Comet Assay

Single cell gel electrophoresis

(SCGE)

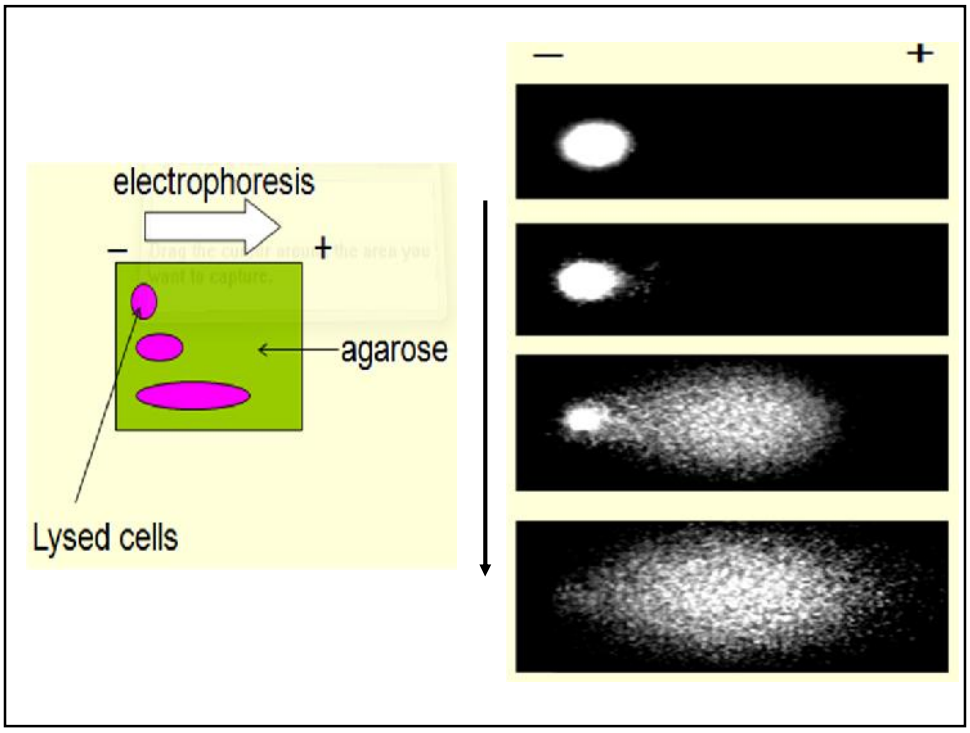
Introduction

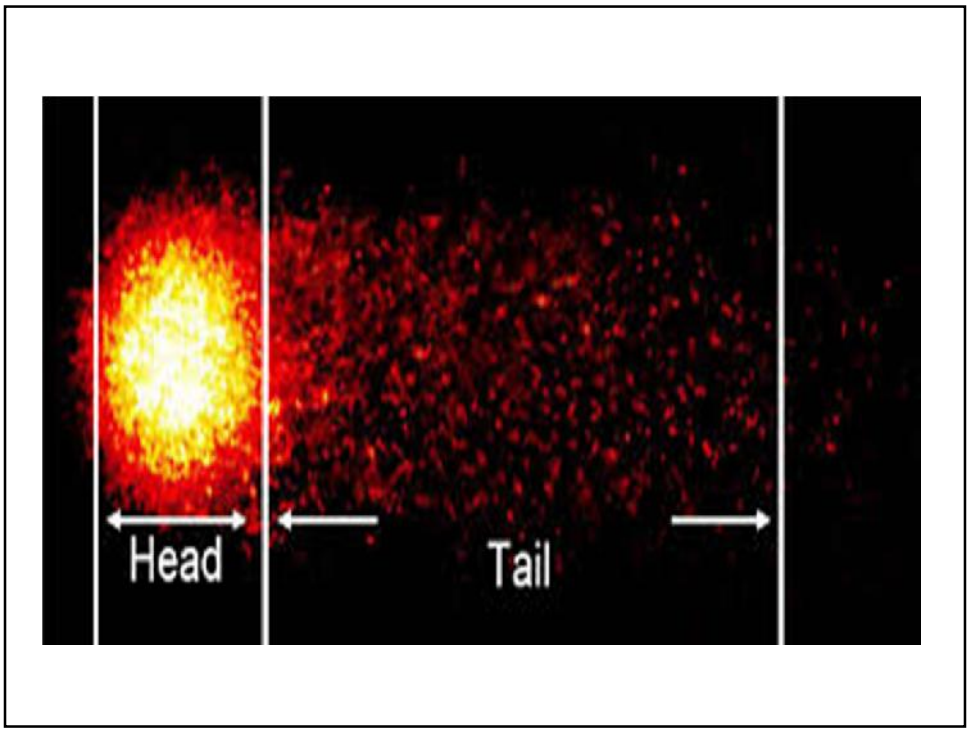
Single cell gel electrophoresis (Comet assay) is a sensitive and rapid method for measuring DNA damage and repair in single eukaryotic cell.

