ROSLIN

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GENOME EDITING: APPLICATIONS IN LIVESTOCK

Simon Lillico

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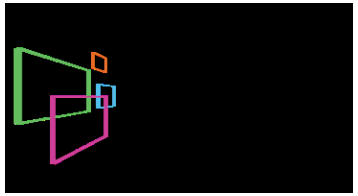
Selective breeding



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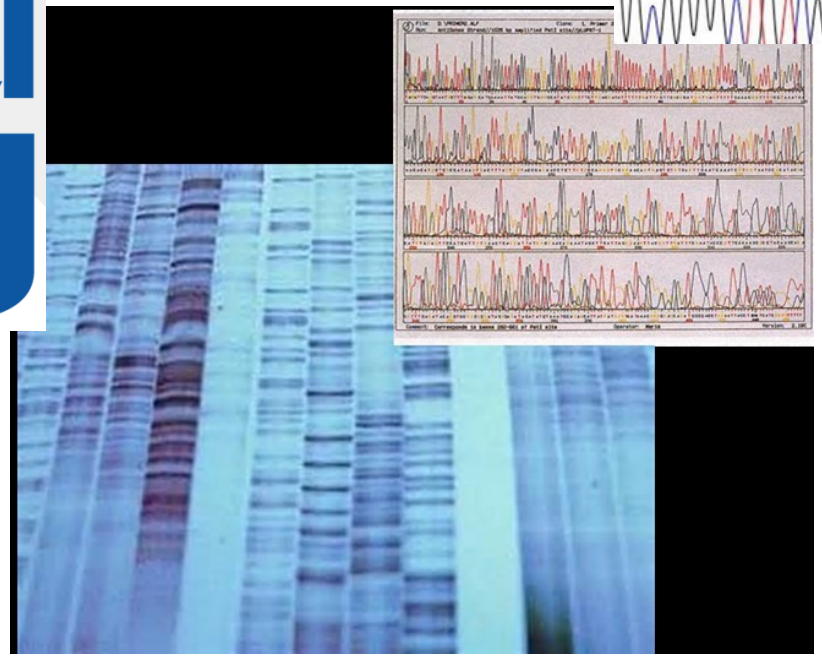
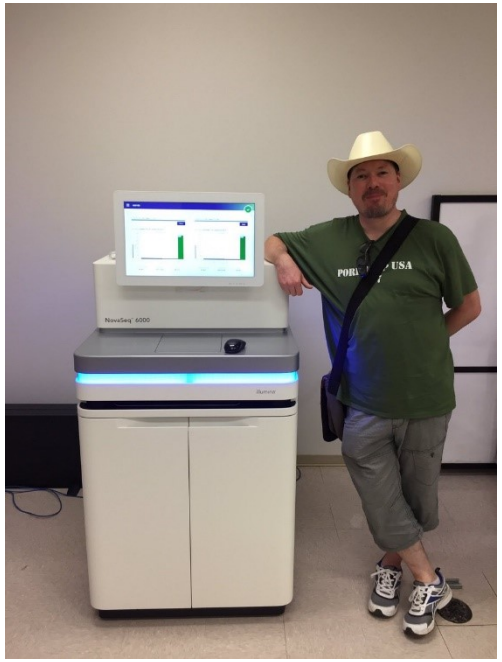
Big data

华大基因
BGI

edinburgh genomics.





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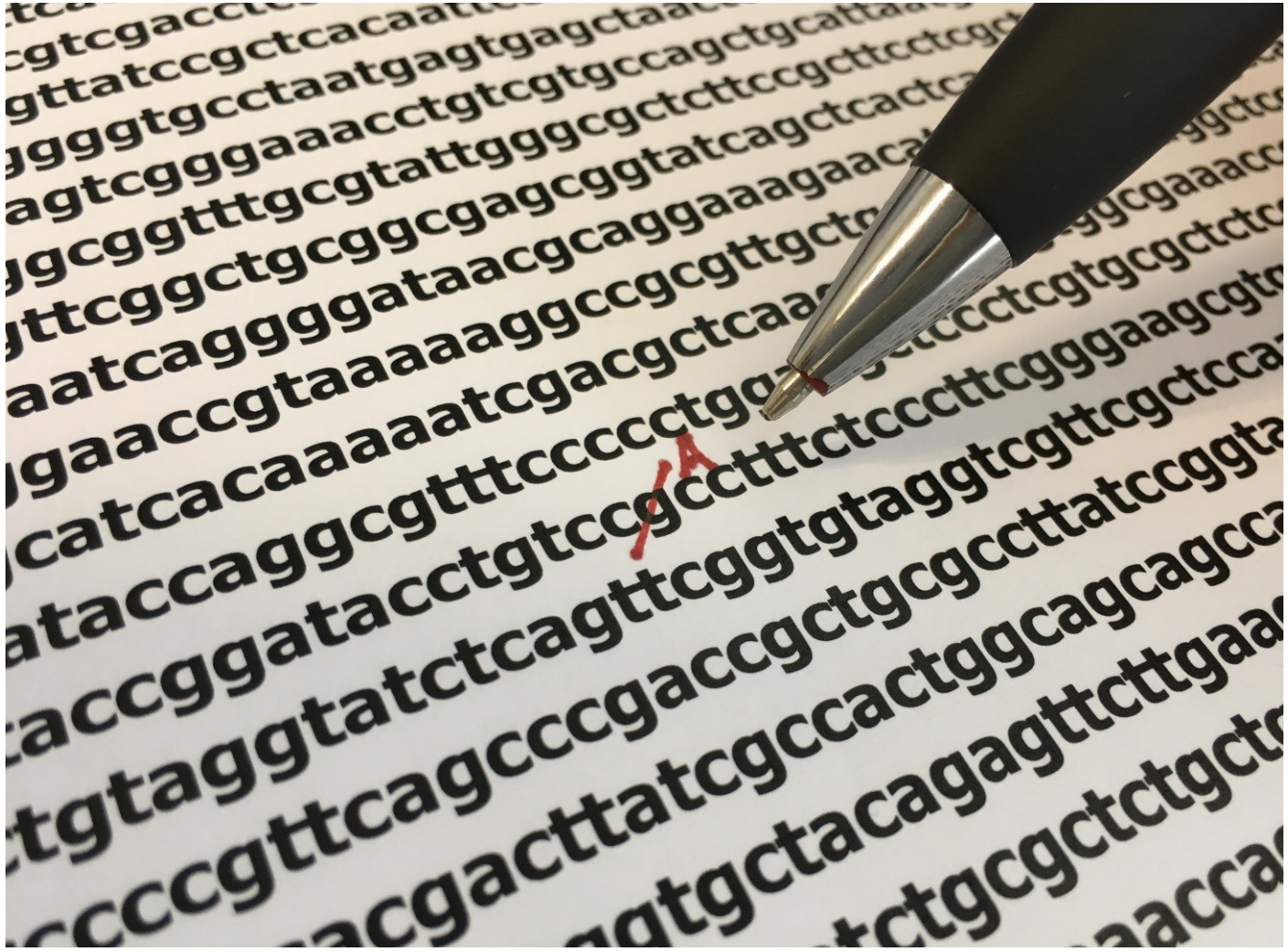
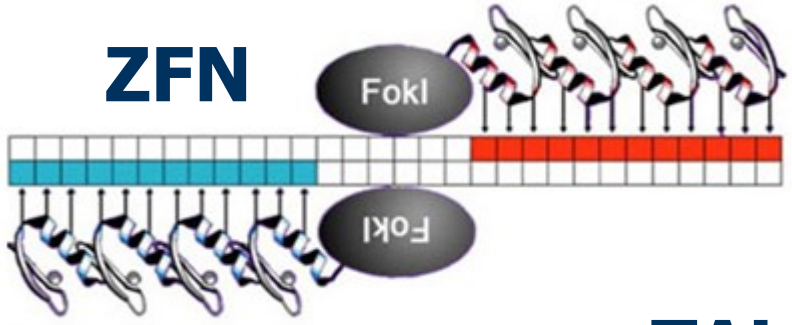
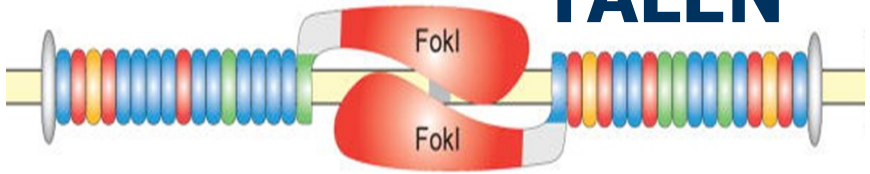
Myriad possibilities



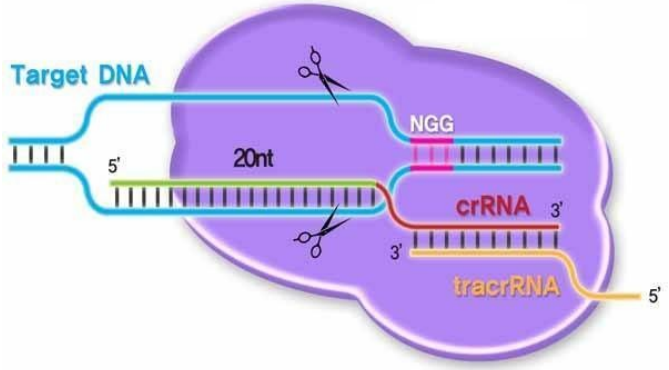
ZFN



TALEN



CRISPR Cas9



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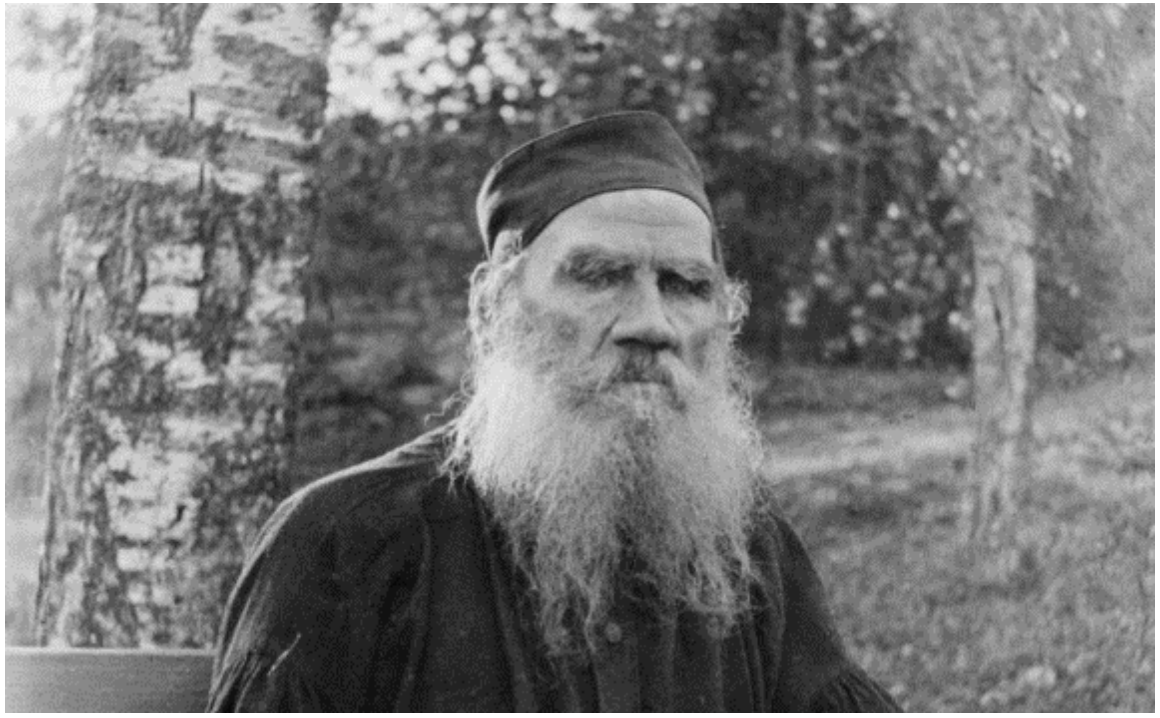
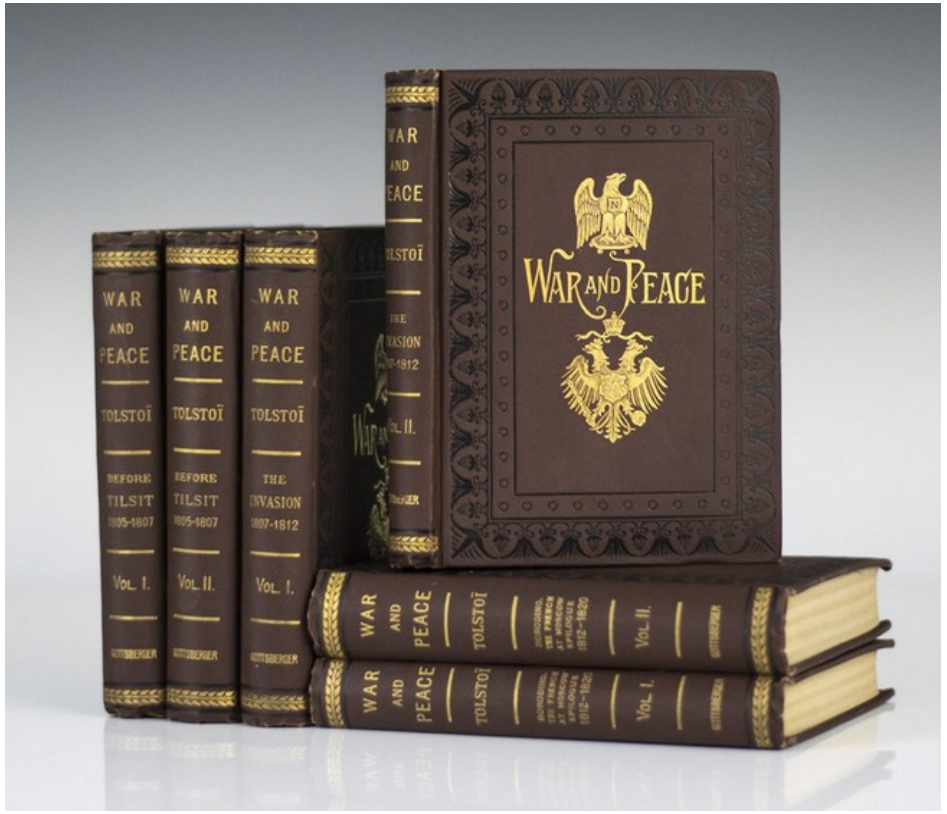
Science fiction





