

## **Supplementary Information**

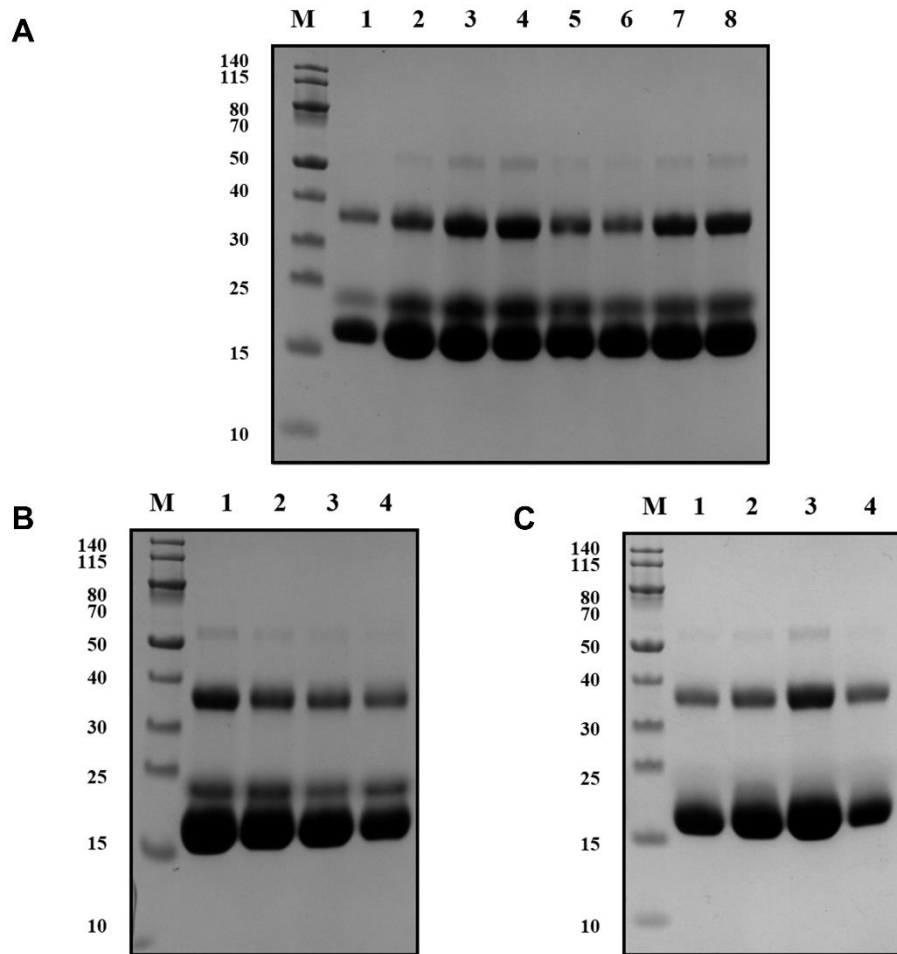
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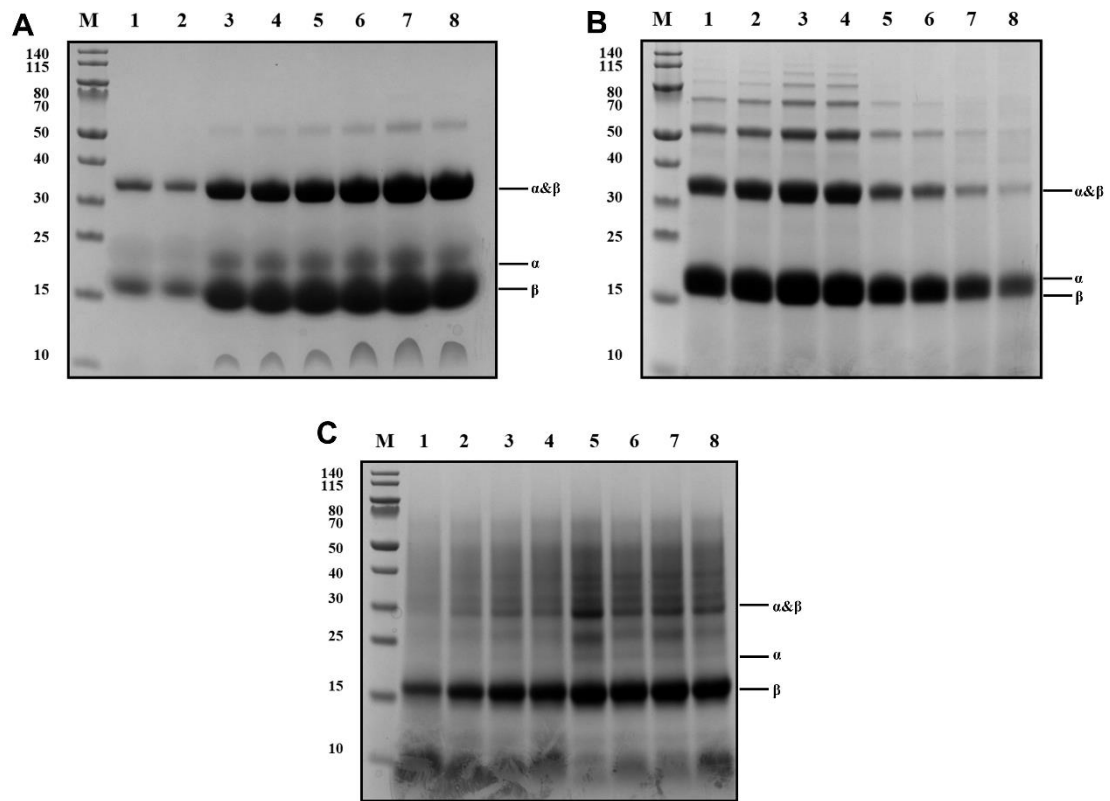
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## Supplementary Figure S1



