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science**



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Real-Life Roslin Science:

Which Little Piggy?



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The Roslin Institute- Improving animal health and welfare



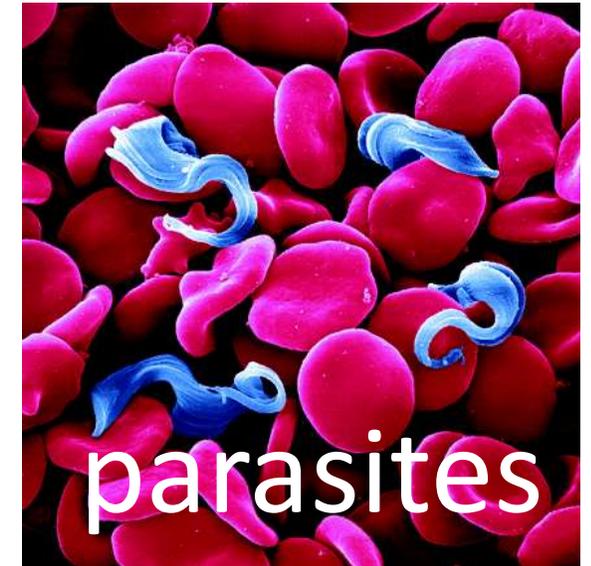
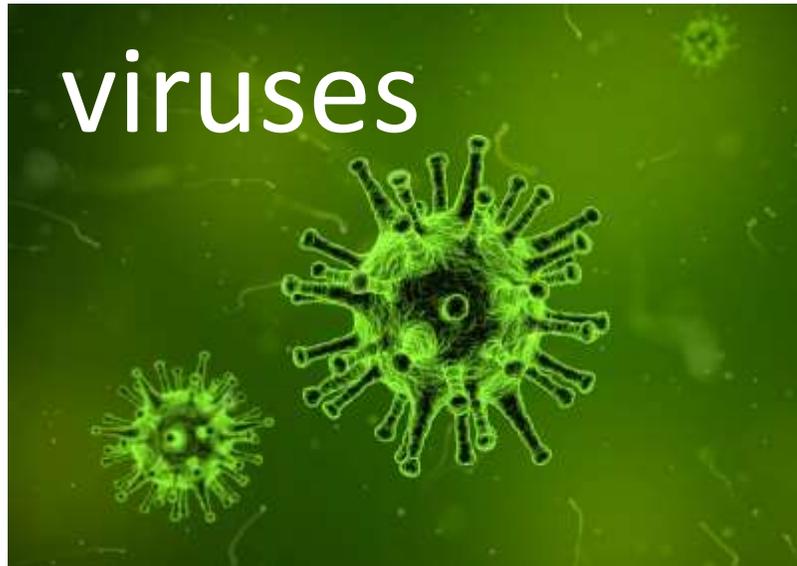
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Control of Infectious Diseases

Our scientists study...



...that infect animals, especially farm animal species

Our scientists are trying to solve a real-world problem

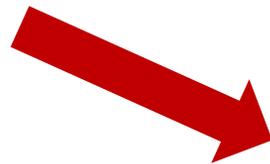


Respiratory disease (pneumonia) in young pigs



Fewer piglets born due to abortions and stillbirths

Porcine Respiratory and Reproductive Syndrome (PRRS)



Vaccines don't work against all strains



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Our scientists are trying to solve a real-world problem

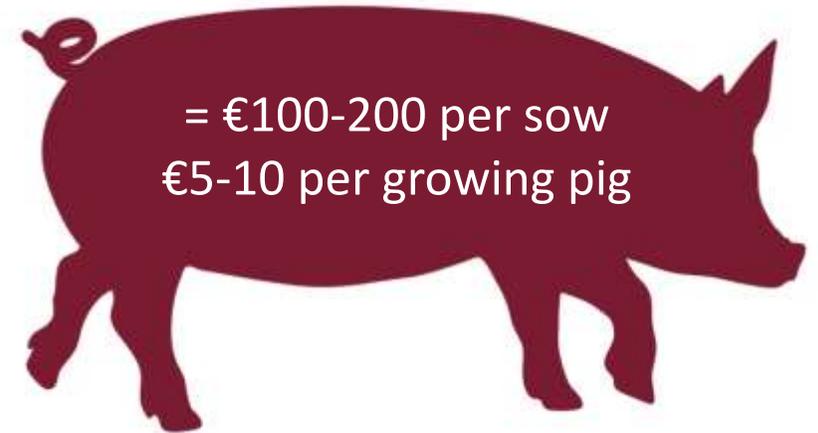


Porcine Respiratory and Reproductive Syndrome (PRRS)



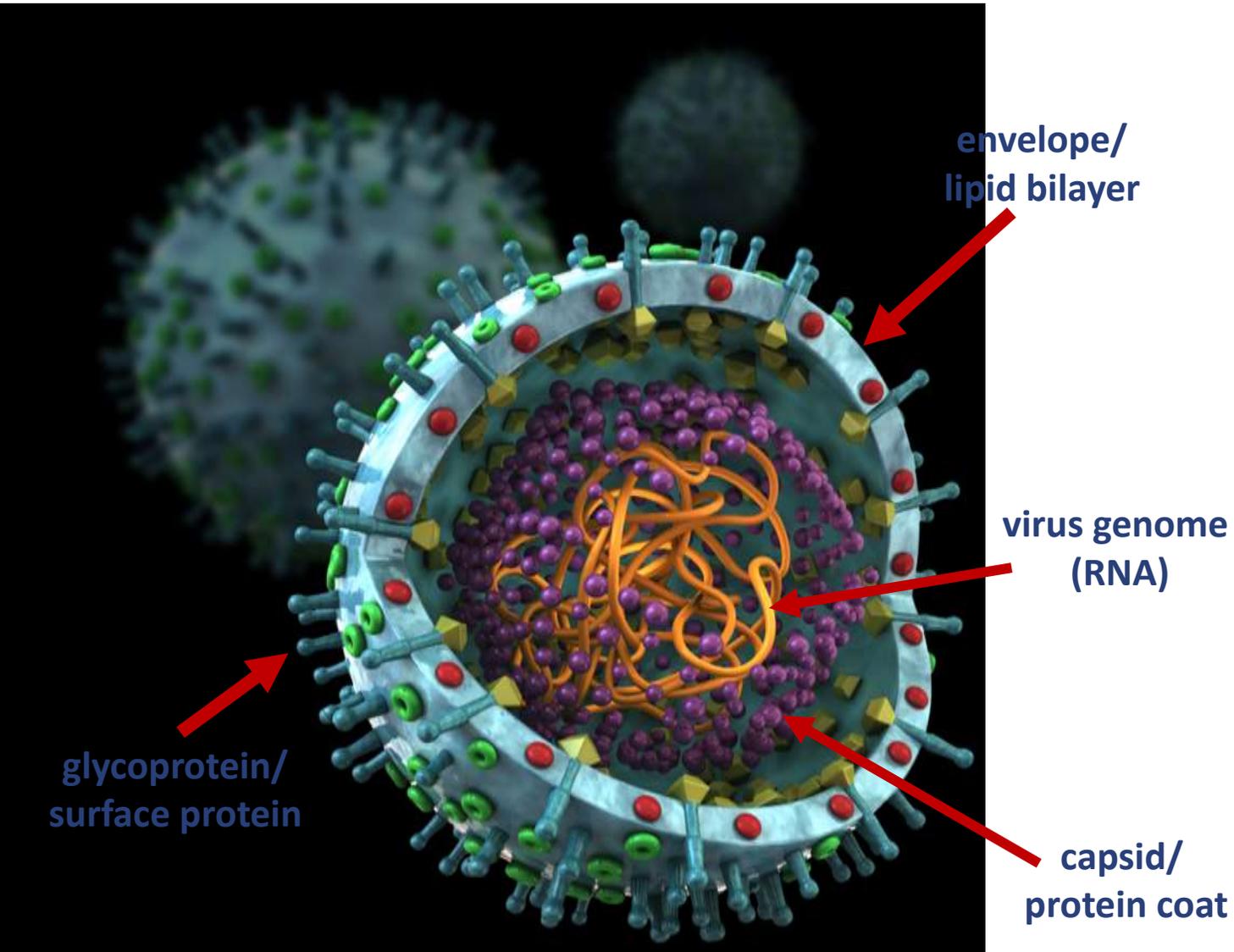
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Cost of PRRS in Europe per year =
€940 million to
€1.5 billion



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What causes PRRS?



Porcine Respiratory and Reproductive Syndrome Virus (PRRSV)

- Infects pigs
- Worldwide spread
- Two virus species and many subtypes
- Infects one pig cell type = macrophages



Meet Christine

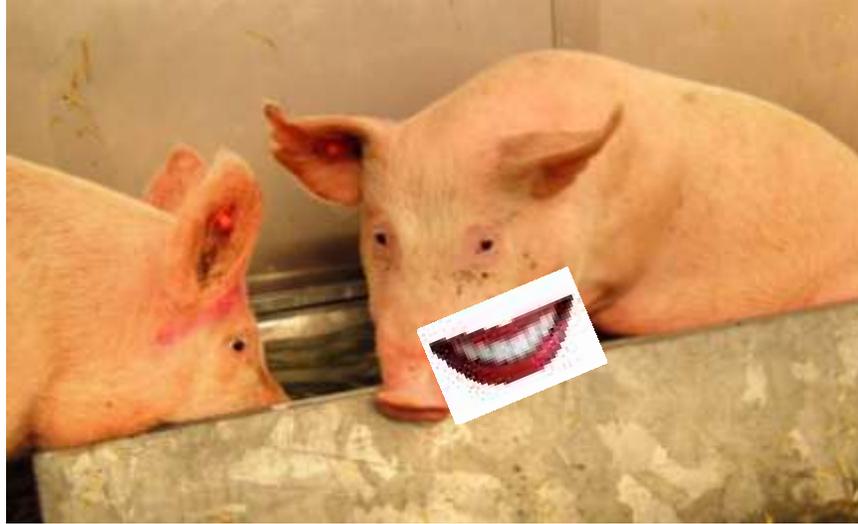


Dr Christine Tait-Burkard



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Christine and her team are trying to protect pigs from PRRSV...

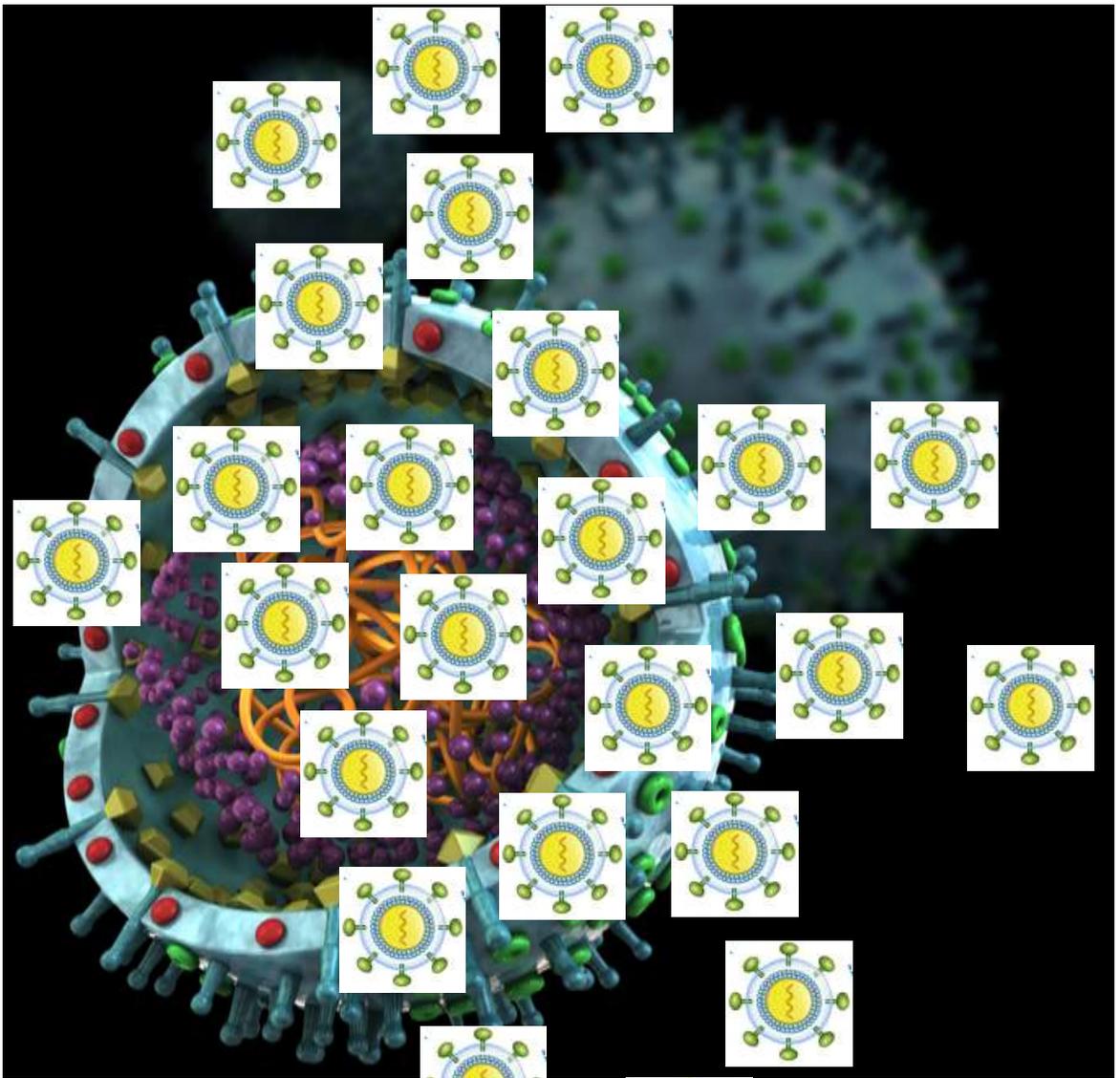
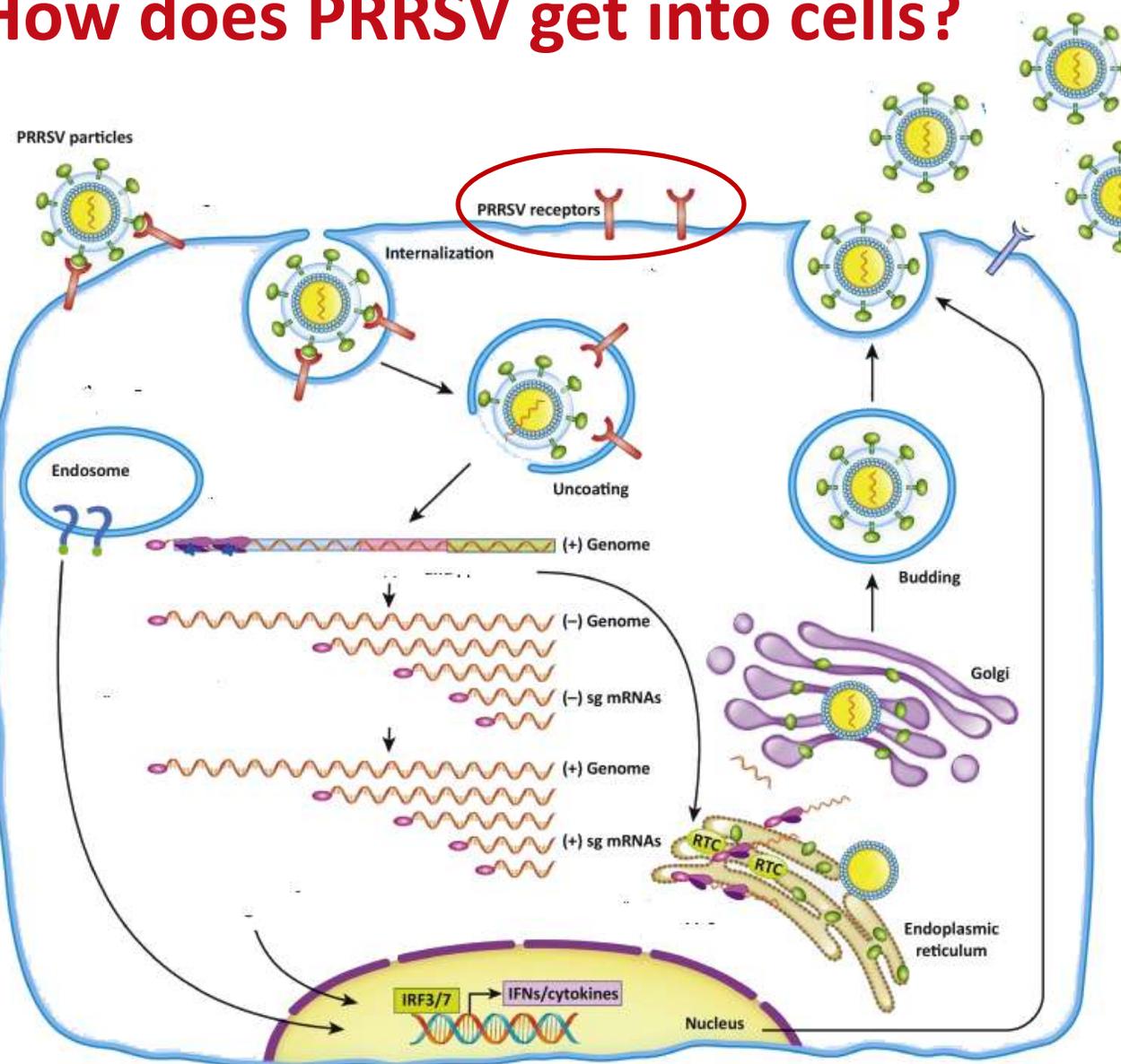


