Escherichia coli con genoma reducido (grEc) y células completas inactivadas (Killed Whole Cell, KWC) como una plataforma vacunal

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Escherichia coli con genoma reducido (grEc) y células completas inactivadas (Killed Whole Cell, KWC) como una plataforma vacunal

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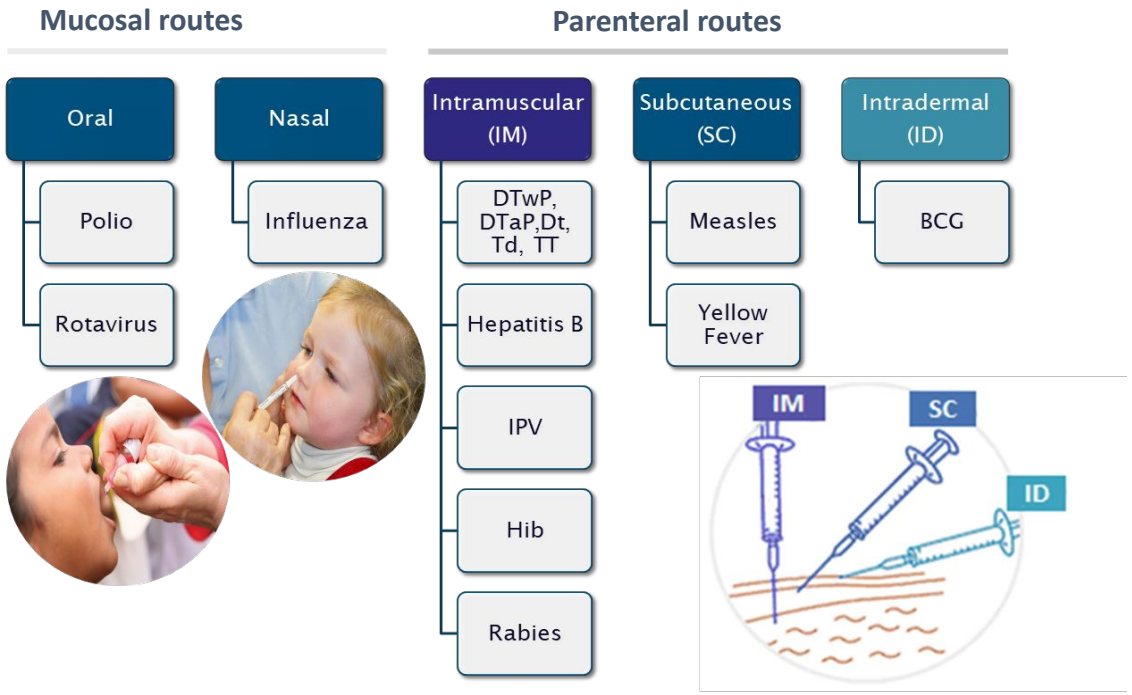
Tipos de vacunas y vías de administración



- **Vacunas de subunidades (SV)**
  - Incluyen únicamente los componentes o antígenos que mejor estimulan el sistema inmunológico;
  - Proteínas recombinantes;Partículas similares a virus;
  - Nanopartículas.
- **Vacunas toxoides (TV)**
- La vacuna toxoide está hecha de toxinas producidas por una bacteria que ha estado inactiva**;**
- **Vacunas de ácido nucleico (NAV)**
  - Plásmido de ADN;
  - ARNm;
- **Vacunas de patógeno completo (WPV)**
  - Inactivadas: versión muerta del agente que causa una enfermedad.
  - Vivas atenuadas: que contienen una versión del microbio vivo que ha sido debilitada en el laboratorio.

https://www.niaid.nih.gov/research/vaccine-types

Problem/Opportunities:

- Current/new vaccines (including mRNA) are safe and highly effective BUT are:
  - Costly
  - Take months to make new vaccines/versions
  - Require elaborate and expensive feedstocks
  - Have complicated production processes, require specialized factories & costly, difficult to source starting materials
  - Have demanding storage and cold chain requirements
  - Less suitable for very fast/custom vaccines, LMIC use
  - Not suitable for agricultural applications (cost/storage)

Why our technology is better:

A New Vaccine Platform Technology: Killed Whole Cell Genome-Reduced Bacteria Expressing Antigens on the Cell Surface

- Synthetic biology-based ($0.09/bp) – $40-50/construct
- Fast, enabling rapid Design-Build-Test-Learn (DBTL) cycles
- Employs available, established technology (killed whole cell vaccine)
- Inexpensive, abundant feedstocks (e.g. yeast lysate/L-Broth)
- Antigens expressed on bacteria (very inexpensive), << $1/dose
- Killed whole cell bacterial vaccines are stable at 2-8 C for 24 months
- Presenting the antigen on the bacterial surface means that the whole cell vaccine would be “auto-adjuvanting”
- Bacterial vectors can be further engineered (enhanced immunogenicity)
- Can express difficult antigens (inducible expression)
- Surface expression mechanism has chaperonin-like properties
- Non-parenteral immunization has been demonstrated (oral/intranasal administration – for example, approved cholera vaccines)
- Factories making old technology killed whole cell bacterial vaccines already exist around the world. Minimal adaptation required

Economic considerations

- With a production cost ≤$1/dose for our vaccines (benchmarked against current old technology killed whole cell bacterial vaccines) the global use case is clear. “No one is safe unless everyone is safe.” Universal vaccination minimizes variant evolution
- Very low cost enables ”One Health” solutions (animal + human)
- Killed whole cell platform technology means many different vaccines can be produced using the platform, unlike vectored live/quasi-live (e.g. Ad vectored) antivector immunity will not adversely affect immunogenicity.

Reduced genome *Escherichia coli*

Molecular Microbiology (2005) **55**(1), 137–149 doi:10.1111/j.1365-2958.2004.04386.x

Cell size and nucleoid organization of engineered *Escherichia coli* cells with a reduced genome

Masayuki Hashimoto,[1] Toshiharu Ichimura,[1] Hiroshi Mizoguchi,[2] Kimie Tanaka,[2] Kazuyuki Fujimitsu,[3] Kenji Keyamura,[3] Tomotake Ote,[1] Takehiro Yamakawa,[4] Yukiko Yamazaki,[4] Hideo Mori,[2] Tsutomu Katayama[3] and Jun-ichi Kato[1]*

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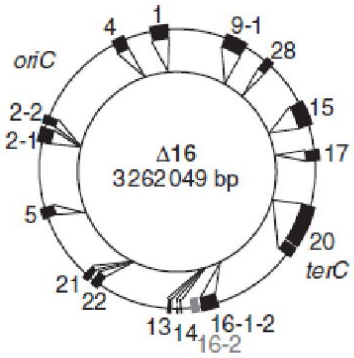

Mutant	Deletion units removed (Number)	(bp)	Cumulative deletion (bp)	(%)	
Δ1	1	111734	111734	2.4	ME5110
Δ2	5	53250	164984	3.6	
Δ3	28	52245	217229	4.7	
Δ4	4	82372	299601	6.5	
Δ5	2-2	46153	345754	7.5	
Δ6	2-1	99304	445058	9.6	
Δ7	16-2	46517	491575	10.6	
Δ8	16-1-2	109112	600687	12.9	
Δ9	17	72554	673241	14.5	
Δ10	22	57798	731039	15.8	ME5119
Δ11	21	42636	773675	16.7	
Δ12	14	47675	821350	17.7	
Δ13	13	41411	816244	17.6	
Δ14	20	300703	1116947	24.1	
Δ15	9-1	125568	1242515	26.8	
Δ16	15	134657	1377172	29.7	ME5125

Fig. 2. Units deleted in the construction of the large-scale deletion (LD) series. The outer and inner circles represent the genomes of MG1655 and the largest deletion mutant (Δ16), respectively. The closed boxes labelled with numbers indicate the sequences that were deleted. *oriC* and *terC* show the map position of origin and terminus of replication, respectively. Note that the construction of the Δ13 mutant (where unit 13 was deleted) was accompanied with the reconstitution of the unit 16-2 region (which is shown in gray and was deleted in the construction of the Δ7 mutant) with the intact wild-type region. Consequently, the Δ7–Δ12 mutants have the unit 16-2 region, while the Δ13–Δ16 mutants lack it.

