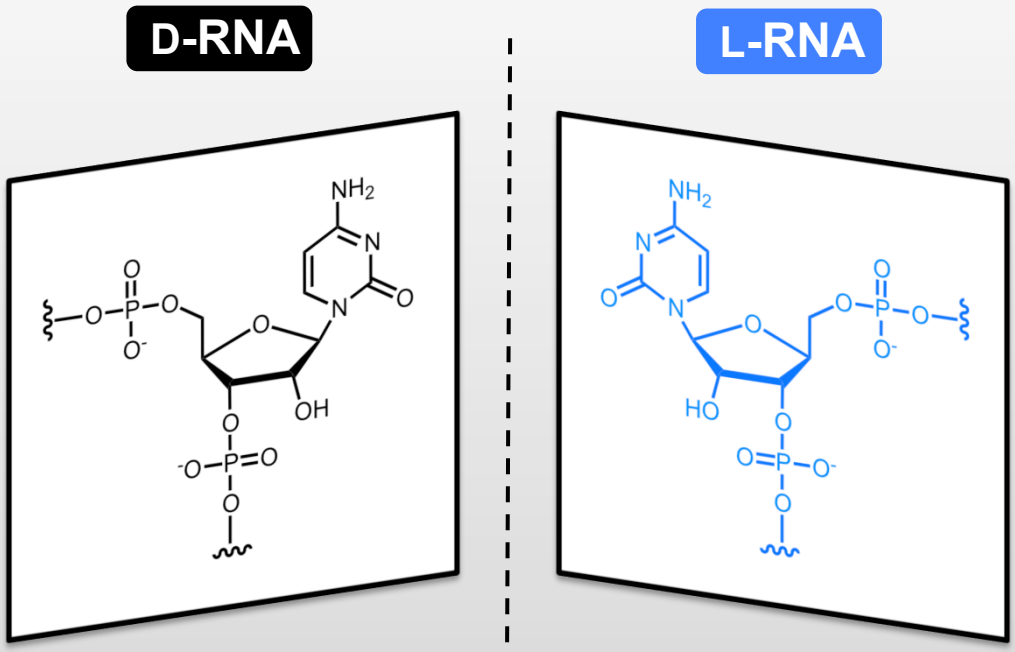
Talking to the Mirror: *In Vitro* Evolution of a Cross-Chiral Ribozyme

Jonathan T. Sczepanski
Texas A&M University

D-RNA vs. L-RNA



- Life on Earth uses exclusively D-nucleic acids
- D-RNA is rapidly degraded *in vivo*

- L-nucleic acids are no longer found in nature
- L- RNA is invisible to natural biology

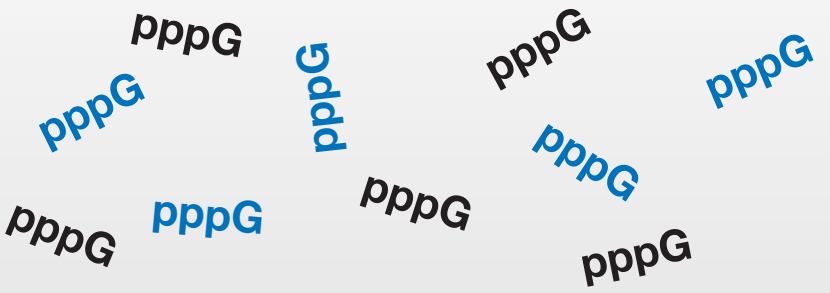
D-RNA and L-RNA do not form contiguous base pairs!

Ashley, G. W. *J. Am. Chem. Soc.* **114**, 9732–9736 (1992).

