56th Annual BIF Research Symposium and Convention

Young Producers Symposium

Biotechnology 101 – A practical, producer guide to gene editing and vaccinology

Jon Beever, PhD

June 10, 2024

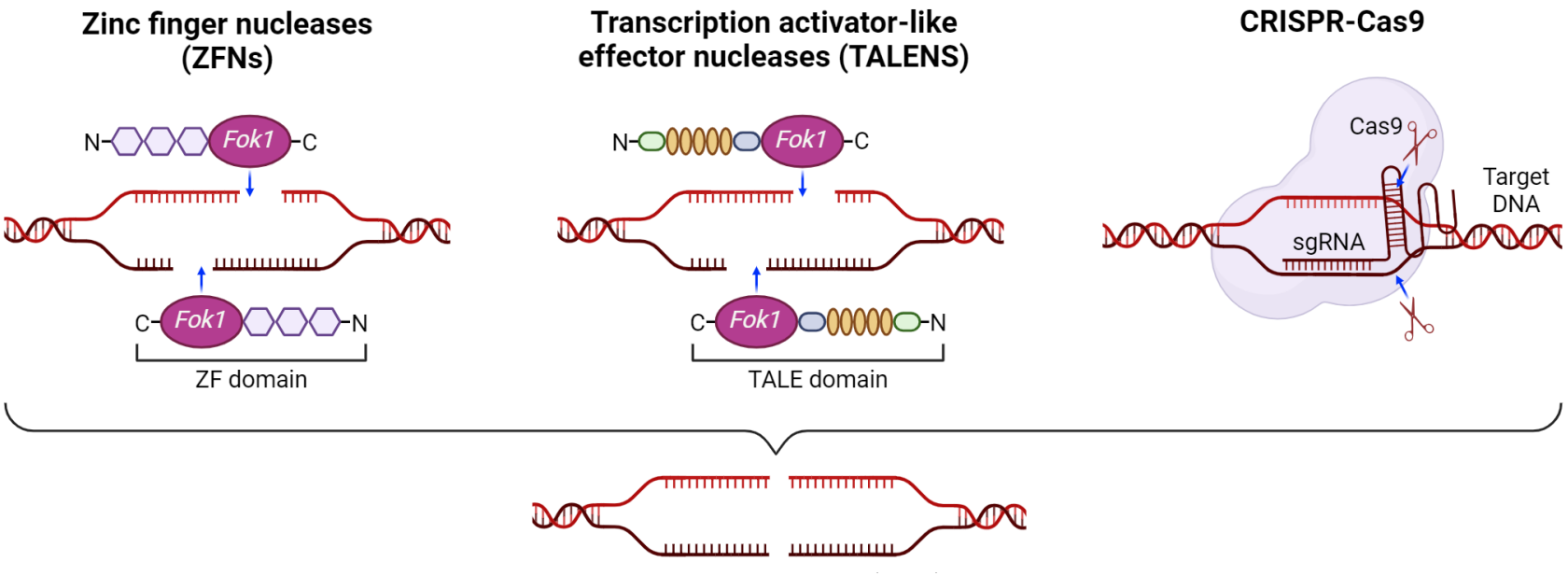
Genome(Gene) Editing

- What is gene editing?
- How is gene editing performed?
- Gene editing and Advanced Reproductive Technologies (ARTs)
- Examples of livestock gene editing
- Regulation of gene edited animals

Genome(Gene) Editing

- The use of modern molecular biology technologies to precisely change the DNA or "genetic blueprint" of an organism
  - Advances in the precision of targeting versus previous technologies
  - Higher efficiency of successful edits
  - Amenable to direct editing in germ cells and embryos