...by blocking PRRS virus entry into pig cells.

Viruses that can't get inside pig cells can't cause disease.

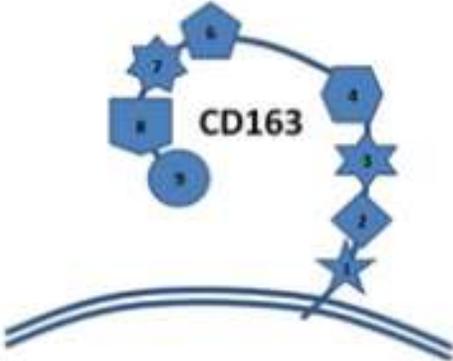


How does PRRSV get into cells?



Trends in Microbiology

How does PRRSV get inside cells?



How can we change the pig receptor protein?



How can we change the pig receptor protein?

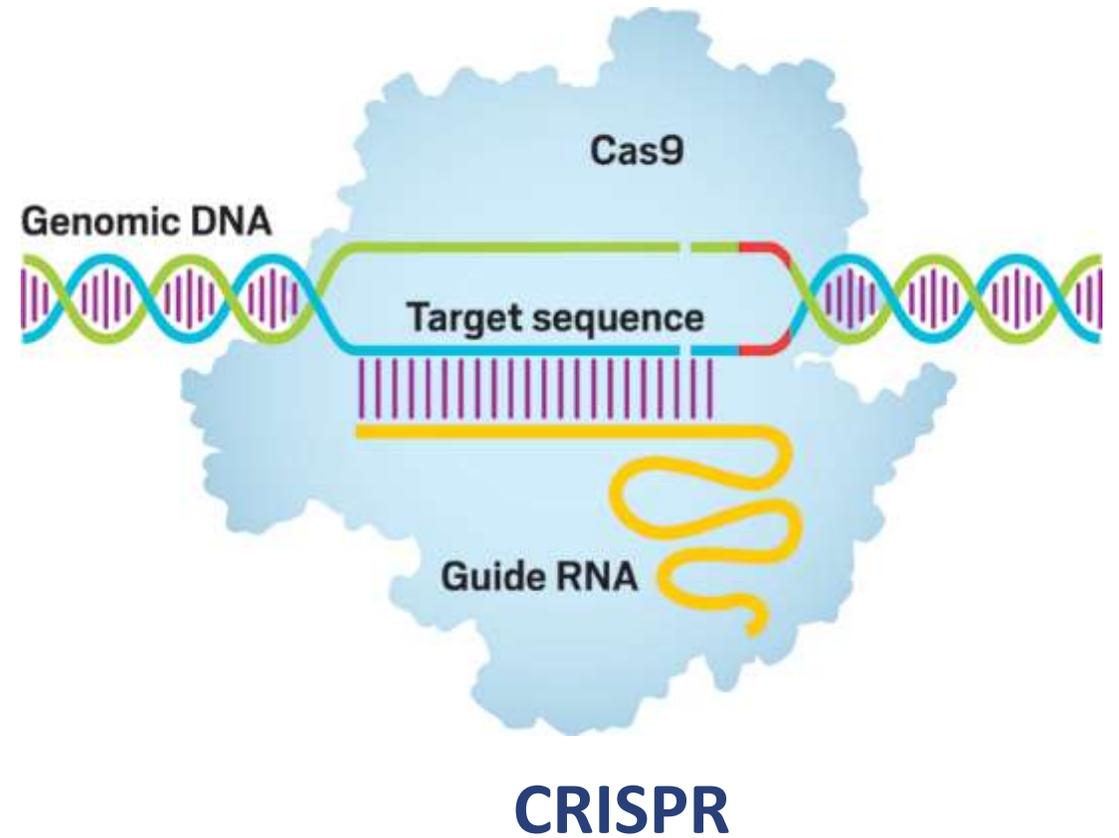
By changing the receptor gene using a technique called
CRISPR genome editing



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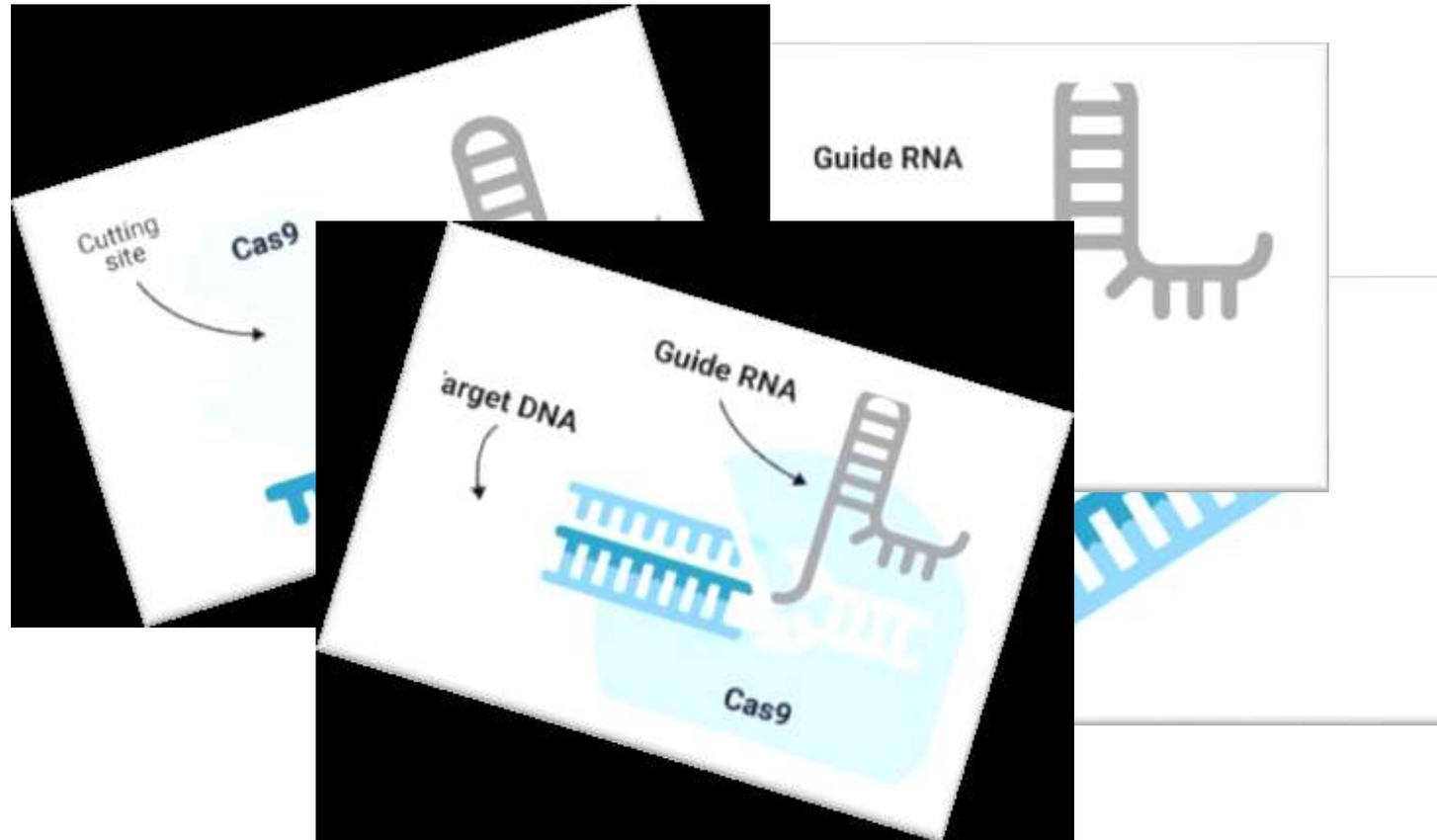
What is genome editing?



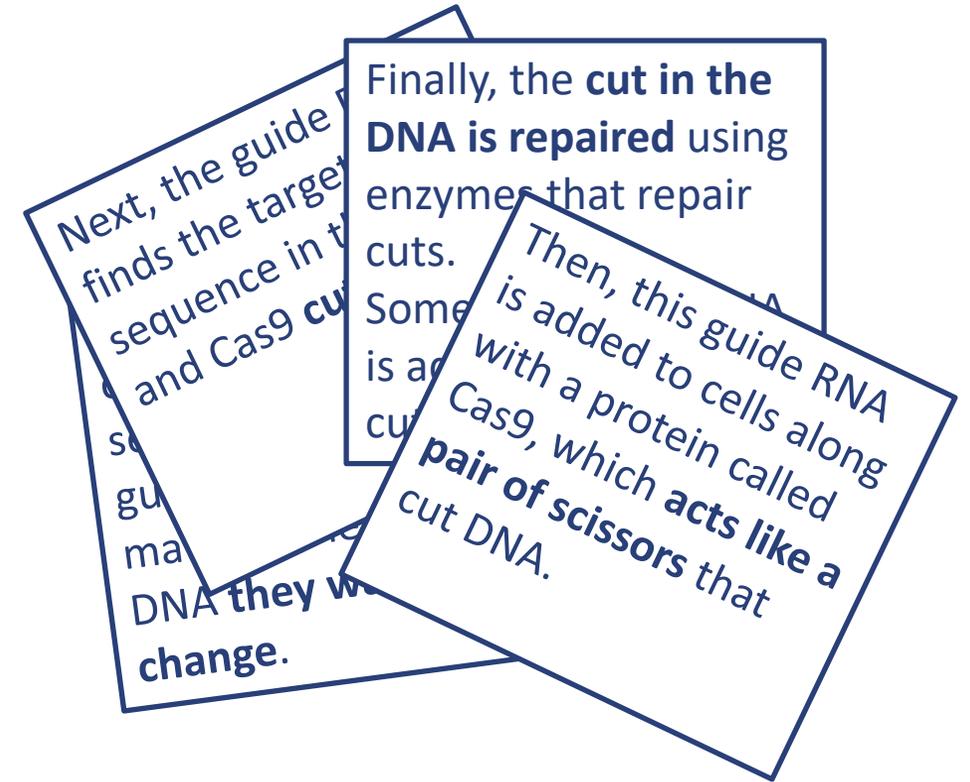
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How does CRISPR work?



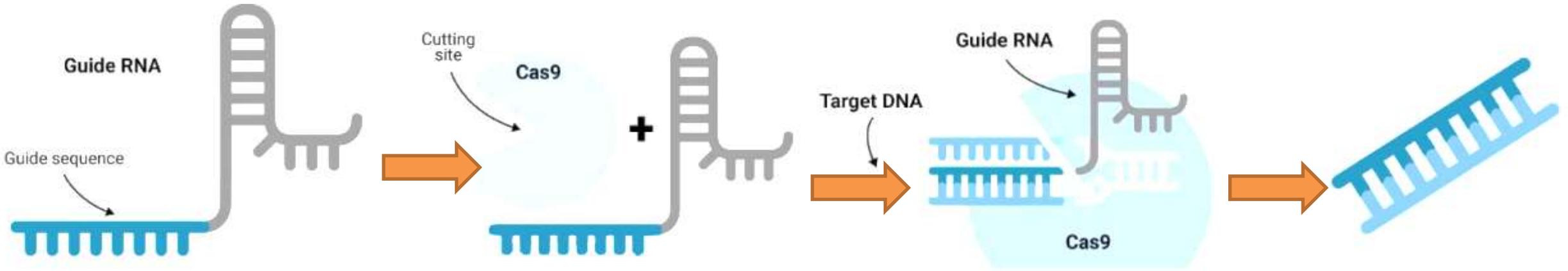
First order the pictures



Then match the descriptions



How does CRISPR work?



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.

