Continued Success, Great Potential To Use C1 As Production Platform For rVaccines In Human & Animals

14xΔ protease deletion strain DNL155 is now available

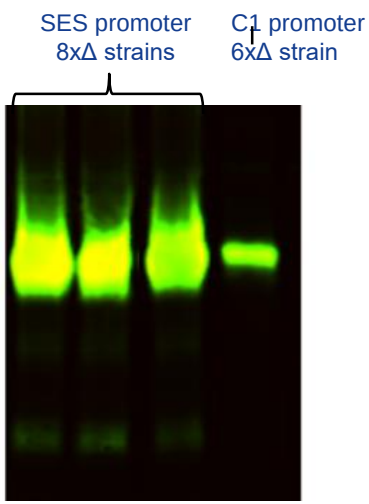
DYADIC®

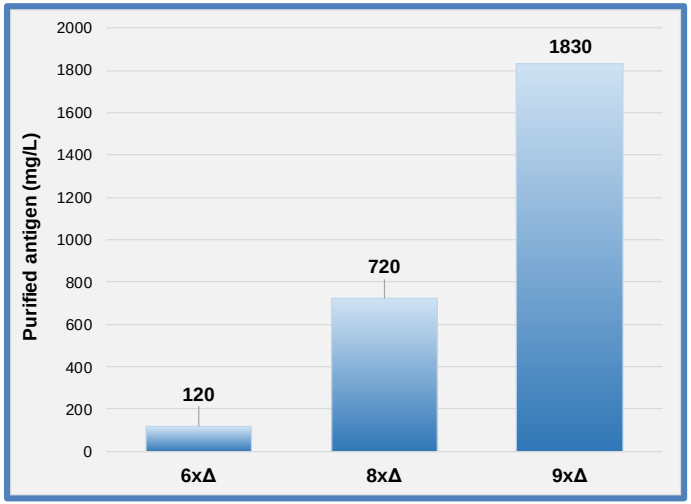
Reinventing biological vaccine and drug development & production

May 15, 2020

The expression of SBV antigen in C1 xΔ protease strains

- Initial strain we used a native C1 promoter in a 5xΔ protease deletion strain
- Second strain we used the synthetic promoter (SES) for expression, higher copy number and 8xΔ protease deletion strain increased production several fold
- Third strain we used was the 9xΔ protease deletion strain and modified fermentation process conditions, which significantly increased the titer



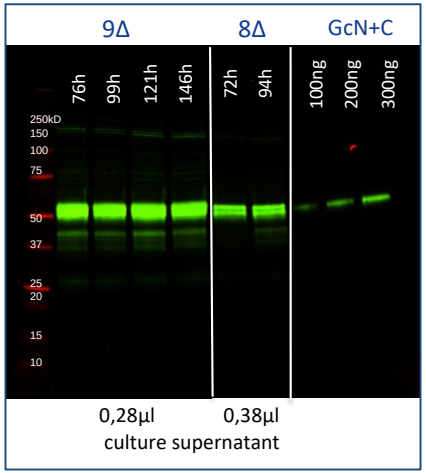


- **The 9xΔ protease strain using SES promoter system significantly increased the production and stability of the target SBV antigen**
- **Up to 1.8 g/L was purified by affinity chromatography**

Fermentation Results of SBV GcN+C-SpyTag with C1

The expression level of SBV GCN+C-SpyTag level produced from the 9xΔ protease deletion strain was 300 fold high than the expression level in Baculovirus

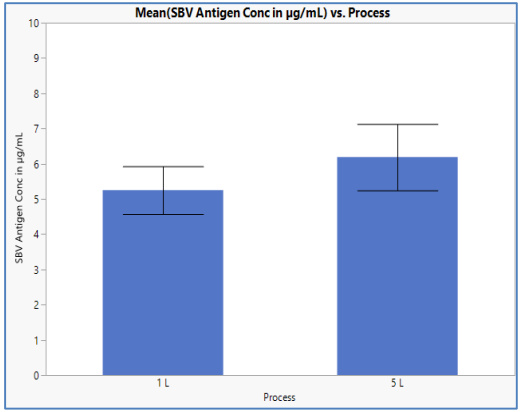
C1 Fermentation



SBV yields: 1, 800mg/l (time point 121h)

