Strains and plasmids

All fermentation work were done in *E. coli* D5α containing the appropriate plasmids. All strains were transformed prior to this experiment using plasmids verified to have the right insert using Sanger sequencing. Detailed information about the strains used can be found in Table 1.

Table 1. The four strains of E. coli used in this study. All TAL genes are of Rhodobacter sphaeroides origin and all plasmids have been sequence verified to contain the right insert

Strain nr.	**Plasmid backbone**	**Plasmid insert**	**Comment**
106.5	pSB3K3	J23110-B0034-TAL	*TAL* CDS with promoter J23110
137.1	pSB3K3	CP8-B0034-TAL	*TAL* CDS with promoter CP8
189.3	pSB3K3	J23101-B0034-TAL	*TAL* CDS with promoter J23101
119.2	pSB1C3	Negative control	No *TAL* CDS or promoter. Two cultures prepared for this strain. One for negative control and one modified to be positive control after sample collection.

P-coumaric acid fermentation

For each *E. coli* strain; one bacterial colony was collected from an agar plate containing the specific strain. Each colony were grown overnight in 37 °C in 6 mL LB containing 6 µL of the appropriate antibiotic. After 17 h of 37 °C incubation, incubation at 30 °C was initiated . 3 h after initiation of the 30 °C incubation all bacterial cultures were pelleted through centrifugation (>12000 g, 10 min) and resuspended in 6 mL fresh LB containing 6 µL of the appropriate antibiotic and 1 mM of tyrosine before being incubated at 30 °C again. Samples were collected 21 and 48 h after initiation of the first 30 °C incubation.

Sample collection, extraction and spectrophotometry

1 mL samples were collected at different times as described above. For the positive controls, 900 µL of a negative control culture was induced with 5 mM p-coumaric acid in LB to create a bacterial culture with a p-coumaric acid concentration of 500 µM.

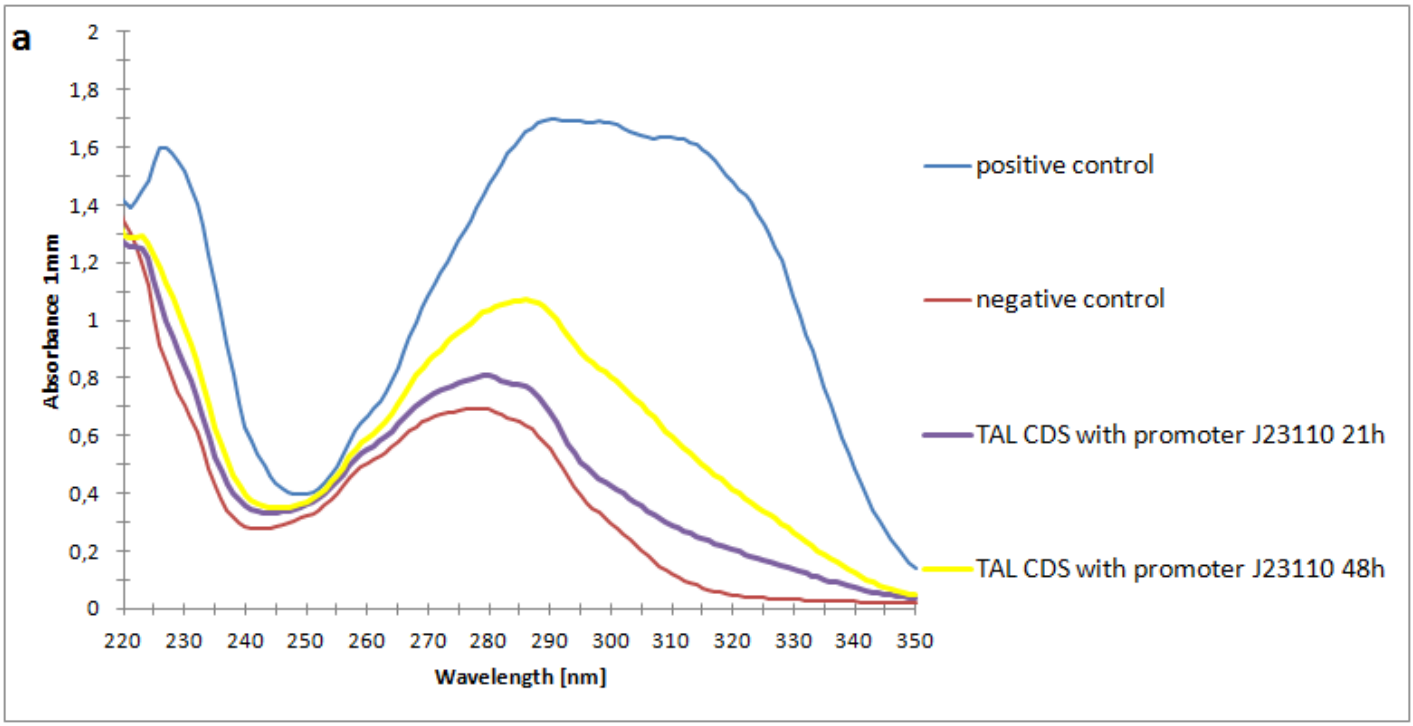
Extraction of all samples followed directly after the sample collection. The samples undergoing lysis were first induced with 100 µL of Lysis buffer and neutralized with 175 Neutralization

