

Larry Loeb - Unnatural Nucleotid 2/4/93  
Sequences that Encode New Proteins

Why make sense to pull out non-natural sequences

① not all possibilities used in current cells

② Nature's limitations

③ can't screen large #'s...

but what about Shifting Balance

④ may not be able to change

Applied Molecular Evolution

① genetic selection

② intro random molecules into proteins

③ select for activity of this protein

② nucleotide amplification = e.g. Joyce  
Schostak & Green

③ screening random phage libraries  
- only currently used for assaying binding

Genetic Selection

① Promoters - M. Horwitz

- tet promoter - replaced 19 bp w/ random 19  
- got some new things

Lessons

① need 99.99% cutting & ligation

② requires intermediates construct

③ need high transfection

④ need stringent assay

② Herpes thymidylate-kinase

dUMP

↑ Thymid. Synthase

- inhibit E. coli's kinase w/ 5-FdU

TdR → dTMP

- transfect w/ herpes thymidine kinase

- soluble

- promiscuous

- slow

- therapy