12. Place the PCR tubes in thermocycle and run cycle (heat block will also work).
    a. Incubate at 37°C for 30 minutes followed by 10 minute incubation at 85°C.

13. Remove PCR tubes from thermocycler and vortex again for ~10sec (pulse w/ vortex); spin to collect sample.

14. At this point you can freeze samples at -20°C until desired number of digestions have been completed.

15. After thawing, or if you continue from Step 12, centrifuge samples for 2 min. at 12,000 rpm.

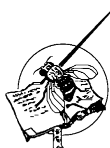
DNA Concentration Quantitation using Qubit® dsDNA HS Kit

1. Prepare the proper amount of Qubit® working solution by diluting the Qubit® reagent 1:200 in Qubit® buffer.
    a. 190 µl of working solution is required for each sample and standard.
    b. 1 µl × N # of samples = X µl of Qubit® Reagent
    c. 199 µl Qubit® buffer × N # of samples = X µl of Qubit® Buffer
2. Aliquot 190 µl of Qubit® working solution into Qubit® ultra-clear assay tubes.
3. Dilute DNA extract 1:20 in Qubit® working solution.
    a. 10 µl of DNA extract into 190 µl of Qubit® working solution.

**Attention*: *Be sure to make samples with Standard #1 and Standard #2 to standardize the Qubit® Fluorometer each time.*

1. Vortex all tubes for 2-3 seconds to mix and spin to collect sample at the bottom of the tube.
2. Incubate the tubes for ~2 minutes at room temperature.
3. Select proper program, dsDNA HS, on Qubit® Fluorometer and standardize machine.
4. Read tubes in the Qubit® Fluorometer.

**Helpful Hint*: *You can automatically calculate the concentration of your original sample by selecting "calculate sample concentration" → GO → 10µl → GO.*



The Citric-Arabic-glycerated gum: An alternative to Fourè gum.

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Introduction

The conservation of biological material for analysis of results in different areas of biology is a process of great importance. Making a microscopic preparation involves enclosing the material of study between two sheets of glass: the slide serving as a support, and the coverslip, used to cover the material.

The material can be mounted in air or in a liquid medium. In the first case material is solidified by cooling or by evaporation. Another side, when the material is put in liquid medium, the preparation has to be locked to avoid mounting fluid evaporation and subsequent drying of the material. In all cases, the object must be placed between the slide and coverslip so that the preparation remains uniformly flat and the front lens of high magnification objectives do not touch

the liquid. The flattening of the material also improves the quality of the image and ease of approach. One of the important features of the mounting medium is the visibility index or index of refraction, which changes according to degree of product purity and origin of the constituents (Sheehan and Hrapchak, 1980; Locquin and Langeron, 1985).

The mounting types are classified into temporary and permanent. There are different hydrophilic mounting media depending on the kind of ingredientes used: glycerinated gelatin, gum chloral, chloral gelatin, and gum syrup of Apathy among others.

Chloral gum, also known as Faure liquid or liquid glycerine Hoyer, has been used for the preservation of different structures and agencies (Upton, 1943). In 1983, Graf *et al*. recommended the use of the Faure's solution (gum arabic 30 g, glycerol 20 ml, chloral hydrate 50 g, water 50 ml) to mount the wings from adults of the fruit fly, in order to analyze the induction of somatic mutation and recombination in the wing cells.

Currently, chloral hydrate is a compound with restricted access so that its availability has been affected. Due to the known preservative properties of the chloral hydrate, we look for an alternative ingredient that remains unchanged in the mounting gum characteristics.

The Citric Acid Monohydrated is a preservative broadly used in industry and pharmacy (Loutit *et al*., 1943; Nielsen and Arneborg, 2007).

Materials and Methods

Faurè – Modified Recipe

Arabic gum 30 g (CAS 9000-01-5, Reasol, IT), Higroscopic glycerin 20 ml (CAS 5681-5, Omnichem, IL), Citric Acid Monohydrate granular 50 gr (CAS 5949-29-1, Omnichem, IL), Distilled Water 50 ml.

The ingredients are weighed separately and mixed dry. Water is added and all is put under constant stirring for 24 h (Rheostat Corning, model PC 351) in the absence of light. The content is then stored in amber bottles.

This mounting liquid has been used for preserving different parts of the flies' body: genital plates, head, thorax, legs, and other structures.

Actually, we make the wing's slides for the Somatic Mutation and Recombination Test (Graf *et al*., 1983) using this gum and after two years, the slides are kept in perfect condition.

References: Graf, U., H. Juon, A.J. Katz, H.J. Frei, and F.E. Würgler 1983, Mutation Research 120: 233-239; Locquin, M., and M. Langeron 1985, *Manual de Microscopia*. Editorial Labor, S. A. Mexico, 372 pp.; Loutit, J.F., P.L. Mollison, and I.M. Young 1943, BMJ 2: 744; Nielsen, M.K., and N. Arneborg 2007**,** Food Microbiology 24(1): 101-105; Sheehan, C.D., and B.B. Hrapchak 1980, *Theory and Practice of Histotechnology*, The C.V. Mosby Company. 480 pp.; Upton, M.S., 1943, Experimental Physiology 32: 183-202.