

Avoiding False Positives in PCR

Physically separating pre and post PCR

Why is contamination ratio so significant in PCR (magnification)

Most significant source: aerosols

From: Previously performed PCR reactions and new samples. The first source is most significant as it often the most concentrated.

The pre and post PCR cabinets are physically separated.
Each cabinet often has own complement of needed supplies kept in the hood.

Hood ventilation is not wanted as to stop aerosols:

Assignment: Make a list of supplies for Pre PCR cabinet.

Autoclave reagents (if appropriate)

Name a few that are good and a few that are bad to autoclave.

Equipment and supplies

Positive displacement pipettes:

Compare and contrast with a diagram of positive and negative displacement pipettes.

Displacement rod will not allow for aspiration into positive displacement pipettes.

Disposable gloves and plastics

Techniques

Aliquot reagents: Following reagents can be pre-aliquoted: water, buffer, dNTPS, mineral oil, primers etc....

Sample to sample contamination

- Again, remember to minimize the # of steps and total time as they both contribute to possible contamination.
- Watch dried products as static charge may cause contamination.
- Rack positioning of tubes. Watch that empty tubes are never in front of tubes being filled.

Positive and negative controls

1. Negative (not target DNA) 2. Positive: template
3. reagent: all substances except for DNA

Add DNA last (preferably after enzyme)
Avoiding splashes. (Mineral oil will, help.)

PCR product destruction

Sources of PRC contamination products: gel rig, razors, UV trans-illuminator tray, microtome blade, centrifuge, speed vacuum, and ice bath

Sterilization involves: 1. HCl 2. NaOH 3. Buffer 4. Water

Depurination: Can bombard with UV light (when Suerol is added to accelerate cross linking.)

Uracil-N-Glycosylase and UV Light

By adding odd base (dUTP) to PCR reaction then PCR products are novel. Uracil-N-Glycosylase (UNG) will destroy uracils and P-S bond so products are fragmented and therefore new PCR reactions will not work.

So if UNG and dUTP are added to cocktail, how does dUTP get in?

At low temperature, the UNG will destroy any PCR products, but after the first cycle the high temperature will destroy the UNG and allow further dUTP incorporation.