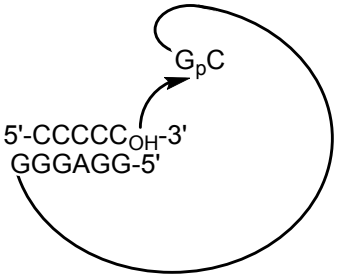
11.12.14 - Lecture 14- RNA catalysis and Protein evolution

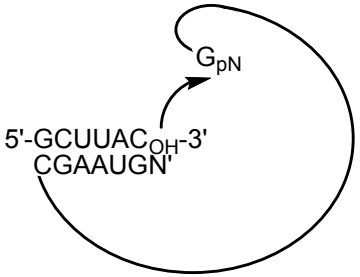
RNA catalysts

Disproportionation

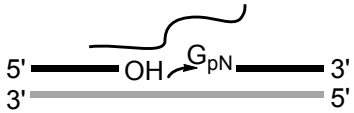


RNA nucleotide ligase

-with guide sequence

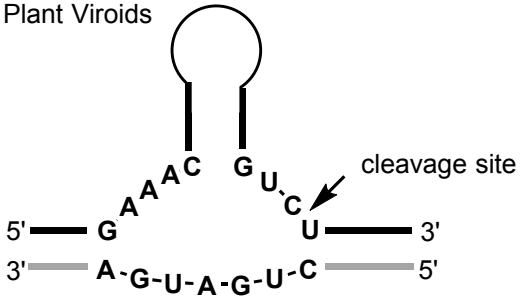


RNA Fragment Ligase

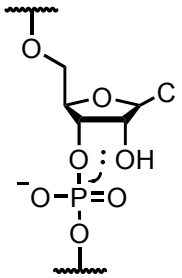


Hammerhead Sequence

From
Plant Viroids

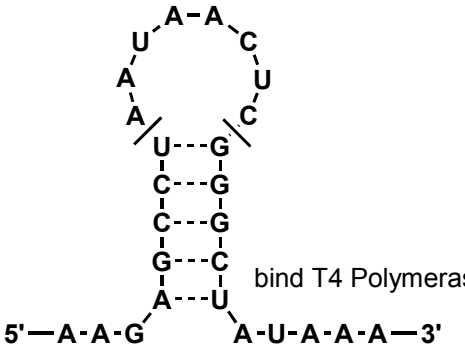


cleavage mechanism



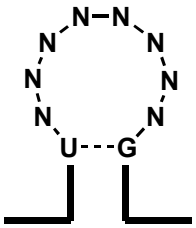
Antibody-like RNAs

-selective receptors: library based approach



bind T4 Polymerase

100 nt



Replace: AAUAACUC with N_8

Library Size: 4^8 ~ 65,000

100nt (1 nt ~ 330 MW)

100 nt ~ 33,000 g

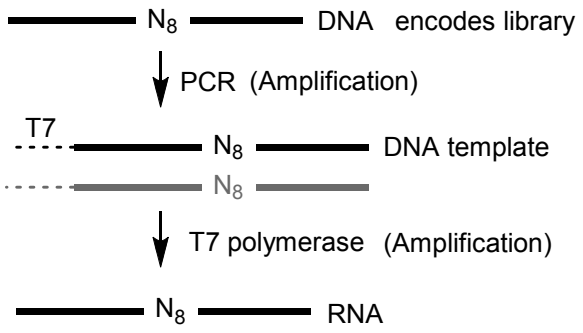
1 ug ~ 30 pmol ~ $2x10^{13}$ molecules

~ Large redundancy in the library

RNA/DNA

Length	Library Size	99% Coverage Passion Distribution	
16 nt	$4.3x10^9$	$1.2x10^{11}$	
20 nt	$1.1x10^{12}$	$3.6x10^{13}$	34 ug (100 mer)
24 nt	$2.8x10^{14}$	$1.1x10^{16}$	584 ug (100 mer)

Library Preparation



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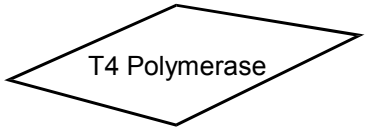
Selection Procedure



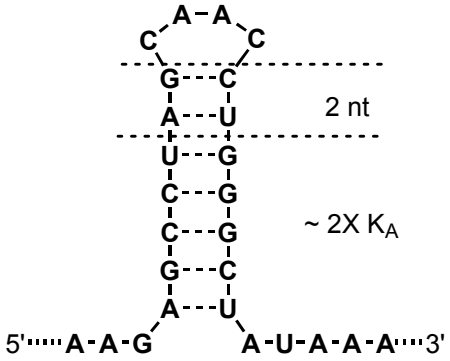
T4 Poly immobilized on nitrocellulose filter Or beads