Strains that had their genomes deleted from 2.4% to 29.7% using a phage λ homologous recombination system

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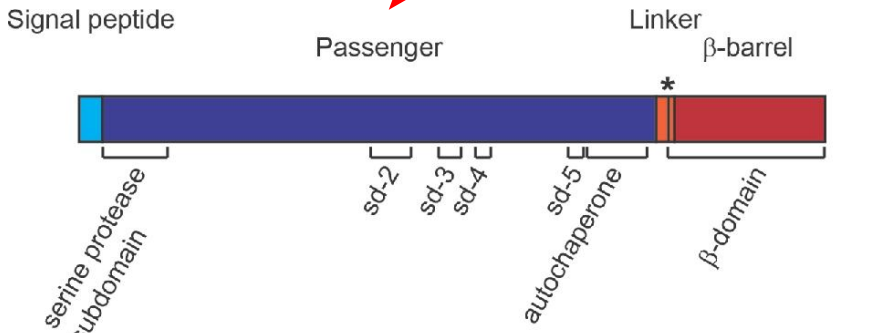
Background: Gram-negative Autotransporters

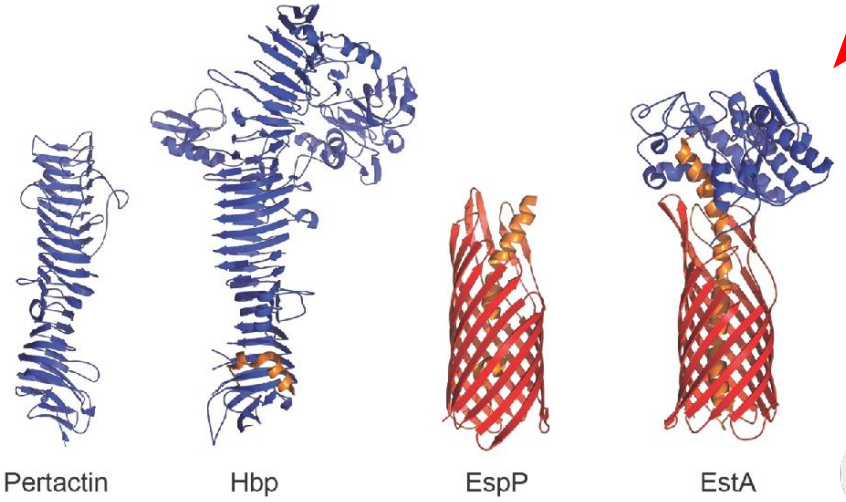
(autotransporter/autodisplay/Type 5 secretion system)

Used to place recombinant antigens on the surfaces of the bacteria, which are then inactivated. **Up to ~100 KDa, ~200,000 proteins/bacteria.** DNA encoding native passenger protein is replaced with DNA encoding antigen.

Antigen coding sequence replaces native Passenger Protein coding sequence



Antigen replaces native Passenger Protein



van Ulsen et al FEMS Microbiol Lett 2018 365 doi: 10.1093/femsle/fny165

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Autotransporter (AT) proteins

- Adhesin involved in diffuse adherence (AIDA): which remains attached to its translocator and/or the cell surface via non-covalent interactions.
- The use of surface displayed epitopes is interesting mostly because of two main reasons.
- Exposed epitopes are more easily accessible for the immune system.
- Second, bacterial cell surface components can serve as an adjuvant thereby eliciting a strong immune response.

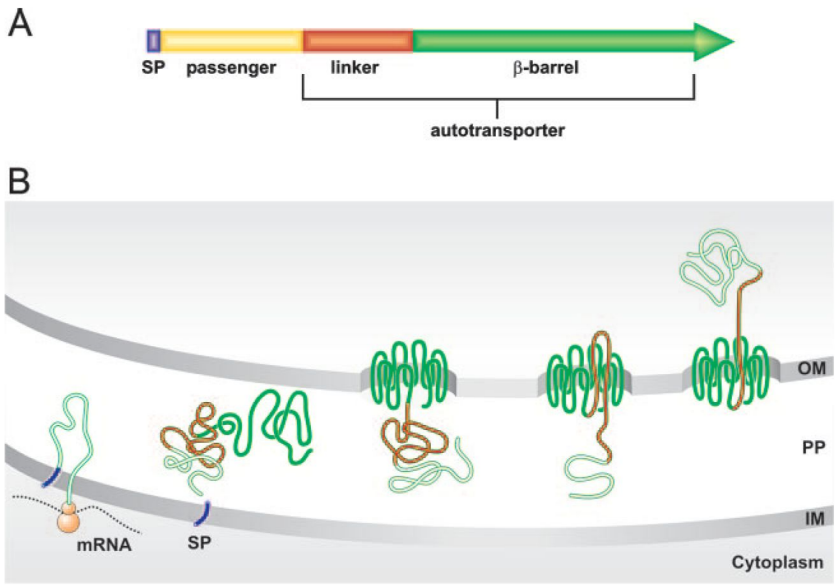


FIG. 1. Secretion mechanism of the autotransporter proteins. (A) Structure of the polyprotein precursor. (B) Transport of the recombinant passenger. By the use of a typical signal peptide, a precursor protein is transported across the inner membrane. After arrival at the periplasm, the C-terminal part of the precursor folds as a porin-like structure, a so-called β-barrel within the outer membrane, and the passenger is transmitted to the cell surface. SP, signal peptide; IM, inner membrane; PP, periplasm; OM, outer membrane.

Koniecnizy et al. Cell surface presentation of recombinant (poly-) peptides including functional T-cell epitopes by the AIDA autotransporter system. FEMS Immunology and Medical Microbiology 27 (2000) 321^332.

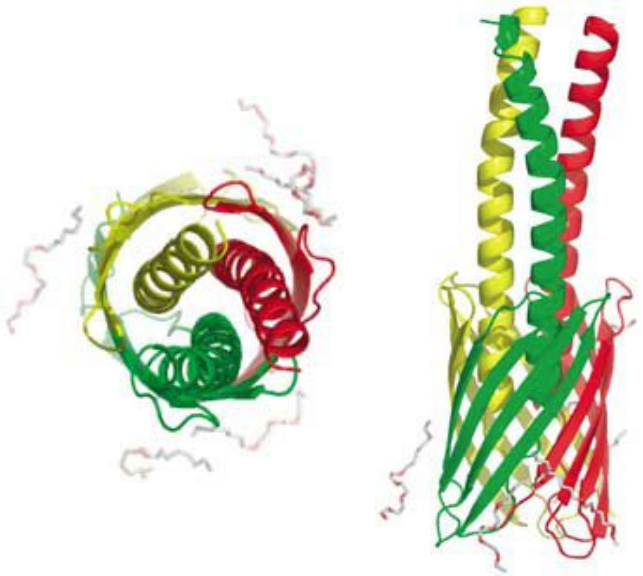
Nicolay et al. Autotransporter-based cell surface display in Gram-negative bacteria. Crit Rev Microbiol, 2015; 41(1): 109–123.

Autotransporter (AT) proteins

- *Haemophilus influenzae* Hia, a class of trimeric ATs, trimerizes in forming the bacterial outer membrane-anchored β-barrel and then translocates 3 passenger proteins through the β-barrel's pore.

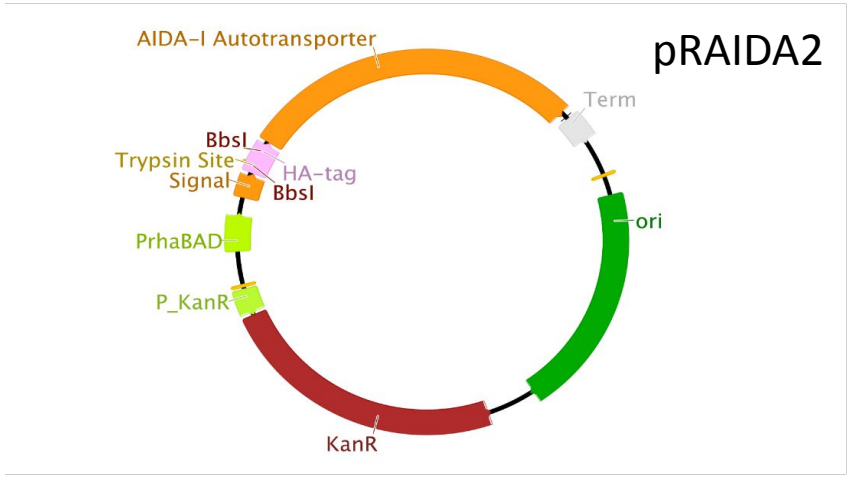
- The structure of these trimeric autotransporters strongly resembles the structure of the HIV envelope trimer embedded in the virion envelope.

