



### Contents

- [1 Using Cytobrush](#)
- [2 PCR with PCR Beads](#)

## Using Cytobrush

A cytobrush is a small brush that is traditionally used for performing pap tests. These are sterile and convenient for single usage inside the cheek of the mouth. Brushes are swirled in a solution of Chelex beads so that the chelating agents in the solution neutralize the action of divalent cations to protect the DNA. Placing these tubes of cells and Chelex onto a boiling heat block is designed to sterilize the solution for safe working conditions. An alternative to this use of Chelex is to displace the cells into a lysis buffer that will burst the cells and protect the DNA.

1. Use sterile cytobrush and insert into mouth
2. brush cytobrush on inside of cheek 25 times
3. Swirl cytobrush in 100  $\mu$ l of Chelex suspension (10% w/v) or lysis buffer containing EDTA
4. Place centrifuge tube with Chelex and cell suspension on 100 °C heat block for 10 minutes
5. Centrifuge tubes at maximum speed for 5 minutes
6. DNA is in the supernatant. (avoid beads at bottom)
7. Store DNA in -20 °C

## PCR with PCR Beads

PCR Beads are prepared mixes of a thermostable polymerase with the appropriate salts (ions), buffering compounds and dNTPs that have been dehydrated into beads in PCR tubes. These beads require hydration with a primer solution and template DNA. Primer solution is usually prepared with sucrose or other dense compound with a dye molecule so that each PCR tube can be directly loaded onto an agarose gel.

1. Add 22  $\mu$ l of primer mix (forward and reverse) to beads
2. Ensure that the the bead is dissolved
3. Add 3  $\mu$ l of DNA (if no amplification, try diluting the DNA sample at least 10x)