



Adult LacZ IMPC_ALZ_001

Purpose

To stain adult mutant and control tissues for bacterial beta-galactosidase (LacZ) activity in order to assess the adult organ, tissue, substructure and cell type of gene expression of IKMC alleles utilizing a LacZ reporter.

Description: Adult mouse tissues are scored for presence of LacZ staining which is distinct from either nonspecific staining observed in wildtype control mice, or is too faint to score as present. Intensity of staining is not required but can be reported. Anatomical terms to localize staining to the organ, structure, substructure, or cell type will use standard mouse anatomy ontology terms.

Experimental Design

Mutants:

- A minimum of two adult animals per mutant line will be stained and annotated
- 1M and 1F will be stained and annotated for each mutant line
- HET mutants will be stained and annotated. If HOM animals are stained and annotated, then they will be in addition to the 1M and 1F HET required
- Age will depend upon the logistics of each center, but all mice will be within ± 7 days of the age stipulated in the center-specific protocols, and all mice will be > 50 days of age;
- The tissues in the agreed parameter list will be stained and annotated for each mutant

Controls:

- A minimum of one control mouse will be stained and annotated for every 10 mutants
- The tissues in the agreed parameter list will be stained and annotated for each control

Procedure

Center-specific methods will be provided and be on file and linked to the data for each mutant line.

[Stock solutions]

- 10% neutral buffered formalin
- 0.1 M ethylene glycol tetraacetic acid (EGTA; adjust pH to 8.0 with NaOH)
- 8% glutaraldehyde (store in a refrigerator at 4°C)

- 1 M MgCl₂
- 10% Nonidet P-40 (NP-40)
- 50 mM K₃[Fe(CN)₆] (store in a freezer at -30°C)
- 50 mM K₄[Fe(CN)₆] (store in a freezer at -30°C)
- 20 mg/ml X-gal in N,N-dimethylformamide (store in a freezer at -30°C)
- 10x PBS without Mg²⁺ and Ca²⁺ (pH 7.4)
- 5x PBS without Mg²⁺ and Ca²⁺ (pH 8.5)
- PBS with 0.1% Tween-20 (PBT; 0.5 ml Tween in 500 ml PBS)
- PBS with 0.02% NP-40 (1 ml 10% NP-40 in 500 ml PBS)

[Working solutions]

Fixative solution

Prepare 150 ml fixative solution per mouse.

	Required volume
Cardiac perfusion	50 ml
Trimming	30 ml
Post-fixation	70 ml
Total	150 ml

Solution	Stock	volume
1% formalin	10%	15 ml
5 mM EGTA	0.1 M	7.5 ml
0.2% glutaraldehyde	8%	3.75 ml
2 mM MgCl ₂	1 M	300 µl
0.02% NP-40	10%	300 µl
10x PBS		15 ml
DW		108.15 ml
	Total	150 ml

Staining solution

Prepare 50 ml staining solution per mouse while washing the tissue slices.

The staining solution should be filtered by using cotton linter cellulose filter paper before adding to sample.

Solution	Stock	volume
5 mM $K_3[Fe(CN)_6]$	50 mM	5 ml
5 mM $K_4[Fe(CN)_6]$	50 mM	5 ml
1 mg/ml X-gal	20 mg/ml	2.5 ml
2 mM $MgCl_2$	1 M	100 μ l
0.02% NP-40	10%	100 μ l
5x PBS (pH 8.5)		10 ml
DW		27.3 ml
	Total	50 ml

[Tissue preparation, fixation, and β -gal staining]

- 1) Deeply anesthetize the mouse with isoflurane in a glass chamber.
- 2) Cut the tip of the right atrium and vena cava in the thoracic cavity and bleed. Insert 27-G butterfly needle into the left ventricle and perfuse with 20 ml PBS with a flow rate of 3.0–4.2 ml/min to remove the blood completely.
- 3) Subsequently perfuse with 50 ml fixative solution.
- 4) Dissect out the organs and slice them into 1 mm-thick slices. The organs should be placed into the fixative solution after removal from the body.
- 5) The tissue slices are grouped into 6 and moved to 20 ml scintillation vials filled with the fixative solution. The grouped tissues are listed in the separate sheet.
- 6) Fix the tissues on a shaker for 10 min at room temperature (RT). Fixation time must not exceed 2 h from the start of perfusion with the fixative.
- 7) Rinse the tissues with PBS.
- 8) Rinse the tissues 3 times on a shaker with PBS/0.02% NP-40 for 30 min at RT.
- 9) Prepare 50 ml staining solution per mouse while washing the tissue slices. Filter the solution just before use.

- 10) Stain the tissues for 3-4 hours on a shaker at RT in the dark.
- 11) Rinse the tissues 3 times with PBT for 10 min.
- 12) Post-fix the tissues overnight in 10% neutral buffered formalin at 4°C.
- 13) Clear the tissues in the following steps.

- Rinse the fixative solution with PBS.

