Sequencing, Finishing, Analysis in the Future Meeting May 28 2009

Next Generation DNA Polymerases & Reverse Transcriptases

David Mead and Tom Schoenfeld

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Polymerases and Sequencing

Sanger	Taq FY
454 Roche	Bst + Taq
ABI Solid	Ligase + Taq
Illumina	Vent + Taq
Polonator	Ligase + Taq
Helicos	Klenow
Complete Genomics	Ligase + phi29
Pacific Biosystems	phi29
Oxford Nanopore	nuclease
Intelligent Biosystems	polymerase

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Polymerase Challenges on Next Gen Sequencers

Solid Phase Surface Chemistry

Adsorption, Steric Hindrance

New Nucleotide Chemistry

Incorporation Bias, Nucleotide Scars, Phasing

Microfluidics

Affinity, Read Length

DNA Amplification

Not uniform nor random – hot and cold spots

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E. coli copies 4 Mb
One error/10^9 bp in 20 minutes

In vitro technical capabilities improve by orders of magnitude

Helicases
SSB
Primosome
DNA Polymerase III
- Core – αεθ
- Sliding clamp - β2
- Clamp loader - γδδ′χψτ2

Type II Topoisomerase (Gyrase)
DNA Polymerase I
DNA Ligase

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Cellular DNAP "DNA repair enzymes"

VS

Phage DNAP "Replicases"

	Phage	Cellular
Strand displacement	High	Low
Fidelity	High	Low
Processivity	High	Low
Analog incorporation	High	Low

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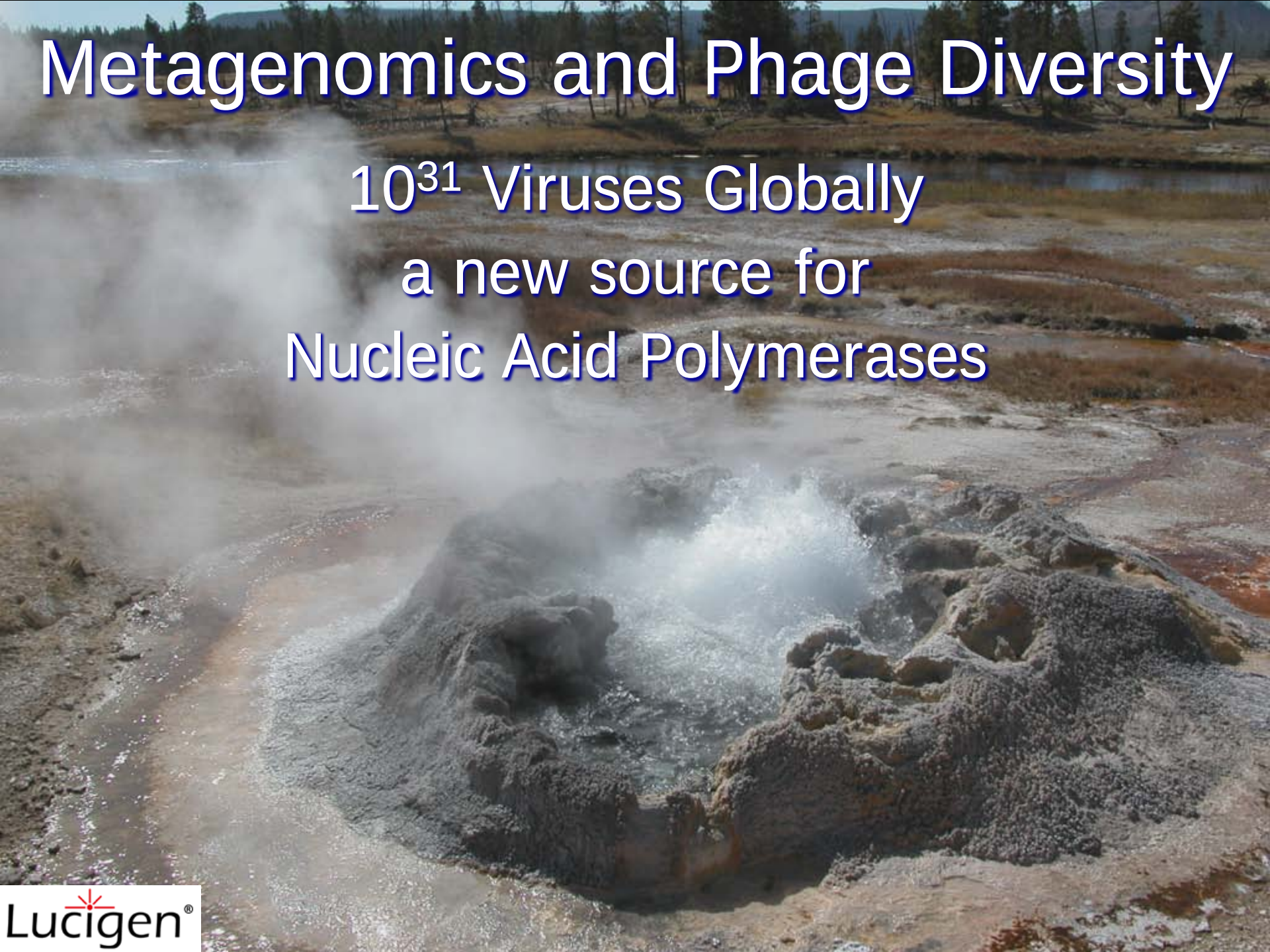
Metagenomics and Phage Diversity

