Real-Life Research

THE UNIVERSITY of EDINBURGH
Easter Bush
Science Outreach Centre

Get hands-on with real-life science

Name ……

Class ……..

Date ……..

REAL-LIFE RESEARCH: WHICH LITTLE PIGGY?

We hope you enjoyed our **Real-life Research: Which little piggy?** workshop and that it gave a real insight and practical hands-on experience on what life in the lab is like here at The Roslin Institute. This workbook will build on the foundation of the experiments you carried out and will give you some more practical experience of interpreting real data. The curriculum links included in this workbook are:

DNA and the genome

- The structure of DNA
- Gene expression
    - Amino acids form polypeptides

Metabolism and survival

- Genetic control of metabolism

Sustainability and interdependence

- Plant and animal breeding
- Animal welfare

This workbook is also a good exercise if you are preparing for exams!

1. What is genome editing?

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

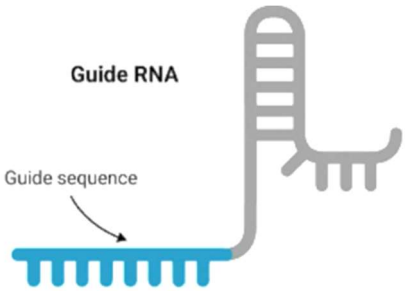
3. Put these phrases in the right order by numbering them 1 to 5.

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

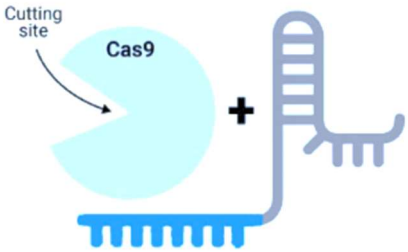
4. What domain of the PRRS receptor, found on the surface of pig macrophages, does PRRS virus bind to?

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

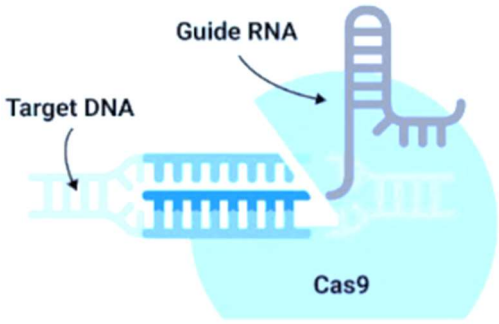
6. Match the pictures with the correct statements.



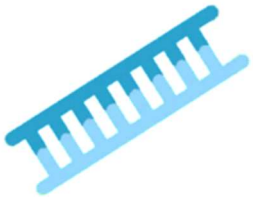
Finally, the **cut in the DNA is repaired** by the cell, sometimes this introduces a **mutation**.



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?

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

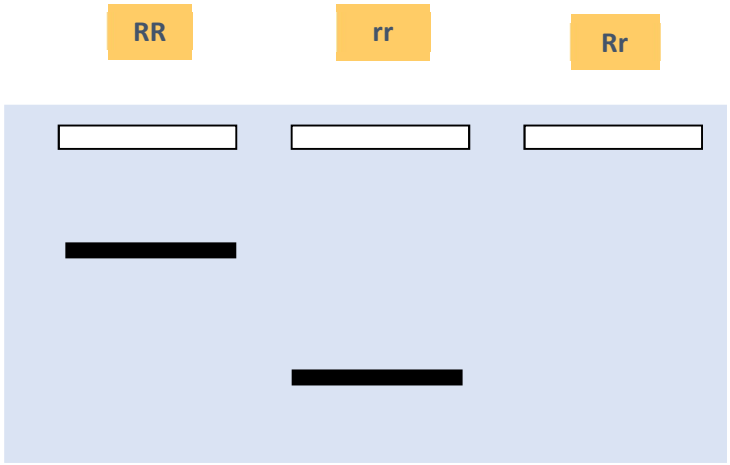
9. How do you generate live pigs with the genome editing technology? (use the words **zygote**, **cas9** and **guide RNA** in your answer)

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

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 1x TAE? How much to make 1L of 0.25X TAE?

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?

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



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

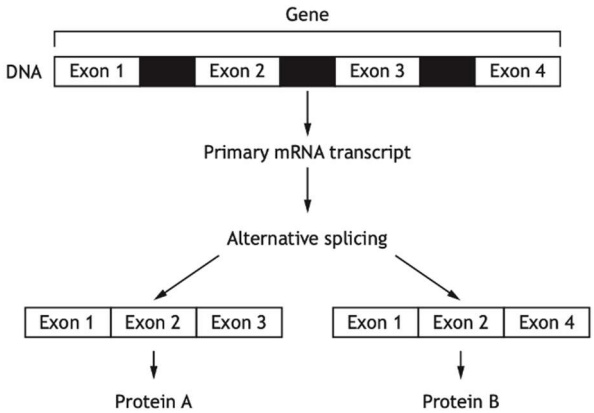
13. What is the mass (grams) of agarose needed to make a 30ml, 2% agarose gel?

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?

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?

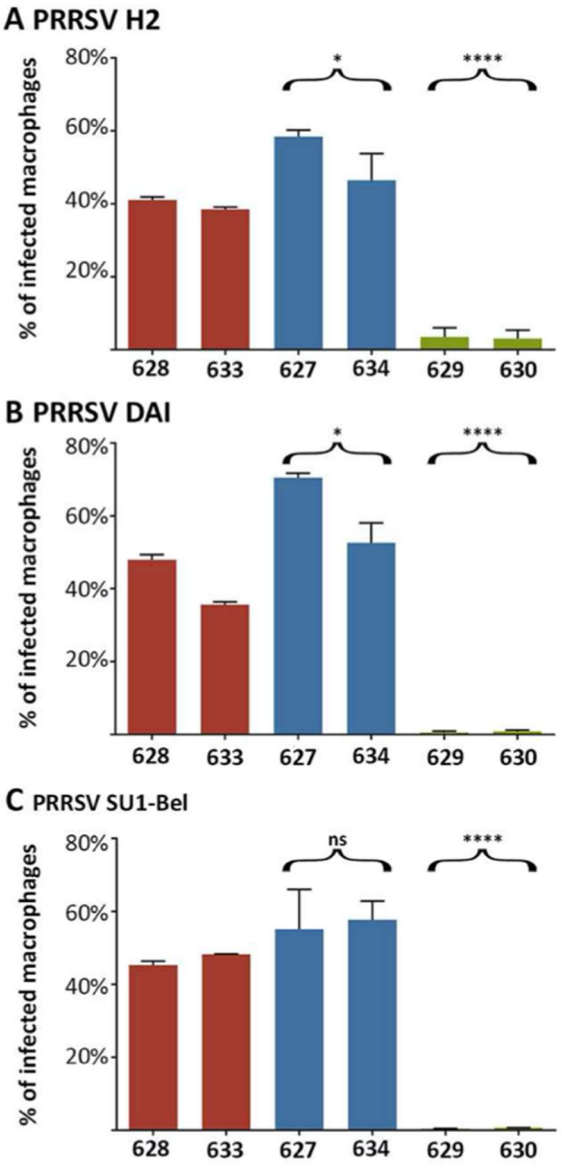
16. The diagram below illustrates the transcription and translation of a gene.
    a. Name the regions always removed from a primary mRNA transcript.

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



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 (one allele edited)

Green bars = homozygous (both alleles edited)

a. What are these three graphs measuring?

b. Describe the results and main findings.

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

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