\+ RNA Library

wash
elute (EDTA)

RT → PCR → In vitro transcription

(EDTA removes Mg^{2+} and unfolds RNA)



$$K_A = \frac{[R \cdot L_B]}{[R] \cdot [L_F]} \qquad [R \cdot L_B] \sim [R]\, K_A$$

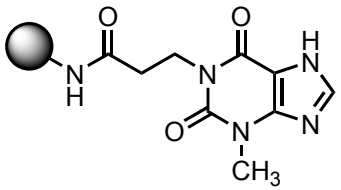
if $K_A \sim 10^8$, $[R] \sim 10^{-8}$

$$\frac{[R \cdot L_B]}{[L_F]} \sim 1$$

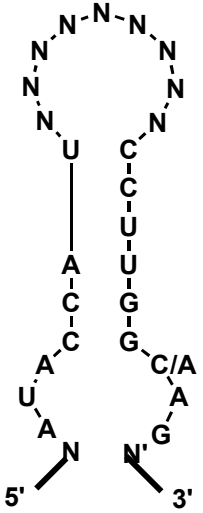
Balance of [R] and K_A is important for selection desgin

10 -100 fold/round enrichment

RNA binds to theophylline

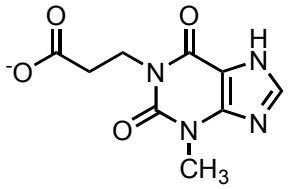


theophylline immobilized on solid support as affinity reagent - used to pan RNA library

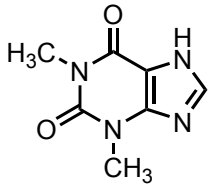


Aptamers bind theophylline

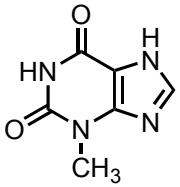
K_d (uM)



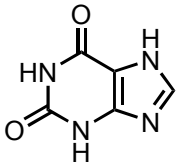
0.93



0.32



2.0



>500

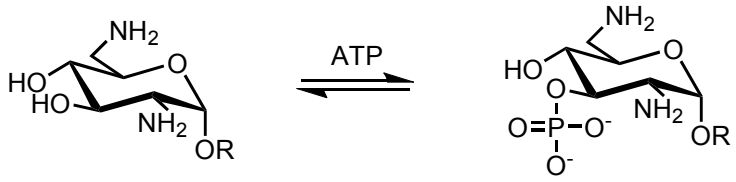
Jenison, R. D.; Gill, S. C.; Pardi, A.; Polisky, B., High-resolution molecular discrimination by RNA. Science **1994**, 263 (5152), 1425-9.

11.12.14 - Lecture 14- RNA catalysis and Protein evolution

Evolution Interesting Functions from Large Libraries

- **Large antibody libraries ~ selective catalysts**
- **Large RNA libraries ~ selective catalysts**
  ~ selective receptors
- **Peptides/Proteins**
- **Small molecules**

Kanamycin nucleotidyl transferase



inactivation by modification

Evolution of Heat-Stable Enzyme

Mutagenesis

mutD5 PolII edit function impaired (mistakes)

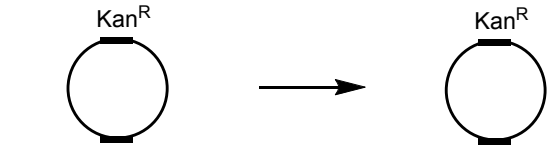
introduce random point mutations in gene

Selection

kanamycin nucleotidyl transferase (stable at 45 ^{o}C, but not 55 ^{o}C)

Evolution of heat stable enzyme

Use thermophile (*Bacillus stearothermophilus* Kan^{R})



Ori (*B. stearothermophilus*)

B. stearothermophilus

Selection at 63 ^{o}C and 71 ^{o}C

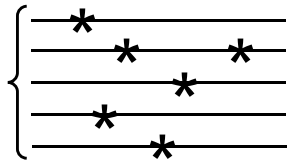
Asp^{80} (GAT) → Tyr (TAT) Point mutation cannot lead to all 20 aa

Liao, H.; McKenzie, T.; Hageman, R., Isolation of a thermostable enzyme variant by cloning and selection in a thermophile. Proceedings of the National Academy of Sciences of the United States of America **1986**, 83 (3), 576-80.