6 books + 2 epilogues

564,364 words

3,202,138 (3×10^6) letters

Pig genome 2.7×10^9 bases

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Myriad possibilities

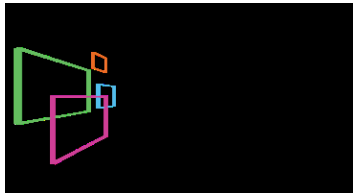
Production traits - meat / milk / eggs

Welfare traits – horns

Improved environmental resilience - heat stress

Disease resistant animals - viruses

Models of human disease - many



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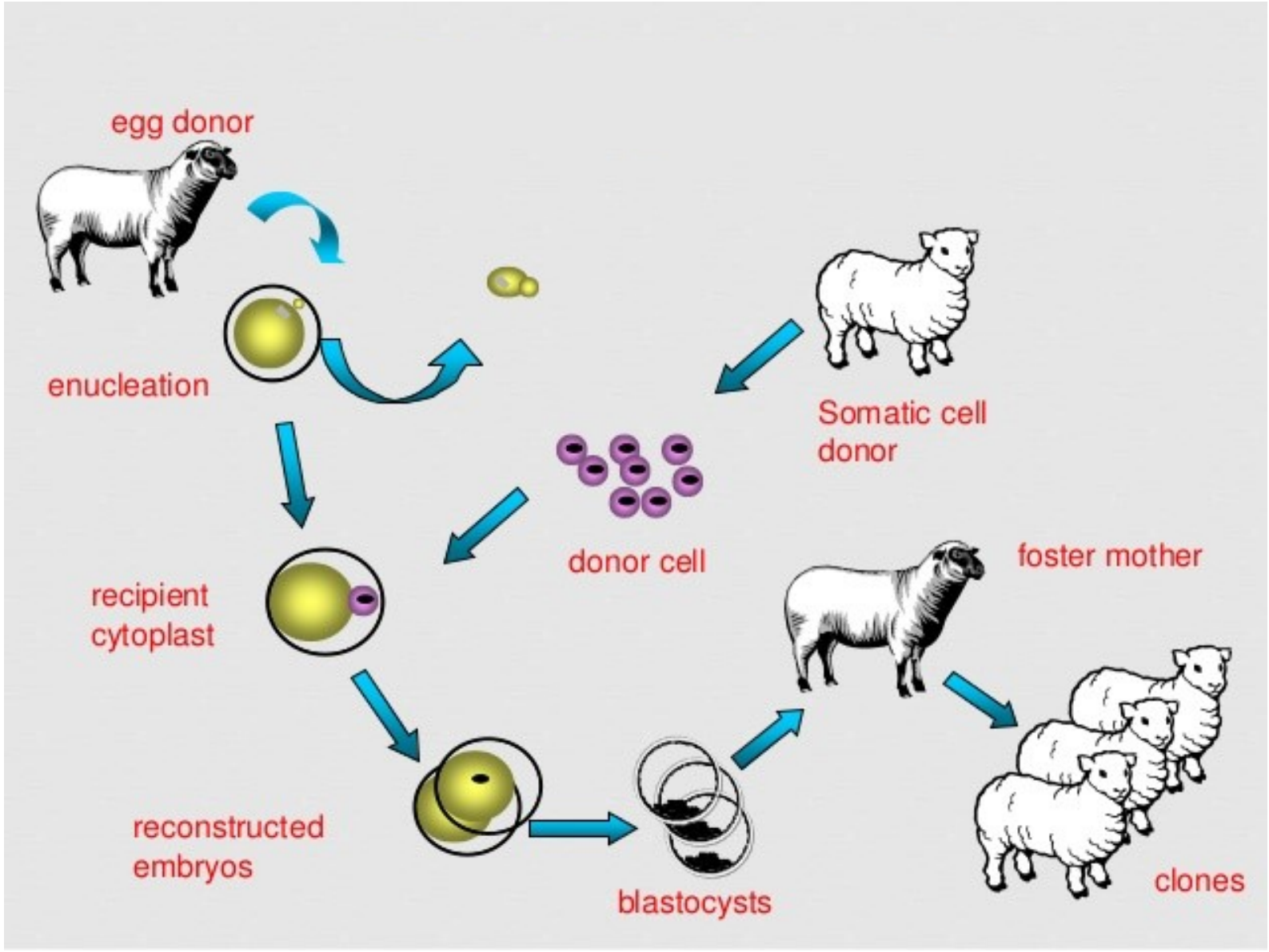
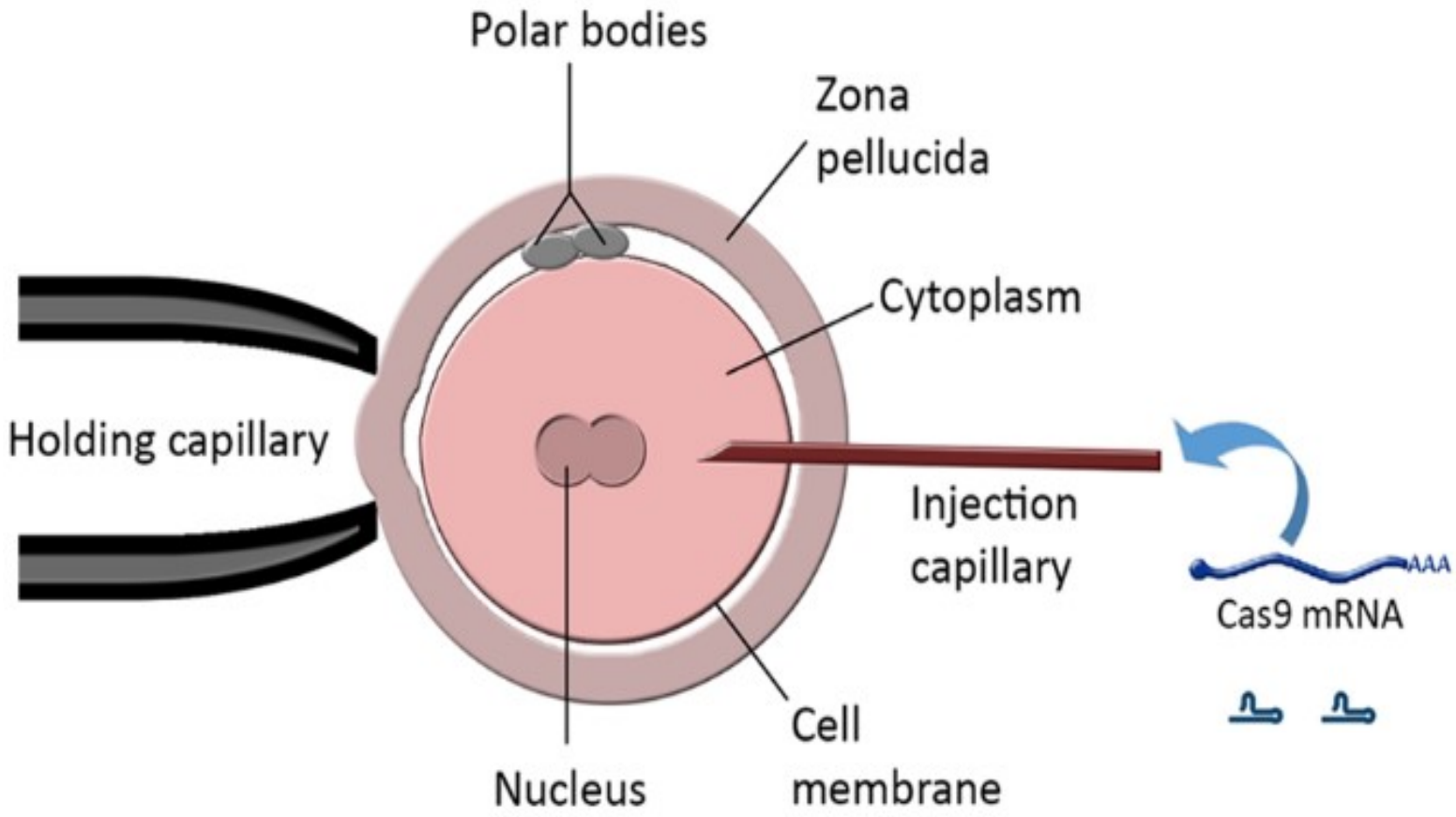
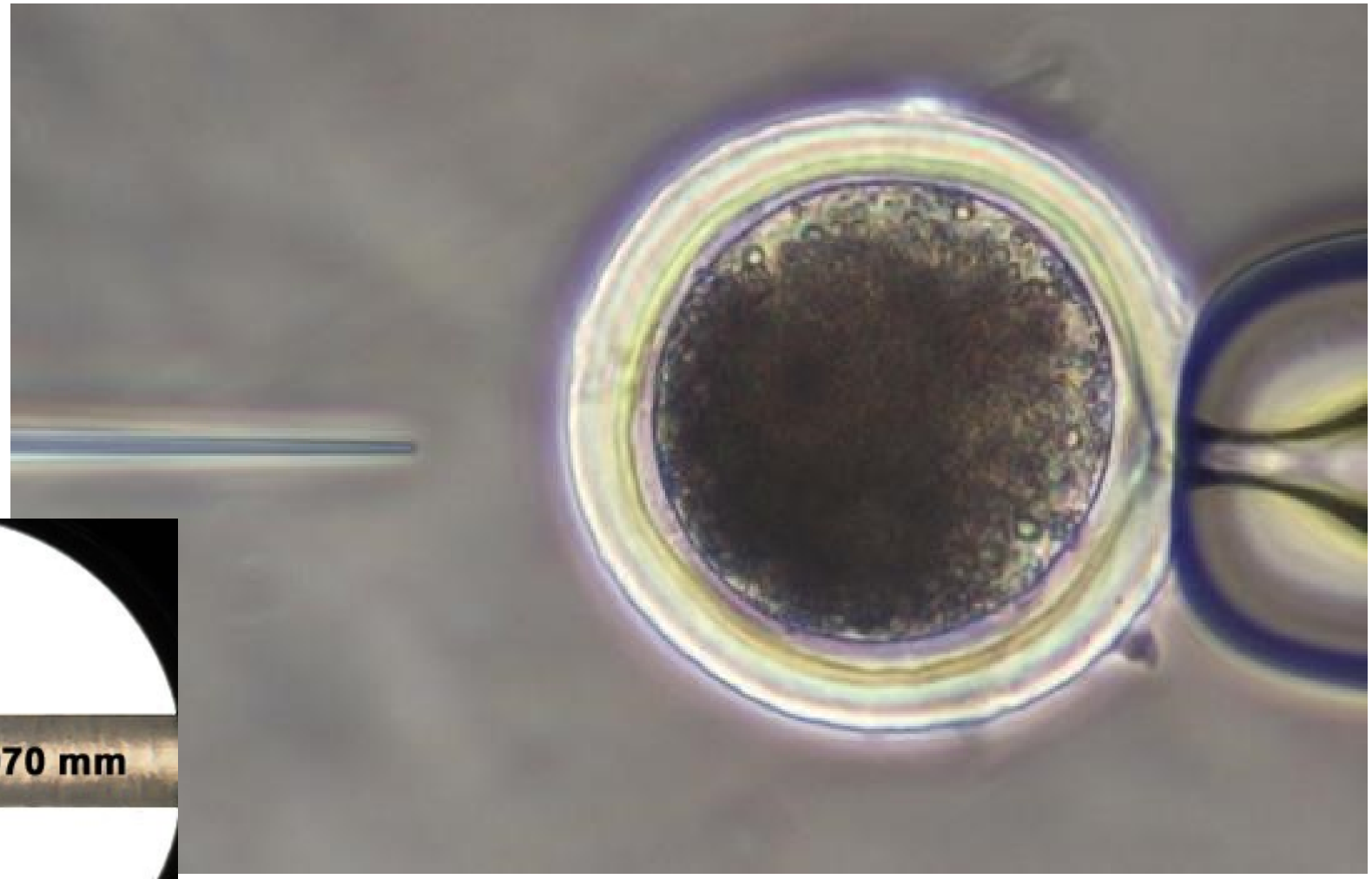
Cloning



