

## Adult *LacZ* Expression

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The *lacZ* reporter gene within the targeting vector was used to determine the whole mount expression profile of the targeted gene. Mice 6 weeks or older were fixed by cardiac perfusion using 4% paraformaldehyde (PFA). Following dissection, the tissues were fixed an additional 30 min in 4% PFA before being rinsed in phosphate buffered saline and transferred to *lacZ* staining solution, containing 0.1% X-gal, for up to 48 hours. After an additional overnight post-staining fixation in 4% PFA, tissues were cleared with 50% glycerol then imaged using a Leica dissection microscope. Only tissues that showed positive staining were imaged. All tissues were transferred to 70% glycerol for long-term storage.

Two heterozygous mice per colony (1 male, 1 female) were examined alongside 2 matching wild-type (1 male, 1 female) mice.

In the original pipeline, 41 wholemount tissues were assessed for *lacZ* expression, including six sex-specific reproductive organs (ovaries, oviduct, uterus, testis, prostate and vas deferens). Aorta was later added to the annotation list bringing the total to 42 tissues.

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, male and female mice representing a colony were closely age-matched, between 8-12 weeks old. The protocol was refined to enable more sub-structures to be discerned, by sub-dissecting specific tissues (brain, kidney, spleen) prior to staining. This allowed 7 sub-structures of the brain to be annotated (brainstem, cerebellum, hypothalamus, hippocampus, striatum, cerebral cortex and olfactory lobe). Nasal epithelia, oral epithelia, colon and vas deferens were removed from the annotation list due to the ambiguity of *lacZ* staining in these areas. In addition, the images taken of *lacZ* stained tissues became standardised to streamline the protocol and facilitate comparison.

### Adult Gene expression pipeline: Annotation list

Adult Gene Expression Pipeline Annotations	Original Pipeline	BaSH Pipeline
Brain	Present	Present
Spinal Cord	Present	Present
Peripheral Nervous System	Present	Present
Pituitary Gland	Present	Present
Skin	Present	Present
Nasal Epithelia	Present	Removed
Oral Epithelia	Present	Removed
Eye	Present	Present
Brown Adipose Tissue	Present	Present
Skeletal Muscle	Present	Present
Bone	Present	Present
Cartilage	Present	Present
Vascular System	Present	Present

White Adipose Tissue	Present	Present
Trachea	Present	Present
Oesophagus	Present	Present
Thyroid	Present	Present
Parathyroid	Present	Present
Lung	Present	Present
Heart	Present	Present
Thymus	Present	Present
Liver	Present	Present
Gall Bladder	Present	Present
Stomach	Present	Present
Small Intestine	Present	Present
Large Intestine	Present	Present
Colon	Present	Removed
Peyers Patch	Present	Present
Spleen	Present	Present
Lymph Node	Present	Present
Pancreas	Present	Present
Adrenal Gland	Present	Present
Kidney	Present	Present
Urinary Tract	Present	Present
Mammary Gland	Present	Present
Ovaries	Present	Present
Oviduct	Present	Present
Uterus	Present	Present
Testis	Present	Present
Prostate	Present	Present
Vas Deferens	Present	Removed
Aorta	Added	Present
Brainstem		Added
Cerebellum		Added
Hypothalamus		Added
Hippocampus		Added
Striatum		Added
Cerebral Cortex		Added
Olfactory Lobe		Added