MGP Expression Pipeline

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Two gene expression pipelines were set-up, with the aim of assessing the gene expression profiles of adult and embryonic mice carrying a *lacZ* reporter gene.

The *lacZ* reporter gene is an integral part of the targeted "knockout-first" allele design used by the IMPC (www.mousephenotype.org). *LacZ* encodes bacterial β-galactosidase, a well-characterized enzyme for in situ localisation which in this case, enables the endogenous expression pattern of each targeted gene to be assessed. The protocol generally uses a simple and robust histochemical approach involving the substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside; Lojda, 1970). When X-Gal is oxidized by β-galactosidase in the presence of iron-containing compounds, a non-diffusible, insoluble, blue precipitate is produced, which can be readily visualised and imaged. Animals heterozygous for the targeted allele were typically used for expression analysis in order to reduce the possible confounding effects of the loss of gene function.

The following 4 outcomes were used to describe each entity that was assessed:

1. 'Present' indicated that reporter gene expression was observed in that tissue.
2. 'Not detected' indicated that reporter gene expression was not observed. Given the snapshot nature of the screen, this outcome is not synonymous with the absence of expression, and may reflect that the target gene was expressed at levels below the detectable threshold of the assay, was not expressed in the context of the age and conditions of the experiment or was never expressed in the tissue being assessed.
3. 'No data' indicated that, for technical reasons, the data were missing.
4. 'Ambiguous' indicated that we were unable to make a confident assessment either because the reporter gene expression was so faint that it was barely perceptible or because of interference from endogenous background staining.

The two expression pipelines were:

- Adult Gene Expression Pipeline
- Embryonic Gene Expression Pipeline

References

1. Lojda, Z. (1970). *Indigogenic methods for glycosidases. II. An improved method for beta-D-galactosidase and its application to localization studies of the enzymes in the intestine and in other tissues.* Histochemie 23, 266-288. doi:10.1007/BF00306428