METHODS FOR THE ISOLATION OF DNA

- **Salting out/salt precipitation method**
- **Phenol/chloroform extraction method**
- **Silica gel extraction method**
- **Proteinase K extraction method**
- **Anion exchange methos**

The choice of method depends on many factors:

- Required quantity
- Purity of DNA
- Time
- Molecular weight of DNA

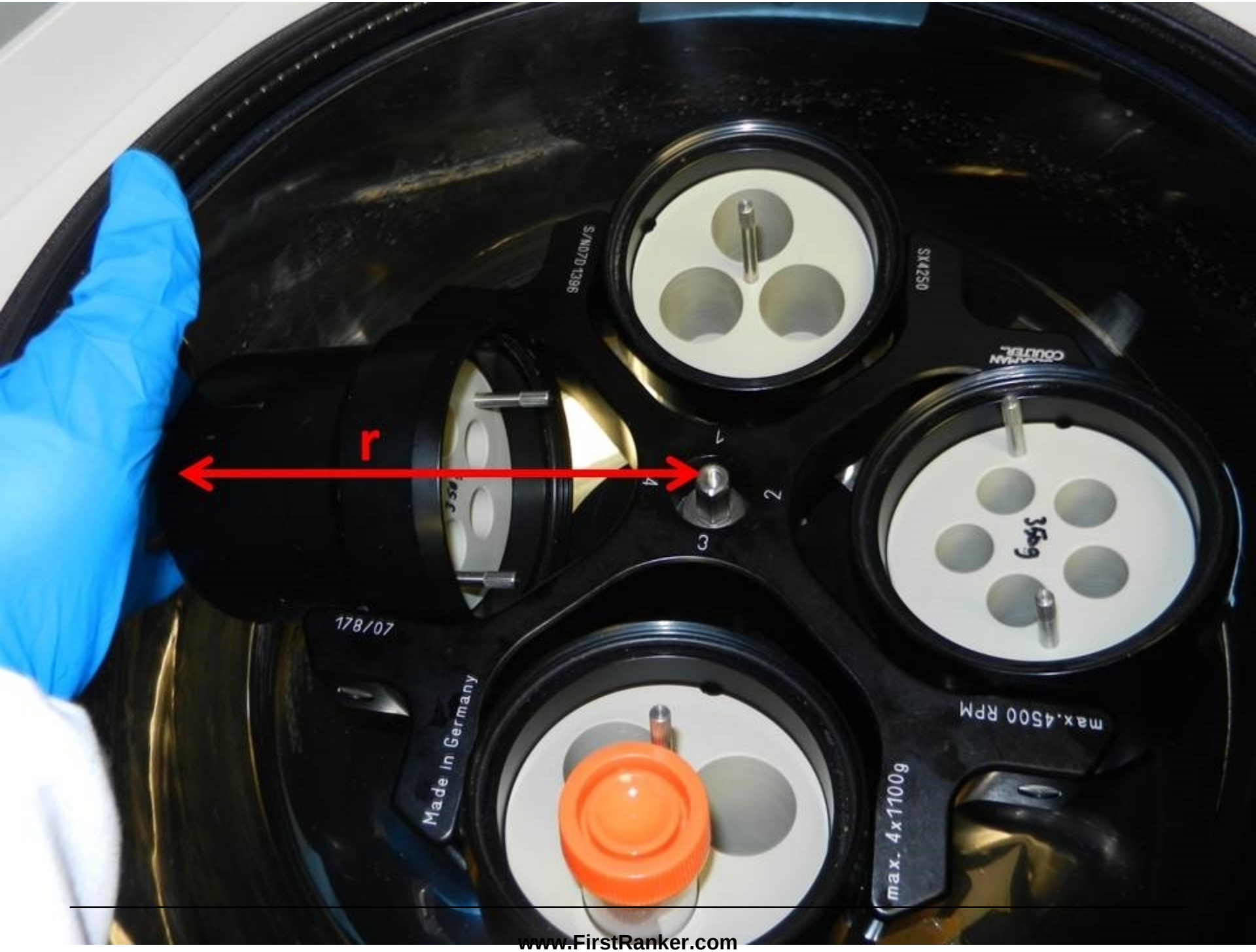
Equipment/reagent requirements

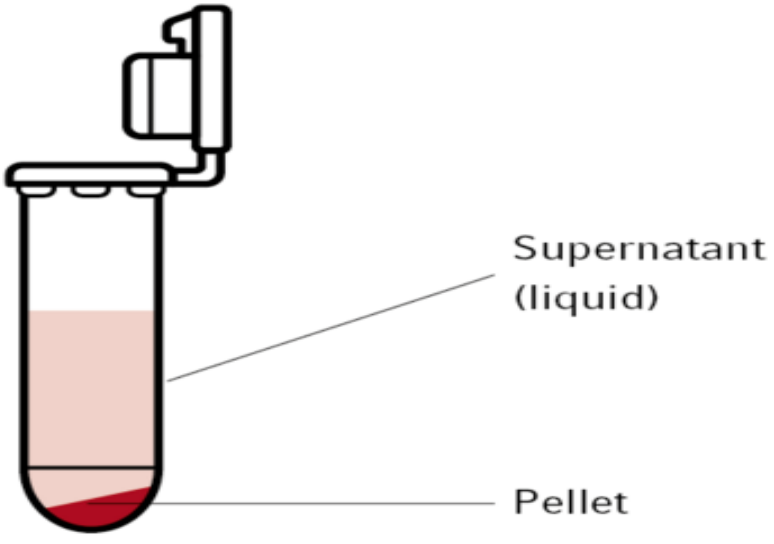
Red blood cell lysis buffer
White blood cell lysis buffer
RNAase
Protein precipitation solution
Isopropanol
DNA hydration buffer
A centrifuge machine
A vortex mixer
-80 degree Freezer
Incubator/Water-bath
A spectrophotometer capable of reading at 260 and 280 nm/Nanodrop

Choice of specimen

- DNA can be isolated from whole blood (EDTA tube) or a cell pellet following plasma separation from blood collected in EDTA tube

- Specimen can be stored at 4 degree Celsius for 48 hours prior to processing.





Precipitate (pellet) stands for the concentrated particles in a tube after successful centrifugation.

Supernatant is the remaining solution above the pellet.

Procedure

Cell lysis

- 1. Dispense 30ml of red blood cell lysis buffer (NH4CL, NaHCO3, EDTA) in to a 50 ml centrifuge tube containing 5-10 ml of whole blood. Incubate at RT for 5 min, inverting occasionally to mix.
