

SUPPLEMENTARY DATA

Improvement in the production of the human recombinant enzyme N-acetylgalactosamine-6-sulfatase (rhGALNS) in *Escherichia coli* using synthetic biology approaches

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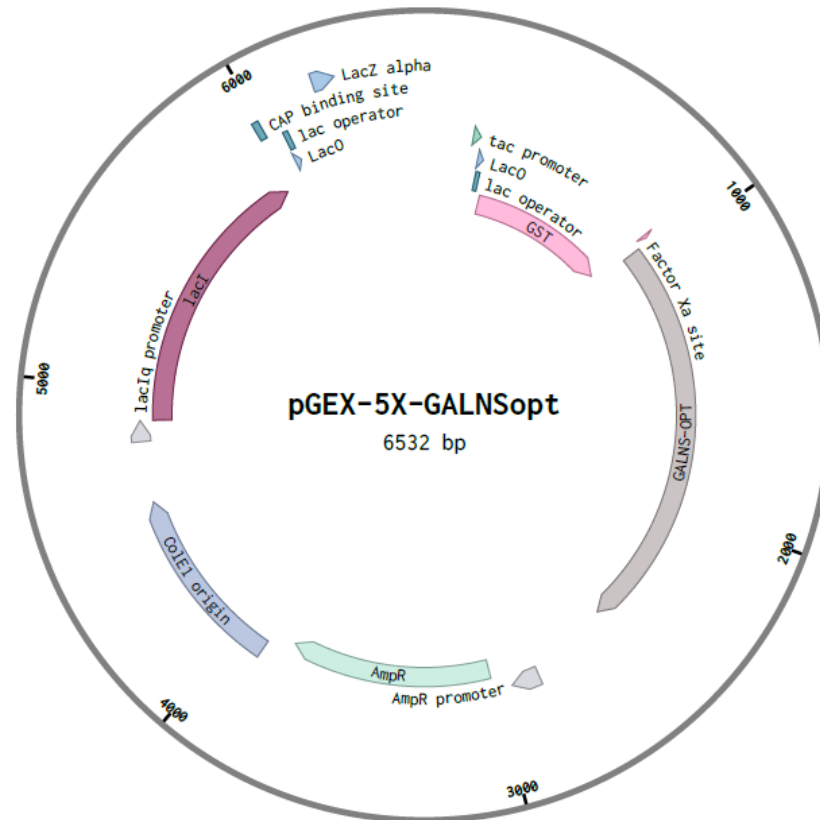
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2. Plasmid maps used in this study

a. pGEX-5X-GALNSopt



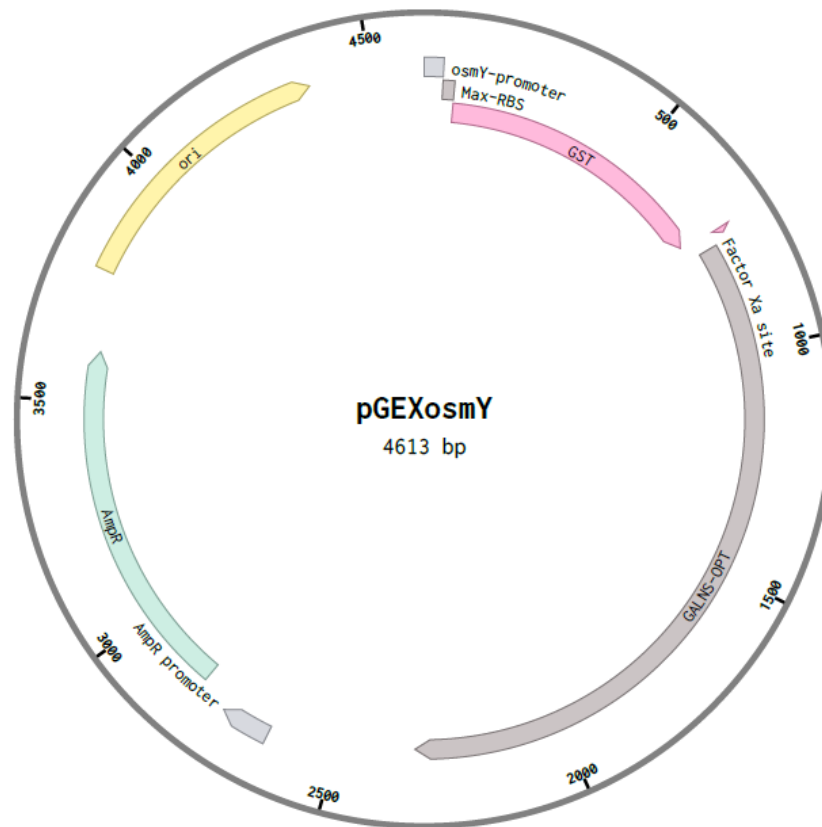
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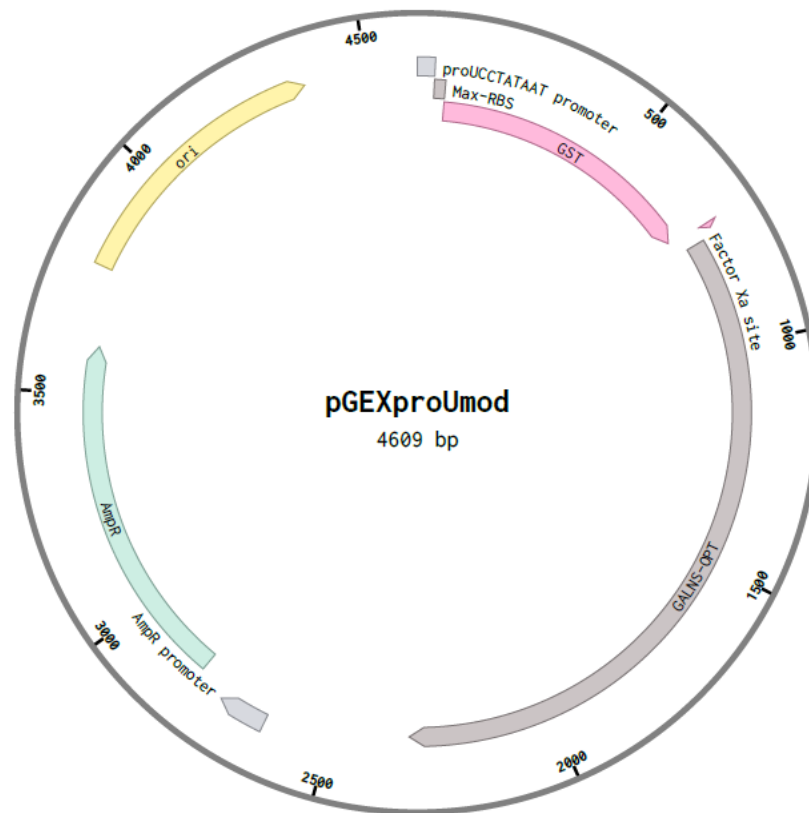