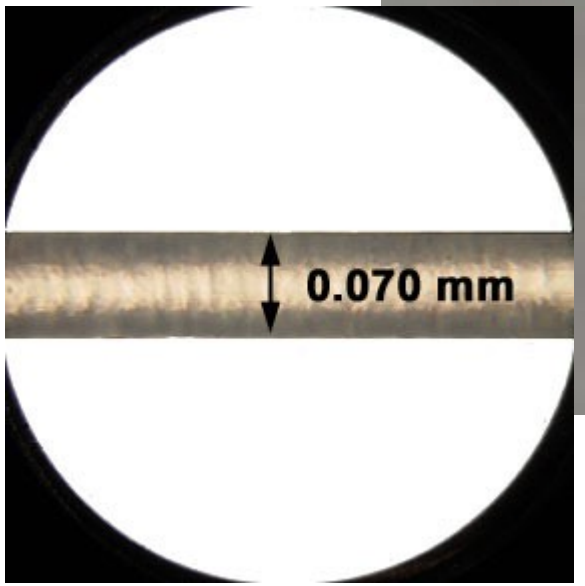
Zygote microinjection



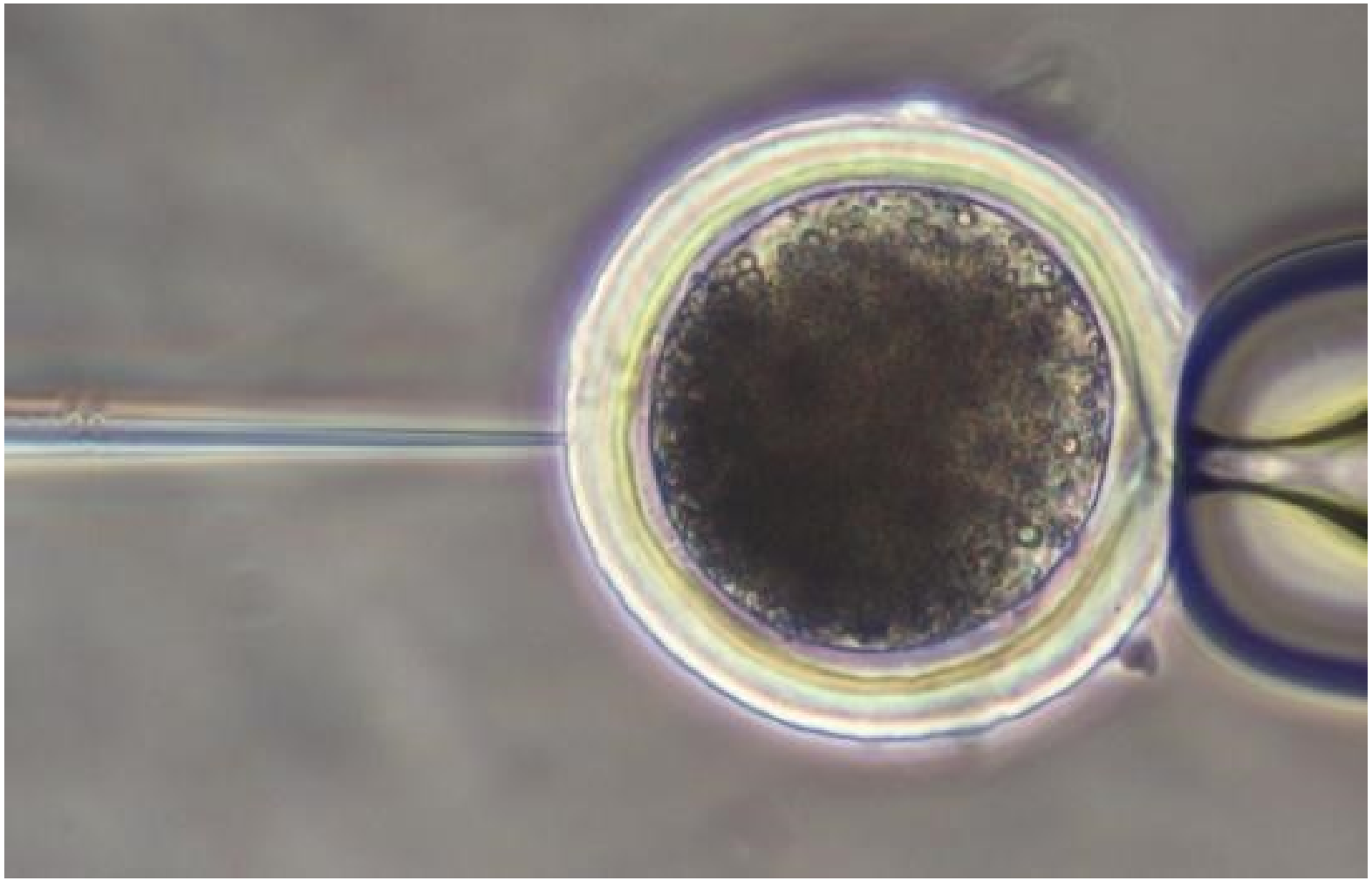
Zygote microinjection



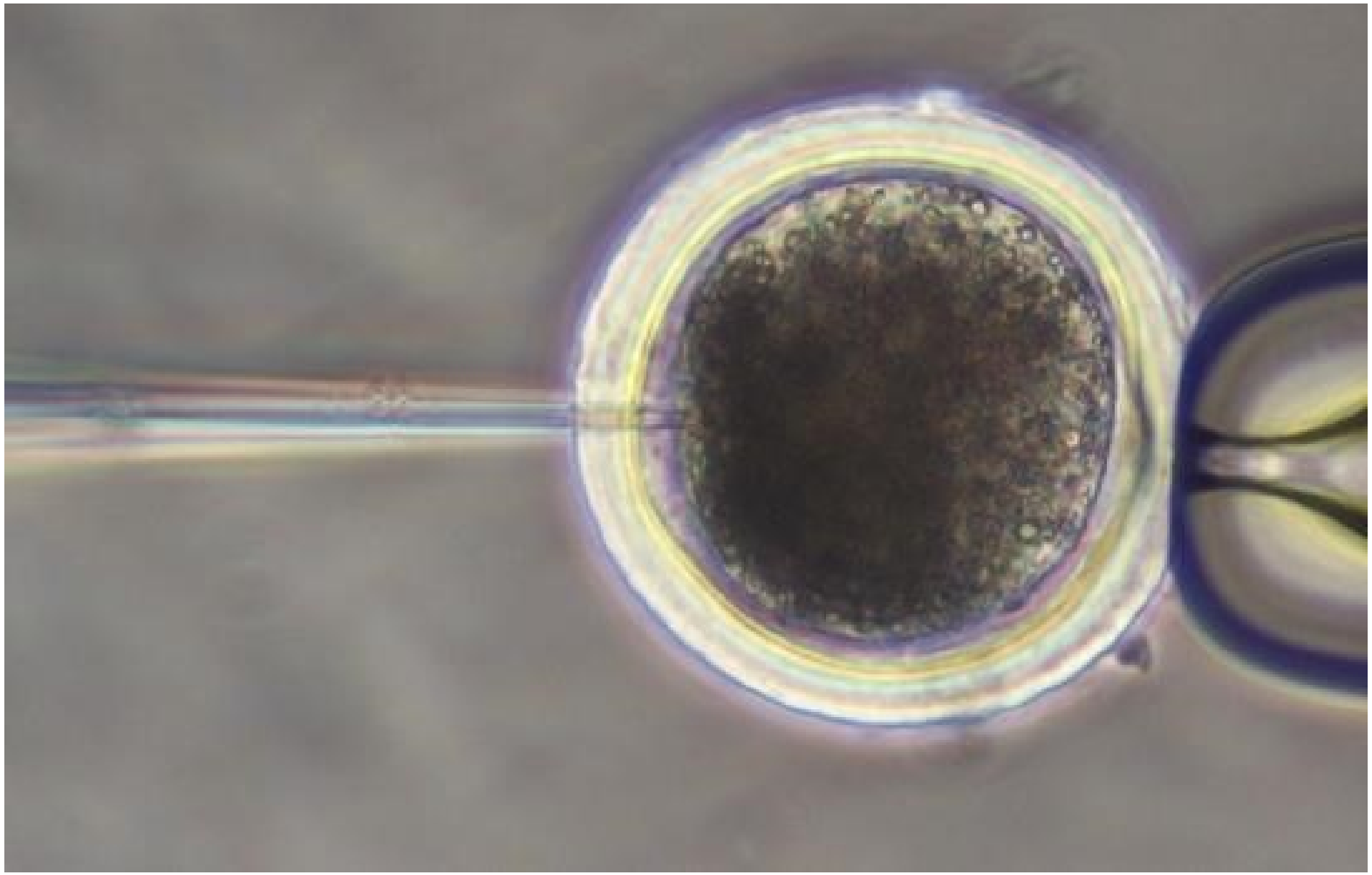
Human hair



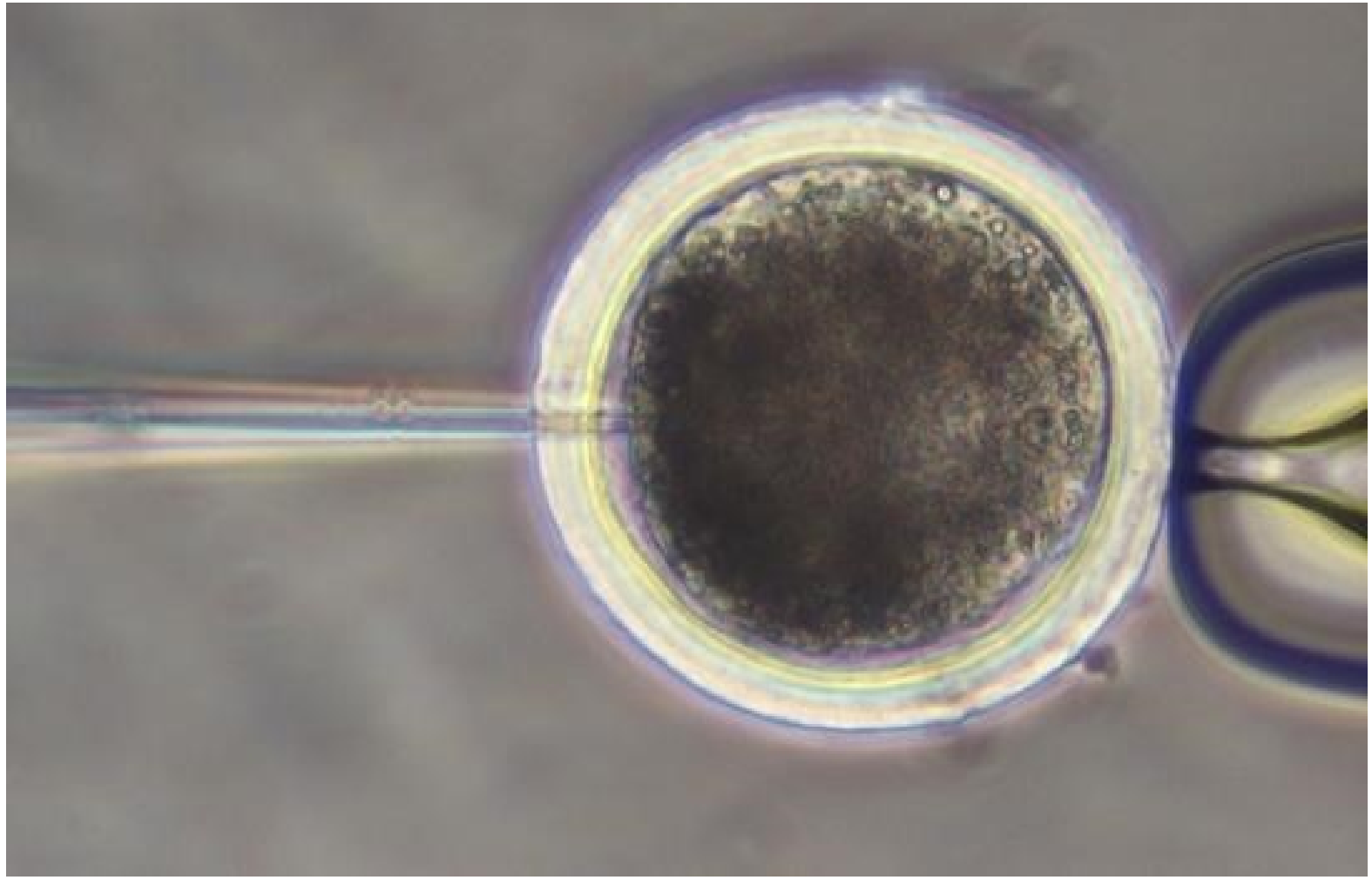
Zygote microinjection



Zygote microinjection



Zygote microinjection



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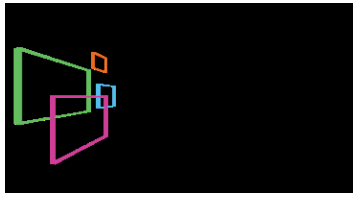
Examples of editing in livestock

1. Muscle mass



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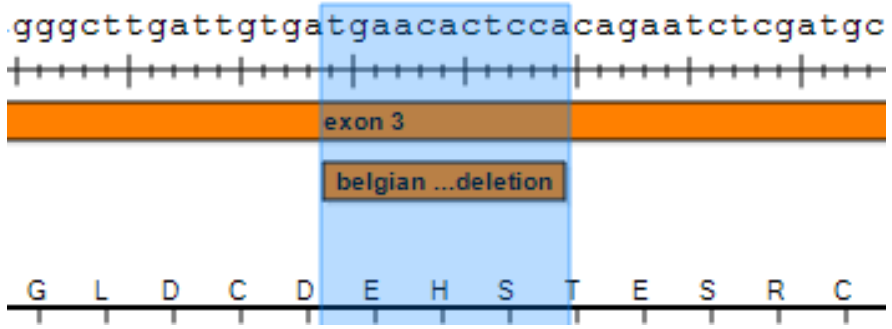
Editing for meat quantity/quality

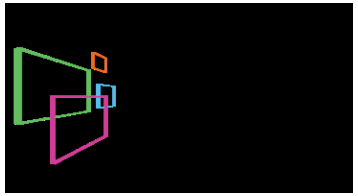
- Myostatin is a negative regulator of muscle
- Belgian Blue cattle have mutation in myostatin
- 11bp deletion
- Double muscle phenotype




```
Bos      DCDEHSTESRCCRYPLTVDF---
Belgian  DCDRISMLSLPSNCGF.
```

Examples of editing in livestock

1. Muscle mass
2. Allergens



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Editing milk allergenicity

SCIENTIFIC REPORTS

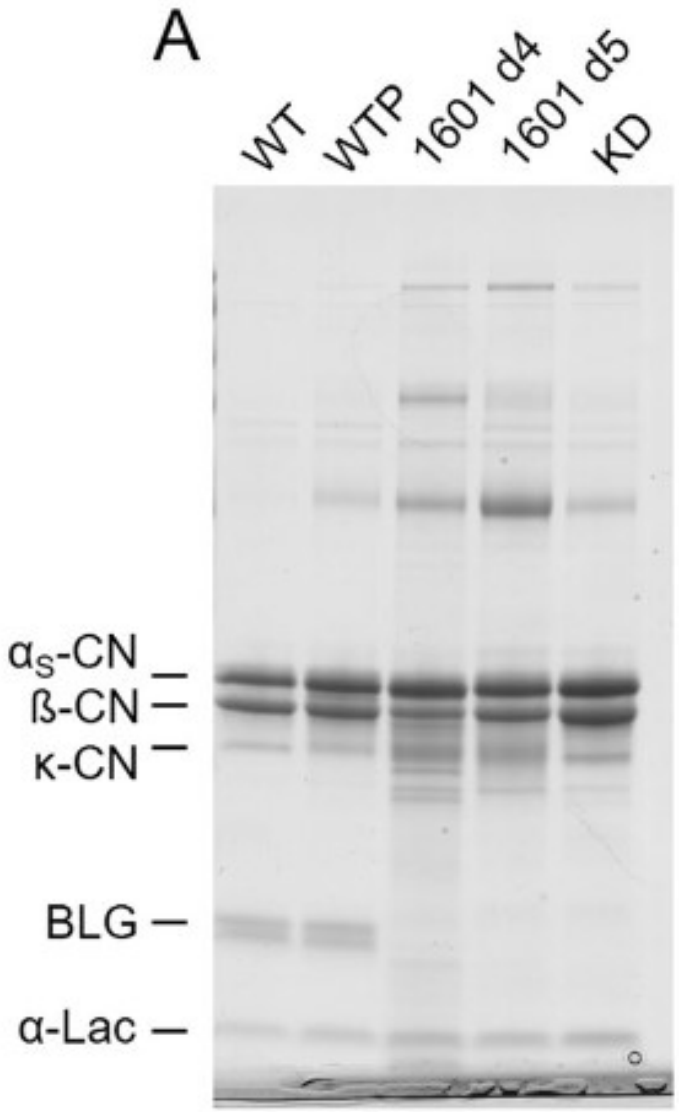
OPEN

Cattle with a precise, zygote-mediated deletion safely eliminate the major milk allergen beta-lactoglobulin

Received: 22 January 2018
Accepted: 19 April 2018
Published online: 16 May 2018

Jingwei Wei[1], Stefan Wagner[1,2], Paul Maclean[1], Brigid Brophy[1], Sally Cole[1], Grant Smolenski[1,3], Dan F. Carlson[4], Scott C. Fahrenkrug[4], David N. Wells[1] & Götz Laible[1]

1601



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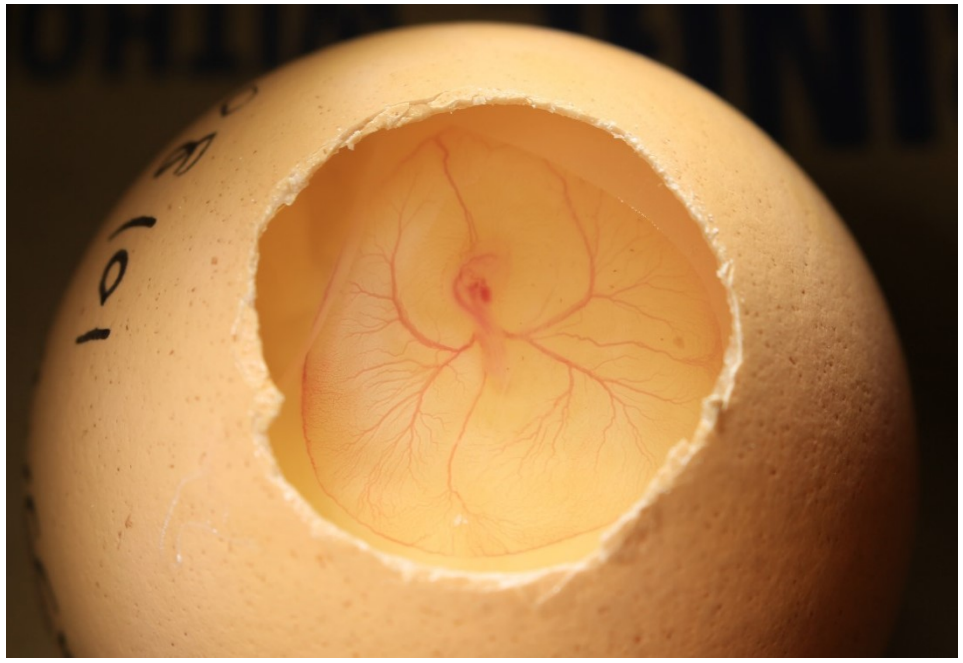
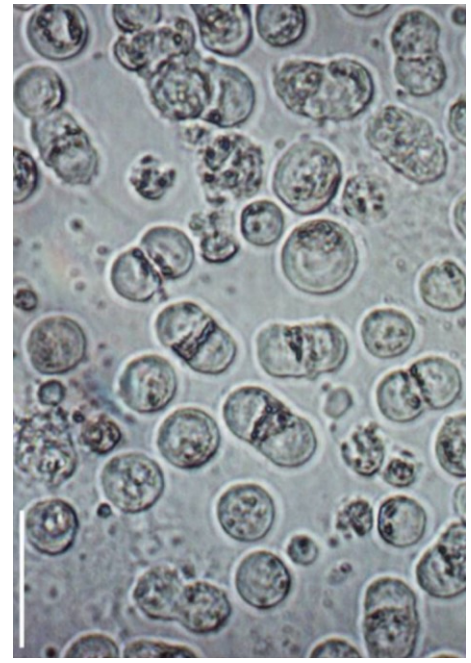
Editing egg allergens

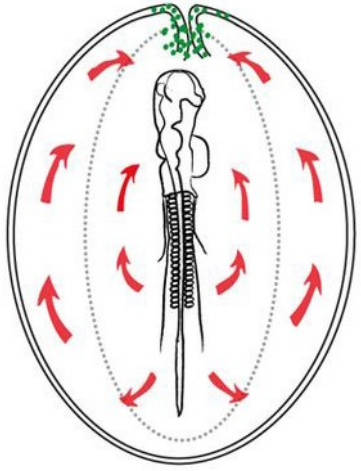
Scientists working at creating allergy-free eggs

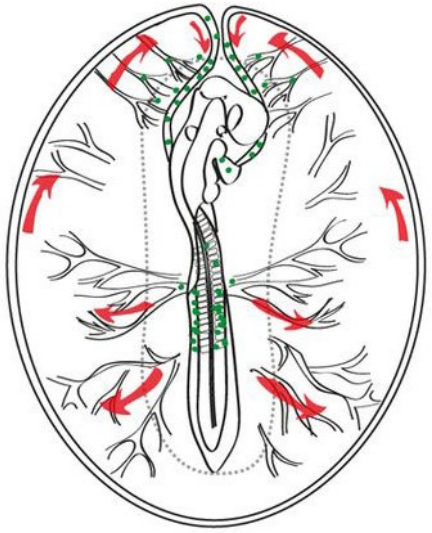
Ben Coxworth | March 14th, 2012

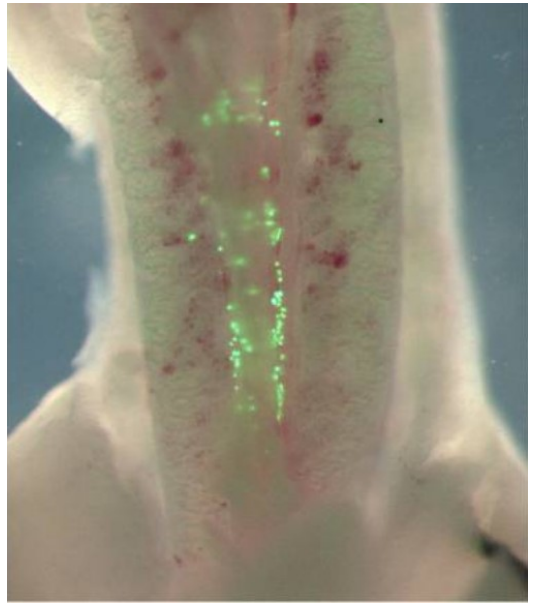
The hypoallergenic egg team: Tim Doran (left), Cenk Suphioglu and Pathum Dhanapala

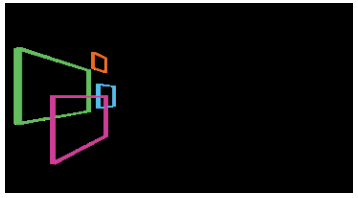












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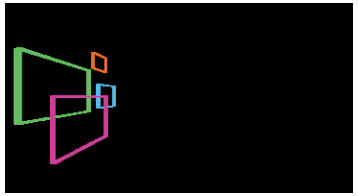
Examples of editing in livestock

1. Muscle mass
2. Allergens
3. Welfare



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Beef

Dairy

Production of hornless dairy cattle from genome-edited cell lines

Daniel F Carlson[1], Cheryl A Lancto[1], Bin Zang[2], Eui-Soo Kim[1], Mark Walton[1], David Oldeschulte[3], Christopher Seabury[3], Tad S Sonstegard[1] & Scott C Fahrenkrug[1]

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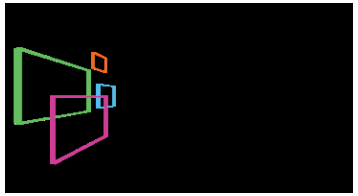
Examples of editing in livestock

1. Muscle mass
2. Allergens
3. Welfare
4. Environmental resilience



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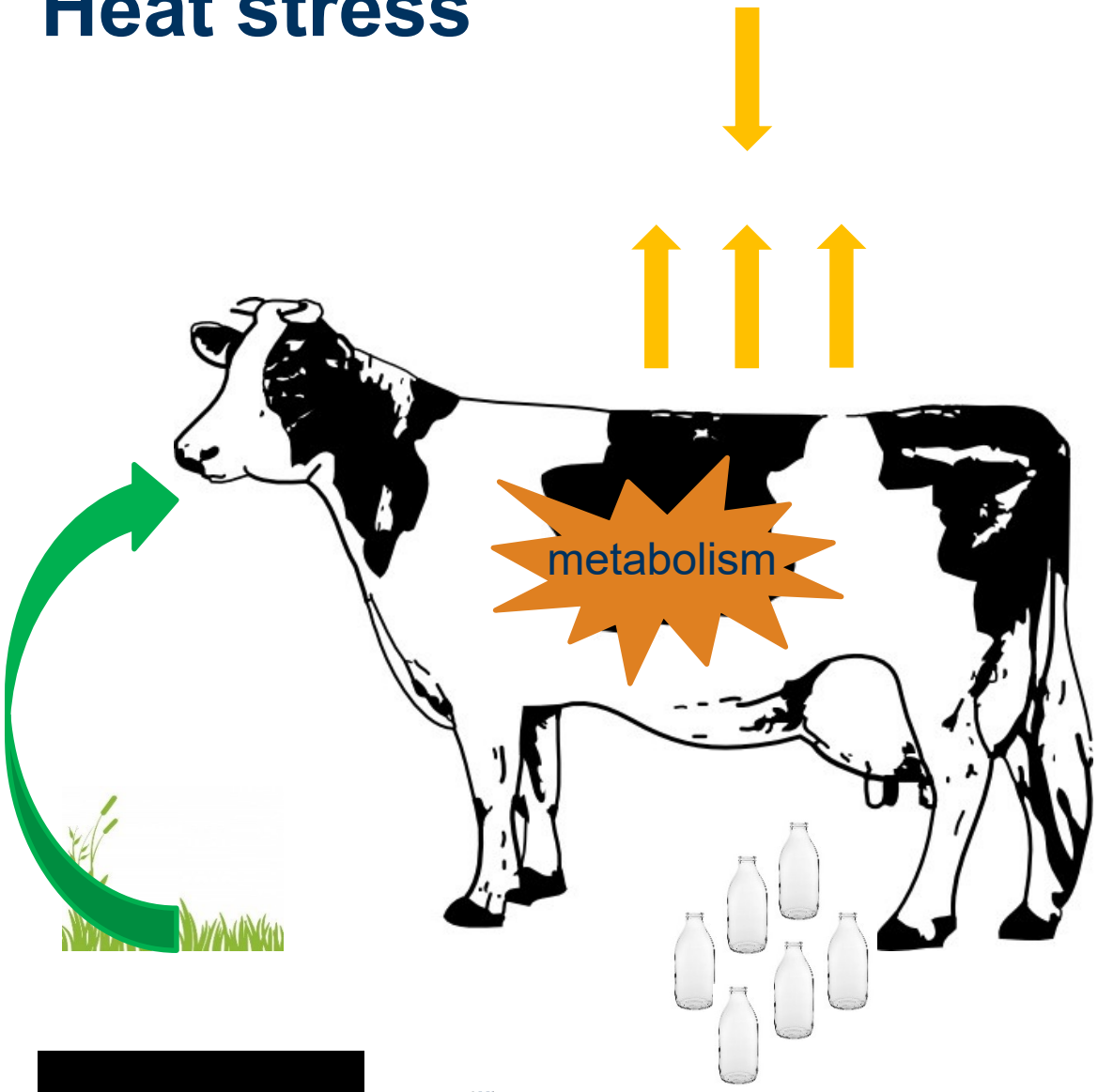
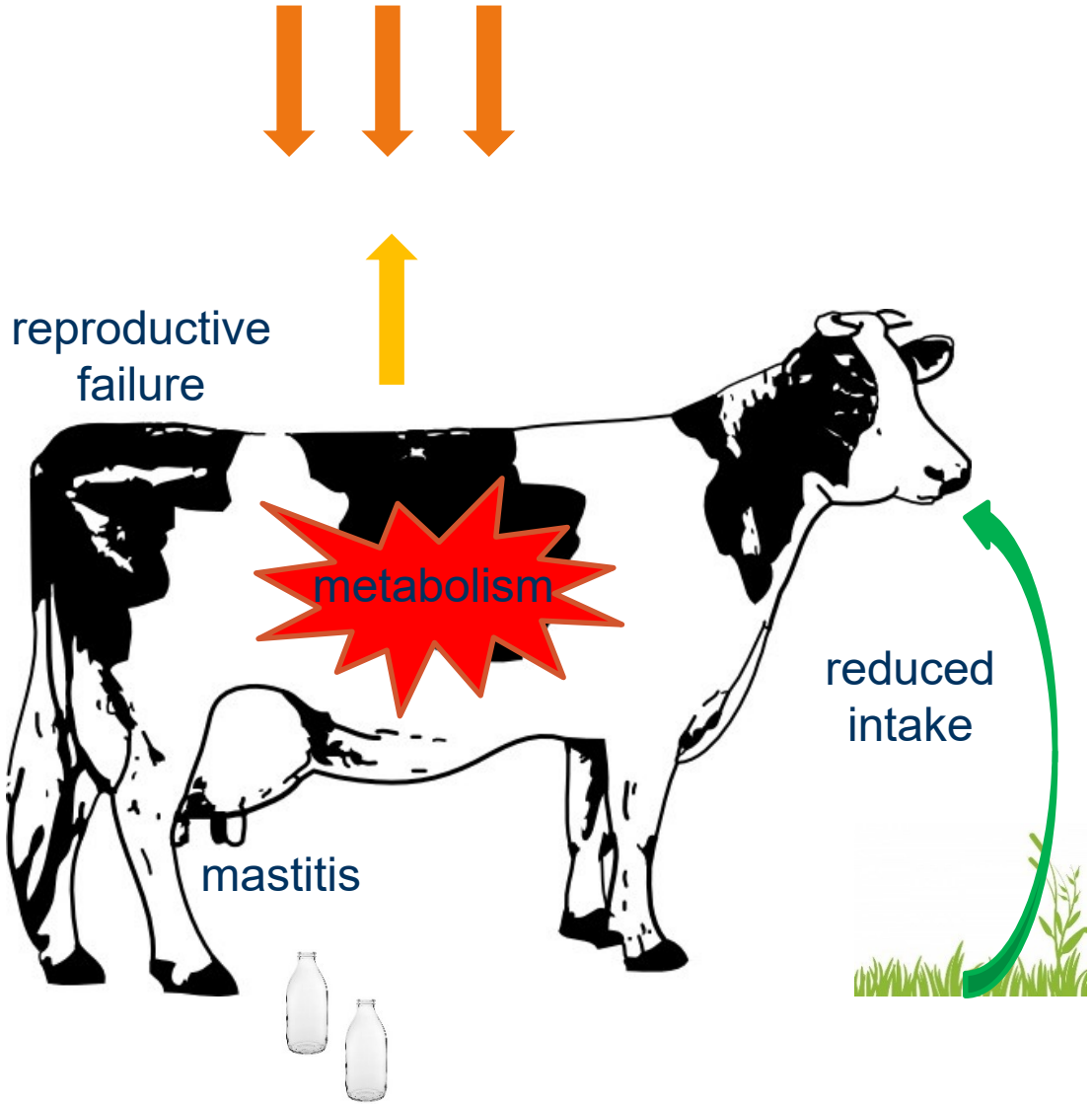
Heat stress