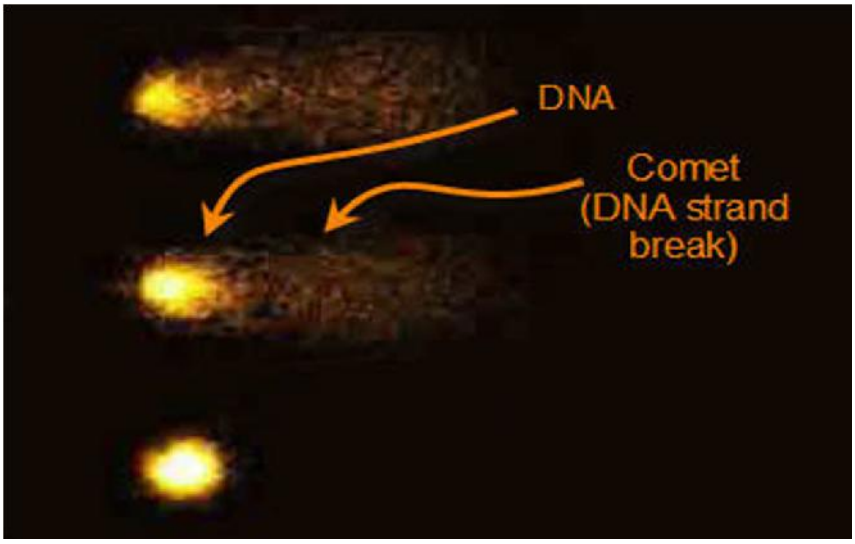
Principle

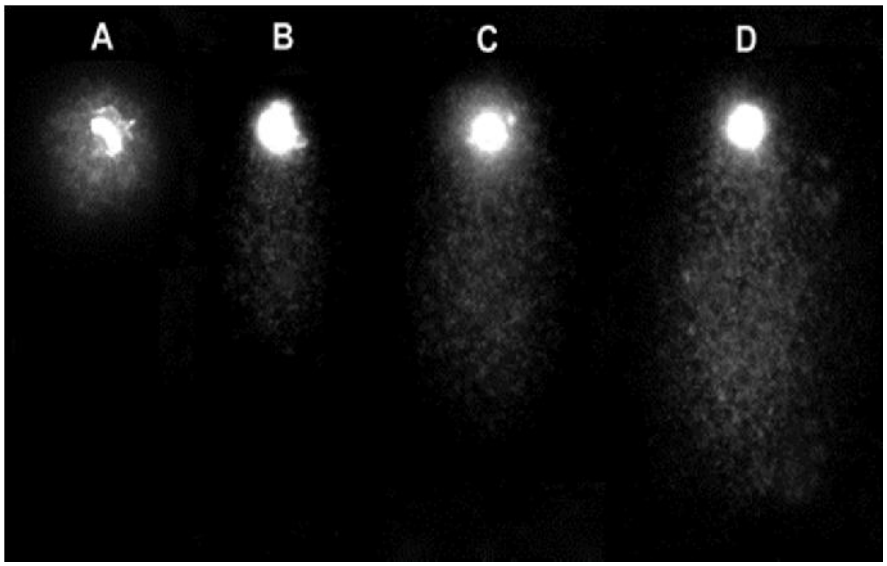
- The basis for this assay is that loops of DNA containing a break lose their supercoiling and become free to extend toward the anode when exposed to current during electrophoresis at high pH.
- The results appear as structures resembling comets observed by fluorescence microscopy.