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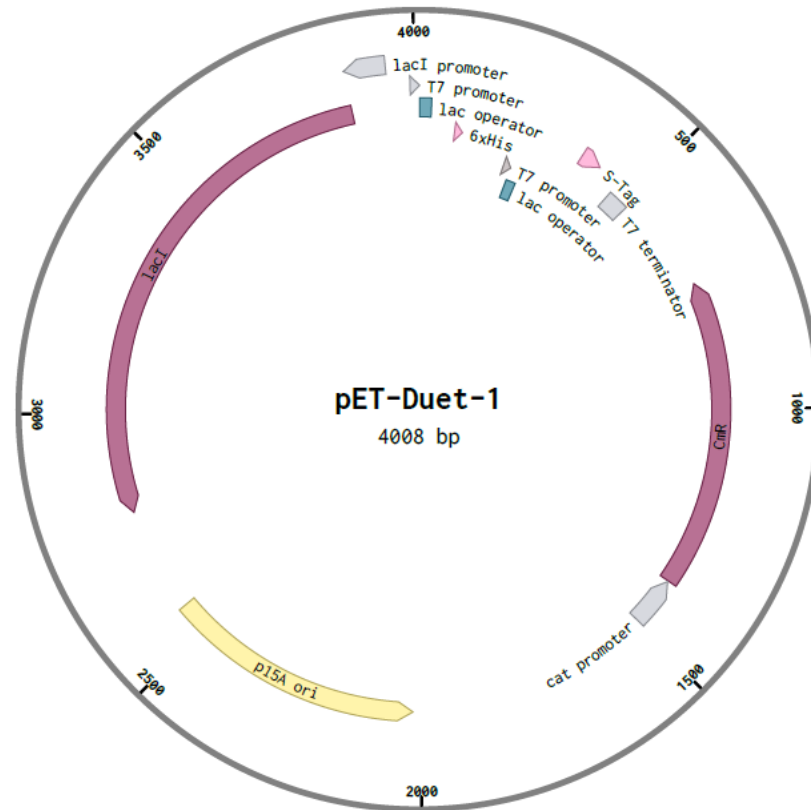


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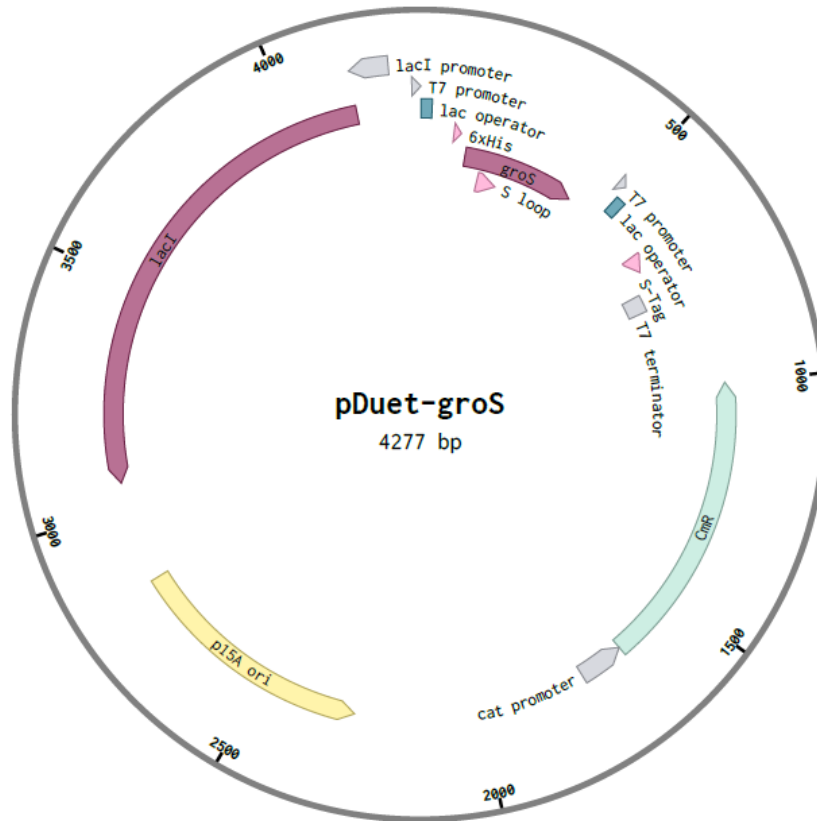