Cross-Chiral Inhibition

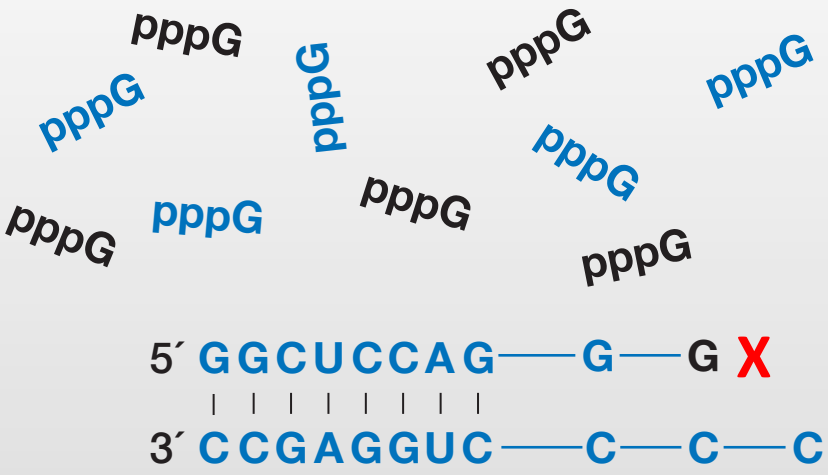
L-RNA

Cross-Chiral Inhibition



L-RNA

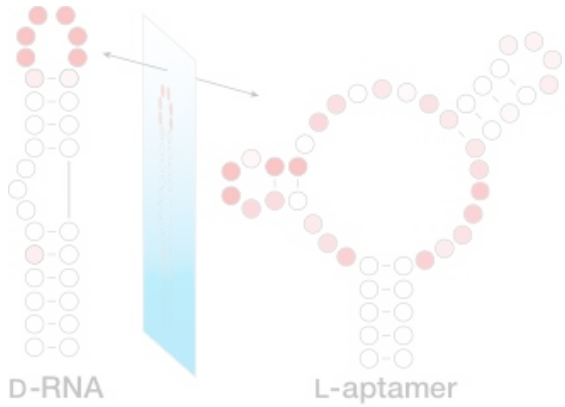
Cross-Chiral Inhibition



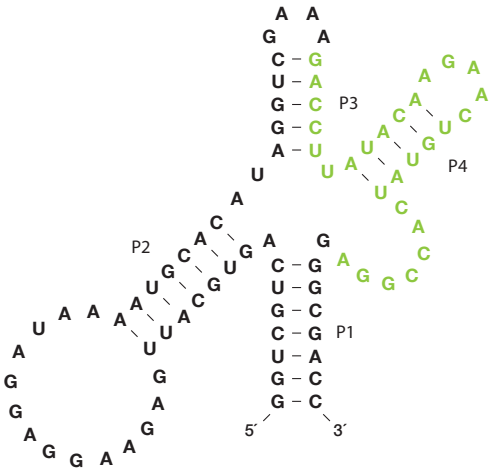
L-RNA

D-RNA vs. L-RNA

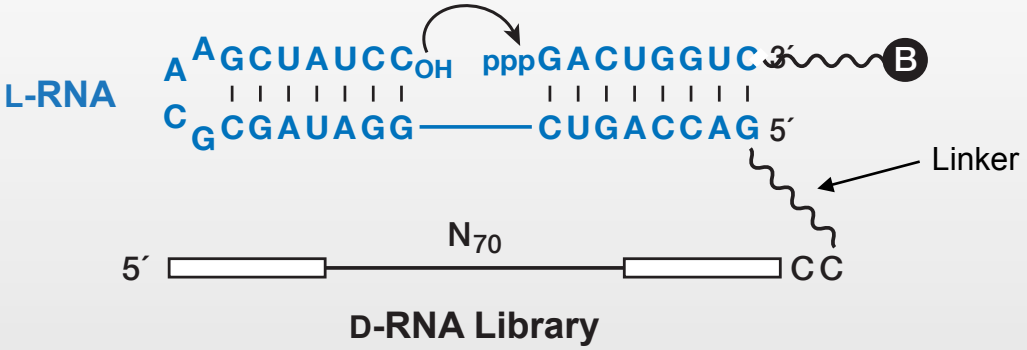
Can we prepare an L-RNA aptamer against a natural D-RNA target?

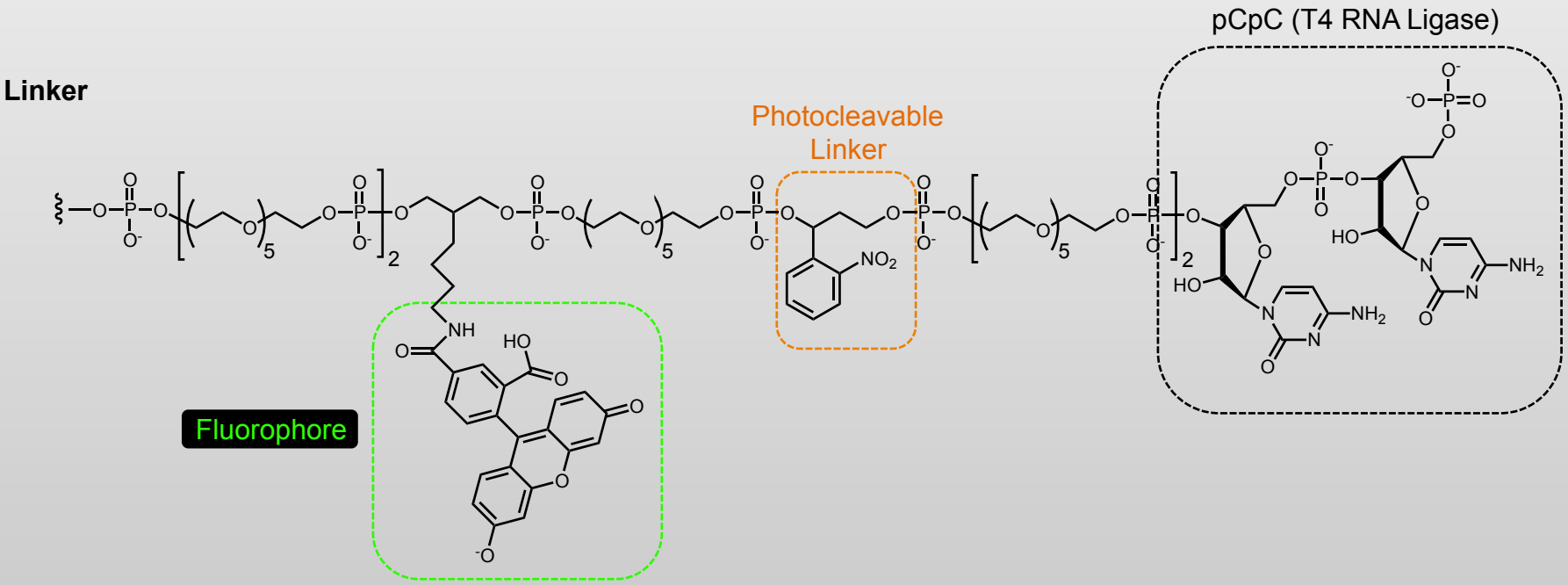


Is cross-chiral catalysis by an RNA enzyme (ribozyme) possible?



In Vitro Evolution of a Cross-Chiral Polymerase Ribozyme





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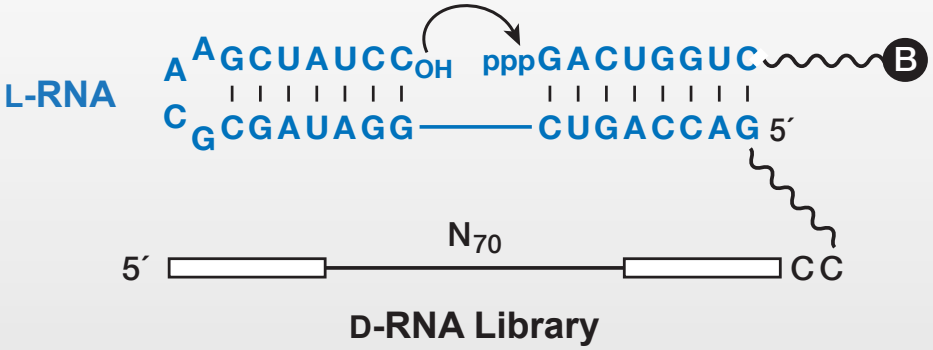