- Transfer the tissue slices to:

- 20% glycerol in 1% KOH for 8 h

- 50% glycerol in 1% KOH overnight or longer

- 80% glycerol in 1% KOH for 24 h or longer

- 100% glycerol for 24 h or longer

Demineralization steps for the spine

- 6') Fix the tissue over night at 4°C.
- 7') Rinse the spine with PBS twice.
- 8') Immerse in 5% EDTA solution (pH6.4) for about 2 weeks.
- 9') Rinse with PBS twice
- 10') Stain overnight on a shaker at RT in the dark.
- 11') Rinse the tissues 3 times with PBT for 10 min.
- 12') Post-fix the tissues overnight in 10% neutral buffered formalin at 4°C.
- 13') Go to the clearing process as above 13).

For each mouse, for each tissue, the scoring system will be: + (specific staining), - (no staining), NS (non-specific staining), or NA (tissue not available)

Where positive, specific, staining is found:

A. A representative image will be taken and uploaded for each mutant line. The image will have a sizing reference bar and optional center-specific watermark embedded into the image.

B. The metadata collected for each mouse will be associated with the image.

C. Each image will have a substructure field for additional annotation provided by that center.

Notes

Tables of results for each tissue from each HET or HOM mouse scored for: presence/absence/non-specific/tissue not-available as defined above.

Representative images for positive staining tissues.

Additional annotation to provide anatomical substructure & cell specificity.

Database of non-specific staining and presentation of images representative of non-specific staining.

Data QC

Quality control for each center's processes and annotation will be established by that center's staff and published as part of their detailed protocol.

A database of control non-specific staining will be maintained by the Data Coordination Center and updated periodically to indicate the frequency of non-specific staining found in wildtype mice by each center. Representative images of non-specific staining will be linked to the data for each mutant line.

Parameters

	Version	Type	Increment	Option	Derived	Unit	Data Type
Adrenal IMPC_ALZ_001_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Lower Urinary Tract IMPC_ALZ_002_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not			

	Version	Type	Increment	Option	Derived	Unit	Data Type
				available			
Brain IMPC_ALZ_003_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
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Eye IMPC_ALZ_004_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Heart IMPC_ALZ_005_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Kidney IMPC_ALZ_006_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Liver IMPC_ALZ_007_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Lymph Node IMPC_ALZ_008_001	1.0	simpleParameter		expression			TEXT
				no expression			

	Version	Type	Increment	Option	Derived	Unit	Data Type
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Mammary gland IMPC_ALZ_009_001	1.0	simpleParameter		expression			TEXT
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Ovary IMPC_ALZ_010_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Oviduct IMPC_ALZ_011_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Skin (with MG) IMPC_ALZ_012_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Stomach IMPC_ALZ_013_001	1.2	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not			

	Version	Type	Increment	Option	Derived	Unit	Data Type
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Testis IMPC_ALZ_014_001	1.1	simpleParameter		expression			TEXT
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Thymus IMPC_ALZ_015_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Trachea IMPC_ALZ_016_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Uterus IMPC_ALZ_017_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Large Intestine IMPC_ALZ_018_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
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				tissue not available			
Esophagus IMPC_ALZ_019_001	1.0	simpleParameter		expression			TEXT
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	Version	Type	Increment	Option	Derived	Unit	Data Type
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Spinal cord IMPC_ALZ_020_001	1.0	simpleParameter		expression			TEXT
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Thyroid IMPC_ALZ_021_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Pituitary IMPC_ALZ_022_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Skeletal Muscle IMPC_ALZ_023_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Small Intestine IMPC_ALZ_024_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not			

	Version	Type	Increment	Option	Derived	Unit	Data Type
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White Adipose Tissue IMPC_ALZ_025_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Lungs IMPC_ALZ_026_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Aorta IMPC_ALZ_027_001	1.0	simpleParameter		expression			TEXT
				no expression			
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				imageOnly			
				tissue not available			
Pancreas IMPC_ALZ_028_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Prostate IMPC_ALZ_029_001	1.0	simpleParameter		expression			TEXT
				no expression			
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				imageOnly			
				tissue not available			
Spleen IMPC_ALZ_030_001	1.0	simpleParameter		expression			TEXT
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	Version	Type	Increment	Option	Derived	Unit	Data Type
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Brainstem IMPC_ALZ_031_001	1.0	simpleParameter		expression			TEXT
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Cartilage IMPC_ALZ_032_001	1.0	simpleParameter		expression			TEXT
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				tissue not available			
Cerebral Cortex IMPC_ALZ_033_001	1.0	simpleParameter		expression			TEXT
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Cerebellum IMPC_ALZ_034_001	1.0	simpleParameter		expression			TEXT
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Hippocampus IMPC_ALZ_035_001	1.0	simpleParameter		expression			TEXT
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	Version	Type	Increment	Option	Derived	Unit	Data Type
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Hypothalamus IMPC_ALZ_036_001	1.0	simpleParameter		expression			TEXT
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Olfactory Lobe IMPC_ALZ_037_001	1.1	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Peripheral Nervous System IMPC_ALZ_038_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Peyers Patch IMPC_ALZ_039_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Striatum IMPC_ALZ_040_001	1.1	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Vascular System IMPC_ALZ_041_001	1.0	simpleParameter		expression			TEXT
				no expression			

