# Supplement to

# Identification of XylR, the activator of arabinose/xylose inducible regulon in *Sulfolobus acidocaldarius* and its application for homologous protein expression

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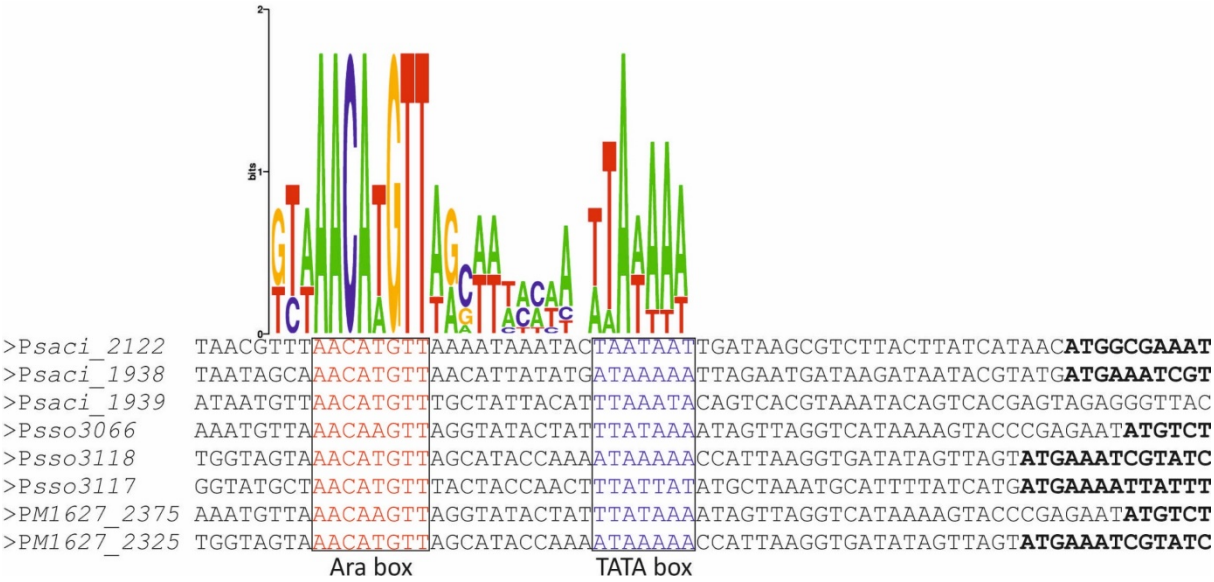



**Figure S1.** Alignment of the promoters of the genes which are regulated by XylR in *S. acidocaldarius* (*saci_2122*, *saci_1938* and *saci_1939*) and the ones containing the Ara-box from *S. solfataricus* (*sso3066, sso 3118 and sso3117*) and *S. islandicus* (*m1627_2375* and *m1627_2325*).

