Lab 3

Level 2

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DNA extraction from human blood

Blood is a complex mixture of cells, proteins, metabolites, and many other substance. About 56% of human blood volume is comprised of cells, more than 99% of which are erythrocytes. Human erythrocytes and thrombocytes (platelets, 0.5% of blood components do not contain nuclei and are therefore unsuitable for preparation of genomic DNA. The only blood cells that contain nuclei are leukocytes (0.3% of cellular blood components). Blood samples may vary widely in the number of leukocytes they contain, depending on th health of the donor. Healthy blood, for example, contains fewer than 107 leukocytes per ml, while blood from an infected donor may have a tenfold higher leukocyte concentration.

Method

Approximately 3-5 ml of blood was taken from each donor by sterile syringe and places in EDYA tubes.

1. Each blood sample was placed into 15 ml tube.
2. Te buffer was added to a volume of about 10 ml.
3. Blood and TE buffer were mixed well by inverting the tubes several times.
4. Mixture was centrifuged at 10000 rpm for 10 minute at 4C.
5. The supernatent was discarded by using a pipette to avoid losing the pellet.
6. The pellet was washed with TE buffer by repeating steps 1-4 until it is pink.
7. The supernatent was discarded by using a plastic pipette (the pellet should not be lost) and pellet was resusbended in 1 ml TBS buffer.
8. 1 ml of Lysis buffer B and 100 µl of proteinase K solution were added.
9. Tubes were incubated in waterbath 55C for 60 min with agitation
10. The samples were taken out from waterbath and 1 ml saturated phenol, and 1 ml of the mixture cloroforme: isoamyle alcohol (24:1) were added.
11. The samples were shaken 5 min by inverting the tubes.
12. The samples were centrifuged at 10000 rpm for 10 min.
13. The upper phase was transfered with plastic pipette to a new tube, the bottom phase should not be touched.
14. 2 ml of chloroforme: isoamyl alcohol (24:1) was added.
15. The samples were shaken 5 min by inverting th tubes.
16. The samples were centrifuged at 10000 rpm for 10 min.
17. The supernatent was transferred to a new tube (the bottom phase should not be touched).
18. Ammonuime acetate solution to a final concentration of 2.5 M was added and then 2.5 volumes of cold (20C) 95% ethanol.
19. The tubes were inverted several times until DNA appeared as a white precipitate.
20. When the DNA concentration was high , DAN standars would forme a visible precipitate, which were colected into a compact mass of material that can easily be removed from the tube by spooling the DNA mass on a pasture pipette. The DAN in this manner was spoold instead of being recoverd it by centrifuge, the DNA was separated from the bluck of RNA which had been co-purifieded but remainded in solution. This eliminated the need to add exogenoous Rnase, which may be contaminated with nucleases.
21. The DNA was dried (it should not be over dried, otherwise it would be difficult to be resuspended) and it was resuspended in 2 ml of TE in 5 ml tube. And alowed to be sat until the DNA was released from the pipette tip. Once the DNA had been released, the tube was let in the room temperature for hours to allow the DNA dissolving in the buffer, and then it was stored in the deep freezer.
22. The samples which do not form a visible precipitate, the DNA was recovereded by 30 min centrifyging and resuspended in TE buffer as discriped before.

Tris-EDTA (TE) Buffer

(10 mM Tris-HCl, 1 mM Na_2EDTA, pH=8)

It was prepared by dissolving 0.2422gm of Tris-Base, 0.0744gm of EDTA in D.W, pH was adjusted to 8.0, volume completed with D.W to 100 ml, sterilized by autoclaving and stored at 4°C.

TBS buffer

(20 mM Tris-HCl, pH= 8, 150 mM NaCl)

It was prepared by dissolving 0.1211gm of Tris-Base, 0.4383gm of NaCL in D.W, pH was adjusted to 8.0, volume completed with D.W to 50 ml, sterilized by autoclaving and stored at 4°C.

B buffer

(400 mM Tris-HCl, 100 mM Na_2EDTA, pH=8, 1% SDS)

This buffer was prepared by dissolving 2.4228gm of Tris-Base, 1.86gm of Na_2EDTA, in D.W, pH was adjusted to 8.0, volume completed with D.W to 50 ml, sterilized by autoclaving then added 0.5gm of SDS.