Real-Life Research

THE UNIVERSITY of EDINBURGH
Easter Bush
Science Outreach Centre

Get hands-on with real-life science

1. What is genome editing?

A technique to edit or modify an organism's DNA.

2. What is the infectious agent that causes Porcine Respiratory & Reproductive Syndrome (PRRS)?

A virus

3. Put these phrases in the right order by numbering them 1 to 7.

5	PRRSV is released out of macrophages by budding and increases infection
1	PRRSV binds to receptors
3	PRRSV is translated inside the macrophage
4	More copies of PRRSV are made
2	PRRSV is taken into macrophages by endocytosis

4. What domain of the PRRS receptor does PRRS virus bind to?

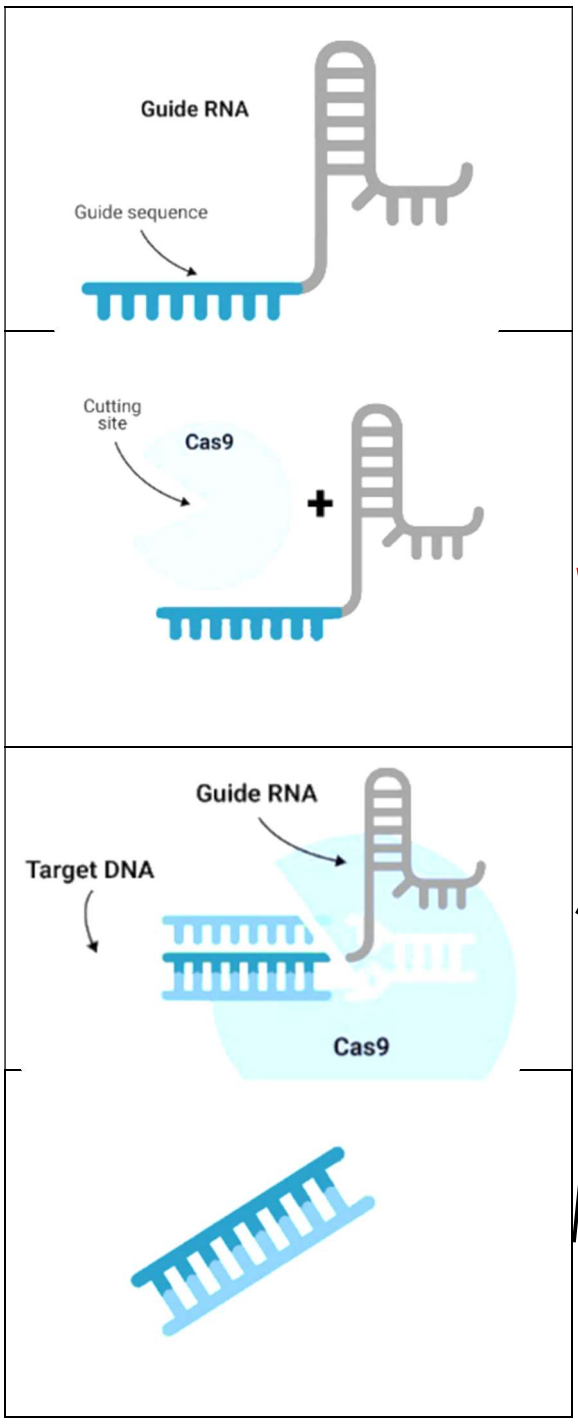
Domain 5

5. What is the hypothesis of the experiment that Christine and her team developed to try to prevent pigs getting PRSS disease?

Example answer:

By editing the pig gene CD163 (the PRSS receptor) and removing the exon (exon 7) that encodes domain 5 of the CD163 protein this will prevent the virus from binding to the receptor. Therefore, the virus will not enter the macrophages and this will prevent infection.

6. Match the pictures with the correct statements.



Finally, the **cut in the DNA is repaired** by the cell, sometimes this introduces a **mutation**.

Next, the guide RNA finds the target DNA sequence in the cells and Cas9 **cuts it out**.

First, scientists create a genetic sequence, called "a guide RNA" that matches the piece of DNA **they want to change**.

Then, this guide RNA is added to cells along with a protein called Cas9, which **acts like a pair of scissors** that cut DNA.

7. What is the difference between and intron and an exon?

Intron – non-coding DNA

Exon – coding DNA

8. When discussing inheritance, what does F0 and F1 mean?

F0 = parent generation

F1= offspring of F0 generation

9. How do you generate live pigs with the genome editing technology? (use the words **zygote**, **cas 9**and **guide RNA** in your answer)

Example answer:

*Use CRISPR to genetically modify the CD163 gene in a pig **zygote** in vitro (in the lab) by adding the CRISPR reagents a **guide RNAs** for exon 7 (2 to make a deletion) and **cas 9**, then implant the edited zygote into surrogate sows.*

10. How does gel electrophoresis work and why was it used in the workshop?

Example answer:

DNA loaded into wells in an agarose gel, placed in a buffer, and connected to power supply. DNA will run down gel from the negative to the positive electrode because DNA is slightly negatively charged. DNA fragments will be separated by size – larger DNA fragments move slower, so stay closer to the top.

