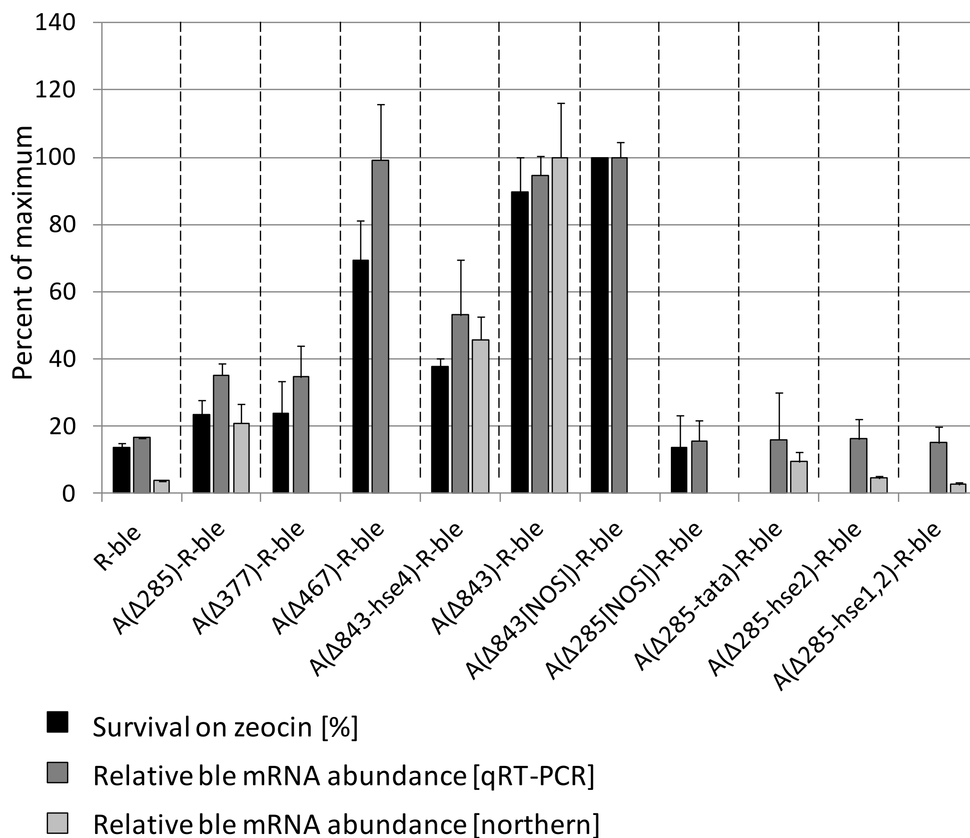
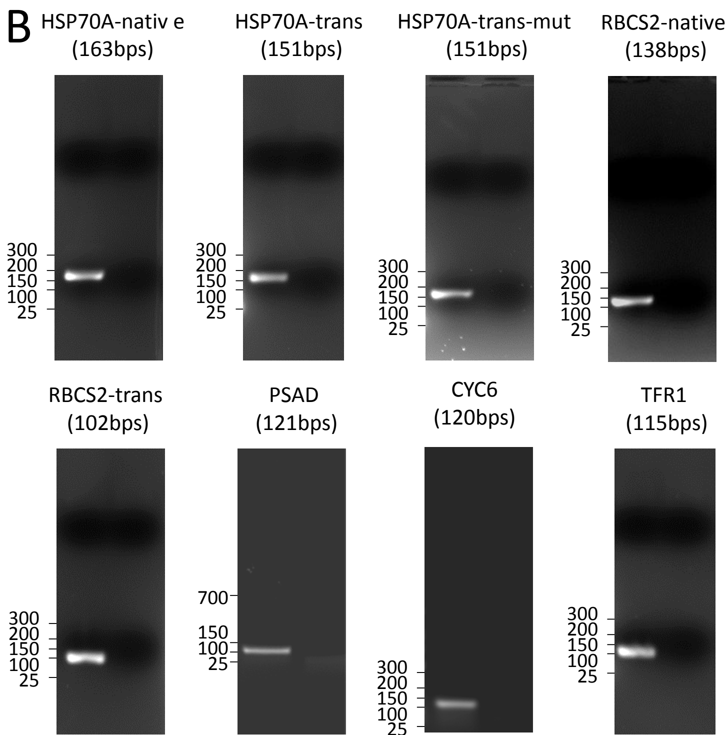


