## PCR

### Background Information

- Genomic DNA and cell lysis
- Components of lysis mixture
- Review DNA replication *in vivo*
- DNA replication *in vitro*
- *Compare in vivo & in vitro DNA synthesis*
- **PCR**
  - Steps
  - Reaction mixture components
- *Human genomic DNA to be amplified*

### Basic Lab Skills

- Genomic DNA preparation
- Setting-up a PCR reaction
- Analysis using DNA Gel Electrophoresis
Compare and contrast DNA Replication *in vivo* with PCR-DNA Amplification *in vitro*. 
DNA Replication

• **Initiation**
  Strand Separation by *helicase*
  Primer synthesis by *primase*

• **Elongation**
  Extension and polymerization of nucleotides by *DNA polymerase* using dNTPs: dATP, dTTP, dCTP, and dGTP

• **Termination**
  Leading strand- End of template strand
  Lagging strand- Replace RNA primer with DNA strand & fill-in gaps with *DNA ligase*
Comparison of *In vivo* and *In vitro* reactions

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<th><em>In vivo</em></th>
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<td>Priming</td>
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Preparation of genomic DNA

• Lyse or break cells by disrupting cell membranes using a detergent

• Digest with a protease to release naked DNA

• Separate DNA from digested proteins
PCR: polymerase Chain Reaction

DNA amplification in a test tube

http://www.sumanasinc.com/webcontent/anisamples/molecularbiology/pcr.html
PCR animation Dolan learning Center

• http://www.dnalc.org/

• http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter16/animations.html#

Polymerase Chain Reaction (PCR)

http://www.biology.arizona.edu/
In prokaryotes

Most of the DNA in a genome codes for protein (or tRNA and rRNA), with a small amount of non-coding DNA, primarily regulatory sequences.

In eukaryotes

97% of human DNA does not code for protein or RNA.

- Non-coding regulatory sequences.
- introns.
- repetitive DNA
  - a. Tandem repeats (satellite DNA)
  - b. Interspersed repeats
Exons (regions of genes coding for protein, rRNA, or tRNA) (1.5%)

Introns and regulatory sequences (24%)

DNA that includes transposable elements and related sequences (44%)

Unique noncoding DNA (15%)

Repetitive DNA unrelated to transposable elements (about 15%)

$Alu$ elements (10%)

Simple sequence DNA (3%)

Large-segment duplications (5–6%)
Origin of tandem repeat families in eukaryotic genomes

Single copy sequence or gene

Gene Duplication

Independent mutations

Family of Identical Tandem repeats (same function)

Family of Homologous Tandem repeats (related functions)
Origin of Interspersed Repeat Families in Eukaryotic Genomes

Single copy sequence

Gene Duplication

Transposition followed by Independent mutations

Family of Homologous Interspersed repeats
Identical Tandem Repeats

**Short** stretches
- within and outside genes

**Long** stretches
- Satellite DNA- structural role at telomeres and centromeres
Interspersed repetitive sequence elements

Several families including retroposons and Alu elements

• Members of Alu family are present within introns, coding regions of genes, or non-coding regions of genes
Target region of PCR amplification

An Alu repeat Element inserted in intron 8 of the Tissue Plasminogen Activator (TPA gene) in some individuals

Position of PCR primers
Individual genotypes
Plasmid Cloning

• cDNA

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter16/animations.html#

• Plasmid cloning

http://www.sumanasinc.com/webcontent/animations/content/plasmidcloning.html

• Steps of gene cloning

http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter16/animations.html#
Recombinant DNA Technology

• [http://present.smith.udel.edu/biotech/rDNA.html](http://present.smith.udel.edu/biotech/rDNA.html)

Plasmid cloning and transformation

• [http://www.sumanasinc.com/webcontent/animal samples/molecularbiology/plasmidcloning_flax.html](http://www.sumanasinc.com/webcontent/animal samples/molecularbiology/plasmidcloning_flax.html)