GSD Bio

PRACTICAL SOLUTIONS FOR GLOBAL HEALTH

GSD Bio is democratizing vaccine production by developing a non-commercial platform to improve immunization options and access.

GSD Bio's Simple Epidemic-Ending Defense (SEED) platform combines helpful bacteriophage and food-making bacteria to transformatively simplify the production and use of immunizations to prevent epidemics.

We are seeking philanthropic financial support and collaborators to evaluate vaccines produced by the SEED platform against currently circulating global threats from highly-pathogenic avian influenza viruses and coronaviruses.

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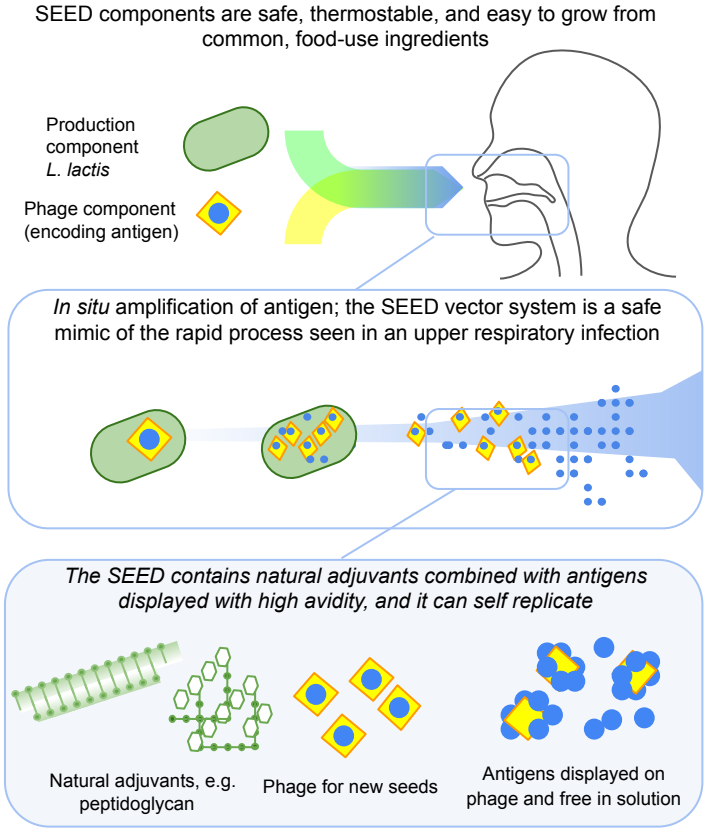
Greater San Diego Biological Solutions is a San Diego, CA not-for-profit 501(c)(3) organization, founded and incorporated in November of 2022. Jonathan Moore, PhD (President/Chairperson) and Joe Warner, PhD (Director of Research and Development) are molecular microbiologists who developed industrial enzymes and microbial cell factories at Genomatica, Diversa, and others.

We are committed to preventing epidemics by enabling low-cost immunizations that can be quickly scaled and produced at point-of-need. We are developing the SEED system for simplified protein production that uses food-safe ingredients and a bacterium used globally in food production.

GSD Bio's Simple Epidemic-Ending Defense (SEED) platform



Why is GSD Bio working on a non-commercial platform for accessible immunizations and why should you support its development?

- Existing commercial models prioritize profit, leaving immunizations out of reach for billions, resulting in ongoing threat to the health and well-being of all.
- Traditional manufacturing requirements and cold chain distribution systems bias vaccine access to urban centers, neglecting rural and resource-limited populations most vulnerable to zoonotic threats.
- Growing risk to animal and human populations from infectious diseases is inadequately addressed by the existing vaccine industry. Innovative, integrated One Health-driven solutions are needed to avoid costly, short-sighted, and reactive responses to human epidemics.

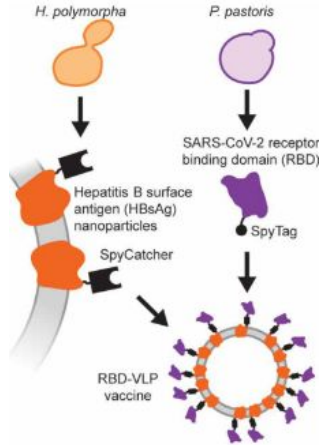
SEED offers a novel and friendly live viral vector vaccine: Self-assembling biomimicry to lower cost and complexity.