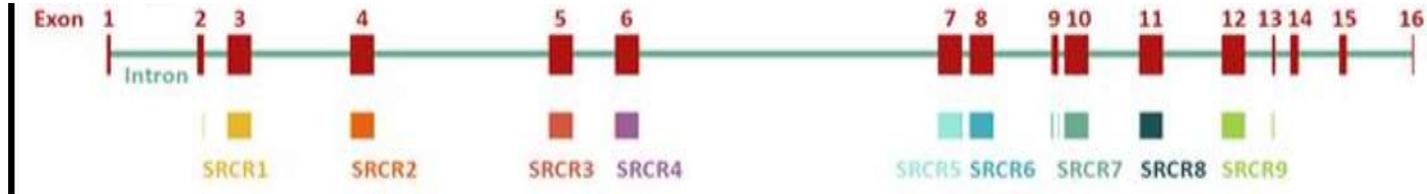
Next, the guide RNA finds the target DNA sequence in the cells and Cas9 **cuts it out**.

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

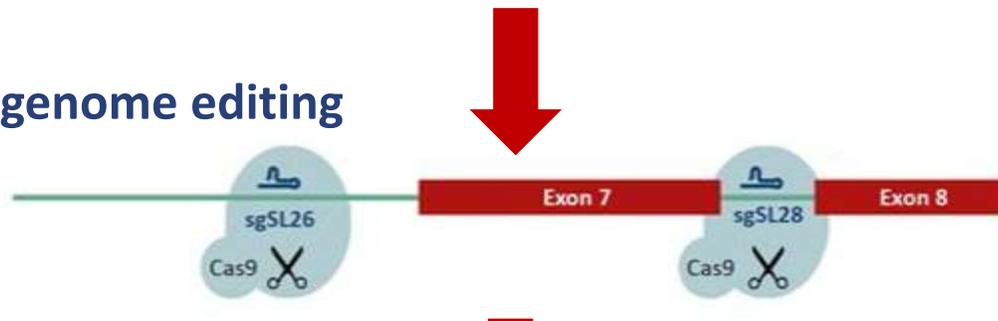


Using genome editing to block PRRSV infection

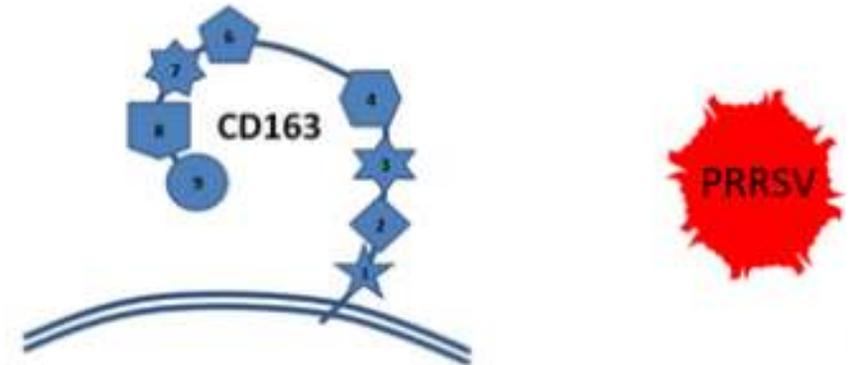
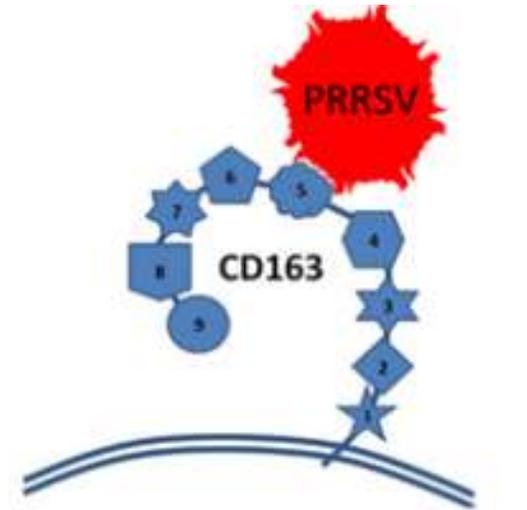
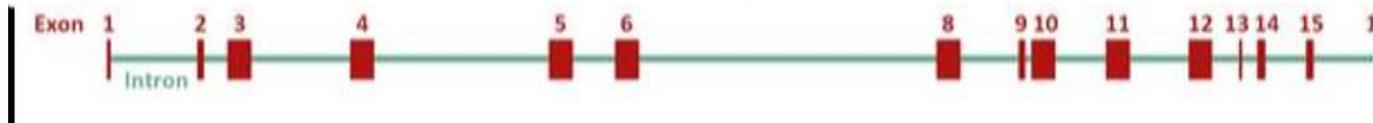
CD163 gene



CRISPR-Cas9 genome editing



CD163 edited gene

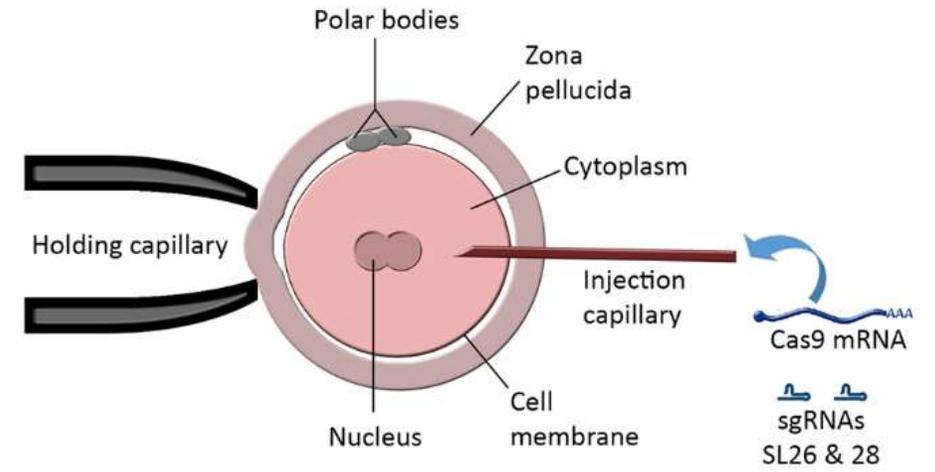
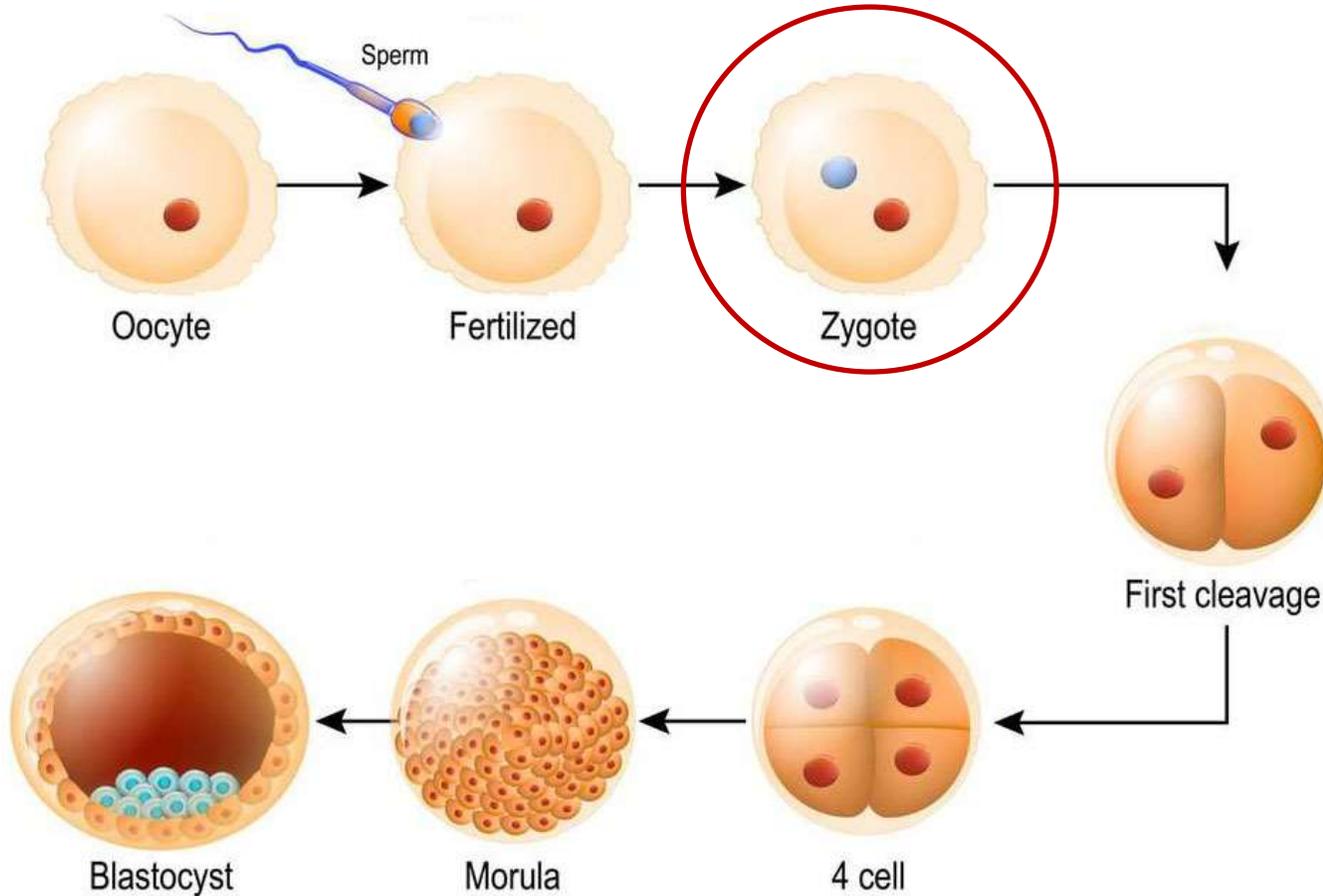




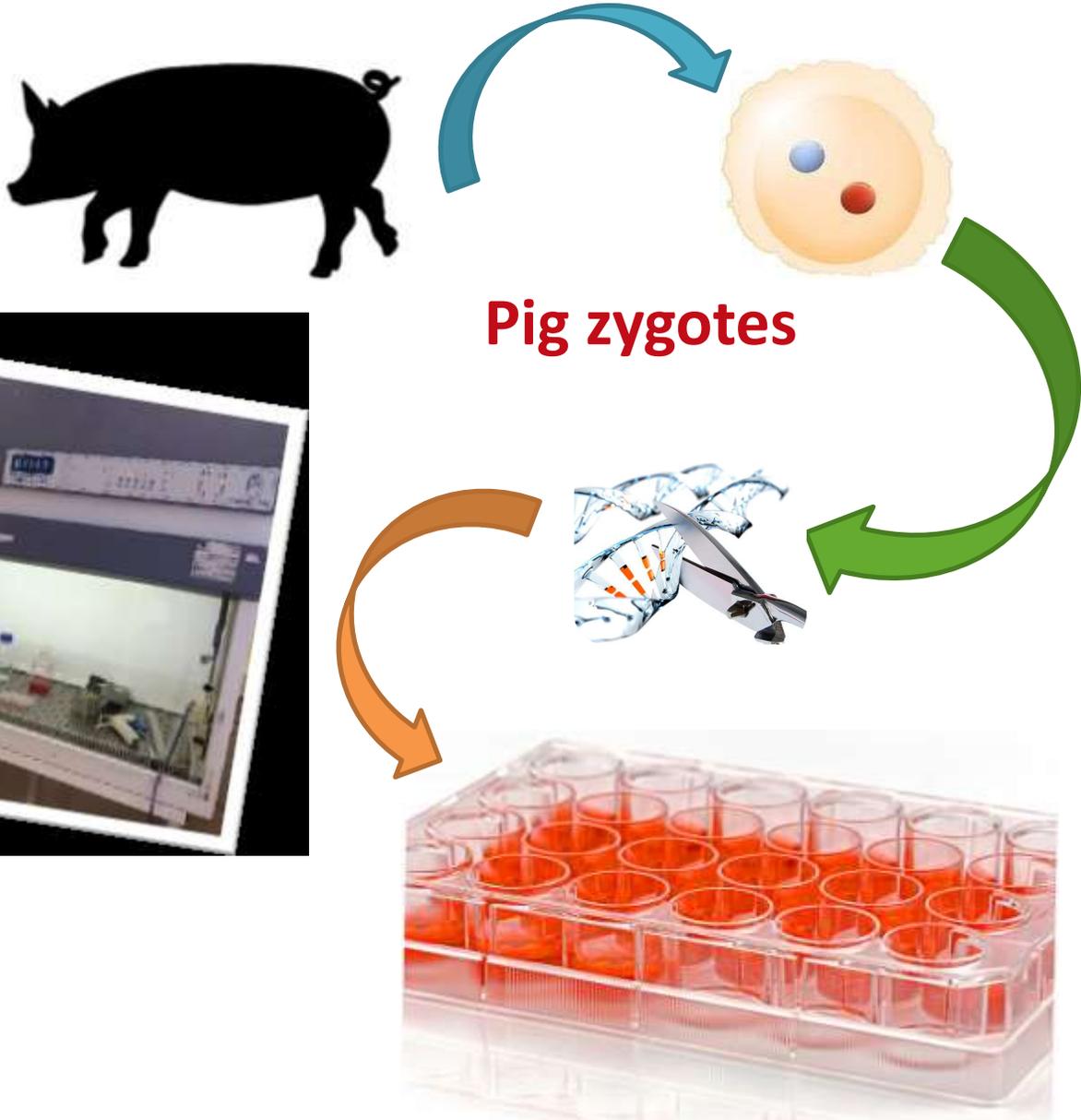
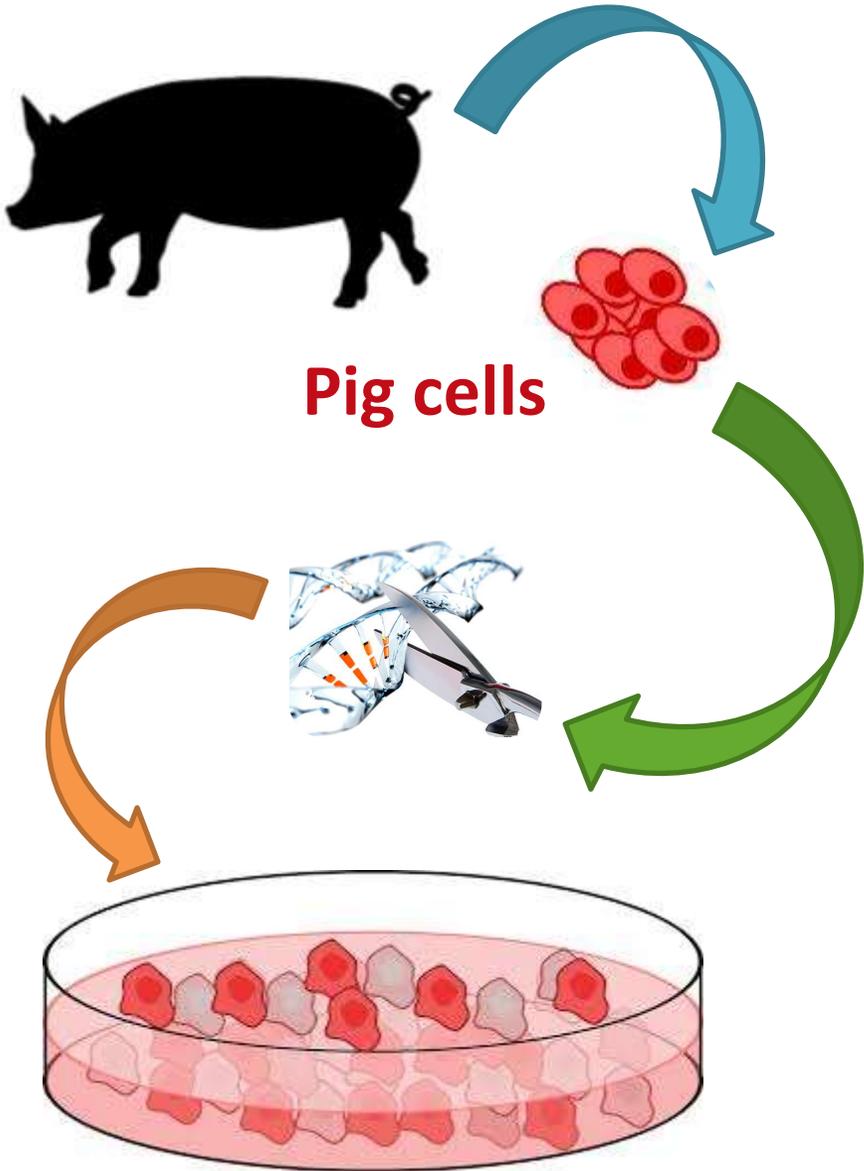
How are Christine and her team doing this?