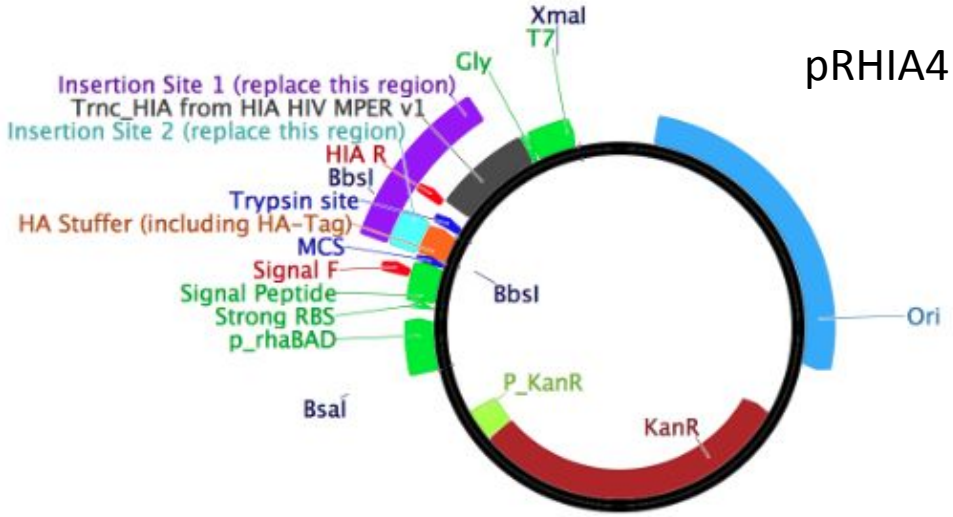
H influenzae Hia Trimeric Autotransporter
Meng et al EMBO J 25:2299 2006



Antigen Surface Expression Plasmid: pRAIDA2 and pRHIA4

- We synthesized pRAIDA2 and pRHIA4
- Has rhamnose-inducible autotranporter expression cassette
- pRAIDA2 has been “on-boarded” with Twist Bioscience. We can order new constructs for **~$40-50/construct with DNA encoding candidate Ag already cloned into the plasmid, turnaround time ~2 weeks.**





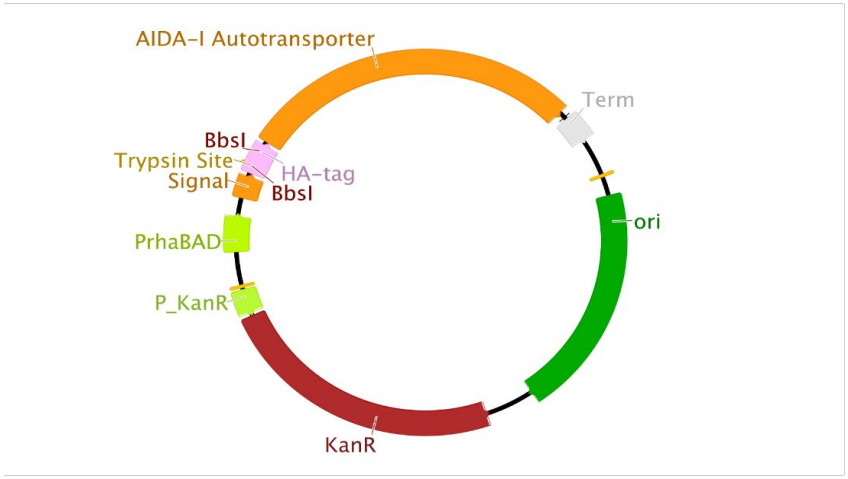
Hypothesis

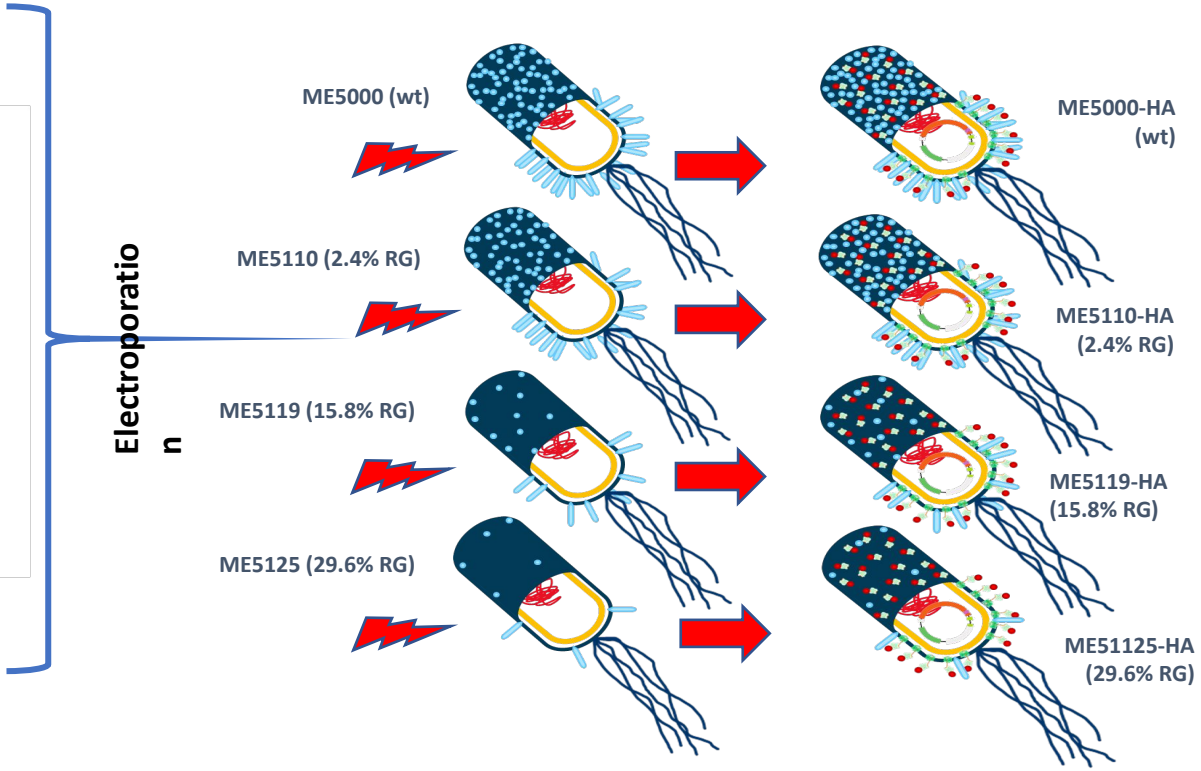
- If we expressing recombinant immunogens on surfaces of RG bacteria, will we elicit a better immune response?

- Will the antigen be more accessible to the immune system?

Vaccine Design

- The HA immuno-tag peptide (YPYDVPDYA) and the AIDA-I autotransporter cloned in plasmid pRAIDA2.