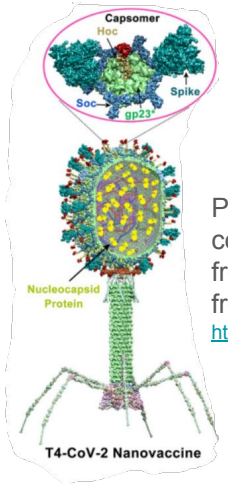
We are inspired by recent reports on the design of virus-like particles (VLPs) and phage particles for immunization.

These designs aim to resemble the pathogen-associated molecular patterns (PAMPs) found on viral pathogens to achieve robust and durable immune responses while avoiding some of the cost or safety concerns associated with use of live viral vectors.

What makes a GSD Bio SEED vaccine unique is its production, by using food-safe bacteria and phage ingredients that can be safely grown in a shirt pocket, in a barn, on a nightstand, or in a GI tract. All of the needed functions are genetically encoded and grown together from simple ingredients that can be easily obtained.

Example of microbial production of VLP, image from Dalvie *et al*. 2022.
https://doi.org/10.1126/sciadv.abl6015



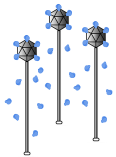


Phage T4 mucosal vaccine containing S and N subunits from SARS-CoV-2, image from Zhu *et al*. 2022
https://doi.org/10.1128/mbio.01822-22

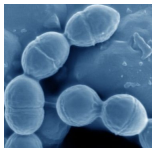
SEED grows beyond common vaccine platforms

Common platforms	Description	Advantages	Limitations
mRNA 	Messenger RNA enters hosts cells and instructs production of protein antigen internally (e.g. Spikevax, COVID-19).	Rapid development and design adaptation to new pathogens/antigens	Relatively complex to produce at scale and cold chain requirements limit access
protein subunit 	Purified antigen protein, usually administered with adjuvant, triggers an immune response (e.g. Corbevax, COVID-19).	Targeted immune response and relatively easy to manufacture	Multiple doses and adjuvants often required
live viral vector 	Weakened or modified live virus induces response similar to natural infection (e.g. JYNNEOS, Mpox).	Strong and long-lasting immune response	Safety risks due to possible side effects or reversion to virulence

GSD Bio's Simple epidemic-ending defense (SEED) system combines advantages of other vaccine platforms

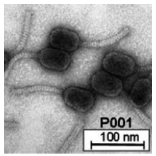
GSD Bio SEED 	SEED platform comprises a probiotic bacterial virus that can display antigens on its surface to deliver protection from disease.	A viral vector without animal infection risk. SEED uniquely enables simple point-of-need production and immunization using food-safe ingredients.	Immunogenicity testing needed

SEED platform, key components



Bacterium *Lactococcus lactis MG1363 (L. lactis)*

- Probiotic bacterium with a long history of safe use in the food industry and for human health purposes (deVos 2011)
- Easy to grow, lacks endotoxin, and has relatively low protease activity
- *L. lactis* cell membranes have been exploited as bacterium-like particles to improve vaccine immunogenicity (Ascough 2019).
- Can be dried for storage and transport at ambient temperature, dried cells can be used directly in GSD Bio's SEED production



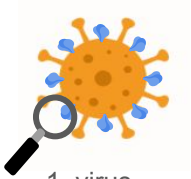
Atamer, 2013

Bacteriophage of *L. lactis*

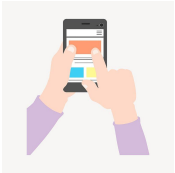
- Well-studied lytic phage of the Siphoviridae family, Sk1 virus genus (Lemay 2019)
- Highly specific for infection and lysis of *L. lactis* subsp *cremoris* MG1363
- Naturally occurring and highly prevalent in dairy products
- Thermostable, retains full viability after 100 days storage at 40 C (internal data)
- 27 Kbp DNA genome can be rapidly modified using CRISPR/Cas9 (Lemay 2018)
  - 42 designs in Year 1
  - Integration of 2.9 Kbp gene achieved, up to ~5 Kbp likely possible

