Real-Life Research

THE UNIVERSITY of EDINBURGH
Easter Bush
Science Outreach Centre

Get hands-on with real-life science

How to build a bone - PHOSPHO1 and the skeleton

Attached dataset: PHOSPHO1 gene expression during embryonic development

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Colin is an expert in all things bone and cartilage and runs a scientific research group at The Roslin Institute. In addition to his research, Colin is Dean of Post-Graduate Research and oversees all research students at Roslin and the Royal (Dick) School of Veterinary Studies. He discovered an enzyme called PHOSPHO1 which is critical for the proper mineralisation of bone during embryonic development and in the early period of life, and continues to work on its biology today!

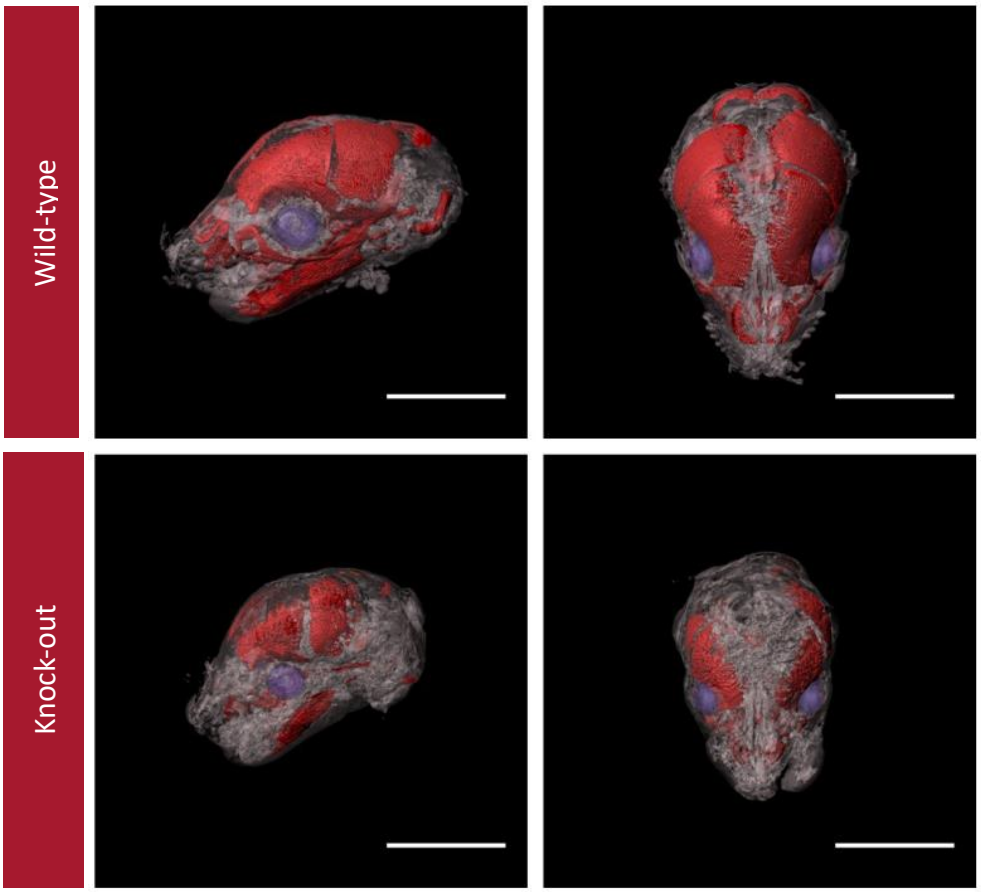
What is PHOSPHO1 (*fos-fo-one*)?

