LYSIS BUFFER

333 µl 1.5M Tris pH 8.8 (final is 50 mM)
20 µl 0.5 M EDTA (final is 1 mM)
500 µl 10% Tween (final is 0.5%)
9.1 ml H2O
Total = 10ml

Or you can use

2.5ml 1M Tris pH 8.8 (final is 50 mM)
0.1 ml 0.5 M EDTA (final is 1 mM)
2.5 ml 10% Tween (final is 0.5%)
Raise to 50ml with H2O.

Cut tail, ear or piece of embryo into a 1.5 ml eppendorf tube or directly into PCR tubes.

For a tail snip (usually smaller than 5mm in length) we use 100 µl buffer + 1 µl proteinase K (we use the Boehringer Mannheim PCR grade)

For ear punches we use 50 µl buffer + 0.5 µl Pro K per ear punch.

For embryos we either take a piece of tail (or any part of the embryo that you will not need) or a piece of the yolk sac and digest in 50 µl buffer + 0.5 µl pro K.

We then digest for at least 6 hours, but we usually like to digest overnight at 60 degrees Celsius.

You then heat your samples for 10 min at 100 degrees to kill the pro K (if you prefer using the PCR strip tubes for your tail/ear samples you have heat in the PCR machine – use the boil program).

You then spin the sample for 30 sec at 13,000 rpm.

For PCR we then use 1 µl taken from the top of your sample (to avoid any chunks ending up in your PCR) to use as template.

Store the remainder of you digested DNA in the fridge at 4 degrees Celsius.