Preparation of embryos for WB

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Anesthetize embryos using Tricaine.

Dechorionate embryos either manually or pronase (1 mg/ml) treatment for 10 min.

Burst open yolk using forceps*. It's not necessary at this step to try to remove all the yolk from the embryos.

This step is not necessary if embryos are over 48 hpf because the amount of yolk is not significant at this stage.

Transfer 30 embryos to a microfuge tube.

Tap the microfuge tube several times, which will detach the yolk still stuck to the embryo proper. Once detached, yolk will dissolve into water.

Spin at 3000 rpm for 1 min.

Carefully scrape off yolk stuck to the wall of the microfuge tube and then remove S/N.

Wash embryos with PBS, spin at 3000 rpm for 1 min and remove S/N.

Add 200 μl of 1x SDS sample buffer and homogenize embryos by passing them through a 1-ml insulin syringe at least 20 times.

Ensure no visible material is left in the tube.

Boil for 5 min, spin at full speed for 2 min and load 30 μl of S/N into a well of SDS gel.

If the protein you want to see is overexpressed in the transgenic line, 2 -3 embryos per lane is usually enough to visualize with WB.

* Removing yolk is important as failure in the removal results in a huge band in around 80-kDa area.