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

We've put together a directory of freely available datasets for you to use in the classroom. These range from open source public and Government material, to case study-linked examples of real scientific data from researchers at the Roslin Institute and Royal (Dick) School of Veterinary Studies.

PHOSPHO1 is an enzyme which is important for the proper development of our skeleton in infancy. You can learn about the function of PHOSPHO1 in the case study which comes with this dataset. Here we've provided you with real-life data on how expression of the *Phospho1* gene changes during development of the mouse skeleton, and how much bone is present at each age.

How can I use data in the classroom?

The resources we've collected here can be used in as many ways as you can dream up and range from simple to complex. Pupils are engaged and motivated when they are involved in planning their own science investigations, and datasets are a great way of challenging pupils to get creative in defining scientific questions and answering them using the data analysis skills they have learned.

Course aims of Higher Biology covered:

- Develop and apply knowledge and understanding of biology
- Develop scientific inquiry and investigate skills
- Develop scientific analytical thinking skills, including scientific evaluation in a biology context
- Develop problem-solving skills in a biology context
- Develop the knowledge and skills for more advanced learning in biology

Key concepts of Higher Biology covered:

- DNA & the Genome
  - Gene expression
  - Mutations
- Metabolism & Survival
  - Metabolic pathways
  - Genetic control of metabolism
- Sustainability & Interdependence
  - Plant and animal breeding

This resource is also intended to demonstrate the importance of system-wide analysis and that the key concepts of the course are interdependent.

Pupils can also be asked to prepare oral or written reports based on what they have found working with data.

How do I use this workbook?

Each spreadsheet contains some challenging questions surrounding the biology of PHOSPHO1 and the skeleton, and a worked example of how we can use data to help answer them. We've also provided you with the raw data so you can design your own investigations – get creative!

We hope you have fun using this resource – get in touch and tell us about what your pupils have been doing with our data!

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Raw data and definitions

The data presented here are real data provided by Roslin Institute scientists working on the biology of the PHOSPHO1 enzyme. All of these data come from analyses performed on the legs of mouse embryos during the development of their skeletons. The age of each embryo is given as embryonic days (i.e. days post-fertilisation).

Bone volume data

Bone volume is measured here by staining the skeletons of embryos with a fluorescent dye, imaging them using a technique called optical projection tomography and analysing those images on a computer. You can see examples of these images in the PHOSPHO1 case study. We've provided you with the volume of bone in mm^3 across all bones in the leg in both wild-type and *Phospho1* knock-out animals, along with the number of bones present at each age. Development of the skeleton begins at day 15 of an embryo in these samples.

Gene expression data

Here we've provided you with the expression of two genes in each sample - *Phospho1* and another that codes for a phosphatase, called Tissue Non-Specific Alkaline Phosphatase (TNAP; *Alpl*). Remember that notation for proteins and genes are different, and when talking about genes we italicise their names (e.g. TNAP protein versus *Alpl* gene).

The expression of each gene is shown as a fold change compared with the average expression at embryonic day 14. A fold change of 1 therefore indicates no change in expression between that sample and the average embryonic day 14 sample, while a fold change of 2 would represent a doubling of gene expression and 0.5 a h

Wild-type- A "normal" mouse that has not been genetically modified.
Knock-out- A mouse which has been genetically modified to remove the function of a gene in order to study its biology.

	Age (embryonic days)	Wild-type bone volume (mm^3)	Wild-type number of bones present	Knock-out bone volume (mm^3)	Knock-out number of bones present
Bone volume	14	0	0	0	0
	14	0	0	0	0
	14	0	0	0	0
	15	0.04419	6	0	0
	15	0.05369	6	0	0
	15	0.03284	6	0	0
	16	0.9354	6	0.05574	6
	16	0.8903	6	0.03259	6
	16	0.9465	6	0.07549	6
	17	1.0916	12	0.2554	6
	17	1.1363	12	0.318	6
	17	1.1871	12	0.2398463	6