Atamer *et al*. 2013. Frontiers in microbiology. 4. 191. https://doi.org/10.3389/fmicb.2013.00191
Ascough *et al*. 2019. Am J Respir Crit Care Med. Aug 15;200(4):481-492. https://doi.org/10.1164/rccm.201810-1921OC
De Vos, WM 2011. Microb. Cell Fact. 10, S2. https://doi.org/10.1186/1475-2859-10-S1-S2
Lemay *et al*. 2018. Bio Protoc. Jan 5;8(1):e2674. https://doi.org/10.21769/bioprotoc.2674
Lemay *et al*. 2019. Mol Cell Proteomics. Apr;18(4):704-714. https://doi.org/10.1074/mcp.RA118.001135

Prototype production for SEED process

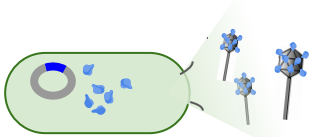


1. virus characterization

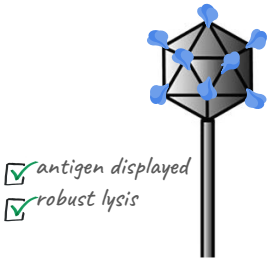


2. design of antigen and phage

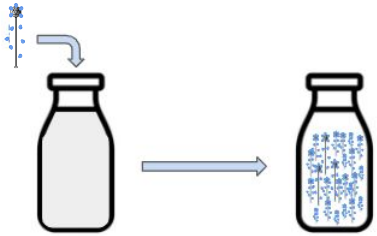
3. DNA synthesis and phage genome assembly



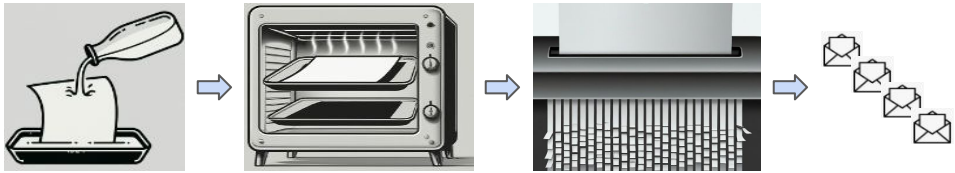
4. transformation into bacteria and 1st lysis



5. isolation of desired phage “Prototype”

