Human cell line authentication

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Protocol for DNA extraction for human cell line authentication

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 exogeneous 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. The cell culture suspension containing 1 to 5 x 10^6 diploid cells is centrifuged in a Safe Lock tube at 2,000 x g for 4 min.
2. The supernatant is removed with a disposable pipette and discarded.
3. The remaining pellet is carefully resuspended in 5 mL PBS using a pipette and centrifuged at 2,000 x g for 4 min.
4. After the washing step, the pellet is resuspended in 200 µL PBS by vortexing. Make sure that even tiny clumps of cells are carefully resuspended.
5. Pre-warm the water bath to 70 °C.
6. For isolation of the genomic DNA, the commercial DNA extraction kit from Roche is recommended (High Pure PCR Template Preparation Kit, Roche, Basel, Switzerland). 200 µL of solution I (guanidinium-hydrochloride) is added to the cell suspension and mixed by pipetting.
7. Add immediately 40 µL proteinase K, mix well using a vortex and incubate at 70 °C for 10 min.
8. Add 100 µL of isopropanol (not included in the kit) to the sample, mix well and apply the whole mixture to a filter tube.
9. Centrifuge for 1 min at 8,000 x g.
10. Discard the flow-through and the Collection tube, add 500 µL of inhibitor removal buffer to the upper reservoir of the Filter tube (with new Collection tube) and centrifuge again for 1 min at 8,000 x g.
11. Discard the flow-through and the Collection tube, add 500 µL of wash buffer with new Collection tube and centrifuge again for 1 min at 8,000 g and repeat this step. Empty the Collection tube and spin the Filter tube-Collection tube assembly for additional 10 sec at 8,000 x g.
12. Replace the Collection tube with a new 1,5 ml Eppendorf Safe Lock tube and add 100 µL of elution buffer preheated to 70 °C and centrifuge for 1 min at 8,000 x g.

13. The purified genomic DNA concentration should be adjusted to approximately 15 to 30 ng/µL, at least 30 µL should be delivered.
14. Label the Safe Lock tube with “MCA” and the “sample name” and your “cost centre” as provided in the submission form
    Note: Don´t seal your Safe Lock tube with Parafilm
15. Store DNA at -20°C
16. Fill out the submission form and deliver DNA sample(s) to the ATV/ICC building INF 242 every Monday, 8-12 a.m. to the doorman. Put the samples in the “SNP-cell-authentication” box