	Version	Type	Increment	Option	Derived	Unit	Data Type
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Bladder IMPC_ALZ_042_001	1.0	simpleParameter		expression			TEXT
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Bone IMPC_ALZ_043_001	1.0	simpleParameter		expression			TEXT
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Cecum IMPC_ALZ_044_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Colon IMPC_ALZ_045_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Duodenum IMPC_ALZ_046_001	1.0	simpleParameter		expression			TEXT
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	Version	Type	Increment	Option	Derived	Unit	Data Type
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Ileum IMPC_ALZ_047_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
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Jejunum IMPC_ALZ_048_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Adipose (gonad) IMPC_ALZ_049_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Vas Deferens IMPC_ALZ_051_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Adipose (Brown) IMPC_ALZ_052_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Adipose (Mesenteric) IMPC_ALZ_053_001	1.0	simpleParameter		expression			TEXT
				no expression			

	Version	Type	Increment	Option	Derived	Unit	Data Type
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Diaphragm IMPC_ALZ_055_001	1.0	simpleParameter		expression			TEXT
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				tissue not available			
Gall Bladder IMPC_ALZ_056_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Hardarian Gland IMPC_ALZ_057_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Hind limb IMPC_ALZ_058_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Muscle (Quad) IMPC_ALZ_059_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				tissue not available			

	Version	Type	Increment	Option	Derived	Unit	Data Type
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Parathyroid IMPC_ALZ_060_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
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Parotid gland IMPC_ALZ_061_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Penis IMPC_ALZ_062_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Pylorus IMPC_ALZ_063_001	1.0	simpleParameter		expression			TEXT
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				tissue not available			
Ribcage IMPC_ALZ_064_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Sciatic Nerve (Quad) IMPC_ALZ_065_001	1.0	simpleParameter		expression			TEXT
				no expression			

	Version	Type	Increment	Option	Derived	Unit	Data Type
				ambiguous			
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Skull IMPC_ALZ_066_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Sublingual Gland IMPC_ALZ_067_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Submandibular Gland IMPC_ALZ_068_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Tongue IMPC_ALZ_069_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Trigeminal nerve IMPC_ALZ_070_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not			

	Version	Type	Increment	Option	Derived	Unit	Data Type
				available			
Vagina IMPC_ALZ_071_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			
Vesicular Gland IMPC_ALZ_072_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Mesenteric Lymph Node IMPC_ALZ_073_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Oral Epithelia IMPC_ALZ_074_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Epididymis IMPC_ALZ_102_001	1.2	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
LacZ Images Wholemount IMPC_ALZ_076_001	1.1	seriesMediaParameter	Minimum: 1				IMAGE

	Version	Type	Increment	Option	Derived	Unit	Data Type
Olfactory Bulb IMPC_ALZ_103_001	1.0	simpleParameter		expression			TEXT
				no expression			
				ambiguous			
				imageOnly			
				tissue not available			
Midbrain IMPC_ALZ_104_001	1.0	simpleParameter		expression			TEXT
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				imageOnly			
				tissue not available			
Thalamus IMPC_ALZ_105_001	1.0	simpleParameter		expression			TEXT
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				ambiguous			
				imageOnly			
				tissue not available			

Metadata

	Version	Type	Increment	Option	Derived	Unit	Data Type
Microscope equipment id IMPC_ALZ_080_001	1.0	procedureMetadata					TEXT
Microscope equipment manufacturer IMPC_ALZ_081_001	1.1	procedureMetadata					TEXT
Microscope equipment model IMPC_ALZ_082_001	1.1	procedureMetadata					TEXT
Date Microscope last calibrated IMPC_ALZ_083_001	1.1	procedureMetadata					DATE
Detector equipment	1.0	procedureMetadata					TEXT

	Version	Type	Increment	Option	Derived	Unit	Data Type
manufacturer IMPC_ALZ_084_001							
Detector equipment model IMPC_ALZ_085_001	1.0	procedureMetadata					TEXT
Objective equipment company IMPC_ALZ_086_001	1.0	procedureMetadata					TEXT
Objective equipment model IMPC_ALZ_087_001	1.0	procedureMetadata					TEXT
Objective magnification IMPC_ALZ_088_001	1.0	procedureMetadata					TEXT
Illumination source manufacturer IMPC_ALZ_089_001	1.0	procedureMetadata					TEXT
Illumination source model IMPC_ALZ_090_001	1.0	procedureMetadata					TEXT
Pixels per millimeter before processing IMPC_ALZ_097_001	1.0	procedureMetadata					TEXT
Pixels per millimeter after processing IMPC_ALZ_098_001	1.1	procedureMetadata					TEXT
Showcase image IMPC_ALZ_099_001	1.0	procedureMetadata					TEXT
Date of Sacrifice IMPC_ALZ_100_001	1.1	procedureMetadata					DATETIME
Date of Perfusion IMPC_ALZ_101_001	1.2	procedureMetadata					DATETIME