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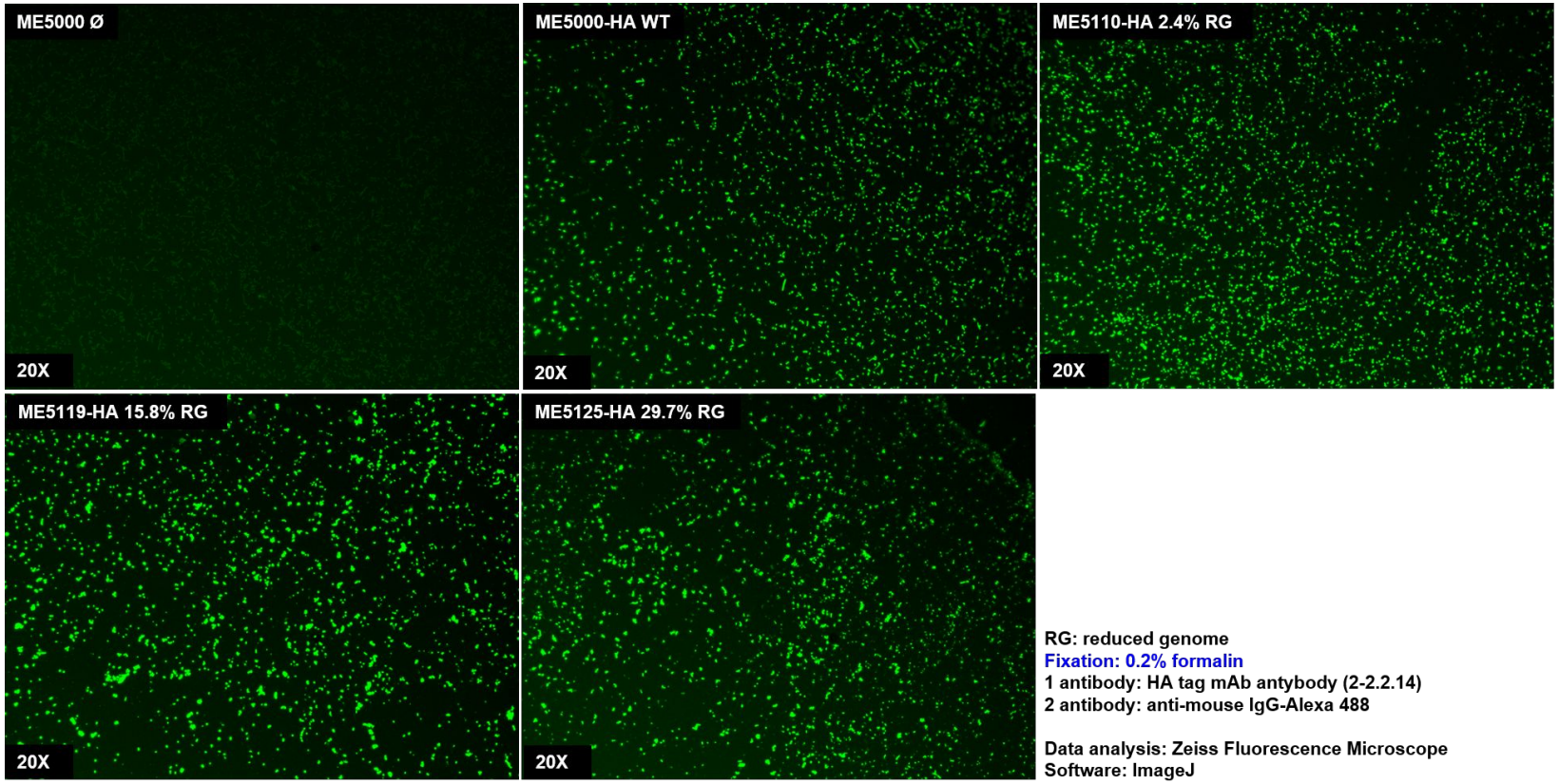
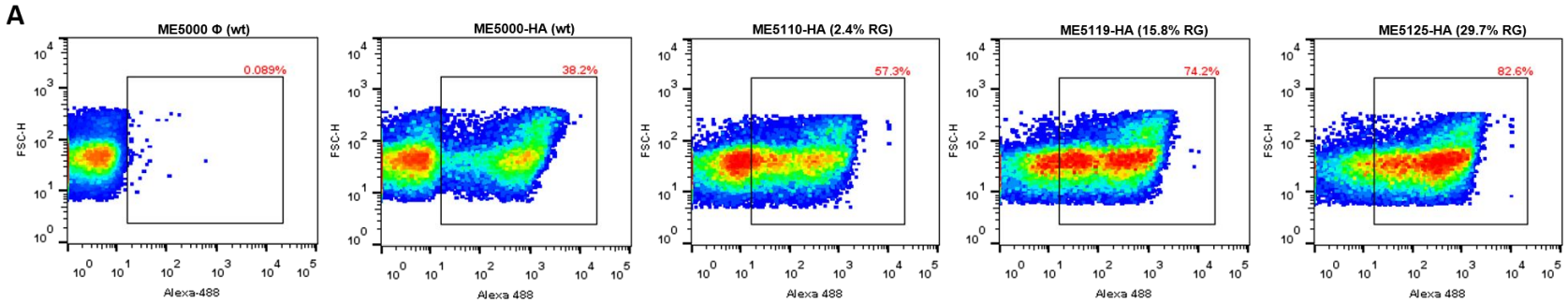
Expression of HA peptide on surface of RG *E. coli* by Immunofluorescence

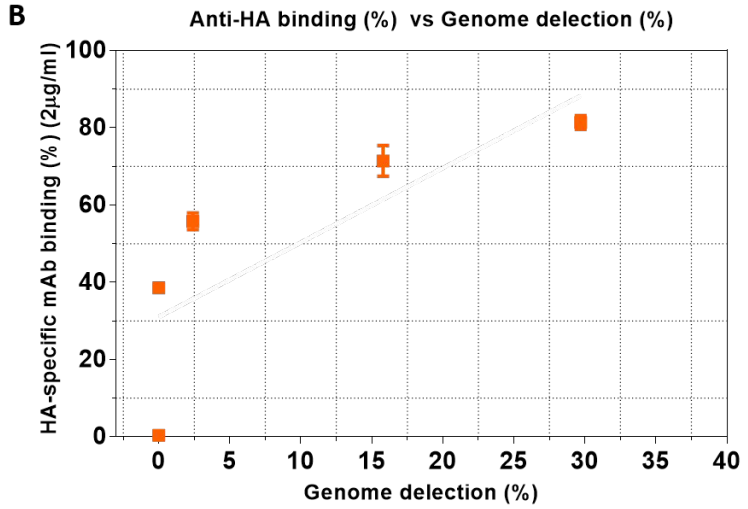


RG: reduced genome
Fixation: 0.2% formalin
1 antibody: HA tag mAb antybody (2-2.2.14)
2 antibody: anti-mouse IgG-Alexa 488