10^{31} Viruses Globally
a new source for
Nucleic Acid Polymerases



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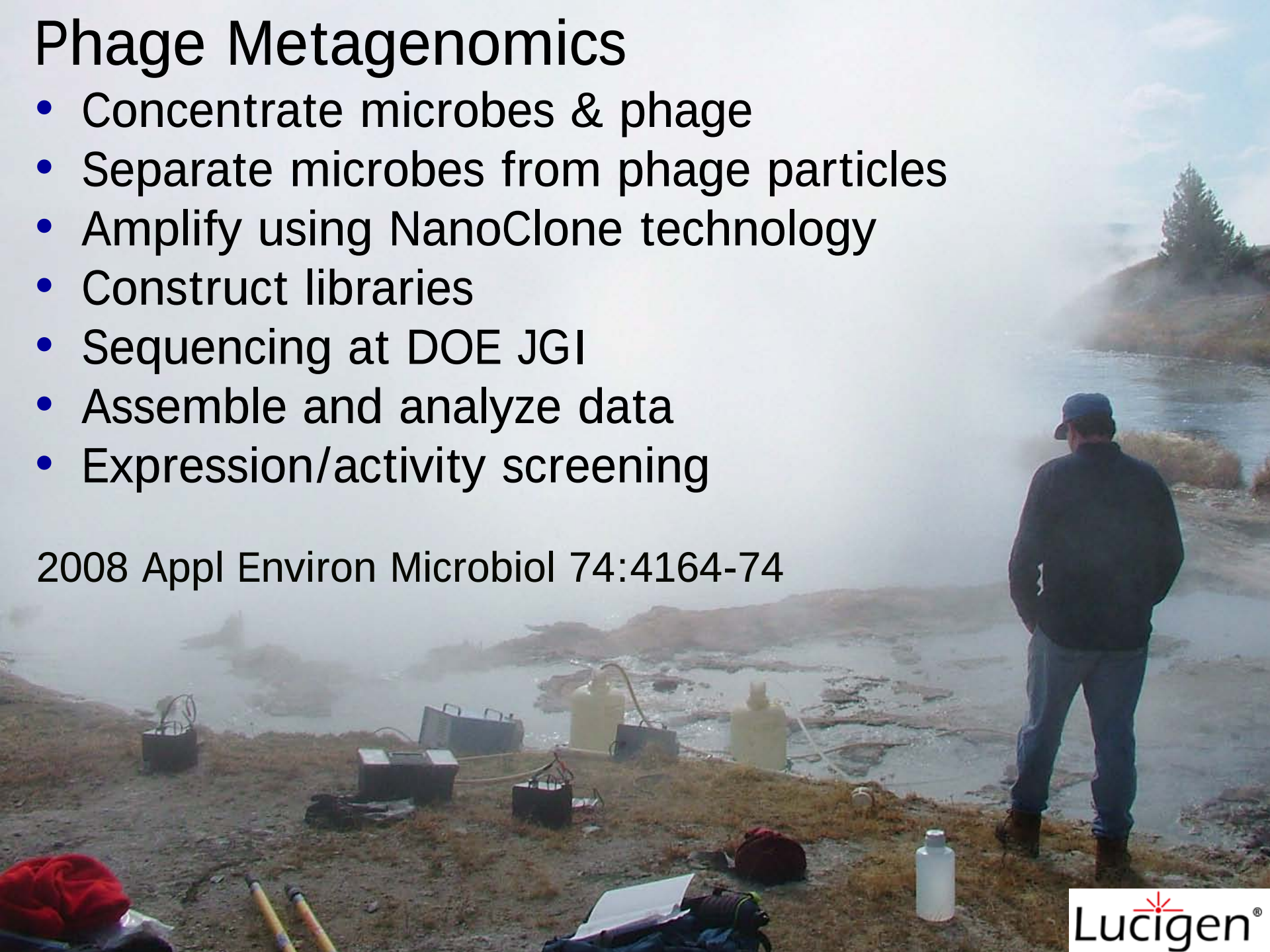
Phage Metagenomics

- Concentrate microbes & phage
- Separate microbes from phage particles
- Amplify using NanoClone technology
- Construct libraries
- Sequencing at DOE JGI
- Assemble and analyze data
- Expression/activity screening

2008 Appl Environ Microbiol 74:4164-74



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Virus Like Elements

LIBRARY	LHC	BEAR PAW	OCTOPUS
HEAD	3	10	15
HELICASE	132	122	274
INTEGRASE	67	30	22
LIGASE	0	11	18
LYSIN	118	260	389
METHYLASE	50	32	127
METHYLTRANSFERASE	55	100	262
NUCLEASE	94	145	232
POLYMERASE	147	235	548
PRIMASE	14	19	21
REVERSE TRANSCRIPTASE	42	35	11
RESTRICTION	71	34	59
RESOLVASE	24	15	87
RIBONUCLEASE	20	13	62
TAIL	17	38	289
TOPOISOMERASE	18	19	21
TRANSPOSASE	208	86	51

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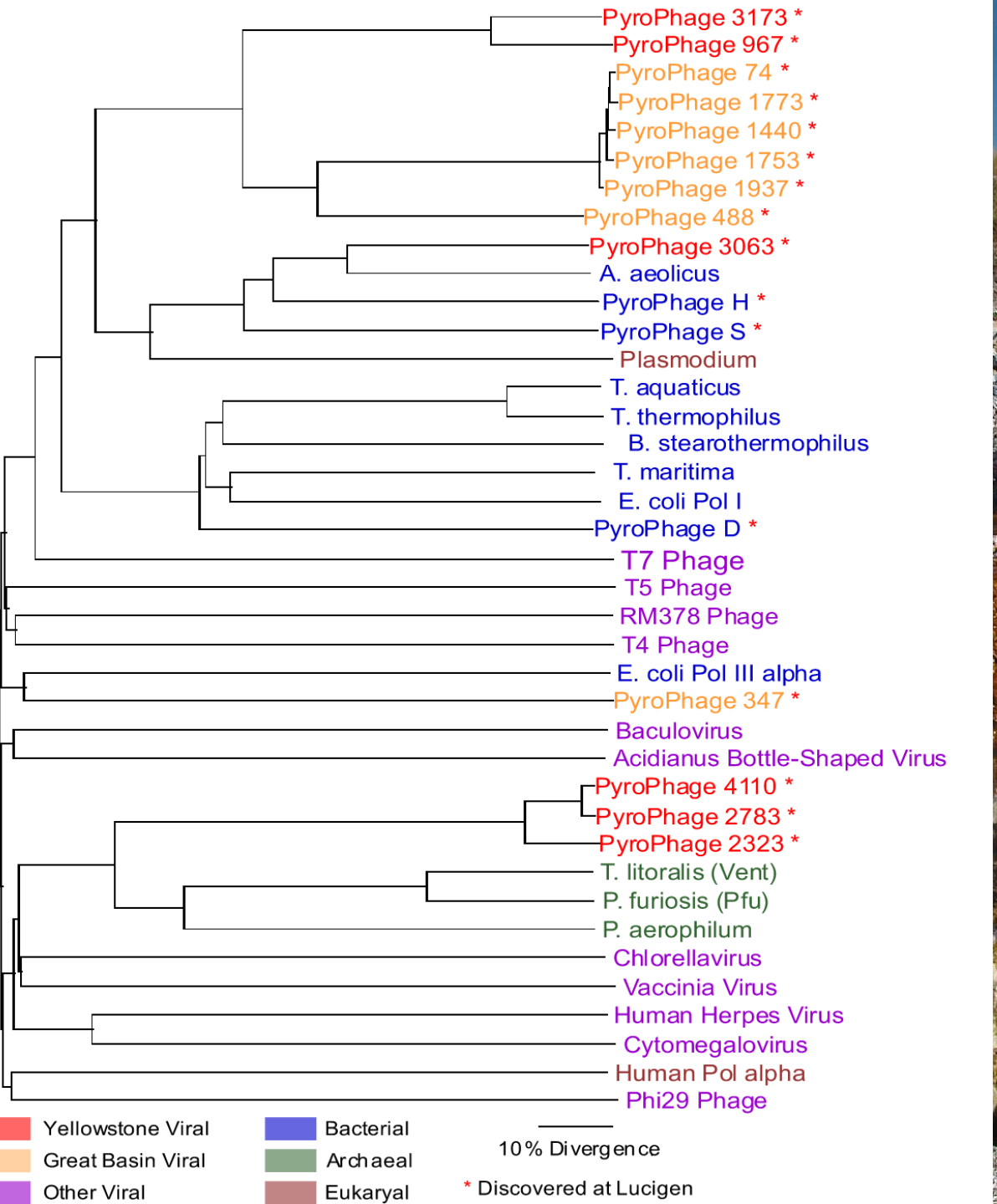

Diversity of Viral DNAP Based on ClustalW Analysis

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Affinity Enhanced Polymerase Improvements

Lower Bias Higher Fidelity
Longer sequence reads
Robust single molecule PCR
Amp/Seq difficult templates
Longer PCR
Better strand displacement
Fast Elongation Rate
Sequence templates directly, without amplification or minipreps.