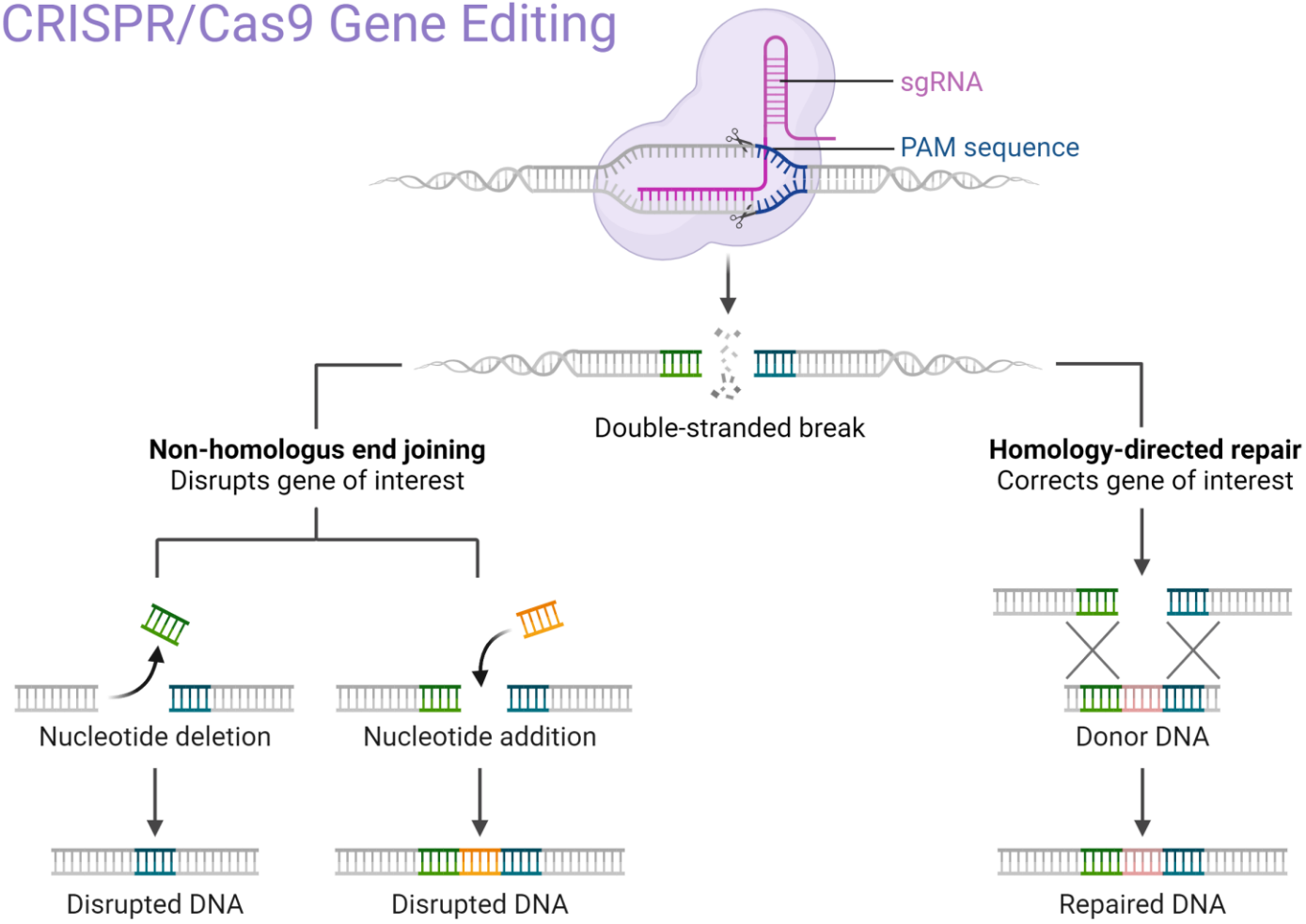
Genome-Editing Technologies



The presence of DSBs in a cell initiate a response from the cell's own repair mechanisms to assist in performing the edit

BioRender

CRISPR/Cas9 Gene Editing



BioRender

Gene Editing and ARTs

- In what cells do you perform gene editing?

- Somatic cells (e.g., fibroblasts)
  - *In vitro* culture of cells during editing
  - Allows preselection of edit prior to producing live offspring
  - Requires somatic cell nuclear transfer (SCNT) or cloning to produce a living edited animal

- Gametes, Embryos or Embryonic Stem Cells (ESCs)
  - Higher "success" rate than SCNT produced animals
  - Little opportunity to select for specific edits
    - Some animals will not have edits
  - Potential for the production of chimeric animals (embryos)

Transgenic Res (2015) 24:147–153
DOI 10.1007/s11248-014-9832-x

ORIGINAL PAPER

Genome edited sheep and cattle

Fig. 3 The *MSTN* editing events. An alignment of the bovine and ovine WT sequences and the alleles present in each of the edited animals. The TALEN binding sites are highlighted on the WT sequences, the ovine mismatch is *underlined* and the corresponding amino acid change is indicated on the *right*

Fig. 2 *MSTN* edited animals. **a** The live born bull (bull #1: *left*) and heifer calf (*right*). **b** The readily observed phenotypic difference between bull #1 (*right*) and the wild-type heifer (*left*). **c** The edited lamb

Precise gene editing paves the way for derivation of *Mannheimia haemolytica* leukotoxin-resistant cattle

Sudarvili Shanthalingam[a], Ahmed Tibary[b], Jonathan E. Beever[c], Poothapillai Kasinathan[d], Wendy C. Brown[a], and Subramaniam Srikumaran[a,1]

[a]Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164; [b]Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA 99164; [c]Department of Animal Sciences, University of Illinois, Urbana–Champaign, IL 61801; and [d]TransOva-ViaGen, Sioux Falls, SD 57106

Edited by Roy Curtiss III, University of Florida, Gainesville, FL, and approved September 29, 2016 (received for review August 11, 2016)

F1000Research F1000Research 2018, 7:1985 Last updated: 09 APR 2019

Check for updates

RESEARCH ARTICLE

A bovine CD18 signal peptide variant with increased binding activity to *Mannheimia hemolytica* leukotoxin [version 1; peer review: 3 approved]

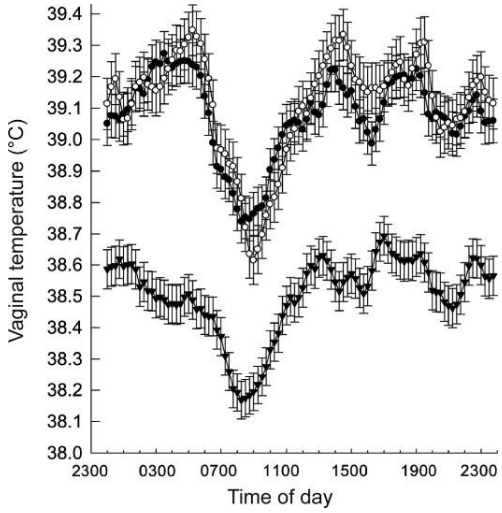
Aspen M. Workman [1], Carol G. Chitko-McKown[1], Timothy P. L. Smith[1], Gary L. Bennett [1], Theodore S. Kalbfleisch[2], Veronica Basnayake[3], Michael P. Heaton [1]

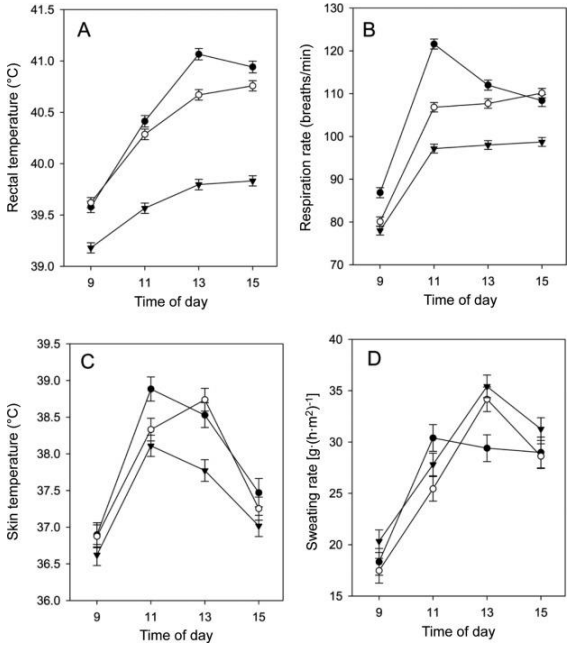
[1]USDA, US Meat Animal Research Center (USMARC), Clay Center, Nebraska, 68933, USA
[2]Department of Biochemistry and Molecular Genetics, School of Medicine, University of Louisville, Louisville, Kentucky, 40292, USA
[3]GeneSeek, a Neogen Company, Lincoln, NE, USA

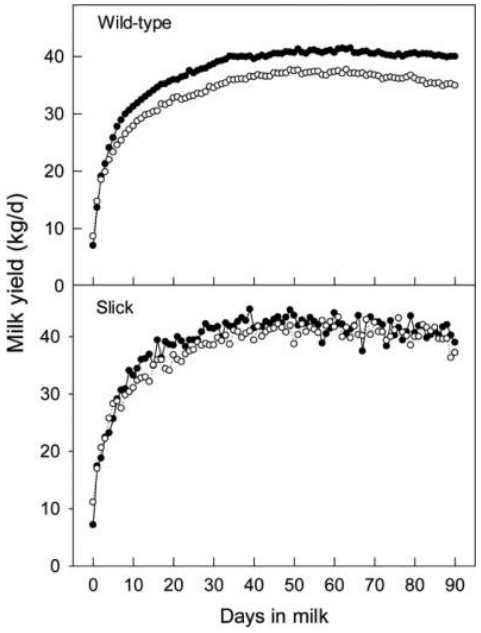
J. Dairy Sci. 97:5508–5520
http://dx.doi.org/10.3168/jds.2014-8087

