Optimization of chondroitin production in *E. coli* using genome scale models

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Supplementary Information

Table SI1. Optimization of chondroitin production using OptFlux. The optimization algorithm was run at least four times for each model. The predicted phenotype for the unmodified and modified strains (from the resulting solutions with highest biomass-product coupled yield (BPCY)) are shown. BPCY is calculated by OptFlux by multiplying biomass by product and then dividing by substrate consumed (in all cases being 10 mmol/g_{DW}/h), as predicted by pFBA simulation. Flux variability analysis (FVA) results are shown as minimum and maximum chondroitin obtained through pFBA for fixed biomass. Predicted biomass and chondroitin values are in units of h^{-1} and mmol/g_{DW}/h, respectively.

Model	BPCY	Genes modified		Predicted phenotype (pFBA)		FVA	
		Underexpression	Overexpression	Biomass	Chondroitin	Min chondroitin	Max chondroitin
iB21_1397_c	-	-	-	0.9756	0.0000	-	-
iB21_1397_c	0.09607	*cmk*	*glmU*	0.3671	2.6168	2.6146	2.6345
	0.09607	*pyrH*	*glmM*	0.3671	2.6168	2.6146	2.6345
	0.09607	*pyrH*	*glmU*	0.3671	2.6168	2.6146	2.6345
	0.09200	*mltC*	*glmU*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltF*	*glmU*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltA*	*glmU*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltB*	*glmU*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltE*	*glmU*	0.6516	1.4119	1.4025	1.4401
	0.09200	*slt*	*glmU*	0.6516	1.4119	1.4025	1.4401
iECBD_1354_c	-	-	-	0.9756	0.0000	-	-
iECBD_1354_c	0.09200	*mltE*	*glmM*	0.6516	1.4119	1.4025	1.4401
	0.09200	*slt*	*glmM*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltC*	*glmM*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltA*	*glmM*	0.6516	1.4119	1.4025	1.4401
	0.09200	*amiABC, ampG*	*glmU*	0.6516	1.4119	1.4025	1.4401
	0.09200	*slt*	*glmU*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltB*	*glmM*	0.6516	1.4119	1.4025	1.4401
	0.09200	*mltF*	*glmM*	0.6516	1.4119	1.4025	1.4401
iEC1356_Bl21DE3_c	-	-	-	0.9767	0.000	-	-
iEC1356_Bl21DE3_c	0.09215	*mltE*	*glmU*	0.6519	1.4135	1.4015	1.4417
	0.09215	*oppC*	*glmU*	0.6519	1.4135	1.3268	1.4417
	0.09215	*mltC*	*glmU*	0.6519	1.4135	1.4015	1.4417
	0.09215	*oppB*	*glmU*	0.6519	1.4135	1.3268	1.4417
	0.09215	*oppF*	*glmU*	0.6519	1.4135	1.3268	1.4417
	0.09215	*amiA*	*glmU*	0.6519	1.4135	1.4015	1.4417
	0.09215	*amiB*	*glmU*	0.6519	1.4135	1.4015	1.4417
	0.09215	*oppD*	*glmU*	0.6519	1.4135	1.3268	1.4417
	0.09215	*mltA*	*glmU*	0.6519	1.4135	1.4015	1.4417
	0.09215	*mltB*	*glmU*	0.6519	1.4135	1.4015	1.4417
	0.09215	*amiC*	*glmU*	0.6519	1.4135	1.4015	1.4417
iJO1366_c	-	-	-	0.9824	0.000	-	-
iJO1366_c	0.09287	*mltB*	*glmM*	0.6531	1.4219	1.4000	1.4501
	0.09287	*mltE*	*glmM*	0.6531	1.4219	1.4000	1.4501

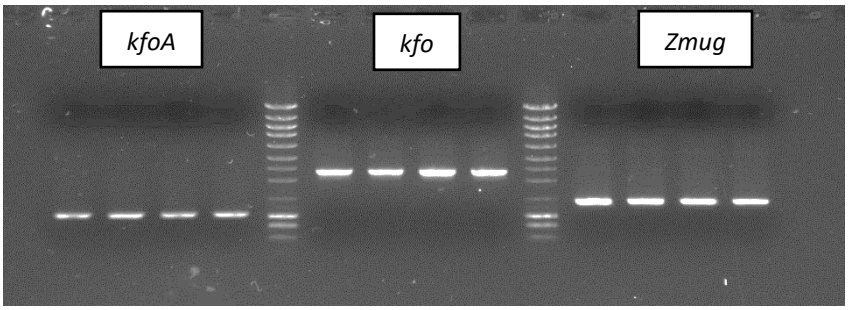

Figure SI1. Agarose gel 0.7% showing polymerase chain reaction (PCR) amplification results of genes for chondroitin biosynthetic pathway construction. Ladder: NZYDNA Ladder III, NZYTech.