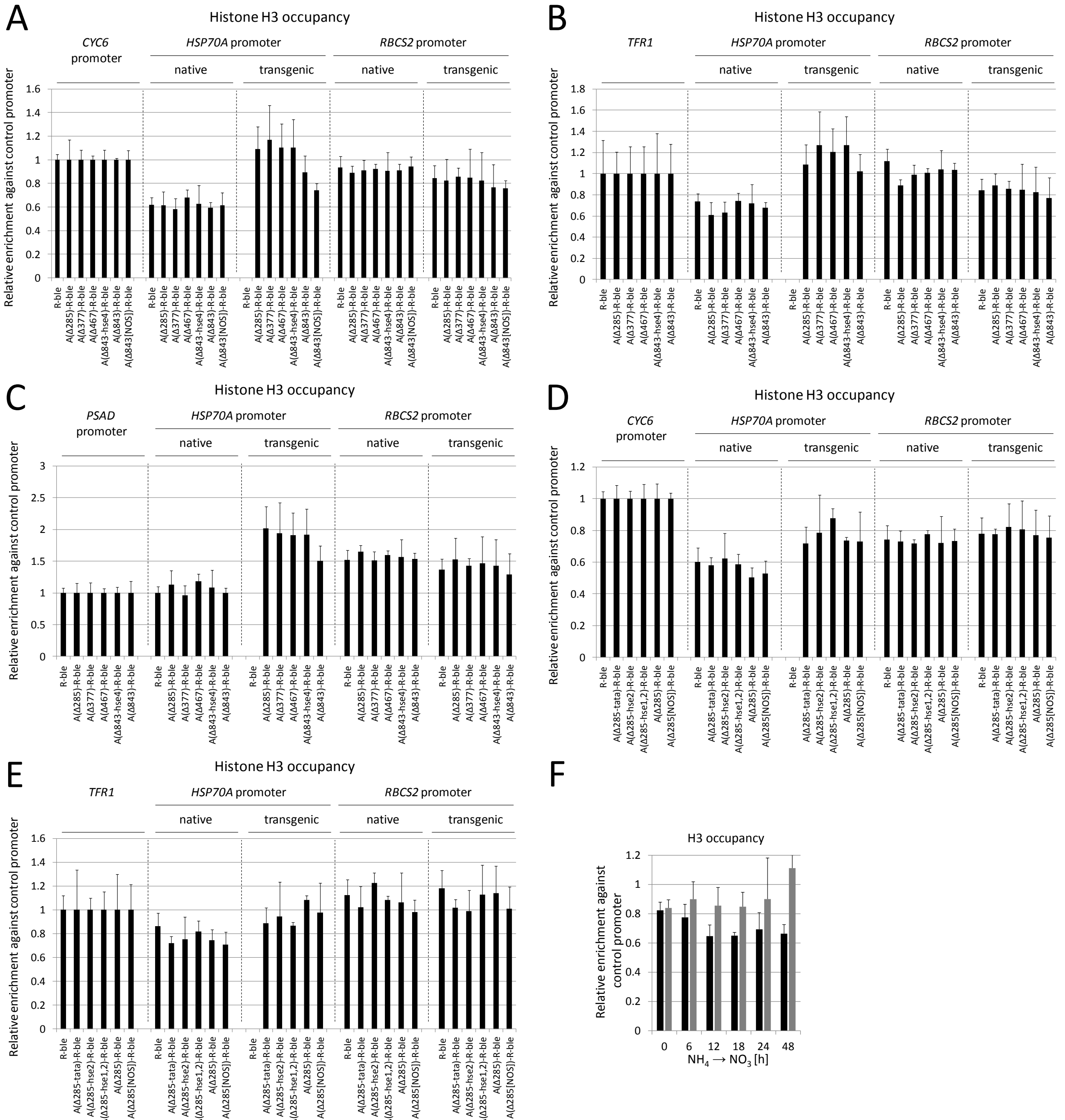
Supplementary Figure S1. Comparison of results from different methods for determining transgene expression levels. Shown is a comparison of the indirect measurement of *ble* expression using growth assays on TAP agar plates containing 1.5 µg/ml zeomycin (in black; data from Figure 2B) and *ble* mRNA levels measured with qRT-PCR (dark grey; data from Figure 2D) or RNA gel blot analyses (light grey; from (33)). To facilitate comparison, all data are given as percent of maximum.