- Comet contain a distinct head and tail.
- The head is composed of intact DNA, while the tail consists of damaged or broken pieces of DNA.
- The intensity of the comet tail relative to the head reflects the number of DNA breaks.


electrophoresis
–
+
agarose
Lysed cells
–
+


Head
Tail





No DNA damage (A) mild (B), moderate (C), and severe damage(D).

Applications of comet assay

- Genotoxicity testing.

✓ It is provides a set of information about safety and genotoxicity of newly developed pharmaceuticals and chemicals.

✓ Study of the protective effect of some phytochemicals on genes when exposed to some genotoxic insults.

✓ This is one of the techniques used in the area of cancer research for the evaluation of genotoxicity and effectiveness of chemotherapy.

- Monitoring environmental contamination with genotoxins.

- Human biomonitoring including:

✓ Monitoring occupational exposure to genotoxic chemicals or radiation.

✓ Assessment of oxidative stress associated with various human diseases.

✓ Detection of DNA damage associated with smoking.

- Nutritional Studies

The comet assay is ideal for investigating nutrient or micronutrient effects at the level of DNA damage in humans.

- Measuring DNA Repair

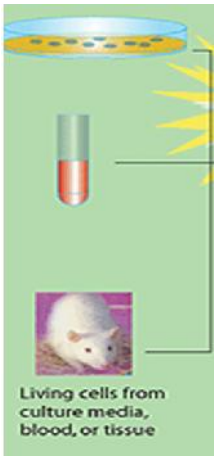
Commet assay is an important determinant

of individuals capacity for DNA repair and their susceptibility to cancer.

What do I need to perform the comet assay

1. Sample

- ✓ Culture media
- ✓ Blood (WBCs)
- ✓ Tissue sample (liver is preferable)



2. Comet assay Kit:

- ✓ Comet Agarose.
- ✓ Hemogenizing solution.
- ✓ Lysis Solution.
- ✓ Alkaline unwinding Solution, pH> 13.
- ✓ SYBR Green staining Solution.
- ✓ Neutralizing Solution.
- ✓ Comet assay slides.

3. Fluorescence microscope.

4. Comet assay scoring software.

5. Laboratory hemogenizer.
6. Centrifuge.
7. Water bath.
8. Horizontal electrophoresis tank.

Procedure

Comet assay-major steps

1. Liver tissue must be fresh.

2. Preparation of lysing solution : 40ml of lysing solution +4ml of DMSO.

3. Homogenization of sample.

4. The homogenized liver tissue centrifuged, the supernatant discardes, resuspende the cells at 1X 10^5 cells/ml in ice cold PBS.

6. Melt agarose

7. Cells will be combined with melting agarose then immediately pipette 75 µl onto
   Comet Slides

8. Slides will be placed at 4°C in the refrigerator for 30 minutes.
9. Immerse slides in prechilled Lysing Solution at 4°C for 60 minutes

