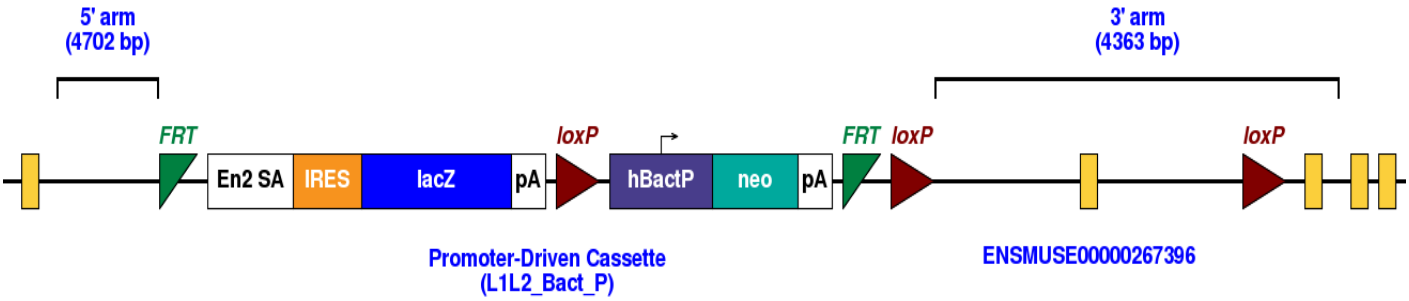
Knockout mouse lines presenting with interesting abnormalities (mammalian phenotype [MP:0000001]) may receive additional testing outside of the normal pipeline in order to maximise information collected on that mouse line. Matched wild-type controls are also processed to identify phenotypic abnormalities arising from the targeted allele.

Cldn16$^{tm1a(KOMP)Wtsi}$

claudin-16, paracellin-1, PCLN1

Genetic Background: C57BL/6Brd-Tyr<c-Brd>;C57BL/6N

ES Cell Clone: EPD0150_2_B12



Genotypes Tested for Secondary Project

Homozygous (*Cldn16*$^{tm1a(KOMP)Wtsi/tm1a(KOMP)Wtsi}$).

Relevant Pipeline Observations

Seven homozygous males and females mutants, with age matched wild-type (WT) controls entered the pipeline between 3-4 weeks of age. They were placed onto a high-fat diet (21.4% crude fat content, Western RD, 829100, Special Diets Services) at 4 weeks of age and commenced the phenotyping screens at that same time.

Test performed of relevance to this report was x-ray morphology.

Mice exhibit:

- X-Ray abnormalities uroliths in bladder; Homozygous males = 100% (7/7), baseline = 0%

Secondary Project Procedure

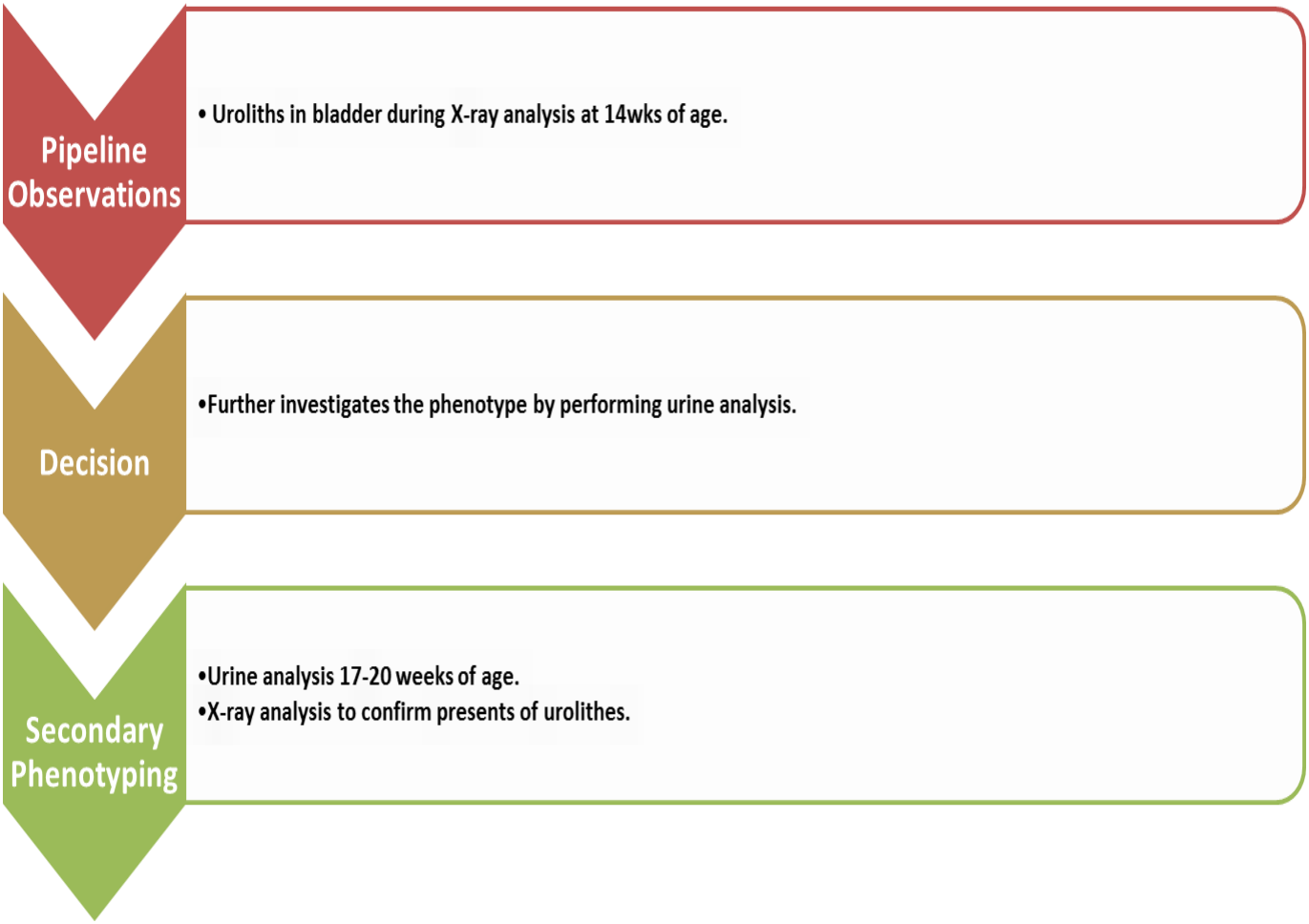
Mice with mutations of Cldn16 are known to show a kidney phenotype.
Mice homozygous for a different null allele display an age-dependent progressive phenotype that includes hypercalciuria and hypomagnesemia, significantly elevated serum parathyroid hormone and calcitriol (1,25(OH)2D3) levels, and a significantly lower urinary pH but no nephrocalcinosis or renal failure (1).