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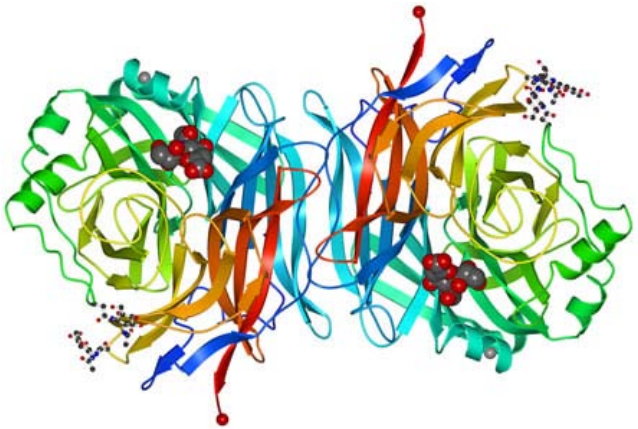
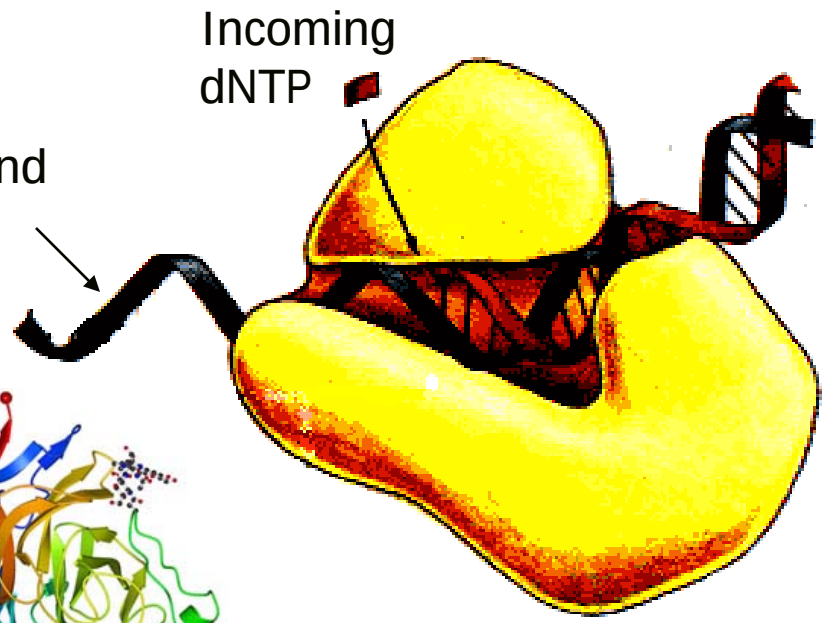
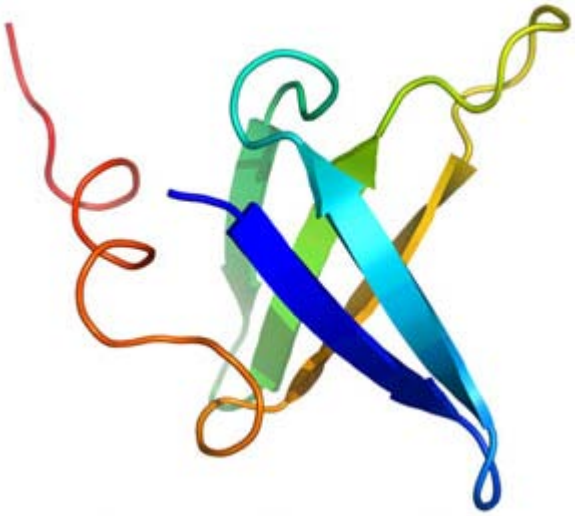
Affinity Partners Influence DNA Binding & Primer Extension Affecting Sequencing and PCR



SSB Domain
Microbial Thermophile
Phage Thermophile



DNA Polymerases
Taq
PyroPhage 3173
T4 exo-
Klenow exo-
Bst



DSB Domains
Microbial Thermophile
Phage Thermophile

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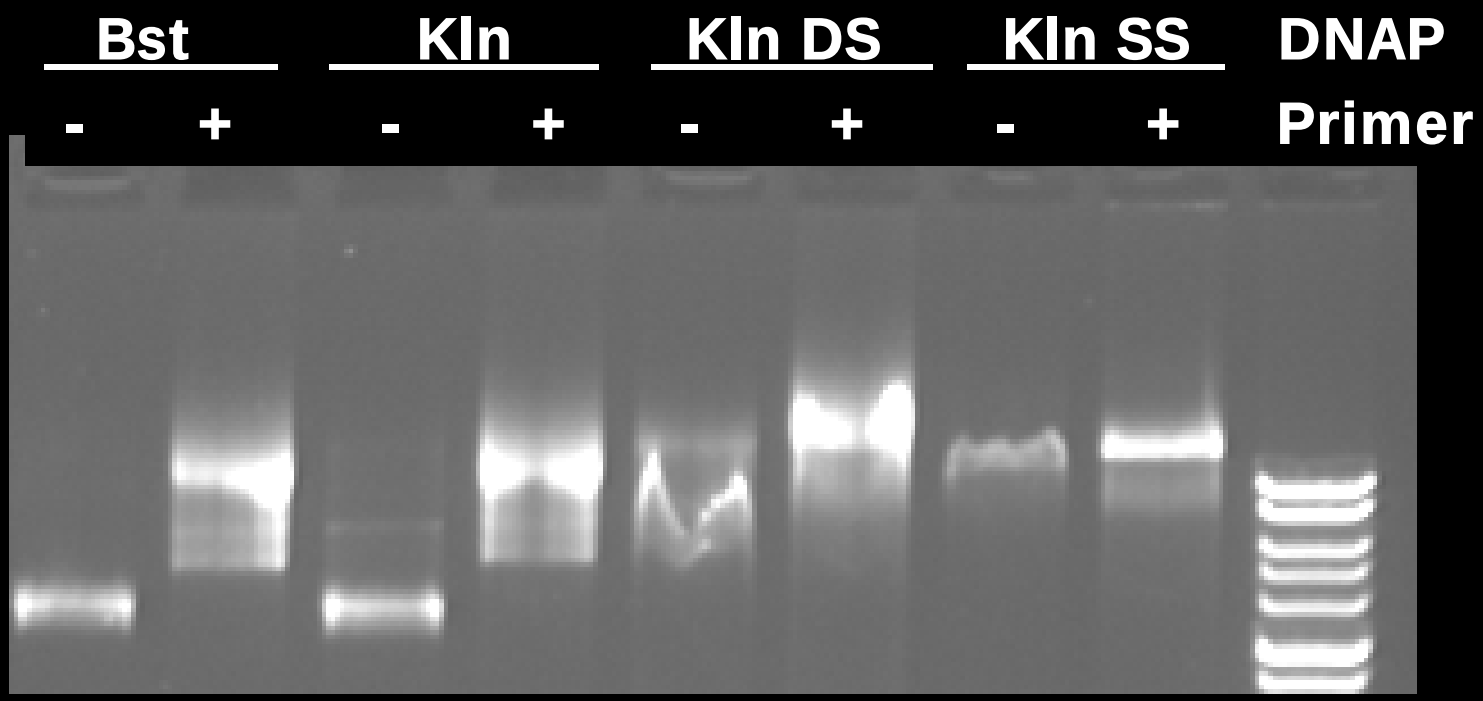
DS and SS Affinity Modified Klenow
M13 Primer Extension Assay



