HBC VLP-BASED PLATFORM FOR THE DEVELOPMENT OF A RECOMBINANT VACCINE PROTOTYPE AGAINST SARS-COV-2

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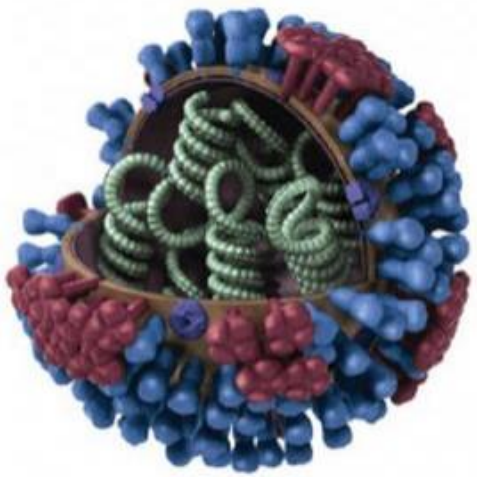
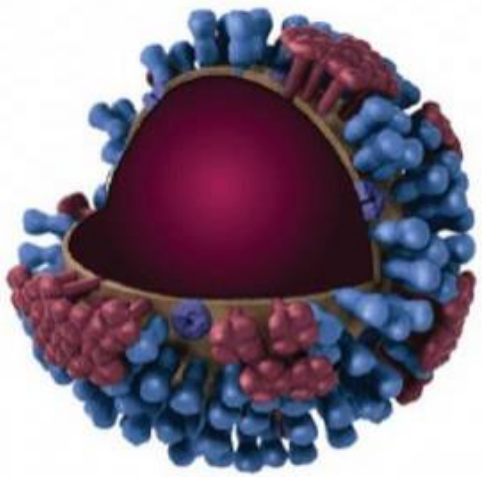
Background. Virus-like particles



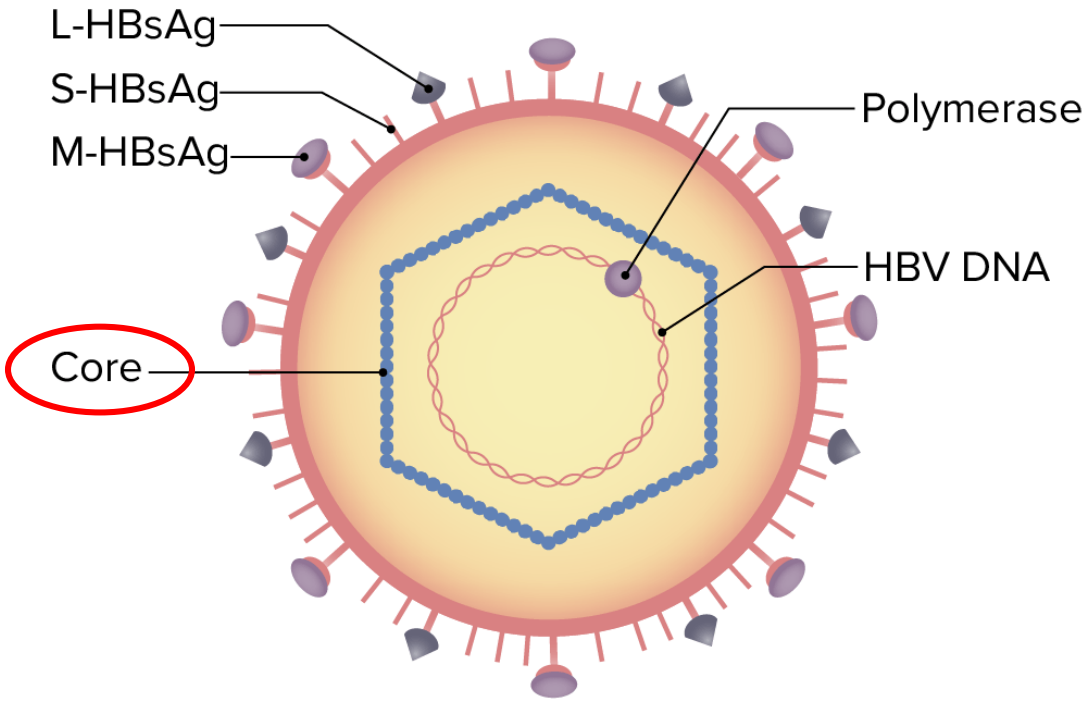
infectious



safe

- lack the viral genome
- caused stronger immune responses
- ensure tissue-specific targeting
- excellent adjuvant properties

