SUPPLEMENTARY INFORMATION

Cooperative [2Fe-2S] cluster-binding regulates the functional transitions of the *Aspergillus fumigatus* iron regulator HapX for adaptation to iron starvation, sufficiency and excess

AUTHORS

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SUPPLEMENTARY FIGURES

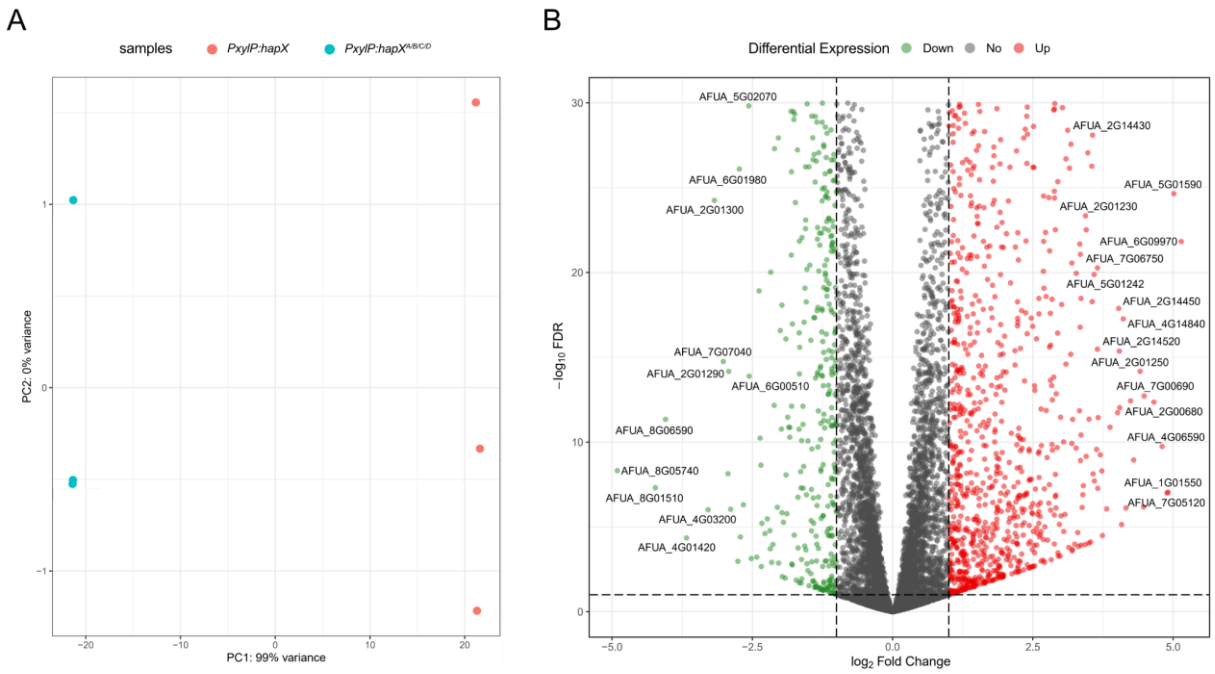

Figure S1: Global comparison of gene expression in strains *hapX* and *hapX*$^{A/B/C/D}$. (A) Principal component analysis showing clustering of biological triplicates and differences between the two strains analysed. (B) Volcano blot analysis illustrating the differences in gene expression in *hapX*$^{A/B/C/D}$ compared to *hapX*. Genes downregulated with a log2 fold change ≤ -1 and upregulated with ≥ 1 in *hapX*$^{A/B/C/D}$ compared to *hapX* are highlighted in green and red, respectively. The log2 fold change values are plotted on the x-axis, while the y-axis represents the -log10 false discovery rate (FDR).

