Engineering dual-glycan responsive expression systems for tunable production of heterologous proteins in *Bacteroides thetaiotaomicron*

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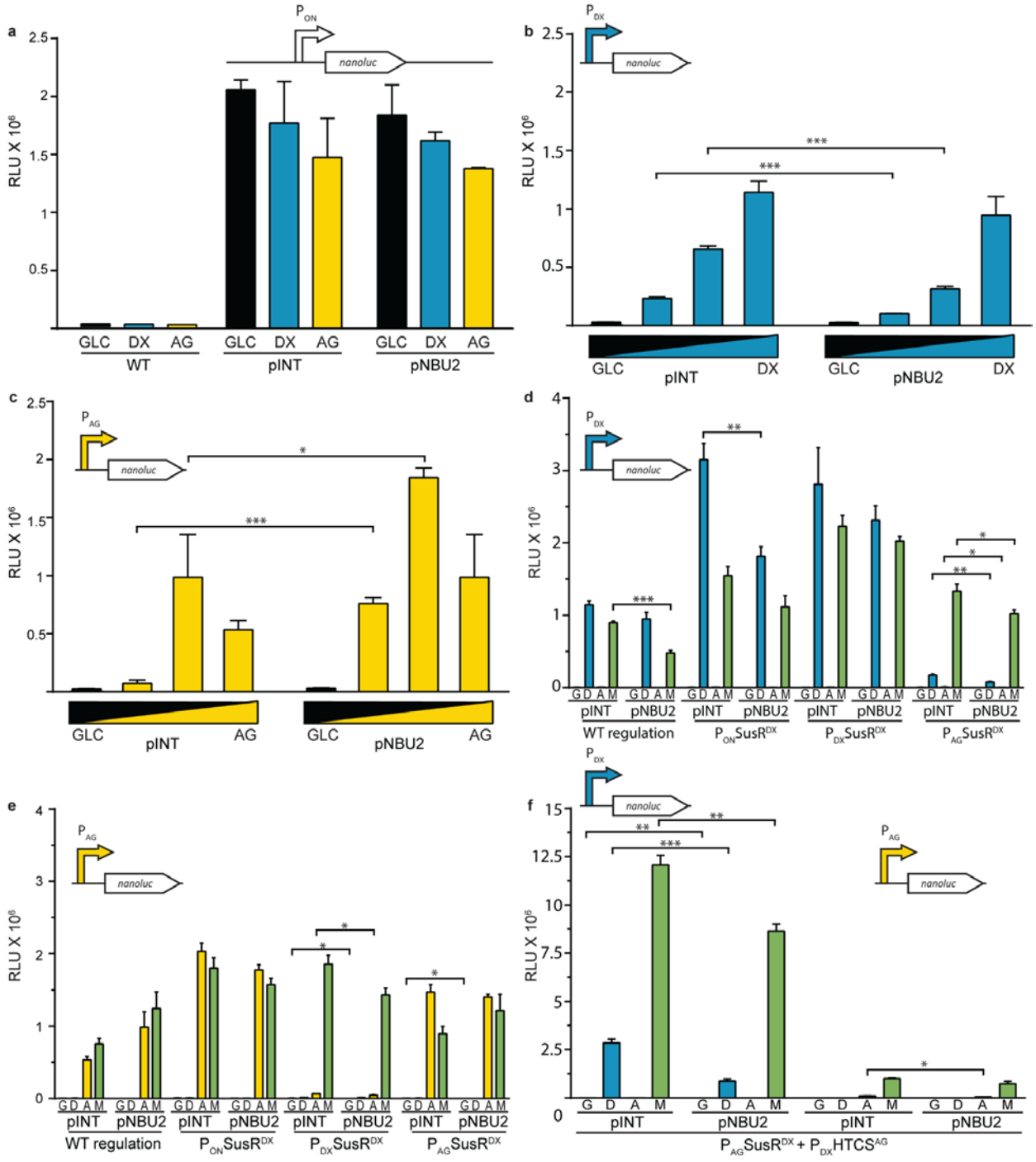
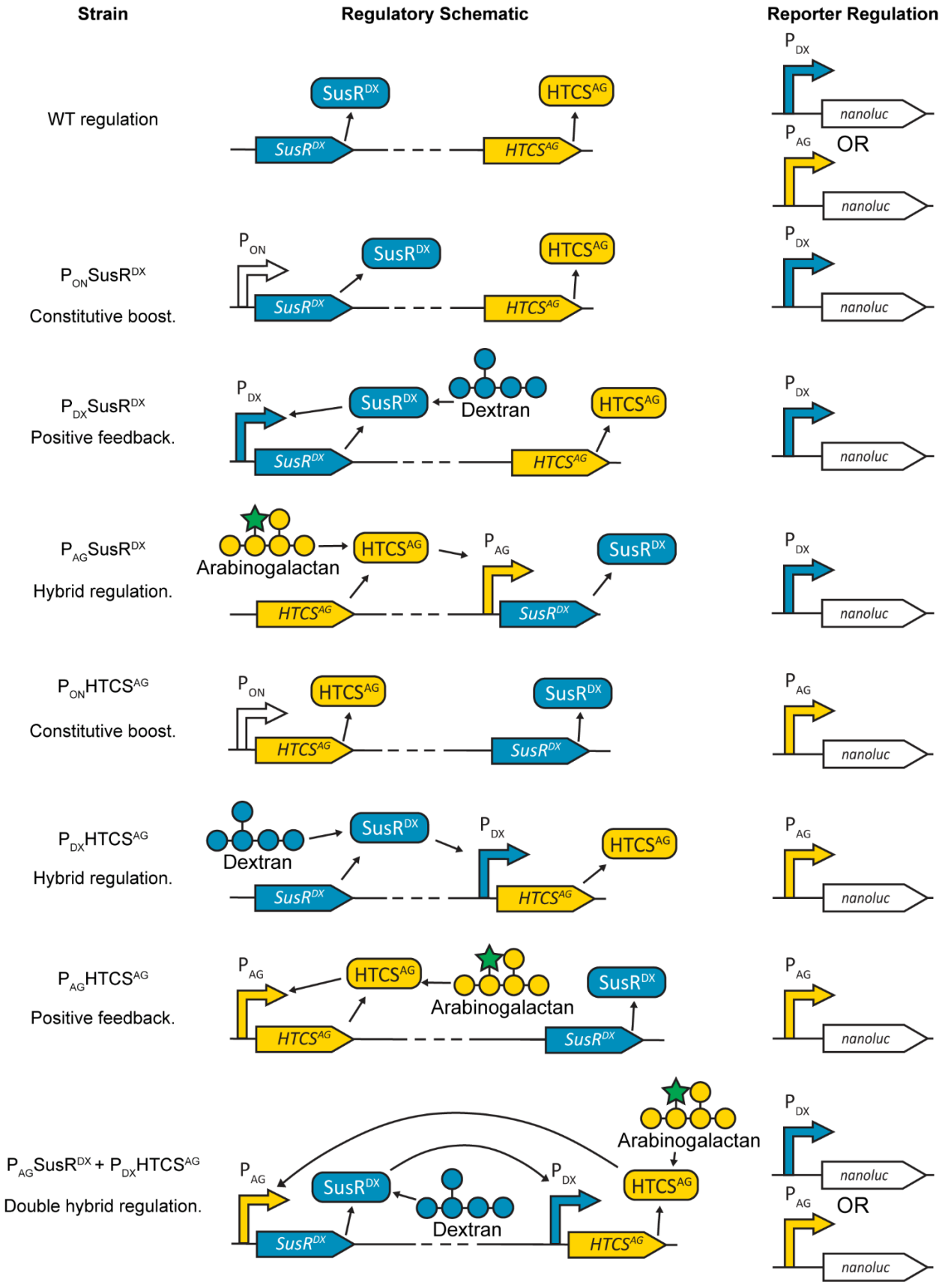
Figure S1: Comparison of NanoLuc activity from two different genomic loci. pINT integrates NanoLuc into the vacant PUL75 locus. pNBU2 integrates NanoLuc, and the entire pNBU2 plasmid, into a $tRNA^{ser}$ locus. To demonstrate that genomic locus did not affect transgene expression, each recombinant pINT strain was compared to its pNBU2 counterpart. **(a)** NanoLuc activity under control of the P_{ON} promoter when induced by GLC (black), DX (blue), or AG (yellow). **(b)** NanoLuc activity under control of the P_{DX} promoter on varying concentrations of GLC and DX. **(c)** NanoLuc activity under control of the P_{AG} promoter on varying concentrations of GLC and AG. **(d)** NanoLuc activity under control of the P_{DX} promoter in modified $SusR^{DX}$ strains on GLC (black), DX (blue), or AG (yellow) or mixed DX and AG (green). **(e)** NanoLuc activity under control of the P_{AG} promoter in modified $HTCS^{AG}$ strains on GLC (black), DX (blue), or AG (yellow) or mixed DX and AG (green). **(f)** NanoLuc activity under