Data analysis: Zeiss Fluorescence Microscope
Software: ImageJ

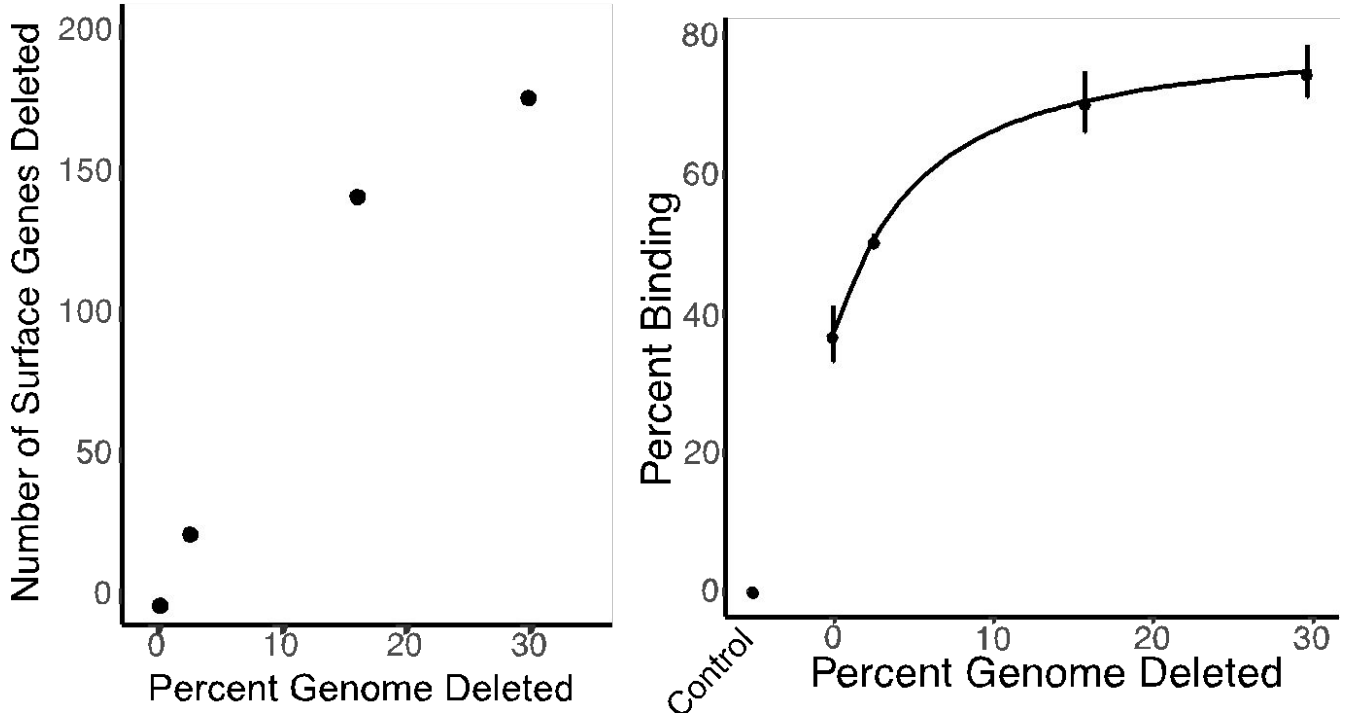
Binding of anti-HA mAb to RG *E. coli*



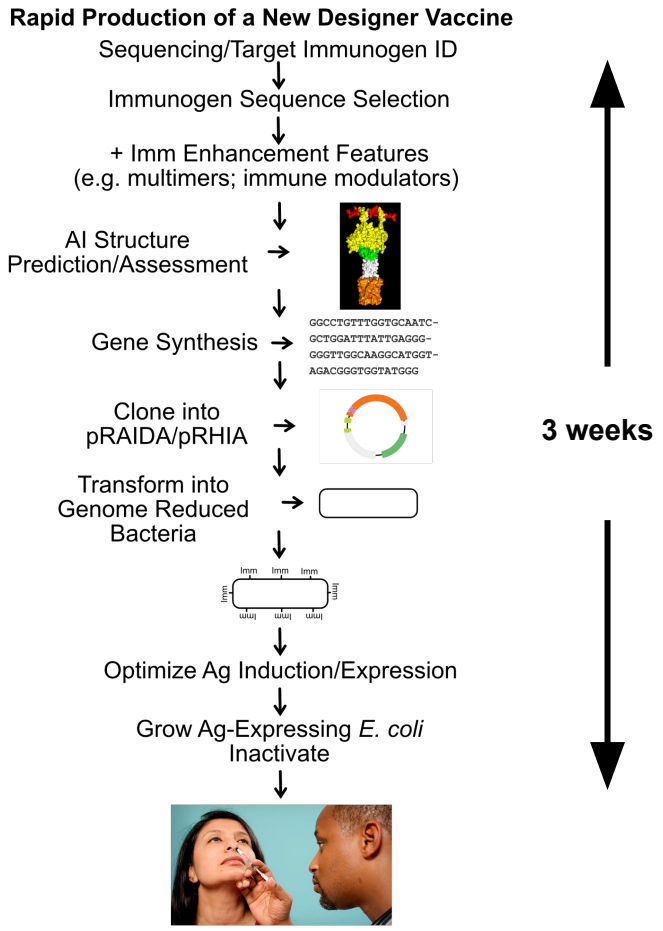


Binding of anti-HA mAb to RG *E. coli*

Number of Genes Removed from the Bacterial Surface in Genome Reduced *E. coli* and Binding of anti-HA MAb to Bacteria Expressing HA Immunotag



New vaccine platform: KWC/GRB



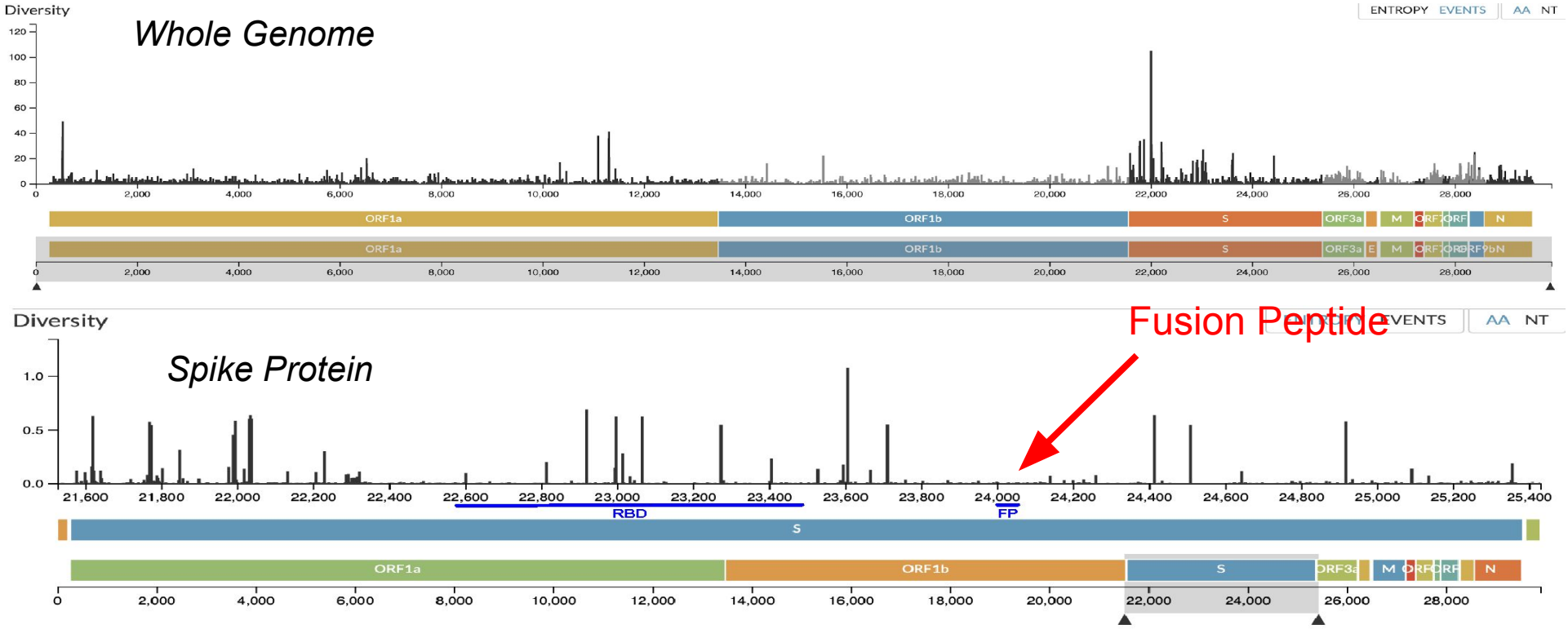
Proof of Concept:

A universal/evolution resistant coronavirus vaccine Humans and Animals

- COVID-19 may/may not be over.
  - New variants continue to emerge.
  - With continued replication in the face of waning vaccine/natural immunity we are conducting a potentially dangerous evolution experiment if a sufficiently different mutation renders previously acquired immunity useless.
- New coronaviruses will almost certainly cross over into humans from animals (e.g. SARS-CoV-1, MERS)
- Coronaviruses are economically important agricultural pathogens.
  - We are also interested in using the platform to make agriculturally useful vaccines (easier initial regulatory path with potential for non-dilutive cash flow to help fund human clinical development).
- As a proof-of-concept we are targeting the **highly conserved** Fusion Peptide (FP) (highly conserved among **all** coronaviruses)