	Age (embryonic days)	*Phospho1* expression	*Alpl* expression
Gene expression in wild-type	14	1.725084064	1.353473524
	14	0.261823531	0.391386708
	14	2.214017563	1.887748625
	15	2.921413689	2.324091174
	15	2.009263349	1.620755722
	15	1.798341071	1.501772904
	16	2.578740617	2.023238881
	16	1.900878955	1.967913307
	16	3.003545807	2.525670902
	17	6.805337288	4.428035126
	17	7.293779908	6.048874241
	17	8.495310432	8.734146117

Calculating and plotting averages

Background

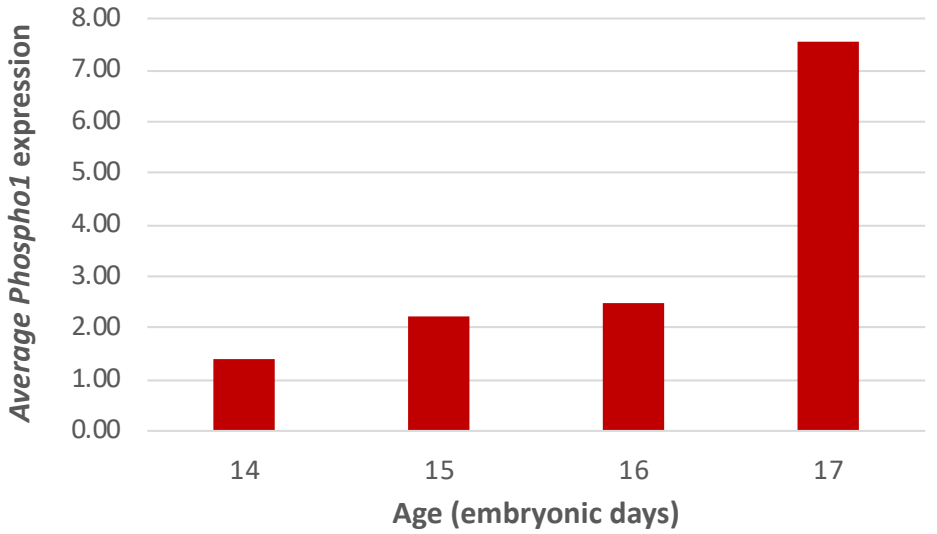
PHOSPHO1 is a phosphatase enzyme. That means it is able to split apart biological molecules to release phosphate ions. Research at The Roslin Institute and elsewhere has proved that it is an extremely important part of making healty bones during childhood.