The *SLICK* hair locus derived from Senepol cattle confers thermotolerance to intensively managed lactating Holstein cows

S. Dikmen,* F. A. Khan,†[1] H. J. Huson,‡[2] T. S. Sonstegard,‡ J. I. Moss,† G. E. Dahl,† and P. J. Hansen†[3]
*University of Uludag, Faculty of Veterinary Medicine, Department of Animal Science, Bursa 16059, Turkey
†University of Florida, Department of Animal Sciences, Gainesville 32611-0910
‡USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD 20705-2350







US FDA clears the way for CRISPR beef cows

Slick coat cattle produced with gene-editing pose low risk, agency says

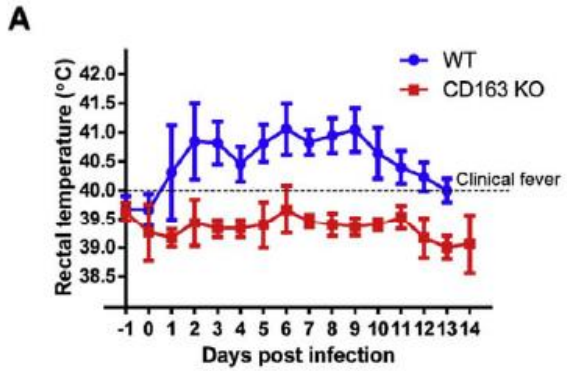
by ***Britt E. Erickson***

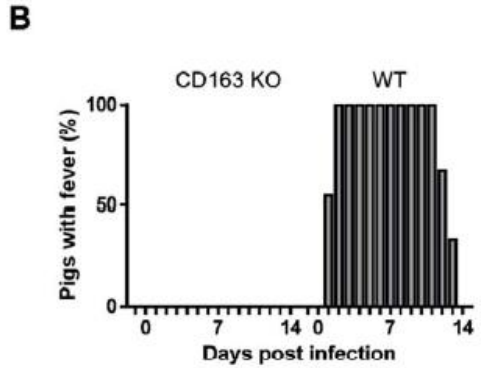
March 16, 2022 | A version of this story appeared in **Volume 100, Issue 10**

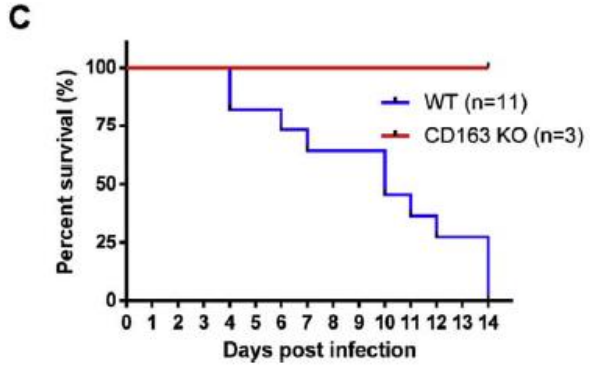
Credit: Shutterstock

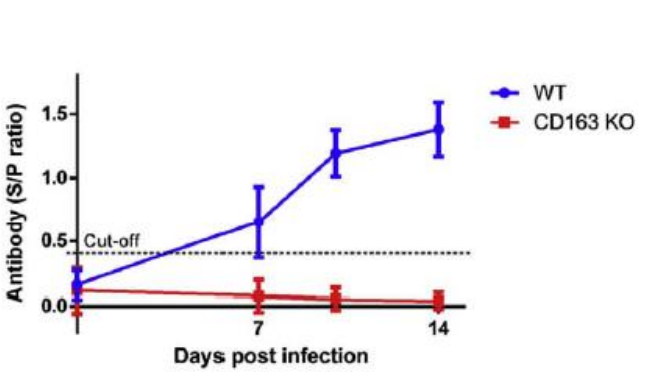
Acceligen has introduced a slick-coat genetic trait, first found in Senepol cattle (shown), into beef cattle to improve their ability to tolerate warm weather.

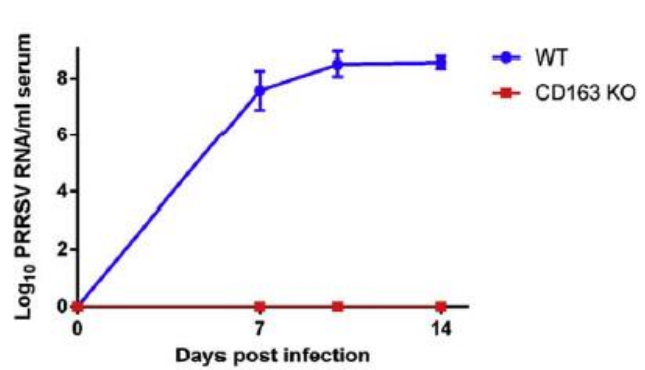
The Food and Drug Administration has paved the way for gene-edited beef to hit the US market. The agency **declared March 7** that two gene-edited beef cattle produced by Acceligen do not raise any safety concerns.


A
WT
CD163 KO
Rectal temperature (°C)
42.0
41.5
41.0
40.5
40.0
39.5
39.0
38.5
Clinical fever
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post infection


B
CD163 KO
WT
Pigs with fever (%)
100
50
0
0 7 14 0 7 14
Days post infection


C
Percent survival (%)
100
75
50
25
0
WT (n=11)
CD163 KO (n=3)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days post infection


D
Antibody (S/P ratio)
1.5
1.0
0.5
0.0
Cut-off
WT
CD163 KO
7
14
Days post infection


E
Log10 PRRSV RNA/ml serum
8
6
4
2
0
WT
CD163 KO
0
7
14
Days post infection


ANTIVIRAL RESEARCH


Check for updates

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

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

Nature Biotechnology **34**, 20–22 (2016) | Cite this article

Agreement targets PRRS-resistant gene-edited pigs

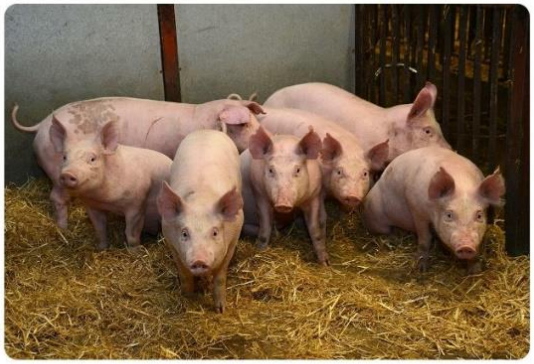
Researchers and commercial partners to continue collaboration on developing pigs resistant to Porcine Reproductive and Respiratory Syndrome.