A

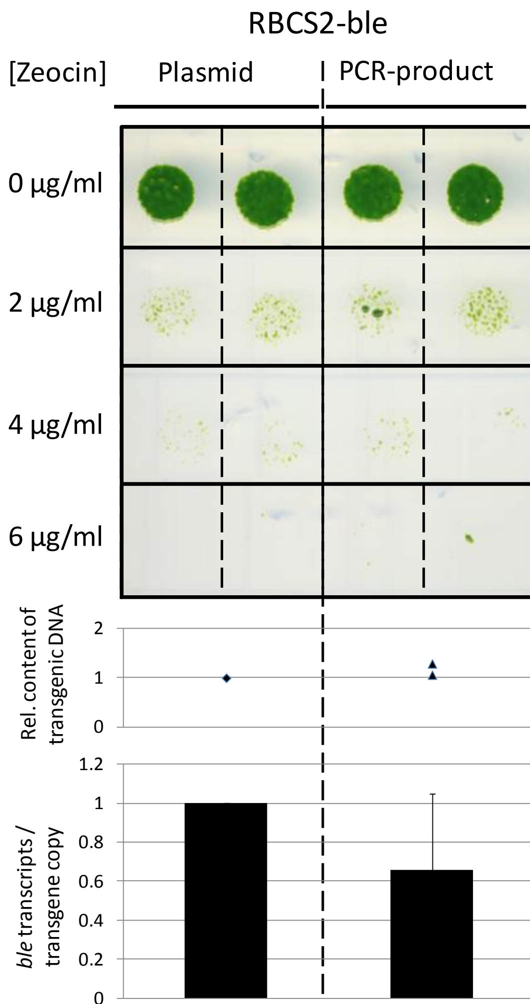
Target	Description	Forward	Reverse	Amplicon size (bps)
HSP70A-native	HSP70A promoter	CGGTATAAAAGCCCGCGAC	GTGCCCAGGTCAATACCGATAG	163
HSP70A-trans	Transgenic HSP70A promoter	CGGCACCCATCCGGTAT	GTTTGGCTGCGCTCCTTC	151
HSP70A-trans-mut	Transgenic HSP70A promoter with TATA-box mutation	CGGCACCCATCCGGgAc	GTTTGGCTGCGCTCCTTC	151
RBCS2-native	Rubisco small subunit 2 promoter	CAATGCAAGCAGTTCGCATG	ACGGAGGACTTGGCAATGAC	138
RBCS2-trans	Transgenic RBCS2 promoter	GCGGTGCCCTCCTGATAAA	AACGGCGCTGGTCAGCTTGGC	102
PSAD	Photosystem I subunit D promoter	CCAGGGTTAGGTGTTGCGCTC	GATGAAGCGCGAGTGGCAG	121
CYC6	Cytochrome c6 promoter	ACACGCCCCTCATTCACAGA	GCACACGAGACACTCCGAGC	120
TFR1	Telomer flanking region 1	GGGTTTTGCAGGGTTTGGA	CCTCATCATGGTCACCCACA	115



Supplementary Figure S2. Primers and amplicons used in this study.
(A) Compilation of primer sequences and amplicon sizes.
(B) PCR end products showing that a single amplicon of the expected size was amplified. Shown is 1/10th of a real-time PCR on DNA extracted from co-transformants generated with pCB412 (ARG7) and pMS188 [$A_{(\Delta\text{-}843)}$-R-ble] (HSP70A-trans, RBCS2-trans and all native targets) and with pCB412 and pMS428 [$A_{(\Delta\text{-}285\text{-}\mathit{tata})}$-R-ble] (HSP70A-trans-mut) in the cw15-302 strain background. Samples were separated on a 1.5% agarose gel that was stained with GelRed (Biotium).



Supplementary Figure S3. Nucleosome occupancy at the promoters investigated in this study. ChIPs were done with an antibody against the unmodified C-terminus of histone H3. The enrichment relative to 10% input DNA was normalized to the values obtained for the respective control promoter. Error bars indicate standard errors of two biological replicates, each analyzed in triplicate. The data was used for the normalization of ChIP data shown in Figure 3A-D **(A)**, Figure 3F **(B)**, Figure 3E **(C)**, Figure 4A and B **(D)**, Figure 4C **(E)**, and Figure 6C **(F)**. In Figure (F), black bars and grey bars indicate nucleosome occupancy at transgenic promoters *HSP70A* and *RBCS2*, respectively.



Supplementary Figure S4. Analysis of transgene expression in pools of co-transformants generated with plasmid DNA or DNA amplified by PCR. Constructs were generated by PCR with the KAPA HiFi-Kit (Peqlab) on pMS171 ($A_{(\Delta\text{-}285)}$-*R-ble*) according to the manufacturer`s protocol. Primers used are as follows: *HSP70A*: CGGTATAAAAGCCCGCGAC and T7: GTAATACGACTCACTATAGGGC. The PCR product and pMS171 were digested with NcoI/NheI to gain a 1108-bp fragment containing the *ble* gene driven by the *RBCS2* promoter. For the generation of co-transformants, 200 ng of pCB412 (*ARG7*) and 250 ng of either PCR-product or plasmid was used. Co-transformant pools were cultured in liquid TAP medium and 10^5 cells were spotted on TAP agar plates with 0, 2, 4 or 6 µg/ml of zeocin and then plates were incubated for 7–10 days under constant white fluorescent light at ~30 µE m^{-2} sec^{-1} and 24°C. In addition, total RNA was extracted from co-transformant pools, transcribed into cDNA and used as template for qPCR. The *ble* mRNA amount of each co-transformant pool was determined with the ΔΔCt method using *CBLP2* transcripts as control. RNA levels were normalized to the amount of transgenic DNA determined with the ΔΔCt method and *CYC6* as control region. Error bars represent standard errors of 2 biological replicates, each analyzed in triplicate.