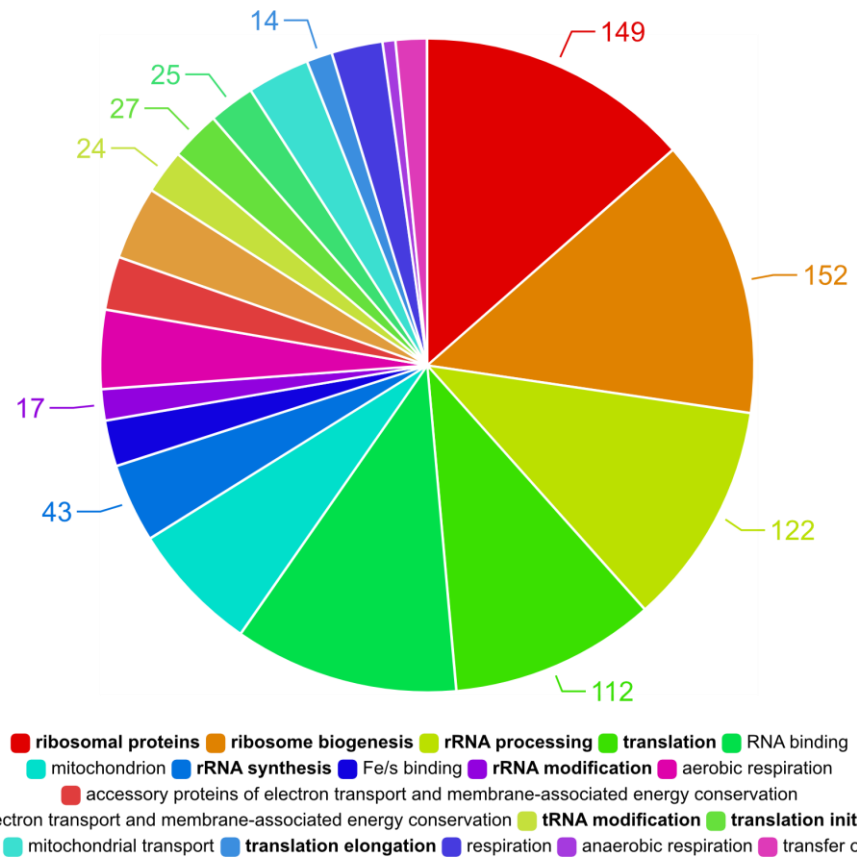

Figure S2: The majority of downregulated genes in strain *hapX*$^{A/B/C/D}$ are related to ribosome biogenesis and translation. Functional annotation of significantly downregulated genes in *hapX*$^{A/B/C/D}$ compared to *hapX* was done using FungiFun2 [1]. Functional categories linked to ribosome biogenesis and translation are in bold. Respective genes are listed in Supplementary Table S1C.

