

Embryo *LacZ* Expression

The *lacZ* reporter gene within the targeting vector was used to determine the whole mount expression profile of the targeted gene. Embryos were fixed using 4% paraformaldehyde (PFA), sub-dissected to enhance penetration of the stain, then transferred into *lacZ* staining solution, containing 0.1% X-gal, for up to 48 hours. After an additional overnight post-staining fixation in 4% PFA, embryos were cleared with 50% glycerol then imaged using a Leica dissection microscope. Only embryos that show positive staining were imaged. Embryos were transferred to 70% glycerol for long-term storage.

Litters were collected from either HET x HET matings (when gene expression analysis was performed as part of a recessive lethality study), or from HOM/HET/HEMI (male) x WT (female) matings to obtain two heterozygous *lacZ* stained embryos per staining method.

Whole litters were processed as either wholemount *lacZ* stained or fixed frozen for cryosections. Upon genotyping, embryos could be selected for imaging (wholemount litters) or cryosectioning and *lacZ* staining (fixed frozen litters) as appropriate.

In the original embryonic gene expression pipeline, embryos only underwent wholemount *lacZ* staining and were predominantly collected at ED14.5. In addition, tail tissue was collected for genotyping. The staining outcome was recorded for the embryo only.

In the BaSH consortium (Baylor College of Medicine, Houston, Texas, Wellcome Trust Sanger Institute, Hinxton, UK, and the Medical Research Council Harwell, UK) gene expression pipeline only ED12.5 embryos were analysed, and yolk sac was used for genotyping. One litter of ED12.5 was wholemount *lacZ* stained and 2 litters of ED12.5 were fixed frozen for *lacZ* stained cryosections. The annotation list was refined to include the placenta and 4 additional regions for wholemount embryos (head, forelimb, hindlimb and tail). A further 25 tissue regions were added as a minimum to be assessed from *lacZ* stained cryosections, with 8 optional regions if these were also present on the stained material.

Embryonic gene expression pipeline: Annotation list

Embryonic Gene Expression Pipeline Annotations	Original Pipeline	BaSH Pipeline
Embryo <i>LacZ</i> staining	Present	Present
Placenta <i>LacZ</i> staining		Present
Head (Whole mount)		Wholemount
Forelimb (whole mount)		Wholemount
Hindlimb (whole mount)		Wholemount
Tail (whole mount)		Wholemount
Brain		Cryosection
Forebrain		Cryosection
Hindbrain		Cryosection
Midbrain		Cryosection
Notochord		Cryosection
Spinal cord		Cryosection

Dorsal root ganglion		Cryosection
Ear (inner ear)		Cryosection
Eye		Cryosection
Vibrissa placode		Cryosection
Oral cavity		Cryosection
Mandibular process		Cryosection
Maxillary process		Cryosection
Tongue		Cryosection
Lung		Cryosection
Rib pre-cartilage condensation		Cryosection
Heart		Cryosection
Atrium		Cryosection
Ventricle		Cryosection
Stomach		Cryosection
Liver		Cryosection
Metanephros		Cryosection
Skin		Cryosection
Somite		Cryosection
Mesenchyme		Cryosection
Yolk sac (optional)		Cryosection (Optional)
Umbilical cord (optional)		Cryosection (Optional)
Pituitary gland (optional)		Cryosection (Optional)
Nasal cavity (optional)		Cryosection (Optional)
Intestines (optional)		Cryosection (Optional)
Metanephros (optional)		Cryosection (Optional)
Mesenchyme (optional)		Cryosection (Optional)
Cartilage (optional)		Cryosection (Optional)