Production of chimeric monoclonal IgG1 in *Escherichia coli* against *Acinetobacter baumannii* BamA protein

Guillem Puigsech Boixeda
Bachelor's Degree in Microbiology – Research Project

Universitat Autònoma de Barcelona

INTRODUCTION

- ***Acinetobacter baumannii*** is a gram-negative coccobacillus that causes nosocomial infections worldwide and belongs to the ESKAPE group. It is currently one of the highest priority pathogenic bacteria for which it is necessary to find new effective alternative therapies to the use of antimicrobials, and passive immunotherapy with monoclonal antibodies against its outer membrane protein BamA, which is essential for its viability, could be a solution.
- **Monoclonal antibodies** are conventionally produced using hybridomas, but they could also be produced more efficiently using *Escherichia coli as s*trains capable of producing such therapeutic agents have been developed. One example is the SHuffle2 strain, which presents deletions in the disulphide bridge reduction pathways (*trxB* and *gor*), a mutation in AhpC peroxidase that restores cell reducing power in essential cases and, in addition, overexpression of disulphide bridge isomerases. Given the production yield of *E. coli*, it could be a great tool to be used widely in the monoclonal antibody industry as its use is becoming more and more widespread in our society.

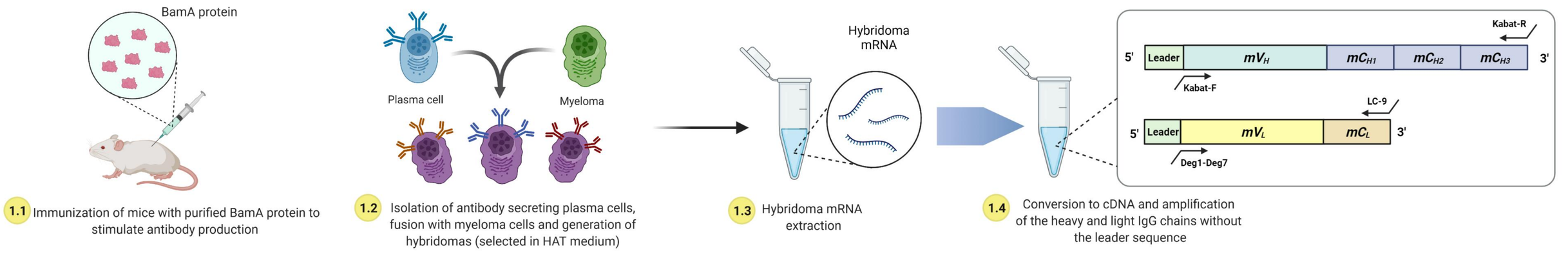
HYPOTHESIS AND OBJECTIVES

Production in Shuffle2 of aglycosilated IgG capable of binding specifically to the BamA protein of *Acinetobacter baumannii* and inducing its phagocytosis could show promising results in *in vitro* assays.

- **To establish a procedure that includes the use of *Escherichia coli* for the screening of the sequences of monoclonal, chimeric and aglycosylated IgG variable regions with affinity for the BamA protein of Acinetobacter baumannii.**
- **To test the recognition of this aglicosylated IgG by phagocytic cells of the human immune system.**