X300

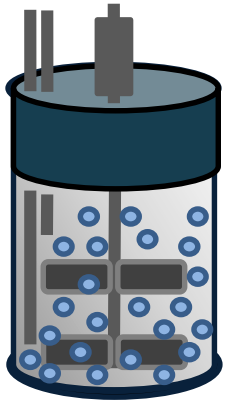
Baculovirus Fermentation



SBV yields: 6mg/l (time point 192h)

Commercial Scale Production of SBV antigen by C1

- The production volume that will be needed to produced 1 batch of 100K, 1,000K and 10,000K SBV doses with C1 (1.75 g/L)
- C1 fermentation is based on Fed-batch technology with glucose feeding and synthetic media
- The fermentation can be run for 5 days at various temperatures (20°C - 42°C) and various pH (5.0 – 8.0)



Doses per batch (20ug/dose)	100 K	1 000 K	10 000 K
Total volume (g)	2	20	200
Productivity (g/L)	1,75	1,75	1,75
Recovery (%)	75	75	75
Working volume (%)	80	80	80
Fermentation volume needed for 1 batch run with C1	**2L**	**20L**	**200L**

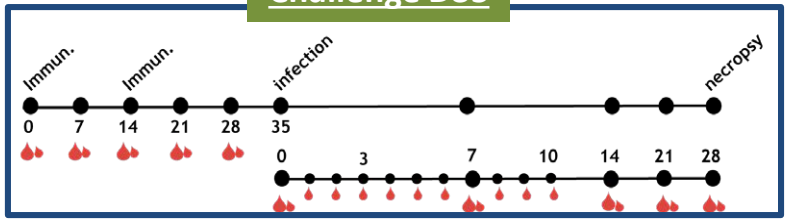
Conclusions

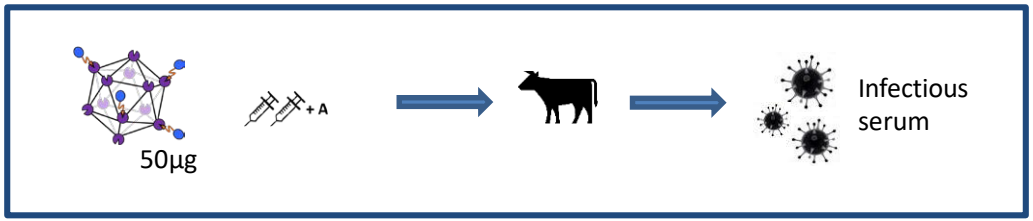
- The 9XΔ protease deletion strain reached a level of 1.75 g/L in 5 days fermentation.
  - The newer 14XΔ protease deletion strain might yield even greater productivity.
- The high productivity that was achieved by C1, will enable significant decrease in fermentation volume and production cost – for example:
  - For 10K, 1M or 10M of SBV doses – 1 batch of 2L, 20L or 200L scale will respectively be needed for commercial manufacturing.
    - Using the newer 14XΔ protease deletion strain might reduce scale even further.
- The predecessor C1 9XΔ protease deletion strain was rapidly constructed for new antigens
- New SBV GcN+C SpyTag strain using SES promoter system and two copies of the expression cassette **In Summary**, C1 technology is able to exceed ZAPI goals for SBV, RFV, etc. :
  - To be fast in producing sufficient amount of doses when the idea is to deliver high number of doses under 4-6 months.
  - To be able to produce sufficient amount of doses at low cost and reduced fermentation capacity.