17 September 2021 2 minute read By: The Roslin Institute
Europe North America Asia

The Roslin Institute and animal genetics company Genus have signed an agreement to produce pigs that are resistant to a respiratory disease which costs around $2.5 billion each year in the US and Europe alone.

Researchers and the company hope the licensing agreement will lead the way to gene-edited, disease-resistant pigs being available to global pork-producing markets.

With the signing of the agreement, facilitated by Edinburgh Innovations, the University's commercialisation service, Genus will continue planned work for testing multiple generations of pigs and conducting studies required for approval by the US Food and Drug Administration (FDA).

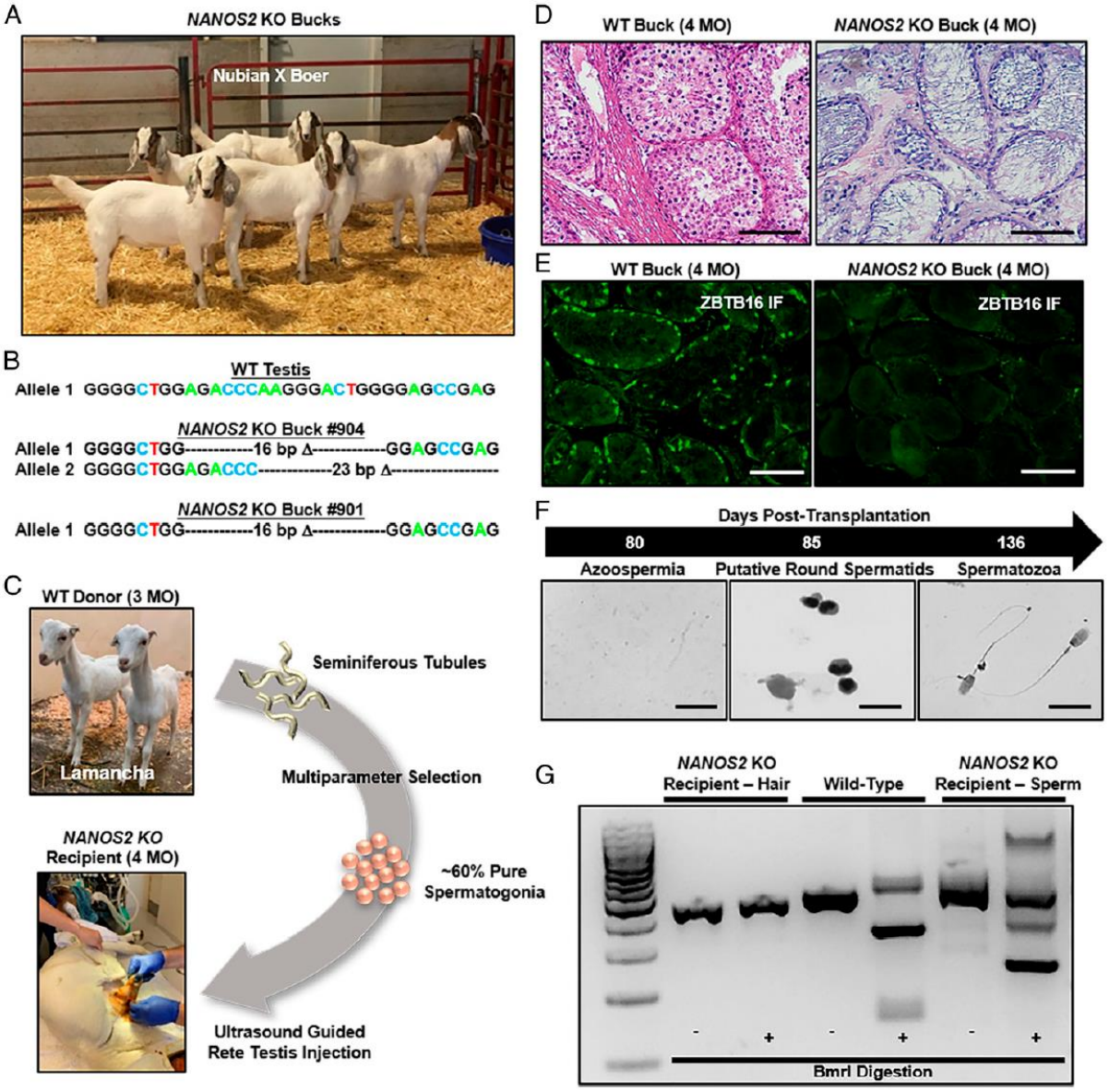


Donor-d … transpla … n cell … ales

Michela Ciccarelli… Blanca Lopez-Bila… Chi-Hun Park[f], Ki… and Jon M. Oatle…

[a]Center for Reproduct… Medicine, Washingtor… Pullman, WA 99164; [d]… [e]Center for Tropical Li… Sciences, University of… Sciences, Utah State U…

…llico[d,e], …olejaeva[g], …llege of Veterinary …ton State University, … United Kingdom; … Animal and Avian …iculture and Applied



https://www.fda.gov/animal-veterinary/biotechnology-products-cvm-animals-and-animal-food/intentional-genomic-alterations-igas-animals

U.S. FOOD & DRUG ADMINISTRATION

Intentional Genomic Alterations (IGAs) in Animals

- **Animal Drugs, Devices and Animal Foods are regulated by the Food and Drug Administration (FDA) under the Federal Food, Drug and Cosmetic Act (FFDCA)**

IGAs in animals are changes to an animal's genomic DNA produced using modern molecular technologies, which may include random or targeted DNA sequence changes including nucleotide insertions, substitutions, or deletions. The IGA can be introduced into the animal's genome using recombinant DNA, genome editing, or other technologies. IGAs in animals have many different intended uses, including applications in human health (e.g., reduced allergenicity; "biopharm" animals that produce substances (generally in their milk or eggs) for use in the production of human therapeutics; animals used to model human disease), in improved animal health, well-being, and husbandry practices (e.g., disease resistance, heat tolerance), and in enhanced production and food quality (e.g., faster growth, feed efficiency, nutritional benefits).