Background. Hepatitis B/G-genotype VLP



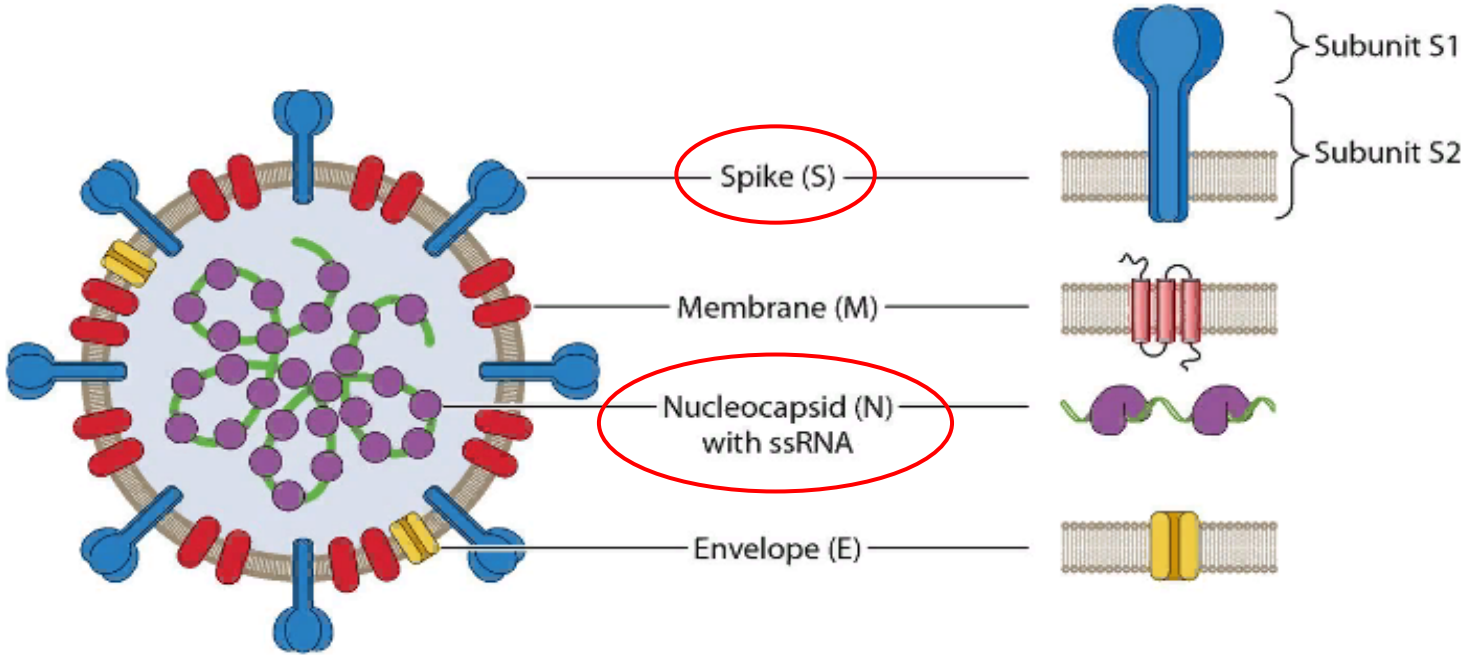
- high outcome in E.coli expression system
- high stability
- high packaging potential in vitro

Background. Hepatitis B/G-genotype VLP

Table 1. Characterization of expression of HBc proteins from different HBV genotypes. The HBc purity is shown for the expression culture obtained from the best transformant.

HBc Origin (HBV Genotype)	HBc Length, aa	Variability of HBc Expression Level in Five Individual *E. coli* Transformants (% of Total Protein)	HBc Purity, %	VLP Outcome, mg/g Wet Cells
A	185	3.1–7.6	>90	8.45
B	183	4.1–7.1	80	5.6
C	183	3.3–4.9	70	4.4
D	183	4.2–10.0	≥90	15
E	183	5.4–6.3	80	8.4
F	183	4.5–5.2	80	7.4
G	195	5.6–7.2	>90	20

Background. SARS-CoV-2



Materials. Plasmids

HBc/G MIR insertions

Spike protein RBM

GS - linker

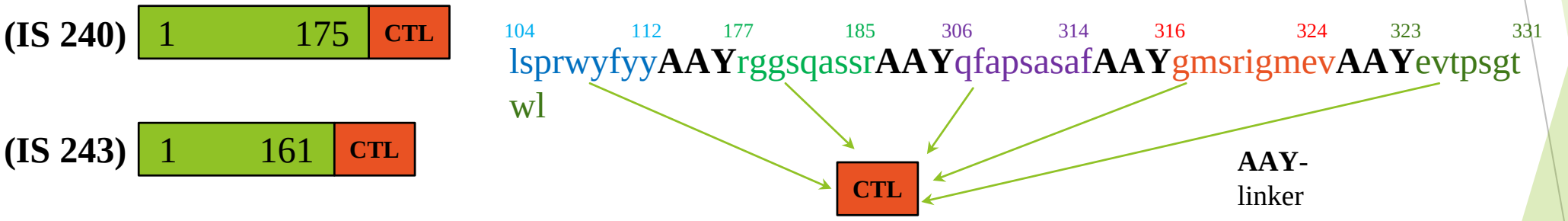
- SARS Wuhan-Hu-1 NC 045512.2 **(IS 236)**
- Cov Omicron/Botswana/R40B60BHP3321001247/2021 **(IS 235)**
- Delta SARS QW 65230.1 **(IS 234)**