SARS-CoV-2 Evolution and Variation

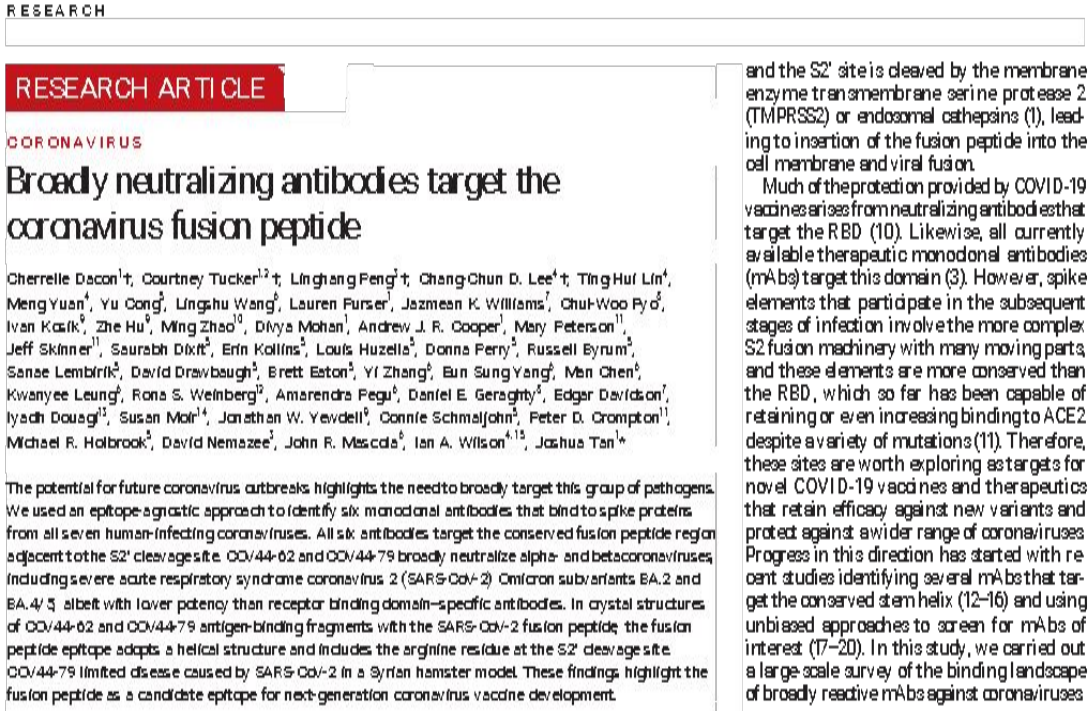
The Fusion Peptide region is very highly conserved.



Source: nextstrain.org

SARS-CoV-2 Evolution and Variation

The FP has also been identified as the target of several broadly-neutralizing monoclonal Abs, confirming concept validity



RESEARCH

RESEARCH ARTICLE

CORONAVIRUS

Broadly neutralizing antibodies target the coronavirus fusion peptide

Cherrelle Dacon[1]†, Courtney Tucker[1,2]†, Linghang Peng[3]†, Chang-Chun D. Lee[4]†, Ting Hui Lin[4], Meng Yuan[4], Yu Cong[5], Lingshu Wang[5], Lauren Purser[1], Jazmean K. Williams[7], Chul-Woo Pyo[8], Ivan Kosik[9], Zhe Hu[9], Ming Zhao[10], Divya Mohan[1], Andrew J. R. Cooper[1], Mary Peterson[11], Jeff Skinner[11], Saurabh Dixit[5], Erin Kollins[5], Louis Huzella[5], Donna Perry[5], Russell Byrum[5], Sanae Lembirik[5], David Drawbaugh[5], Brett Eston[5], Yi Zhang[6], Eun Sung Yang[6], Man Chen[6], Kwanyee Leung[6], Rona S. Weinberg[12], Amarendra Pegu[6], Daniel E. Geraghty[6], Edgar Davidson[7], Iyadh Douagi[13], Susan Moir[14], Jonathan W. Yewdell[9], Connie Schmaljohn[5], Peter D. Crompton[11], Michael R. Holbrook[5], David Nemazee[1], John R. Mascola[6], Ian A. Wilson[4,15], Joshua Tan[1]*

The potential for future coronavirus outbreaks highlights the need to broadly target this group of pathogens. We used an epitope-agnostic approach to identify six monoclonal antibodies that bind to spike proteins from all seven human-infecting coronaviruses. All six antibodies target the conserved fusion peptide region adjacent to the S2′ cleavage site. CoV44-62 and CoV44-79 broadly neutralize alpha- and betacoronaviruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron subvariants BA.2 and BA.4/5, albeit with lower potency than receptor binding domain–specific antibodies. In crystal structures of CoV44-62 and CoV44-79 antigen-binding fragments with the SARS-CoV-2 fusion peptide, the fusion peptide epitope adopts a helical structure and includes the arginine residue at the S2′ cleavage site. CoV44-79 limited disease caused by SARS-CoV-2 in a Syrian hamster model. These findings highlight the fusion peptide as a candidate epitope for next-generation coronavirus vaccine development.

and the S2′ site is cleaved by the membrane enzyme transmembrane serine protease 2 (TMPRSS2) or endosomal cathepsins (1), leading to insertion of the fusion peptide into the cell membrane and viral fusion.

Much of the protection provided by COVID-19 vaccines arises from neutralizing antibodies that target the RBD (10). Likewise, all currently available therapeutic monoclonal antibodies (mAbs) target this domain (3). However, spike elements that participate in the subsequent stages of infection involve the more complex S2 fusion machinery with many moving parts, and these elements are more conserved than the RBD, which so far has been capable of retaining or even increasing binding to ACE2 despite a variety of mutations (11). Therefore, these sites are worth exploring as targets for novel COVID-19 vaccines and therapeutics that retain efficacy against new variants and protect against a wider range of coronaviruses. Progress in this direction has started with recent studies identifying several mAbs that target the conserved stem helix (12–16) and using unbiased approaches to screen for mAbs of interest (17–20). In this study, we carried out a large-scale survey of the binding landscape of broadly reactive mAbs against coronaviruses

A Novel Animal Model for a SARS-CoV-2 Vaccine

Proc Natl Acad Sci U S A. 118(18):e2025622118. doi: 10.1073/pnas.2025622118

- Porcine Epidemic Diarrhea Virus (w Meng lab Virginia Tech)
  - Useful proof-of-principle with a real market
  - A very serious coronavirus disease that poses a significant risk to agriculture
  - Entered the US pig population in 2007 with a ~10% mortality rate)
- A unique opportunity to study safety/efficacy of a vaccine against a pathogen in its native host
- A second, non-rodent species for regulatory reviews for human vaccines.
- **An early opportunity for a useful agricultural vaccine that can yield non-dilutive revenue for development of other vaccines.**
- **Licensed pig vaccines have US annual sales >$100M**
  - **Virginia Tech has licensed other Meng lab pig vaccines to Zoetis**

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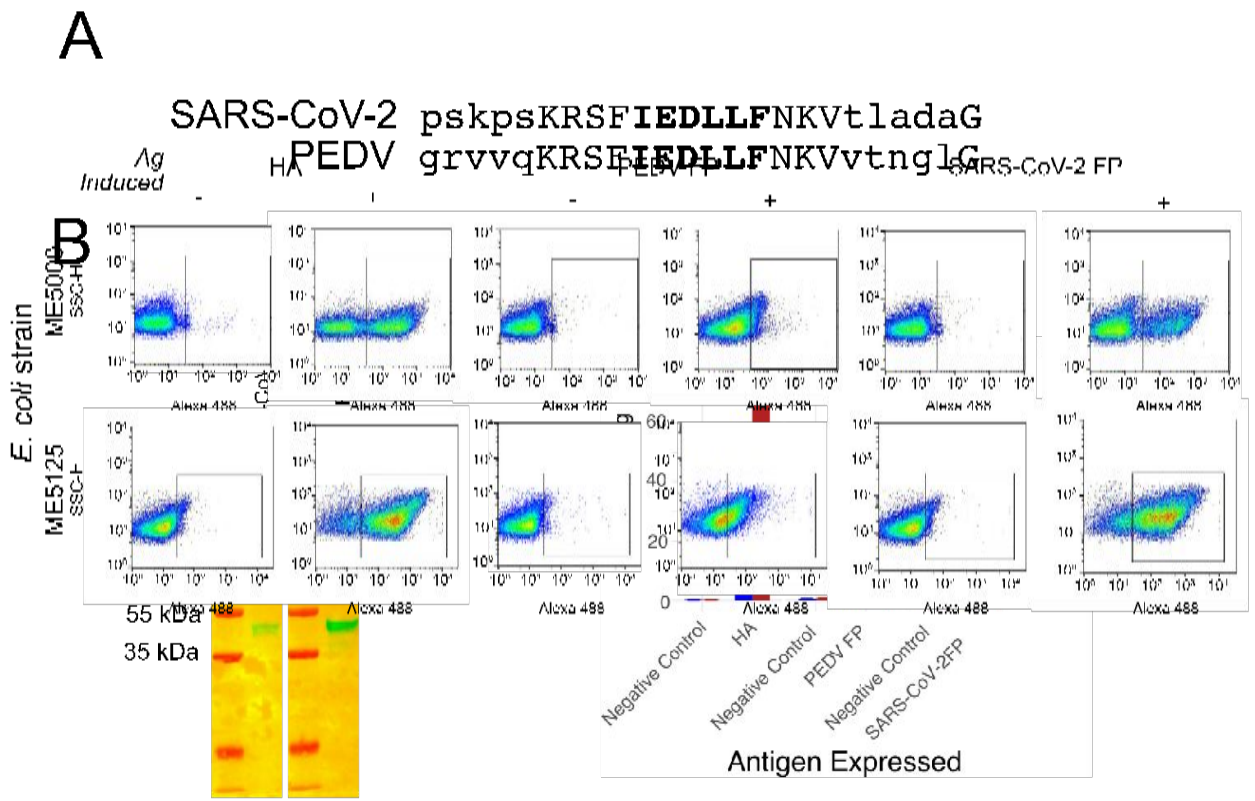
Preparation and QC of coronavirus fusion peptide vaccines

A. The FP sequences are very highly conserved for all coronaviruses (**BOLD** sequences are universally conserved for ALL coronaviruses. CAPS are conserved for SARS-CoV-2 and PEDV).