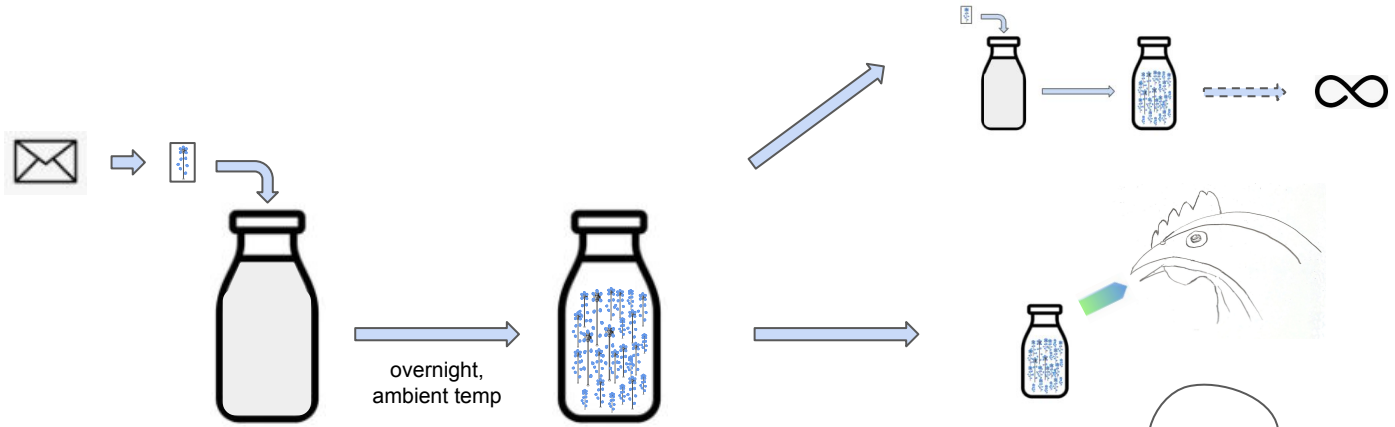


6. SEED process (phage, *L. lactis*, common food ingredients) for phage prototype propagation



7. Preparation of prototype for storage and distribution for point-of-need SEED production

Point-of-need SEED process for propagation & production



SEED phage prototype addition to *L. lactis* culture

SEED lysate ready for use and for further propagation

Simple and flexible mucosal administration via food, water, nasal gel/spray

SEED details and accessible production

SEED lysis process

- *Lactococcus lactis* cells are grown or rehydrated in commonly available nutrients followed by addition of GSD Bio's SEED phage. The phage specifically attach to the bacterial cell walls of *L. lactis* and insert the phage DNA including the programmed DNA encoding clinically relevant antigen(s). The phage repurposes the bacterial cell to produce new phage particles and the desired antigens. After an hour, some of the bacterial cells are lysed and the antigens are released along with a burst of new bacteriophage. This process continues until all the bacterial cells are lysed, typically 5-10 hrs. The end user receives the vaccine dose via an easily applied gel/spray or via food/drink addition.

- SEED lysates are envisioned to contain all ingredients needed for a targeted and robust immune response and without the cost, complexity, or risk of purification and processing.

Accessible production at various scales

- Growth and lysis of fresh ingredients at point of use (lower cost option good for large populations, produced and used in timely manner)

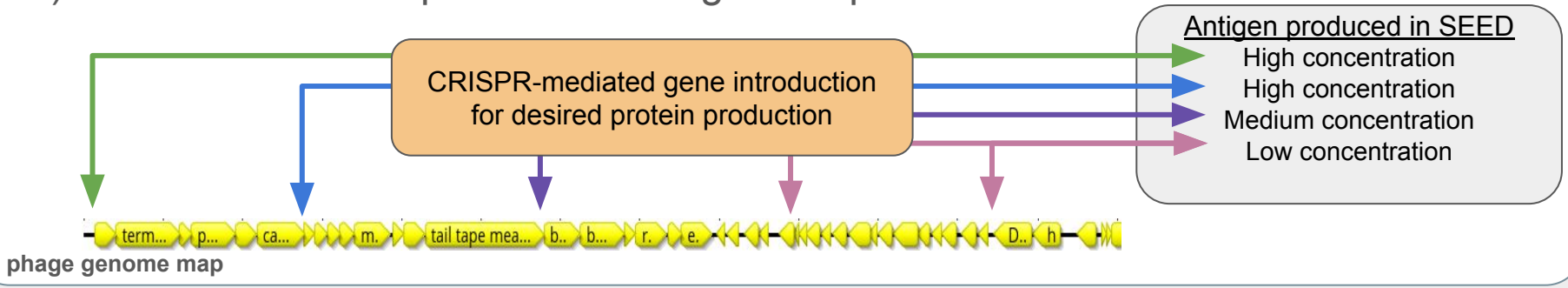
- Alternatively, aliquots of dried ingredients can be distributed for individuals in various environments. Rehydration and self-administration is possible without specialized training or equipment.

Protein production using *L. lactis*

- Antigens currently produced by the SEED system are free of glycosylation. Numerous protein subunit vaccine candidates produced from *L. lactis* have been described in peer-reviewed literature (deVos 2011). These antigens/targets are substantially de-risked for production by the SEED platform.

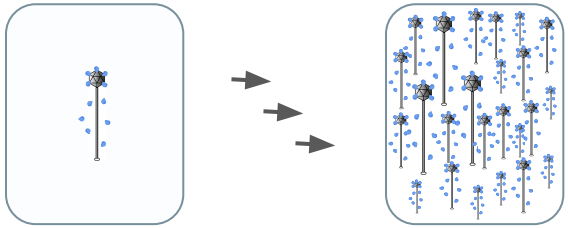
GSD Bio's SEED platform improvements

1) Identification of optimal sites for gene expression

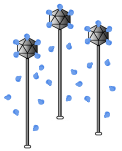


2) SEED formulation was optimized, increased antigen 40-fold.