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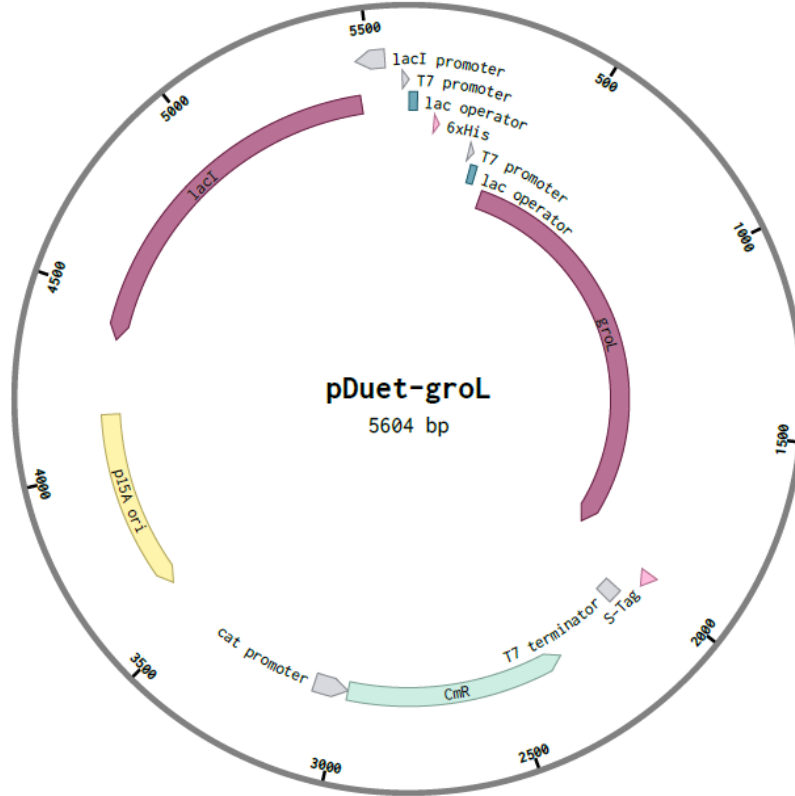


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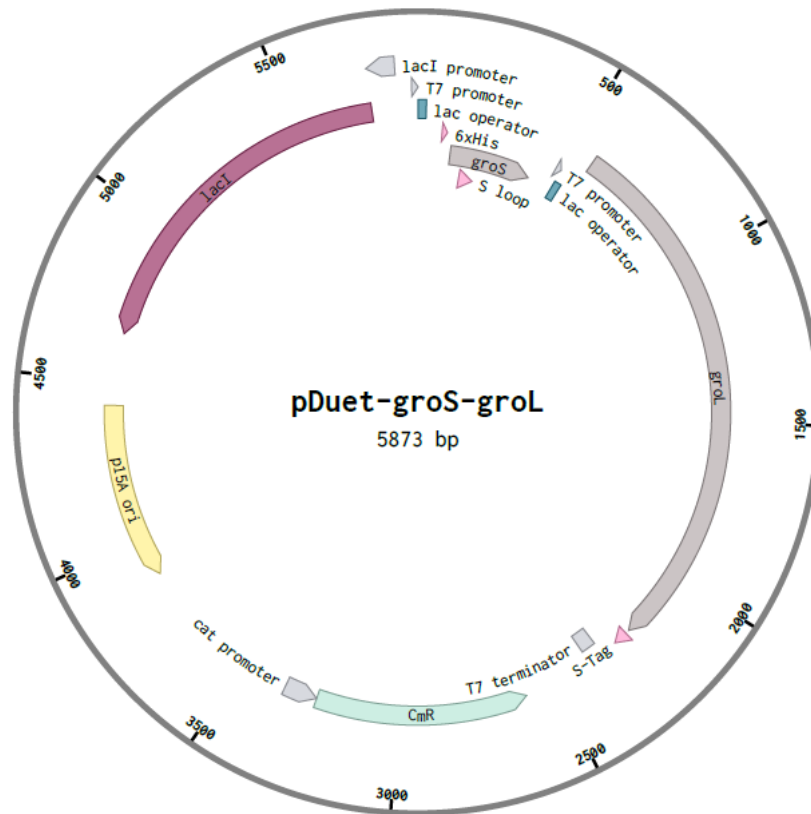
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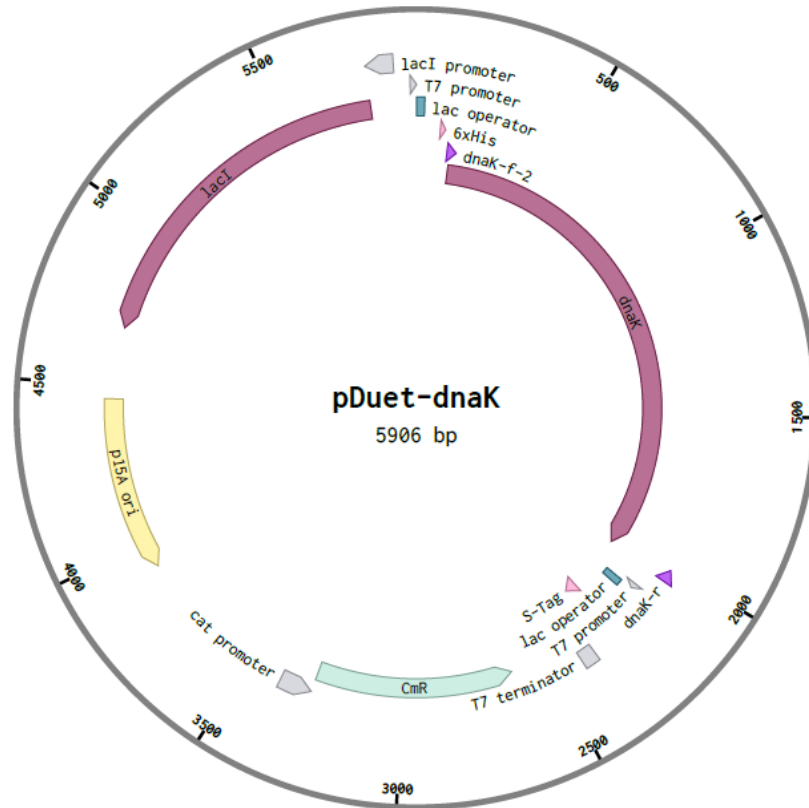
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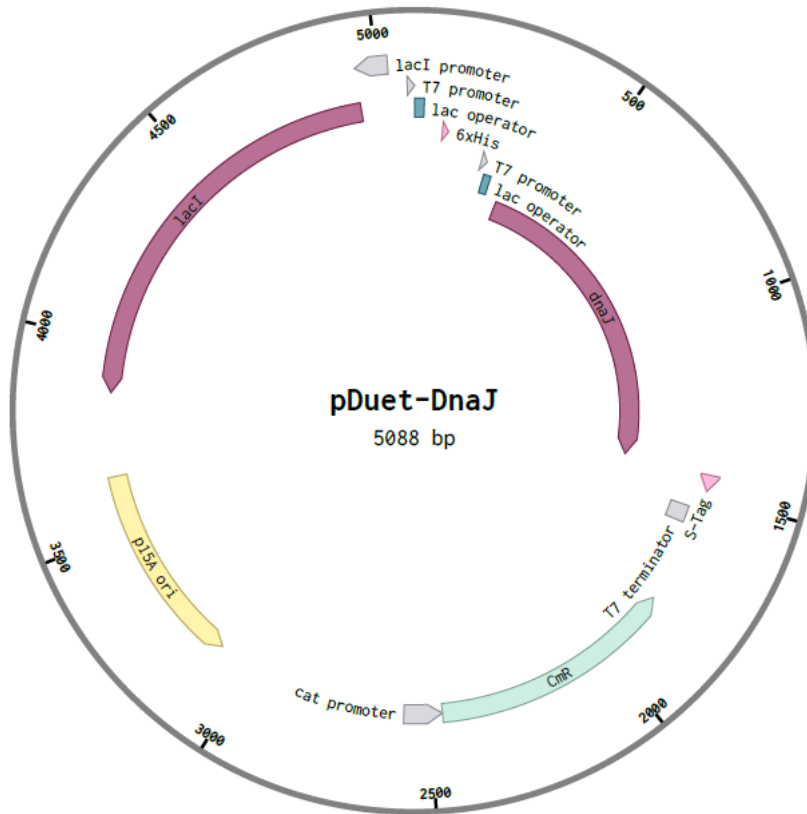
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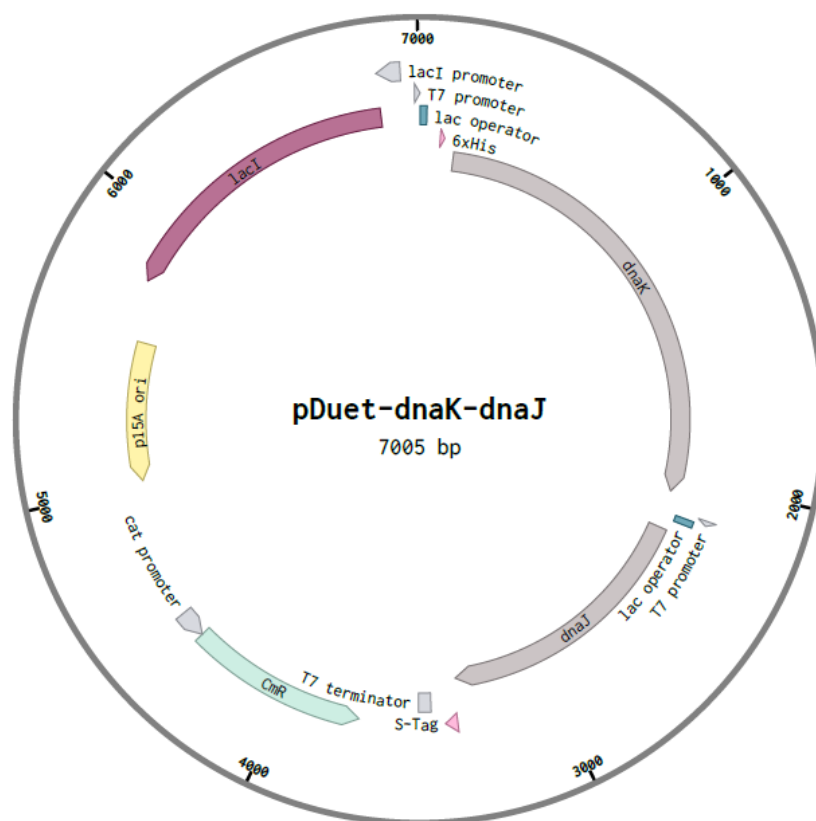