PHOSPHO1 was discovered 20 years ago and is a phosphatase enzyme. This means it is able to split certain kinds of molecules apart to make phosphate ions (PO_4^{3-}). We know that when the skeleton is developing in embryos, the specialised cells which make bone known as osteoblasts increase the amount of PHOSPHO1 they produce by about 100 times, and so Colin and his team thought that this enzyme must be extremely important in the process of making bone. 60% of your bones by weight are made of up a mineral called hydroxyapatite which contains mainly calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions, so PHOSPHO1 could be responsible for generating one of the building blocks needed to make bone.

To prove this Colin and his collaborators changed the mouse genotype to see the effect on its phenotype. Which gene do you think that they changed? They made a knock-out mouse

with a mutation in the *Phospho1* gene which made it non-functional and examined changes in the skeleton of these animals. The scientists saw that much less bone was made in the knock-out mice compared to the normal mice (we call these the wild-types). You can see this in Figure 1.

Figure 1 – Colin and his team created the images below to demonstrate that mice without PHOSPHO1 form much less bone than the wild-types. The red-stained areas make up the bone in the skulls of these mice while the grey areas are the surrounding soft tissues. The scale bars represent 20mm. Adapted from Dillon *et al* (2019).



How do we tell which mouse is which?

In the lab, before we start doing any experiments, we have to make absolutely sure that the animals we are working with are the correct genotype – i.e. whether they are knock-out or wild-type. To achieve this we use a method called genotyping.

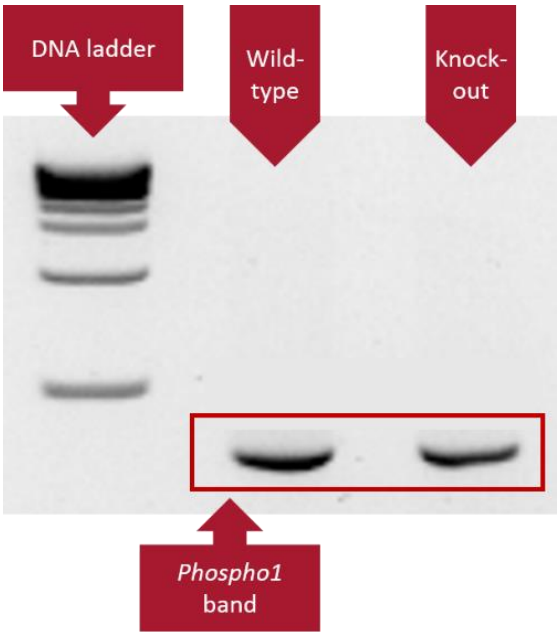
In Colin's lab, the genotyping protocol is based on a technique known as polymerase chain reaction (PCR) which you will have learned about in school.

Test your knowledge: What 5 components are required for a successful PCR?

In order to generate the PHOSPHO1 knock-out mouse, scientists introduced a single nucleotide polymorphism (SNP) into the *Phospho1* gene which made the gene non-functional. In this case, a single C (cytosine) was replaced with a T (tyrosine):

TCCTCCTCACCTTCGACTTCGATGAGACCATCGTGGACGAGAACAGCGACGACTCGATCGTGCGTGCCGCG
CCAGGCCAGCAACTGCCCGAGAGCCTGCGTGCCACCTATCGCGAGGGCTACTACAATGAGTACATGCAAC/
TGAGTCTTTAAGTACCTGGGTGAGCAGGGTGTACGGCCCCGGGACCTGCGCGCTGTCTACGAGACCATCCC
CCTGTCGCCAGGCATGGGCGATTTGTTGCAGTTCATAGCCAAACAGGGCTCCTGCTTCGAGGTTATTCTCAT
TTCGGATGCCAACACCTTCGGTGTGGAGAGTGCCCTGCGTGCCGCTGGCCACCACAGTTTATTCCGCCGCA
T

The team were able to design primers against this specific section of the gene, and perform a PCR. We can see our PCR product using gel electrophoresis, but this still throws up a problem. As we have introduced a SNP, both the wild-type and the modified gene have the same number of base pairs. When we run out our gel therefore, both PCR products look exactly the same since they are the same size (Figure 2).