10. Slides will immersed in freshly prepared alkaline solution, pH>13 then left it for 20 minutes at room temperature

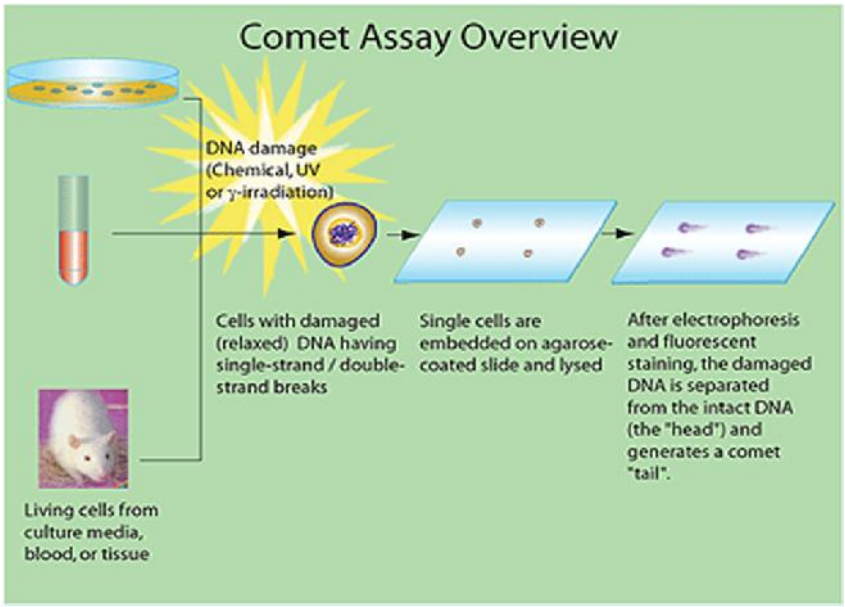
11. Slide will be put in a horizontal electrophoresis apparatus. The Alkaline Solution will be carefully poured until level just covers samples

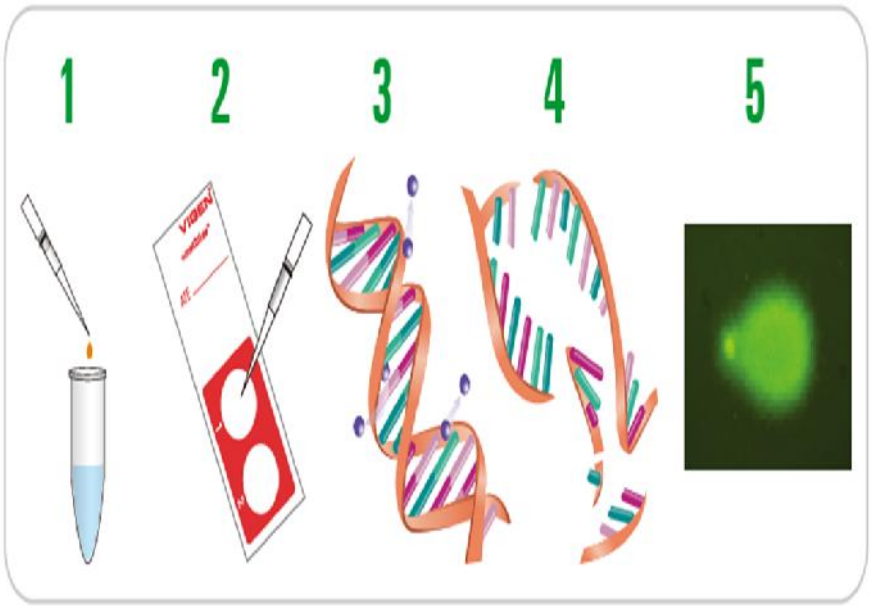
12. Adjust the apparatus on 21 Vol and 300 m.A.

13.Electrophoresis will be performed for 20 minutes

14. Slides will be neutralized by dipping several times in distalled water , then immerse it in 70% ethanol for 5 minutes

14. The slides will be stained with 50 µl of diluted SYBR Green ,then examined by fluorescence microscope.





Scoring of DNA damage

Scoring of DNA damage was done by Comet Assay IV software

- Transferred live video picture using a high resolution video camera attached to the microscope
- Fifty randomly selected non overlapping cells per sample will be scored
- Tail length and tail moment will be made.