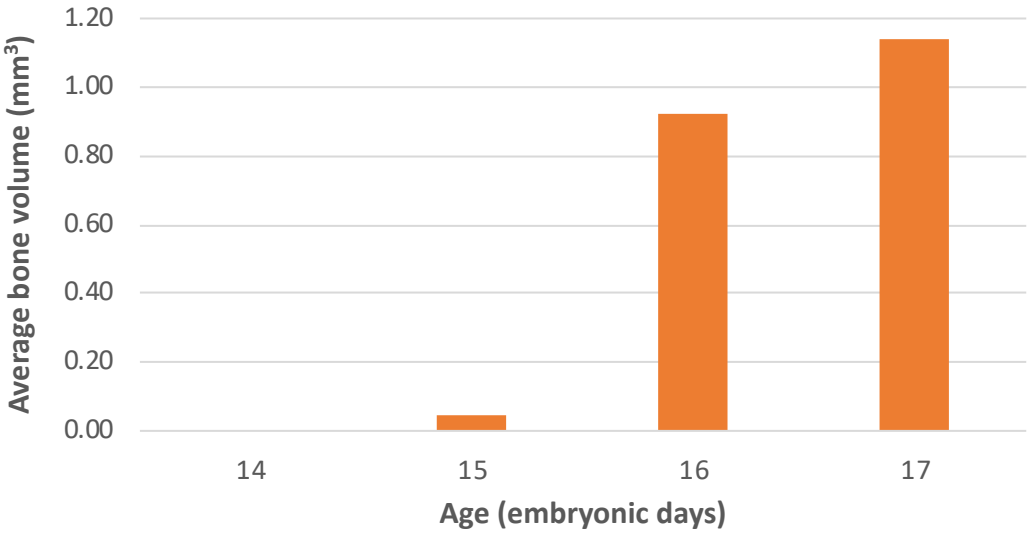
Your challenge

Some of our scientists have worked out how much of the PHOSPHO1 gene is expressed in the legs of mice at several ages during their development. They've also calculated how much bone is present at each age.

First, can you work out the average gene expression and the average bone volume and plot each on a graph?

Age (embryonic days)	Average *Phospho1* expression	Average bone volume (mm^3)
14	1.40	0.00
15	2.24	0.04
16	2.49	0.92
17	7.53	1.14



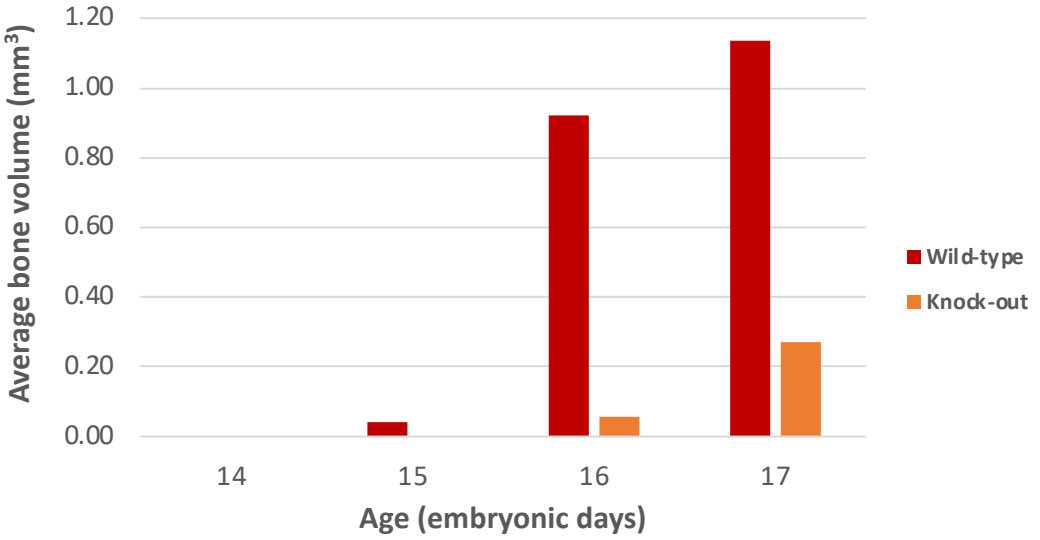


Too easy for you?

What we're really interested in is differences between wild-type animals and those which lack the PHOSPHO1 enzyme. We have been able to make mice with no PHOSPHO1 by modifying the gene and introducing a mutation (knock-out animals). The data opposite shows measurements of bone volume at different ages during mouse development from either wild-type or knock-out animals.

Calculate the average bone volume in wild-type and knock-out animals and plot both on the same bar chart.

Age (embryonic days)	Average wild-type bone volume (mm^3)	Average knock-out bone volume (mm^3)
14	0.00	0.00
15	0.04	0.00
16	0.92	0.05
17	1.14	0.27



Follow up questions:

1) On average, what volume of bone was present at 17 days of development in wild-type and knock-out animals?

The average bone volume at 17 days of development was 1.14mm³ in wild-type and 0.27mm³ in knock-out mice.

2) What do these data tell us about the skeletons of animals which lack PHOSPHO1, compared to wild-type controls?

The data indicates that the skeletons of animals with lack the PHOSPHO1 enzyme, due to the gene being mutated, have much less volume.