MATHERIALS AND METHODS

1. Generation of the hybridoma and amplification of anti-BamA IgG coding genes.



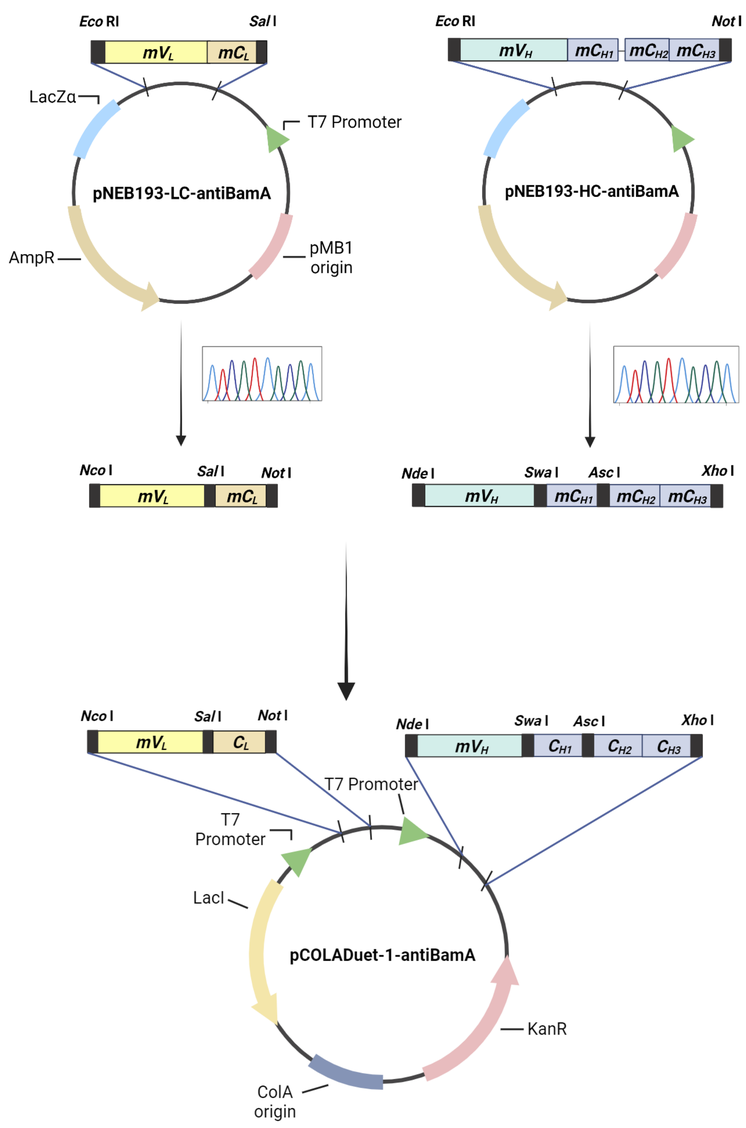
1.1 Immunization of mice with purified BamA protein to stimulate antibody production

1.2 Isolation of antibody secreting plasma cells, fusion with myeloma cells and generation of hybridomas (selected in HAT medium)