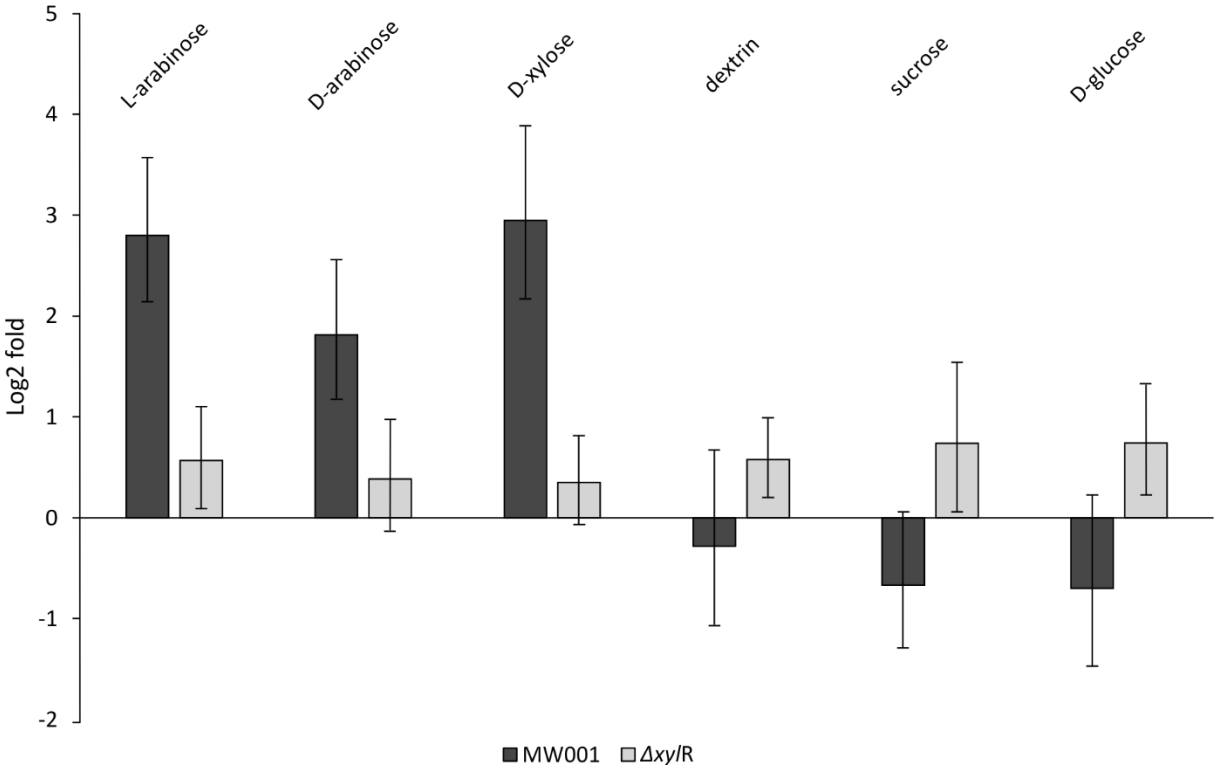



**Figure S2.** Transciption levels of the gene encoding KDXD/KDAD (*saci_1939*) in the parental strain MW001( dark grey) and ΔxylR deletion mutant (light grey) when grown on different sugars. The strains were cultured on 0.1% N-Z-Amine and supplemented with 0.2% L-arabinose, 0.2% D-arabinose, 0.2% D-xylose, 0.2% dextrin, 0.2% sucrose or 0.2% D-glucose. Bars indicate the sugar-specific transcription compared to cells only grown on N-Z-Amine on $\log_2$-fold

