β-Gal Staining of *Xenopus* Embryos

1) Fix embryos for 30 minutes to1 hour (no more!) in MEMFA (while nutating).
2) Rinse 2 times with PBS + 2 mM $MgCl_2$.
3) Transfer to staining solution (max. volume).
4) Transfer to X-gal mix: add 100 λ of the 50 mg/ml stuff (it is a small brown bottle in the -20 °C freezer) per 5 ml of embryo sample in staining solution. Use a minimal volume (5 ml is more than enough for 1 scint. vial) – this stuff is expensive. To develop more rapidly and with a deeper purple color, add 5 λ of NBT (NBT stock as for *in situs*: 75 mg/ml in 70% dimethyl formamide / 30% water) to 10 ml of final developing mix.
5) Incubate at **37 °C** in the dark (wrapped in tin foil) until desirable stain develops (a few hours to 24 hours).
6) Rinse in PBS, and twice in Methanol. Samples can be stored here.
7) Clear in Benzyl Benzoate : Benzyl Alcohol (2:1) if desired. BB:BA is very toxic; it is not water mixable (clean glass with ethanol) and melts plastic, so use it in glass only. It will also leech the stain out of embryos (which become brittle) in a while. You should photograph your samples in a watch-glass.

Staining solution (kept wrapped in foil in fridge at 4 °C):

	10 ml =	1 L =
20 mM $K_3Fe(CN)_6$	0.06 g	6 g
20 mM $K_4Fe(CN)_6 \cdot 3H_2O$	0.08 g	8 g
0.01% deoxycholic acid	0.001 g	0.1 g
0.02% NP-40 in PBS + 2 mM $MgCl_2$	2 λ	200 λ