3) What kind of experiments would you do next to find out more?

Students' own answers. Example answer *Look to see what the role of PHOSPHO1 is in the development of bone. Use microscopy to study the bone more closely and to compare the bone of wild-type and knock-out mice.*

Calculating Standard Deviation

Background

Standard deviation is a number that can be calculated, it tells us how measurements for a group are spread out from the average (mean), or expected value. A low standard deviation means that most of the numbers are close to the average. A high standard deviation means that the numbers are more spread out.

Error bars can be added to a graph using the standard deviation number, this means we can see the standard deviation at a glance.

Your challenge

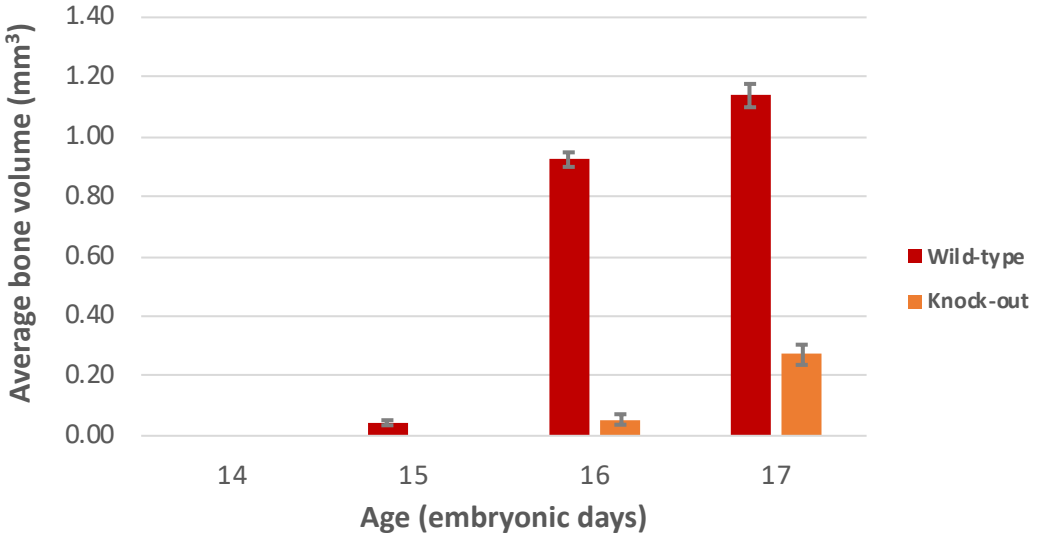
Calculate the standard deviation and add error bars to your graph on page 4.

Can you calculate the standard deviation for the average bone volume for wild-type and knock-out mice?

$$s = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}} = \sqrt{\frac{\Sigma x^2 - (\Sigma x)^2/n}{n-1}}$$, where *n* is the sample size

Can you add error bars to the data on the graph on page 4? How good is this data? Is it reliable?

Age (embryonic days)	Standard deviation	Standard deviation
14	0.00	0.00
15	0.01	0.00
16	0.02	0.02
17	0.04	0.03



Working with trends

Background

In biology, while we often only study the function of one gene at a time, in reality many genes are functioning together to bring about the development of the skeleton.