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Environmental adaptation

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Environmental adaptation

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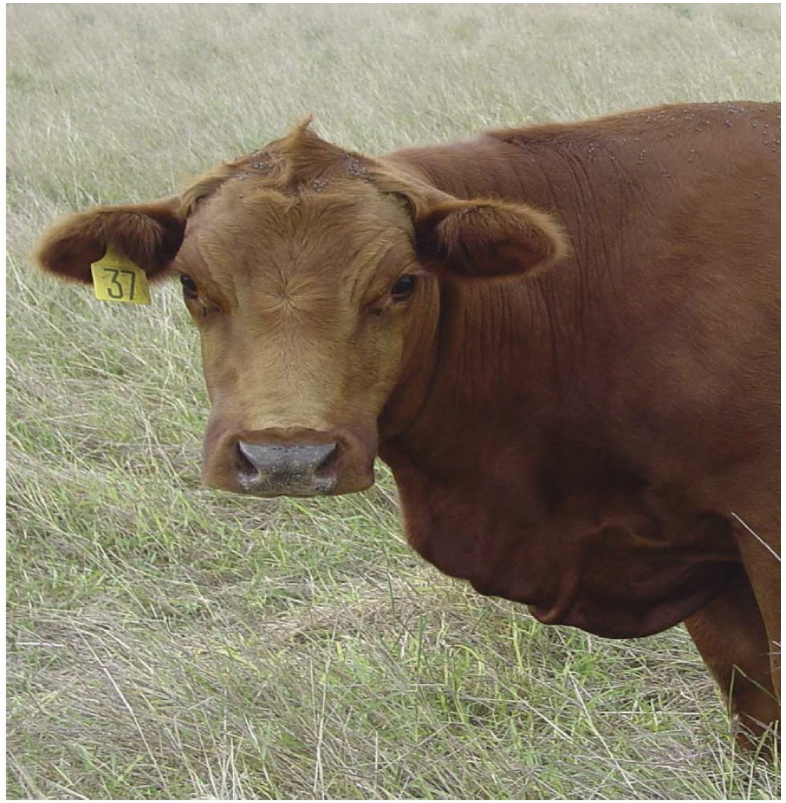
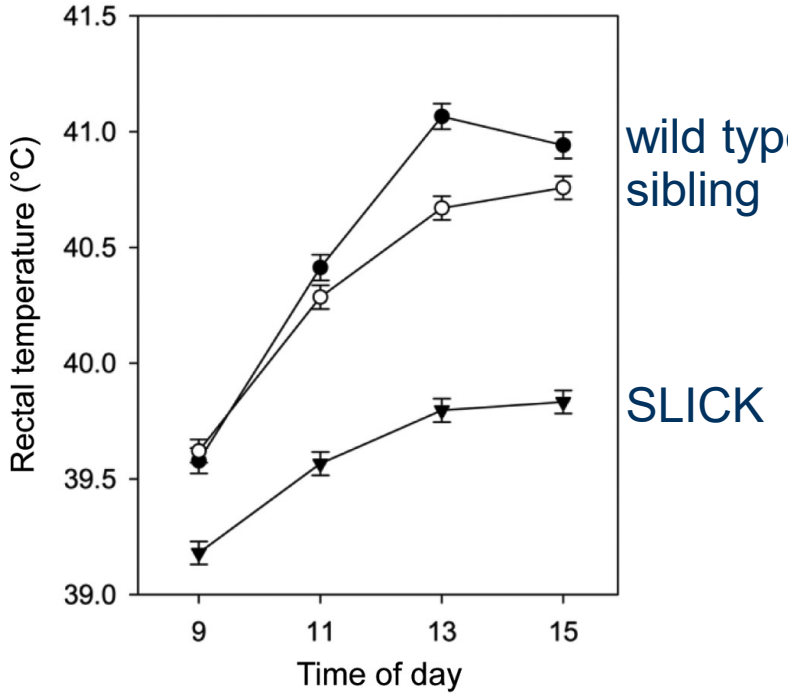
Environmental adaptation – the SLICK phenotype

B. taurus D Q H A L K A • • • • Prolactin receptor

Senopol D Q H V *





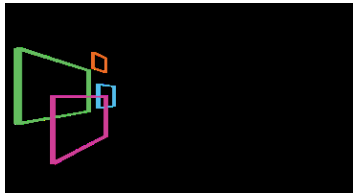
Centre for Tropical Livestock Genetics and Health

acceligen

A RECOMBINETICS COMPANY

Examples of editing in livestock

1. Muscle mass
2. Allergens
3. Welfare
4. Environmental resilience
5. Diseases



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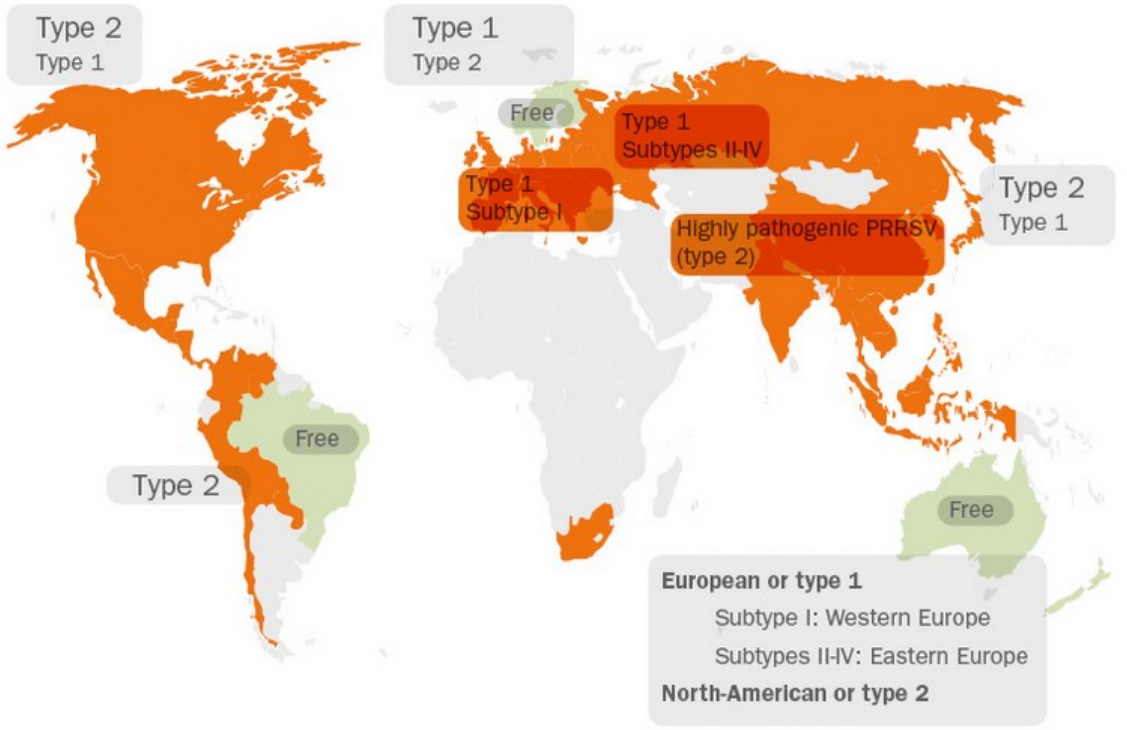
Porcine Reproductive and Respiratory Syndrome



HIPRA

Vaccines not cross-protective

1.5B € annually

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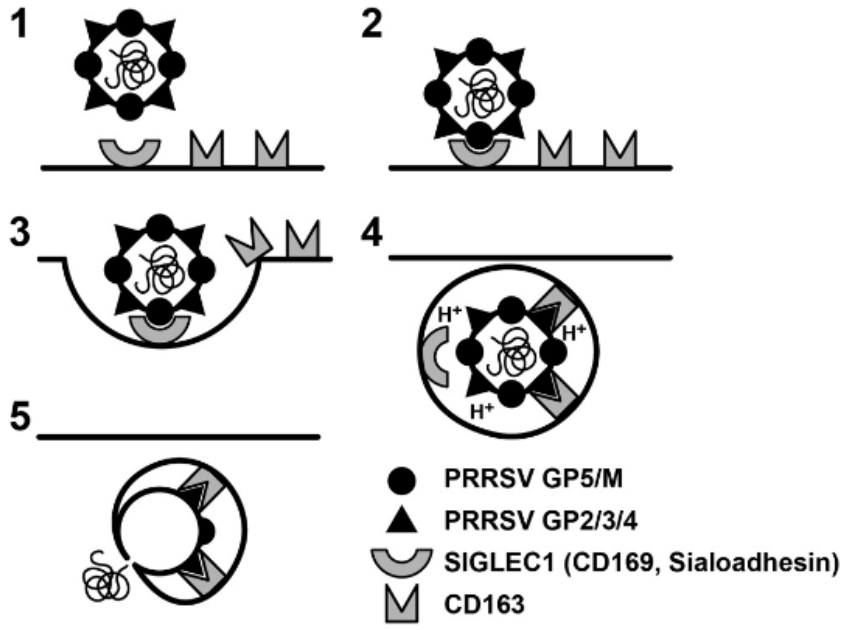
Porcine arterivirus attachment to the macrophage-specific receptor sialoadhesin is dependent on the sialic acid-binding activity of the N-terminal immunoglobulin domain of sialoadhesin.

Abstract

The sialic acid-binding lectin sialoadhesin (Sn) is a macrophage-restricted receptor for porcine reproductive and respiratory syndrome virus (PRRSV). To investigate the importance of pSn sialic acid-binding activity for PRRSV infection, an R(116)-to-E mutation was introduced in the predicted sialic acid-binding domain of pSn, resulting in a mutant, pSn(RE), that could not bind sialic acids. PSn, but not pSn(RE), allowed PRRSV binding and internalization. These data show that the sialic acid-binding activity of pSn is essential for PRRSV attachment to pSn and thus identifies the variable, N-terminal domain of Sn as a PRRSV binding domain.

Peter L. Delputte,[1]*† Wander Van Breedam,[1]† Iris Delrue,[1] Cornelia Oetke,[2] Paul R. Crocker,[2] and Hans J. Nauwynck[1]



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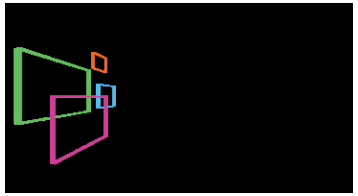
An Intact Sialoadhesin (Sn/SIGLEC1/CD169) Is Not Required for Attachment/Internalization of the Porcine Reproductive and Respiratory Syndrome Virus

Randall S. Prather,[a] Raymond R. R. Rowland,[b] Catherine Ewen,[b] Benjamin Trible,[b] Maureen Kerrigan,[b] Bhupinder Bawa,[b] Jennifer M. Teson,[a] Jiude Mao,[a] Kiho Lee,[a] Melissa S. Samuel,[a] Kristin M. Whitworth,[a] Clifton N. Murphy,[a] Tina Egen,[a] Jonathan A. Green[a]

Division of Animal Science, College of Food Agriculture and Natural Resources, University of Missouri, Columbia, Missouri, USA[a]; Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA[b]

Surface expression of SIGLEC1, also known as sialoadhesin or CD169, is considered a primary determinant of the permissiveness of porcine alveolar macrophages for infection by porcine reproductive and respiratory syndrome virus (PRRSV). *In vitro*, the attachment and internalization of PRRSV are dependent on the interaction between sialic acid on the virion surface and the sialic acid binding domain of the *SIGLEC1* gene. To test the role of SIGLEC1 in PRRSV infection, a *SIGLEC1* gene knockout pig was created by removing part of exon 1 and all of exons 2 and 3 of the *SIGLEC1* gene. The resulting knockout ablated SIGLEC1 expression on the surface of alveolar macrophages but had no effect on the expression of CD163, a coreceptor for PRRSV. After infection, PRRSV viremia in *SIGLEC1*$^{-/-}$ pigs followed the same course as in *SIGLEC1*$^{-/+}$ and *SIGLEC1*$^{+/+}$ littermates. The absence of SIGLEC1 had no measurable effect on other aspects of PRRSV infection, including clinical disease course and histopathology. The results demonstrate that the expression of the *SIGLEC1* gene is not required for infection of pigs with PRRSV and that the absence of SIGLEC1 does not contribute to the pathogenesis of acute disease.