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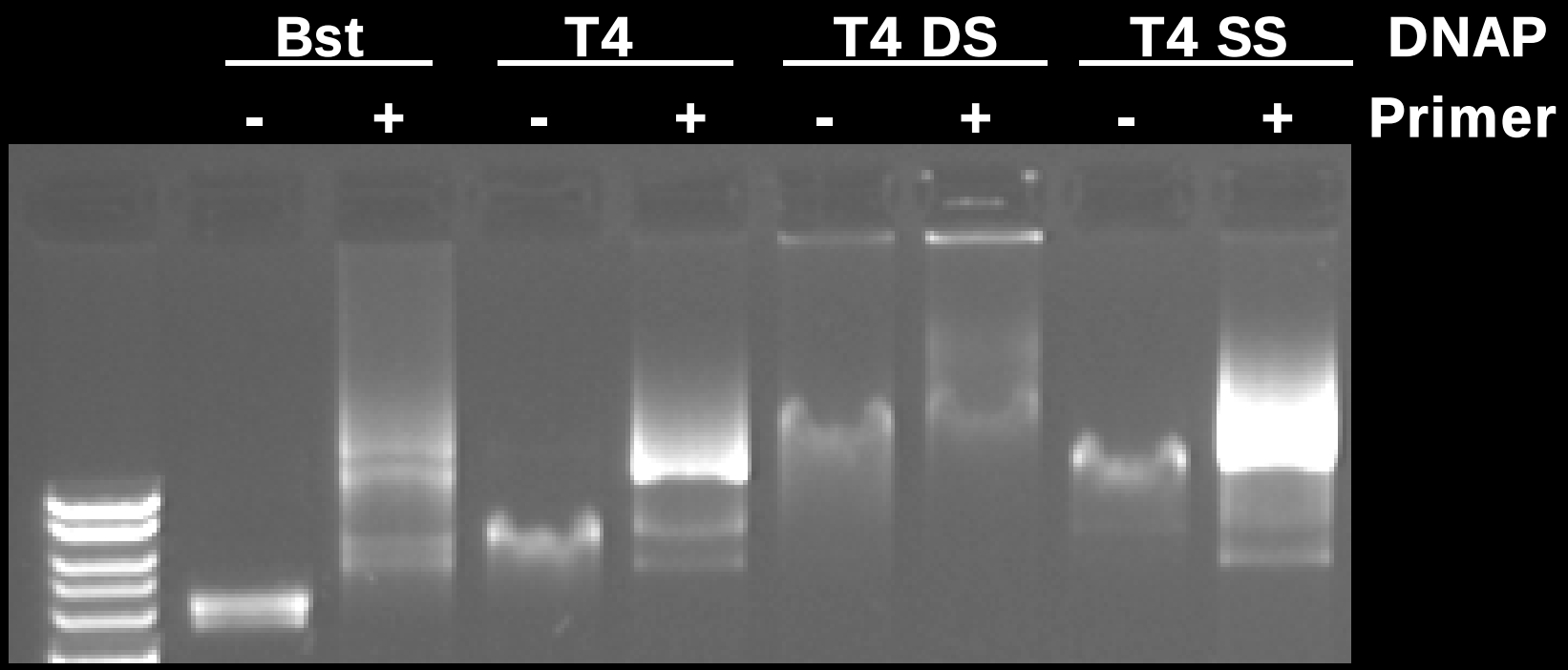
DS and SS Affinity Modified T4 DNAP
M13 Primer Extension Assay



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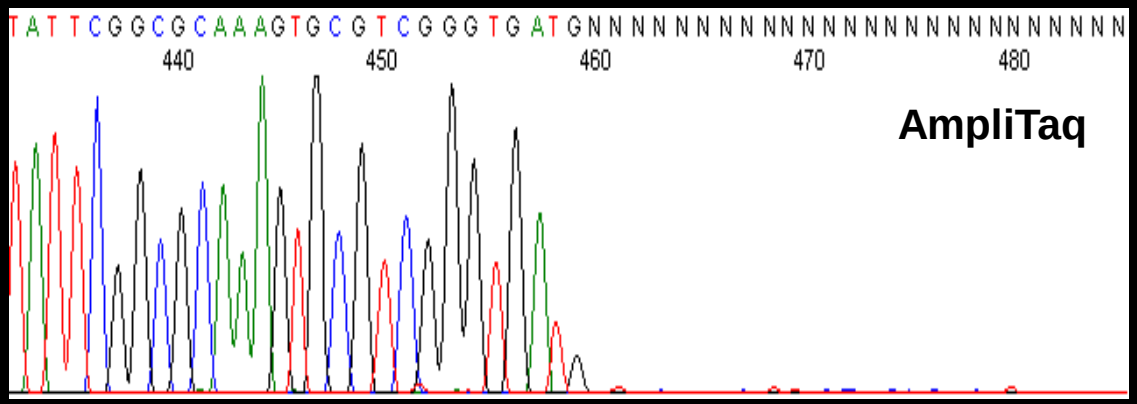
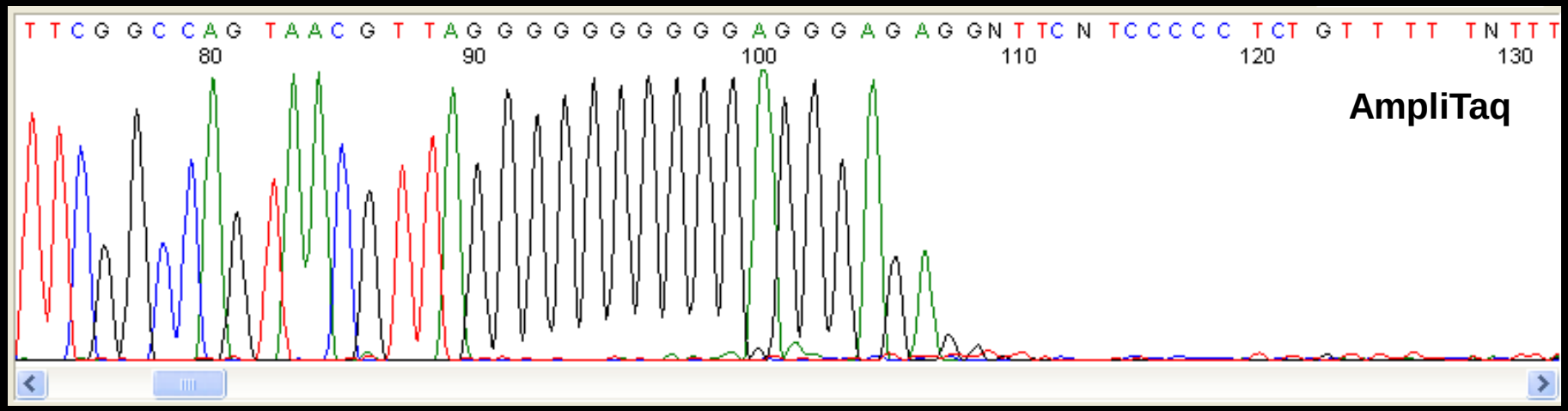
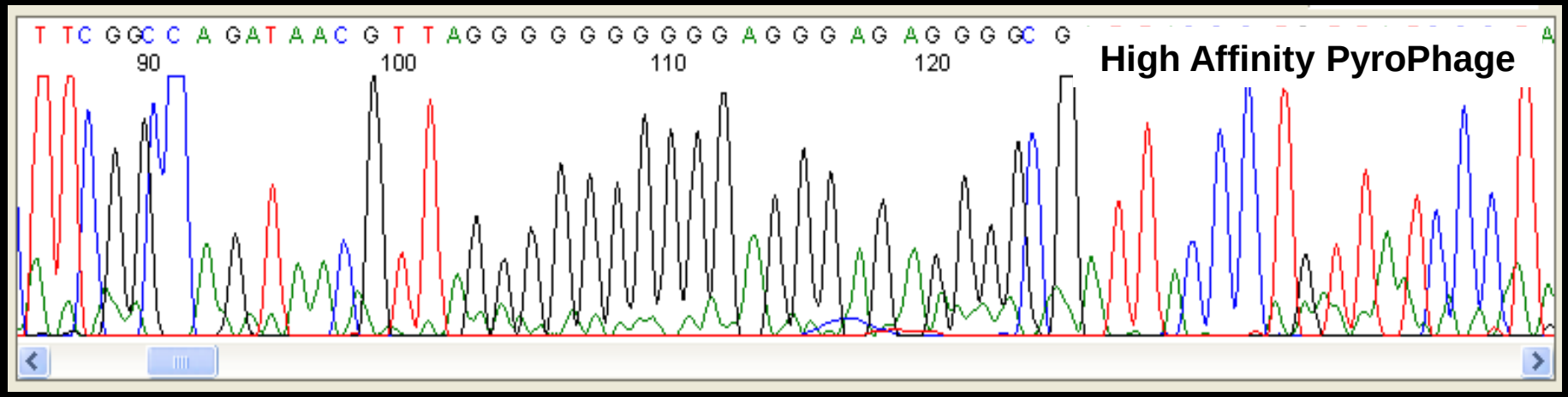
Improved Dye Terminator Sequencing