B epitope of protein N

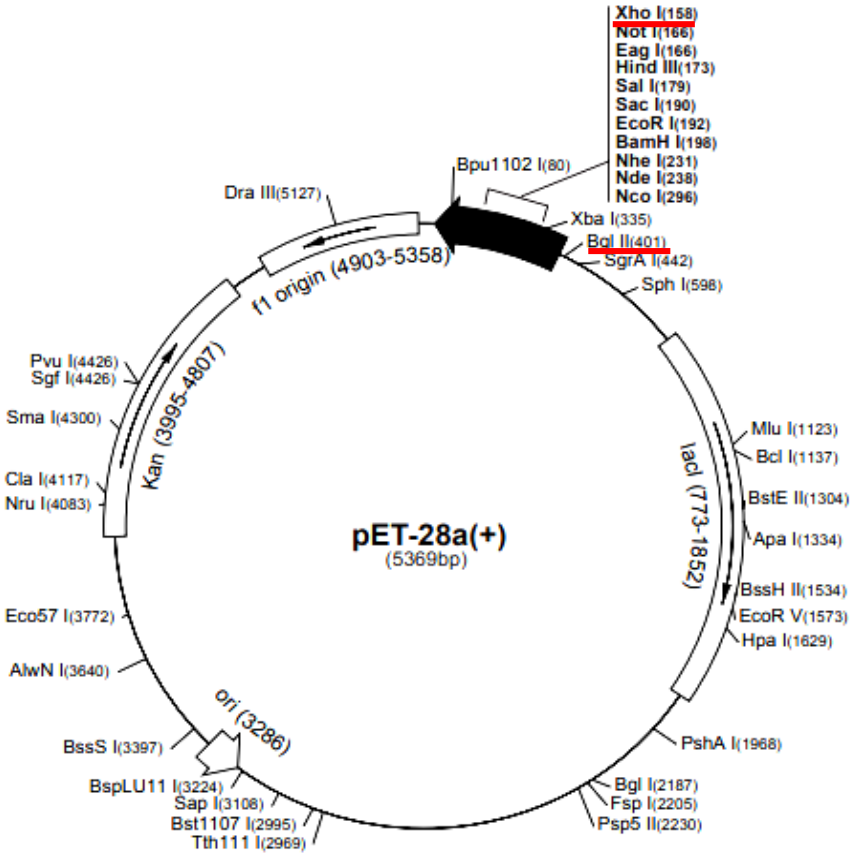
- SARS Wuhan-Hu-1 NC 045512.2 **(IS 239)**
- Cov Omicron/Botswana/R40B60BHP3321001247/2021 **(IS 238)**
- Delta SARS QW 65230.1 **(IS 237)**