Guidance for Industry (GFI) #187A and #187B

In May 2024, FDA CVM released GFI #187A "Heritable intentional Genomic Alterations in Animals: Risk Based Approach" and draft GFI #187B "Heritable Intentional Genomic Alterations in Animals: The Approval Process." GFI #187A describes FDA's risk-based regulatory approach to the oversight of heritable IGAs in animals and draft GFI #187B describes how the approval process applies to heritable IGAs in animals. FDA CVM plans to finalize GFI #187B in the future based on feedback from stakeholders.

Information for Developers and Consumers

CVM is committed to engaging with industry, academia, animal owners/producers, and other stakeholders to increase the transparency of our regulatory process. You can find additional information on the regulation of IGAs in animals below.

- EPA, FDA, and USDA Issue Joint Regulatory Plan for Biotechnology
- CVM's FDA-TRACK goals for emerging technologies
- Questions and Answers (Q&A) on FDA regulation of IGAs in animals
- Q&A for Developers of Intentional Genomic Alterations in Animals
- Q&A on Intentional Genomic Alterations in Animals for Consumers
- The Unified Website for Biotechnology Regulation

GUIDANCE DOCUMENT

CVM GFI #187A Heritable Intentional Genomic Alterations in Animals: Risk-Based Approach

MAY 2024

Download the Final Guidance Document | Read the Federal Register Notice

Final

Share | Post | Linkedin | Email | Print

Docket Number: FDA-2008-D-0394

Issued by: Center for Veterinary Medicine

This guidance clarifies FDA's requirements and recommendations with respect to heritable intentional genomic alterations (IGAs) in animals.

IGAs in animals are intentional genomic alterations made using modern molecular technologies, which may include random or targeted DNA sequence changes including

GUIDANCE DOCUMENT

CVM GFI #187B Heritable Intentional Genomic Alterations in Animals: The Approval Process

MAY 2024

Download the Draft Guidance Document | Read the Federal Register Notice

Draft

Not for implementation. Contains non-binding recommendations.

This guidance is being distributed for comment purposes only.

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Submit Comments by 07/31/2024

Submit Comments Online

Although you can comment on any guidance at any time (see 21 CFR 10.115(g)(5)), to ensure that the FDA considers your comment on a draft guidance before it begins work on the final version of the guidance, submit either online or written comments

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cvm-gfi-187b-heritable-intentional-genomic-alterations-animals-approval-process

MOU 225-24-010

Memorandum of Understanding between the U.S. Department of Agriculture and the U.S. Department of Health and Human Services Food and Drug Administration

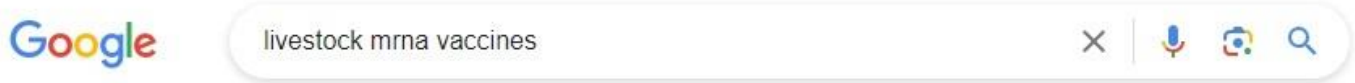
concerning information sharing and regulatory cooperation related to intentional genomic alterations in animals subject to USDA jurisdiction.

I. Purpose

This Memorandum of Understanding (MOU) between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services Food and Drug

Vaccinology

- Current topics in vaccine development (mRNA vaccines)
- Why do we vaccinate?
- Types of vaccines
- The Central Dogma of Molecular Biology
- What is an mRNA vaccine?



UT Beef and Forage Center
https://utbeef.tennessee.edu › Articles › Dr. Lew Strickland

Livestock Health: mRNA Vaccine vs Conventional Vaccines

Jun 7, 2023 — Dr. Lew StricklandAssociate Professor and Extension **Livestock** VeterinarianDepartment of **Animal** ScienceP: 865-974-3150 A couple of months ago ...

ABC News - Breaking News, Latest News and Videos
https://abcnews.go.com › Health › wireStory › scientists-t...

Scientists are testing mRNA vaccines to protect cows and ...

May 31, 2024 — The bird flu outbreak in U.S. **dairy cows** is prompting development of new, next-generation **mRNA vaccines** — akin to the shots deployed during ...

Tennessee Farm Bureau
https://tnfarmbureau.org › mrna-vaccines-in-livestock

mRNA Vaccines in Livestock

Jul 27, 2023 — Currently, none of the commonly used **vaccines** licensed by USDA for **cattle** utilize **mRNA** technology. SEQUIVITY, developed by Merck **Animal** Health ...

National Cattlemen's Beef Association
https://www.ncba.org › news-releases › news › details

NCBA Statement Correcting Internet Falsehoods About ...

Apr 5, 2023 — "There are no current **mRNA vaccines** licensed for use in **beef cattle** in the United States. **Cattle** farmers and ranchers do vaccinate **cattle** to ...