TATTCGGCGCAAAGTGCGTCGGGTGATGNNN

Improved Dye Terminator Sequencing
GC Clamp





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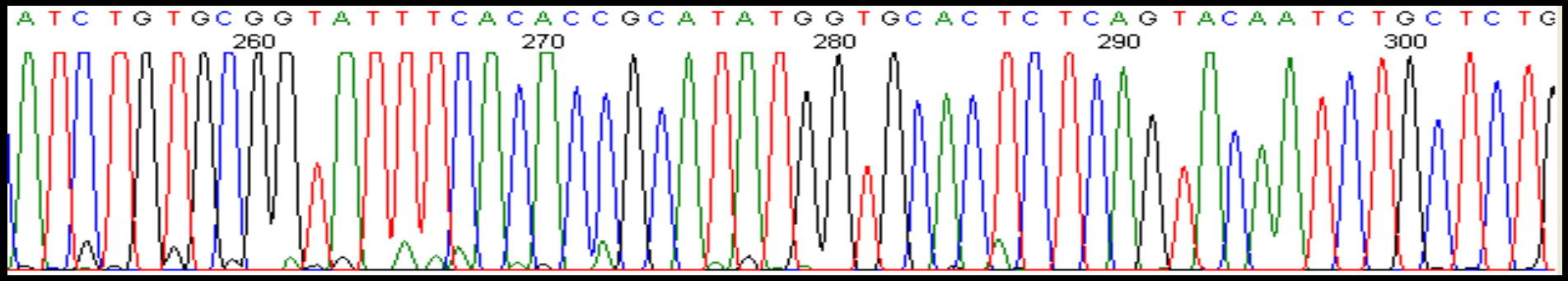
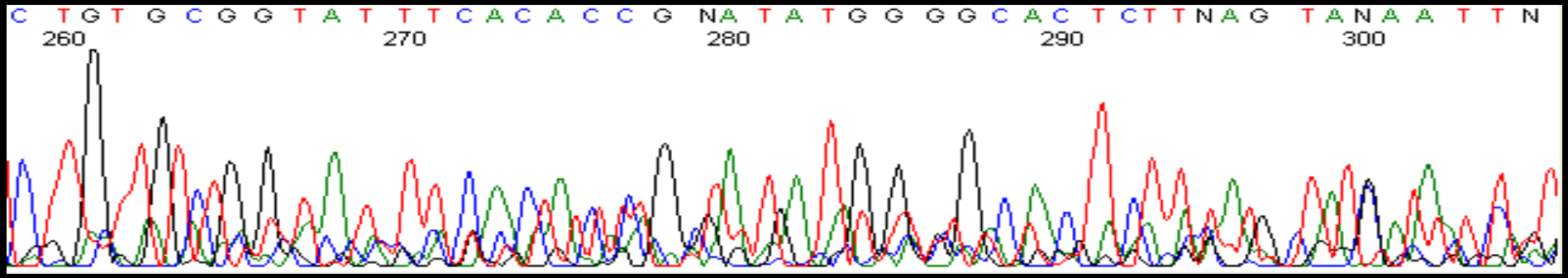
Direct Sequencing from Liquid Culture

High Affinity Taq



AmpliTaq



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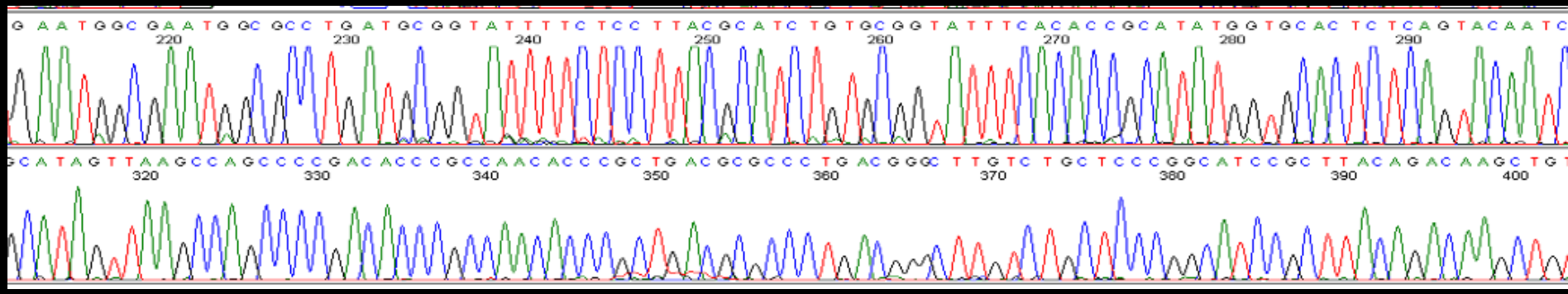
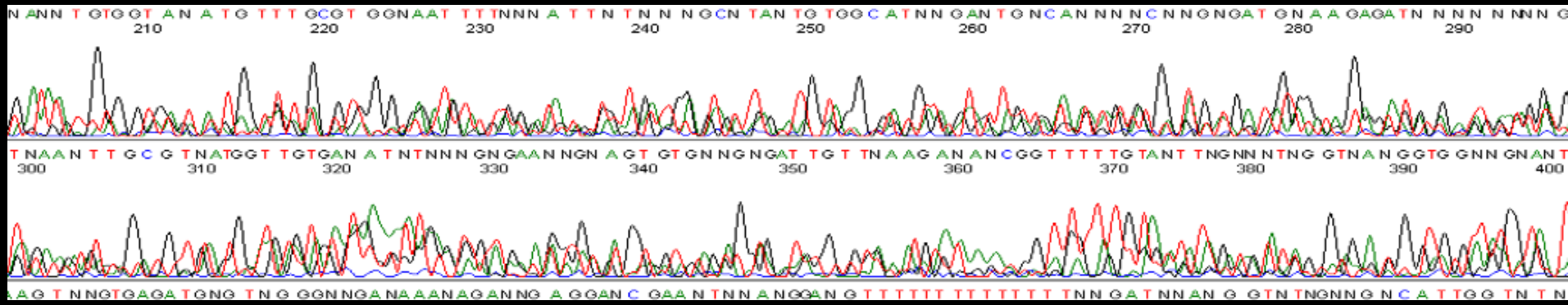
Direct Single Colony Sequencing