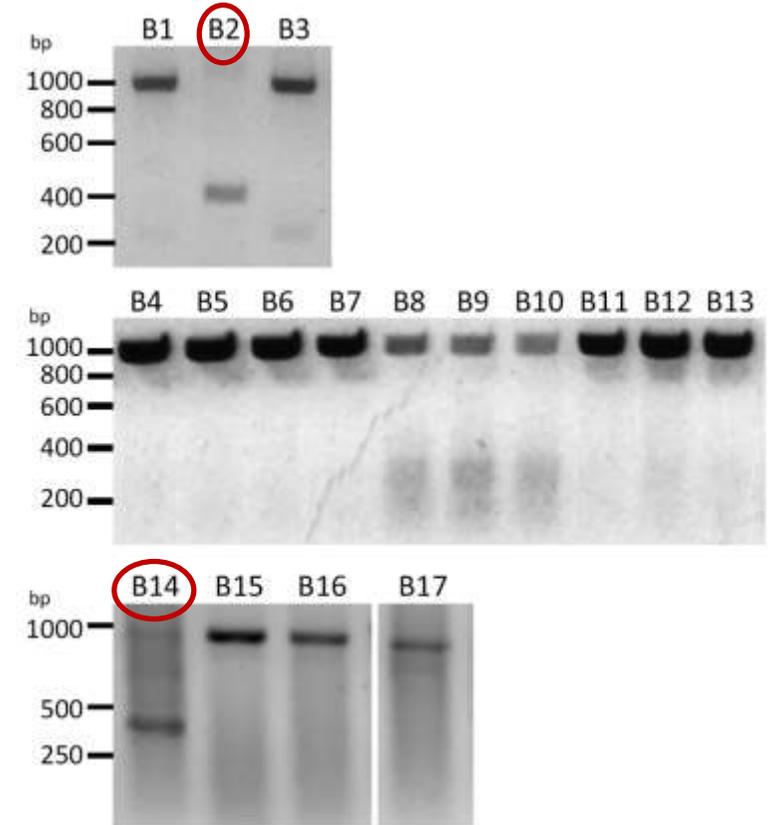
How can we edit all of the cells in our pigs?



First the team tested the CRISPR reagents in vitro...



DNA from the genome edited cells was checked for the edit



This is proof that the CRISPR technique works to edit pig CD163!



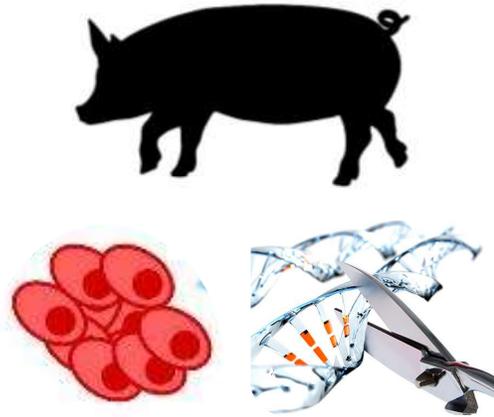
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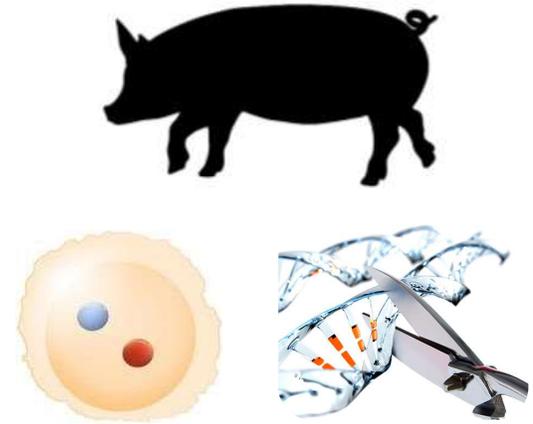
So far...Christine and her team have...



Designed the reagents
for the CRISPR process



Tested the CRISPR
reagents in pig cells



Tested the CRISPR
reagents in pig zygotes

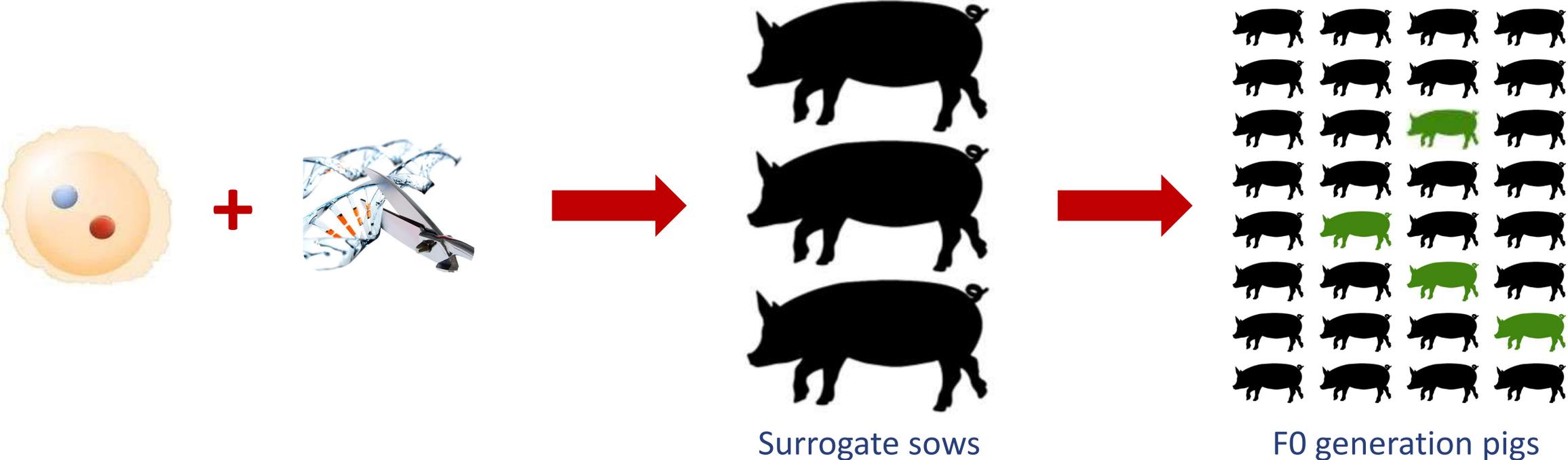
What would you do next?



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Creating edited pigs



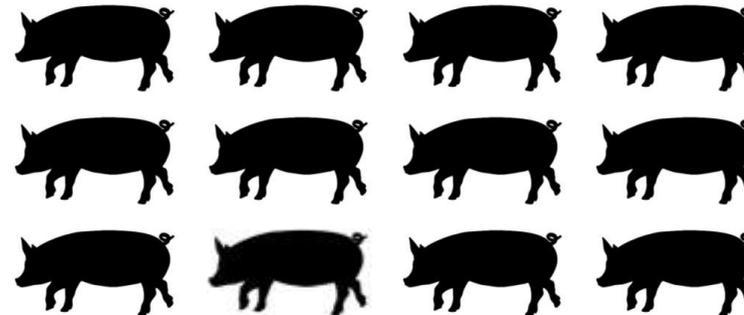
But, DNA results showed that the edited pigs each only have **one copy** of the edited gene...

Creating edited pigs – the next generation

F0 edited pigs



F1 generation piglets



You will be testing DNA from four of the F1 piglets to assess their genotype



Safety first!



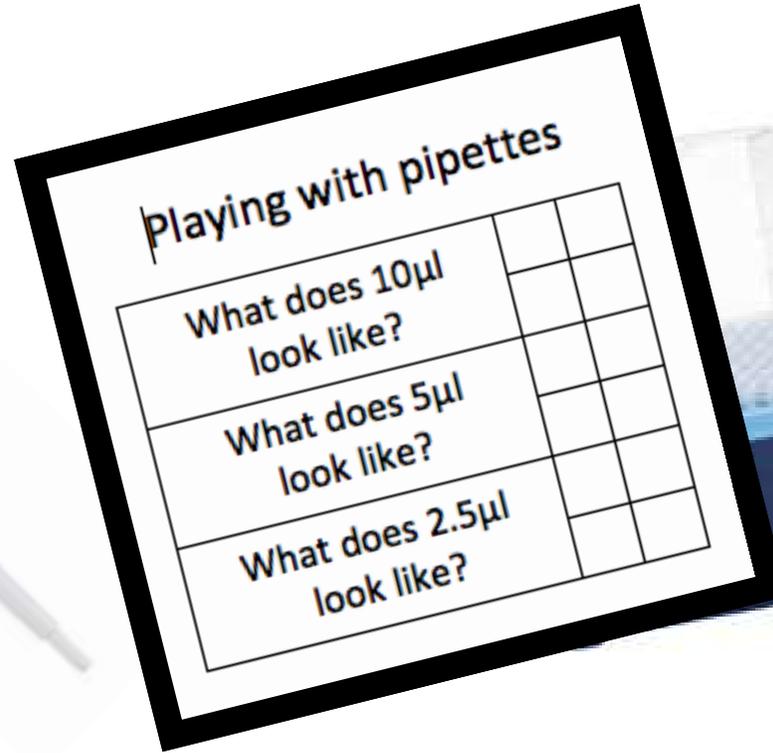
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Using Micropipettes



P20 pipette



Tips



What is the genotype of our F1 edited piglets?

You are going to get **DNA samples** from four piglets...



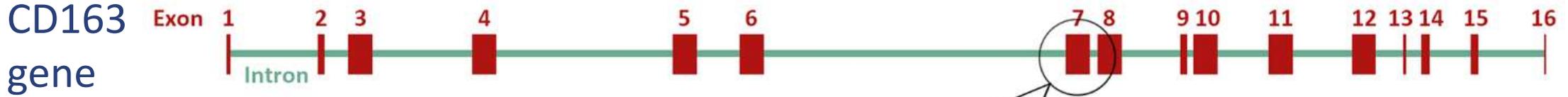
...to identify their **genotype**.



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How can we tell if the CD163 gene has been edited?



Editing sites



DNA Analysis



Unedited = longer DNA fragment

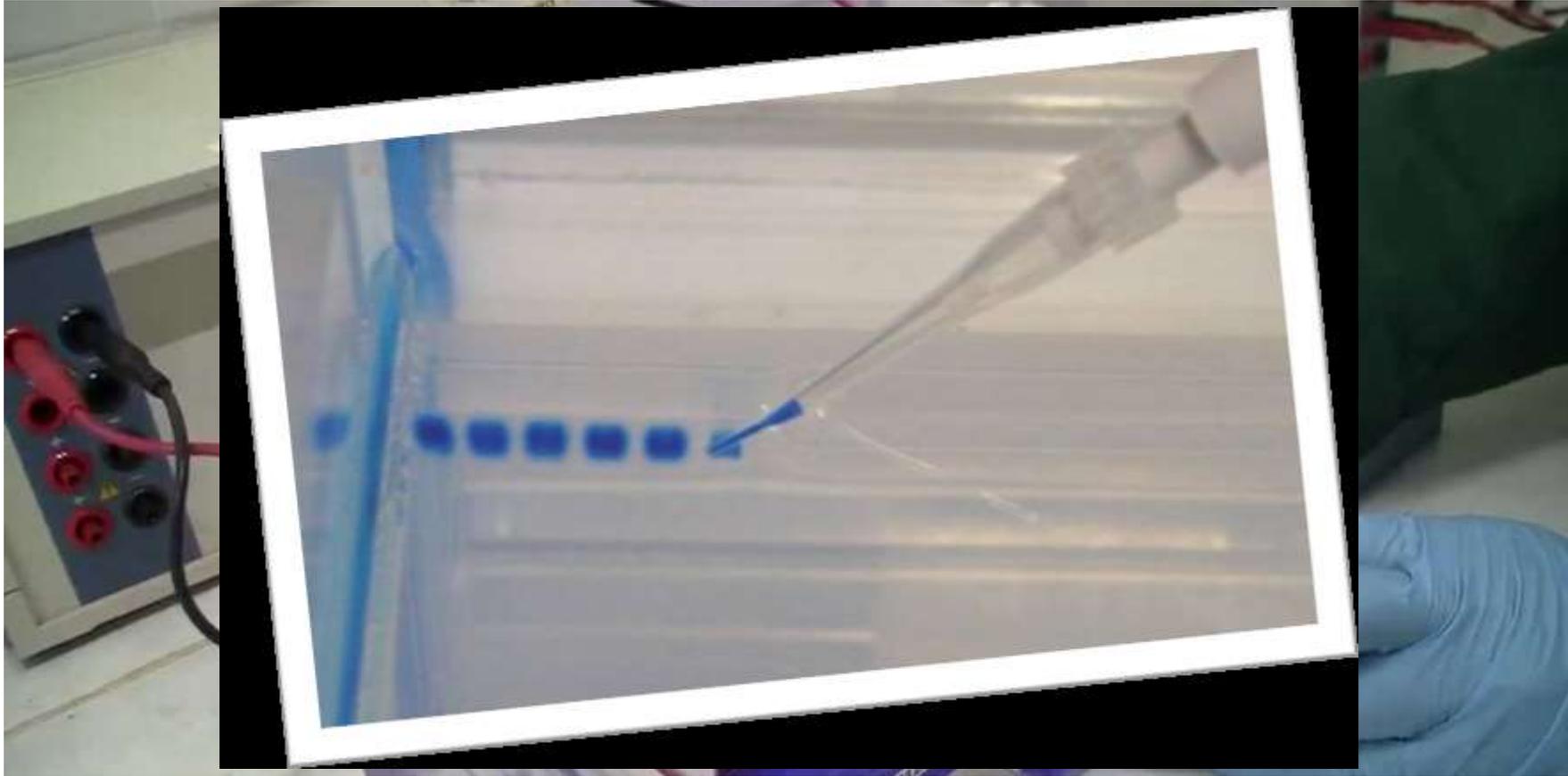
Edited = shorter DNA fragment



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How can we see the lengths of the DNA fragments?