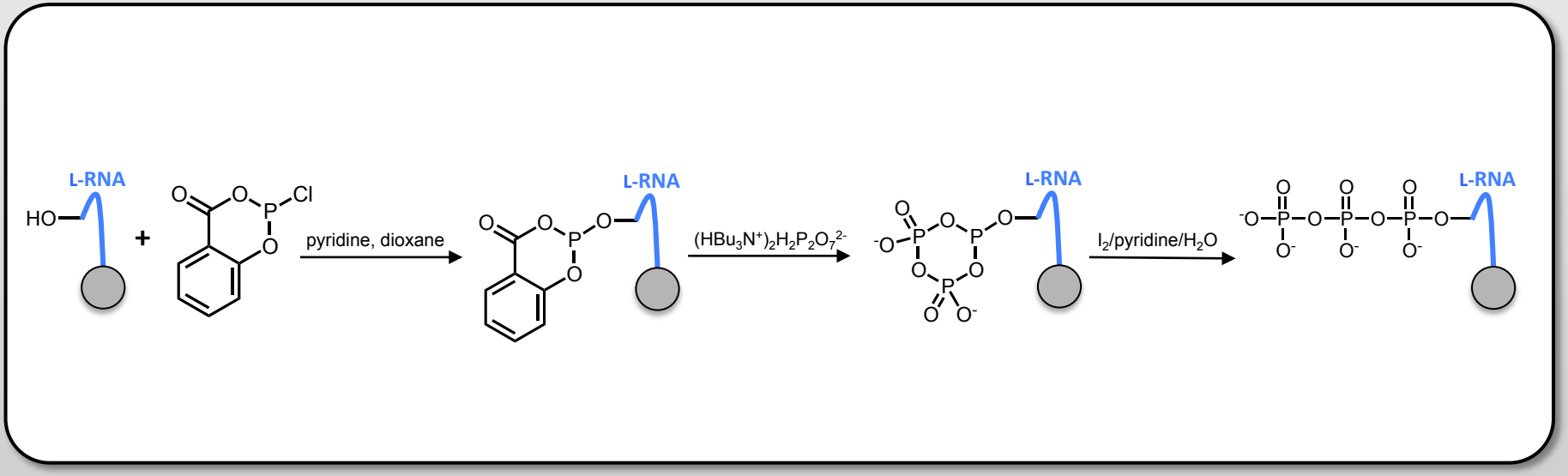

Ludwig, J & Eckstein F. *J. Org. Chem.* **54**, 631 (1989)

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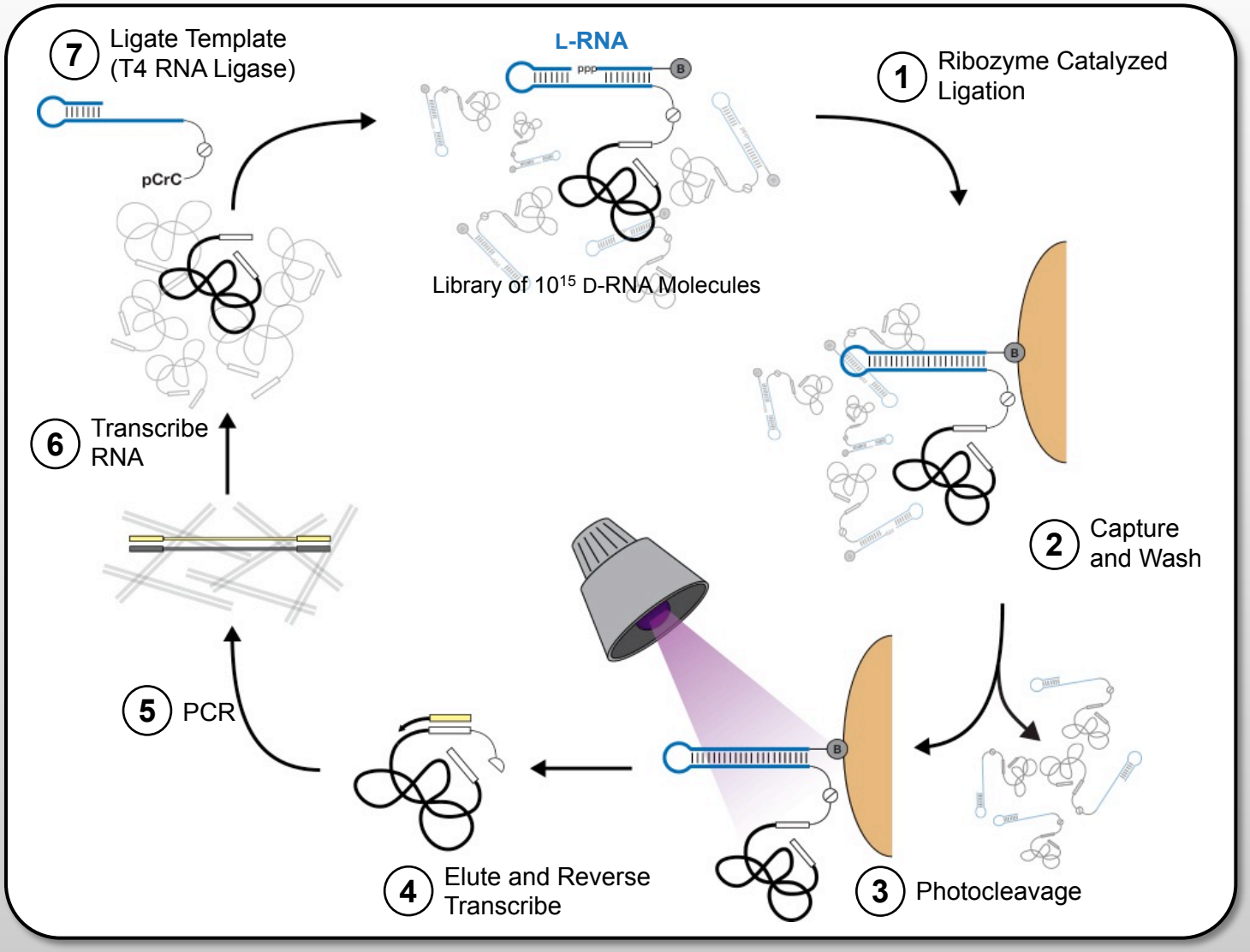