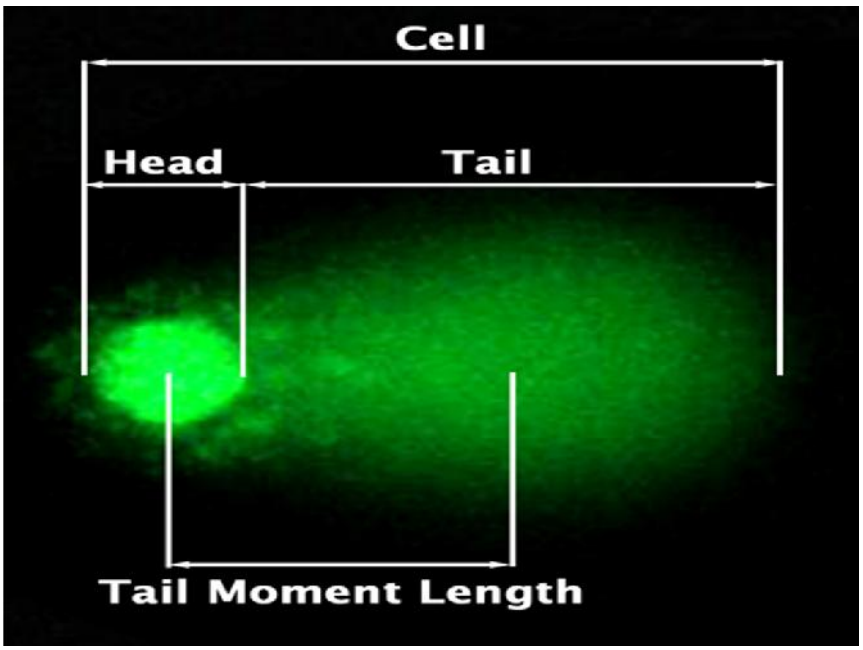


Image analysis

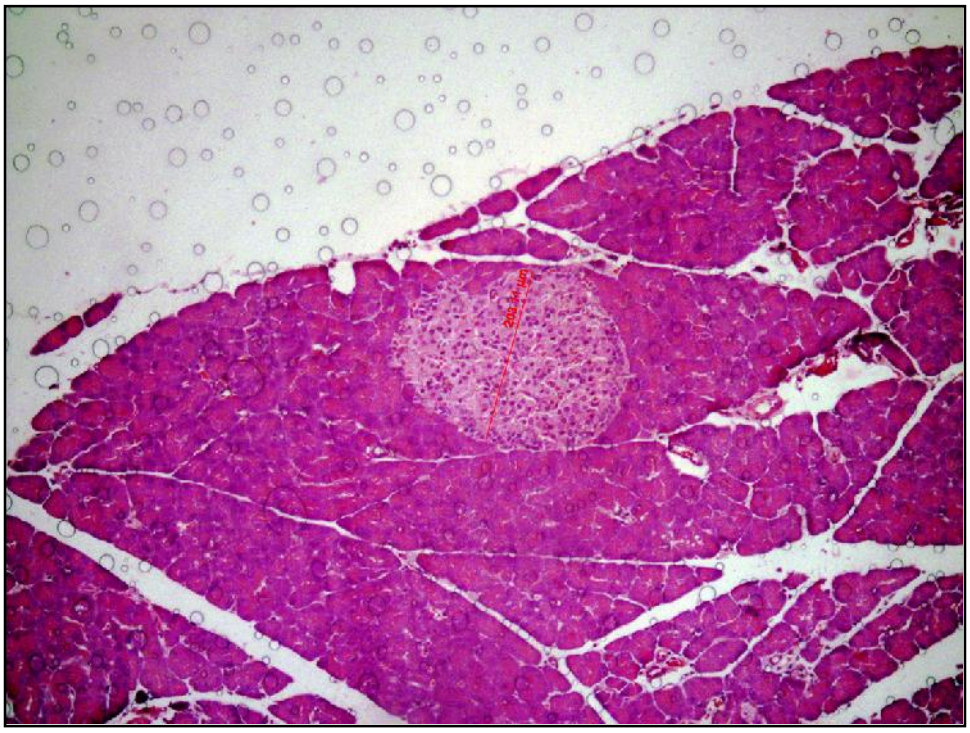
- Most current pathology diagnosis is based on the subjective opinion of pathologists, there is clearly a need for quantitative image-based assessment of digital pathology slides.