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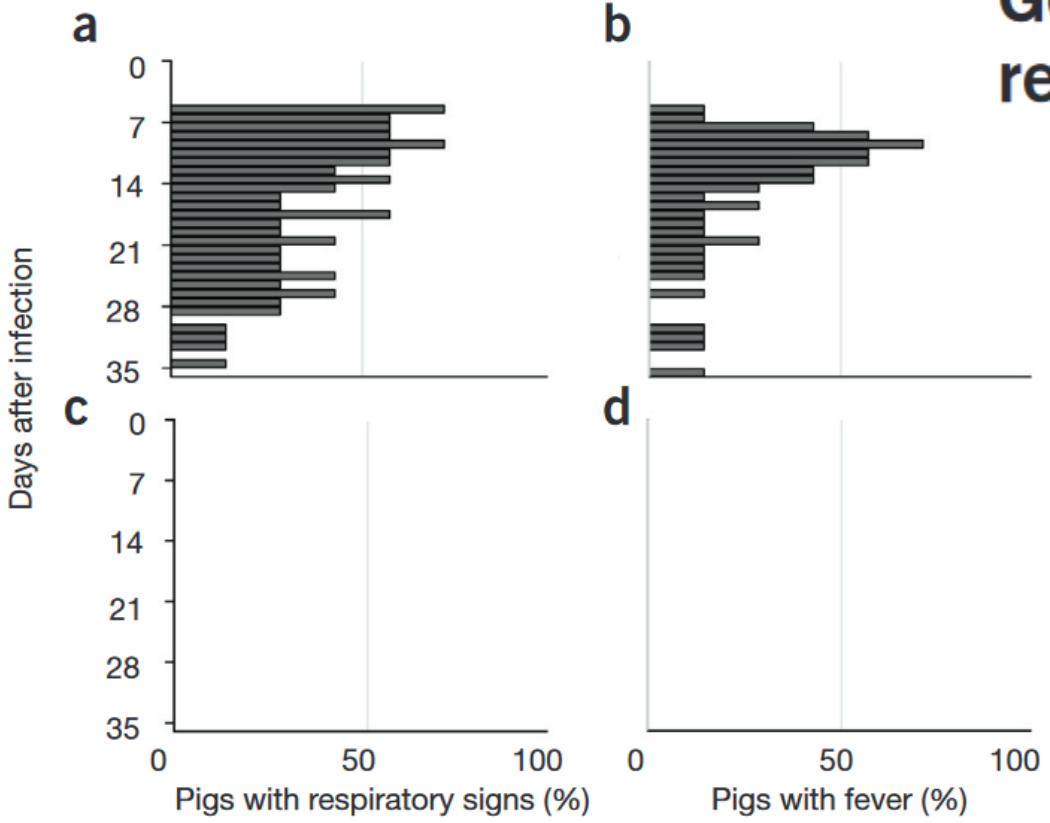
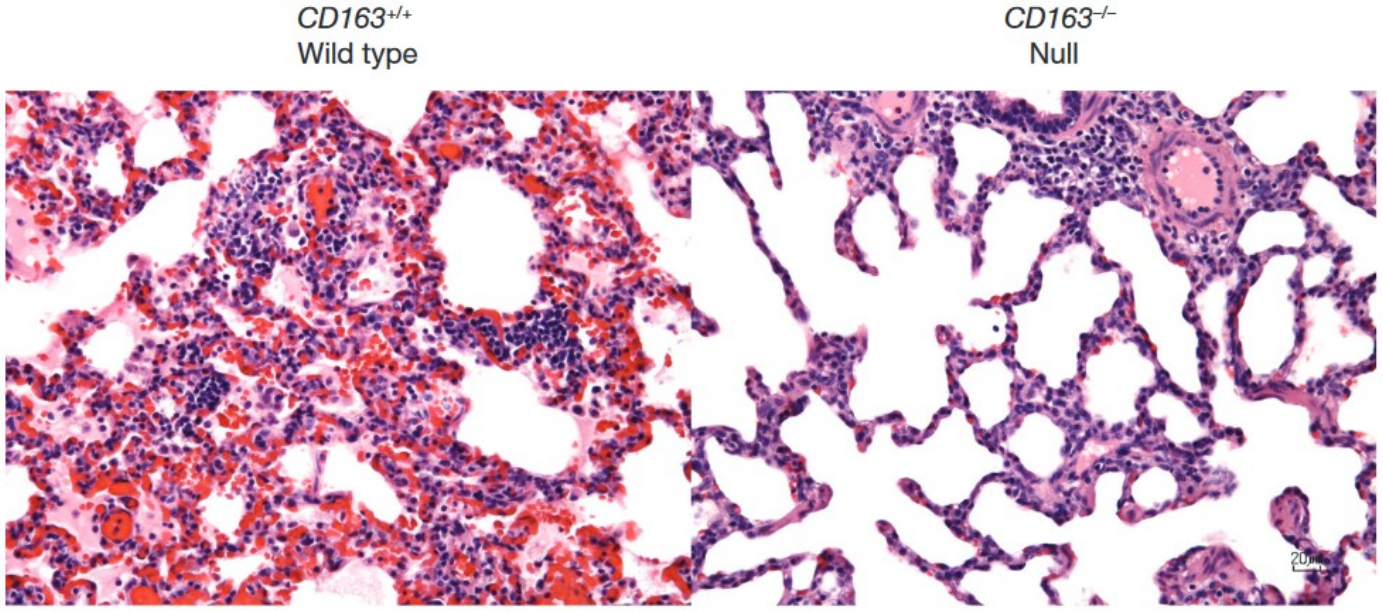
Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus

Kristin M Whitworth[1], Raymond R R Rowland[2], Catherine L Ewen[2], Benjamin R Trible[2], Maureen A Kerrigan[2], Ada G Cino-Ozuna[2], Melissa S Samuel[1], Jonathan E Lightner[3], David G McLaren[3], Alan J Mileham[3], Kevin D Wells[1] & Randall S Prather[1]

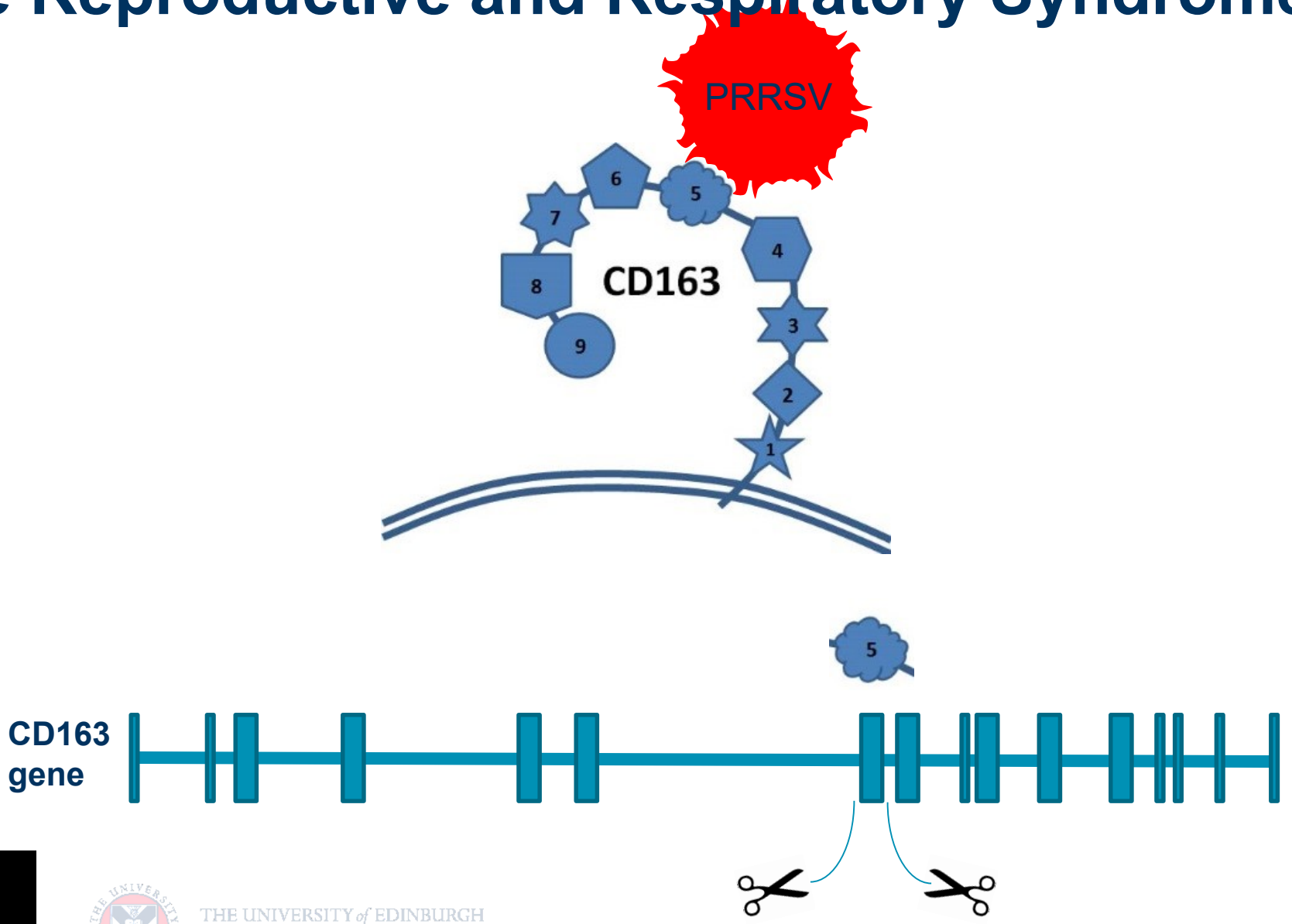


CD163$^{+/+}$
Wild type

CD163$^{-/-}$
Null



Porcine Reproductive and Respiratory Syndrome



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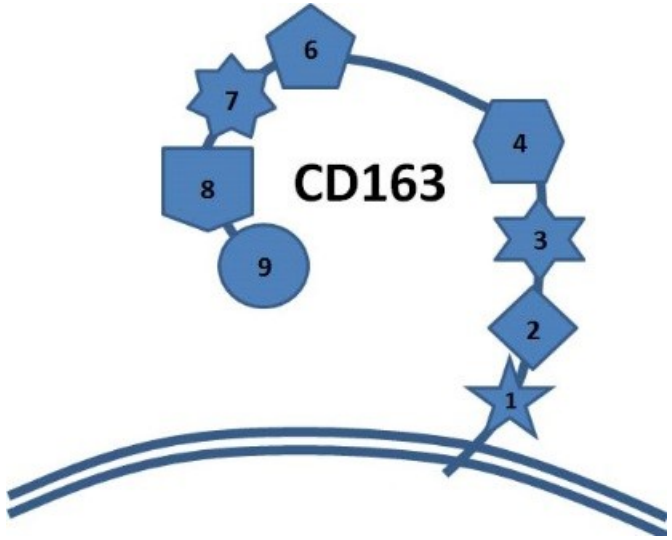

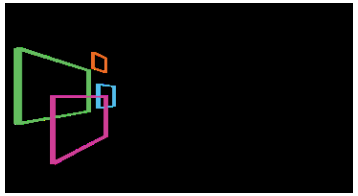

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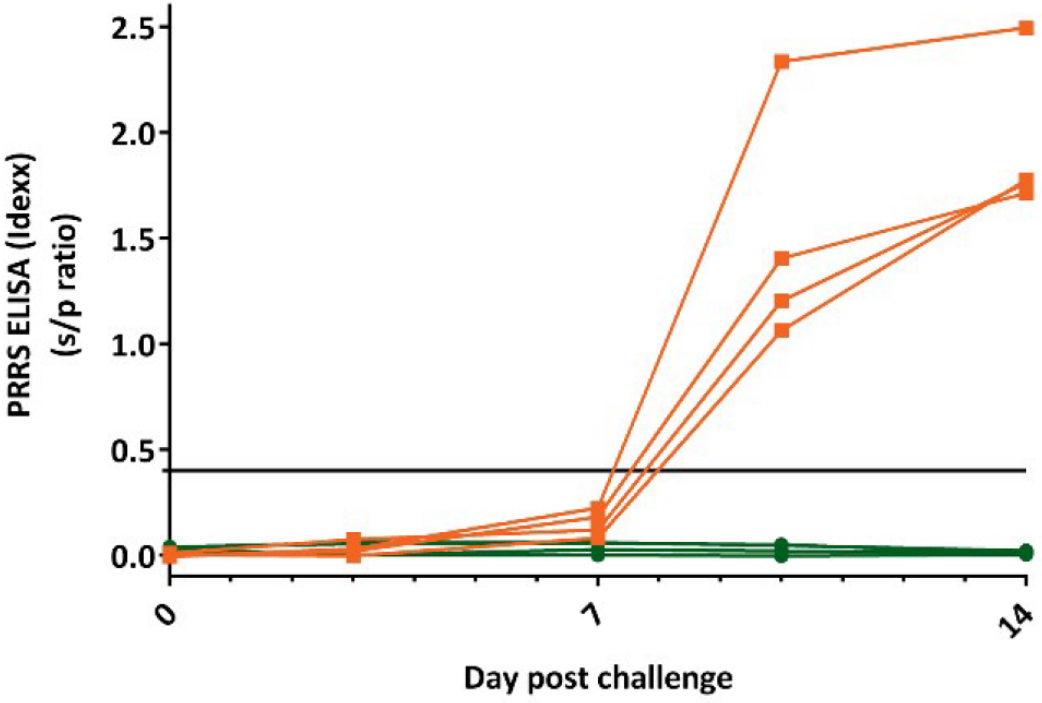

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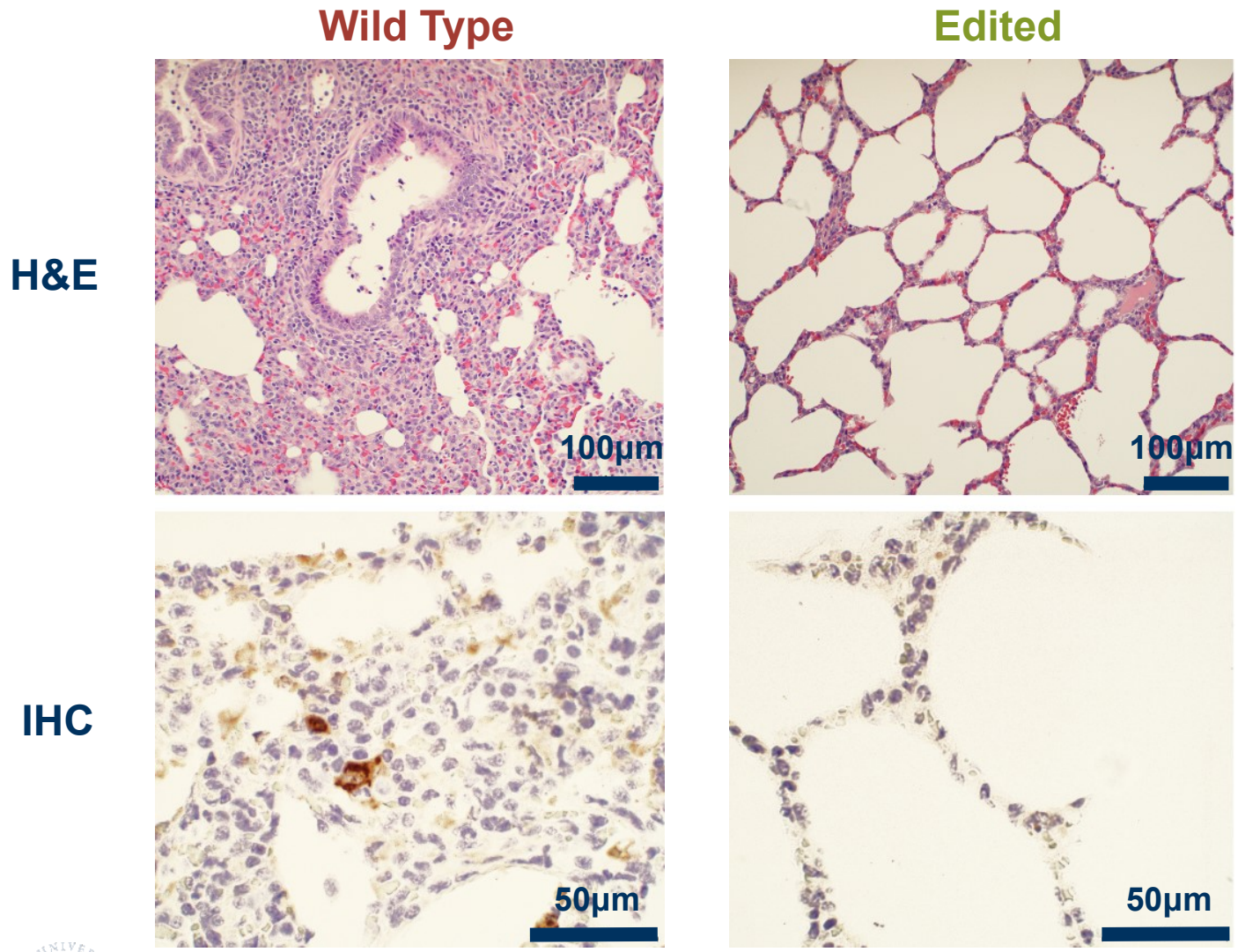
Porcine Reproductive and Respiratory Syndrome



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Genus

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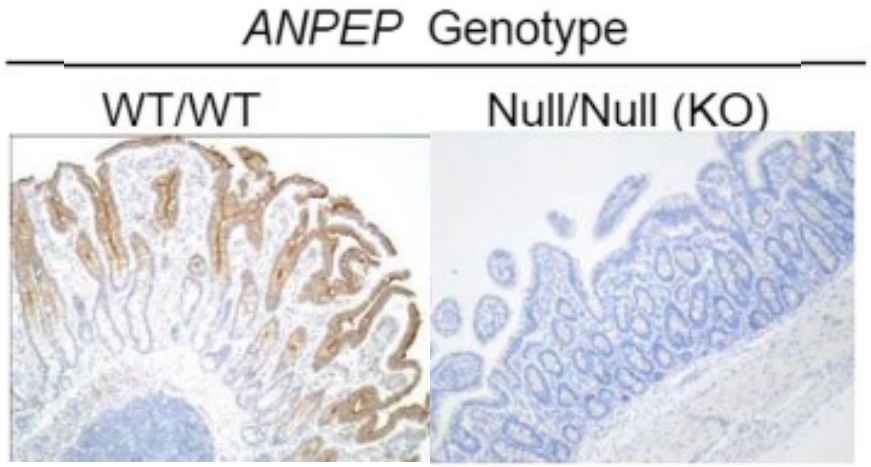
Transgenic Res (2019) 28:21–32
https://doi.org/10.1007/s11248-018-0100-3

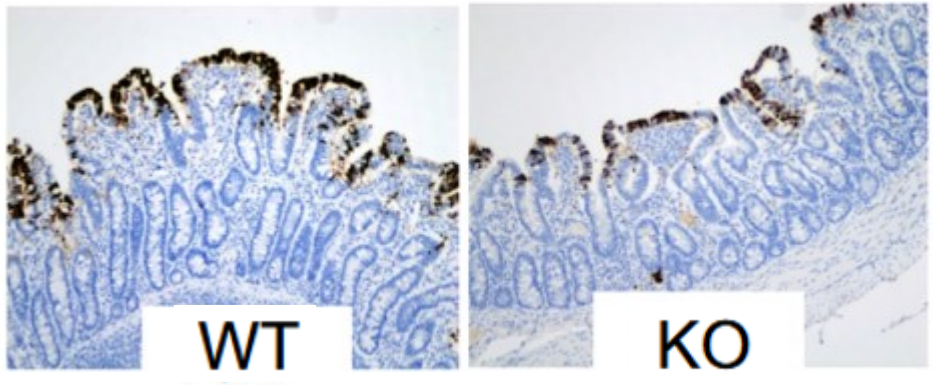
ORIGINAL PAPER

Resistance to coronavirus infection in amino peptidase N-deficient pigs

Kristin M. Whitworth · Raymond R. R. Rowland · Vlad Petrovan · Maureen Sheahan · Ada G. Cino-Ozuna · Ying Fang · Richard Hesse · Alan Mileham · Melissa S. Samuel · Kevin D. Wells · Randall S. Prather