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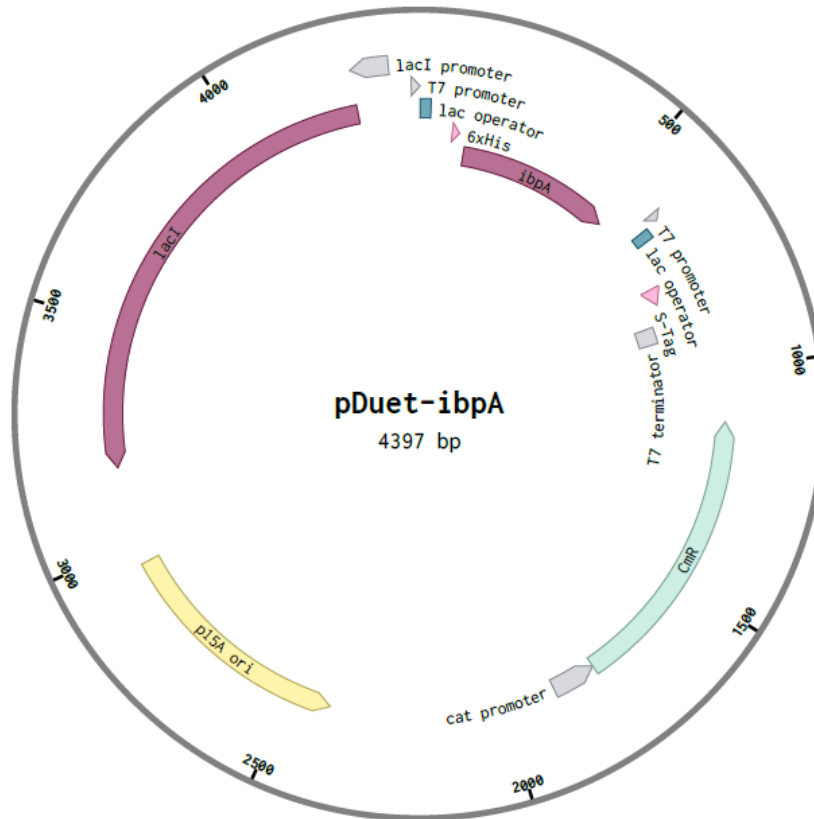
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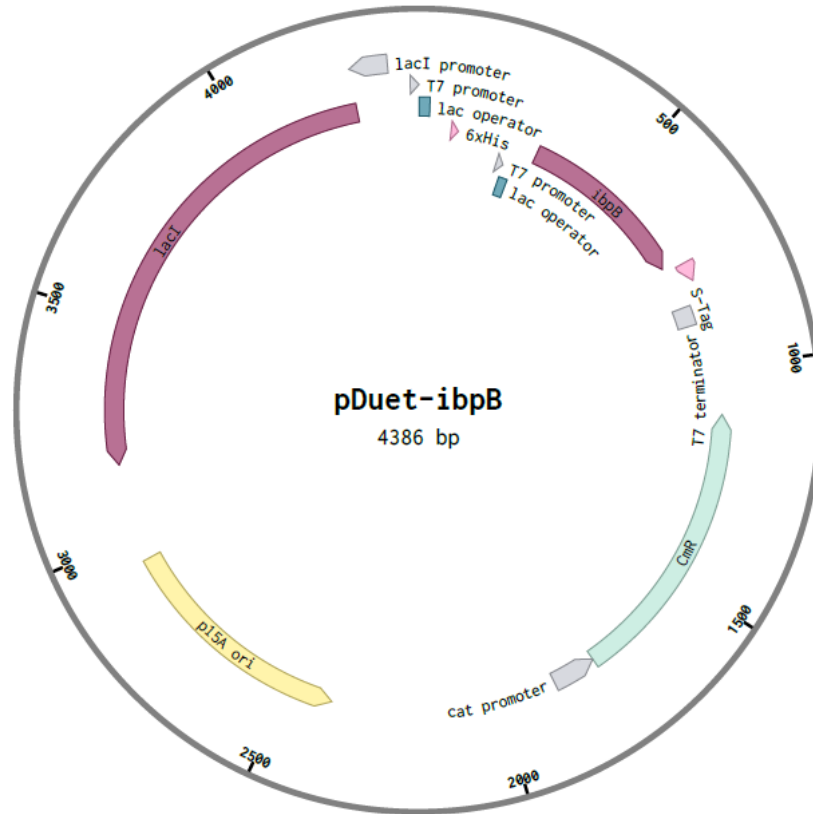