B. We can produce the antigens.

C. Antibodies against the FPs bind to bacteria expressing the FPs on their surfaces, and the binding is better for genome-reduced bacteria.



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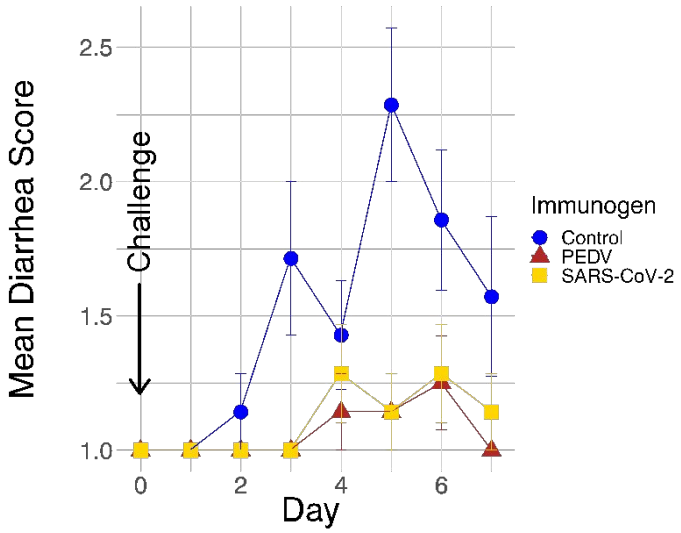
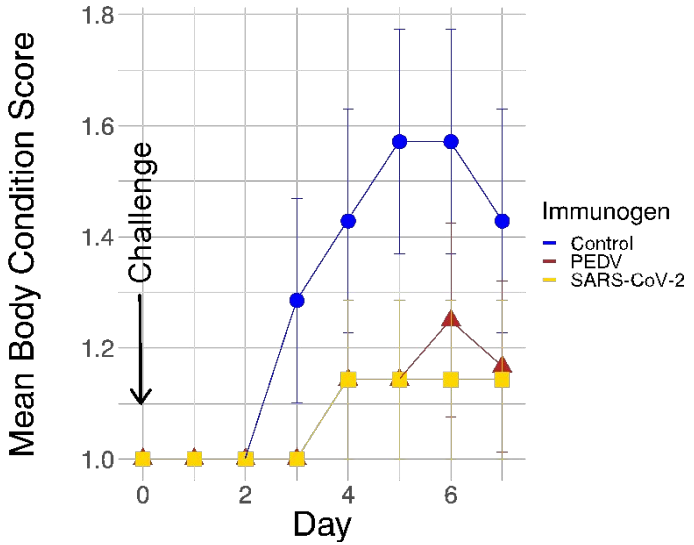
Pig Clinical Responses – Clinical Proof of Principle

Either the SARS-CoV-2 FP vaccine OR the PEDV FP vaccine protect the pigs against clinical disease. The vaccines are cross-protective, indicating the possibility of a universal coronavirus vaccine.



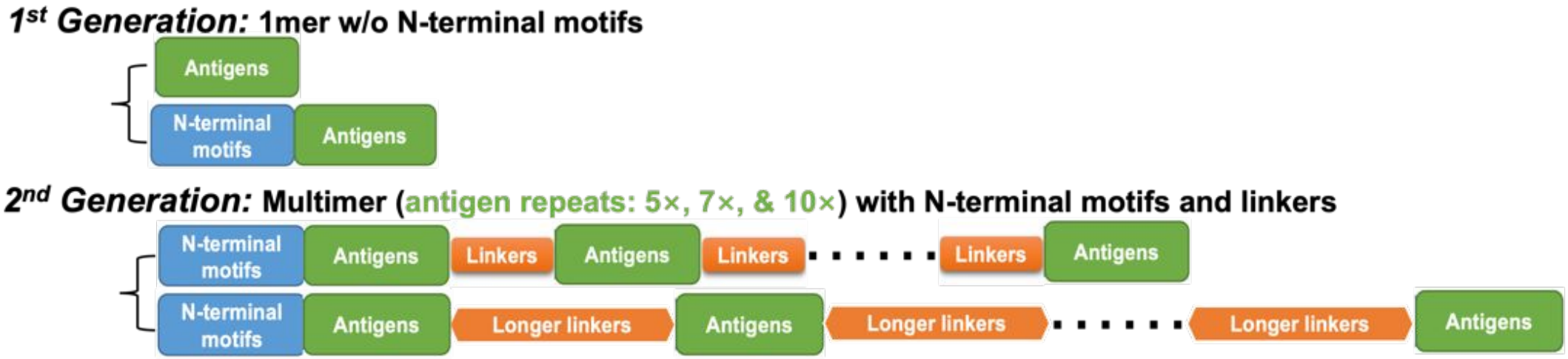


After challenge, pigs were monitored daily for clinical symptom of PEDV infection. About two days post-challenge (dpc), pigs in control group showed clinical symptom. In totally, 6 pigs in control group showed symptom of PEDV infection. In two vaccine groups, only 1-2 pigs showed symptom. Significant difference between vaccinated and control groups after 2 dpc.

1. Body condition scores (1-3). 1, undetectable spinous processes and hook bones. 2, spinous processes and hook bones were slightly felt. 3, spinous processes and hook bones were easily felt and visible.
2. Diarrhea scores (1-3). 1, normal to pasty feces. 2, semi-liquid diarrhea with some solid content. 3, liquid diarrhea with no solid content