Changes in salts, pH, carbon source, vessel…



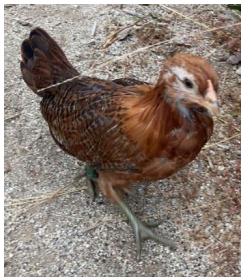
3) 20-50% of antigen can be displayed on phage surface

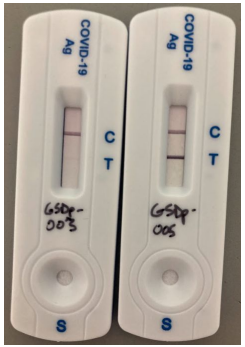


through genetic fusion of antigen & structural genes,

or post-translational coupling of antigen and structural proteins

Applications, vaccines needed for HPAI, COVID19, FCOV

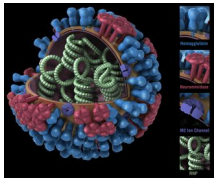




- **HPAIV**: H5N1 (clade 2.3.4.4b) is the prevalent highly pathogenic avian influenza (HPAI) virus, spreading rapidly on almost every continent since first detection in 2020 (Parr 2023). It is devastating wild and domestic bird populations, as well as many marine mammals. The zoonotic disease risk is increasing with frequency and density of exposures. There is an urgent unmet need to protect animal health and minimize risk of a deadly human epidemic.
- **SARS-CoV-2**: The SARS-CoV-2 pandemic is ongoing, causing death, suffering, mass disability from post-acute sequelae of COVID-19 (PASC, or “long COVID”), while unabated spread enables further immunity-evasion and prolonged global harm.
- **Feline coronavirus**: “Cross-species transmission of coronaviruses (CoVs) poses a serious threat to both animal and human health ..the emergence of a novel, highly pathogenic FCoV-CCoV recombinant responsible for a rapidly spreading outbreak of feline infectious peritonitis (FIP), originating in Cyprus**.”** (from Charalampos 2023).

Charalampos A. *et al*. 2023 bioRxiv.11.08.566182, https://doi.org/10.1101/2023.11.08.566182
Parr, Lillian. “Briefer: The Avian Influenza Outbreak—Ecological and Biological Security Implications.” Council on Strategic Risks, 14 March 2023, https://councilonstrategicrisks.org/2023/03/14/briefer-the-avian-influenza-outbreak-ecological-and-biological-security-implications/

SEED vaccine candidates for highly pathogenic avian influenza (HPAI)



CDC, 2022

- Hemagglutinin (HA) is the most abundant and immunogenic protein on the surface of the influenza virus and plays a role in the initial steps of host infection. Additionally, Influenza A virus contains a highly conserved matrix protein 2 (M2) exposed on the surface of the virion, an attractive target for the development of vaccines that induce broad protection.
- Mucosal administration and production of a protein from H5N1 (HA1, from H5 hemagglutinin, fused to M2) by Lactococcus lactis was shown to provide a protective immune response in chickens against HPAI and dramatically improve survival rates (Ren 2022).
- Building upon these promising results, we've incorporated DNA encoding the HA1 subunit, from H5 gene of the H5N1 Influenza A virus (A/goose/Czech Republic/18520-1/2021(H5N1)), and M2 gene into lactococcal phages of the SEED platform.
- GSD Bio has constructed 8 vaccine candidates, comprising combinations of the HA1 subunit, or HA1 subunit fused to M2, or the HA1 subunit fused to SpyTag/SpyCatcher for display on the phage surface.
- Preliminary safety evaluations for 4 vaccine candidates was conducted by feeding 3 doses each to a small flock of 5 hens. No changes in behavior or deviation in weight gain was observed.