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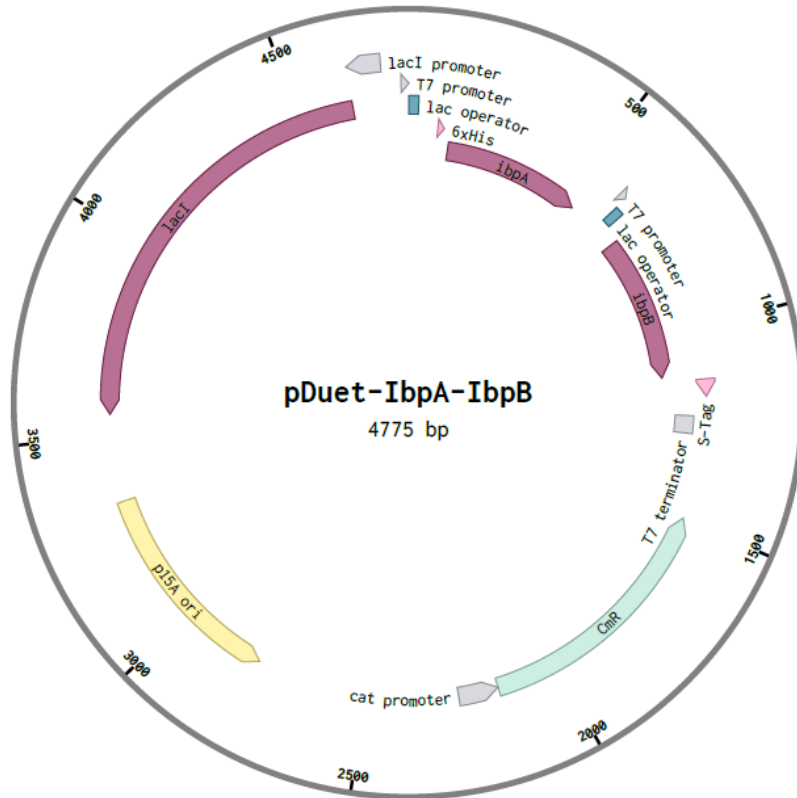


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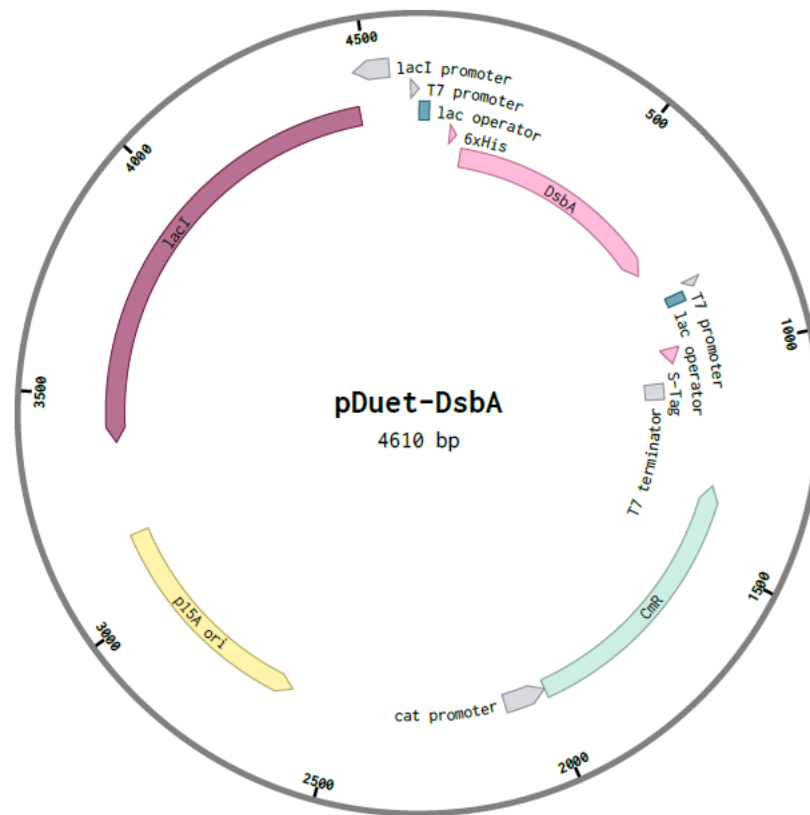