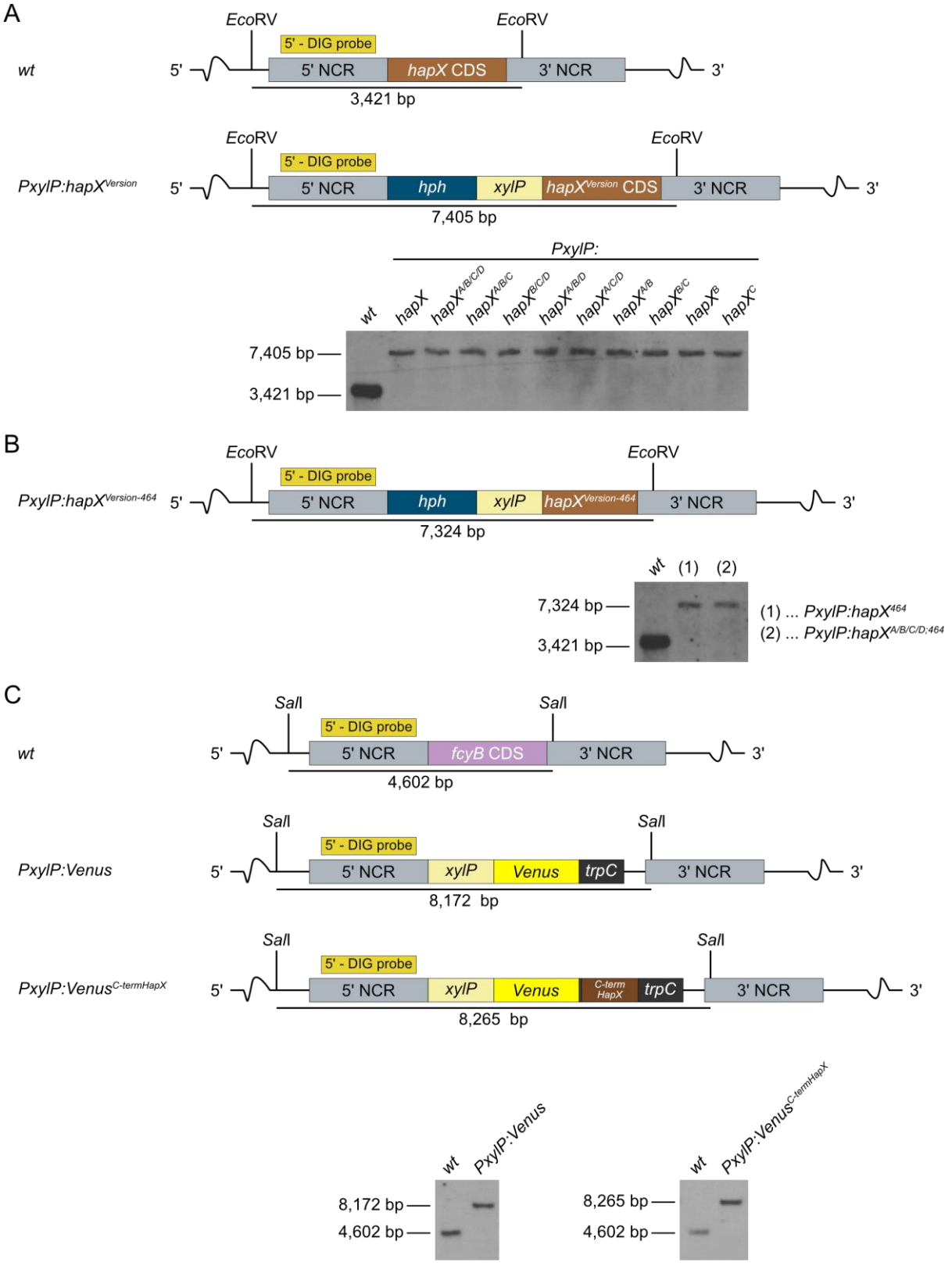

Figure S3: Schemes of the targeted genomic loci and Southern blot confirmation of the generated mutant strains. Genomic DNA was digested using specific restriction enzymes and then subjected to Southern blot analysis. (A) *hapX* locus in *wt* and transformed *hapX* CRR mutant strains; digestion using *Eco*RV led to a detected fragment length of 3,421 bp in *wt* and in a 7,405 bp fragment in the mutant strains; (B) in strains encoding C-terminally truncated *hapX* alleles the detected fragment in the mutant strains was slightly smaller, i.e. 7,324 bp. (C) *wt fcyB* locus, used for marker-free integration of the conditionally expressed *Venus* constructs into the *wt* background. *Sal*I digestion resulted in a detected *wt* fragment length of 4,602 bp which increased to 8,172 bp in the *Venus* and to 8,265 bp in the *Venus*$^{C\text{-}termHapX}$ mutant strains, respectively. All Southern blot results are in line with the *in silico* predictions.

SUPPLEMENTARY TABLES

Table S1: Transcriptome data of comparative analysis between strains *hapX* and *hapX*$^{A/B/C/D}$.

Supplementary Table S1A – DESeq2 normalized transcriptome dataset

Supplementary Table S1B – Selected differentially expressed genes in *hapX*$^{A/B/C/D}$ compared to *hapX* in +Fe

Supplementary Table S1C – Functional annotation of significantly downregulated genes using FungiFun2

Table S2: Strains used in this study.

strain	genotype	reference
AfS77 (wt)	*ATCC46645, ΔakuA::loxP*	[2]
ΔhapX	*AfS77,* Δ*hapX::ptrA*	[3]
PxylP:hapX	*AfS77, 5'hapX::hph, PxylP:hapX*	this study
PxylP:hapX$^{A/B/C/D}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{A/B/C/D}$	this study
PxylP:hapX$^{A/B/C}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{A/B/C}$	this study
PxylP:hapX$^{B/C/D}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{B/C/D}$	this study
PxylP:hapX$^{A/B/D}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{A/B/D}$	this study
PxylP:hapX$^{A/C/D}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{A/C/D}$	this study
PxylP:hapX$^{B/C}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{B/C}$	this study
PxylP:hapX$^{A/B}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{A/B}$	this study
PxylP:hapX^{C}	*AfS77, 5'hapX::hph, PxylP:hapX*^{C}	this study
PxylP:hapX^{B}	*AfS77, 5'hapX::hph, PxylP:hapX*^{B}	this study
PxylP:hapX^{464}	*AfS77, 5'hapX::hph, PxylP:hapX*^{464}	this study
PxylP:hapX$^{A/B/C/D;464}$	*AfS77, 5'hapX::hph, PxylP:hapX*$^{A/B/C/D;464}$	this study
PxylP:Venus	*AfS77, 5'fcyB::PxylP:Venus*	this study
PxylP:Venus$^{C\text{-}termHapX}$	*AfS77, 5'fcyB::PxylP:Venus*$^{C\text{-}termHapX}$	this study

Table S3: Summary of generated plasmids in this study and the encoded *hapX* alleles. Plasmids were either generated applying the NEBuilder or the site-directed mutagenesis (SDM) approach.