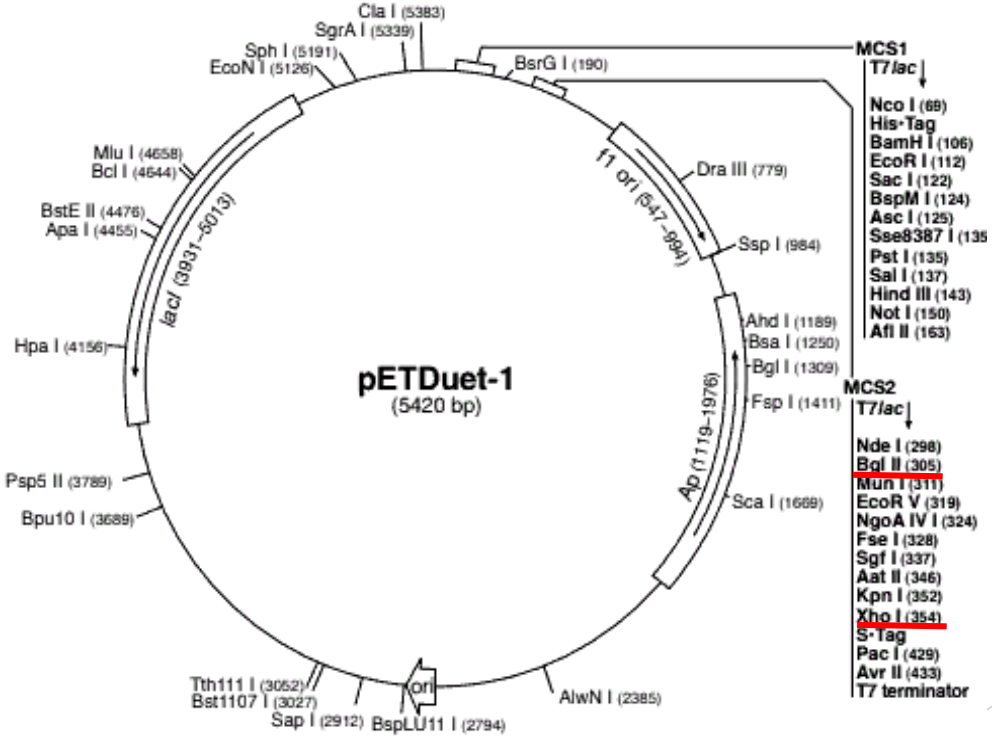
Materials. Plasmids

Shortened HBc/G C-terminus insertions

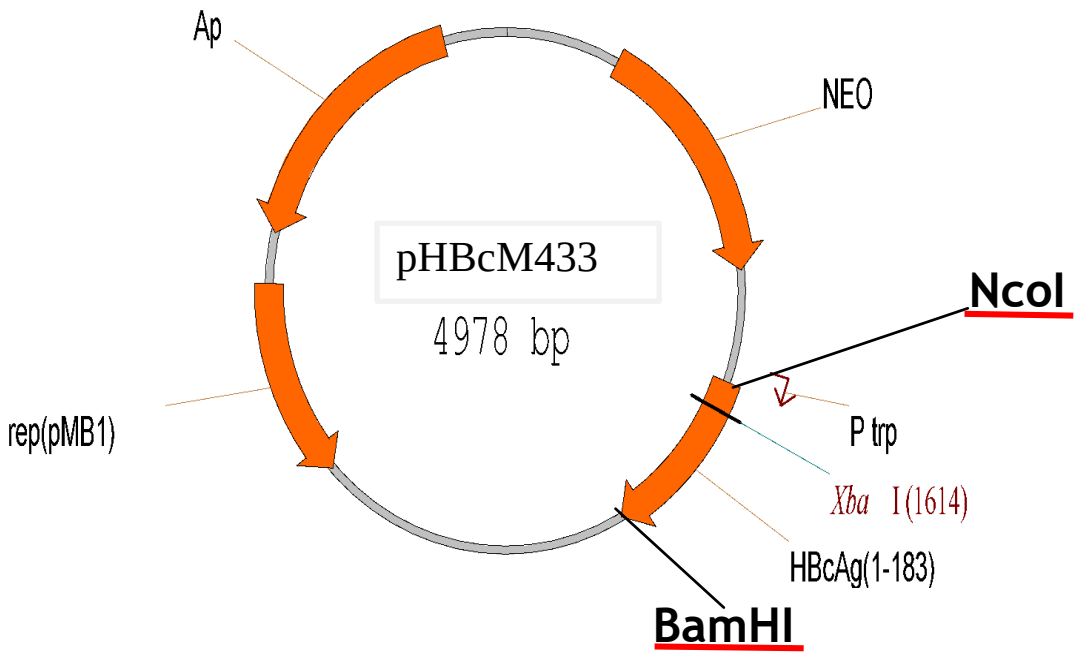


Materials. Vectors





Materials. Vectors



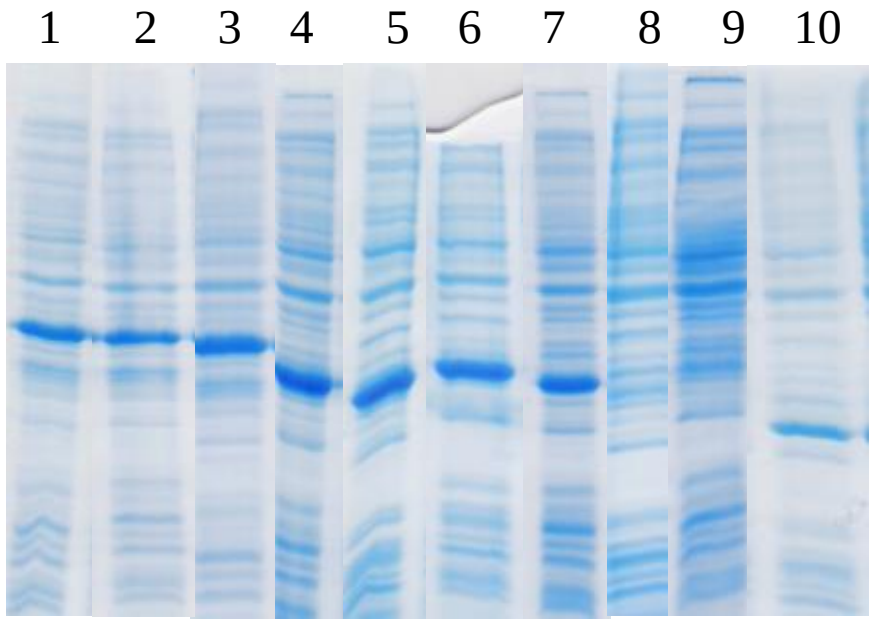
Materials. Competent cells

- XL1-Blue
- BL21 (DE3)
- BL21
- K802

Methods.

