

## Sample Preparation for PCR

### Type of Sample

Three types of samples include:

General strategy: Quick (as longer steps equal more chances for contamination)

Short: As fewer steps will result in fewer potential mistakes.

### Tissue Processing

General considerations:

- Centrifugation to keep products deep in tube to avoid contamination.
- Tubes spaced far apart on racks
- Fresh gauze is used when opening tubes.
- Procedure is done in dedicated hood.
- Vortexed to loosen reagents (as rotation is often not enough)
- Tubes marked on top as sides tend to smear.

**Remove parafilm** (Organic solvent removal)

**Fixative degradation** Variable ideas and specific removal

**RBC:** Actually a contaminate of blood as WBCs are source of DNA. Heme group of RBC's interferes with PCR.

**Urine:** Sterile solution, so is quite a good source (provided cells are washed.)

### Other Inhibitors

#### Polyanions in Agarose

#### Metal Cations

**Proteinase K Digestion:** Digests peptides and helps to free DNA and will destroy DNAases.

3-parts to solutions: proteinase, detergent (non ionic, why not stronger?) and buffer.

Water baths are not usually used, (helps avoid contamination.)

**Yield:** Proteinase K time is watched very closely as too little time will result in inadequate digestion, and too much will degrade DNA

### PCR Modifications

Temperature regimes are usually modified in light of the quality of DNA with poor quality DNA requiring longer temperature regimes. Why?