control of either P_{DX} or P_{AG} in strains with modified $SusR^{DX}$ and $HTCS^{AG}$ on GLC (black), DX (blue), or AG (yellow) or mixed DX and AG (green). Significant differences are indicated with brackets. Lack of brackets indicates no significant difference. 32/48 (67%) of comparisons between the two loci do not result in statistically significant difference. Asterisks represent level of significance (*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$).

Figure S2: Schematic regulatory maps of strains engineered in this study. Promoters (colored arrows) are placed upstream of open reading frames (pointed rectangles) on the genome. Blue arrow = dextran responsive (bt3091), yellow arrow = arabinogalactan responsive (bt0268). Regulatory proteins (rounded boxes) are induced by carbohydrates and effect downstream expression of reporter proteins.


a
b
c
d
e
f
RLU X 10^6
nanoluc
GLC
DX
AG
WT
pINT
pNBU2
WT regulation
P_ONSusR^DX
P_DXSusR^DX
P_AGSusR^DX
P_AGSusR^DX + P_DXHTCS^AG


Strain
Regulatory Schematic
Reporter Regulation
WT regulation
SusR^DX
HTCS^AG
P_DX
nanoluc
P_AG
OR
P_ON SusR^DX
Constitutive boost.
P_DX SusR^DX
Positive feedback.
Dextran
P_AG SusR^DX
Hybrid regulation.
Arabinogalactan
P_ON HTCS^AG
P_DX HTCS^AG
P_AG HTCS^AG
P_AG SusR^DX + P_DX HTCS^AG
Double hybrid regulation.