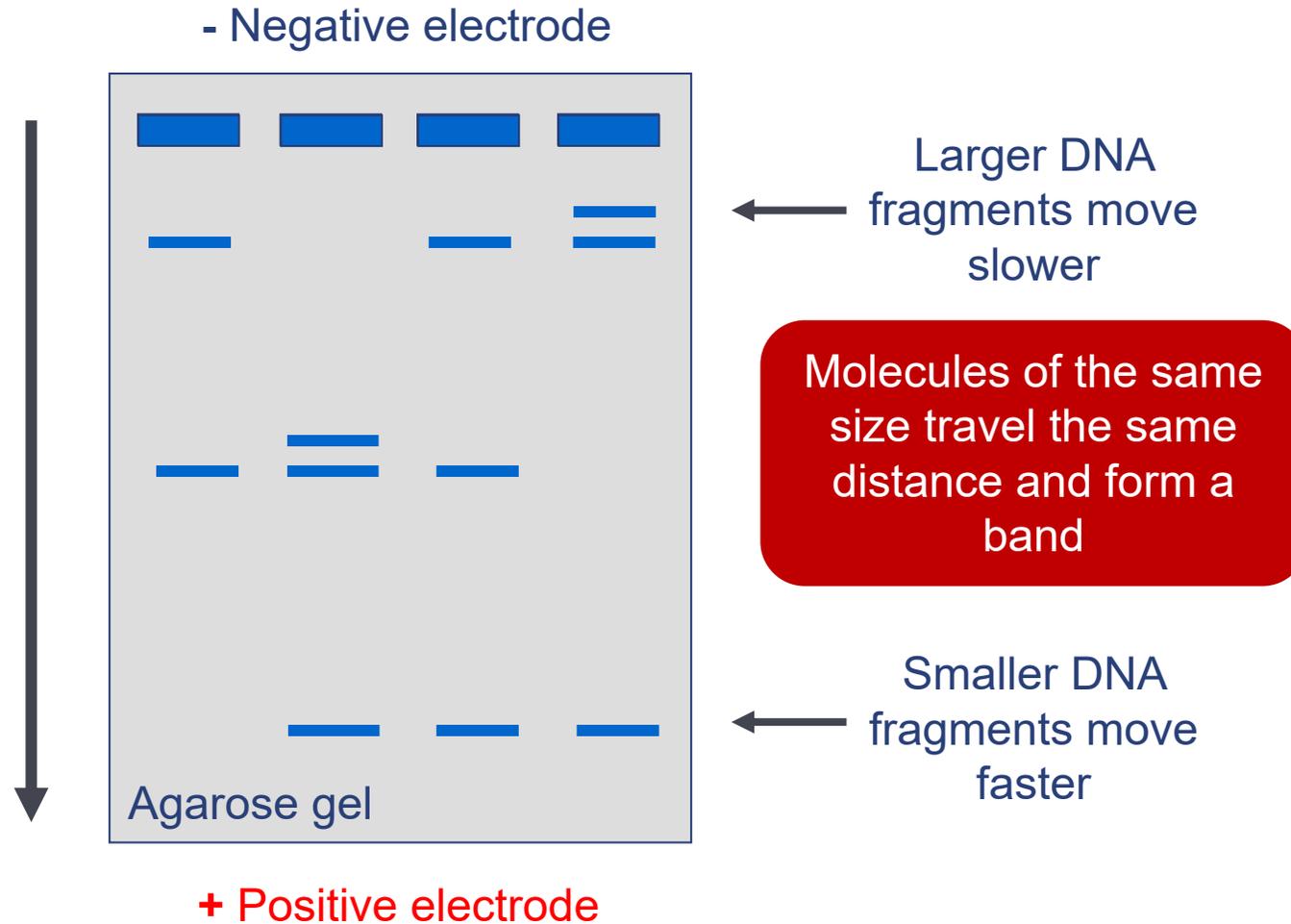
Gel Electrophoresis



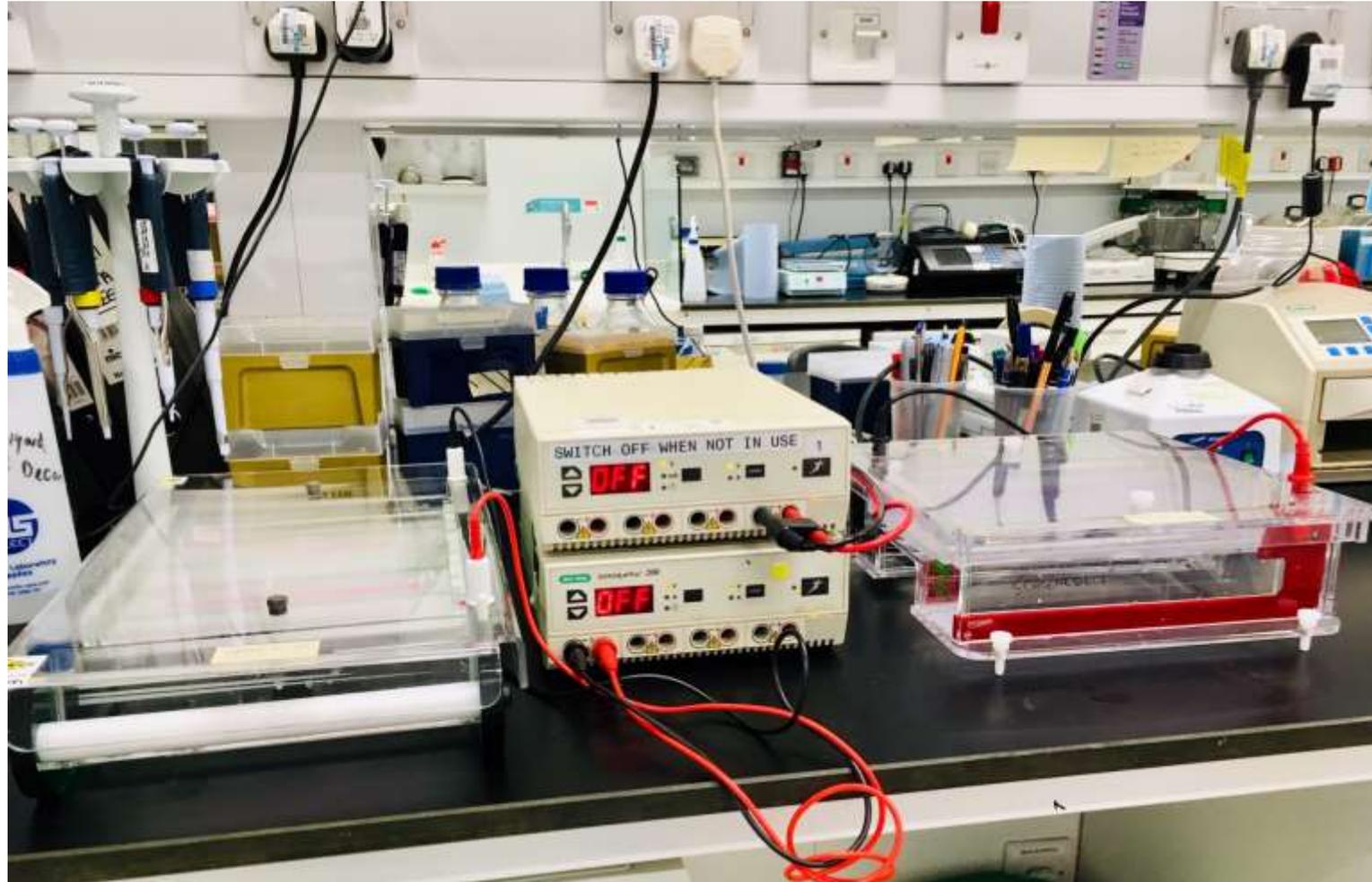
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Gel Electrophoresis of DNA



Running DNA samples in the lab



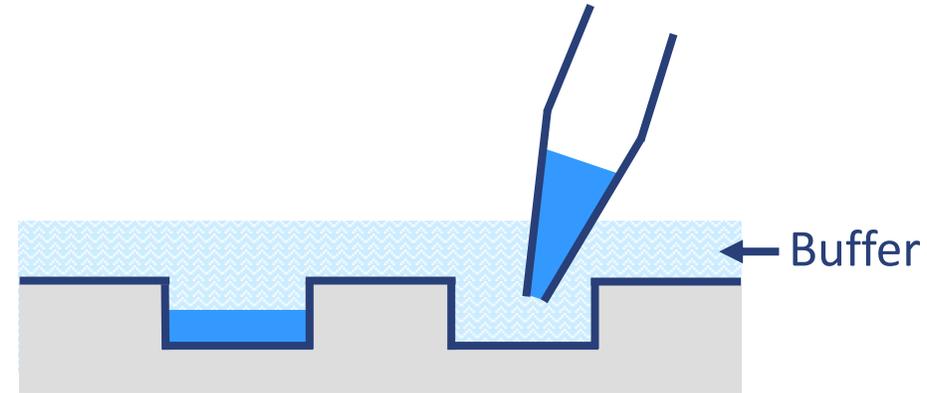
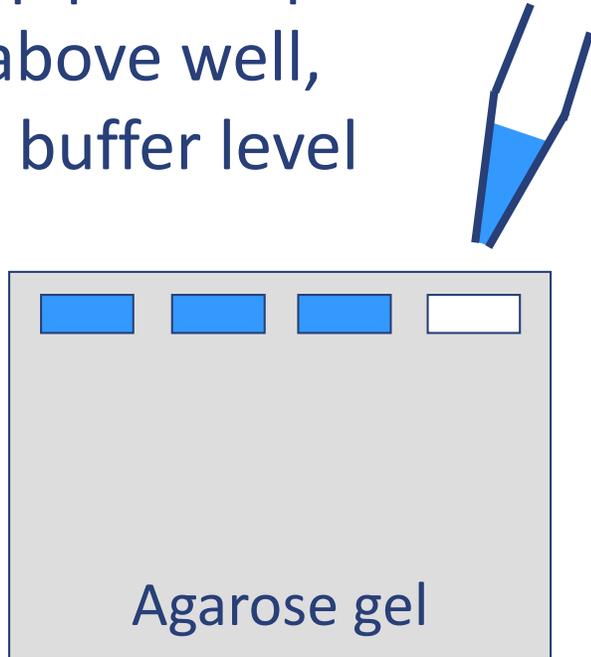
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Loading your samples onto an agarose gel



Hold pipette tip
just above well,
below buffer level



Be careful not to pierce
the bottom of the well
with your pipette tip!

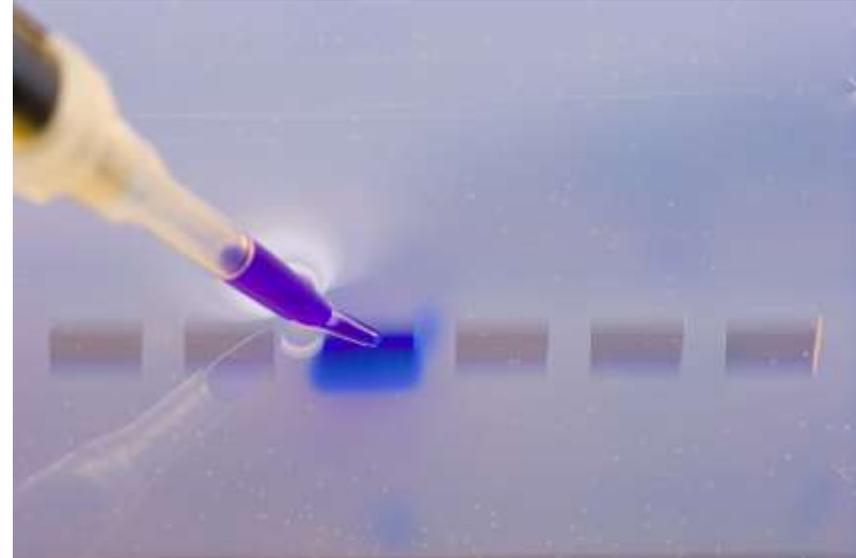
N.B. Only push down to the 1st stop to fill the well with the DNA sample



Have a go with a practice gel!



Load **10 μ l practice dye** (tube marked **P**) into each well.



N.B. Only push down to the 1st stop to fill the well with the DNA sample



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Centrifuge your piglet DNA samples



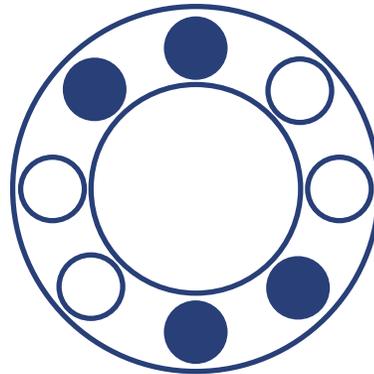
Open the centrifuge



Put in the tubes, make sure they are balanced!