Ren, Yi *et al*. "Protective immunity induced by oral vaccination with a recombinant *Lactococcus lactis* vaccine against H5Nx in chickens." BMC veterinary research vol. 18,1 3. 3 Jan. 2022, https://doi.org/10.1186/s12917-021-03109-z

Vaccine candidates for SARS-CoV-2 (COVID-19)

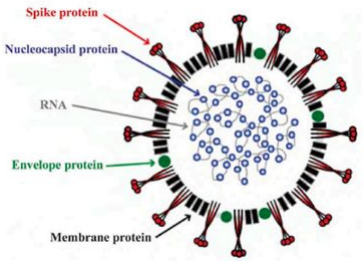
Taghinezhad 2021

- Lactic acid bacteria are being explored as vaccine delivery vehicles for a number of respiratory diseases including SARS-CoV-2 (Taghinezhad 2021).
- The spike (S) protein and S1 RBD domain of SARS-CoV-2 are the focus of many vaccine strategies owing to their importance in the viral life cycle and well known immunogenicity.
- Nucleocapsid (N) protein plays a role in RNA packaging and viral assembly and is another attractive antiviral target. It is the most abundant protein in virions and a high immunogenicity antigen (Wu 2023). The N protein sequence is also well conserved among coronavirus variants and for this reason is an attractive target for pan-coronavirus vaccine development.
- GSD Bio has constructed SARS-CoV-2 vaccine candidates featuring expression of the S protein RBD domain, the N protein, or the N protein fused to the phage capsid gene for display on the particle surface.
- SEED-based production of phage particles containing N protein have been characterized* and we are looking for partners to advance preclinical studies of this unique SARS-CoV-2 vaccine candidate.

Taghinezhad-S, S. *et al*. 2021 Vaccines 9, 466. https://doi.org/10.3390/vaccines9050466
Wu, W. *et al*. 2023 *Virol J* 20, 6. https://doi.org/10.1186/s12985-023-01968-6

* dose (0.5 mL) contains 10^{11} phage particles and 1 ug N protein (by immunoassay)

Vaccine candidate for feline coronavirus (FCoV)



Gao 2023

- "FCoV is divided into two genotypes according to its antigenicity, FCoV I and FCoV II, and each of them can be divided into two biotypes based on its pathogenicity: FECV and FIPV" (excerpt from Gao 2023). Both FCoV I and FCoV II belong to the genus *Alphacoronavirus* of the family *Coronaviridae*, whereas SARS and SARS-CoV-2 belong to the genus *Betacoronavirus*.
- Studies have shown that FCoV-infected cats develop antibodies that cross-react with SARS (SCoV1) nucleocapsid (N), and another study showed the sera from both FCoV I- and FCoV II-infected cats developed cross-reactive antibodies to SCoV2 RBD (Yamamoto 2023).
- Late in 2023, a novel and highly pathogenic CoV-Canine CoV recombinant (FCoV-23) was identified and is responsible for a rapidly spreading outbreak of FIP in Cyprus and the UK.
- In parallel with our SARS-CoV-2 designs, GSD Bio has constructed vaccine candidates featuring expression the N protein of FCoV, or the N protein fused to the phage capsid gene for display on the particle surface.
- These vaccine candidates may provide a route to low-cost and potent protection against currently circulating feline coronaviruses.

Gao, Y. *et al*. 2023 Virus Research, 326, https://doi.org/10.1016/j.virusres.2023.199059.
Yamamoto, J.K. *et al*. 2023 Viruses, 15, 914. https://doi.org/10.3390/v15040914
Charalampos A. *et al*. 2023 bioRxiv.11.08.566182, https://doi.org/10.1101/2023.11.08.566182

Unknowns and Next Steps

We have developed GSD Bio's SEED platform as part of a low-cost, scaleable, and field-deployable immunization strategy.

Key questions remain:

- Does administration of a SEED vaccine protect against disease? Or lower the rate of transmission?
- What is the appropriate dose and route of administration?
- Are additional adjuvants needed in SEED composition?

The immunostimulatory effect of vaccines featuring probiotic bacteria (or components of which) can differ drastically depending upon route of administration (Sudo 2023).

We are seeking preclinical testing to evaluate oral and nasal administration of GSD Bio vaccine candidates. We are also seeking support to continue improving the lactococcal phage platform (e.g. incorporate genetically-encoded adjuvant in the SEED design).

Sudo H, *et al*. 2023, Front. Immunol. 14, https://doi.org/10.3389/fimmu.2023.1082273