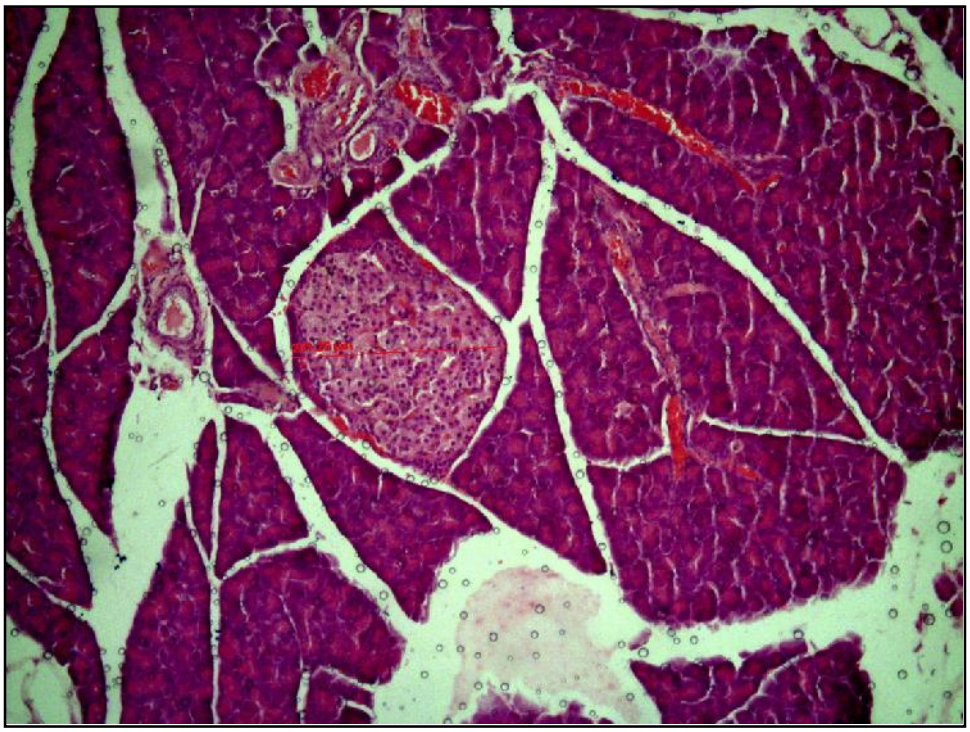
- Quantitative Image Analysis used also for Disease Grading.

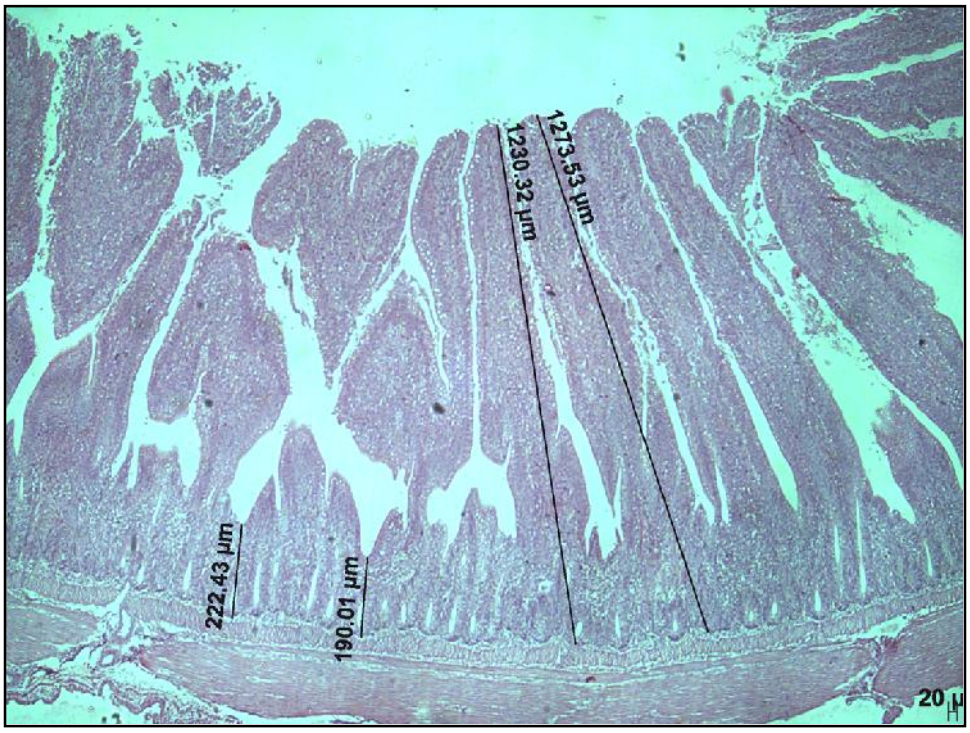
✓ Histopathological tissue analysis by a pathologist represents the only definitive method (a) for confirmation of presence or absence of disease, and (b) disease grading, or the measurement of disease progression.