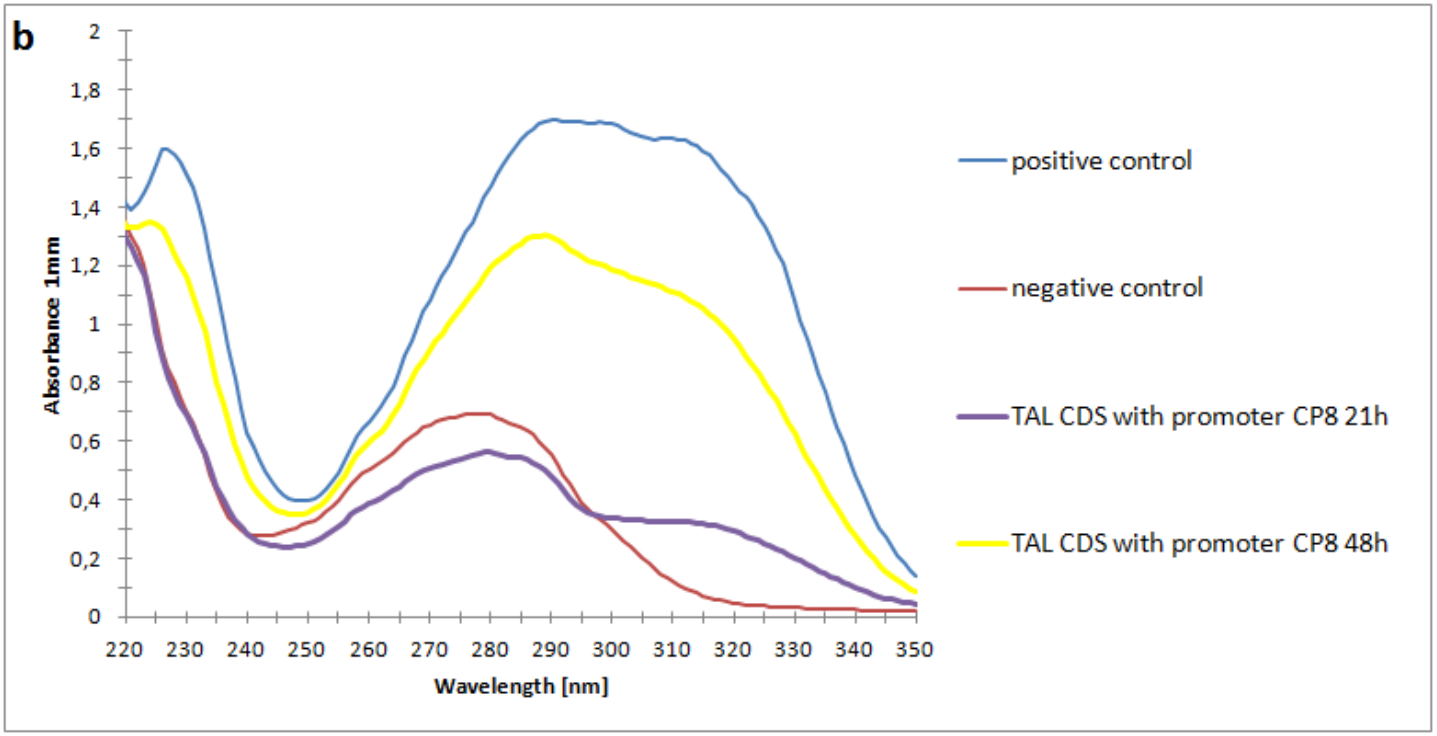
buffer (Thermo Plasmid MiniPrep). All samples were then acidified in 50 µL concentrated acetic acid and vortexed. Samples were then induced with 200 µL n-octanol (the organic phase in the two-phase extraction). Vortexing and centrifugation (>12000 g, 1 min) followed and the upper organic phase was then collected for measurement. All extracts were diluted three times by 400 µL n-octanol before measurement.