High Affinity Taq



AmpliTaq



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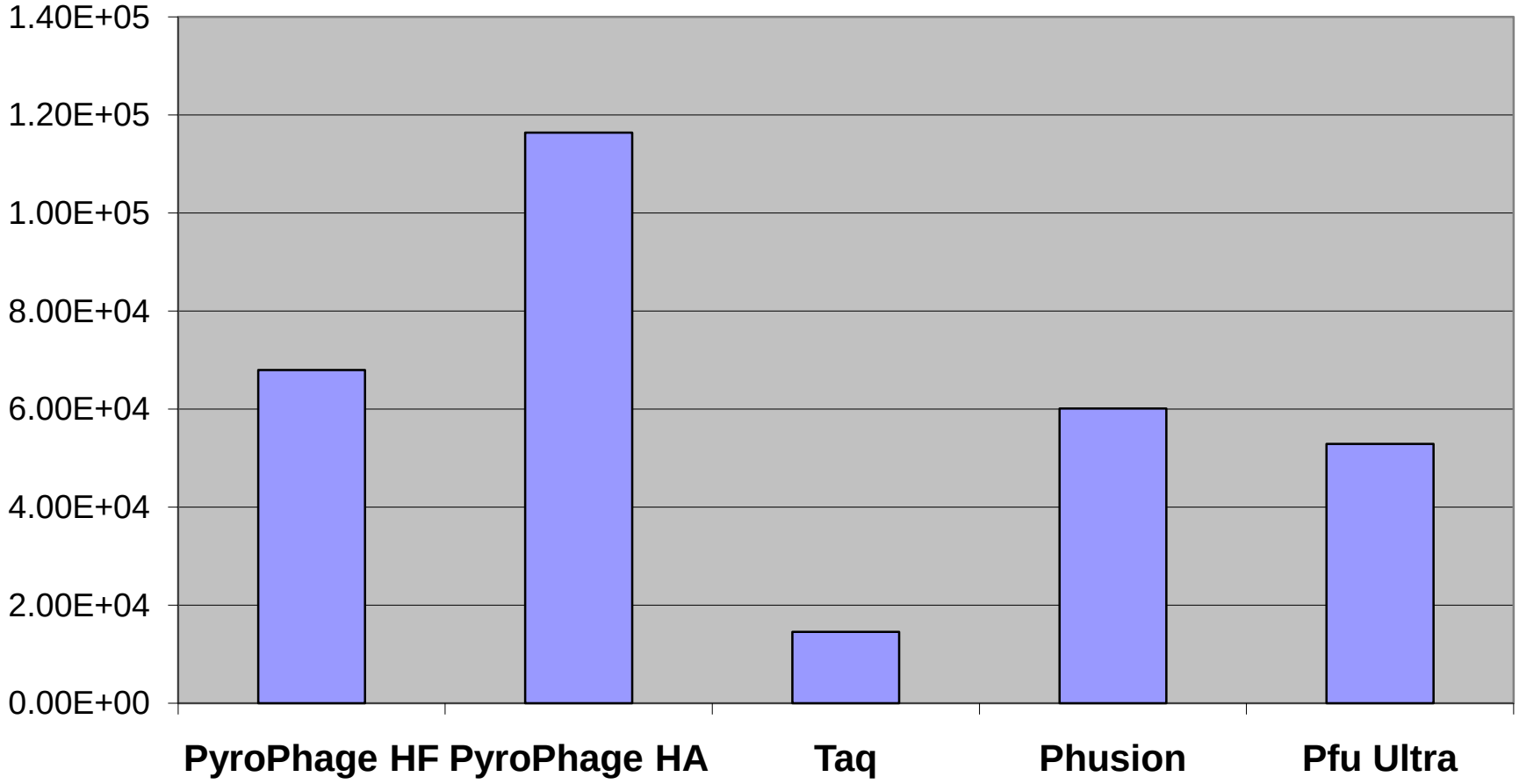
Fidelity Assay of DNA Polymerases



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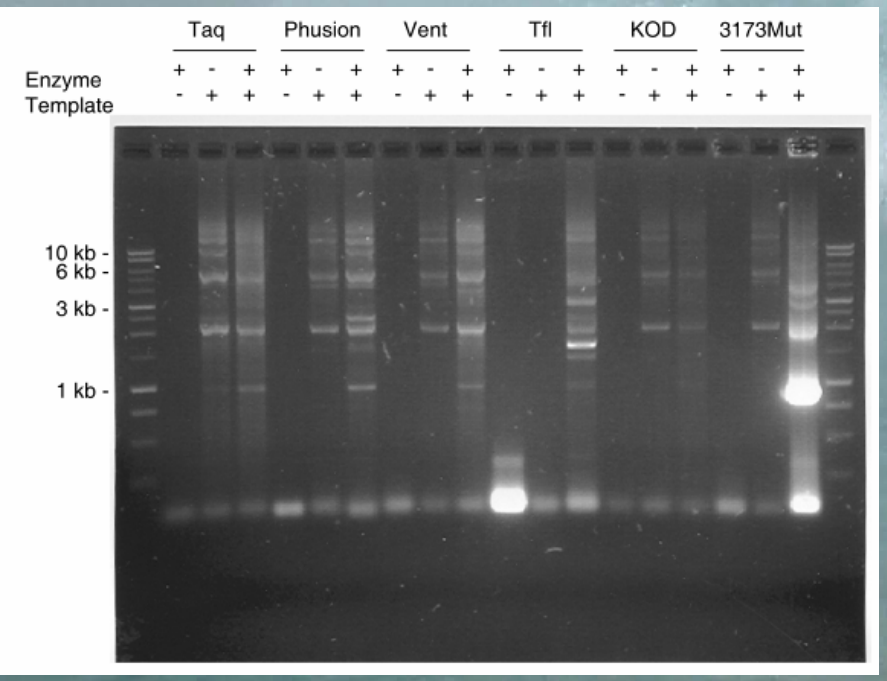
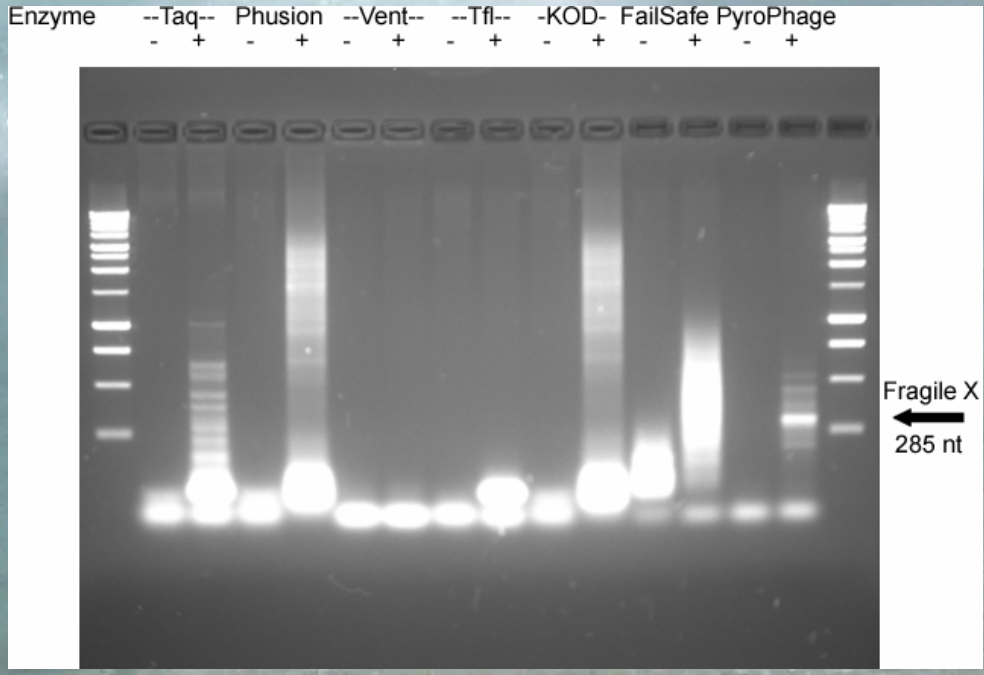
PCR Amplification of Difficult Templates