✓Grading scheme is used to predict cancer prognosis and help guide therapy.

- Image analysis of histopathological glass slide stained with H&E to study different components of the tissue.
- Analysis of cytopathological image for studying the cells.
- Immunofluorescence image analysis using molecular markers based on chromogen dyes (e.g., DAB) or fluorescent dyes.



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1230.32 µm
222.43 µm
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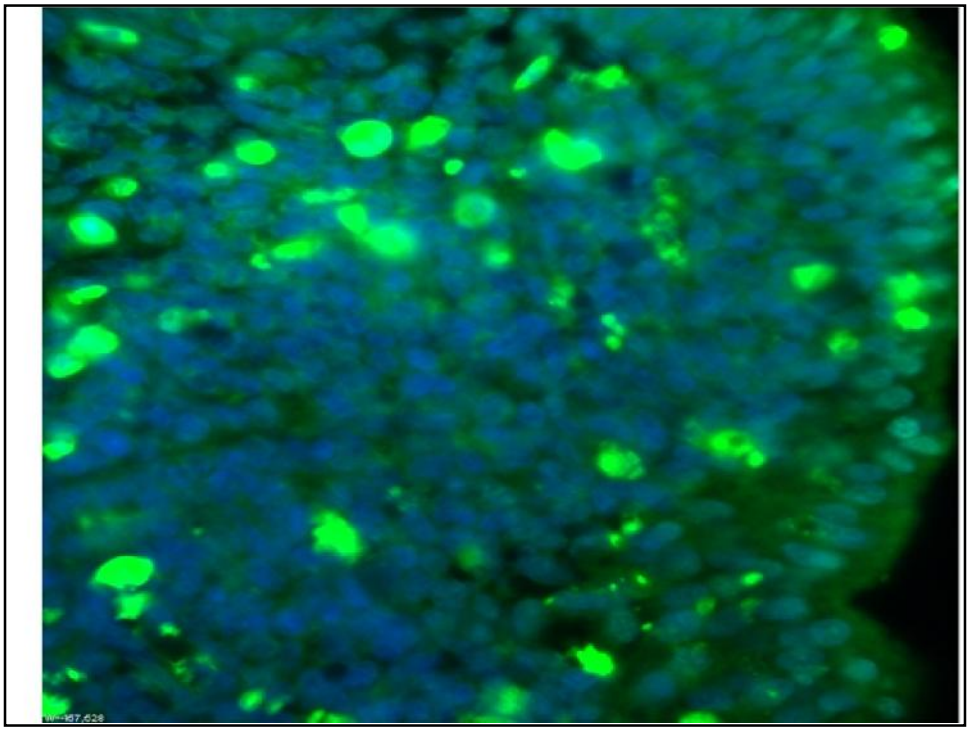
20 µ




THANKYOU
DNA FINGERPRINTING