It was used in the workshop to determine which pigs had been edited (exon 7 removed).

11. The buffer used is called TAE and was at 0.25x concentration, made using 50x TAE stock solution and water. How much 50x TAE stock solution do you need to use to make 1L of 0.25x TAE? How much to make 300ml?

1L of 1xTAE:

20ml of 50x TAE

980ml of H_2O

1L of 0.25xTAE:

250ml 1x TAE

750ml of H_2O

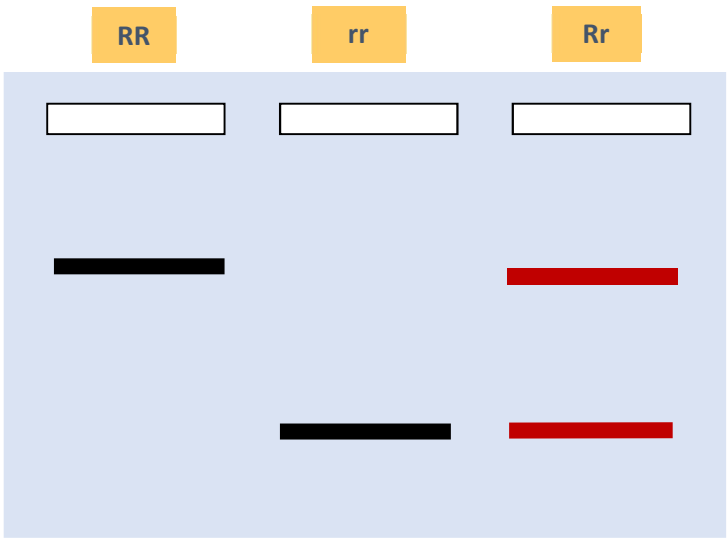
a. The gel also contains a dye called SYBER SAFE. It is used at a concentration of 1:10 000. How much SYBR SAFE (in µl) do you need to use if you make up 300ml of gel?

300ml / 10 000 = 0.03ml = 30µl

12. What bands would you expect in the last lane? Draw them!

R= not edited

r = edited



a. What do these different genotypes mean? Which of these pigs will have domain 5 in PRRS receptor?

RR = homozygous, not edited

rr = homozygous, edited

Rr= heterozygous, one allele with edited gene, one allele with non-edited gene.

RR and Rr will have domain 5 of the CD163 protein.

13. What is the mass (grams) of agarose needed to make a 30ml, 2% agarose gel?

To make a 1% gel that is 30ml:

30ml/100% = 0.3g

To make a 2% gel that is 30ml

0.3g x 2 = 0.6g

14. ***None of the people that looked after the pigs, carried out the virus infection or studied samples from the pigs knew which pigs were edited and which were wild-type.*** Why is it important that the researchers were blinded when testing their hypothesis in this experiment?

Example answer:

So they are not biased and do no influence the results. They might unconsciously behave differently or treat the pigs differently depending on the outcome they are hoping for.

15. A section of double-stranded DNA contains 80 guanine and 40 adenine molecules. What is the total number of deoxyribose sugars in this section?

Remind students that guanine pairs with cytosine, and adenine pairs with thiamine.

80G + 80C

40A + 40T

= 240 deoxyribose sugars

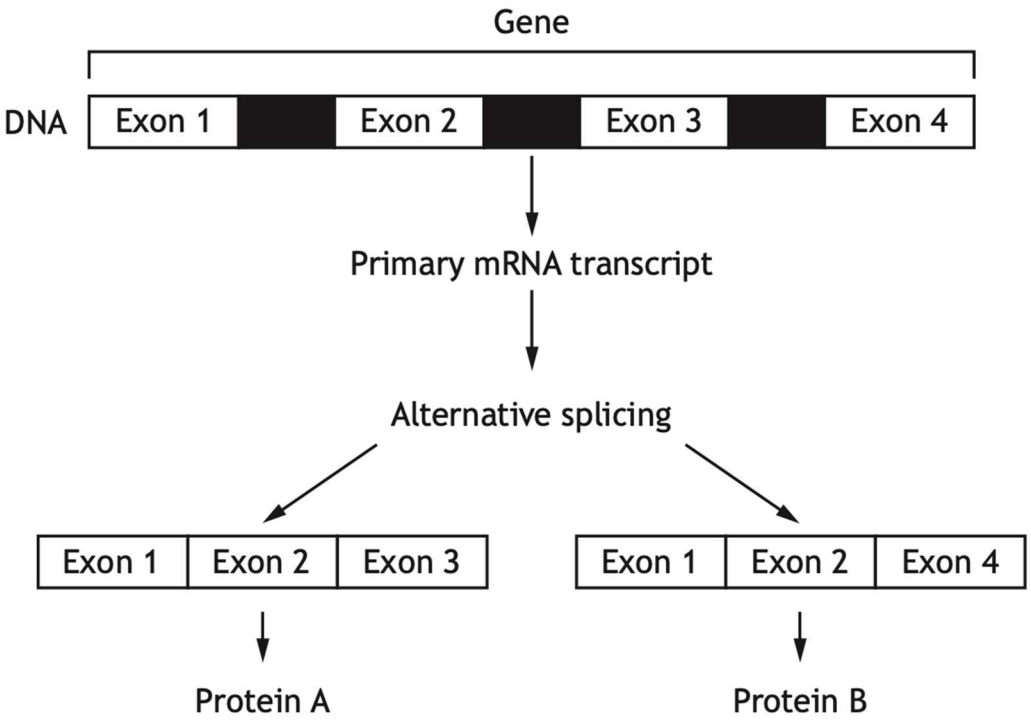
16. The diagram illustrates steps in the transcription and translation of a gene.