- Transformation/retransformation
- DNA purification
- Expression
- Electrophoresis – PAAGE and NAGE
- Western Blot
- PCR
- Restriction digestion
- Preparative gel
- DNA isolation from TAE
- Ligation reaction
- Sequencing

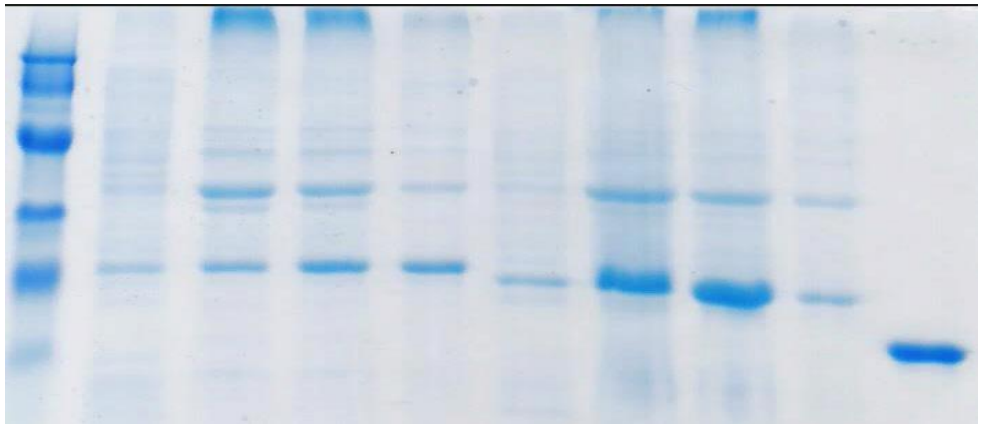
Results. Primary clone selection and expression



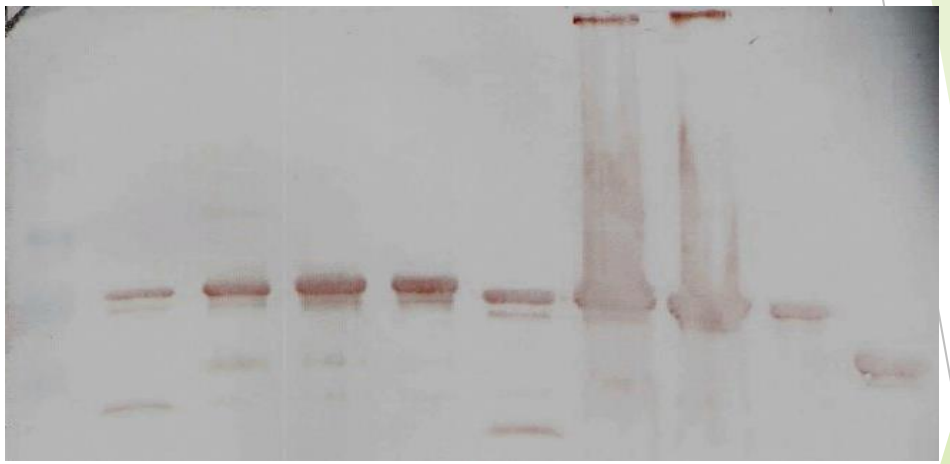
1	pET28a + HBc/G opt RBM Delta	IS234
2	pET28a + HBc/G opt RMB Omicron	IS235
3	pET28a + HBc/G opt RMB Wuhan	IS236
4	HBc/G opt Bep Delta	IS237
5	pET28a + HBc/G opt Bep Omicron	IS238
6	pET28a + HBc/G opt Bep Wuhan	IS239
7	HBc/G opt Cterm CTL (long)	IS240
8	pET28a + HBc/G opt Nterm CTL	IS241
9	HBc/G opt del Nterm CTL	IS242
10	pET28a + HBc/G opt VV CTL (short)	IS243