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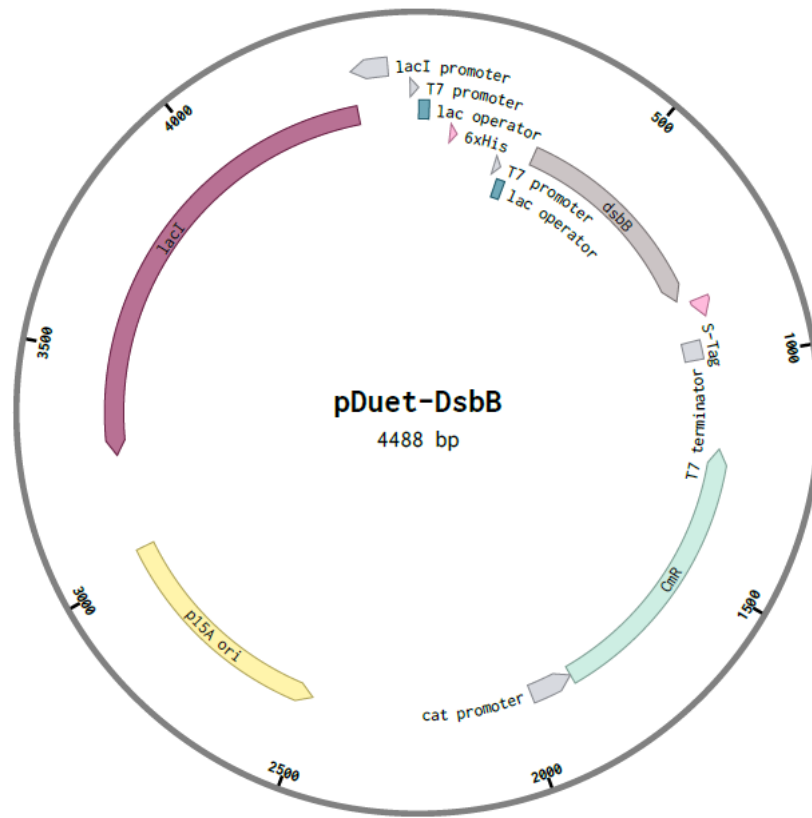


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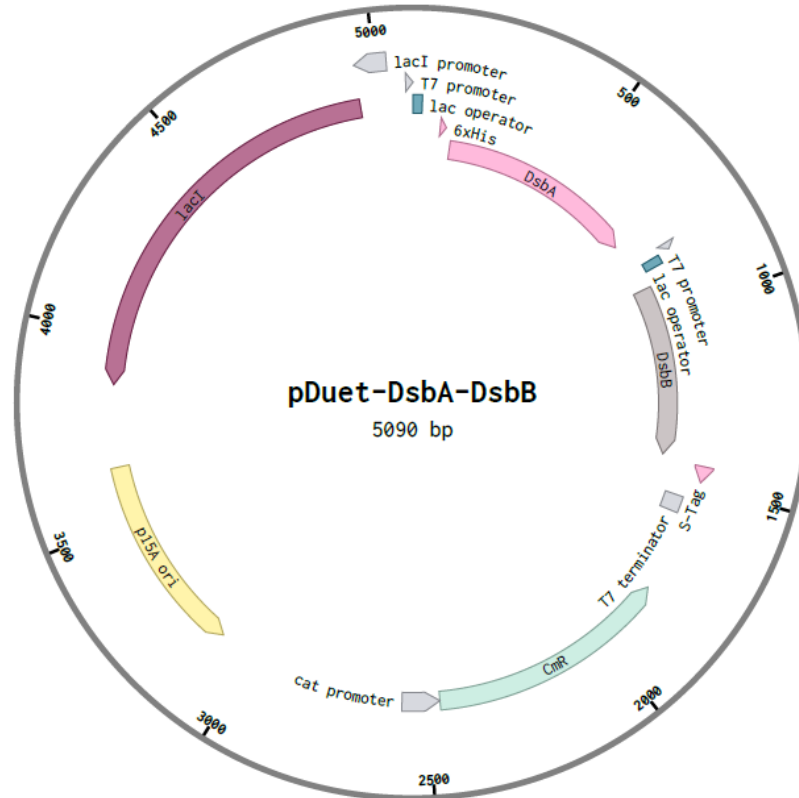