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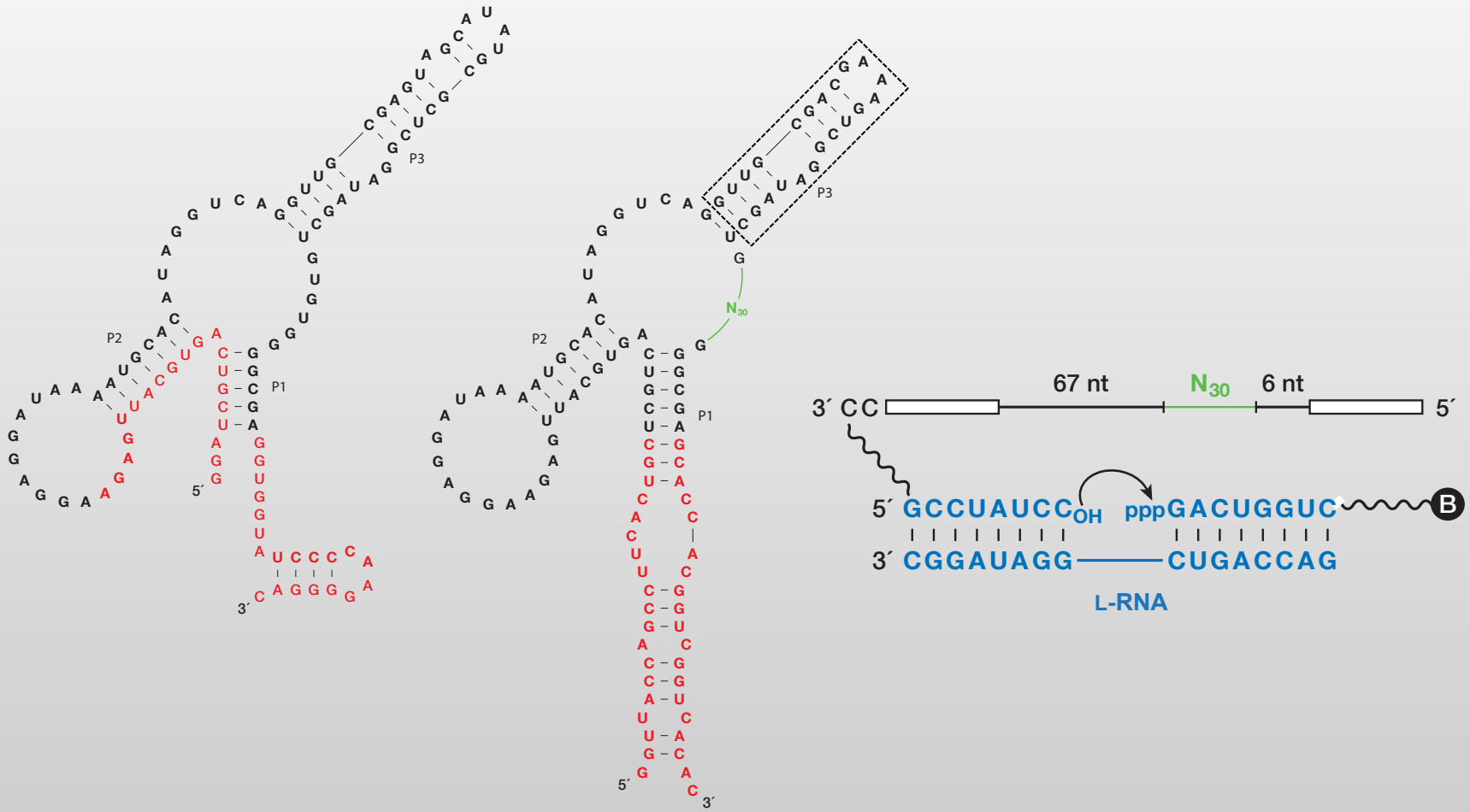

Sczepanski, J. T. & Joyce, G. F. *Nature,* **515**, 440-442 (2014)

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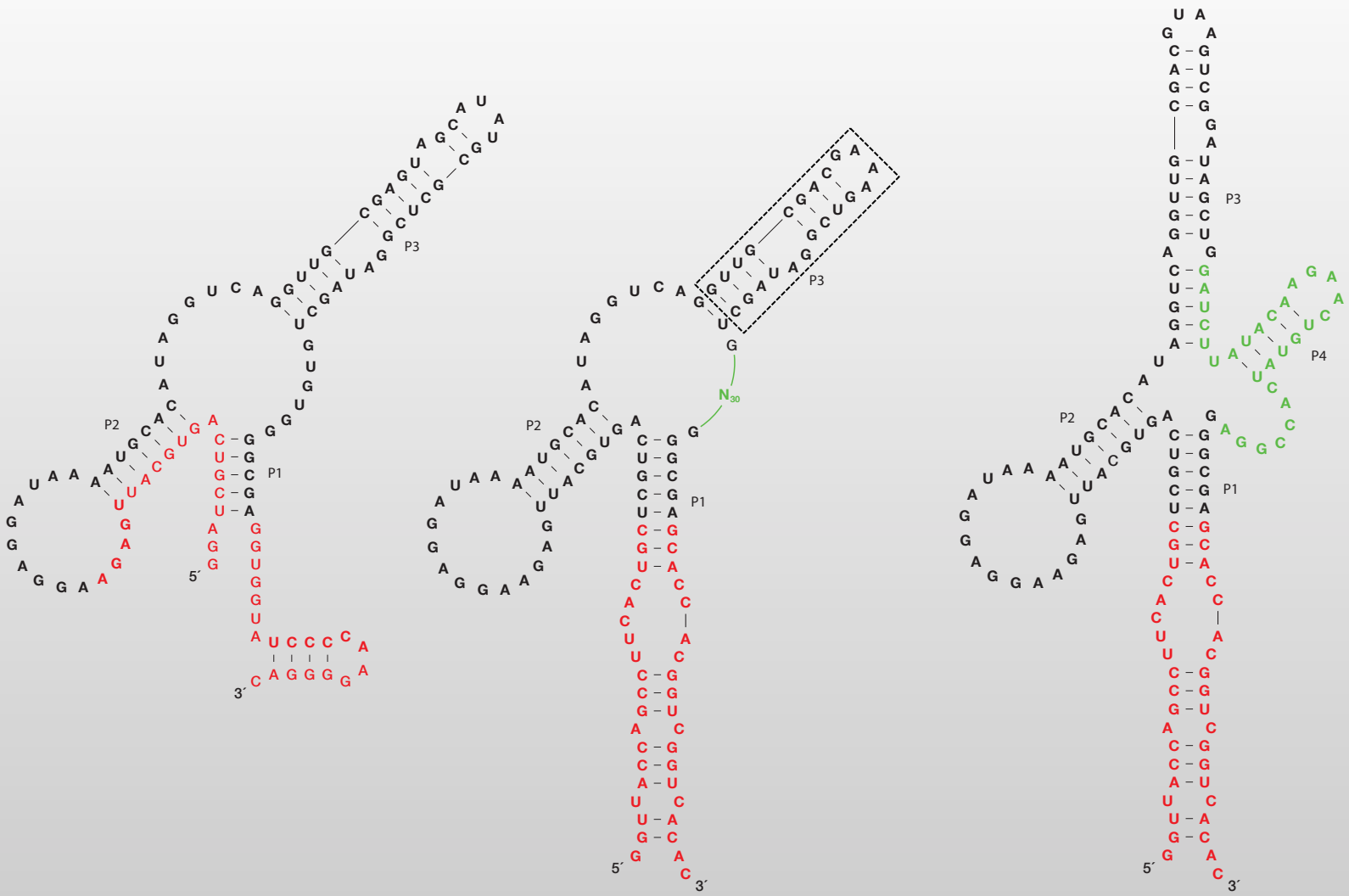

