University San Diego

Polymerase Chain Reaction (PCR)

PCR can be used to amplify millions of copies of a single fragment of DNA in a short period of time.

PCR uses a reaction mixture containing

- template DNA
- primers
- heat stable polymerase
- deoxynucleotides







TABLE 6.1. Thermostable DNA polymerases differ in their enzymatic activities					
Relative efficiency ^a	Error rate ^b	Processivity	Extension rate ^d	3' to 5' exo	5' to 3' ex
88	2×10^{-4}	55	75	no	yes
70	4×10^{-5}	7	67	yes	no
60	7×10^{-7}	n.d.	n.d.	yes	no
n d	n d	30	60	no	ves
	Relative efficiency ^a 88 70 60 n.d	Relative efficiency ^a Error rate ^b 88 2 × 10 ⁻⁴ 70 4 × 10 ⁻⁵ 60 7 × 110 ⁻⁷ n.d. n.d.	Relative efficiency" Error rate ^b Processivity" 88 2 × 10 ⁻⁴ 55 70 4 × 10 ⁻⁵ 7 60 7 × 10 ⁻⁷ n.d. nd nd ⁻⁷ 30	Thermostable DNA polymerases differ in their enzym Relative efficiency" Error rate ⁶ Processivity Extension rate ⁴ 88 2×10^{-4} 55 75 70 4×10^{-5} 7 67 60 7×10^{-7} n.d. n.d. n.d. 30 60	Thermostable DNA polymerases differ in their enzymatic activities Relative efficiency Error rate ^b Extension Processivity Extension rate ^d 3' to 5' exo 88 2×10^{-4} 55 75 no 70 4×10^{-5} 7 67 yes 60 7×10^{-7} nd. nd. yes

































- Sequencing
- Data Analysis







DNA Cloning

Restriction Enzymes

- Bacteria protect themselves from phage infection by enzymes which bind to foreign DNA and cleave it.
- Many different types (>4,000) each with different binding and cutting characteristics
- We will focus on sticky or blunt end restriction enzymes













Plasmids = ve (filled plasmid often called co	Actors s are onstructs)		
Plasmid Element	Description (Map and table from addgene)		
Origin of Replication (ORI)	DNA sequence which allows initiation of replication within a plasmid by recruiting transcriptional machinery proteins		
Antibiotic Resistance Gene	Allows for selection of plasmid-containing bacteria.		
Multiple Cloning Site (MCS)	Short segment of <u>DNA which</u> contains several restriction sites allowing for the easy insertion of DNA. In expression plasmids, the MCS is often downstream from a promoter.		
Insert	Gene, promoter or other DNA fragment cloned into the MCS for further study.		
Promoter Region	Drives transcription of the target gene. Vital component for expression vectors: determines which cell types the gene is expressed in and amount of recombinant protein obtained.		
Selectable Marker	The antibiotic resistance gene allows for selection in bacteria. However, many plasmids also have selectable markers for use in other cell types.		
Primer Binding Site	A short single-stranded DNA sequence used as an initiation point for PCR amplification or sequencing. Primers can be exploited for sequence verification of plasmids		











Transfection Defined

University San Diego Transfection is the process of getting pieces of DNA into a eukaryotic cell.

Eukaryotic cells can be infected with a virus or transfected using electroporation, cationic lipids, or microinjection.

Transient vs Stable Transfection: integrating DNA into host genome involves selection antiboitic

most plasmids are transient - plasmids are not replicated nor shared with daughter cell







