PROTOCOL FOR PI STAINING OF DNA

1) Fix cells in 4% paraformaldehyde with 0.05% gluteraldehyde in PBS for 15 minutes.
2) Wash in PBS with 0.1% triton for 5 minutes.
3) Wash in PBS 2 x 5 minutes.
4) RNA digestion: 400ug/mg RNAse A at 37°C for 30 minutes.
5) Wash in PBS 2 x 5 minutes.
6) Stain with PI (0.25mg/ml for 30 minutes) at RT.
7) Wash with PBS 3 x 5 minutes.
8) Fix in 2% gluteraldehyde for 5-15 minutes.
9) Wash in cacodylate then photooxidize with DAB 1mg/ml.
10) Wash in cacodylate 3 x 5 minutes.
11) Postfix in 1% OsO4 in cacodylate for 30 minutes.
12) Standard EM procedure.