1.3 Hybridoma mRNA extraction

1.4 Conversion to cDNA and amplification of the heavy and light IgG chains without the leader sequence

2. Cloning of the IgG coding genes in pCOLADuet-1 plasmids

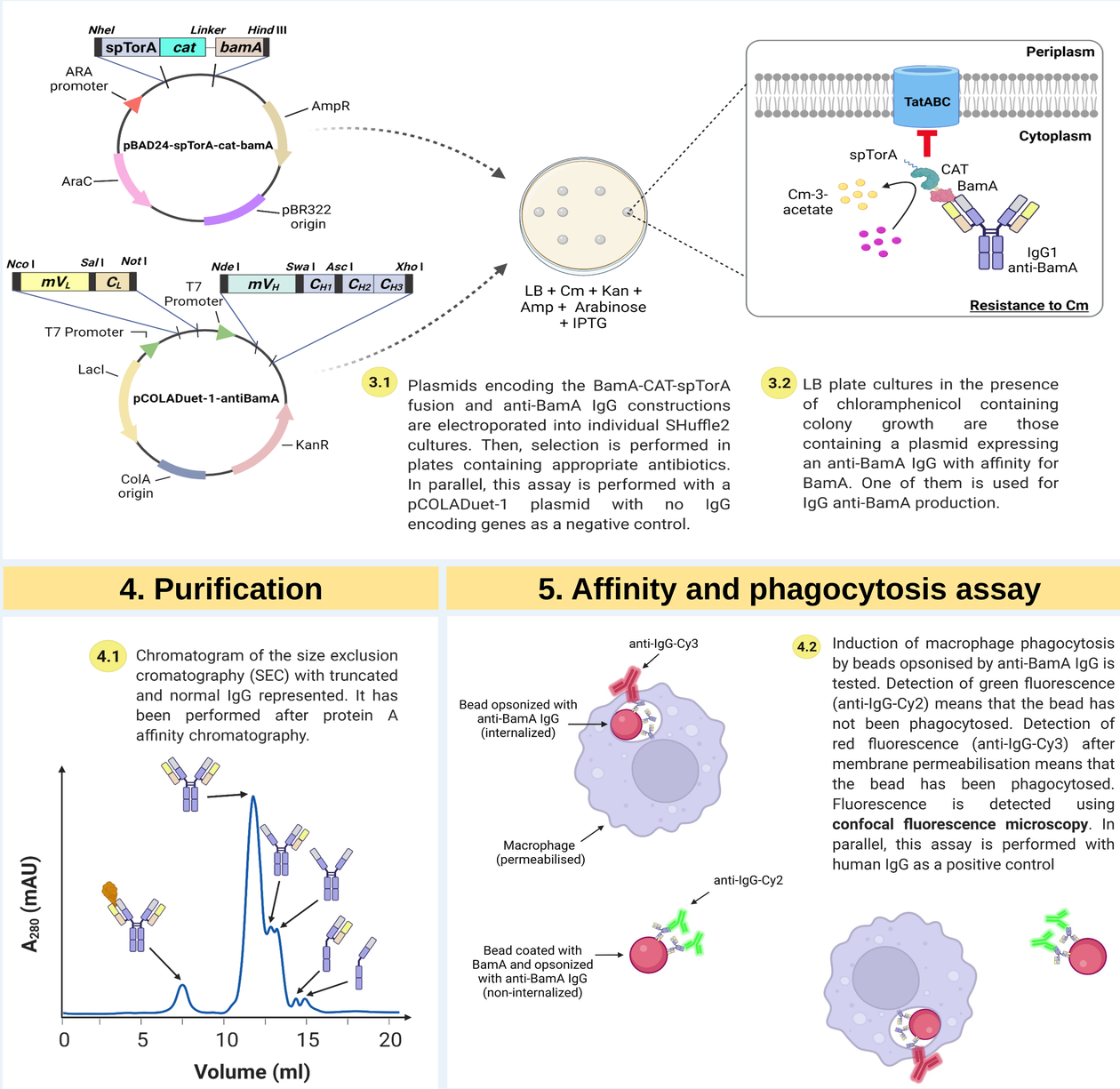


2.1 The amplified genes encoding the mouse IgG chains are cloned into pNEB193 plasmids for subsequent sequencing.

2.2 Additional restriction sites are added to the IgG genes by synthetic gene synthesis and overlap extension for cloning to the pCOLADuet-1 plasmid.

2.3 **pCOLADuet-1-antiBamA** plasmids are constructed and the genes encoding the constant regions of mouse IgG are replaced with the human constant regions (C_L, C_{H1-3}). Moreover, three mutations in C_{H1} and C_{H3} Fc encoding genes are introduced in order to favour antibody and phagocytes interactions: **E382V**, **M428I** and **V214 (GTG to GTT)**.

3. BamA-affinity IgG screening and production



3.1 Plasmids encoding the BamA-CAT-spTorA fusion and anti-BamA IgG constructions are electroporated into individual SHuffle2 cultures. Then, selection is performed in plates containing appropriate antibiotics. In parallel, this assay is performed with a pCOLADuet-1 plasmid with no IgG encoding genes as a negative control.

3.2 LB plate cultures in the presence of chloramphenicol containing colony growth are those containing a plasmid expressing an anti-BamA IgG with affinity for BamA. One of them is used for IgG anti-BamA production.

4. Purification

4.1 Chromatogram of the size exclusion cromatography (SEC) with truncated and normal IgG represented. It has been performed after protein A affinity chromatography.

5. Affinity and phagocytosis assay

4.2 Induction of macrophage phagocytosis by beads opsonised by anti-BamA IgG is tested. Detection of green fluorescence (anti-IgG-Cy2) means that the bead has not been phagocytosed. Detection of red fluorescence (anti-IgG-Cy3) after membrane permeabilisation means that the bead has been phagocytosed. Fluorescence is detected using **confocal fluorescence microscopy**. In parallel, this assay is performed with human IgG as a positive control

EXPECTED RESULTS

- **To increase the availability of treatments for *Acinetobacter baumannii* infections that do not require antibiotic administration.**
- **Provide sequences of antibodies against the BamA protein to be studied for humanization.**
- **Motivate other scientists to start new projects in order to improve this strategy of monoclonal antibody production.**
- **Application of the exposed procedure to the generation of monoclonal antibodies for other purposes.**

DISSEMINATION PLAN

- Publications in **biotechnology** and **applied microbiology** journals.
- Presentation of the poster at national and international conferences to show this alternative to the use of hybridomas.

REFERENCES

(1) Robinson MP, Ke N, Lobstein J, Peterson C, Szkodny A, Mansell TJ, et al. Efficient expression of full-length antibodies in the cytoplasm of engineered bacteria. Nat Commun. 2015 Nov;6(1):8072. (2) Lénon M, Ke N, Szady C, Sakhtah H, Ren G, Manta B, et al. Improved production of Humira antibody in the genetically engineered Escherichia coli SHuffle, by co-expression of human PDI-GPx7 fusions. Appl Microbiol Biotechnol. 2020 Nov;104(22):9693–706. (3) Isolation of engineered, full-length antibodies from libraries expressed in Escherichia coli | Nature Biotechnology (4) Choy CH, Botelho RJ. Quantifying Phagocytosis by Immunofluorescence and Microscopy. In: Botelho R, editor. Phagocytosis and Phagosomes: Methods and Protocols. **Images created with Biorender.com**