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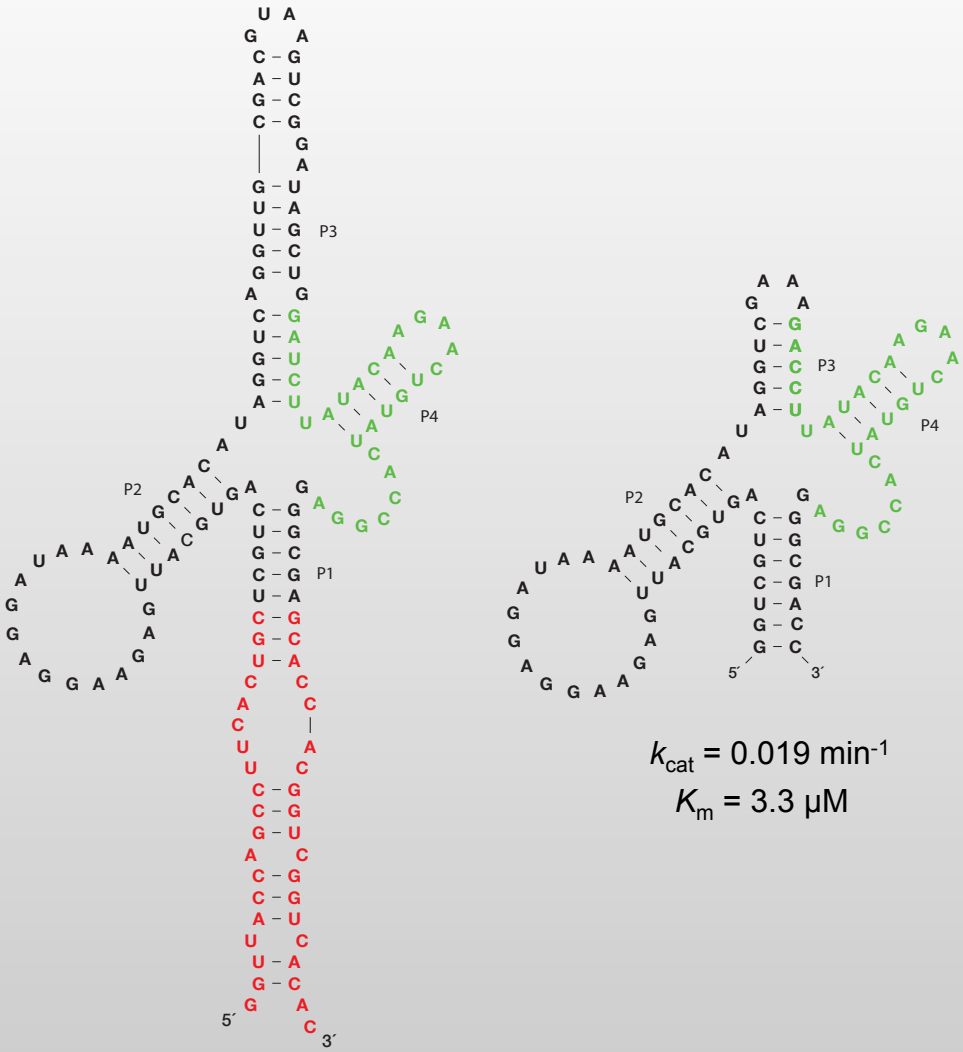

k_{cat} = 0.019 min^{-1}

K_m = 3.3 μM

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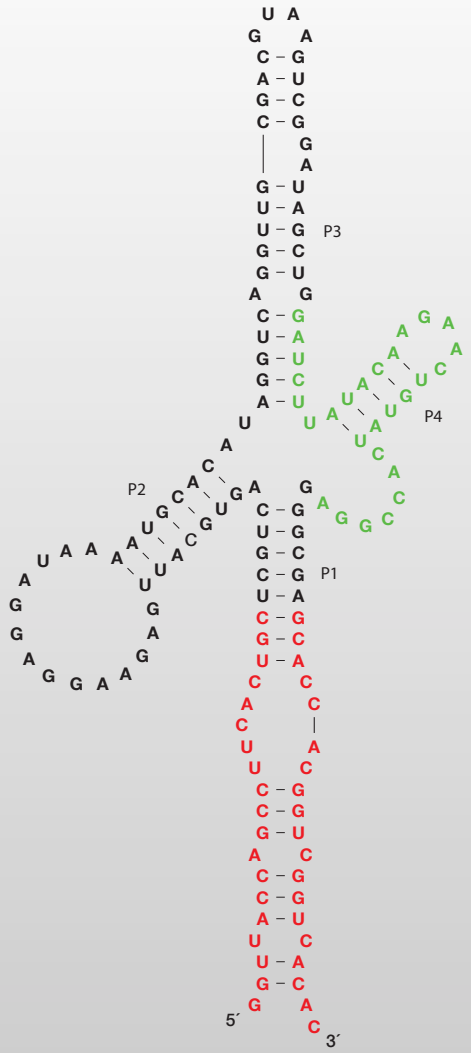
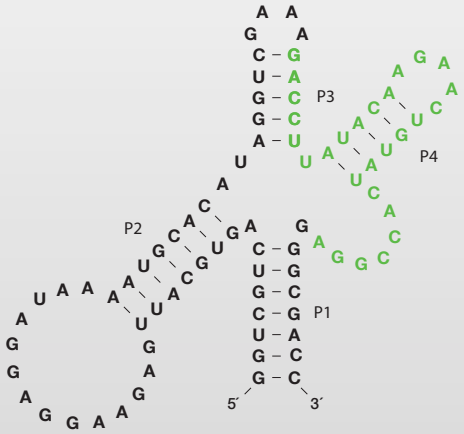
In Vitro Evolution of a Cross-Chiral Polymerase Ribozyme





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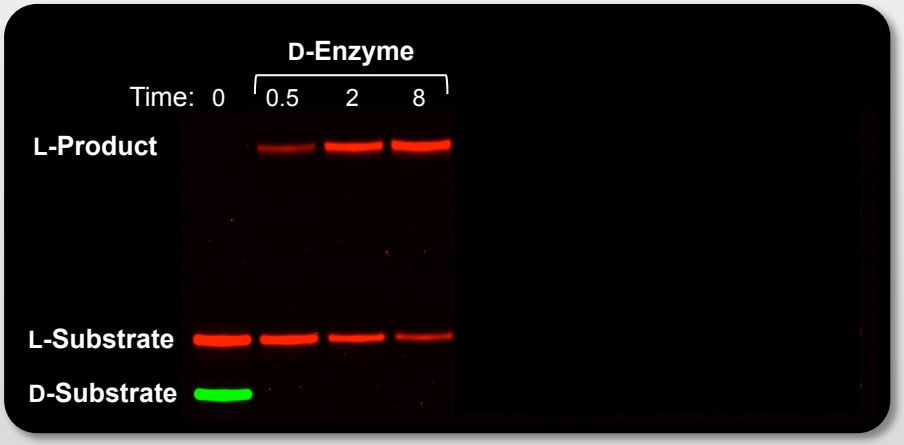