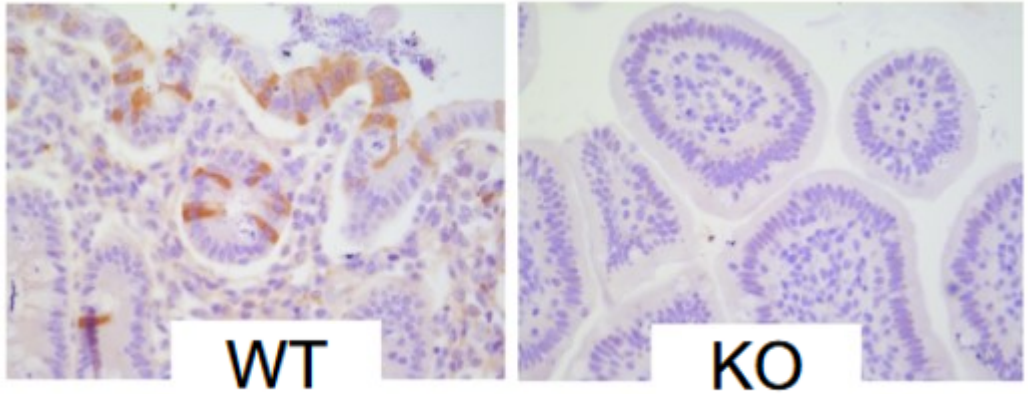


PEDV



Alphacoronaviruses:
Porcine Epidemic Diarrhea Virus (PEDV)
Transmissible Gastroenteritis Virus (TGEV)

TGEV



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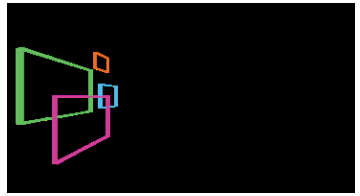
Examples of editing in livestock

1. Muscle mass
2. Allergens
3. Welfare
4. Environmental resilience
5. Diseases
6. Discovery



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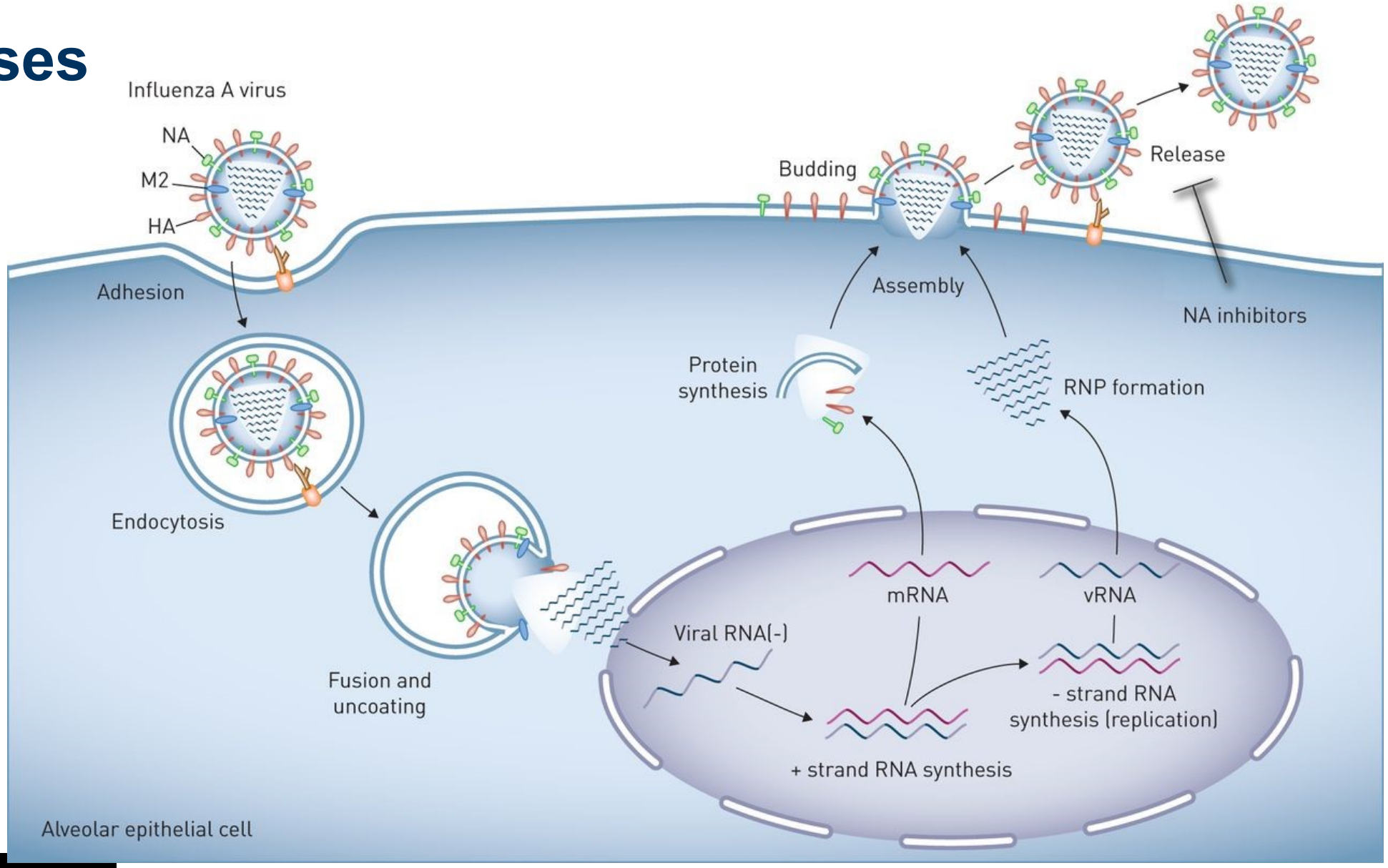
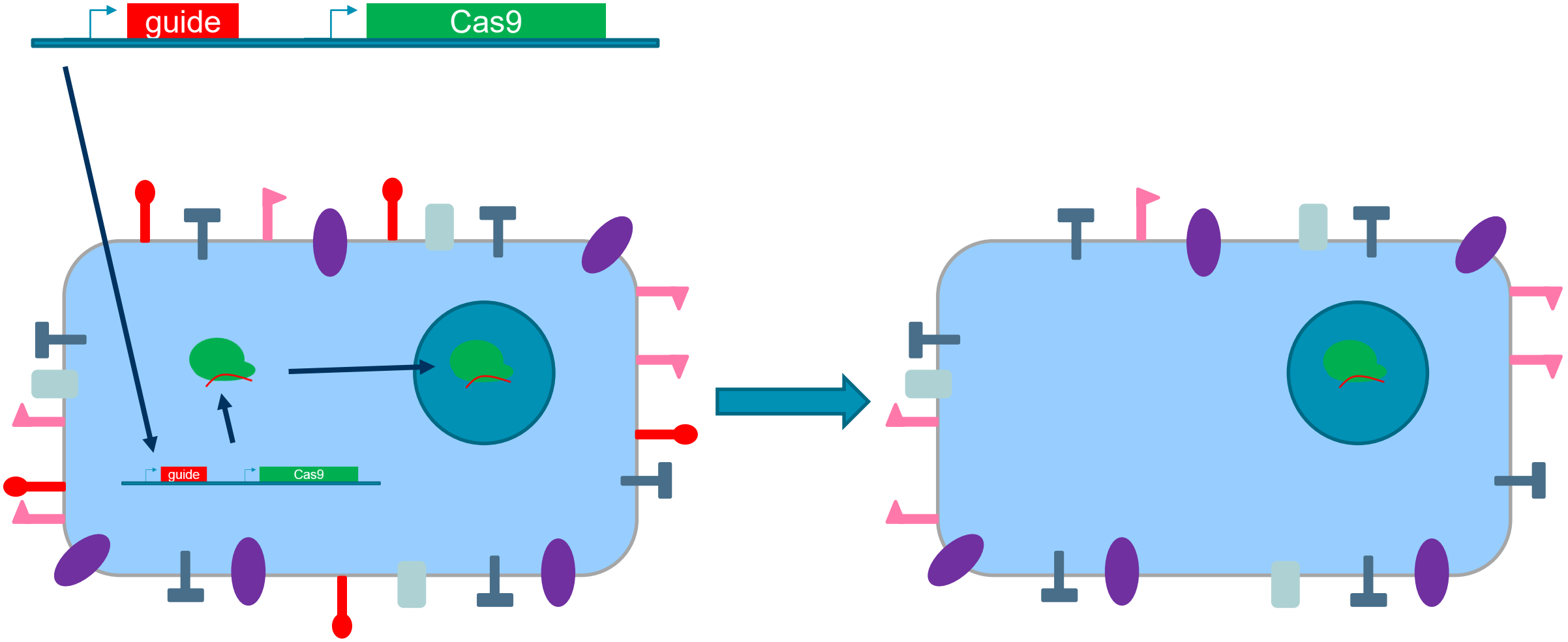
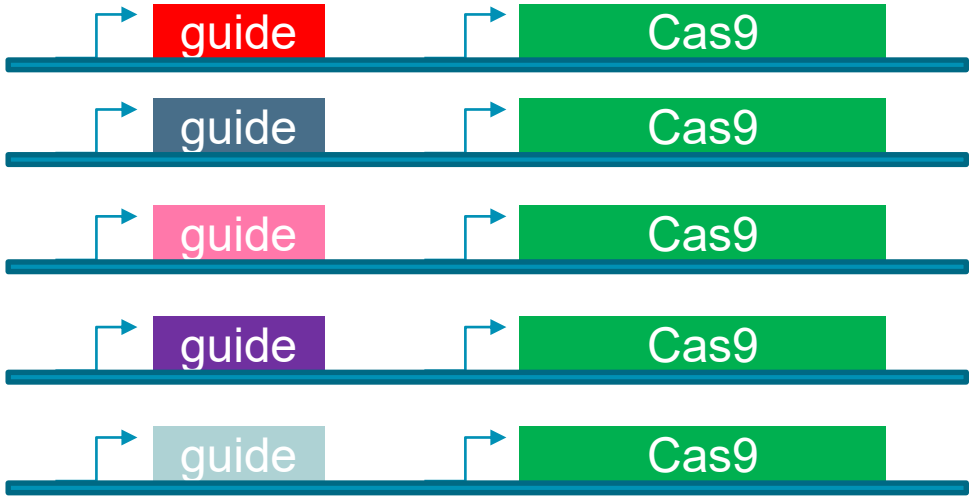
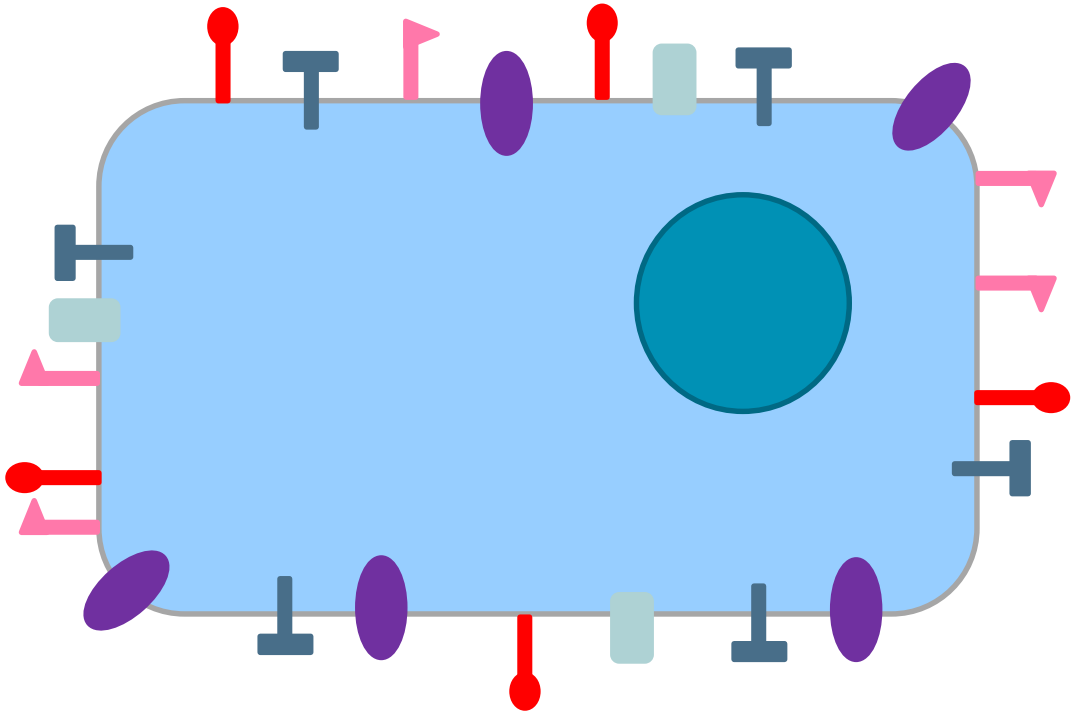
Viruses


Influenza A virus
NA
M2
HA
Adhesion
Endocytosis
Fusion and uncoating
Viral RNA(-)
mRNA
vRNA
+ strand RNA synthesis
- strand RNA synthesis (replication)
Protein synthesis
RNP formation
Assembly
Budding
Release
NA inhibitors
Alveolar epithelial cell



GeCKO
Genome-scale
CRISPR Knock-Out





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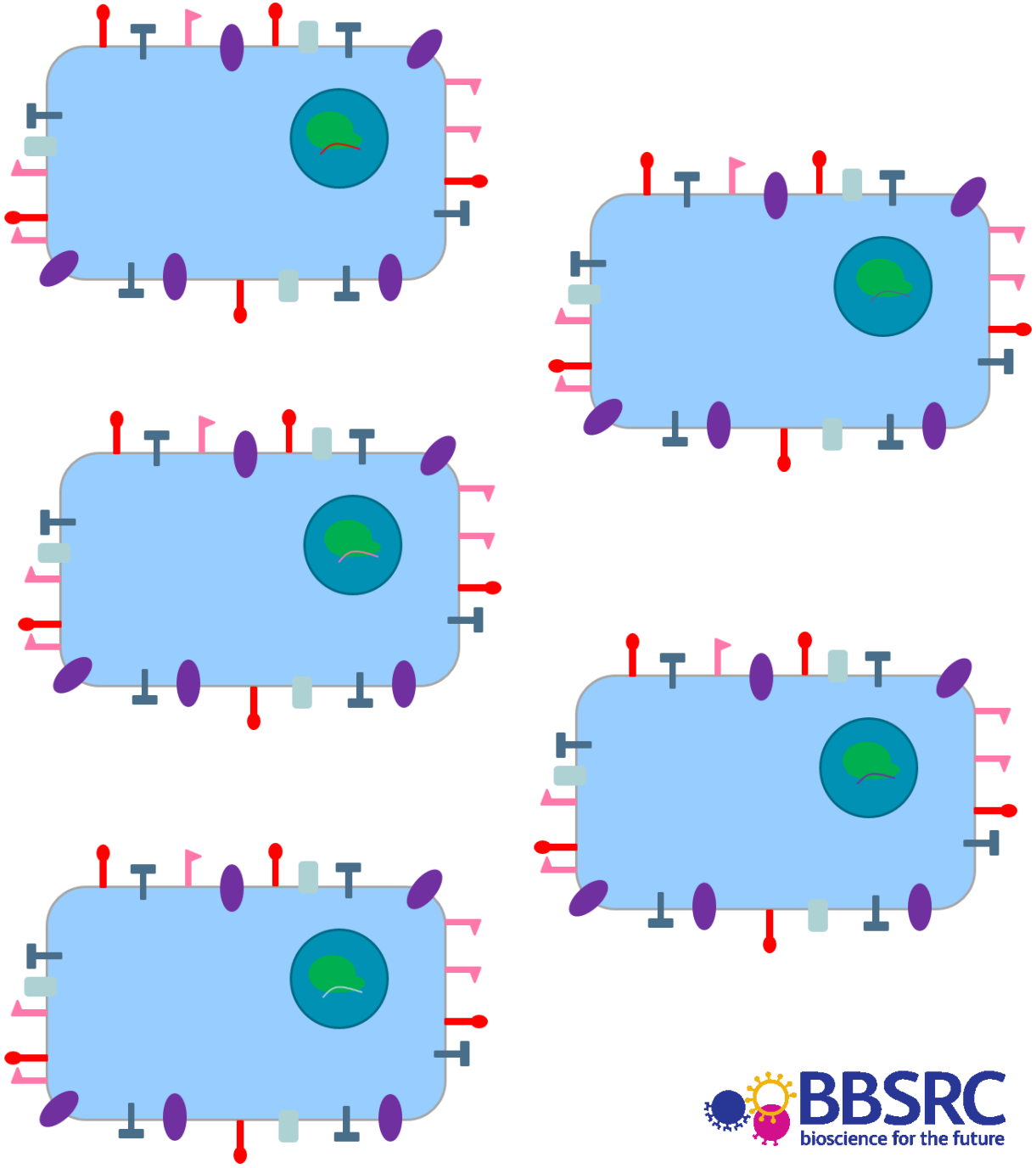

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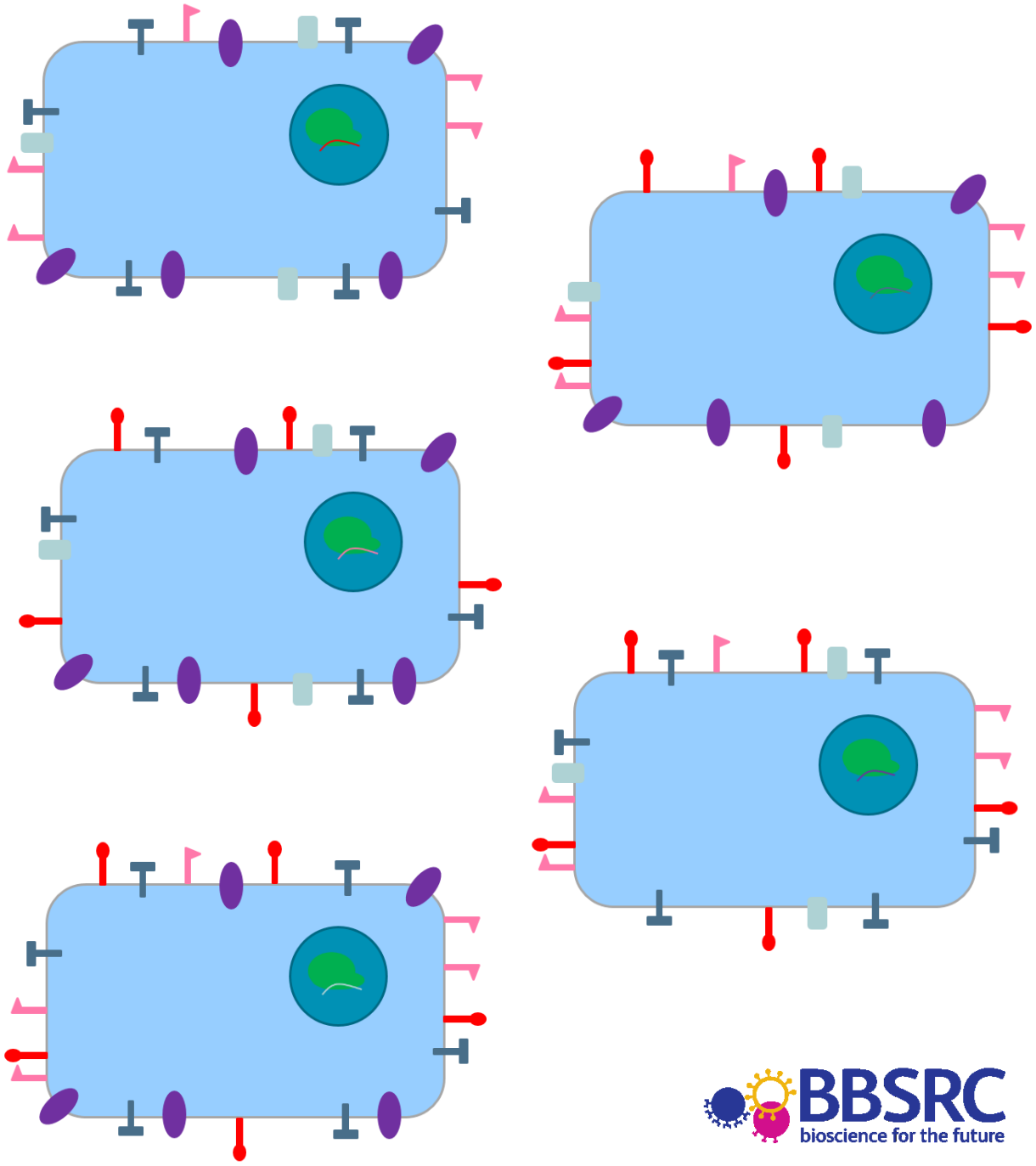