Design-Build-Test-Learn Process for KWC/GRB Vaccine Immunogens

- HIV, Sars-CoV2, PEDV, Influenza and Cancer



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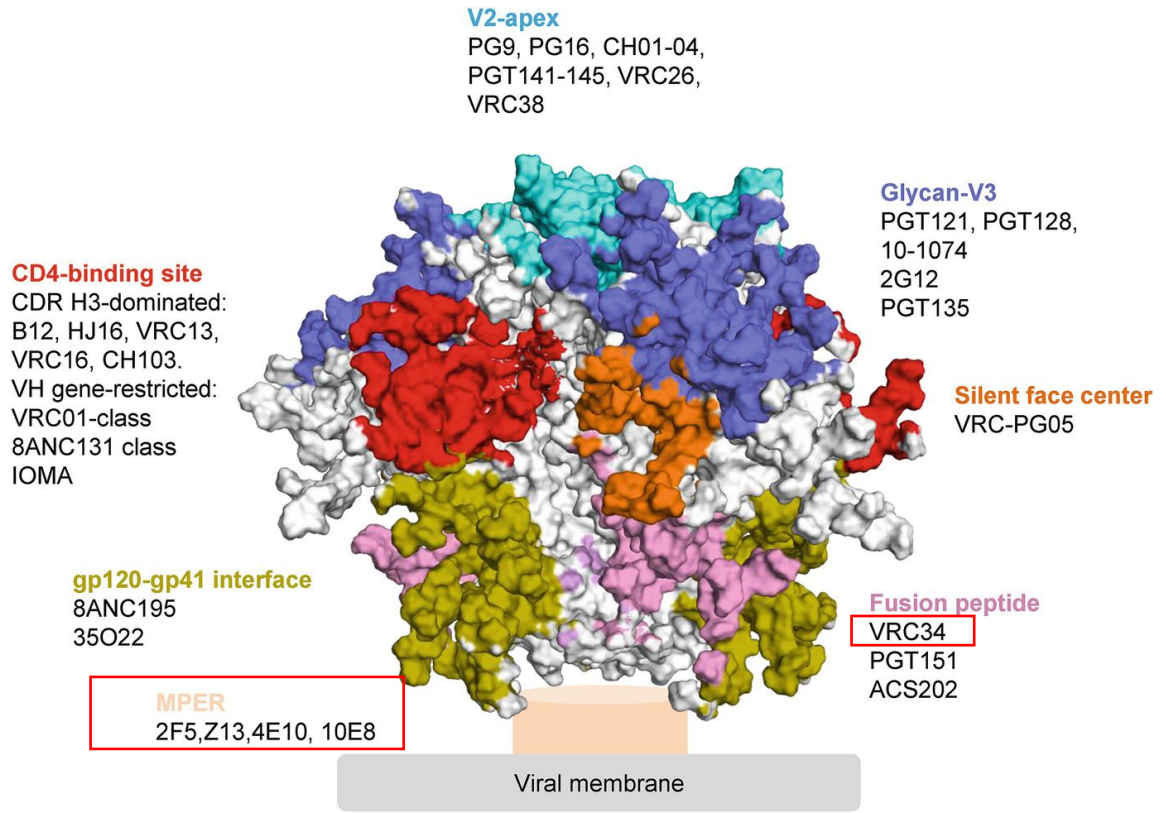
How can we make HIV vaccine?

- Conventional vaccines such as live attenuated and killed virus fail against the highly variable and evasive HIV.
- Sera from a subset of chronically-infected HIV patients was found to neutralize multiple HIV strains.
- This sera contained broadly neutralizing antibodies (bnAbs).
- Broadly neutralizing monoclonal antibodies (bnMAbs) were produced using B-cells from these patients.
- Structural biologist maps the binding sites of the broadly neutralizing antibodies to the envelope protein.
- If we can make the antigen look like the antigen that binds to neutralizing antibodies and use that antigen for immunization, possibly we can stimulate a specific immune response.
- Optimal template for vaccine design.



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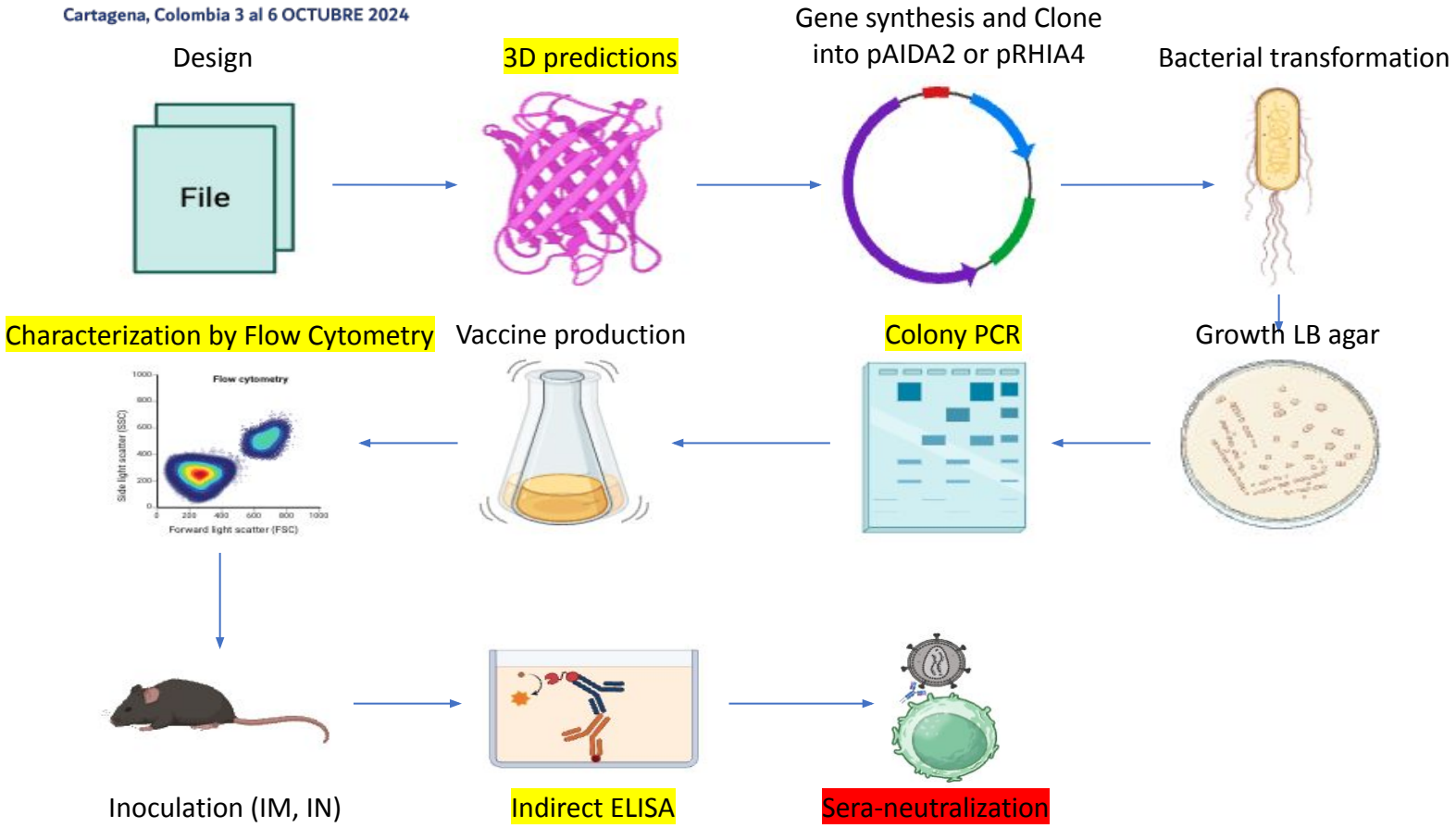
Currently work KWC/GRB HIV

Currently we are testing 28 vaccines against HIV

(9 FP, 11 MPER and 8 bivalent)

All vaccines are in sera neutralization assay



The industrial capacity to make killed whole cell vaccines is already in place in factories around the world

This slide shows a 1,500-L Bioreactor at Incepta Vaccine (Savar, Dhaka, Bangladesh).

This bioreactor makes all the DTP vaccine for Bangladesh (P component is a Killed Whole Cell Vaccine). The UNICEF price for DTP is ~$0.25/dose. 1,500-L bioreactors are available for ~$100,000. Feedstock is bacterial growth medium ~ yeast lysate – available and inexpensive.

Conclusions/Take-aways

- We have a new disruptive synthetic biology vaccine platform technology that:
- Can produce a new candidate vaccine in **~3 wk from Ag candidate identification.**
- For infectious disease vaccines – pandemic response and biothreats, conventional vaccines, humans and veterinary applications (“One Health”)
- We have demonstrated clinical proof-of-principle in pig model
- For personalized therapeutic cancer vaccines
- At a cost of **<<$1/dose**, based on costs of existing killed whole cell bacterial vaccines (cholera)
- Animal vaccines have a $100M/y and human vaccines >$1B/y sales potential
- Vaccines capable of being made in **existing factories** around the world, **including LMICs**
- We have additional strategies to enhance antigen production/immunogenicity
- We have a clear path to early commercialization (PEDV vaccine), licensed to an animal health company, that would provide abundant non-dilutive capital for development of more vaccines, animal and human.

Selected Links

- We published in the scientific literature
  - https://www.pnas.org/doi/abs/10.1073/pnas.2025622118

- Some coverage in lay press

•https://jamanetwork.com/journals/jama/fullarticle/2781521

•https://www.usnews.com/news/health-news/articles/2021-04-26/is-a-cheap-universal-coronavirus-vaccine-on-the-way

•https://leaps.org/virus-protection/

•https://www.salon.com/2021/04/25/the-quest-for-a-universal-coronavirus-vaccine/

•https://knowablemagazine.org/article/health-disease/2022/what-nextgen-covid-19-vaccines-might-look-like

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