OUR COW
Eat better meat

Build Your Box | Curated Boxes | Explore

Check your next delivery day →

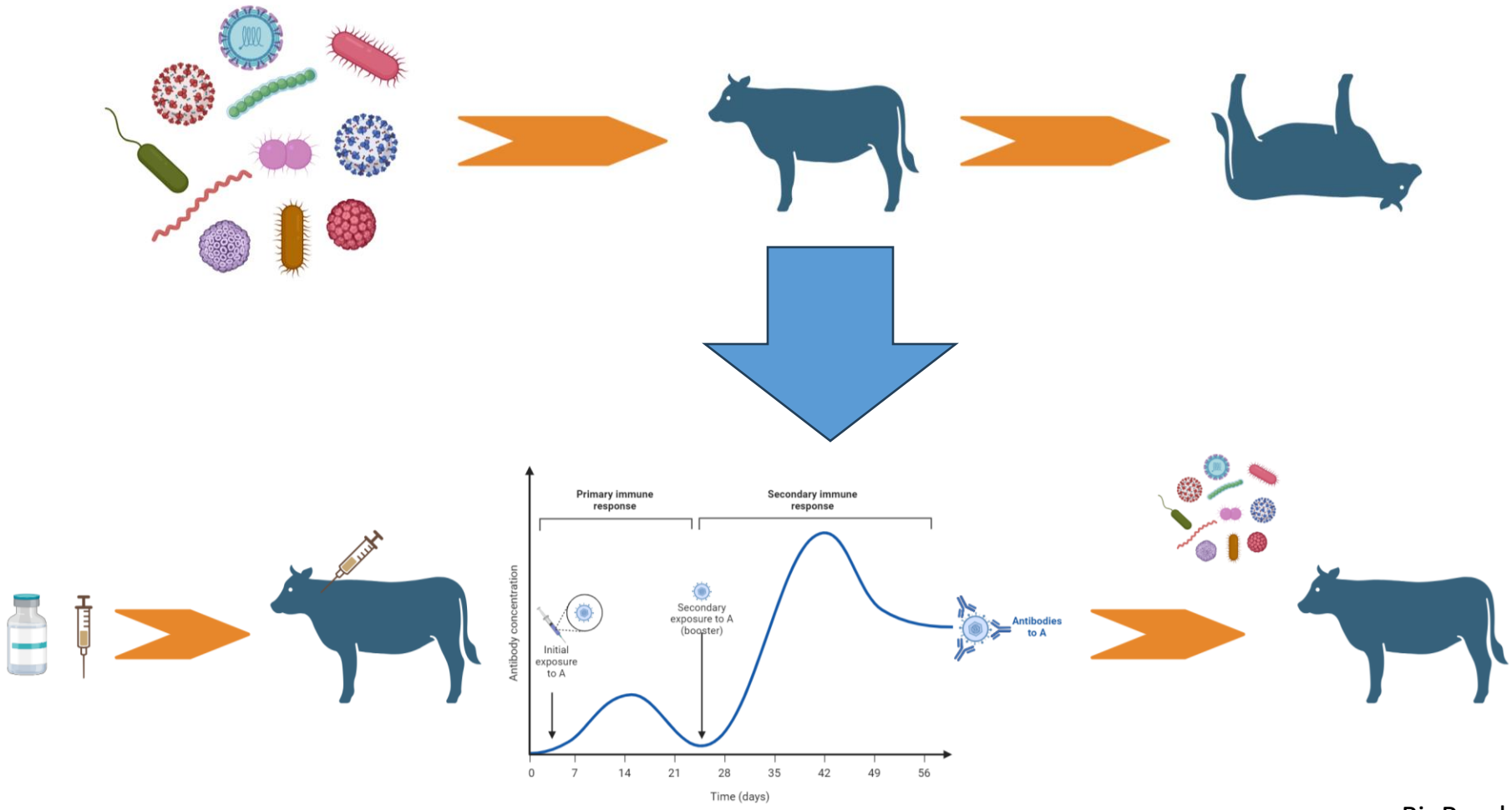
mRNA Free Meat

Friday 22 September 2023 • Share

Understanding the mRNA Vaccine in Australia and Our Commitment to Livestock.

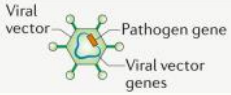
In recent times, the topic of vaccines has taken center stage worldwide. One

Why do we vaccinate?



BioRender

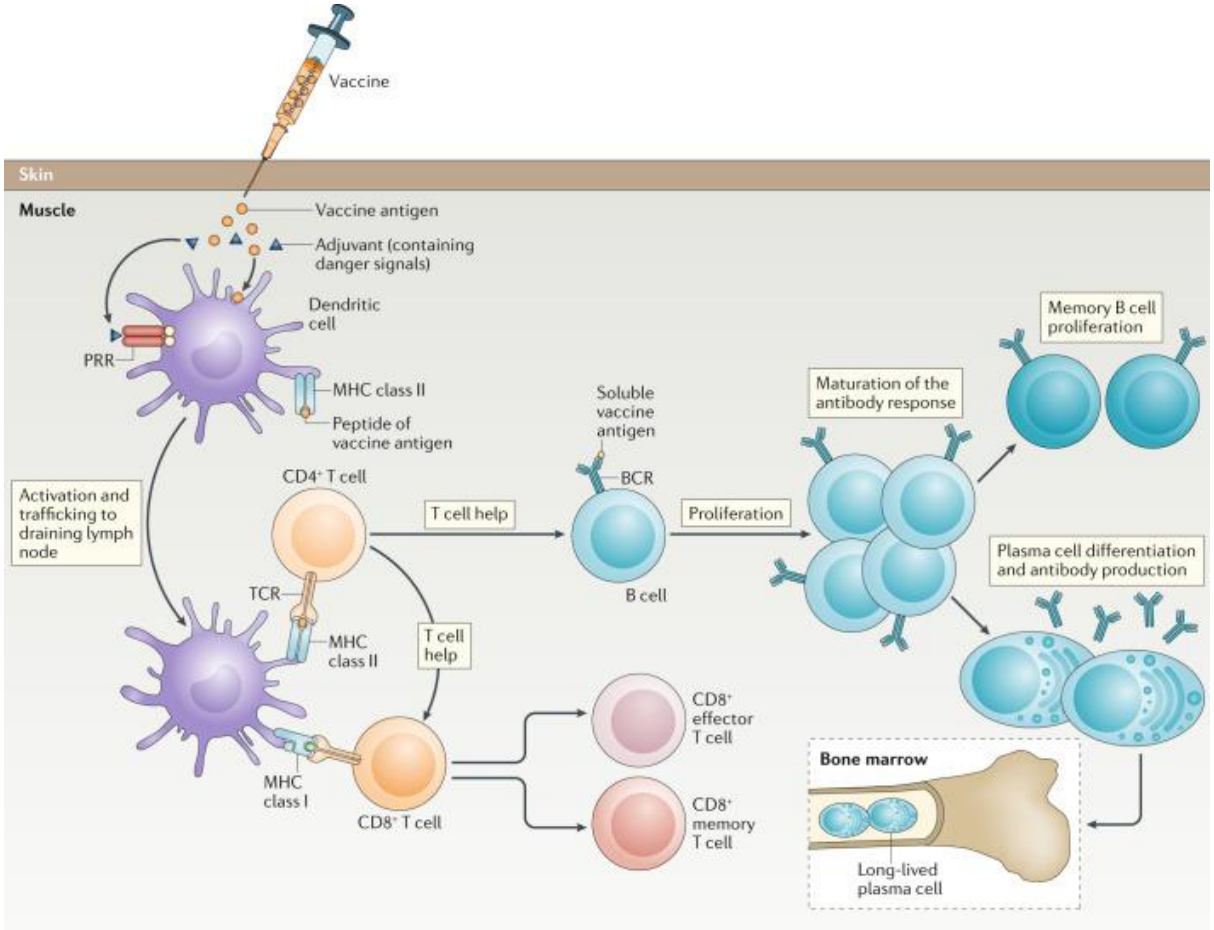
Types of vaccines

Type of vaccine		Licensed vaccines using this technology	First introduced
Live attenuated (weakened or inactivated)		Measles, mumps, rubella, yellow fever, influenza, oral polio, typhoid, Japanese encephalitis, rotavirus, BCG, varicella zoster	1798 (smallpox)
Killed whole organism		Whole-cell pertussis, polio, influenza, Japanese encephalitis, hepatitis A, rabies	1896 (typhoid)
Toxoid		Diphtheria, tetanus	1923 (diphtheria)
Subunit (purified protein, recombinant protein, polysaccharide, peptide)		Pertussis, influenza, hepatitis B, meningococcal, pneumococcal, typhoid, hepatitis A	1970 (anthrax)
Virus-like particle		Human papillomavirus	1986 (hepatitis B)
Outer membrane vesicle		Group B meningococcal	1987 (group B meningococcal)
Protein–polysaccharide conjugate		*Haemophilus influenzae* type B, pneumococcal, meningococcal, typhoid	1987 (*H. influenzae* type b)
Viral vectored		Ebola	2019 (Ebola)
Nucleic acid vaccine		SARS-CoV-2	2020 (SARS-CoV-2)