Cattle trial: Conclusions

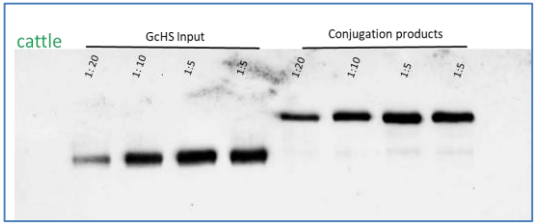
- **MPSP complex (particle-display) vaccines:**
  - protection after 1 immunization in mice
  - sterile immunity in cattle target species
- **Success/performance:**
  - LS-GcH + LS-GcHS: complete protection, sterile immunity!
  - C1-expressed SBV GcHS fully functional for protection in cattle

Challenge D35



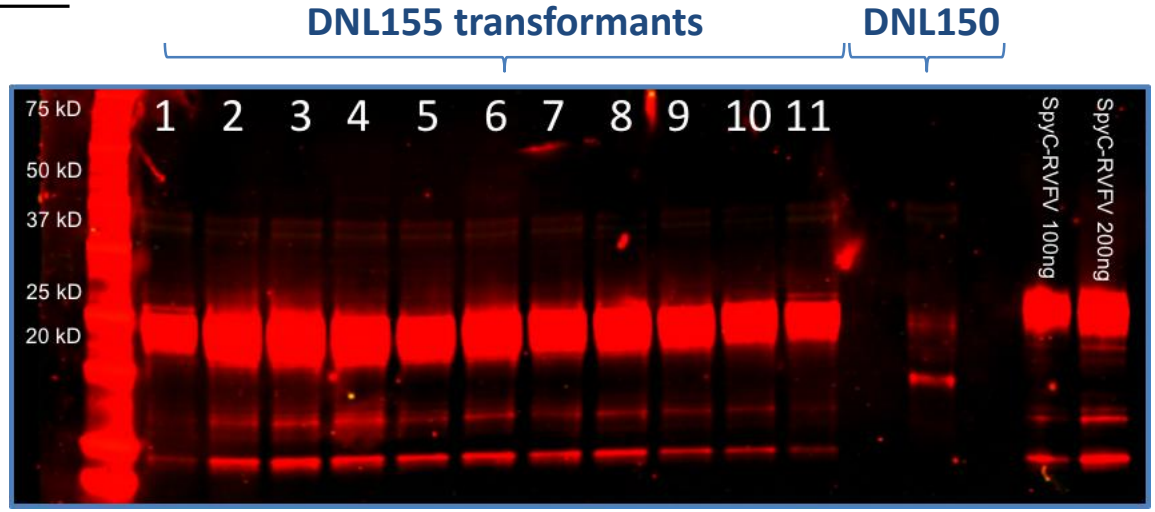


Conjugation products Nanoparticles + SBV



DNL155 demonstrates that C1 can serve as the production platform for ZAPI project

24 MTP results



- Production strains obtained from the RVFV antigen expression vector were grown in 24-well plates and production of the antigen was analyzed with Western blotting with antibody against RVFV antigen.
- 11 transformants from DNL155 and one transformant from DNL150 are shown, control protein at the right

- **The vaccine antigen protein from Rift Valley Fever Virus (RVFV) was expressed from the same expression vector in a 13xΔ protease deletion strain DNL150 and in the 14xΔ protease deletion strain DNL155.**
- **In DNL 150 the expression level is very low and most of the product is truncated.**
- **In DNL 155 the expression level is much higher and the full-length RVF is produced.**

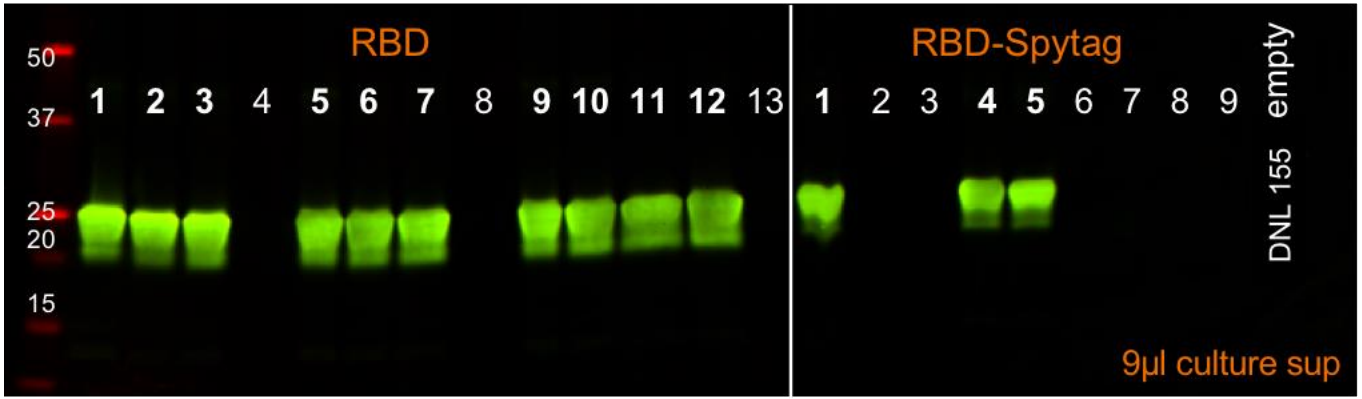
*** Note: The RVF was not able to be expressed in baculovirus**