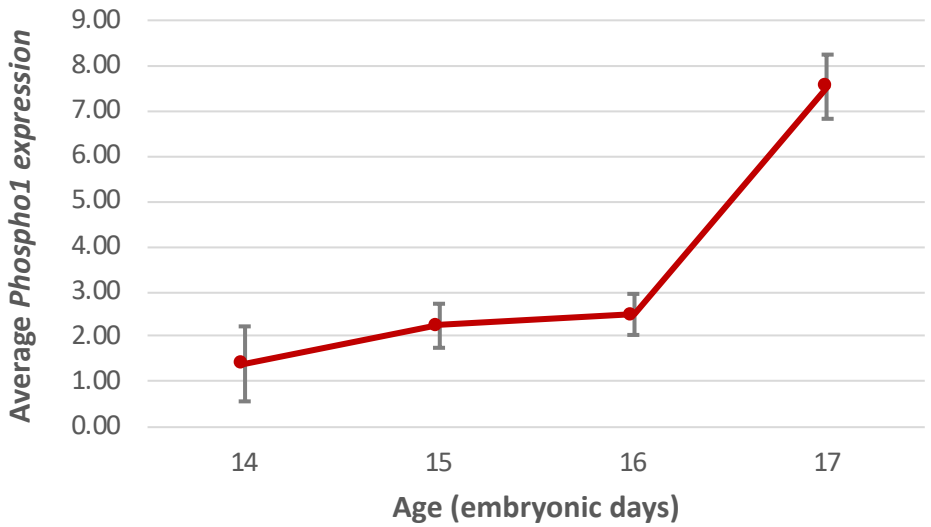
Tissue Non-specific Alkaline Phosphatase (or TNAP for short) is another phosphatase enzyme which we know helps to make bone. In fact when we knock-out the genes *Phospho1* and *Alp1*, that make PHOSPHO1 and TNAP, they have no skeletons at all.

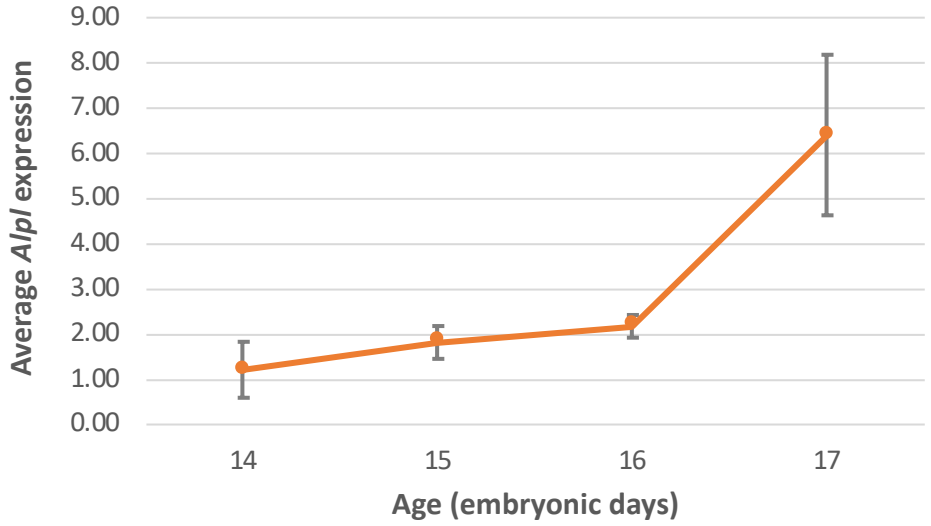
Your challenge

Our scientists have measured how much of each gene is expressed at a given age during mouse development so that we can work out how bone cells make bone.

Plot the average gene expression of the *Phospho1* and *Alpl* genes over time as line graphs, showing the standard deviation using error bars. Describe how the expression of these genes changes over time.

Age (embryonic days)	Average *Phospho1* expression	Average Alpl expression
14	1.40	1.21
15	2.24	1.82
16	2.49	2.17
17	7.53	6.40
	Standard deviation	Standard deviation
14	0.83	0.62
15	0.49	0.36
16	0.45	0.25
17	0.71	1.78



