Xanthine Dehydrogenase Knockout Genotyping protocol:

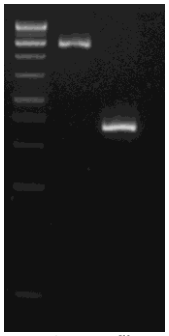
We obtain tail snips to isolate gDNA for genotyping using the Sigma REDExtract-N- Amp PCR tissue kit (catalog # XNAT). The samples are analyzed with the following primers:

For the wildtype:
WT5p: cac cgt gat gat ctc caa gt
WT3p: cct atg cct tcc aca gtt gt
For the knockout:
KO5p: atg cga tgt tcg ctt ggt gg
KO3p: cta ttc ggc tat gac tgg gc

We run separate reactions for the WT and KO. For the WT, you should see a band of around 1.5 kb, for the KO it should be around 350 bp. In the past we used the Advantage cDNA polymerase kit to genotype these mice and we could not obtain clear bands for the KO reaction if we ran both reactions together. We switched to the Sigma kit and we have observed much cleaner and faster isolation/PCR reactions at a fraction of the cost when compared with the Clontech kit. I can try to do a couple of trial runs to see if with the Sigma kit we can run both reactions together. The PCR reaction conditions are:

1st cycle: 94 degrees- 3 minutes
2nd cycle: 94 degrees- 30 seconds
60 degrees- 30 seconds
72 degrees- 1 minute
Repeat 35 times
3rd cycle: 72 degrees- 7 minutes
Hold at 4 degrees-indefinitely.

The total volume of the reaction is 20 μL, and the final concentration of primers is 0.4 μM. We use 4 μL of DNA, as required in the kit's instructions.



PCR profile
XDH-heterozygote mouse