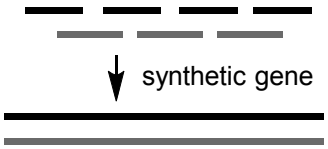
Kanamycin nucleotidyl transferase

		55 ^{o}C	55 ^{o}C	55 ^{o}C
63 ^{o}C selection	Asp80Tyr	>60 mins	16.5 mins	<1 mins
71 ^{o}C selection	Asp80Tyr Thr130Lys	stable	>60 mins	15 mins

Natured mutation (Somatic mutation/recombination)

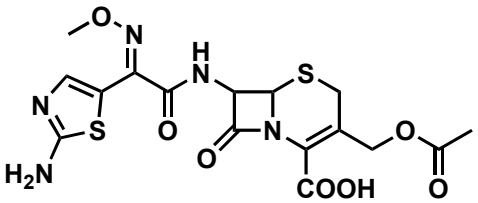


Error Prone PCR Amplification



Introduce mutations
Control mutation sites / frequency

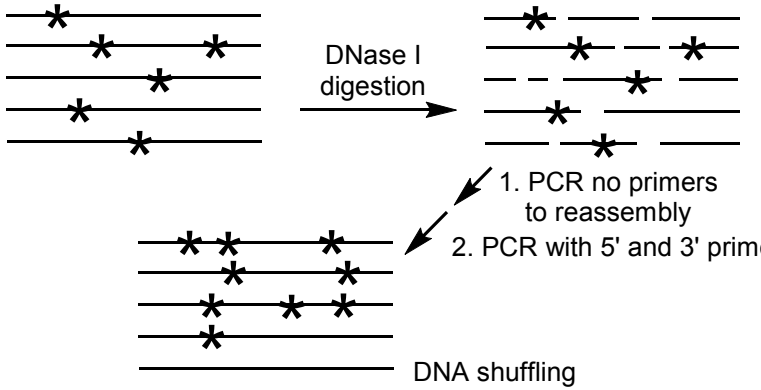
β-lactamase



Cefotaxime (CTX)

MIC ~ wt TEM β-lactamase 0.02 ug/mL

Selection : growth on increasing antibiotics



DNA shuffling

11.12.14 - Lecture 14- RNA catalysis and Protein evolution

Rapid evolution of β-lactamase

DNA shuffling

↓

Point mutation/Genetic recombination

Selection Scheme:

wt TEM-1 → 6.4 ug/mL → 10 ug/mL → 80 ug/mL → 160 ug/mL

E104K/G238S/M182T
A42G/G92S/R241H

MIC
640 ug/mL

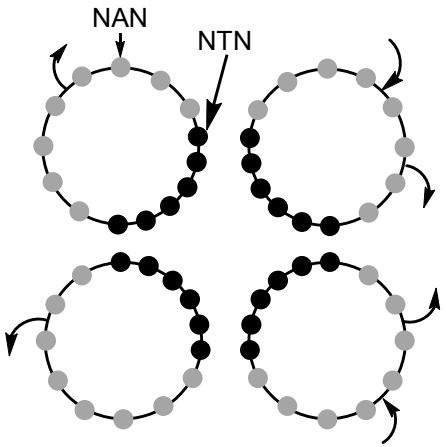
Remove silence mutation (shuffle with wt sequence)

Stemmer, W. P., Rapid evolution of a protein in vitro by DNA shuffling. Nature **1994**, 370 (6488), 389-91.

Protein evolution: 4 Helix Bundle Protein

For protein (6 sites): $20^6 \sim 6.4 \times 10^7 \sim 10^8$ to 10^9 clones for full coverage

For nucleotide (20 sites): $4^{20} \sim 1.1 \times 10^{12} \sim 10^{13}$ clones for full coverage



● hydrophobic
● hydrophilic

L_1: Pro Asp Ser
L_2: Pro Ser Gly
L_3: Pro Arg Ser

● hydrophilic = NAN
Glu, Asp, Lys, Asn, Gln, His

● hydrophobic = NTN
Phe, Leu, Ile, Met, Val

Selection:
soluble, stable to proteolysis
any folded structure
molten globules

Take 48 colonies, 29 satisfied criterion
purify 3 proteins, 2 were stable to denaturant

$\Delta G\_unfolding \sim 3\text{-}4$ kcal/mol
staph nuclease ~ 6.1 kcal/mol

$5^{24} \times 6^{32} = 4.7 \times 10^{41}$ possible library members

Hecht, M. H.; Richardson, J. S.; Richardson, D. C.; Ogden, R. C., De novo design, expression, and characterization of Felix: a four-helix bundle protein of native-like sequence. Science **1990**, 249 (4971), 884-91