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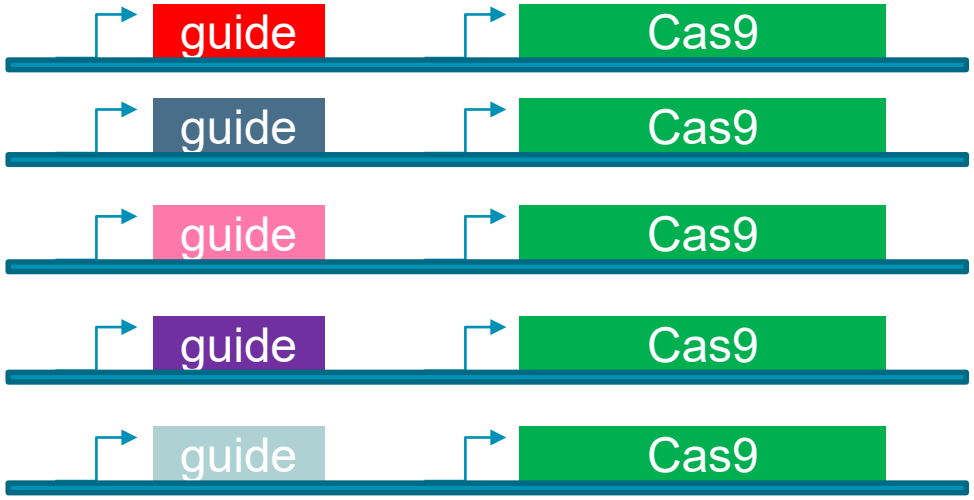

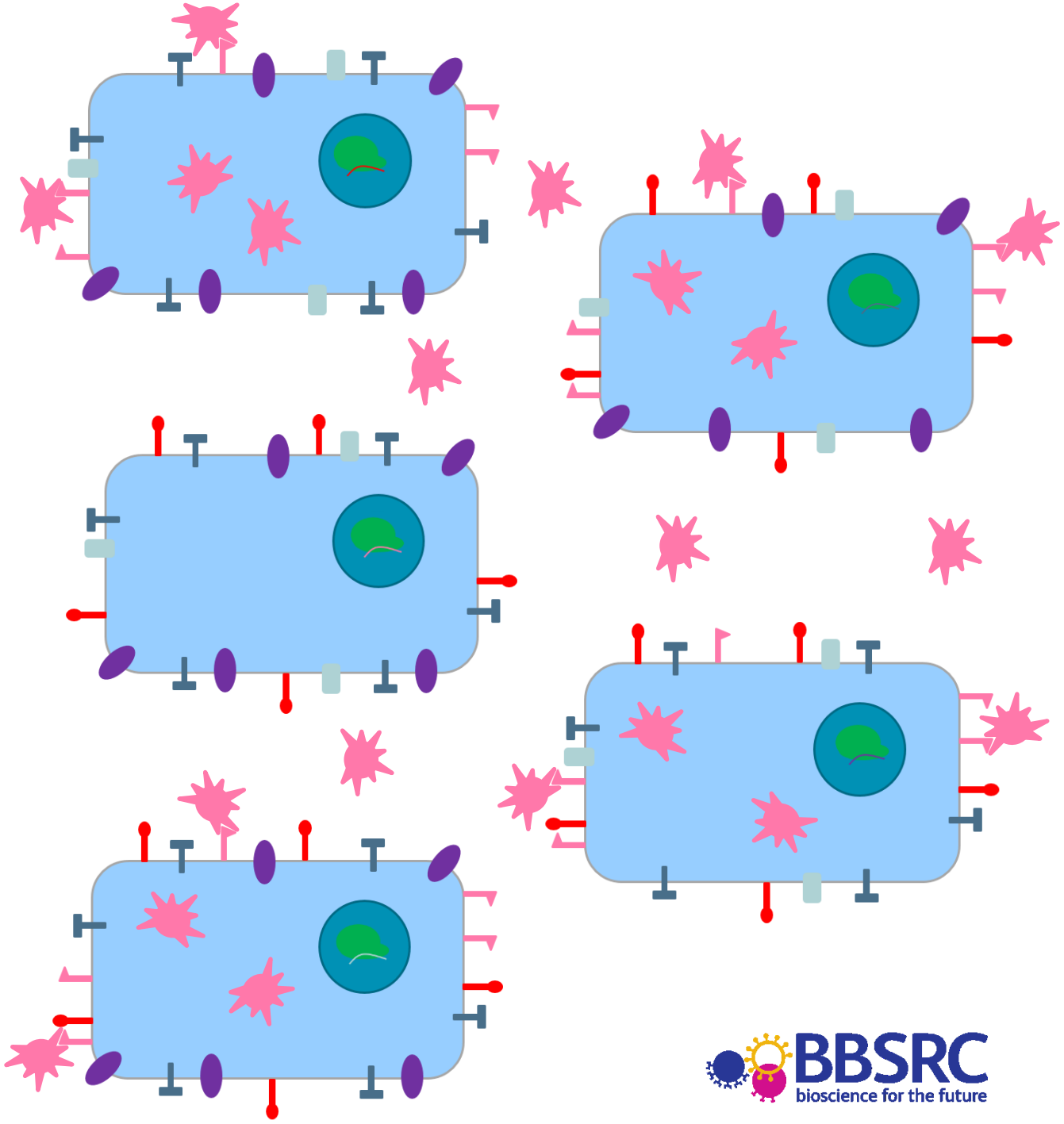

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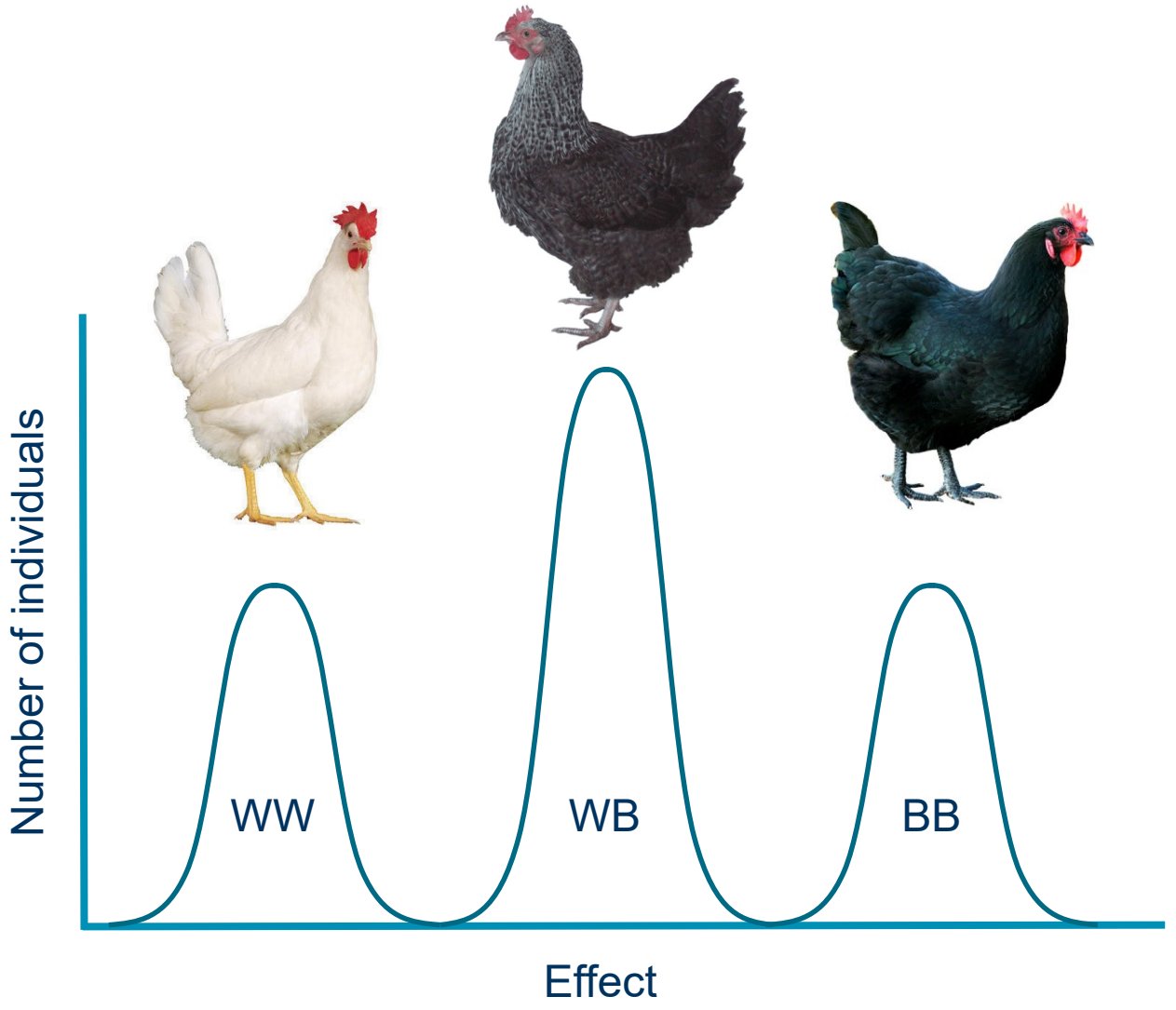
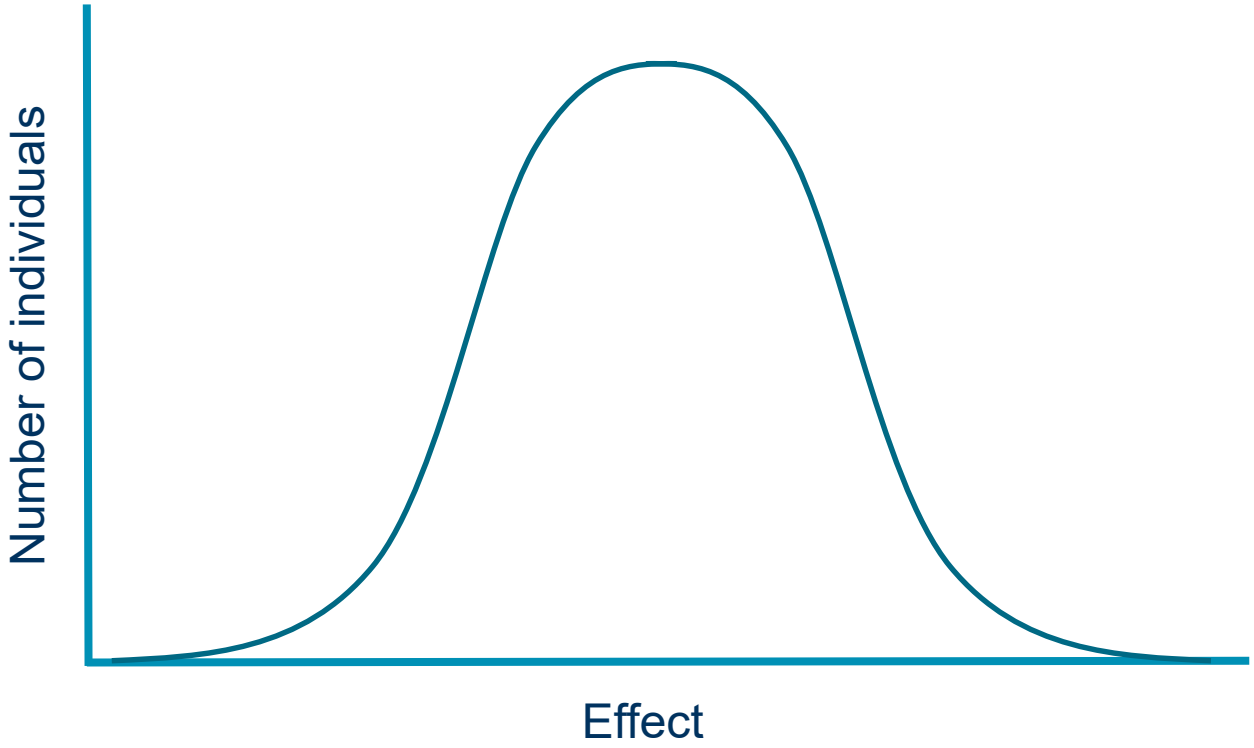
Monogenic traits



Polygenic traits



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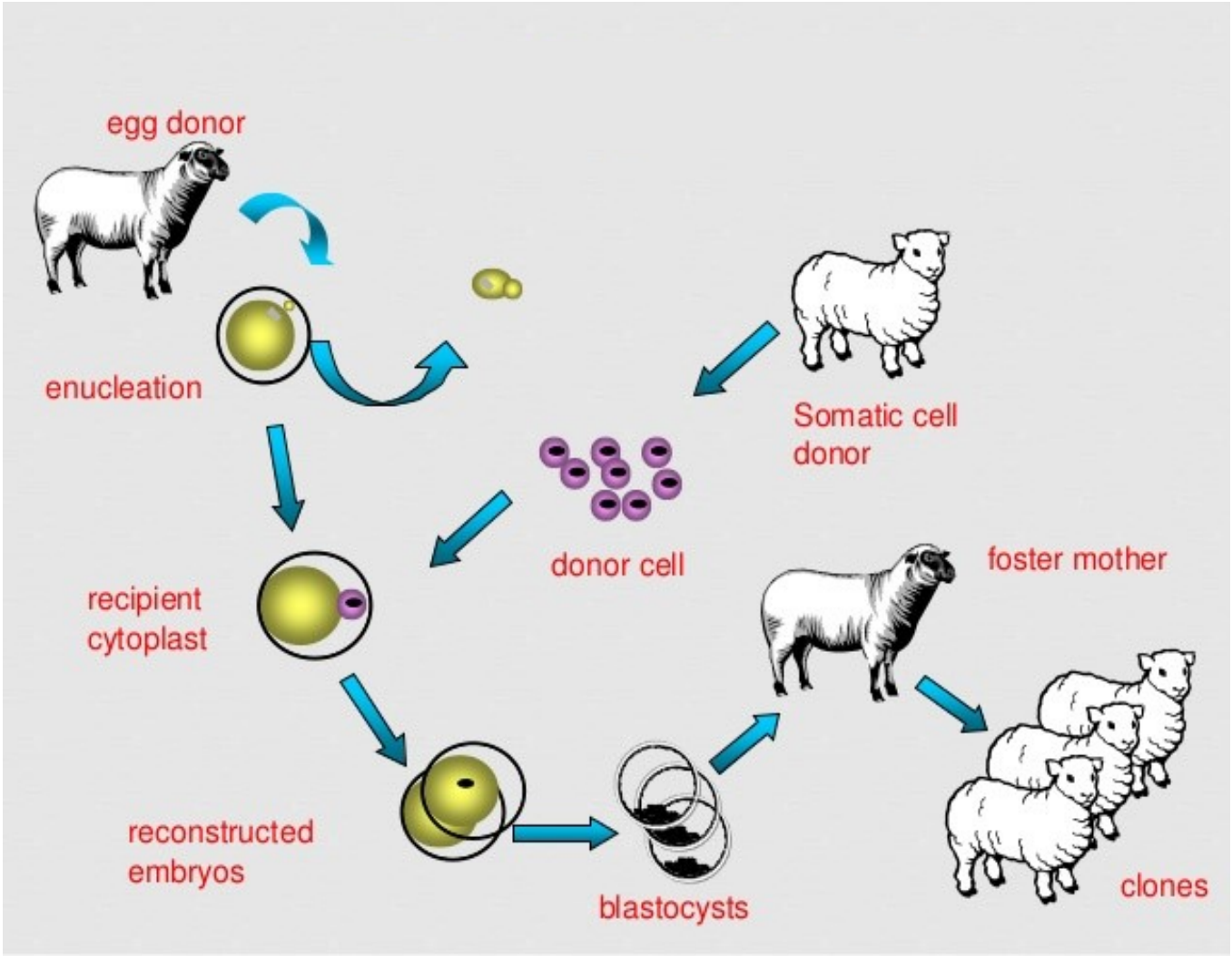
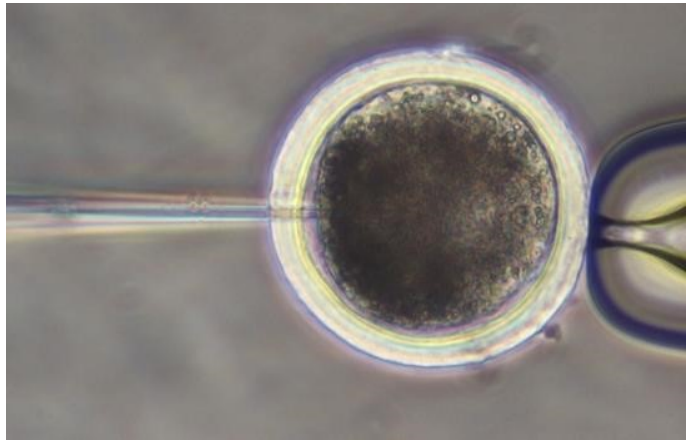
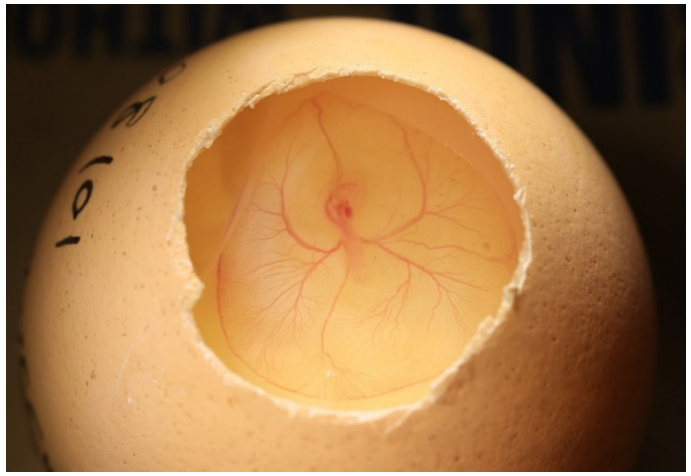
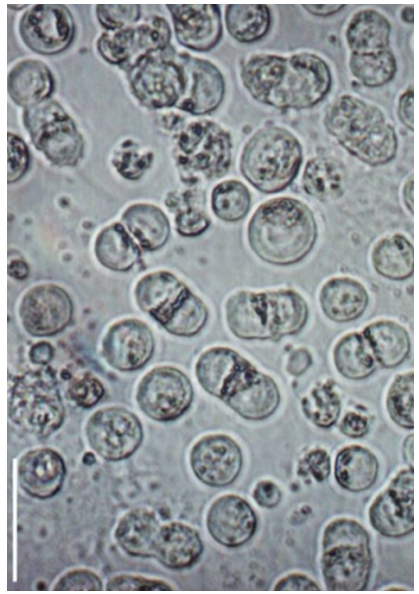
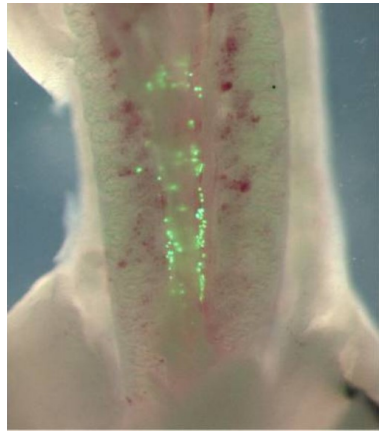
Polygenic traits