The absorbance in the UV-area (190-350 nm) of the octanol extracts were measured in a spectrophotometer (Nanodrop 2000). The spectrophotometer was tared with pure n-octanol. All measurements were done on 2 µL of the diluted extracts with a cuvette length of 1 mm. Absorbance measurements were done in the same way on standards of p-coumaric acid in octanol prepared from solid p-coumaric acid (Sigma Aldrich).

Detection of p-coumaric acid in bacterial cultures with additional tyrosine

The absorbance spectra of the extracts collected at different times after incubation at 30 °C shows the change of absorbance over time in the UV-Area (Figure 1). All cultures with *TAL* containing constructs shows an increase in absorbance around 305 nm at 48 h compared to 21 h and to the negative control.





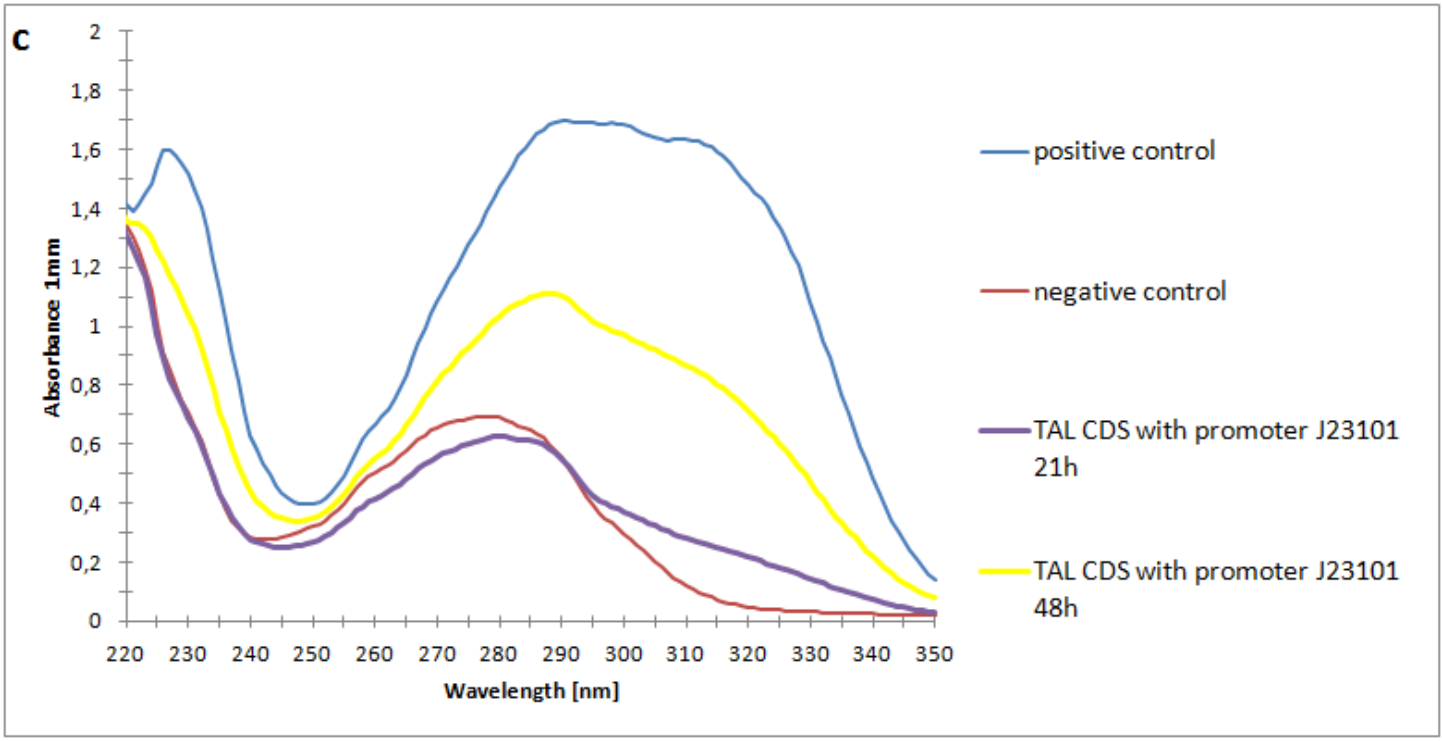


Figure 1. Absorbance spectra of extracts collected from bacterial cultures. Samples were collected 21 h and 48 h after 30 °C incubation. The negative control is an extract from a strain with no *TAL* gene on transformed plasmid. The positive control is an extract a culture of the same strain as the negative control but with added p-coumaric acid to a concentration of 500 µM before extraction. (a) Spectra from the strain with *TAL* CDS with promoter J23110. (b) Spectra from the strain with *TAL* CDS with promoter CP8. (c) Spectra from the strain with *TAL* CDS with promoter J23101.