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Protocols: A guide to methods and applications, Innis et al. eds. Academic Press, 1990.

Proteinase K: make fresh solution @ 20 mg/ml in 10 mM Tris-HCl, pH 7.5

K buffer: 10 mM Tris-HCl, pH 8.3
50 mM KCl
1.5 mM MgCl2
0.5% Tween 20
100 ug/ml proteinase K (freshly dissolved enzyme)

1. Pellet cells from PBS
2. Remove supernatant
3. Optional: wash again
4. Resuspend in about 4 volumes (50-300ul) K buffer (volume is such that there will be 1000 - 5000 cells per ul)
5. Transfer to an Eppendorf tube and incubate @ 55C for 1 hr.
6. Heat at 95C for 10 min to inactivate the proteinase K.
7. Store the sample at -20C until ready for use.
8. For PCR, thaw, vortex, use 5-10ul per 100ul reaction.