Close the centrifuge, turn on for five seconds and then turn off



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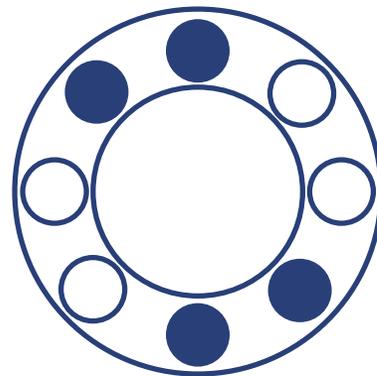
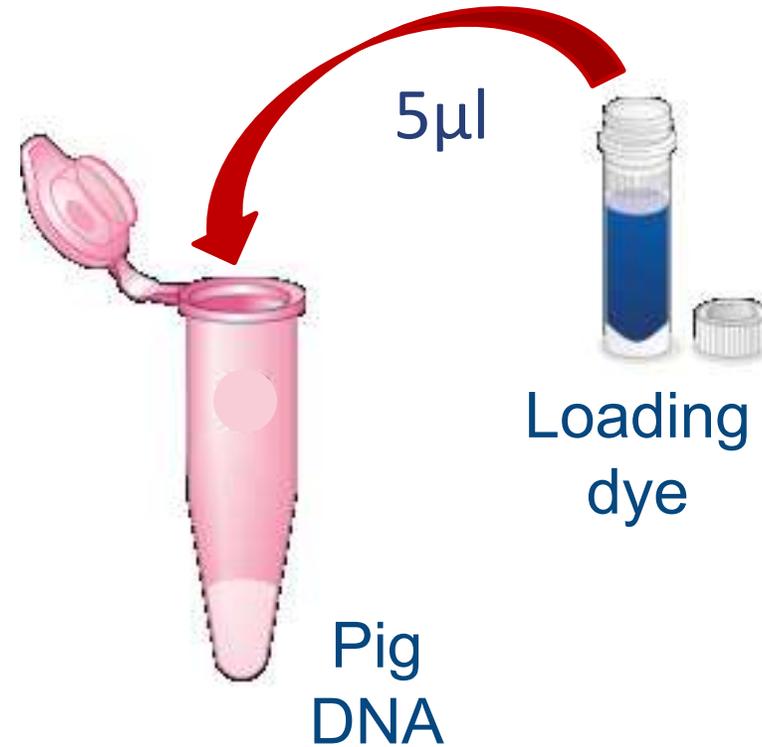
Prepare the DNA for analysis



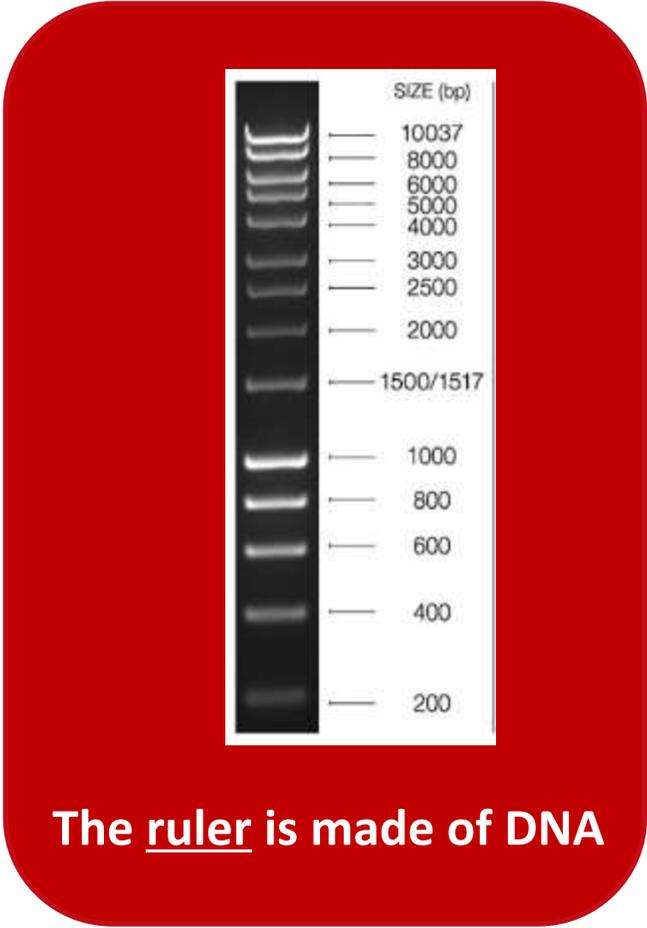
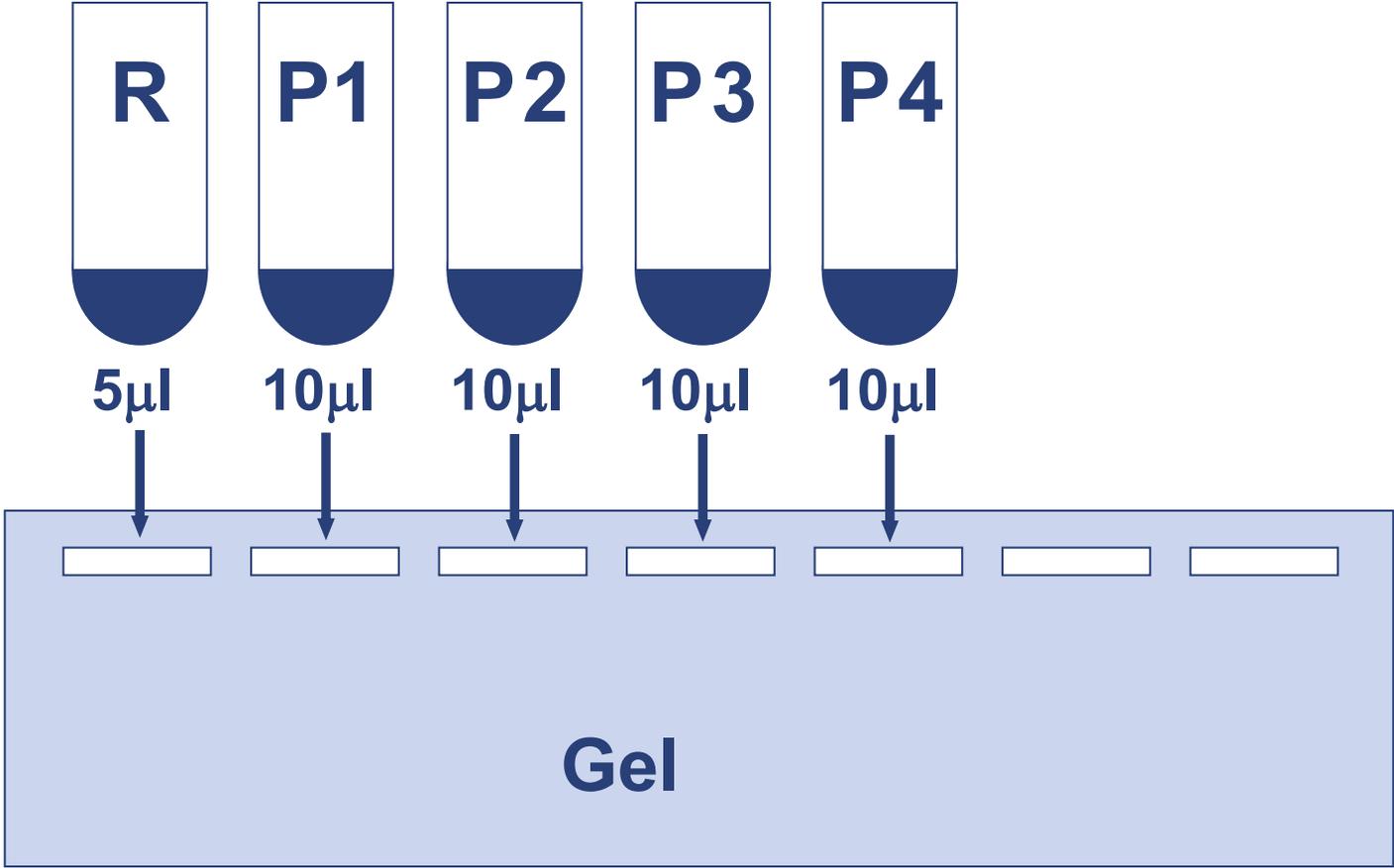
1. Add **5 μ l blue loading dye** (tube marked LD) to each DNA sample #1-4

2. **Change the pipette tip** for each sample

3. Vortex tubes, then centrifuge again for a few seconds (**make sure they are balanced**)



Gel loading plan



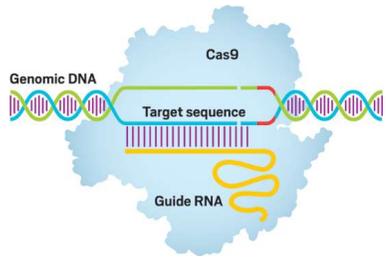
The ruler is made of DNA



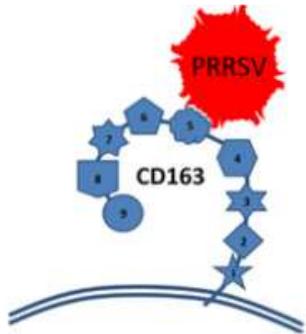
Meet the Scientists



What have you done so far?



Learned about **CRISPR-Cas9 genome editing** and how it can be used to **change genes** and their protein products

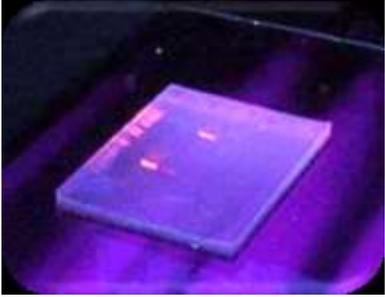


Found out about a gene (CD163) and its protein product that could be changed to make pigs resistant to PRRSV



Loaded DNA samples from 4 piglets onto an agarose gel

What will you do next?



Analyse the DNA to work out the genotype of the 4 pigs



Think and discuss the next steps



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Recap – what do we expect to see on our gels?

CD163 gene



Editing sites



DNA Analysis



Unedited = longer DNA fragment

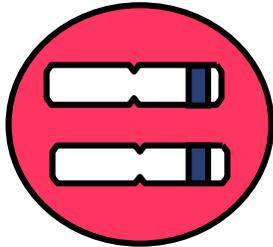
Edited = shorter DNA fragment



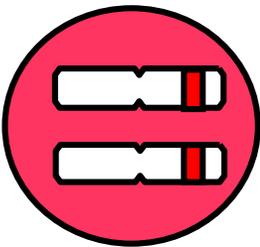
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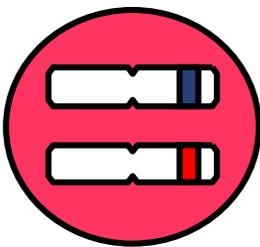
What do you expect to see on the gel?



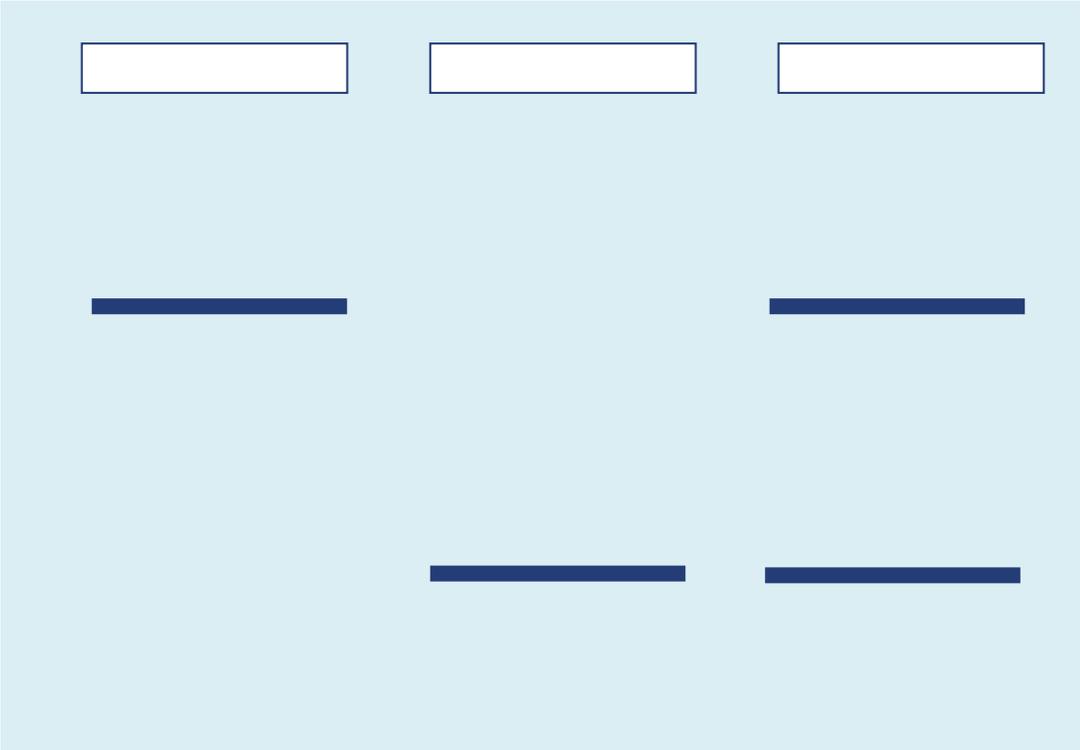
RR



rr



Rr



longer (not edited)
DNA fragment

shorter (edited)
DNA fragment

Safety check!



