**UW Medicine - Pathology**

400-03-01-03

NOR Staining

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| Adopted Date: 09/04/91Review Date: 06/12/09Revision Date: 03/16/07 |

PURPOSE

The nucleolar organizer regions (NOR) contain genes for 18S and 28S rRNA. Regions in which the genes are thought to be actively transcribing can be selectively stained using silver nitrate (NOR staining). In humans, the rRNA genes are located on the short arms of the acrocentric chromosomes (13, 14, 15, 21, 22). Not all of the chromosomes may stain; there may also be considerable variation in the size of the region stained among individuals, and this polymorphism is a heritable characteristic.

PROCEDURE

### Materials and Equipment

1. Square plastic Petri dish, 4" x 4".
2. Slide warmer at 60°C.
3. Light microscope.

### Reagents and Solutions

1. Formaldehyde, 37% reagent grade (Baker Cat. #5016).
2. Formic acid, reagent grade (Baker Cat. #0128).
3. Silver nitrate, reagent grade (Baker Cat. #3426).
4. Developer
	1. 2 ml of 37% formaldehyde.
	2. 98 ml distilled H2O.
	3. Adjust pH to 2.6-2.7 with formic acid.
	4. Store in brown bottle. Expires in 2 yr.
		1. Silver nitrate solution
			1. (50% W/V) 50 g/100 ml distilled H2O.
			2. Store in brown bottle. Expires in 2 yr.

6. Wright’s stain (Sigma W3000)

### Procedure

1. Staining time is 3-6 min for blood leukocyte slides, which are fresh or moderately aged (2-3 wk old).
2. Place a Petri dish with a wet paper towel or gauze on the bottom on the slide warmer (at 60°C). Place the slide on top of a piece of wet gauze or paper towel.
3. Using separate pipettes for each solution, pipette 1 drop of developer at one end of the chromosome preparation on the slide, then pipette 7 drops of silver nitrate on top of the developer.
4. Mix with a coverslip and cover the slide; place the cover on the pre-warmed Petri dish and incubate the moist chamber until the preparation becomes yellowish-brown in color (about 2.5-3 min). Do not allow it to get too dark (brownish-black) in color, since this results in high background of silver grains.
5. After the desired intensity is attained, stop the development by rinsing off the coverslip in dH2O and air dry the slide.
6. Counter-stain horizontally positioned slide with a 1:4 dilution of Wright's stain in pHydrion buffer for 2-3 min. Rinse with distilled H2O.

***Note****:* For G-bands after NOR staining: follow the above procedure then, using the solutions for G-bands, trypsinize for 15 min in 1 ml trypsin stock solution in 50 ml PBS without Ca+2 or Mg+2. Stain for 2 min in Wright's stain.

REFERENCES

1. Bloom S, An improved method for staining chromosomal nucleolar organizer regions with silver solution. *Hum. Genet.*, 34:199-206, 1976.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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 Cytogenetics Supervisor