Bacillus cobA



Human fragile X



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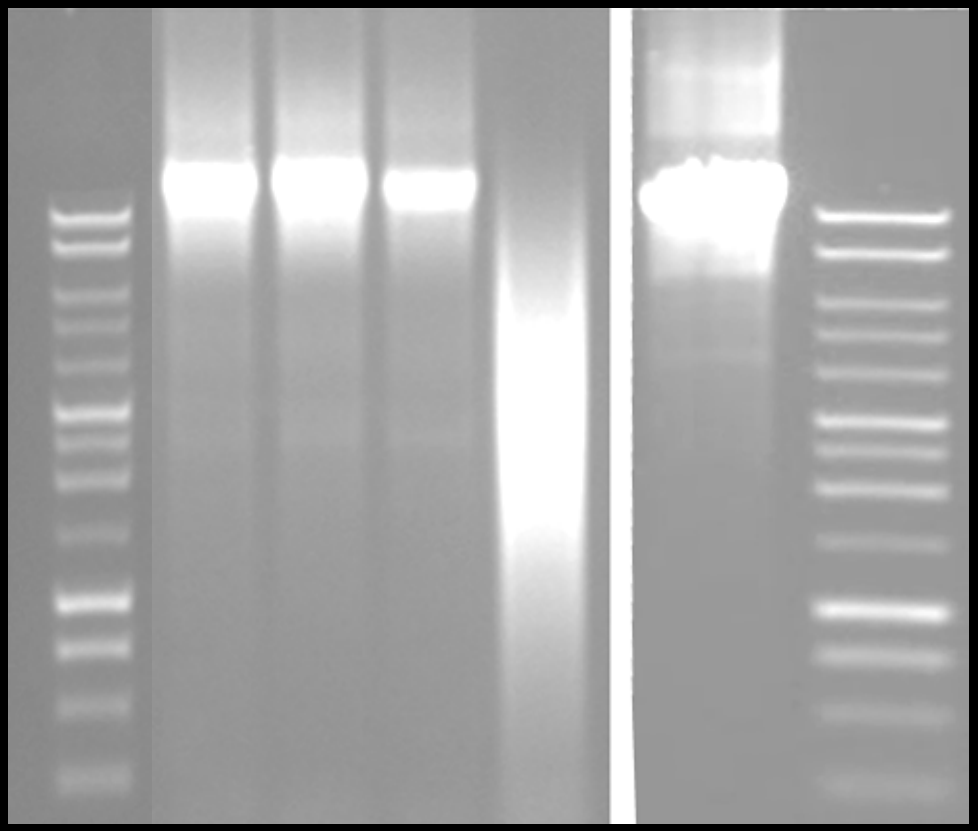
10kb Long PCR

HA Taq	Wt Taq	HA PyroPhage



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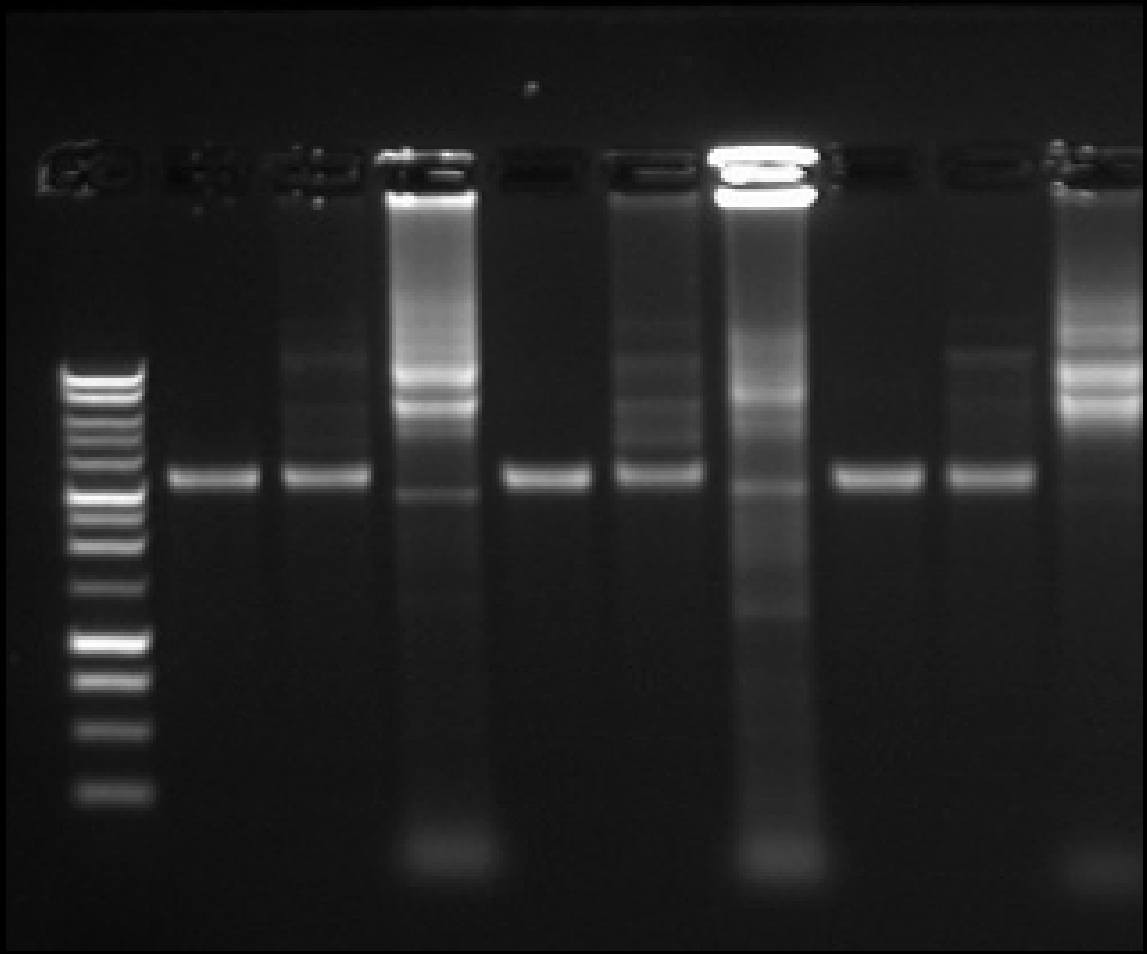
Bst DNAP is Next



