

## STAINING FOR $\beta$ -GALACTOSIDASE

### Materials and Reagents:

#### *Staining solution*

	[stock]	Volume added ( $\mu$ L)	[final]
Na-Phosphate buffer pH 7.5	0.8M	250	0.2mM
MgCl <sub>2</sub>	1M	1	1mM
SDS	1%	4	0.004%
Redox buffer	100mM	100	10mM
Kanamycin	5mg/mL	15	75 $\mu$ g/mL
DAPI	1mg/mL	2	2 $\mu$ g/mL
X-gal (in dimethyl formamide)	5%	8	0.04%
dH <sub>2</sub> O	-	620	-

Redox buffer: equal volumes 100mM potassium ferricyanide and potassium ferrocyanide (CAUTION: contains cyanide, wear gloves). Make fresh each time from 100mM stocks.

### Procedure:

- 1) Wash worms off the plate in water, PBS, or M9.
- 2) Wash 2 times to remove excess bacteria (spin down at 3000rpm X 1min).
- 3) Remove as much supernatant as possible.
- 4) Freeze tubes in liquid nitrogen.
- 5) Lyophilize worms by spinning in speed vac (~45 minutes).
- 6) Add 250 $\mu$ L cold acetone and put in freezer 3 min.
- 7) Remove acetone with pipetman (or aspirate carefully) and speedvac to remove residual acetone.
- 8) Resuspend worm pellet in ~200 $\mu$ L staining solution (depending on amount of worms).
- 9) Stain in the dark at RT or 37°C. Examine a small aliquot periodically on the microscope to assess progress of staining.
- 10) When staining is satisfactory, wash animals twice in PBS.
- 11) Mount on an agar pad and examine under the microscope.

### Tips/Troubleshooting

- 1) Instead of lyophilization and acetone, fixation in 1-3% formaldehyde for 10 min at RT (followed by 3X wash with PBS) works well.
- 2) If high background is a problem, try washing the worms more at step 1/2 to remove more bacteria. Alternatively, grow worms on  $\lambda$ -gal deficient bacteria (worms seem to grow fine on DH5 $\lambda$ ).
- 3) Kanamycin in the staining solution does not seem to be necessary. Also, unless you intend to examine animals on a microscope equipped to see DAPI, you can also omit this. Make up volumes with dH<sub>2</sub>O.

**References:**

- 1) This protocol is adapted from Michael Koelle's (<http://cobweb.dartmouth.edu/~ambros/worms/index.html>)
- 2) Fire, A. 1992 . Genet Anal Tech Appl. Oct-Dec;9(5-6):151-8.

**Submitted by:**

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