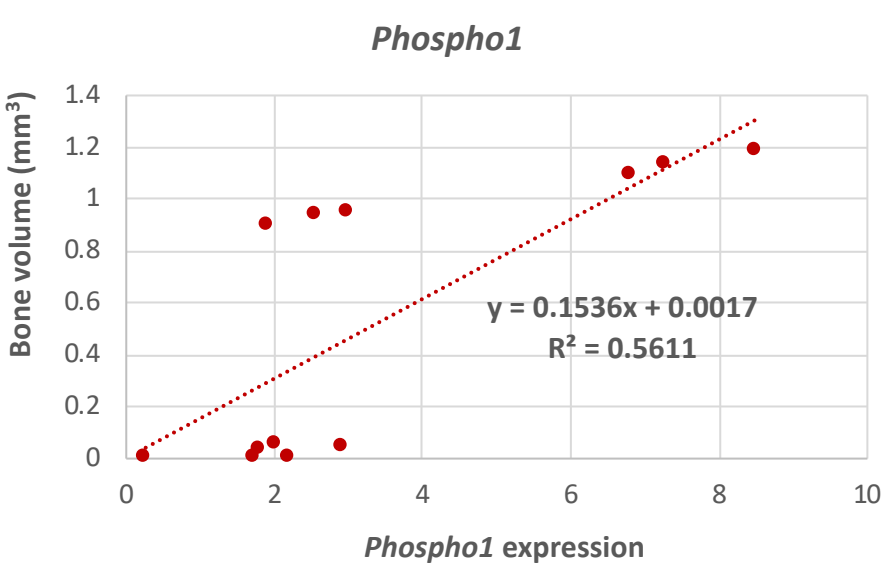


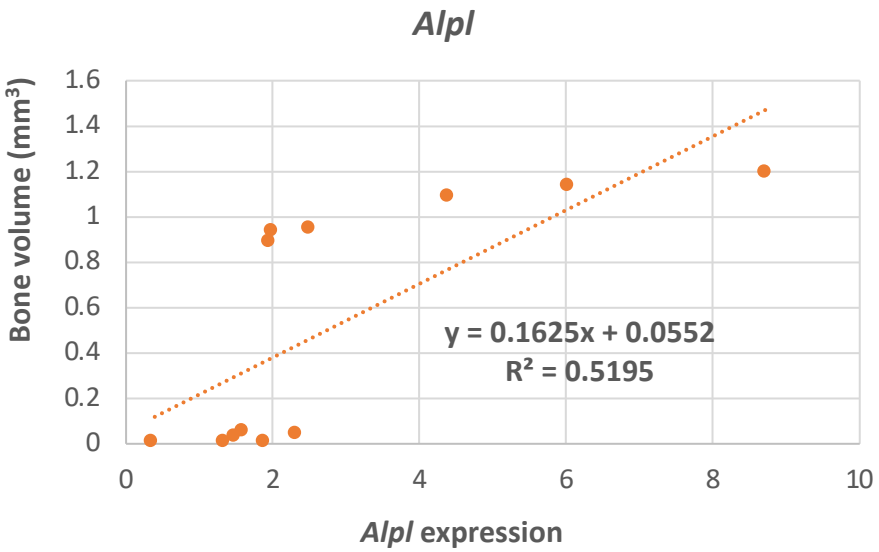
Let's dig a little bit deeper

So, now we know how much of each gene is expressed over time by the bone cells while the skeleton is getting made. One of the first steps we can make when trying to understand the role of these genes is to see whether their expression correlated with what we're interested in.

Scatter graphs are a good way of displaying two sets of data to see if there is a correlation, or connection.

Plot the average expression of each gene against average bone volume in the wild-type animal as a scatter graph. Then draw a linear line of best fit





Follow up questions:

1) What are the y-intercepts of the lines of best fit predicting about *Phospho1* and *Alpl* expression and bone volume?

They predict the level of *Phospho1* and *Alpl* required to start bone development.

2) Describe the correlations between the two genes we have been studying and bone volume in the lower limb. What might these correlations indicate about the biology of these enzymes?

They follow similar expression patterns, indicating that they could both be regulated the same way i.e. switched "on" together.

3) What kind of experiments would you do next to find out more?

Students' own answers: Example answer: *Create a knock out mouse of Alp1 to study its bone volume in embryonic development. The Phospho1 and Alp1 mice could be bred together to create a double-knock out, this could help us understand the relationship between theses two genes in bone development.*