

DNA Sequencing using PCR

Review this video only in an overview sense, as it is somewhat outdated, but good to show you progress and some techniques!! *Note, this technique is rarely used, as thermal cycling PCR reaction is preferred.*

Single stranded (SS) DNA Template

PCR Synthesis and Purification

Theory ideas (cite relevant text and or Cold Spring Website.)

Gel electrophoresis is used to isolate fragments of desired length (from other non target DNA.). Agarose (w/ DNA) is excised from gel and spun free of agarose.

Wash Magnetic Beads

Sense and non sense strand is incubated with biotin (which attaches to DNA)

Biotin labeled DNA is added to magnetic beads coated with streptavidin and so biotin is attracted to beads.

By using a magnet, beads are collected at side of tube and washed.. the non-polymerized DNA, Mg, TAQ, etc. are removed.

So beads are pure DSDNA attached by one strand with biotin/streptavidin cross-linker.

Add PCR to Magnetic Beads, Incubate Beads

Separate Beads from Solution , Denature and spin down while warm (so now you have SSDNA in the supernatant and SSDNA in the pellet.)

Labeling and Termination Reactions

Labeling Reactions

Termination Reactions

Add Stop Solution

Electrophoresis

Different polymerases can be used: TAQ T7, AMV polymerase and Klenow

Rational for different polymerases:

- Ability to perform at different temperature.
- Elongation rate
- Processivity (how well the enzyme attaches to the template.
- Exonuclease activity
- Nucleotide analogs and likelihood of integration.