

PCR and Detection

Name and describe 3 applications of PCR

- 1.
- 2.
- 3.

Avoid Contamination

List the significant steps to take:

- a.
- b.
- c. Why acid and base?

Make Master Mix

Components: Buffer, dNTPs, TAQ, water, primers

Role of dUTP avoid contamination, How? See other videos

- DNTPs conc.
- Role of filter tip in plug?
- TAQ origin

Master mix has no specificity, so added to tube as first part of cocktail

Primers: recall needed for polymerase to have free three prime end.

Set Up PCR Reactions

-**add mineral oil:** Can use repeat pipet. Why added first? To avoid aerosol formation

- **add Master Mix**

- **Add Controls and Samples: Three primary types**

1. negative control: lacks DNA target.
2. positive reaction: target template.
3. reagent control: all reagents except for template DNA.

Amplify DNA

Review Cold Spring Harbor URL if necessary

Why is inward synthesis crucial (as opposed to outward synthesis?)

True/False: DNA must be fresh for PCR reactions to work:

Cite three ideas to support your assertion.

1.

2.

3.

Detect PCR Products

-Et-Br staining: Basically a review: What are a few disadvantages?

- a. requires high amount of product
- b. can not tell for sure if it is target DNA

- Southern Blotting Hybridization

Probe with a complementary DNA Advantages: sensitivity and verification.

Disadvantages: Time and money

- Oligomer Hybridization Most sensitive and specific

*P probe allows for specificity

Electrophoresis is used to separate non-bonded oligomer

Kinase equilibrium will phosphorylate with * ATP.

Gel Filtration: Can be used to separate *ATP from labeled probe with probe emerging first.

PCR reaction products are denatured and mixed with *P (probe) and cooled to allow annealing.

Onto PAGE and different bands can be seen (note these bands are known (unlike Et-Br))

Oligomer Hybridization is usually optimized by varying concentration/degree of: enzyme, T, Mg [] and primers.

Low Fidelity: Causes mismatch and subsequent mis-extension

High Specificity: Has low balanced dNTP[] (if too high, may cause mismatch (as a function of saturation)

High Annealing temperature will avoid non-specific bonding.

Low enzyme concentration [] too.....

Final thoughts: Avoid contamination, set up competent master mixes, set up reactions to avoid contamination, amplify, detect, optimize.