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Viewing the DNA



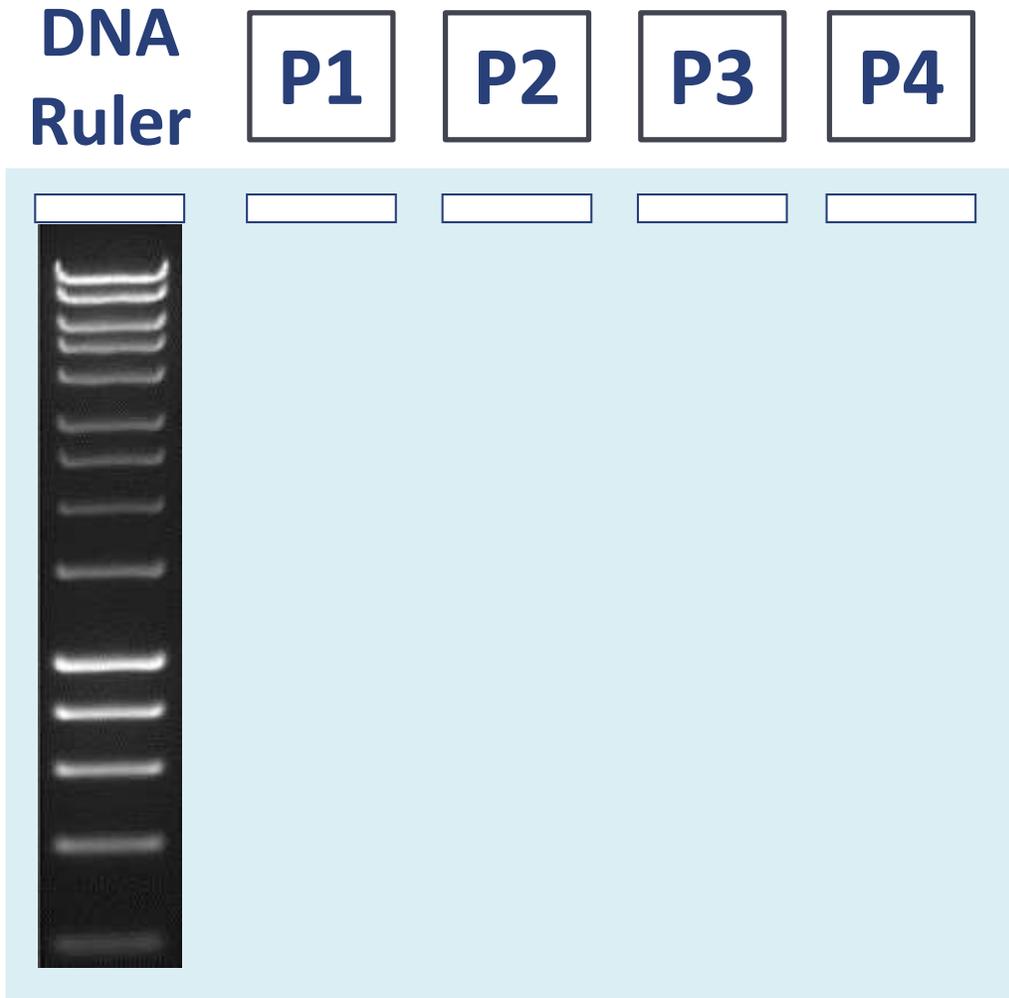
A DNA stain was added to the agarose when the gels were made



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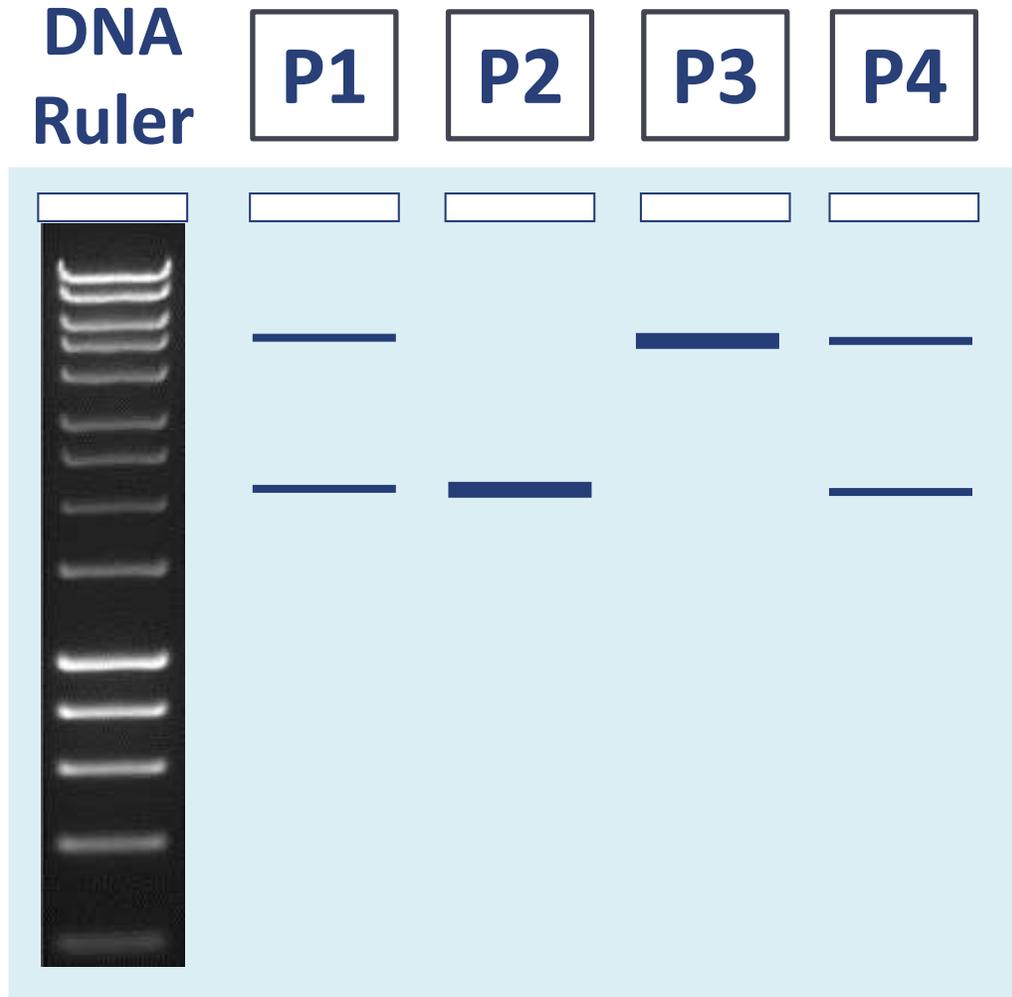
DNA Analysis



View the DNA fragments and draw the bands you can see.



DNA Analysis



Longer (not edited) fragment

Shorter (edited) fragment



Pedigree Analysis



R = wildtype receptor gene (CD163)
r = edited receptor gene (CD163)

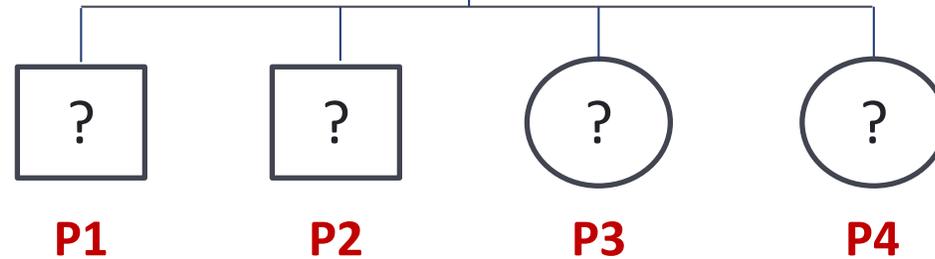
 = Male

 = Female

F0:



F1:



- Use the results from your gel to add the genotype of each pig to the pedigree
- Can you work out the genotypes of the parents (F0)?



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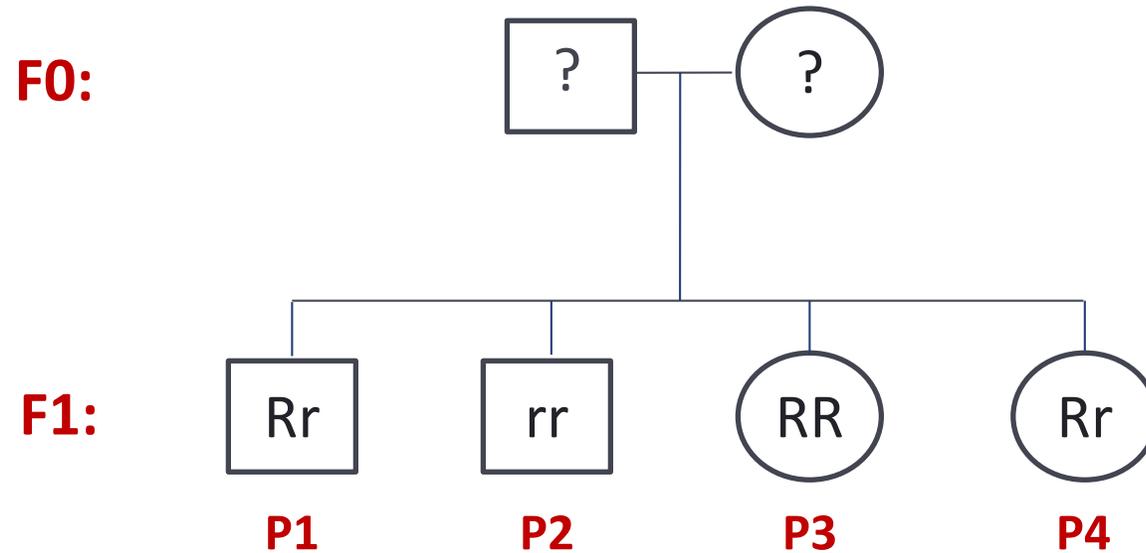
Pedigree Analysis



R = wildtype receptor gene (CD163)
r = edited receptor gene (CD163)

 = Male

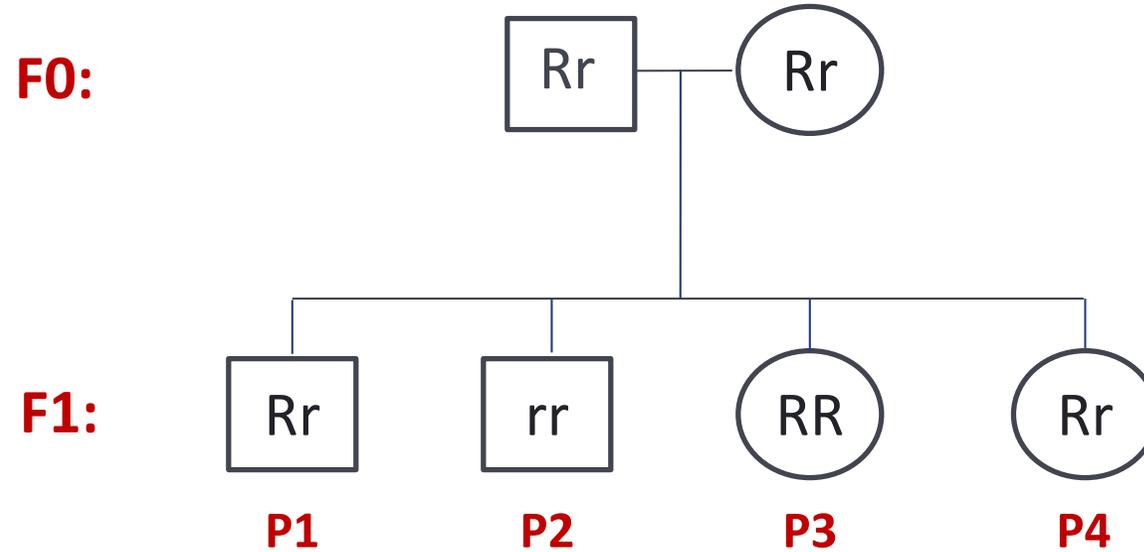
 = Female



- Use the results from your gel to add the genotype of each pig to the pedigree.
- Can you work out the genotypes of the parents (F0)?



Pedigree Analysis

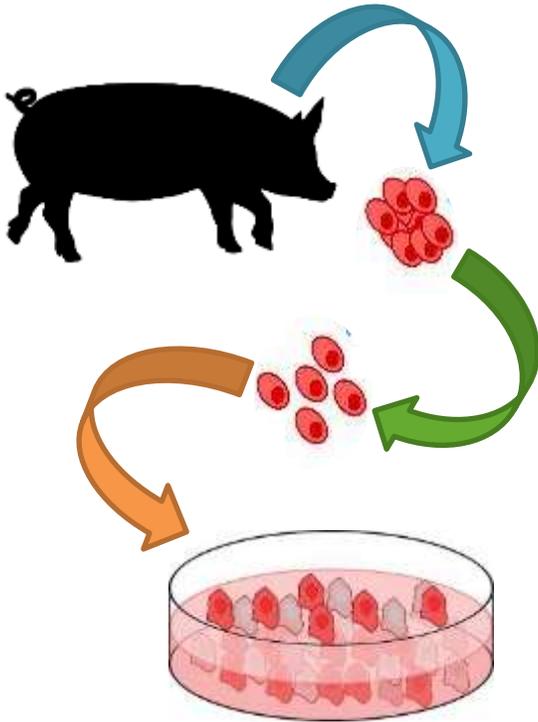


- 1) Which pigs might be resistant to PRRSV?
- 2) What is the genotype of the parents (F0)?



Further Investigations

How would you test if the **rr** pig is resistant to PRRSV?