Results. Western Blot

M 240 Sepharose 4FF 243 Sepharose 4FF
US S 1.fr 2.fr 3.fr US S 1.fr 2.fr 3.fr C



M 240 Sepharose 4FF 243 Sepharose 4FF
US S 1.fr 2.fr 3.fr US S 1.fr 2.fr 3.fr C

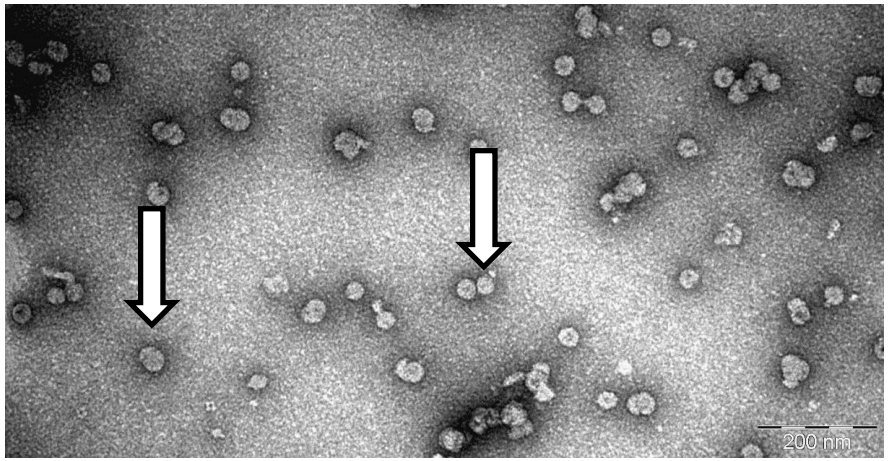


M	Protein Marker
240 US S	Supernatant of CTL epitope in HBc C-terminus (IS240) after ultrasonification
240 Sepharose 4FF 1fr.-3fr.	CTL epitope in HBc (IS240) after gel filtration on Sepharose 4FF in different fractions
243 US S	Supernatant of CTL epitope in HBc C-terminus after (IS243) after ultrasonification
243 Sepharose 4FF 1fr.-3fr.	CTL epitope in HBc (IS243) after gel filtration on Sepharose 4FF in different fractions
C	Control - HBc/D genotype (183 aa)

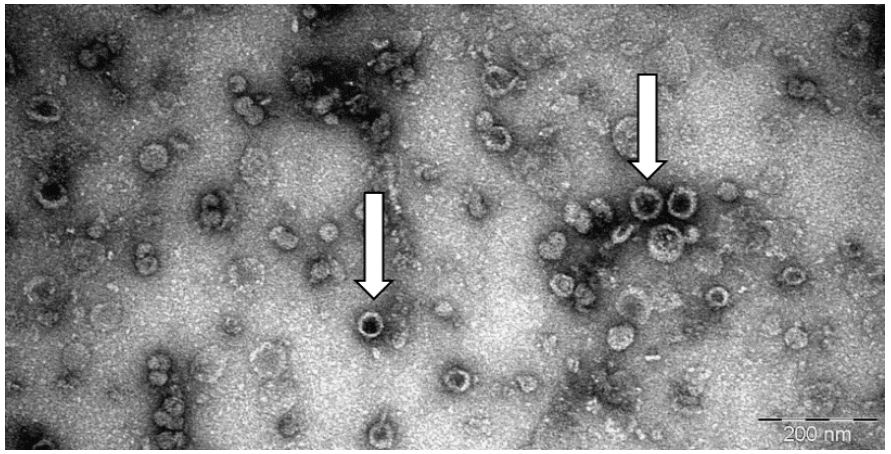
Results. Electronmicroscopy

CTL epitope string at HBc/G C-terminus (175 aa) - IS240.
June, 2022: *E.coli* was cultivated in 2TY media at 20˚C. Capsids after gel filtration and anion exchange chromatography on Fracto-DEAE.
September, 2022: *E.coli* was cultivated in AIB media at 20˚C. Capsids after gel filtration.

June, 2022



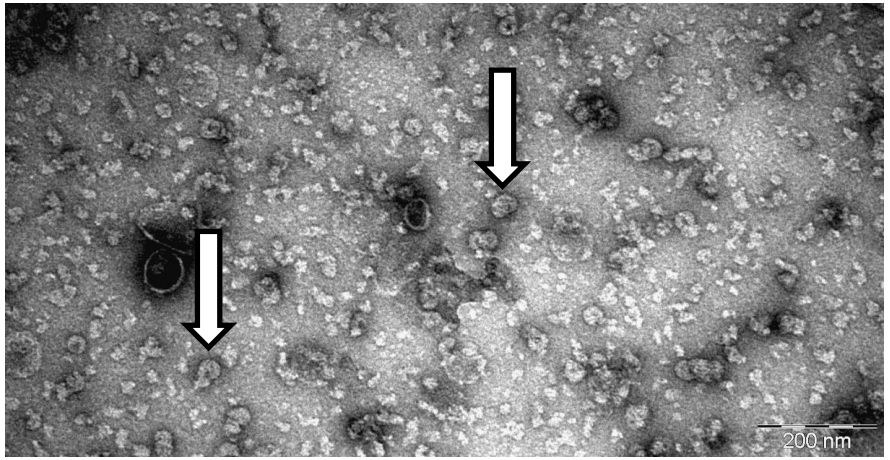
September, 2022



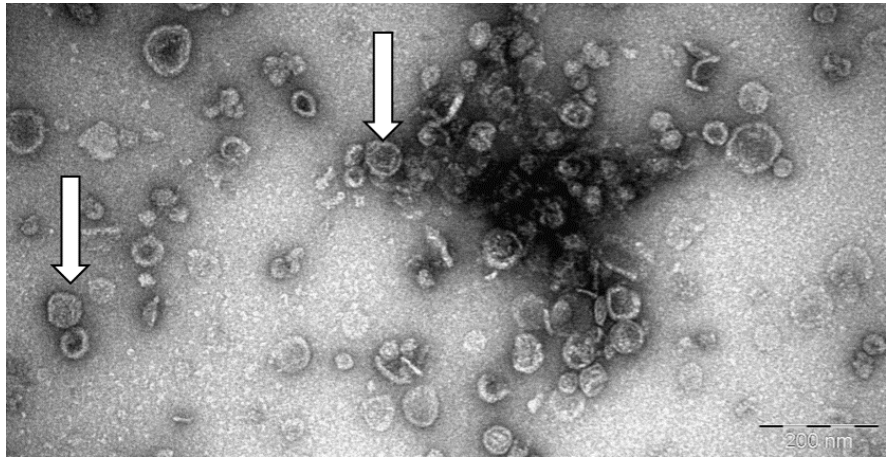
Results. Electronmicroscopy

CTL epitope string at HBc/G C- terminus (161 aa) - IS243.
June, 2022: *E.coli* was cultivated in 2TY media at 20˚C. Capsids after gel filtration
September, 2022: *E.coli* was cultivated in AIB media at 20˚C. Capsids after gel filtration.