    a. Name the regions always removed from a primary mRNA transcript.

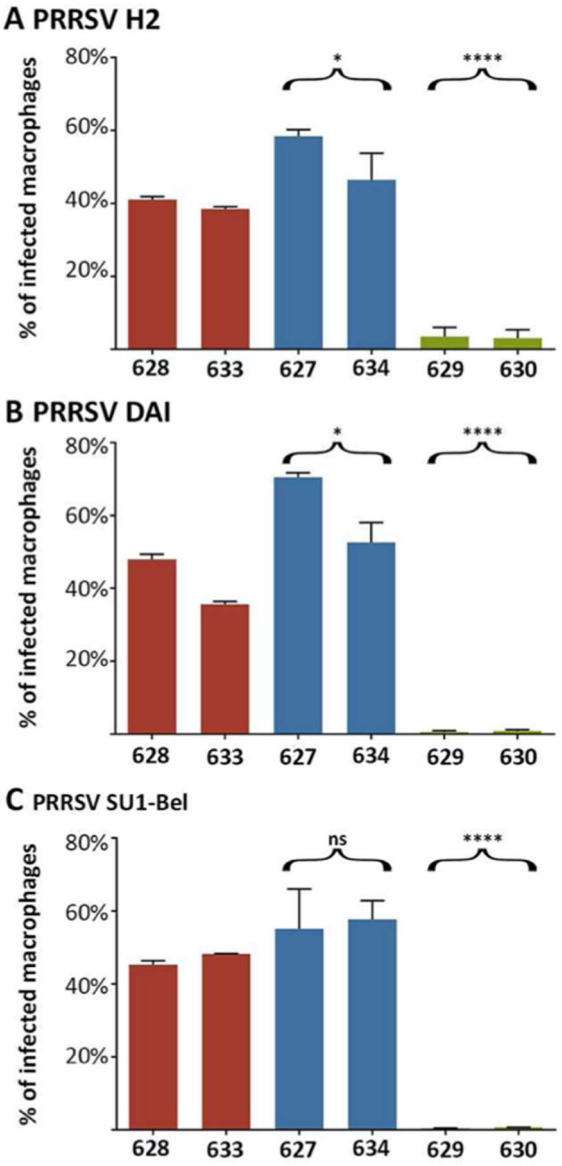
Introns

    b. Which three exons in this gene could be translated to produce a protein which is different from proteins A and B.

Exons 1, 3, 4 or exons 2, 3, 4,



17. This is a figure from the research paper that was published by Dr Christine Burkard, the researcher leading these experiments. There are three subtypes of the PRRS virus called **H2**, **DAI**, and **SU1-Bel**.
    In this experiment, Christine and her team tested their genome edited pigs (wild type, heterozygous and homozygous edited pigs) with these three virus subtypes, the numbers along the *x* axis are individual pig identification numbers.



KEY

Red bars = wild type (no genome editing)

Blue bars = heterozygous (only one allele with deleted exon 7)

Green bars = homozygous (both alleles with deleted exon 7)

Burkard *et al.* (2017) *Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function* PLOS Pathogens

a. What are these three graphs measuring?

The percentage of infected macrophages.

b. Describe the results and main findings.

Example answer:

Homozygous genome edited pigs had <5% / significantly less infected macrophages in all three strains of the PRRS virus compared to the wild type and heterozygous.

Heterozygous pigs and wild type had a high percentage (40-60%) of infected macrophages in all three strains of the PRRS virus.

These results indicate that gene editing of the CD163 gene, to remove exon 7, prevents infection of macrophages regardless of strain. However, one edited allele alone is not enough to prevent infection.