

Detection of cell culture contaminations using McCT

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Protocol for boiling extraction of genomic DNA from cultured cells

Important: In order to avoid cross-contamination during DNA extraction use aerosol-resistant filter tips and change tips after every pipetting step! Cabinets should be used for DNA extraction in order to protect staff from being infected with hazardous material and to protect the specimens from exogenous contaminations. Such cabinets should contain systems for filtered circulating air (equivalent to airflow within a class II biological safety cabinet), as well as UV light facilities for internal decontamination of the work area, air and pipettes.

One must be aware of the importance of maintaining the working efficiency of such cabinets, as these may become a source of contamination if the filters are not serviced regularly. Furthermore, the efficiency of UV light production should be monitored regularly.

1. count cells of interest (either suspension or trypsinated adherent cells) and pellet cells (10^6 to 10^7) by centrifugation (600 g, 5 min in tabletop centrifuge) using a labelled 1.5 ml safe-lock tube
2. resuspend the pellet into 100 μ l of PBS
3. place tubes at **95°C for 15 minutes**
4. centrifuge at > 10.000 g for 5 min to pellet the cellular debris
5. transfer supernatant (lysate) into a properly labelled (with sample name) **1.5 ml safe-lock tube** (Label tubes with 'KstSt' AND 'specific sample name' as provided in the Submission Form)
6. store lysate at -20 to 4°C
7. fill out the submission form and deliver lysate(s) to the ATV building INF 242 every Monday, 10-12 a.m. to the doorman