



Biology 475 - Molecular Biology Lab Genomic Isolation & PCR Detection



Goals

Understand how to isolate total genomic DNA from bacterial communities (red or green layers)
Compare/contrast these methods with plasmid isolation
Understand PCR and primer sets, comparing/contrasting with DNA Sequencing

*Bear in mind, full-length product will be analyzed via Southern Blot and probed over next 2 labs.
DGGE product will be analyzed after that. BE CAREFUL because everything hinges on today.*

ASSUMED BACKGROUND

	DNA Replication	Contrast PCR
Start	Origin bound by primer	Man-made primers FLANK TARGET - (16S, here)
Unwound by	Helicase	Boiling
Copied by	DNA Polymerase	Thermo-tolerant Taq DNA Polymerase
Monomers	dNTPs (N = A, T, G, C)	dNTPs (N = A, T, G, C)
Products	Two full-length genomes	Millions of ONLY TARGET

We will be using the Promega PCR MasterMix kit that contains buffer, monomers, and Taq all mixed in a 2X concentration. What does that mean you will be doing?

Our PCR Cycling Conditions

Denaturing: 94°C for 1 minute.

Annealing: 1 minute for 60°C. Depends on primer composition. Roughly = $3(\# \text{ G+C}) + 2(\# \text{ A+T})$

Extension: 3 minutes at 72°C. Depends on the product length. Roughly = 1 minute per 1000 bases

Number of Cycles: 35 repetitions. This is variable and somewhat contentious.

Troubleshooting PCR - False Negatives

Amplifying DNA from many samples is tricky - trace metals, acids, bases inhibit Taq

Even too much starting template DNA can inhibit PCR

We will address by amplifying both diluted and undiluted template DNA

Troubleshooting PCR - False Positives

PCR is SOOOOO sensitive - many reactions will detect 1 MOLECULE of starting template

Areas where template DNA is purified and PCR reactions are set up should be P-Free

All chemical stocks, water, equipment should also be P-Free

The P stands for plasmid and PCR product (and also phage - not used at WOU)

Although you will be working in temporary P-Free zones (treated with UV/Bleach), most labs (including my back office) have a dedicated P-Free space for generating product to be cloned.

GENOMIC DNA ISOLATION ACTIVITIES

Carry Out In Fume Hood - You Should Be Done With This By 1:30

Obtain ice from organic chemistry lab

You will be provided with a Bead Beater (BB) vial that is half-full of 0.1 mm Zirconium Beads

Add 800 ul Phenol/Chloroform to BB vial, cap, and invert to "wet" the beads

Add mat sample (up to 0.1 g), 66 ul 10% SDS; Top off with GTE until no airspace (600 – 700 ul)

Tightly cap vial and place in BB, beating at max speed (48) for 1 minute (1 increment = 10 seconds)