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In Vitro Evolution of a Cross-Chiral Polymerase Ribozyme

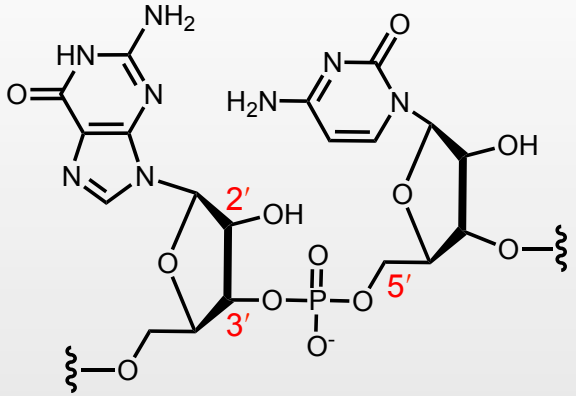
Regiospecificity

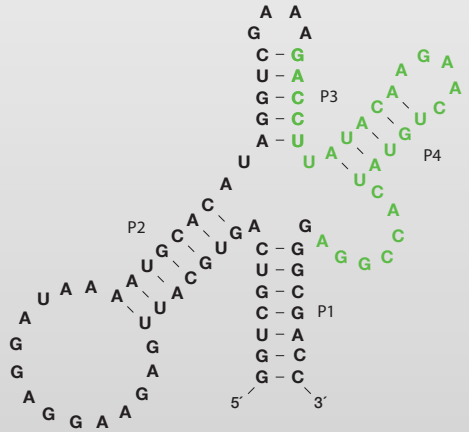
D-RNA

5′ GCCUAUCC ● GAAGGGAA

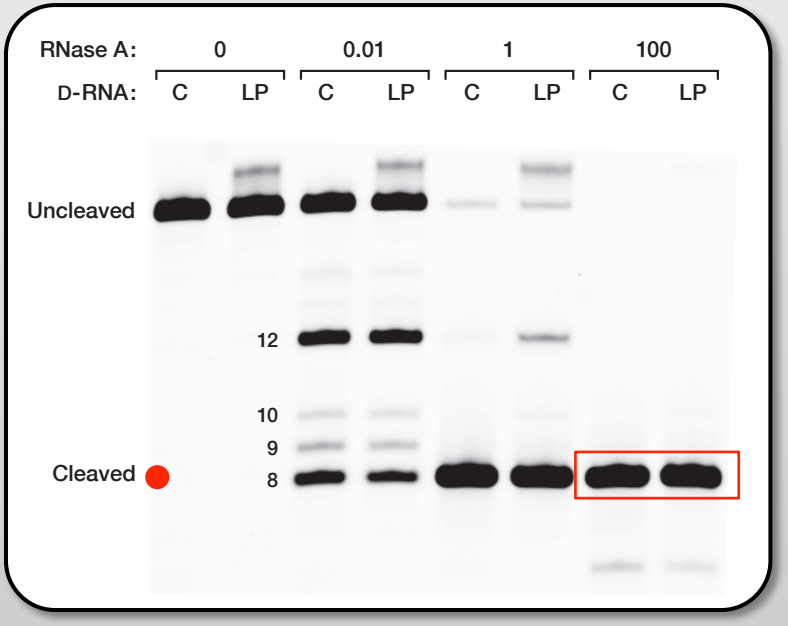
3′ CGGAUAGG—CUUCCCUU 5′

Exclusively 3′-5′ Linkages





L-Enzyme



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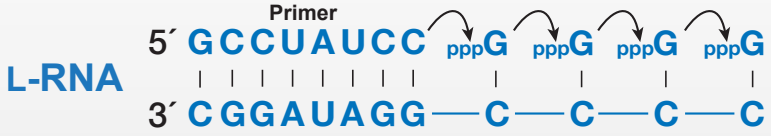

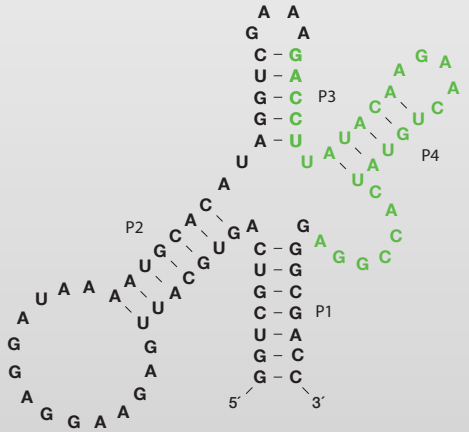

D-Enzyme

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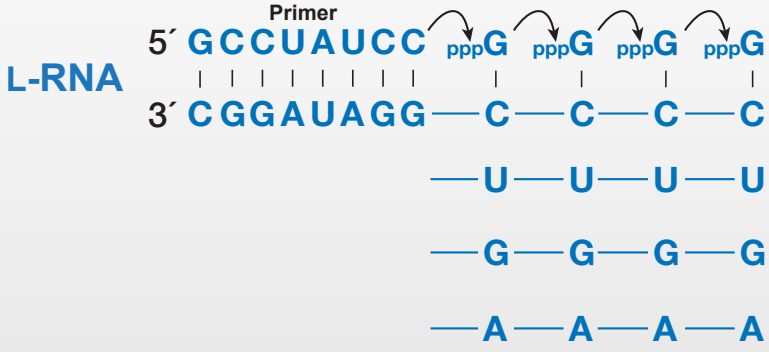