plasmid	encoded *hapX* allele	construction method
pMMHL79	*hapX*^{B}	SDM
pSO01	*hapX*$^{A/B}$	SDM
pSO02	*hapX*$^{A/B/C}$	SDM
pSO03	*hapX*$^{A/B/C/D}$	SDM
pSO04	*hapX*$^{A/C/D}$	SDM
pSO05	*hapX*$^{B/C/D}$	SDM
pSO06	*hapX*$^{A/B/D}$	SDM
pSO07	*hapX*$^{A/B/C/D;464}$	SDM
pSO08	*hapX*$^{B/C}$	SDM
pSO09	*hapX*^{464}	SDM
pSO11	*hapX*	NEBuilder
pSO28	*hapX*^{C}	SDM
pSO52	*Venus:hapX*	NEBuilder
pSO53	*Venus*	SDM
pSO54	*Venus*$^{C\text{-}termHapX}$	SDM

Table S4: Primers used in this study. Small letters indicate primer overhangs for plasmid construction or introduction of mutations via site-directed mutagenesis (SDM). PC, plasmid construction; TCA, transformation cassette amplification; SB, Sothern blot probe; SC, sequencing.

primer	sequence [5' - 3']	used for
pJet1.2 backbone_fwd	ATCTTTCTAGAAGATCTCCTAC	**PC** → **pSO11** [*hapX*]
pJet1.2 backbone_rev	ATCTTGCTGAAAAACTCG	**PC** → **pSO11** [*hapX*]
5'hapX flank_fwd	ctcgagtttttcagcaagatAGCGACTATAGCCGGATG	**PC** → **pSO11** [*hapX*]
5'hapX flank_rev	taaatggtacGATTACGGATGATGAGAC	**PC** → **pSO11** [*hapX*]
hph-xylP cassette_fwd	atccgtaatcGTACCATTTAATTCTATTTGTGTTTG	**PC** → **pSO11** [*hapX*]
hph-xylP cassette_rev	gtgtagacatGGTTGGTTCTTCGAGTCG	**PC** → **pSO11** [*hapX*]
hapX-3'flank_fwd	agaaccaaccATGTCTACACCTTCAATAGC	**PC** → **pSO11** [*hapX*]
hapX-3'flank_rev	aggagatcttctagaaagatCCTTGGGTCTTGAAGCTTG	**PC** → **pSO11** [*hapX*]
oAfHapX-B1.f	CGTGGATCCCgccGGCTTCTGTTC	**PC – SDM of CRR-B in pSO11** → **pMMHL-79** [$hapX^{B}$]
oAfHapX-B1.r	GCAGGAGACGAAACG	**PC – SDM of CRR-B in pSO11** → **pMMHL-79** [$hapX^{B}$]
oAfHapX-A2.f	CTGCAATGATgcaTCCACATCGCATTG	**PC – SDM of CRR-A in pMMHL79** → **pSO01** [$hapX^{A/B}$]
oAfHapX-A2.r	CCTAACGGTACCTCC	**PC – SDM of CRR-A in pMMHL79** → **pSO01** [$hapX^{A/B}$]
oAfHapX-C3.f	TGCGCGCAGgcgCTTGCAGATCCG	**PC – SDM of CRR-C in pSO01 & pSO11** → **pSO02** [$hapX^{A/B/C}$] & **pSO28** [$hapX^{C}$]
oAfHapX-C3.r	TGTGCCCGGCCCGTTGGC	**PC – SDM of CRR-C in pSO01 & pSO11** → **pSO02** [$hapX^{A/B/C}$] & **pSO28** [$hapX^{C}$]
oAfHapX-D2.f	GTCGGGATGCgcaGGAGGTAAAGGCGC	**PC – SDM of CRR-D in pSO02** → **pSO03** [$hapX^{A/B/C/D}$]
oAfHapX-D2.r	GGGGCAGCGCTAGGG	**PC – SDM of CRR-D in pSO02** → **pSO03** [$hapX^{A/B/C/D}$]
oAfhapX-SO3	CGTGGATCCCtgtgggTTCTGTTCGGATG	**PC – reverse SDM of CRR-B in pSO03** → **pSO04** [$hapX^{A/C/D}$]
oAfhapX-SO4	GCAGGAGACGAAACGGAC	**PC – reverse SDM of CRR-B in pSO03** → **pSO04** [$hapX^{A/C/D}$]
oAfhapX-SO1	CTGCAATGATtgcTCCACATCGCATTGC	**PC – reverse SDM of CRR-A in pSO03 & pSO02** → **pSO05** [$hapX^{B/C/D}$] & **pSO08** [$hapX^{B/C}$]
oAfhapX-SO2	CCTAACGGTACCTCCTC	**PC – reverse SDM of CRR-A in pSO03 & pSO02** → **pSO05** [$hapX^{B/C/D}$] & **pSO08** [$hapX^{B/C}$]
oAfhapX-SO5	AgtgtCTTGCAGATCCGCGGAGGAC	**PC – reverse SDM of CRR-C in pSO03** → **pSO06** [$hapX^{A/B/D}$]
oAfhapX-SO6	GCGCGCATGTGCCCGGCCCG	**PC – reverse SDM of CRR-C in pSO03** → **pSO06** [$hapX^{A/B/D}$]
oAfhapX-SO7	TGATTTATCGCATCTCTGCTTG	**PC – SDM (C-terminal truncation) in pSO11 & pSO03** → **pSO09** [$hapX^{464}$] & **pSO07** [$hapX^{A/B/C/D;464}$]
oAfhapX-SO8	CCCACGATCGGTTAAGGG	**PC – SDM (C-terminal truncation) in pSO11 & pSO03** → **pSO09** [$hapX^{464}$] & **pSO07** [$hapX^{A/B/C/D;464}$]
fcyB-bb_trpC_fwd	CCATGGCAGCAGTGATTTC	**PC** → **pSO52** [*Venus:hapX*]
fcyB-bb_xylP_rev	GGTTGGTTCTTCGAGTCG	**PC** → **pSO52** [*Venus:hapX*]
Venus_fwd	atcgactcgaagaaccaaccATGGTCAGCAAGGGCGAG	**PC** → **pSO52** [*Venus:hapX*]
Venus_linker-seq_rev	gtgtagacatGGTTACGGATGACTTGTACAGCTCGTCCATG	**PC** → **pSO52** [*Venus:hapX*]