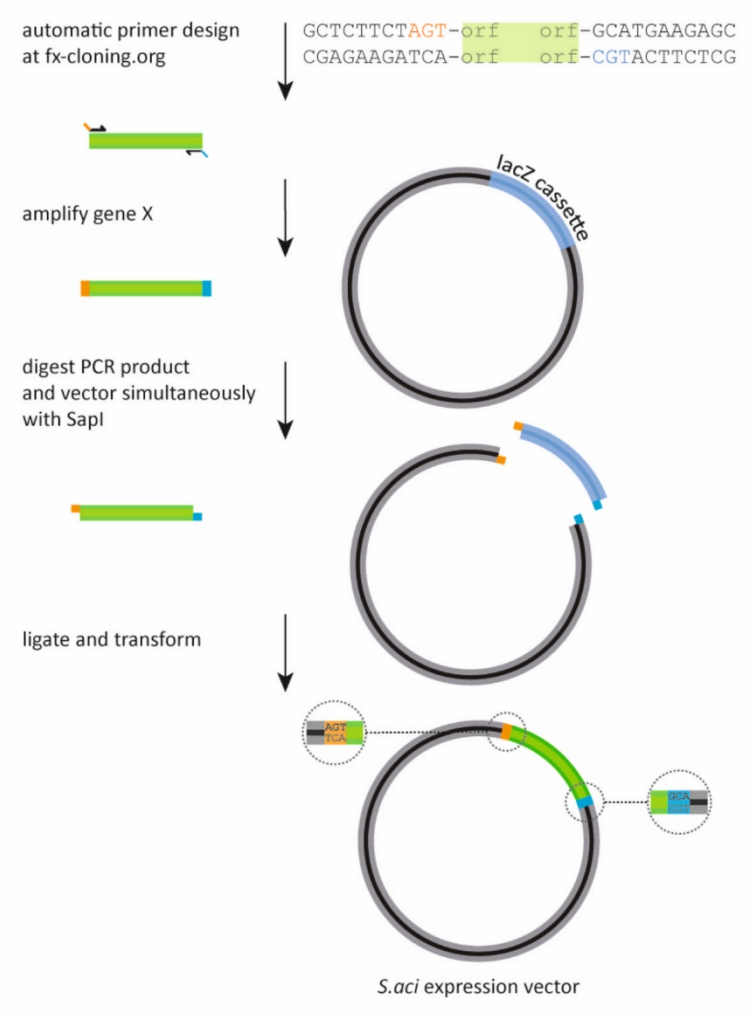
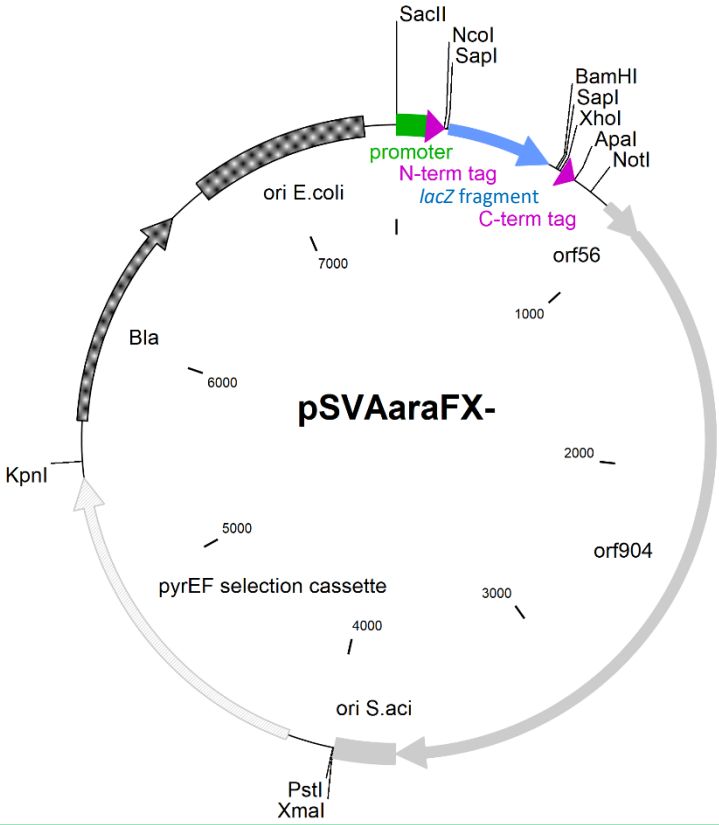



**Figure S3. Scheme of the FX cloning method.** Adapted from [18]

Light grey: *S.aci* plasmid replication elements
Dark grey: *E.coli* replication elements
Green: promoters
Blue: cloning cassette with lacZ fragment
Pink: N- or C-term tags




Saci_2122 (ara)

***SacII*** **CCGCGG**TATAAATACTAACTGCAATATTATATCTATAATTATTACTCAGCGTTTATAACGTTTAACATGTTAAAATAAATACTAATAATTGATAAGCGTCTTACTTATCATA

Saci_1938 (xyl)

***SacII***
**CCGCGG**TTATTGTAACCCTCTACTCGTGACTGTATTTACGTGACTGTATTTAAATGTAATAGCAAACATGTTAACATTATATGATAAAAATTAGAATGATAAGATAATACGTA

Saci_1165 (mal)

***SacII*** **CCGCGG**CTAATTAATAATACTAATATCATTTCGTAAACTACATCTTATAATACTTAAGTTGACATGTTCAACGGAGGTGTCCTTAAGTTTAGACCTTAAATATTTTA
TATATATATTAAGTTTATAAATAATTACGTGATTAAGTTAA