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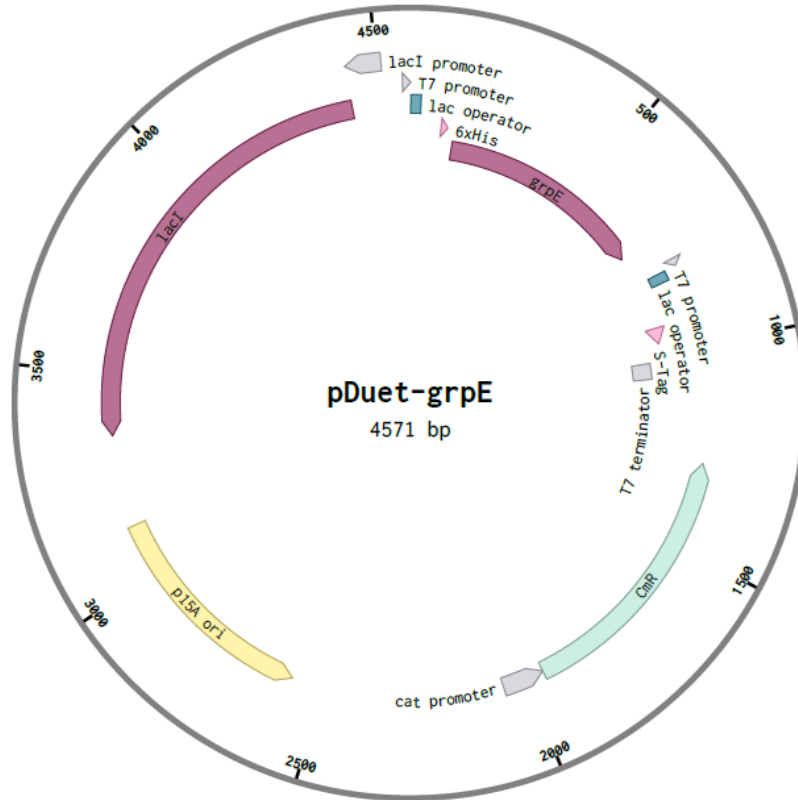


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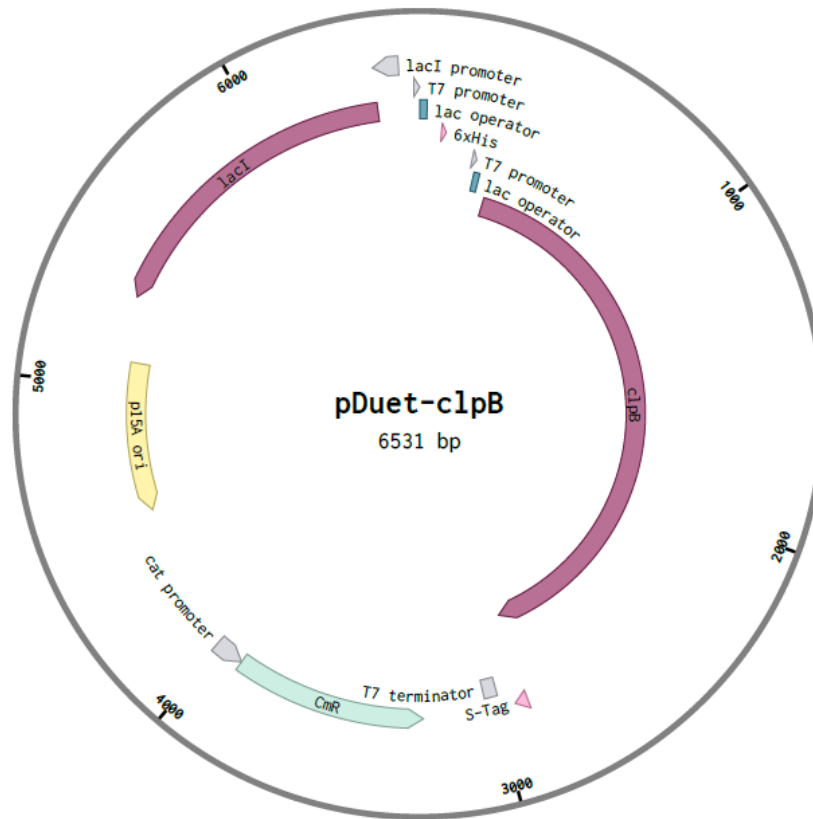


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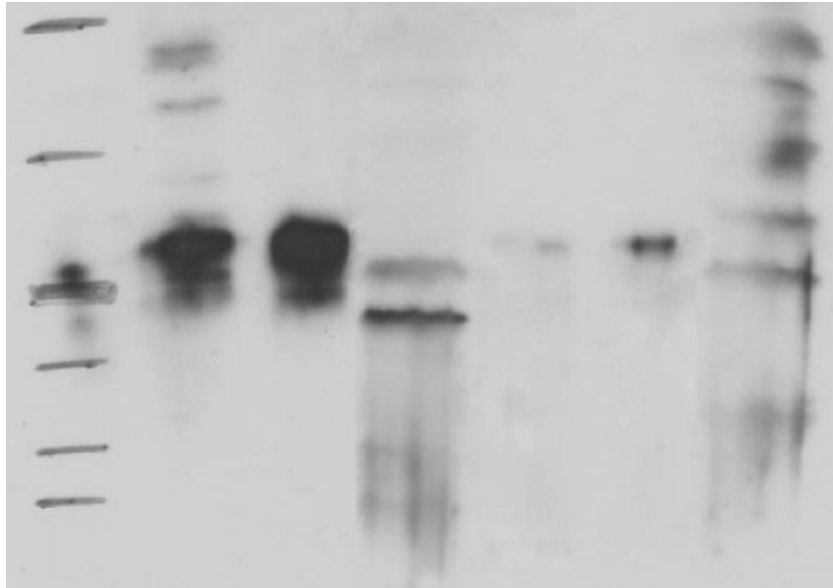
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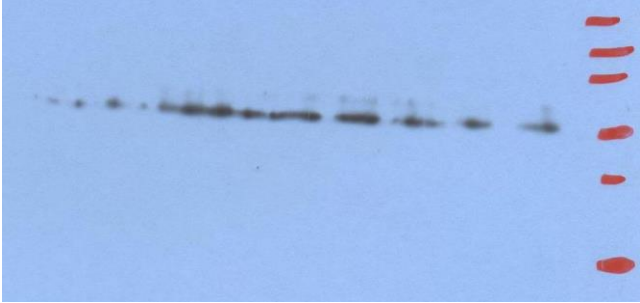
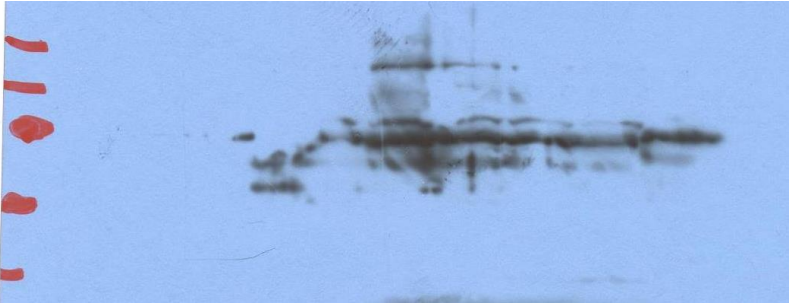
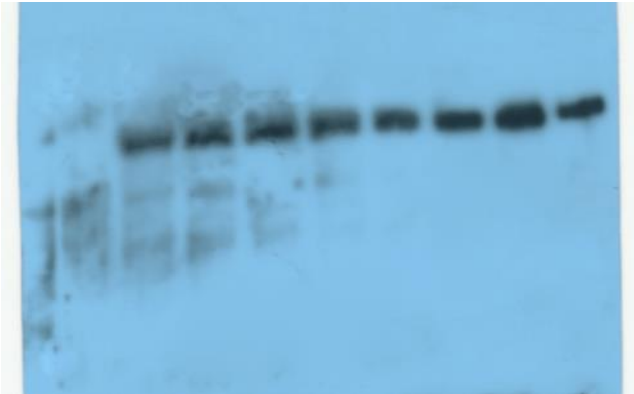
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3. Non-cropped Western Blots