When homozygous Cldn16$^{tm1a(KOMP)Wtsi/tm1a(KOMP)Wtsi}$ were tested at the MGP males showed uroliths in bladder at X-rays (7/7).

In order to further investigate the phenotype urine analysis was performed. Four additional homozygous males and age matched controls were used for urine analysis (COMBUR –TEST/Chemstrip (Roche)) at 17-20 weeks.
They also underwent X-ray analysis to confirm the presence of urolithes.

1. Will C et al., "Targeted deletion of murine Cldn16 identifies extra- and intrarenal compensatory mechanisms of Ca2+ and Mg2+ wasting." Am J Physiol Renal Physiol 2010 Feb 10;

Schematic Outline of Secondary Project



Pipeline Phenotyping Heat Map

Colony Prefix	Allele Name	Viability at weaning	Recessive Lethal Study	Fertility	Embryo LacZ Expression	Adult LacZ Expression	General Observations	Weight Curves	Open Field	Modified SHIRPA	Grip Strength	Hot Plate	Dysmorphology	Indirect Calorimetry	Glucose Tolerance (ip)	Auditory Brainstem Response	Body Composition (DEXA)	X-ray Imaging	Stress Induced Hyperthermia	Eye Morphology	Plasma Chemistry	Plasma Immunoglobulins	Haematology (CBC)	Peripheral Blood Lymphocytes	Micronuclei	Tissue Biobank	Heart Weight	Heart Histology	Brain Histopathology	Eye Histopathology	Salmonella Challenge	Citrobacter Challenge
MAPG	Cldn16<tm1a(KOMP)Wtsi>																															