- 2. Centrifuge the samples at 3,000g for to pellet the white blood cells. Pour the supernatant to waste.
- 3. Add 10ml of white cell lysis buffer (SDS, EDTA) to white blood cell pellet and vortex vigorously for 10 sec.
- 4. RNAase is added to remove RNA from the preparation .Incubate at 37 degree Celsius for 15 minutes.

Protein Precipitation

- 6. Add 3.3ml of ammonium acetate protein precipitation solution, and vortex vigorously for 20 sec at high speed.
- 7. Centrifuge for 5 min at 3,000g. The precipitated proteins should form a tight,dark brown pellet.

DNA precipitation

- 8. Dispense 10ml isopropanol in to a clean 50ml centrifuge tube and add the supernatant from the previous step.
- 9.Mix by inverting gently 50 times until DNA is visible as thread or clump
- 10. Centrifuge for 5 min at 3,000g. Carefully discard the supernatant
- 11. Wash the DNA pellet by adding 10 ml of ethanol and centrifuge for 5 minutes at 3,000g and remove the supernatant.

DNA Hydration

- 12. Add 0.3-1.0 ml of DNA hydration buffer and vortex for 5 sec at medium speed to mix. Incubate at 65 degree C for 1 hour to dissolve DNA.

- 13. Incubate at RT overnight with gentle shaking.

- 14. The absorbance of the DNA at 260 nm and 280 nm should be measured using a quartz cuvette to assess purity as well as to known the approximate concentration of DNA