If you use this protocol, please reference: Faith L.W. Liebl, Kaiyun Chen, Julie Karr, Qi Sheng, and David E. Featherstone. (2005) Increased Synaptic Microtubules and Altered Synapse Development in Drosophila Sec8 Mutants. BMC Biology 3:27

Featherstone Lab GluR Western Blotting Protocol

- Pick 20 embryos and homogenize in 40ul of SDS sample buffer on ice. The samples were boiled for 5 min and centrifuged briefly.

- 20ul of the supernatant is loaded on an 8% SDS-PAGE gel and separated at 100V for 1.5 hr.

- Soak the membrane first in 100% MeOH for a few seconds until it turns grey, then soak the membrane together with the gel, two filter paper, two fiber pads in transfer buffer for 30 min.

- Set up transfer sandwich, remove any air bubble, add cooled transfer buffer, stir bar, cooling device and transfer for 30 min at 100V.

- Wash membrane for 1hr with IBLOCK, change solution every 15min.

- Incubate membrane with primary antibody at certain concentration O/N with shaking at 4 ºC. Save primary antibody for reuse(8B4D2 (concentrated form) at 1:200 for glur2A, 781-1 at 1:4000 for glur2B**, 1:2000 for glur2C).

**Note that the anti-GluRIIB that works well for in situ staining does NOT work well for Westerns. The anti-GluRIIB ab that works well on westerns was raised against the GluRIIB C-terminus, last 17 amino acids.

- Wash membrane for 1hr with IBLOCK, change solution every 15min.

- Incubate with secondary antibody for 1hr at RT and wash for 1hr, change solution every 15min (anti-mouse at 1:1000 for glur2A, anti-rabbit at 1:5000 for glur2B and glur2C).

- Film development. Glur2A has a specific band at ~MW 110kD, Glur2B has a specific band at ~120kD.