**NcoI** **SapI** **AatI** **NheI** **SalI** **BamHI** **SapI** **XhoI**

**CCATGG** CTAGTTG **AAGAGC** … lacZ fragment 416 bp … **GACGTC GCTAGC GTCGAC GGATCC** G **GCTCTTC** AGCA **CTCGAG** TAA

**ApaI** **Terminator region** **NotI**

**GGGCCC** ACTTTCTCAAGTCTCACTATACCAAATGAGTTTTCTTTTAATCTTATTCTAATCTCATTTTCATTAGATT **GCGGCC**

NtS

AC**ATG**GCGTGGAGTCATCCACAATTTGAGAAGG

NtHA

AC**ATG**GCGTACCCGTATGACGTTCCGGACTACGCGG

NtH10S

AC**ATG**GCGCATCACCATCATCACCATCATCACCATCATTCCGGATGGAGTC
ATCCACAATTTGAGAAGG

NtH6

AC**ATG**GCGCATCACCATCATCACCATG

NtSS

AC**ATG**GCGTGGAGTCATCCACAATTTGAGAAGGGTGGAGGTTCCGGAGG
TGGATCGGGAGGTTCTGCATGGTCACATCCTCAATTCGAAAAGGGAGGTT

**SH10**

TGGAGTCATCCACAATTTGAGAAGCATCACCATCATCACCATCATCACCAT
CATTGA

**S**

TGGAGTCATCCACAATTTGAGAAGTAA

**HA**

TACCCGTATGACGTTCCGGACTACGCGTAA

**H6**

CATCACCATCATCACCATTAA

**CtSS**

AGCGCTTGGTCACATCCTCAATTTGAGAAAGGTGGAGGTTCCGGAGGTG
GATCGGGAGGTTCTGCATGGAGTCACCCACAGTTCGAAAAGTAA

**Fig S4.** Detailed vector map of all FX cloning vectors. The vector map is shown and in the inserts the sequences of the different tags dependend on which vector is described.

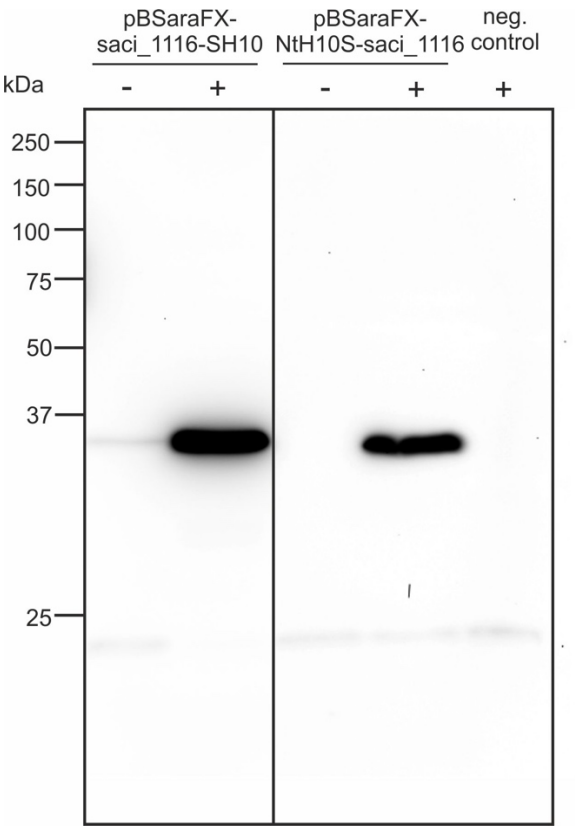



**Figure S5.** Immunodetection of the esterase Saci_1116 after cloning in pBSaraFX-SH10 and pBSaraFX-NtH10S and expression in *S. acidocaldarius*. Cells were grown in Brock medium supplemented with 0.1% N-Z-Amine and 0.3% dextrin (-) or D-xylose (+). Similar amounts of whole cells were boiled for 5min in SDS loading buffer and separated via SDS-PAGE. Western blot transfer occurred on PVDF membrane using the Trans-Blot Turbo machine from Bio-Rad. For immunodetection Anti His-tag antibody (Abcam ab184607) was used in 1:10000 dilution.

## Supplementary tables

Table S1 strains

| **Strain** | **Background strain** | **Genotype** | **Reference** |
|---|---|---|---|
| MW001 | *S. acidocaldarius* DSM639 | Δ*pyrE* (Δ91-412) | (Wagner et al., 2012) |
| MW413 | *S. acidocaldarius* MW001 | Δ*xylR (Δsaci_2116)* | This study |