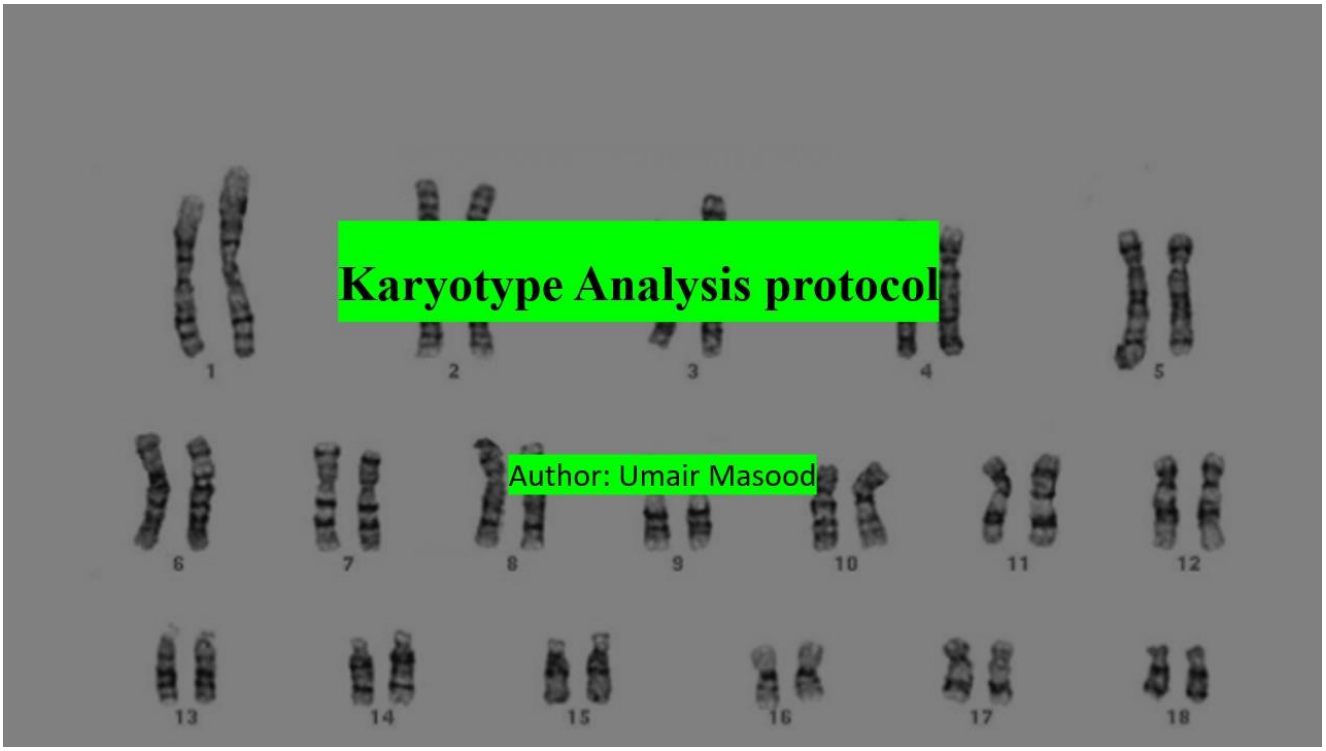

[karyotype Analysis protocol]

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karyotype Analysis protocol:

Material and reagents:

- Cell lines (FaDu)
- Trypsine
- 10% fetal bovine serum
- Colcemid (10 µg/ml)
- Hypotonic solution
- Glacial acetic acid
- Fixative (Methanol and glacial acetic acid 3:1)
- Growth medium (10% fetal bovine serum, 1% Penicillin Streptomycin)

Procedure:

- put-on 0.1 ml colcemid, which can cave in mitotic spindles and prevent the completion of mitosis, to each dish and mix lightly.
- Incubated at 37 °C, 5 % CO2 for 2 h.
- shift medium to centrifuge tubes from the cell culture dishes. utilize PBS to wash the dishes, and remove PBS.
- put-on 1 ml 0.1% Trypsine into the dishes at 37 °C, 5% CO2 for 2m.
- Also, shift the mixture (Trypsine and cells) into the centrifuge tubes and mix with the medium which is shifted to centrifuge tubes before we use PBS to wash the dishes.
- centrifuge at 100 RCF for 10m
- Reject the supernatant and leave 0.5 ml medium to mix the pellet lightly
- Resuspend the pellet in 5-7 ml 37 °C hypotonic solution and mix completely
- incubate at 37 °C for 10 min.
- centrifuge at 100 RCF for 10m
- Rejected the supernatant and leave 0.5 ml solution to mix the pellet lightly
- Resuspend the pellet in 5 ml cold fixative
- Set the centrifuge tube on ice minimum 20 min
- Centrifuge at 100 RCF for 10m.....(a)
- Rejected the supernatant and put-on 3-5 ml cold fixative.....(b)
- Repeat step a and b

- After the final centrifugation, suspend the cells in a few drops of cold fixative to give a slightly non-transparent suspension.
- 1-2 drops of cold fixative
- Dry the slides at 37°C temperature
- Notice the chromosomes with the microscope.