linker-seq_hapX_fwd	atccgtaaccATGTCTACACCTTCAATAGC	**PC** → **pSO52** [*Venus:hapX*]
hapX_rev	tgaaatcactgctgccatggTCATTTGTCGGCAAACCG	**PC** → **pSO52** [*Venus:hapX*]
pSO53_fwd	TGACCATGGCAGCAGTGATTTC	**PC – SDM (truncation) in pSO52** → **pSO53** [*Venus*]
pSO53_rev	CTTGTACAGCTCGTCCATGC	**PC – SDM (truncation) in pSO52** → **pSO53** [*Venus*]
pSO54_fwd	GTCCCAAGGGCCGCTTTG	**PC – SDM (truncation) in pSO52** → **pSO54** [*Venus*$^{27aaC\text{-}termHapX}$]
pSO54_rev	GGTTACGGATGACTTGTACAGCTC	**PC – SDM (truncation) in pSO52** → **pSO54** [*Venus*$^{27aaC\text{-}termHapX}$]
oAfhapX-seq1	TCGGTGGAAAGAAGTGCC	SC
oAfhapX-seq2	CGAGTCCGTTTGGGTATC	SC
oAfhapX-S3	TTTGTCGGCAAACCGTCG	SC
oAfhapX-1	AGCGACTATAGCCGGATG	TCA, SB
oAfhapX-2	CCTTGGGTCTTGAAGCTTGCG	TCA
oAfhapX-4	ATCAGAGCTGGAGAGGCA	SB
oAfhapX-xylP.seq	GTATAAGTATCGCCTCCATC	SC
*5'fcyB*_fwd	CAGAGAATTGCCAAGCTGGT	SB
*5'fcyB*_rev	TAGTTCTGTTACCGAGCCGGCCTGAGTCAATCCCCACCAC	SB

Table S5: Primers used for generation of DIG-labelled Northern blot probes.

primer	**sequence [5' - 3']**	**targeted gene**	**short description**
oAfhapX-seq1	TCGGTGGAAAGAAGTGCC	*hapX* Afu5g03920	iron regulatory transcription factor
oAfhapX-S3	TTTGTCGGCAAACCGTCG		
oAfmirB1	AAGCCGAGAAAAAGGGGG	*mirB* Afu3g03640	TAFC transporter
oAfmirB2	AACCCAGATGAAGCCCAG		
oAfsreA5	CTCAGTACGATCGCTTCC	*sreA* Afu5g11260	iron regulatory transcription factor
oAfsreA6	GTTGGACGAGTAGGTAGC		
oAfhemA-f	CAAAGGCAAGACTCCACG	*hemA* Afu5g06270	heme biosynthesis
oAfhemA-r	GGCATCACCAACCAGAAG		
Venus_probe_fwd	GACGTAAACGGCCACAAGTT	*Venus*	yellow-fluorescent protein, Venus
Venus_probe_rev	GAACTCCAGCAGGACCATGT		

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