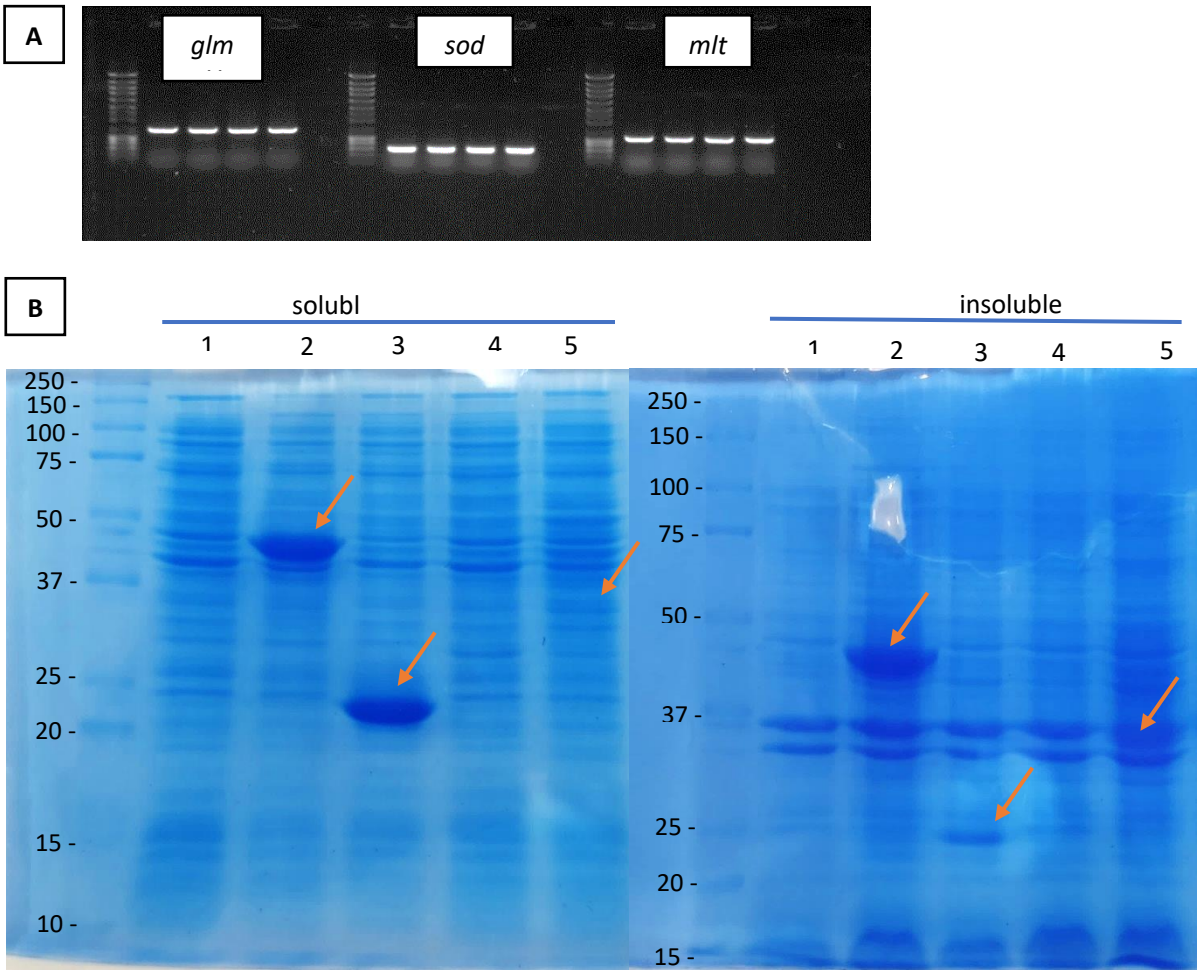

Figure SI2. Cloning and expression of identified targets for chondroitin production enhancement: *glmU*, glucosamine-1-phosphate acetyltransferase/*N*-acetylglucosamine-1-phosphate uridyltransferase; *mltB*, membrane-bound lytic murein transglycosylase; *sodA*, superoxide dismutase. **A.** Agarose gel 0.7% showing PCR result of amplification of genes *glmU*, *sodA* and *mltB* from *E. coli* K-12 MG1655 (DE3) genome. **B.** SDS-PAGE gel showing overexpression of genes *glmU*, *sodA* and *mltB* in *E. coli* K-12 MG1655 (DE3). 1 – pETDuet-1, 2 - pETDuet_glmU, 3 – pETDuet_sodA, 4 – pCDFDuet-1, 5 – pCDFDuet_mltB. The predicted sizes were: GlmU 49.2 kDa; SodA 24.91 kDa; MltB 41.2 kDa (Slt35 36 kDa).

