Feulgen staining of nuclear DNA in eukaryotic cells.

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MOLECULAR BIOLOGY PRACTICALS.

Practical 1. Feulgen staining of nuclear DNA in eukaryotic cells.

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Theoretical background. The Feulgen procedure was invented by German physician and chemist Joachim Wilhelm Robert Feulgen during 1914-1924 [6, 7]. He also discovered animal and plant DNA (*"thymonucleic acid"*) congeniality and estimated the nature of nucleic acids as a polymers of nucleotides with four kinds of nitrogenous bases [11]. It is, probably, the most simple cytochemical method allowing indication and evaluation of DNA levels into cells. Schiff's reagent is the stain used in this technique. It specifically stains the DNA due to reaction of Schiff's (or Schiff's-like) reagent with aldehyde groups exposed at C1 as the result of the nitrogen bases cleavage from deoxyribose by 1N HCl hydrolysis at 60°C within about 10 minutes.

Usually, a sulfite rinse followed the hydrolysis, but this is now considered worthless [4]. Optionally, the sample can be counterstained with Light Green SF yellowish [5]. DNA should be stained red. The background, if counterstained, is green. To avoid the bacground fading, Light Green SF can be replaced with Fast Green FCF [3], which also has more brilliant color. Then, it is dehydrated with alcohol, cleared with xylene, and mounted in a resinous medium [4].

The pink color intensity correlates with DNA content in cell. However, Feulgen technique is a semi-quantitative. It is becoming quantitative for DNA, if the only aldehydes remaining in the cell are those produced in hydrolysis of DNA. It is used for DNA quantification in nuclei by image cytometry for ploidy evaluation in oncology [1, 2, 8].

The DNA content cannot be measured directly from microscopy. It is not easy to discern visually differences in the intensity of the Feulgen stain reaction within the nuclei. The microdensitometer or microscpectrophotometer are used to measure the intensity of the pink color for a given organelle [10]. Using this method, it was early determined that interphase cells were composed of two populations, those with diploid and those with tetraploid levels of DNA. The nuclei looked identical, but one contained twice as much DNA within it. This gave rise to the division of the interphase period of the cell cycle to a G1, an S, and a G2 based on the synthesis of that extra DNA [4].

After quantitative DNA-staining, the nuclear Integrated Optical Density (IOD) is the cytometric equivalent of its DNA content. The quantitation of nuclear DNA requires a rescaling of the IOD values by comparison with those from cells with known DNA content. Therefore the DNA content is expressed in a "c" scale in which

1c is half the mean nuclear DNA content of cells from a normal (non-pathological) diploid population in G0/G1 cell cyle phase [1].

Because the intensity color in the Feulgen reaction depends from many variables in its conduction, several attempts were made in standardization of the method [1, 9, 10].

The application of the Feulgen staining in electron microscopy allowed to study of the structural organization of DNA *in situ* [2].

As DNA from any of organisms has the same nature, we can use the cells from any source (humam, animals or plants) to demonstrate its presence in nuclei. We are using the onion roots for teaching purposes in this practical.

Materials:

Schiff's reagent, 1N HCl, sodium or potassium metabisulphite, freshly prepared bleaching solution (5 ml of 10% sodium metabisulphite + 5 ml of 1N HCl + 90 ml of distilled water), 45% acetic acid, mixture glycerol:water (1:1, v/v), hydroponically growing onion.

Preparation of Schiff's reagent

1. Dissolve 0,5g of basic fuchsin in 90 mL of boiling distilled H_2O
2. When cooled to approximately 45^{o}C, add slowly 10 mL of 1N HCl
3. Cool to room temperature and add 1g of $K_2S_2O_5$ or $Na_2S_2O_5$ (metabisulfite can be substituted on sulfite, hydrosulfite or sulfurous acid).
4. Shake for 3 minutes and leave it the dark at room temperature overnight or until a light straw or faint pink color develops.
5. Add 0,5 g of fine activated charcoal and shake for 3 minutes.
6. Filter solution (should be transparent).
7. Store at 4^{o}C in a tightly-stoppered bottle in the dark.

Procedure:

1. Fresh onion root tips are transfered to distilled water through alcohol:
2. Absolute ethanol 5 min, than 90%, 70%, 50% and 30% ethanol 5 min each
3. Distilled H_2O 5 min
4. Cool 1N HCl 2 min
5. 1N HCl (at 60ºC in water bath) 6 min
6. Cool 1N HCl quick rinsing
7. Distilled H_2O, rinse once
8. Schiff's reagent 5 min
9. Bleaching solution, 1 min
10. Distilled H_2O, rinse once
11. 45% acetic acid, 5 min.
12. Place the root tip in a drop of 45% acetic acid onto glass slide (or in a drop of 50% glycerol (distilled H_2O + glycerol 1:1), cover it with cover slip and make a pressured preparation by gently pressing between finger and thumb.

13. Observe cells bright pink cytoplasm and dark pink nuclei at objective 40× (Fig.1).

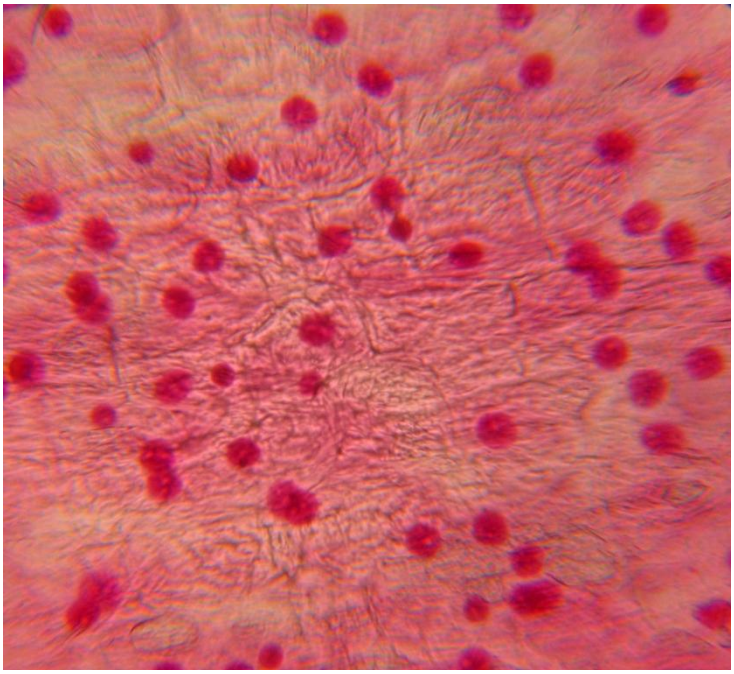

Fig. 1. Feulgen staining of nuclei in onion tip cells.
The tissue consists of more than one layer of cells. This resulted in numerous dark pink nuclei on the field of view of light microscope. Magnification 400 ×.
By B.M.Sharga & D.B.Pylypiv

14. Compare pink color intensity in tissue of tip end and its middle part. It is directly proportional to the amount of DNA present per unit area.

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