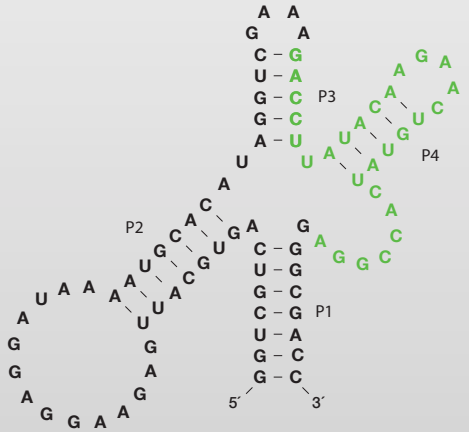

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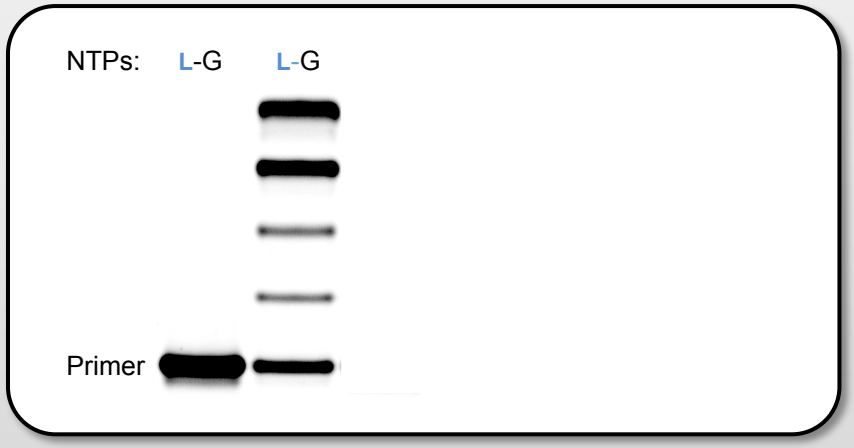

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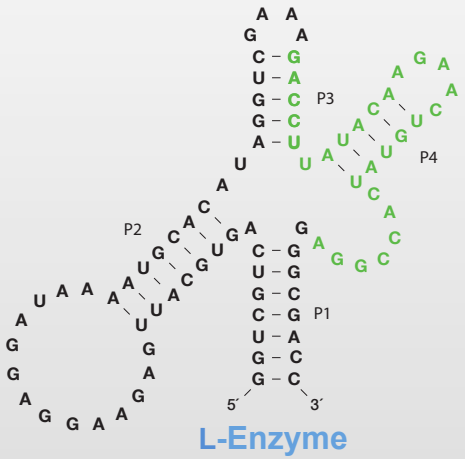
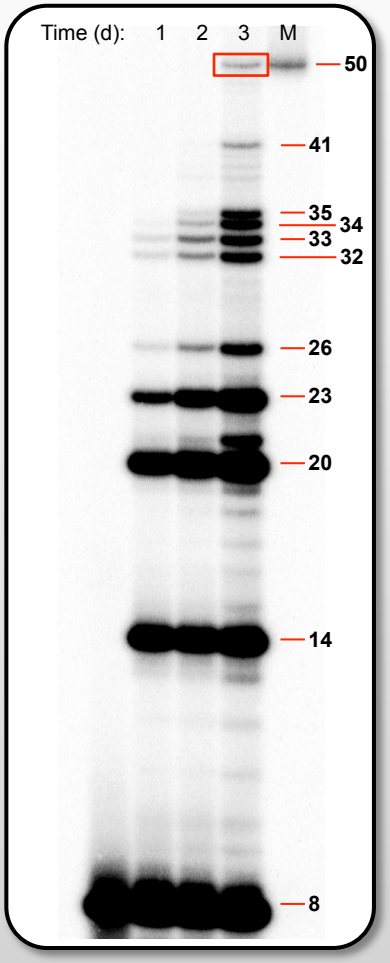


L-Enzyme



D-RNA

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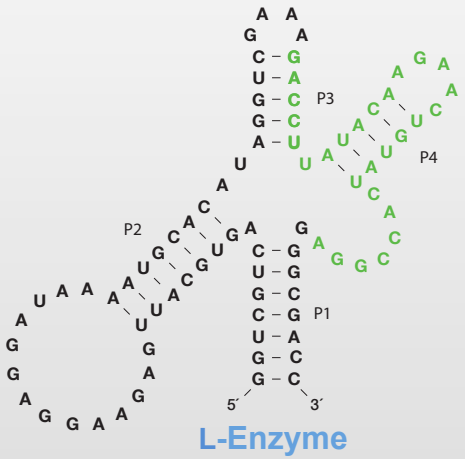
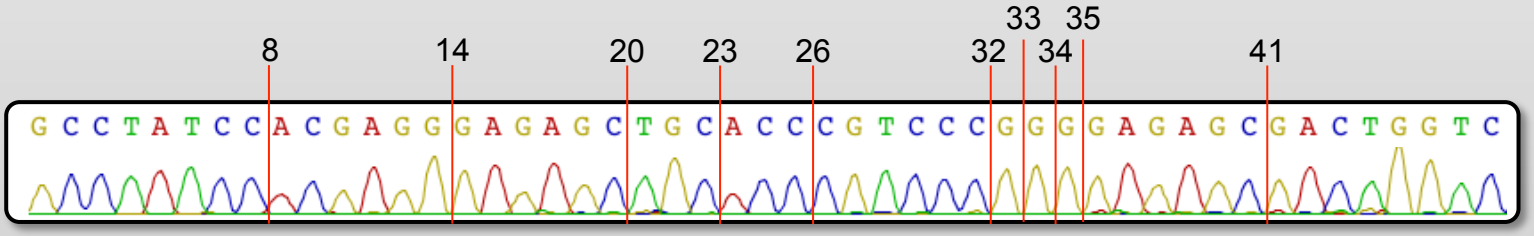


L-Enzyme



D-RNA

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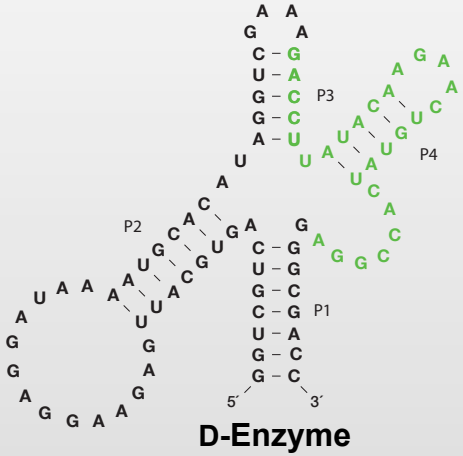