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Transcriptome Sequencing

Deficiencies in Retroviral RTs

- Low thermostability
- Low fidelity
- Frequent rearrangements
- Secondary activities
- High bias for specific primers
- Bias for templates
- Interference with PCR enzymes

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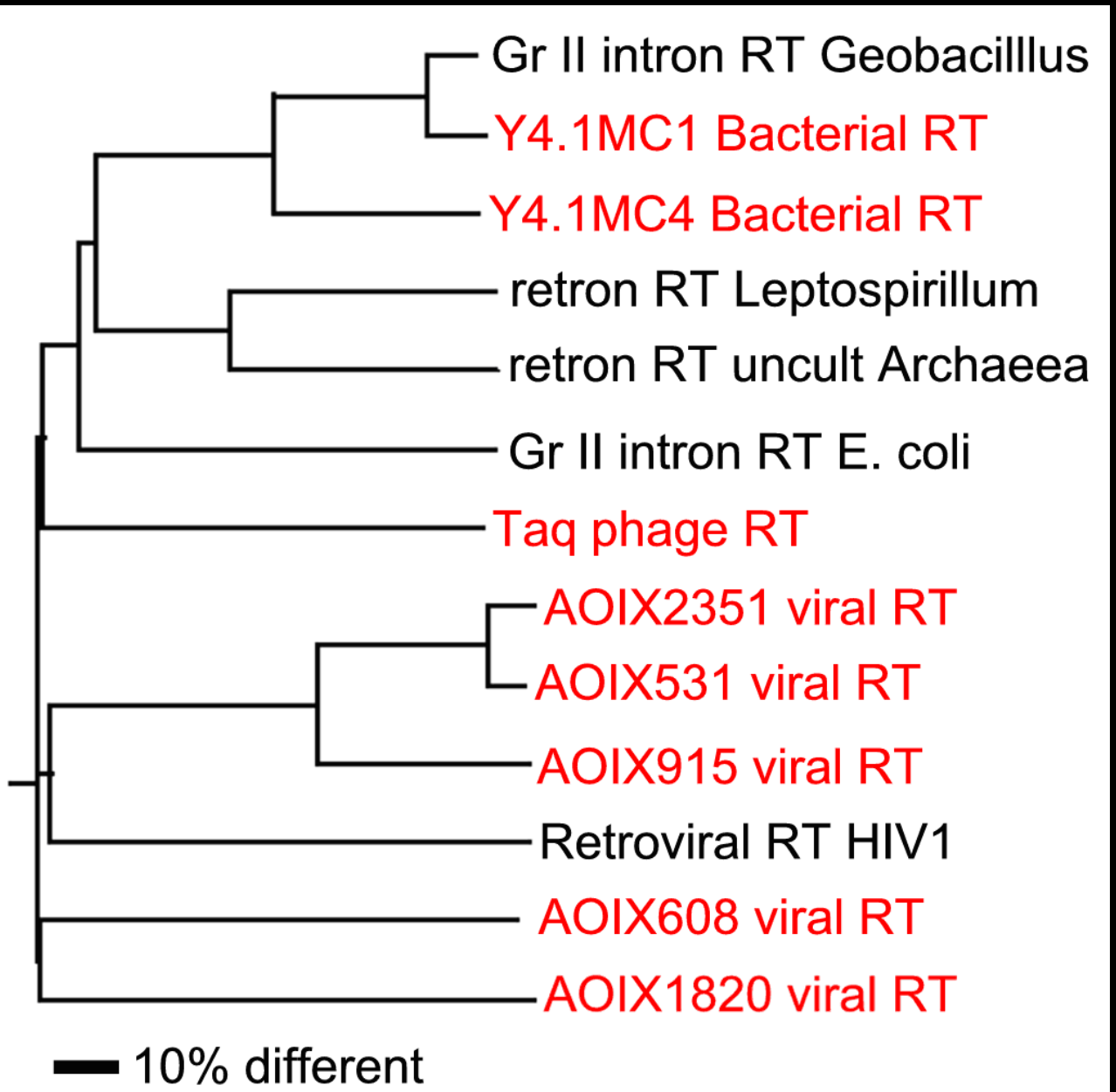
New Thermostable Reverse Transcriptases



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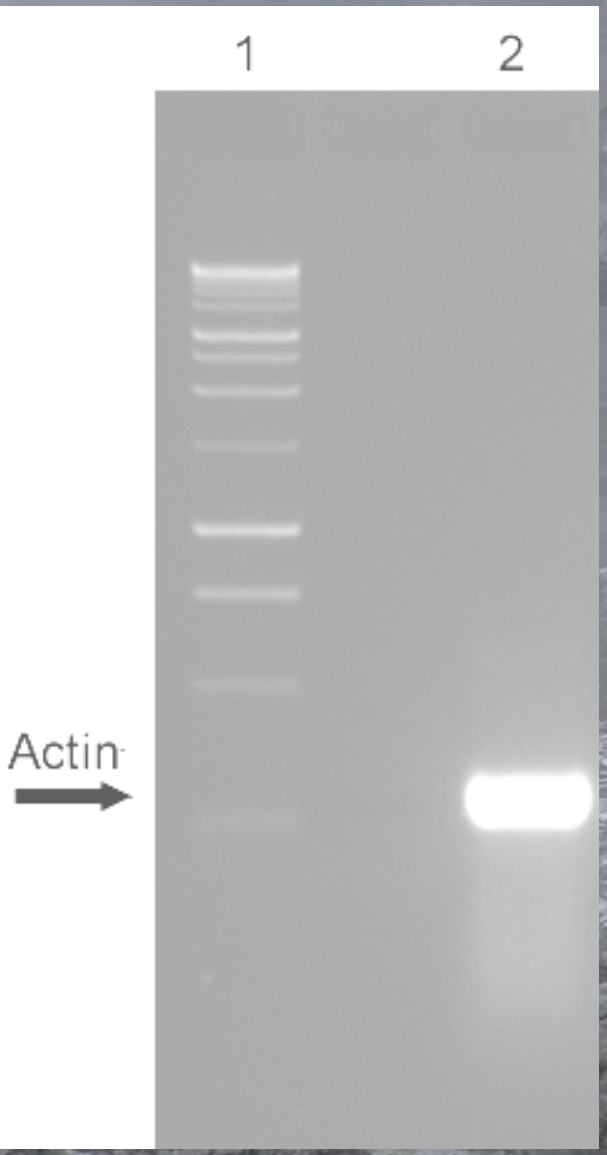
Single Enzyme RT PCR



Template: Mouse liver RNA

Primers: Actin

Conditions: Preincubate 60 min., PCR

Enzyme: PyroPhage RT

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Lucigen Scientists

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NIH NSF JGI NPS

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