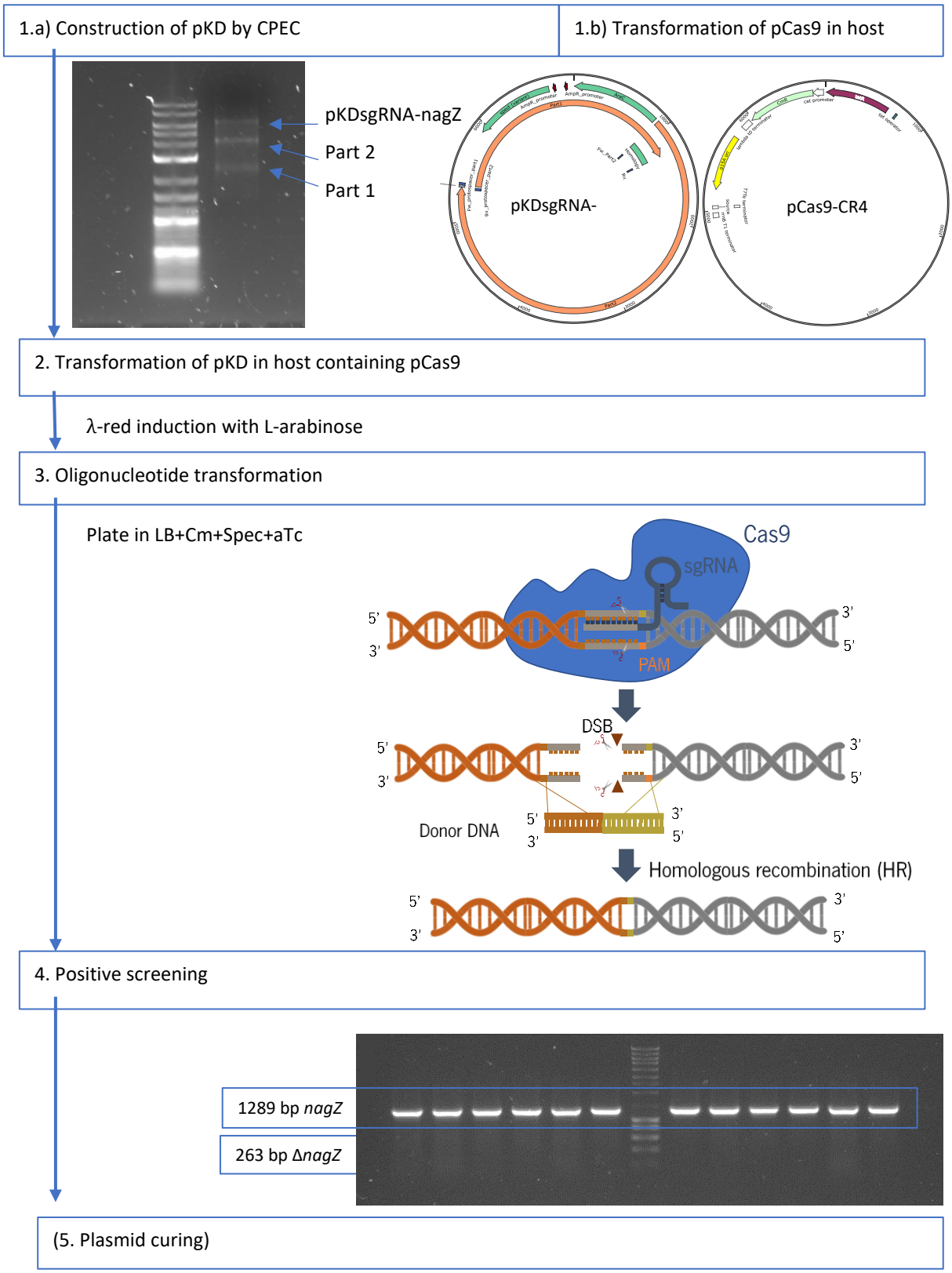

Figure SI3. Attempted CRISPR-Cas9 strategy for *nagZ* deletion. This methodology consists in the cleavage of double stranded DNA by Cas9 and λ-Red recombinase system-facilitated genomic integration of donor DNA. The single-guide RNA (sgRNA) encoding plasmid pKDsgRNA-nagZ to target the *nagZ* has been constructed. The plasmids for the expression of Cas9 and of the sgRNA were transformed. Then, the transformation of the oligonucleotide (donor DNA) that should induce the gene deletion was performed. During the subsequent screenings, no positive colonies were found despite several attempts of this procedure.