Production of RBD SARS-CoV-2 protein in DNL 155 (14x Δ Protease deletion strain)

24 MTP results / (1) Fermentations of both of these RBD strains has begun

Proteins: all expressed with C-tag

- **RBD** =Receptor Binding Domain (23kDa) of SARS-CoV-2 spike protein
- **RBD-Spytag** = RBD with Spytag (23kDa)



- 24-well MTP screening.
- Cultivation for 4 days in production medium.
- Ambr or 1L fermentation will come next

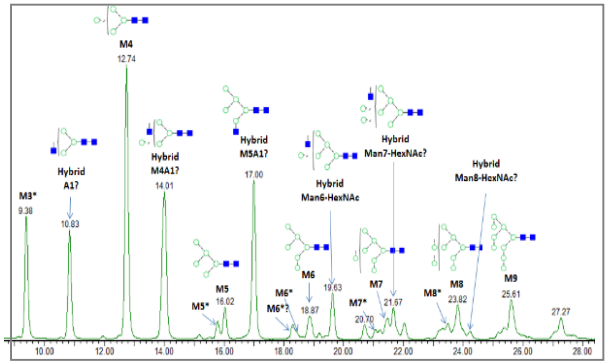
(1) RBD and RBD-SpyTag are produced – estimation level 1-3 g/L

The Rapidly Growing C1 Glycoform Cell Lines / Dialing up & Down Immunogenicity

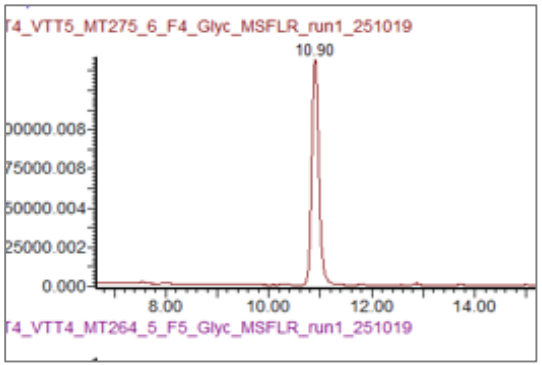
Achievements so far: **The Glycoengineering of C1 has generated good results to date**

Four C1 Strains (Glyco structures on C1 native proteins)

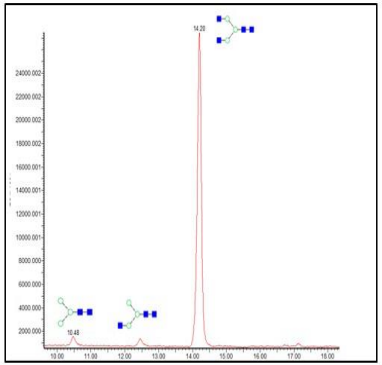
Native C1 Man_{3-9} Strain



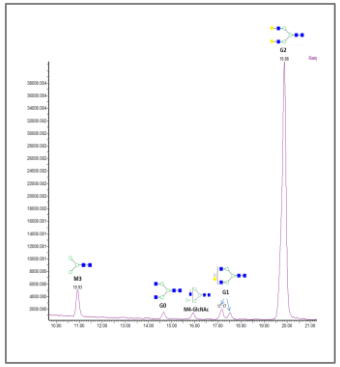
C1 M3 Strain



C1 G0 Strain



C1 G2 Strain



Man_{3-9} with hybrid glycoforms

99 % M3

95%G0

76 % G2

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Thank You

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