June, 2022



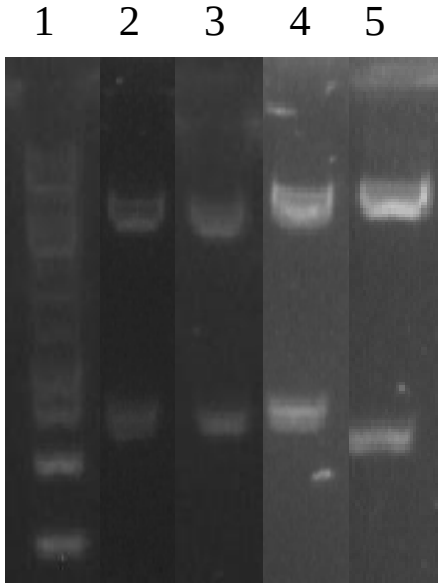
September, 2022



Results. Synthesis

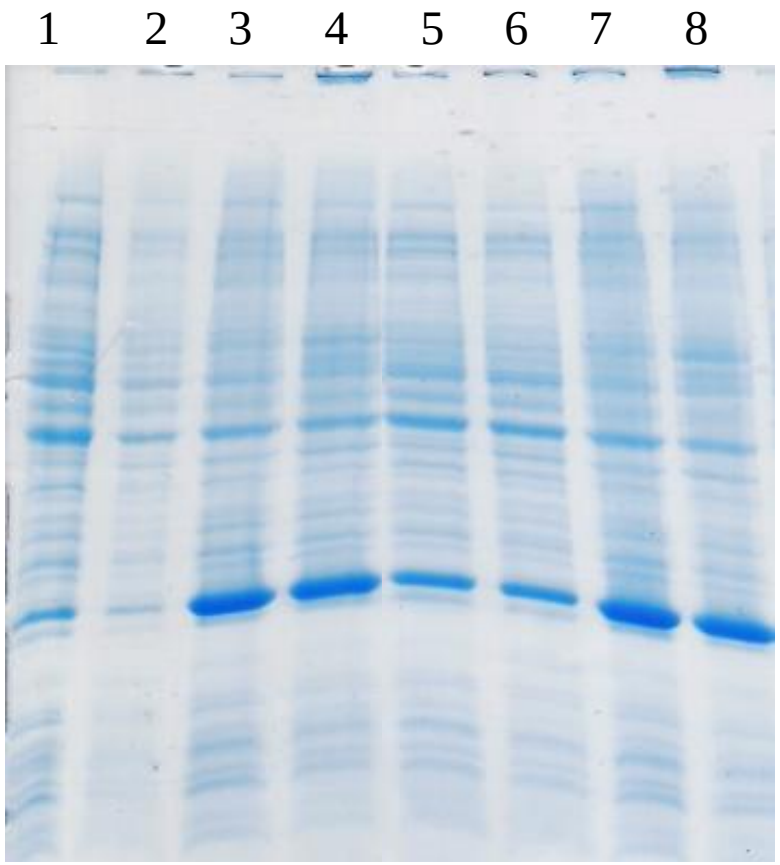
AN1	IS234 fragment BglII/XhoI + vector pETDuet HBc/G BglII/XhoI
AN2	IS235 fragment BglII/XhoI + vector pETDuet HBc/G BglII/XhoI
AN3	IS236 fragment BglII/XhoI + vector pETDuet HBc/G BglII/XhoI
AN4	IS239 fragment BglII/XhoI + vector pETDuet HBc/G BglII/XhoI
AN5	IS234 fragment NcoI/BamHI + vector pHBc/D (M433) NcoI/BamHI
AN6	IS235 fragment NcoI/BamHI + vector pHBc/D (M433) NcoI/BamHI
AN7	IS236 fragment NcoI/BamHI + vector pHBc/D (M433) NcoI/BamHI
AN8	IS237 fragment NcoI/BamHI + vector pHBc/D (M433) NcoI/BamHI

