Bacterial Species Analysis Using 16S rRNA Gene Sequences

Bio300/CMPSC300
Lab Week 3
Spring 2016
From DNA to DNA
From DNA to DNA
Bacteria identification

DNA extraction

PCR

Bioinformatics

DNA sequencing

Gel electrophoresis

ACAGATGTCTTGTAATCCGGC
CGTTGGTGACATAGGGAAAG
GACATTATGGAAGAAATTGTG
ATGCGATGCGGTGAGATCGTG
GCTTATGCTATCGATCAATCA
GGAAATCAATTTAGAGTACTT
AATAGTAGCAAGGAGAGCTGC
TGTAGAGCAACACGTGCTCA
GGCAGATAAAATATTATATCG
TCAAGCATACTAGTAGTATCT
TGAATATCAAATTTTGTGGT
GTTATTCA
Cells

Genomic DNA

Target gene of interest

PCR

DNA sequencing

Gel electrophoresis
PCR – *In vitro* DNA Replication

1. Place genomic DNA in a solution that mimics cellular conditions (pH, salt)
2. Make millions of copies of *region of interest* (ex 16S rRNA gene) using DNA polymerase

http://www.sumanasinc.com/webcontent/animations/content/pcr.html
3. Run DNA on an agarose gel to confirm amplification success
   - is the DNA the expected size?
   - do we have enough DNA?
Cells

Genomic DNA

PCR

Target gene of interest

Bioinformatics

DNA sequencing

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ACAGATGCTCTTGAAATCCGGC
CGTTGGTGCCATAGGGAAAG
GACATTACGAAAGAAAAATTG
ATGCCGATGGTGATCGATG
GCTTAGCTACTGATCAATCA
GGAAATCADTTTAGAGTACCT
ATAGTAGCAAAGGAGCTGCT
TGTTAGAGCAACAGCTGCTCA
GGCGAGATTTATTTATATCG
TCAGGAACTACGGATATCT
TGAAATCCAAAAATTGGTTGTG
GTTATCCA
From DNA to DNA

Total/Genomic DNA

Gene of Interest DNA
From DNA to DNA

Total/Genomic DNA

Gene of Interest DNA

DNA Chromatogram
DNA Chromatogram
DNA Chromatogram
Dye-Labeled Nucleotides
DNA Sequencing – Modified *in vitro* DNA Replication

Replicate DNA using mix of regular and dye labeled nucleotides

- Incorporation of dye-labeled nucleotide terminates sequencing
DNA Sequencing – Modified in vitro DNA Replication

Single-stranded DNA to be sequenced

Add:
- DNA polymerase I
- dATP
- dGTP
- dCTP
- dTTP
- Plus limiting amounts of fluorescently labeled ddATP, ddGTP, ddCTP, ddTTP

Electrophoresis using laser to activate the fluorescent dideoxy nucleotides and a detector to distinguish the colors

Larger fragments

Smaller fragments
DNA Sequencing

Perform DNA sequencing........ to generate chromatograms

Export text files for analysis
Cells

Genomic DNA

Target gene of interest

PCR

Bioinformatics

DNA sequencing

Gel electrophoresis
Bacterial Species Analysis Using 16S rRNA Gene Sequences

Bacteria
- Single-celled, prokaryotic organisms
- Everywhere!!
- 100,000 different species?
Bacterial Species Analysis Using 16S rRNA Gene Sequences

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Bacterial Genomic DNA

16S rRNA Gene DNA
Bacterial Species Analysis Using 16S rRNA Gene Sequences

16S ribosome RNA
- sequence of RNA
- contained within the ribosome
- helps ribosome engaged with RNA during transcription
Bacterial Species Analysis Using 16S rRNA Gene Sequences

**CONSERVED REGIONS:** universal applications

**VARIABLE REGIONS:** group or species-specific applications
Bacterial Species Identification Using 16S rRNA Gene Sequences

**CONSERVED REGIONS:** universal applications

**VARIABLE REGIONS:** group or species-specific applications

Less nucleotide variation

More nucleotide variation
Bacterial Species Analysis Using 16S rRNA Gene Sequences

1. Download and inspect chromatograms
Bacterial Species Analysis Using 16S rRNA Gene Sequences

2. Export in FASTA format
Bacterial Species Analysis Using 16S rRNA Gene Sequences

3. Species Identification and Divergence Time Estimates Using NCBI’s Genbank database and BLAST (Basic Local Alignment Search Tool)