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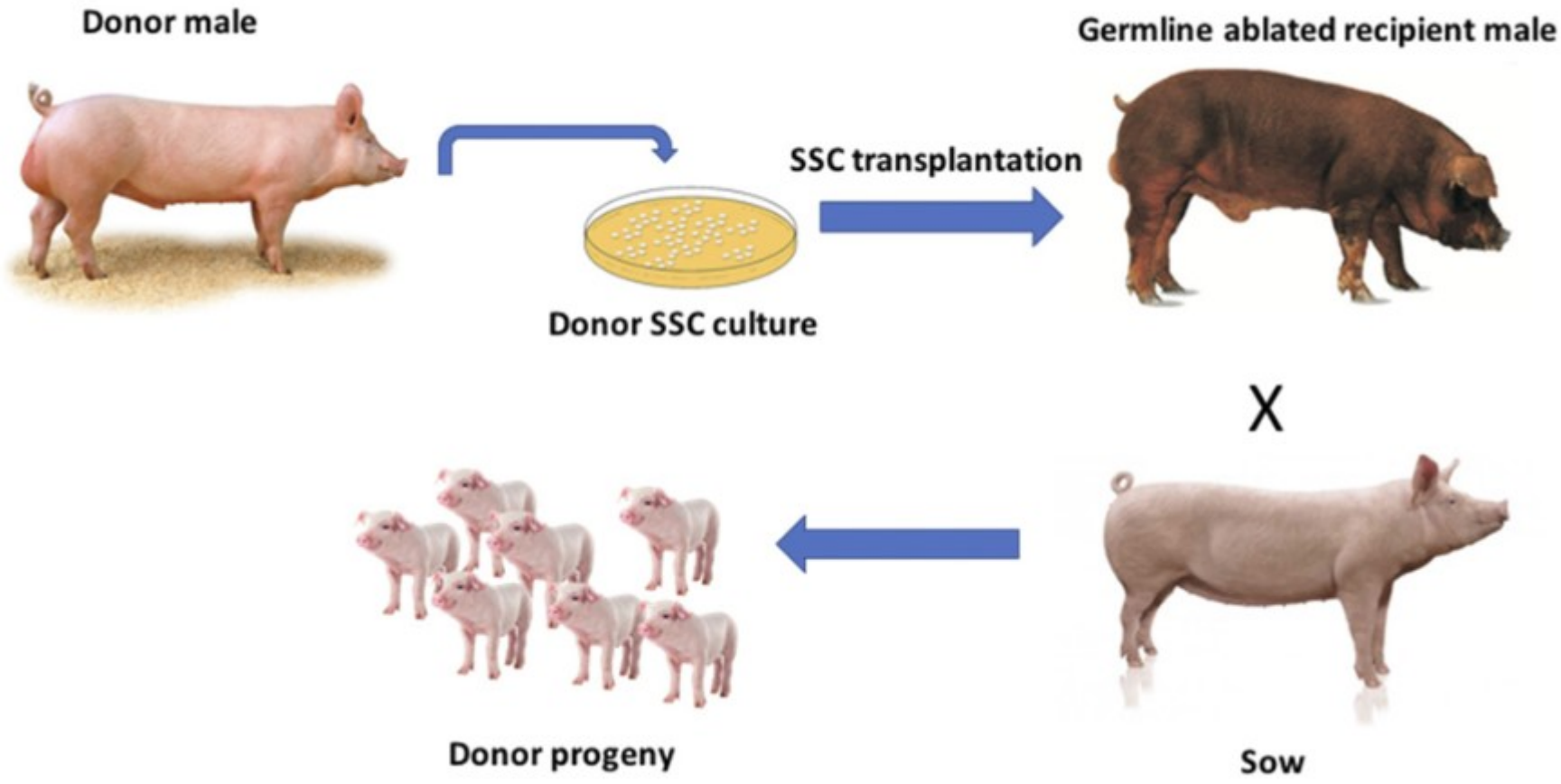
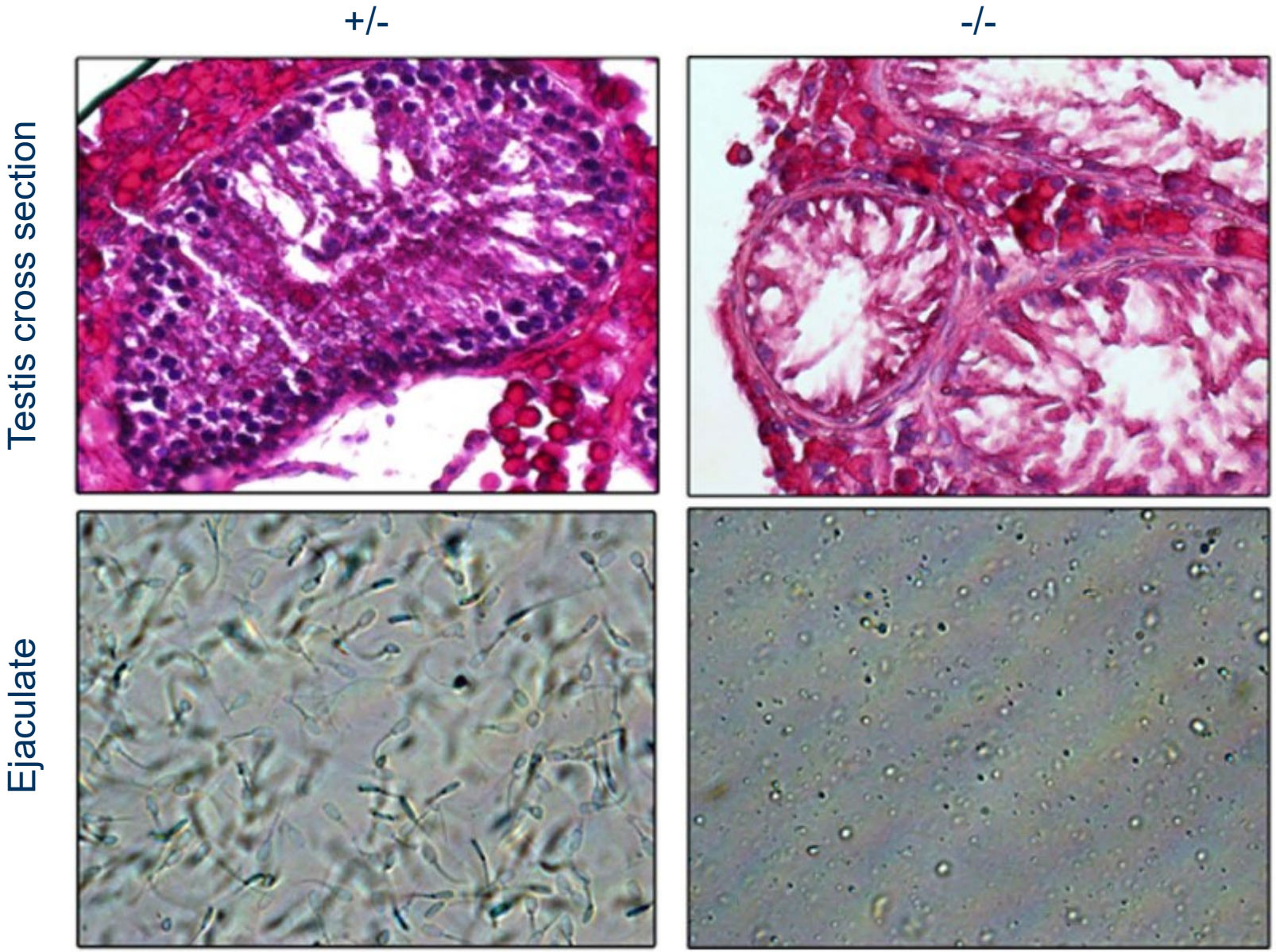
Spermatagonial stem cells

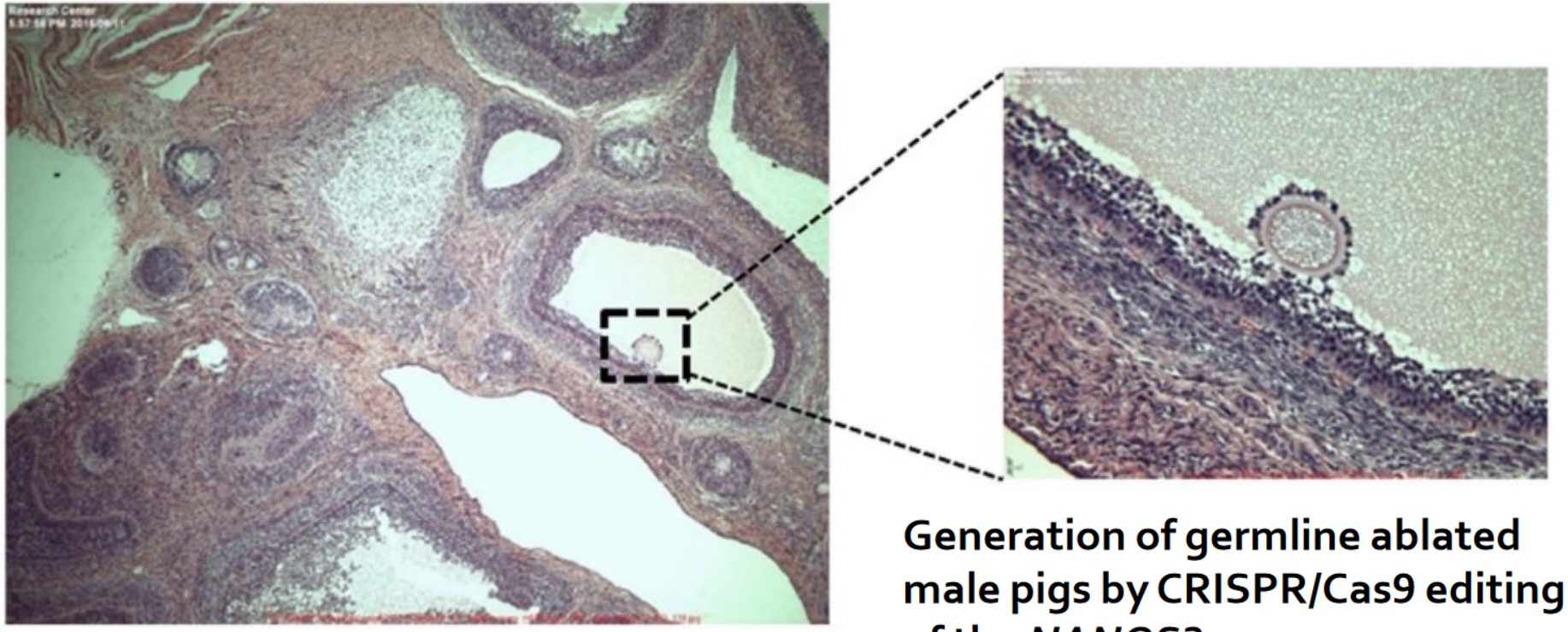


NANOS2 required for male fertility



NANOS2 not required for female fertility

NANOS2 is dispensable for germline development and fertility in female pigs



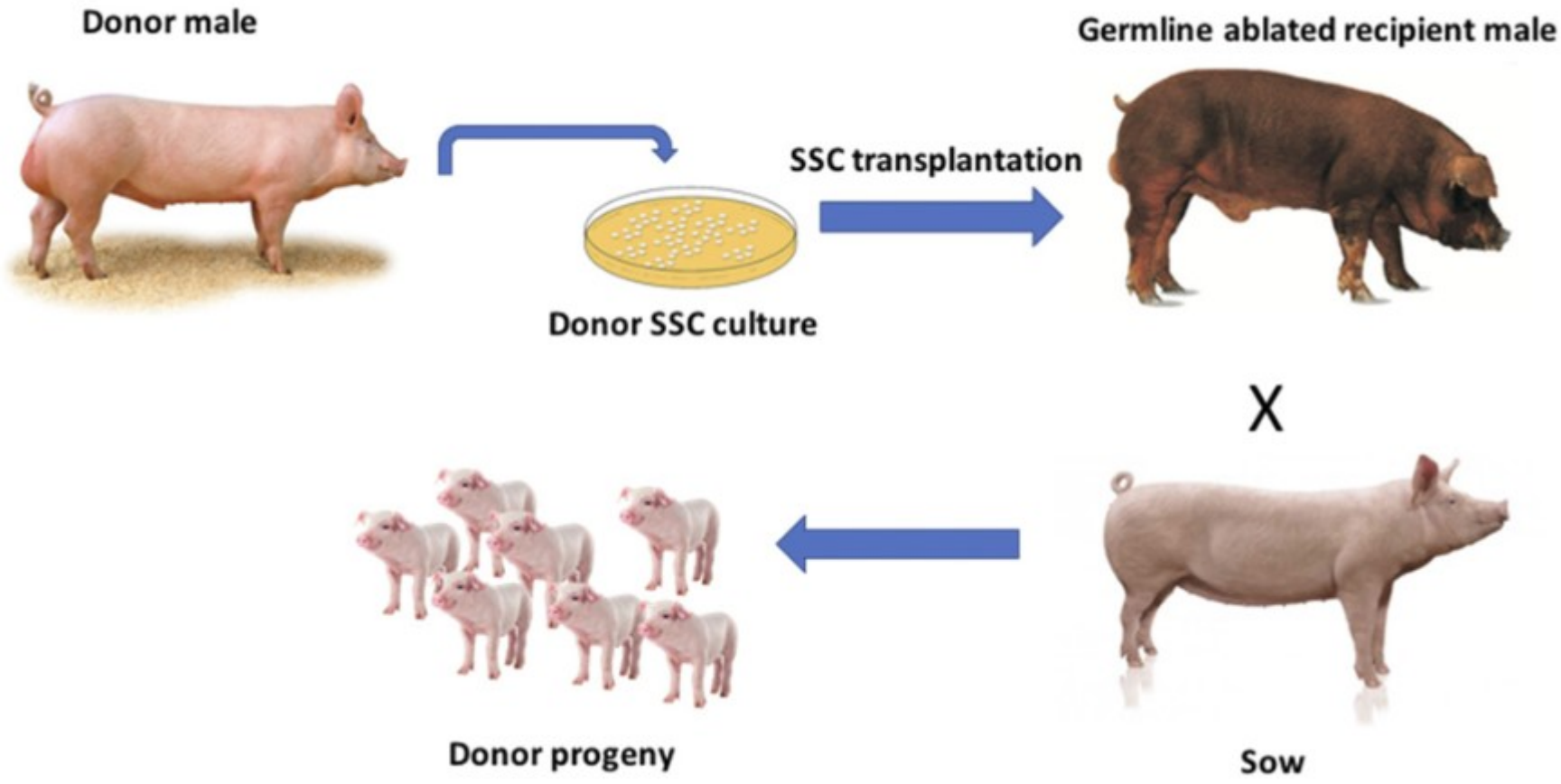
Generation of germline ablated male pigs by CRISPR/Cas9 editing of the *NANOS2* gene

Ki-Eun Park[1,2,3,*], Amy V. Kaucher[4,*], Anne Powell[2], Muhammad Salman Waqas[4], Shelley E.S. Sandmaier[1,2], Melissa J. Oatley[4], Chi-Hun Park[1,2], Ahmed Tibary[4], David M. Donovan[2], Le Ann Blomberg[2], Simon G. Lillico[5], C. Bruce A. Whitelaw[5], Alan Mileham[6], Bhanu P. Telugu[1,2,3] & Jon M. Oatley[4]

SCIENTIFIC REPORTS | 7:40176 | DOI: 10.1038/srep40176

Cross -/- females with -/+ males to propagate

Spermatagonial stem cells



Public acceptance

There is no such thing as THE public