- Vaccines are not new
- Modified Live
  - Mostly viral
  - Non-pathogenic or weakened form
- Killed
  - Most bacteria, some viruses
- Only one mRNA vaccine for livestock
  - SEQUIVITY, Merck Animal Health
    - Prescription vaccine for several viral pathogens

Pollard, A.J., Bijker, E.M. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol 21, 83–100 (2021). https://doi.org/10.1038/s41577-020-00479-7

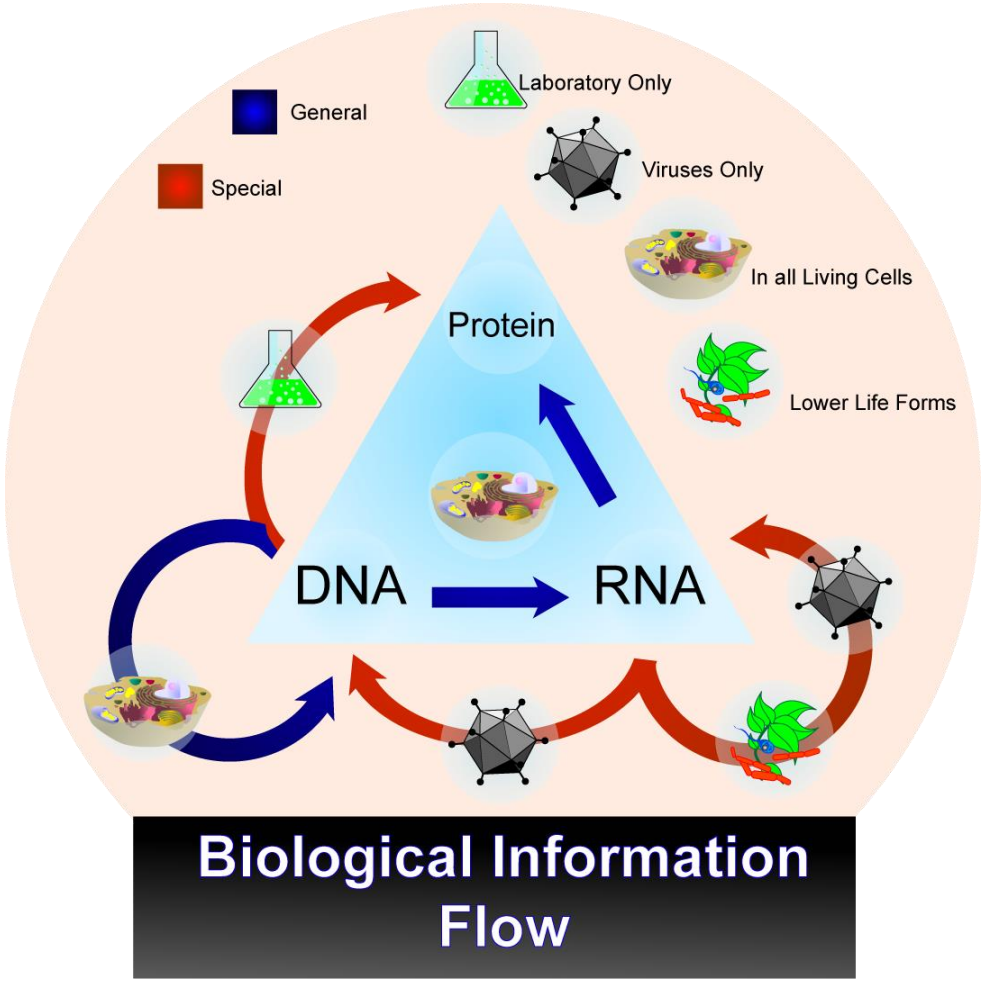
Response to vaccination

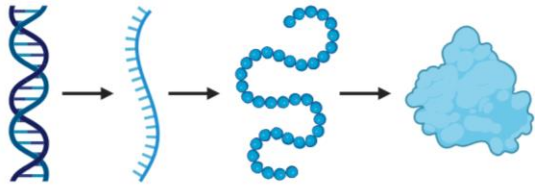


- Mediated by immune cells
- Detection of "foreign" material
  - antigen
  - majority are proteins
- Activation of several cell types
  - leads to antibody production
  - "memory"

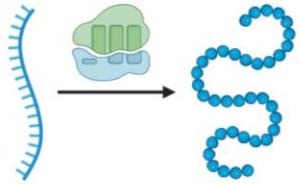
Pollard, A.J., Bijker, E.M. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol 21, 83–100 (2021). https://doi.org/10.1038/s41577-020-00479-7

Central Dogma of Molecular Biology





Gene expression
(central dogma)



Translation
(mRNA to amino acid)