Significant / Interesting
Not significant
Resources available

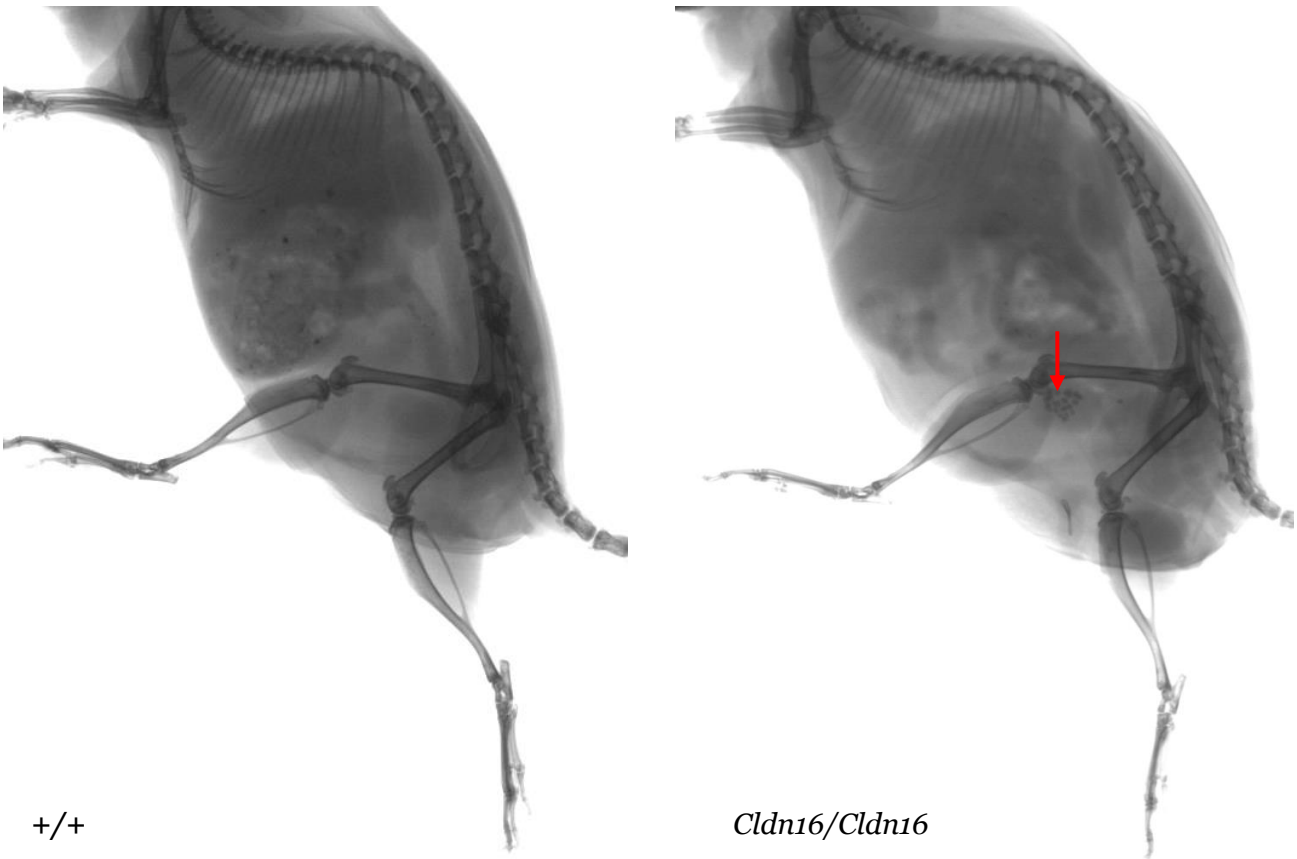
Material & Methods for Non-Pipeline Test(s)

Urine analysis

The mouse was gently restrained and allowed to urinate onto a piece of parafilm. A pipette was used to apply urine to the test strip (Combur-Test/Chemstrip (Roche)). The test paper was placed in a horizontal position for one minute and the colour of the test paper was compared to the colour scale sheet attached to the test paper container.

Phenotyping Data of Interest (Significant Changes)

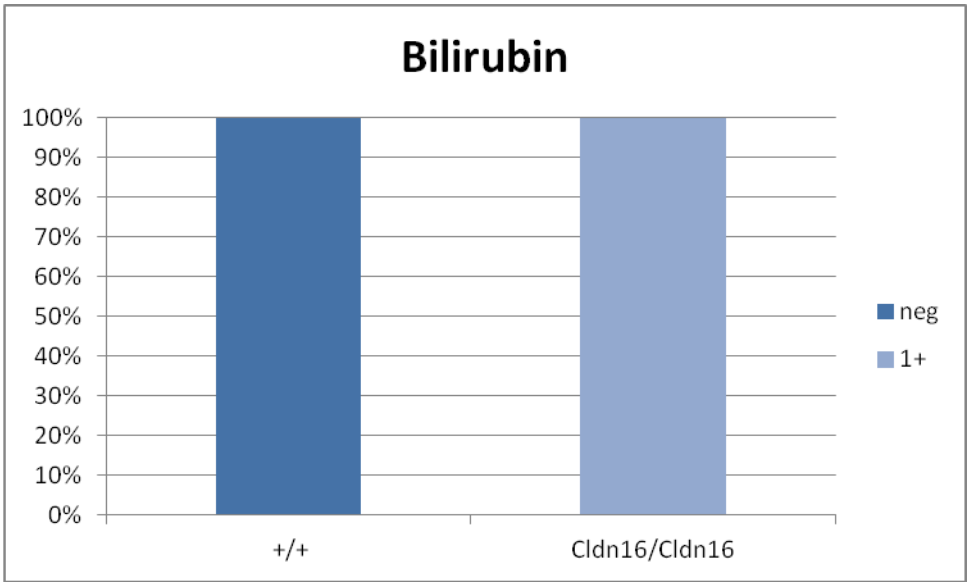
In-Life Phenotyping



a b

Figure 1. Lateral whole body radiograph of (a) wild-type and (b) homozygous mouse showing uroliths in the bladder (urolithiasis [MP:0005360]).

Ex-Vivo Phenotyping



Males – bilirubinuria [MP:0011464]

Figure2. Presence of Bilirubin was observed in the urine of homozygous male mice.