1. Isolate macrophage cells from pigs



2. Infect cells with PRRSV

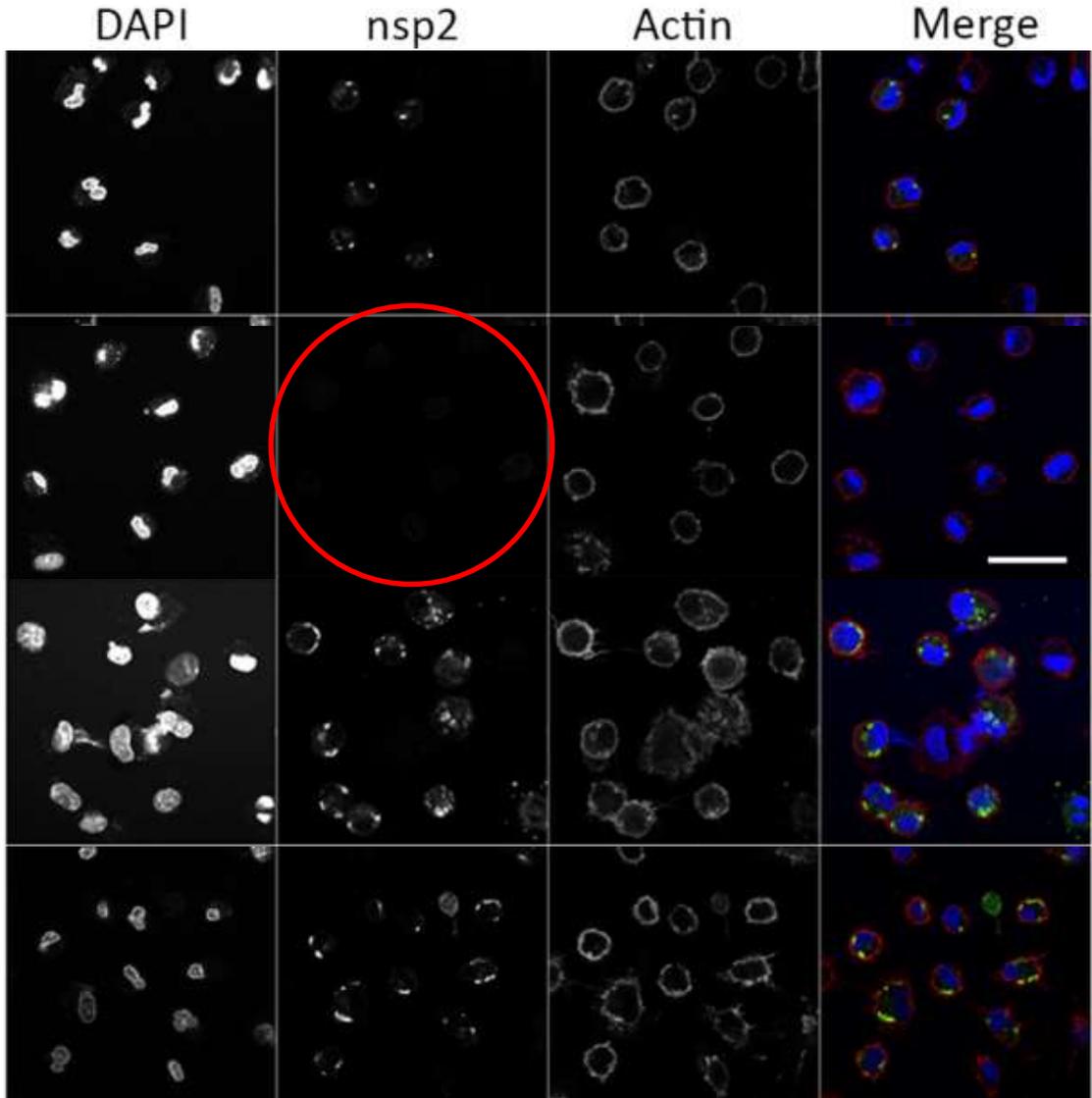
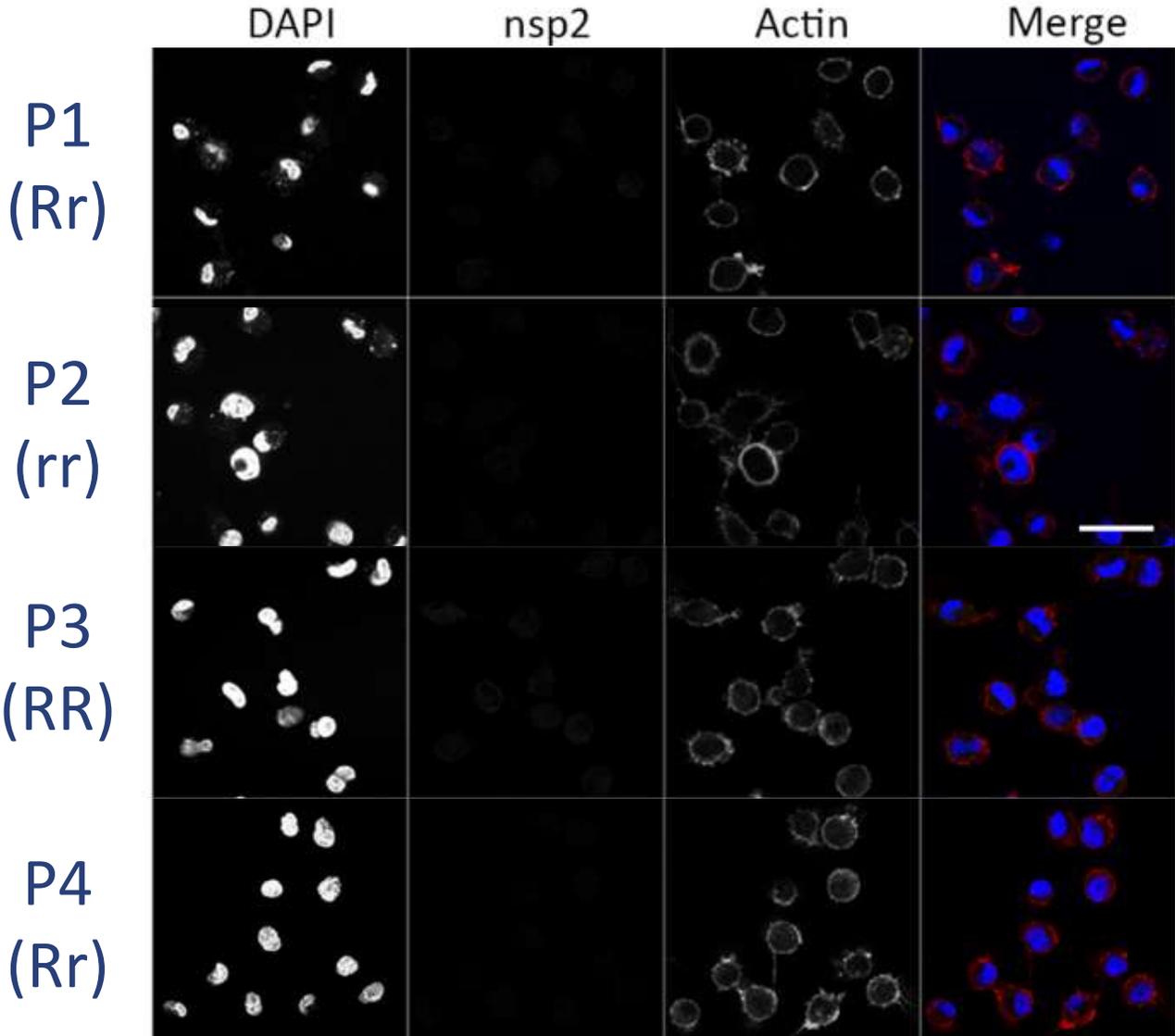


3. Use microscopy to see the effect of the virus

Microscopy results

Uninfected

Infected

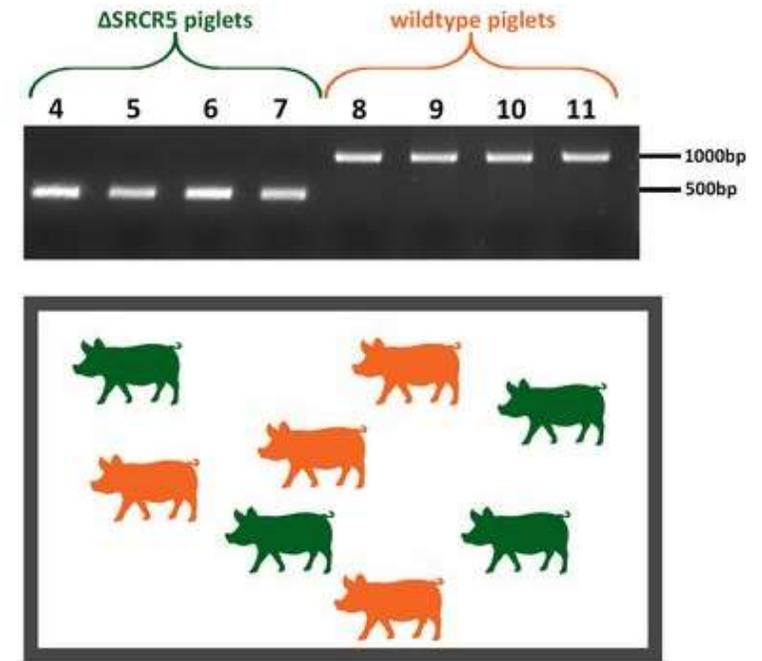


What's the next step after cells?

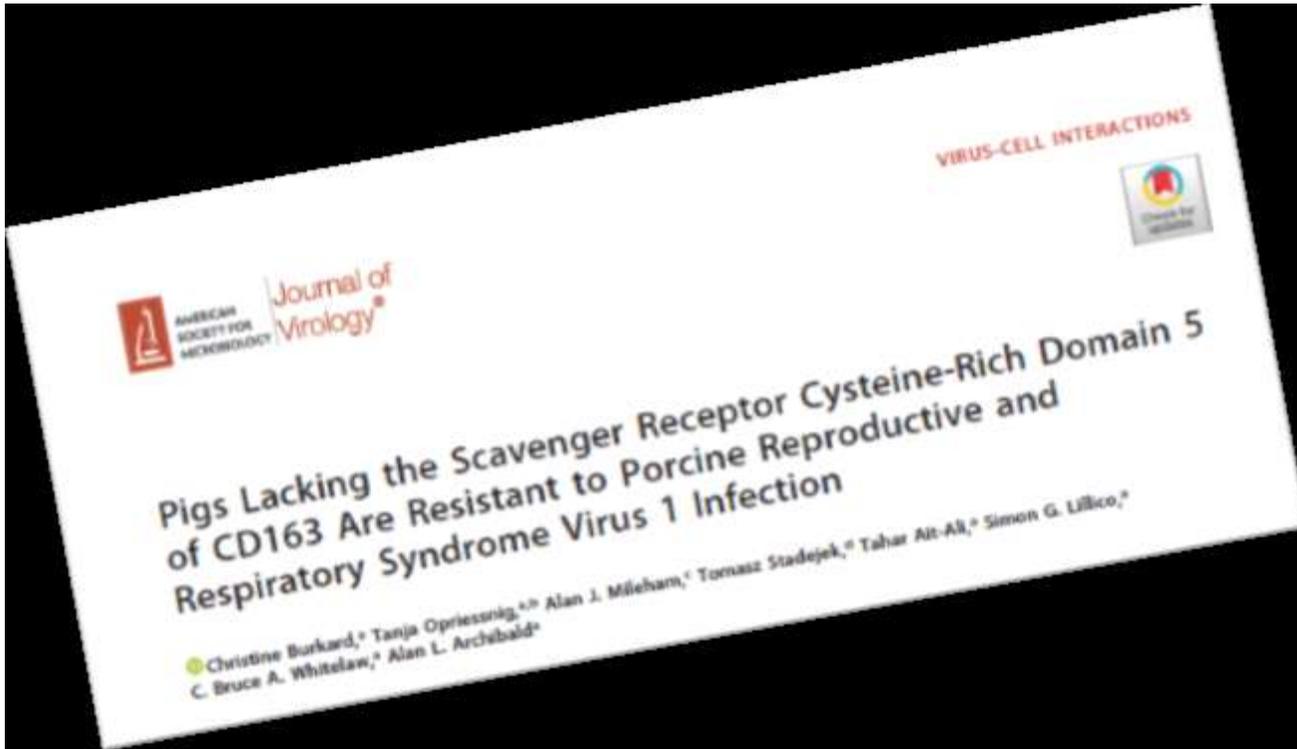
Produce more edited pigs!

- The team mated pigs 1 & 4 from the F1 generation with each other and with wildtype (non-edited) pigs
- Then they infected all eight of the pigs with PRRSV-1

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 wildtype – why?



Results!



21st June 2018

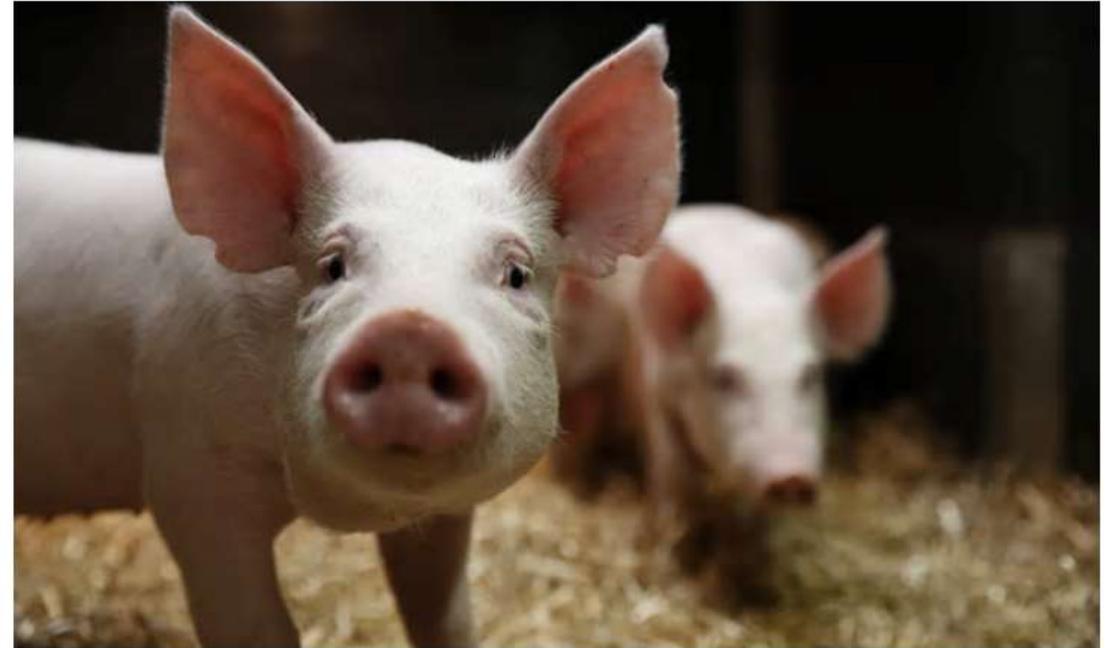


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UK edition
The Guardian

Scientists genetically engineer pigs immune to costly disease

Gene-editing technology could be propelled into commercial farms within five years

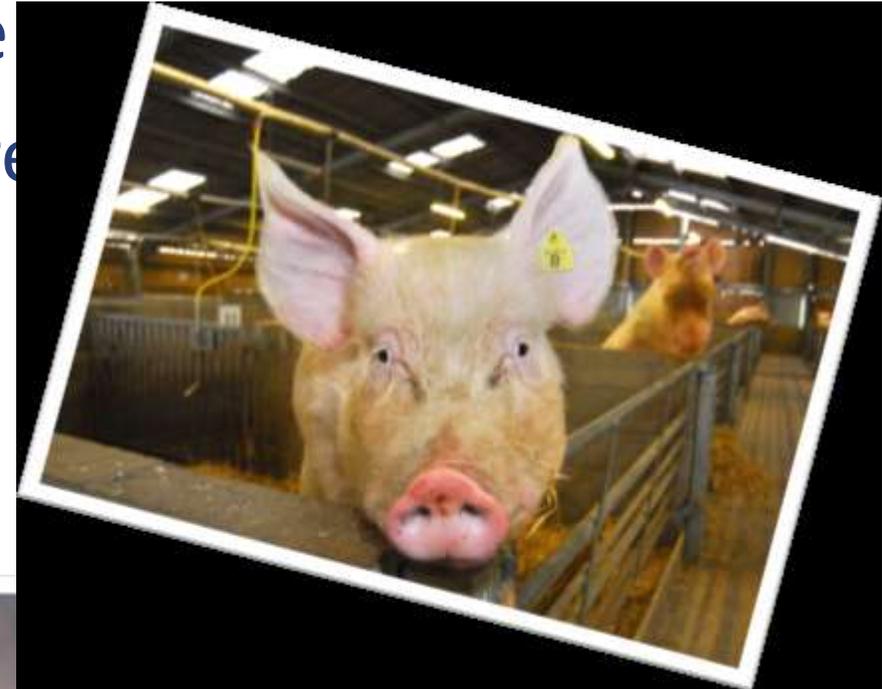


▲ Pigs that have been gene-edited to be resistant to the PRRS virus. Photograph: Murdo Macleod for the Guardian

Food for Thought...



Is it okay to use genome editing in animals that are used to produce food?



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