PROTOCOL FOR CRESYL VIOLET STAINING ("NISSL STAIN")

Cresyl violet solution:	0.1 g cresyl violet acetate 100 ml ddH_2O 250 ul glacial acetic acid (final concentration 0.25% v/v)

Stir overnight at room temperature and filter before using. Lasts 6 months at room temp. in the dark.

For frozen sections (perfused or frozen tissue):

1) Remove the slides from the freezer and leave them to dry at least 60 min at room temperature. (cover the sections so airborne dust does not settle on them).

2) If the sections had not been fixed, use any of these fixatives for post-fixation:
- Normal fixative: 4% paraformaldehyde (or 10% formalin) in 100 mM phosphate buffer, pH 7.4
- Volatile fixative: 100% methanol or cold 100% acetone
- Strong fixative: 70% ethanol + 10 % formalin + 5% glacial acetic acid in ddH_2O

(dip the slide 10 min in the fixative)

3) Rinse the sections 2 x 5 min in 10 mM PBS.

4) Rinse the slides 1min in ddH_2O.

5) Dip the slides 20min in Nissl stain.

6) Rinse the slides 2 x 5min in ddH_2O.

7) Dip the slides in 90% ethanol x 3min.

8) Dip the slides in 95% ethanol x 3min.

9) Dip the slides in two changes of 100% ethanol, 3 min each.

9) Dip the slides in three changes of 100% xylene, 3 min each.

10) Mount the slides with Permount or similar mounting media (keep the slides in xylene during the mounting, do not let them dry). Let the Permount cure before observing the slides under the microscope.

To mount the slides: take the slide still wet with xylene and add a drop of permount with a glass rod or glass pipette, quickly add the coverslip to the Permount to extend it over the sections.
SPREAD WELL THE PERMOUNT AND DO NOT ALLOW BUBBLES TO FORM!
If the mounting fails, put the slide in a separate Coplin jar with clean xylene and gently let the coverslip fall off the slide. Repeat the mounting.

Discard xylene in a large metal pallet, inside the fume hood.

USE XYLENES ONLY INSIDE THE FUME HOOD!!