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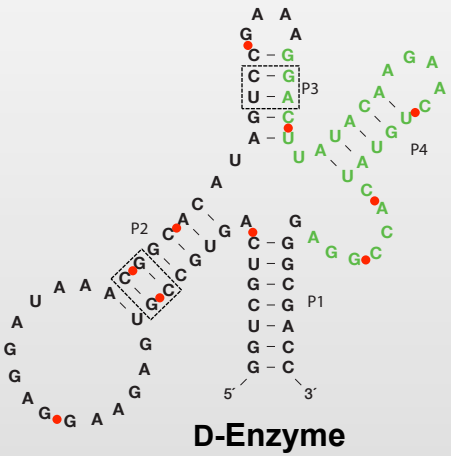
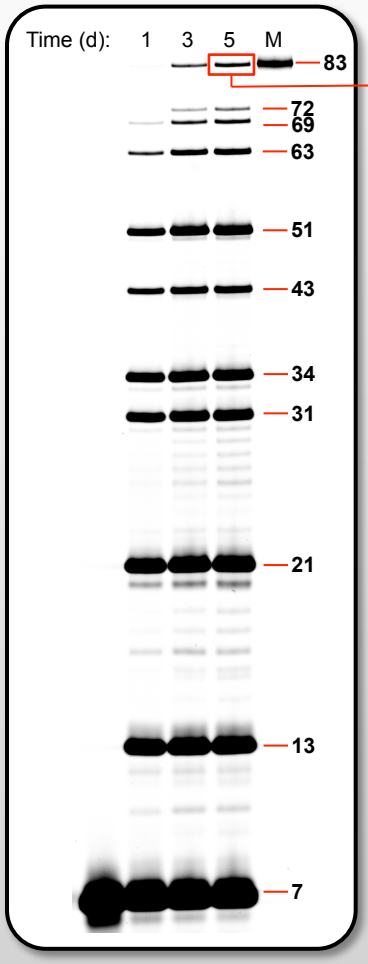
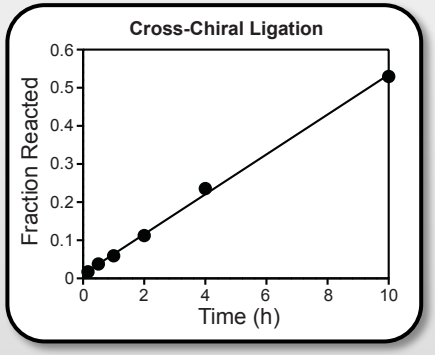


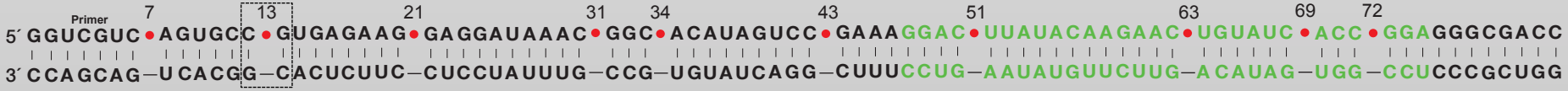
D-Enzyme

Cross-Chiral Replication





L-RNA



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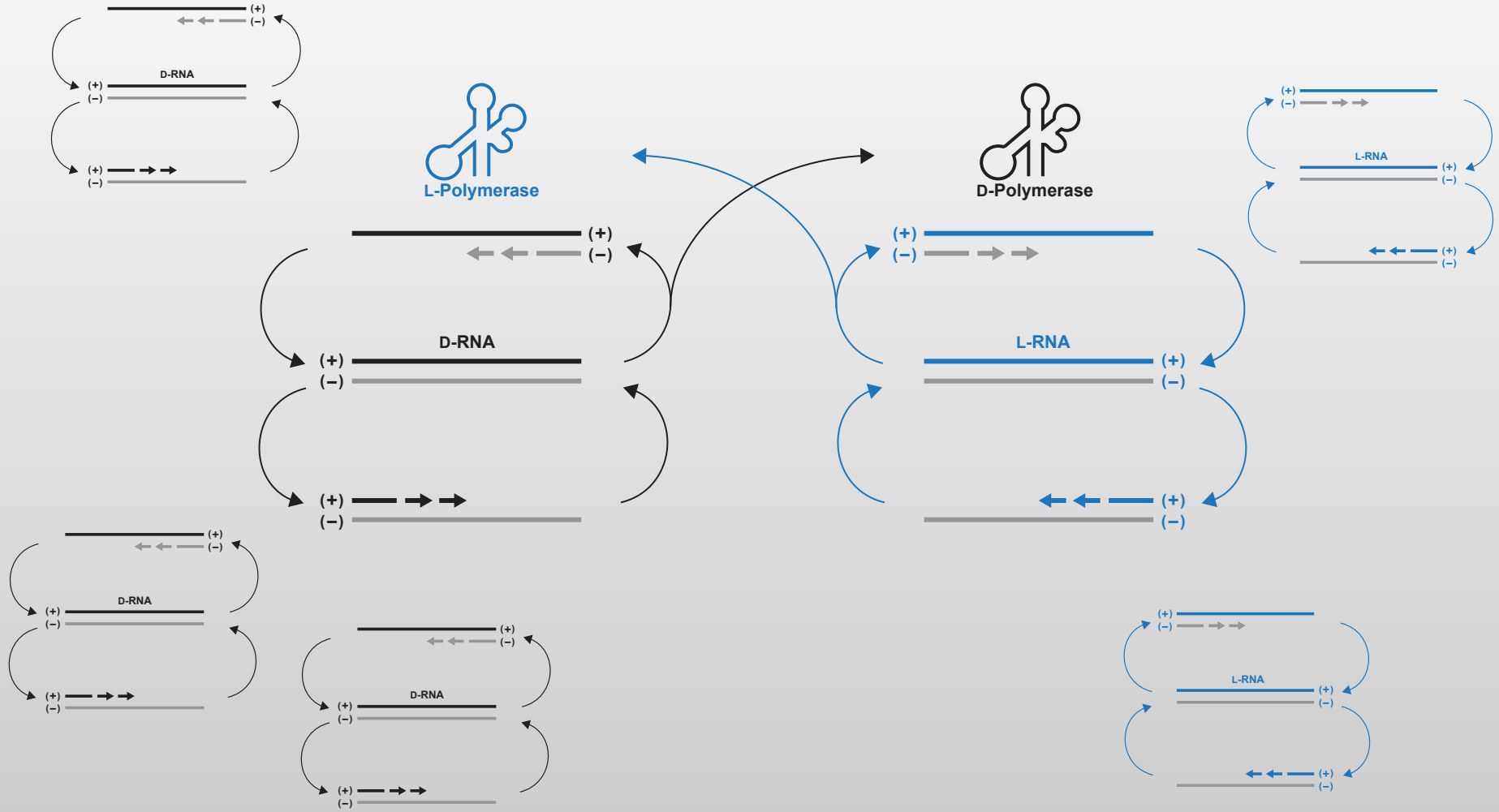
Self-sustained cross-chiral replication system (model for heterochiral evolution)



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Acknowledgments

Joyce Lab

Professor Gerald F. Joyce

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Advancing Research in Basic Science and Mathematics

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CANCER PREVENTION & RESEARCH INSTITUTE OF TEXAS

Questions?