This protocol works on tubule wholemounts. It is based on:

Sulphonylurea sensitivity and enriched expression implicate inward rectifier K^+ channels in *Dros ophila melanogaster* renal function. Jennifer M. Lee, Adrian K. Allan, Shireen A. Davies, Julian A.T. Dow J.exp.Biol. 2005

1. Tubules were dissected, fixed and subjected to *in situ*hybridization according to published protocols, (Siviter et al., 2000) and the BDGP 96 well *in situ* protocol (http://www.fruitfly.org/about/methods/RNAinsitu.html).

2. In situ probes were directed towards the 3'UTR of each gene to minimise cross-hybridisation between related genes. PCR products derived from the 3' UTR of each gene were cloned into pBluescript or pCRII vectors and DIG-labelled RNA probes were generated by *in vitro* transcription.

3. Adult tissues comprising of gut, testes, ovaries and Malpighian tubules were dissected in Schneider's medium (Invitrogen) and placed into wells of a Millipore 96 well plate(MAGVN22 or MAGVS22)
with 100
m
l Schneider's medium.

4. Schneider's medium was removed using a vacuum pump and postfix solution (10 mM potassium phosphate buffer (pH 7.0) containing 140 mM NaCl, 0.1% Tween 20, and 5% v/v formaldehyde) was added for 20 min, followed by three washes with PBT
(10 mM potassium phosphate buffer (pH 7.0)
containing
140 mM NaCl and 0.1% v/v Tween 20).

5. The tissues were incubated with proteinase K in PBT (4 µg/ ml) for 3 min at room temperature, the reaction was stopped with two washes of PBT containing 2 mg/ml glycine.

6. The samples were washed twice with PBT before incubatingwith postfix for a further 20 min at room temperature.

7. The tissues were washed with five changes of PBT, followed by one wash with 50% hybridisation buffer (5×SSC containing 50% v/v formamide, 10 mM KPO_4,
140 mM NaCl, 1 mg/ml glycogen, 0.2 mg/ml sheared salmon sperm
DNA, and 0.1% v/v Tween 20 (pH 7.0)) plus 50% PBT.

8. The samples were washed once with hybridisation buffer, prior to a 1 h preincubation with hybridization buffer at 55°C, and subsequently incubated for 43 h at 55°C with 100 µl of hybridisation buffer containing 10-500 ng of either the sense or antisense riboprobe, taking care to seal the wells with parafilm to prevent evaporation.

9. Following hybridisation, the samples were washed four times with hybridization buffer at 55°C, followed by a final wash overnight with hybridization buffer at 55°C.

10. Samples were washed once with 50% v/v hybridisation buffer and 50% v/v PBT, followed by four washes with PBT and then incubated for overnight at room temperature with 100 µl of pre-absorbed alkaline phosphatase-conjugated anti-digoxigenin Fab fragment (Roche Molecular Biochemicals) diluted 1:2000 with PBT.

11. The unbound antibody was removed with extensive washing in PBT (at least 10 times for about 5-10 min).

12. The samples were incubated with DIG detection buffer (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$) for 5 min then repeated again.

13. The colour reaction was initiated by the addition of DIG detection buffer + BCIP & NBT and left 10 min - 2 h at room temperature.

14. Development was stopped with extensive washing with PBT containing 50 mM EDTA and the tissues were removed from the wells and mounted on slides with 70% glycerol, and viewed with on an Axioscope microscope, equipped with an Axiocam imaging system (Zeiss, Welwyn Garden City,
UK).