a. Production of rhGALNS in *E. coli* BL21(DE3) using different promoters



b. Dynamics of the expression of rhGALNS under the control of the promoter *proU_{mod}*.



c. Production of rhGALNS using the promoters *tac* and *proU_{mod}*, with two different *E. coli* strains: BL21(DE3) and SHuffle®



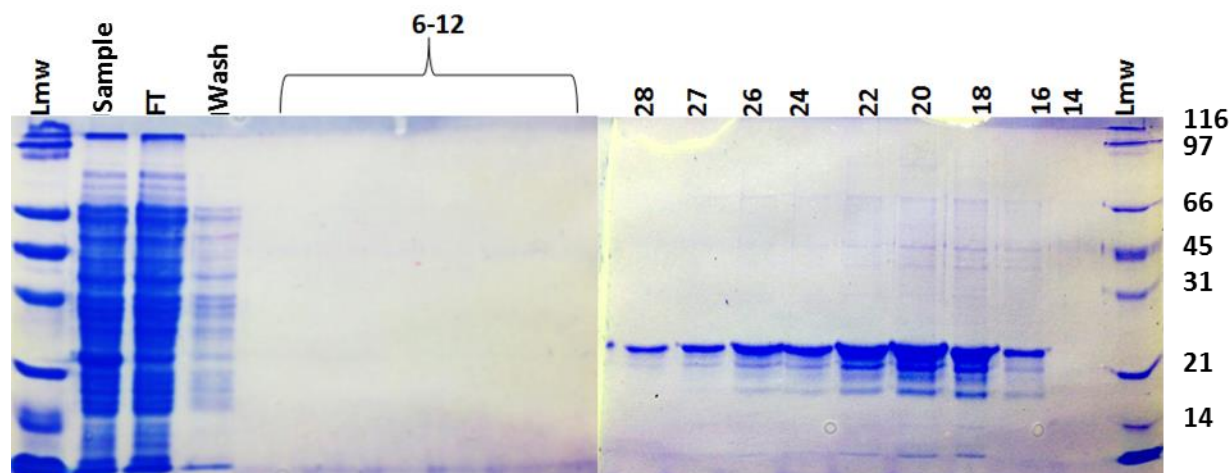
4. Purification via affinity chromatography

Cell lysates were applied to a Glutathione Sepharose 4B with peristaltic pump, under manufacturer instructions (GE Healthcare). In short, the column was equilibrated with approximately ten column volumes of PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂ HPO₄, 1.8 mM KH₂PO₄, pH 7.3). Then, the column was incubated for 1 hour with the sample at room temperature, followed by a washing step with ten column volumes of PBS pH 7.3 until no material appears in the flow-through. Finally, the proteins were eluted with elution buffer (50 mM Tris-HCl, 10 mM reduced glutathione). The pH was continuously increased from pH 8.0. Fractions of 0.5 ml were collected and visualized using a SDS-PAGE.

The following table indicates the protein amounts obtained after each purification step.

	mL	Concentration (mg/mL)	Total protein mg	Bound protein (mg/ml)	Bound protein after washing (mg/ml)	Bound protein after washing + eluted (mg/ml)
Protein load	5	24.870	124.350			
Flow-through	5	18.470	92.850	32.000	14.325	15.268
Wash	50	0.354	17.675			
Eluted	25	0.034	0.9425			

After the samples were visualized in a SDS-PAGE (Figure shown below), it was evident that the protein fusion was not expressed. Instead, a band corresponding to the GST peptide (26 kDa) was found in the different fractions, prominent in the 20th fraction. These samples were tested for enzyme activity but it was not present.



5. Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

An indirect ELISA was carried out in order to validate the extracellular amounts rhGALNS, according to the protocol described by Rodriguez *et. al.* 2010. A calibration curve between 0 and 1 $\mu\text{g/ml}$ was created using a recombinant human GALNS enzyme produced in CHO cells (stock concentration of 551 $\mu\text{g/ml}$). The samples quantified correspond to culture medium of BL21(DE3)/pGEX-5X-GALNSopt after 24 hours cultivation, exposed to 5% (w/v) sucrose, 0.05N NaCl and absence of osmotic stress.

The results indicate (Figure shown below) that the amounts of rhGALNS in the extracellular fraction were larger when the cells were exposed to osmotic stress, either in the presence of sucrose or sodium chloride, with increments of 349% and 265% of the secreted protein, respectively.