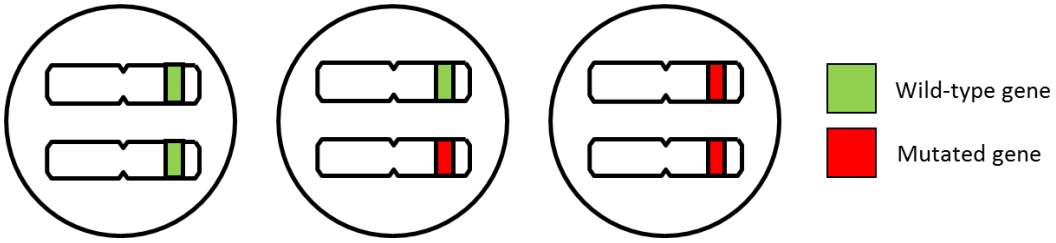
> Figure 2 – After PCR, the DNA which is produced can be separated on an agarose gel using gel electrophoresis. In this gel we can't distinguish between the two samples because they are the same size.

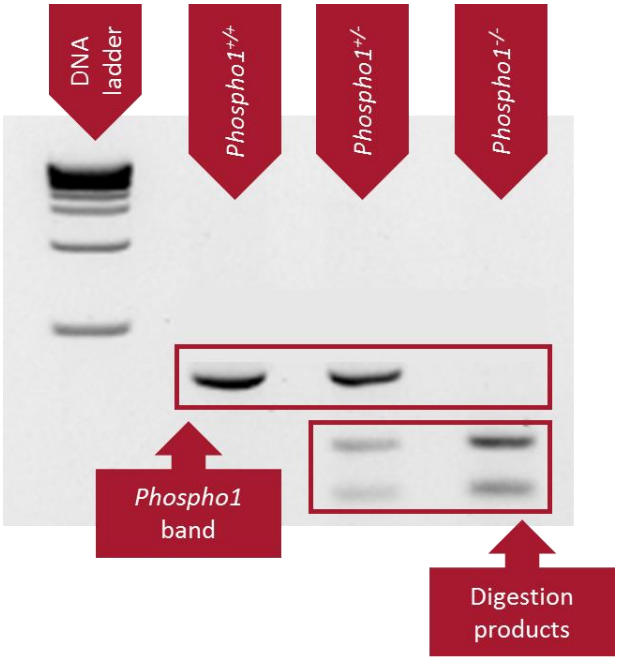
So how do we tell the difference?

Mice have 20 pairs of chromosomes and, just like us, have two copies of nearly every gene known as alleles – one on each chromosome in a pair. Therefore we have to be able to tell the difference between animals with 2, 1 and 0 copies of the *Phospho1* gene! Animals which have 2 identical copies of any one gene are called homozygous, while those which have different copies are called heterozygous. To distinguish between all of these genotypes we use the following notation:

- Homozygous wild-type – *Phospho1*$^{+/+}$
- Homozygous knock-out – *Phospho1*$^{-/-}$
- Heterozygous – *Phospho1*$^{+/-}$

Test your knowledge: Which of the cells below are homozygous and which are heterozygous for the *Phospho1* gene?





To separate all of these genotypes, we can use restriction endonucleases. These are enzymes which cut DNA at very specific sequences. For *Phospho1* genotyping we use an enzyme called BseMI which recognises the sequence GCAATG. The enzyme will therefore recognise the mutated version of the *Phospho1* gene, but not the wild-type version which reads GCAACG! In solution we would therefore expect any mutated DNA copies to be cut neatly into two fragments while the wild-type copies remain as long single strands.

This means we can now work out the genotype of our samples (Figure 3).

< Figure 3 – Once we have broken up our PCR products with the restriction enzyme BseMI we can tell our genotypes apart based on whether they have been cut or not. This gel shows the differences between the genotypes.

Feeling confident? See if you can genotype the litter of mice below. Can you work out what size each of the fragments are?