Results. Electrophoresis – NAGE



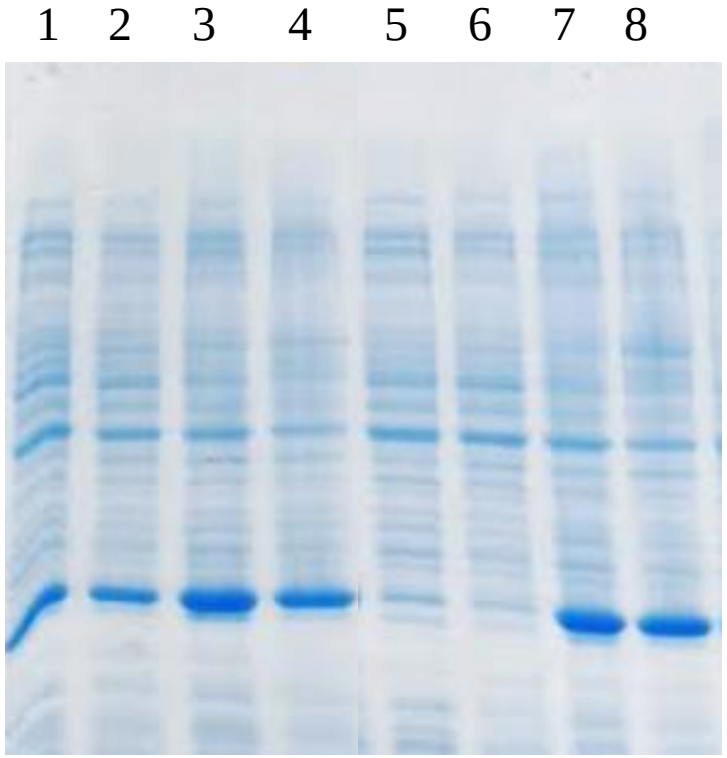
1	M	Marker GeneRuler 1kb
2	AN5	HBc/G + HBc/G opt RBM Delta (IS234)
3	AN6	HBc/G + HBc/G opt RBM Omicron (IS235)
4	AN7	HBc/G + HBc/G opt RBM Wuhan (IS236)
5	AN8	HBc/G + HBc/G opt Bep Omicron (IS237)

Results. Electrophoresis – PAAGE



1	AN1-1	pETDuet-1 HBc/G opt + RBM Delta (IS234). Individual clone cultivated in 2TY media, sample - 3 hours after induction.
2	AN1-1B	pETDuet-1 HBc/G opt + RBM Delta (IS234). Individual clone cultivated in 2TY media, sample - 24 hours after induction.
3	AN1-1	pETDuet-1 HBc/G opt + RBM Delta (IS234). Individual clone cultivated in autoinduction media, sample - 24 hours after induction.
4	AN1-1B	pETDuet-1 HBc/G opt + RBM Delta (IS234). Individual clone cultivated in autoinduction media, sample - 48 hours after induction.
5	AN2-4	pETDuet-1 HBc/G opt + RMB Wuhan (IS235). Individual clone cultivated in 2TY media, sample - 3 hours after induction.
6	AN2-4B	pETDuet-1 HBc/G opt + RMB Wuhan (IS235). Individual clone cultivated in 2TY media, sample - 24 hours after induction.
7	AN2-4	pETDuet-1 HBc/G opt + RMB Wuhan (IS235). Individual clone cultivated in autoinduction media, sample - 24 hours after induction.
8	AN2-4B	pETDuet-1 HBc/G opt + RMB Wuhan (IS235). Individual clone cultivated in autoinduction media, sample - 48 hours after induction.

Results. Electrophoresis – PAAGE



1	AN3-7	pETDuet-1 HBc/G opt + RMB Wuhan (IS236). Individual clone cultivated in 2TY media, sample - 3 hours after induction.
2	AN3-7B	pETDuet-1 HBc/G opt + RMB Wuhan (IS236). Individual clone cultivated in 2TY media, sample - 24 hours after induction.
3	AN3-7	pETDuet-1 HBc/G opt + RMB Wuhan (IS236). Individual clone cultivated in autoinduction media, sample - 24 hours after induction.
4	AN3-7B	pETDuet-1 HBc/G opt + RMB Wuhan (IS236). Individual clone cultivated in autoinduction media, sample - 48 hours after induction.
5	AN4-10	pETDuet-1 HBc/G opt + VV CTL (IS243). Individual clone cultivated in 2TY media, sample - 3 hours after induction.
6	AN4-10B	pETDuet-1 HBc/G opt + VV CTL (IS243). Individual clone cultivated in 2TY media, sample - 24 hours after induction.
7	AN4-10	pETDuet-1 HBc/G opt + VV CTL (IS243). Individual clone cultivated in autoinduction media, sample - 24 hours after induction.
8	AN4-10B	pETDuet-1 HBc/G opt + VV CTL (IS243). Individual clone cultivated in autoinduction media, sample - 48 hours after induction.

Conclusion

- The capsid formation was obtained in case of CTL- string insertions at the C-terminus of shortened HBc /G till 175 and 161 aa
- Plasmids on the basis of pET28a showed good expression in *E. coli* cells.
- Plasmids on the basis of pETDuet-1 plasmids where as the first was HBc/G and the second HBc/G with insertion didn’t show equal expression of both proteins.
- It is expected that the creation of the novel virus-like particles stimulates design of new vaccine prototypes against SARS-Cov-2.

References

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