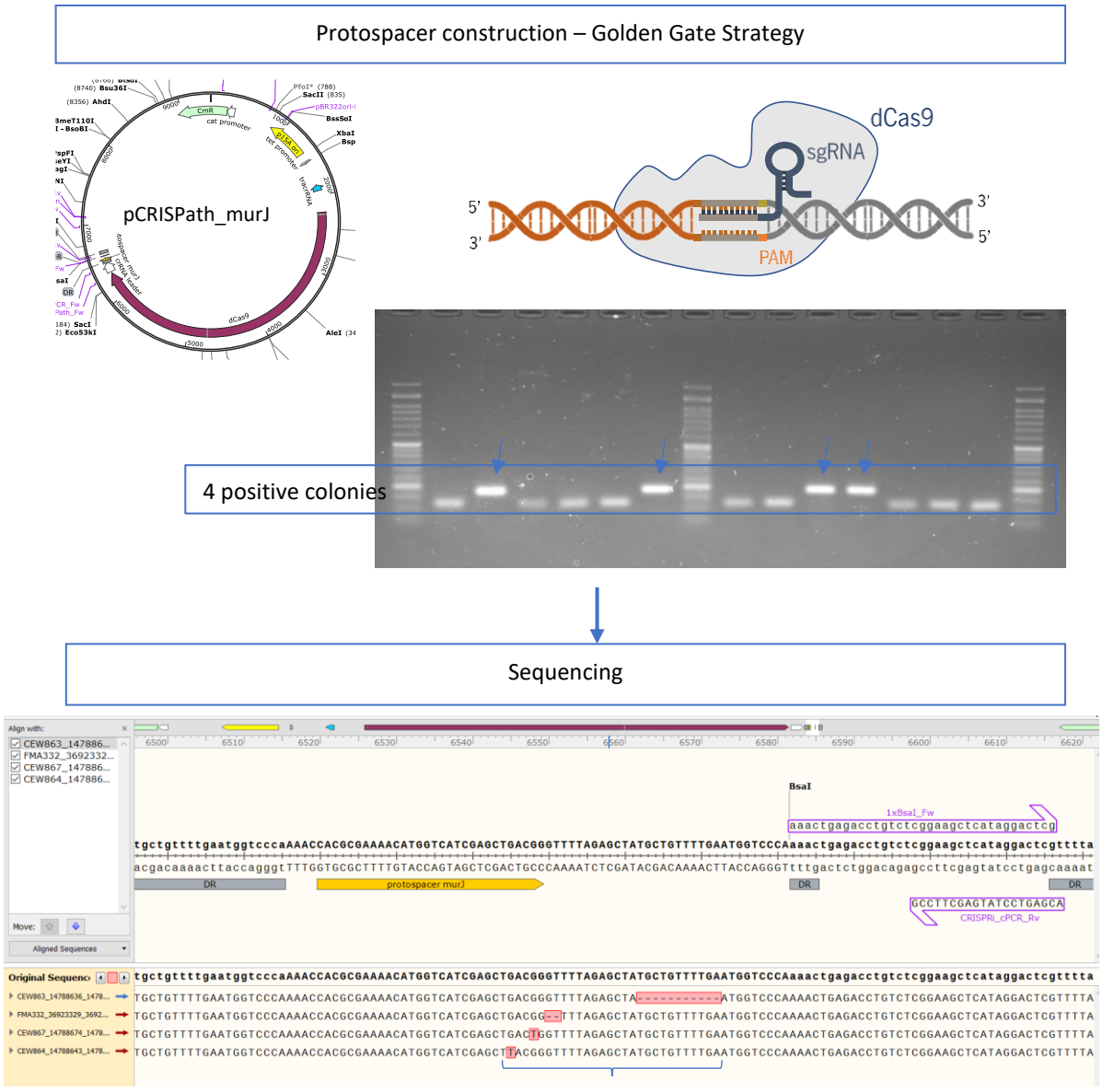

Figure SI4. Attempted CRISPR interference (CRISPRi) strategy to underexpress *murJ*. In this strategy, a modified version of the caspase 9 protein, commonly referred as dead Cas9 (dCas9), is expressed to target the *murJ* gene, ultimately repressing its expression. The dCas9 variant lacks nuclease activity but maintains the capability to specifically bind to double stranded DNA sequences. The cloning of the protospacer in the pCRISPathBrick was performed by Golden Gate strategy. The resulting pCRISPath_murJ revealed that the constructed plasmids constantly exhibited errors in or next to the protospacer.