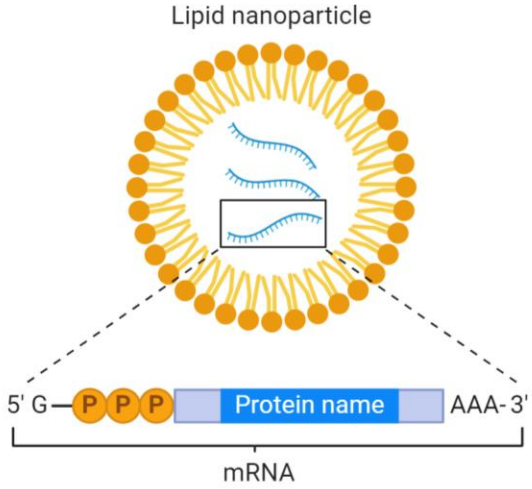
Ribosome with mRNA

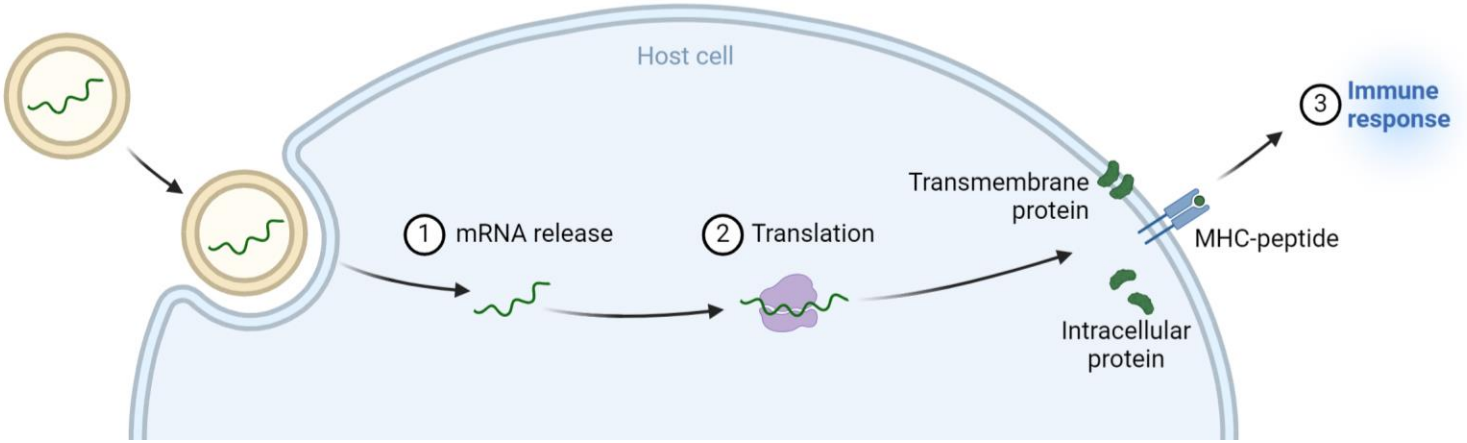
https://www.wikidoc.org/index.php/Central_dogma_of_molecular_biology

BioRender

What is an mRNA vaccine?

- Synthetic RNA molecule with specific properties
  - Modified ribonucleotides (stability)
  - 5′ cap, protein coding sequence, polyA tail for recognition of cellular machinery
- Encapsulated in a lipid “membrane”





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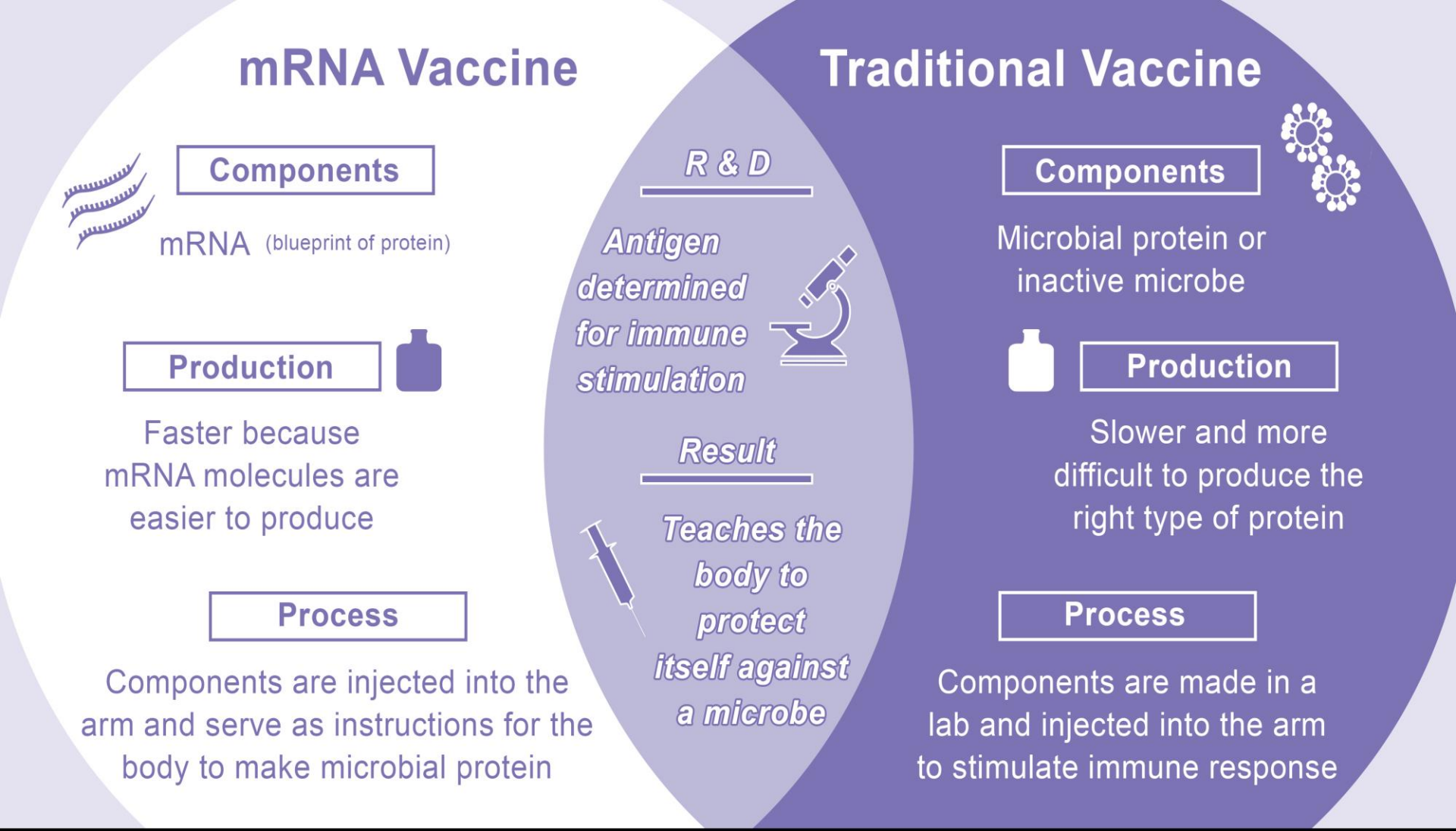

Vanderbilt Vaccine Research Program | Vanderbilt Institute for Infection, Immunology and Inflammation

https://www.vumc.org/viiii/infographics/how-does-mrna-vaccine-compare-traditional-vaccine

Questions?