**Figure S1.** The SDS-PAGE analysis for optimization of bHB fermentation conditions at shaking-flask level. **(A)** Impact of hemin supplementation and methanol concentration on bHB expression. Lanes 1-4 (hemin supplementation): 20 mg/L, 60 mg/L, 100 mg/L, 140 mg/L; Lanes 5-8 (methanol concentration): 0.5%, 1%, 2%, 4%; **(B)** Effect of culture temperature on bHB expression. Lanes 1-4: 24°C, 27°C, 30°C, 33°C **(C)** Effect of initial pH in the medium on bHB expression. Lanes 1-4: 6.5, 6.0, 5.5, 5.0. M indicates the protein ladder.

## Supplementary Figure S2



**Figure S2.** The SDS-PAGE results of purified hemoglobin samples at 5-L fermenter scale. (A) Bovine hemoglobin. (B) Human hemoglobin. (C) Porcine hemoglobin. Lanes 1-8 represent for 60 h, 72 h, 84 h, 96 h, 108 h, 120 h, 132 h, and 144 h.

# Supplementary Tables S1-S6

**Table S1 Plasmids, genes, and promoters used in this study**

Plasmids/Genes /Promoters	Description	Source
<b>Plasmids</b>		
pPICZαA	<i>P. pastoris</i> inducible expression plasmid; Zeo <sup>R</sup>	Invitrogen
pGAPZαA	<i>P. pastoris</i> constitutive expression plasmid; Zeo <sup>R</sup>	Invitrogen
pPICZαA-αF-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and α-Factor as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PpPIR2-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PpPIR2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BGL2 -LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BGL2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_03304T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_03304T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-MSB2 -LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and MSB2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PP7435_Ch3-1225 -LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PP7435_Ch3-1225 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-FPR2-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and FPR2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-CTS1 -LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and CTS1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PP7435_Ch2-0752 -LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PP7435_Ch2-0752 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-FLO5-1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and FLO5-1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-SUN4-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and SUN4 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-RCE3-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and RCE3 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-SCW10-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and SCW10 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-GAS3-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and GAS3 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-FLO11-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and FLO11 as the SP; Zeo <sup>R</sup>	This study

pPICZαA-BA75_04148T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_04148T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-CDA2-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and CDA2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-GAS1-1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and GAS1-1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-EXG1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and EXG1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-UTH1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and UTH1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-KAR2-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and KAR2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_00070T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_00070T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PRY2-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PRY2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PDI1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PDI1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PpPIR1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PpPIR1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_04419T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_04419T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-YPS1-1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and YPS1-1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_02630T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_02630T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PP7435_Chr3-1213-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PP7435_Chr3-1213 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PEP4-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PEP4 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-SCW11-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and SCW11 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PP7435_Chr4-0690-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PP7435_Chr4-0690 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_0002T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_0002T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-DSE4-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and DSE4 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-EPX1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and EPX1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_04741T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_04741T0 as the SP; Zeo <sup>R</sup>	This study

pPICZαA-BA75_04680T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_04680T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-TOS1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and TOS1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_02525T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_02525T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-YMR321C-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and YMR321C as the SP; Zeo <sup>R</sup>	This study
pPICZαA-MPD1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and MPD1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-CPR5-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and CPR5 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_00206T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_00206T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-NCR1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and NCR1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_01378T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_01378T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_04539T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_04539T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_01990T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_01990T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_02958T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_02958T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_02022T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_02022T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_01121T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_01121T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-ADP1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and ADP1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-UTR2-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and UTR2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-YBR139W-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and YBR139W as the SP; Zeo <sup>R</sup>	This study
pPICZαA-OST1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and OST1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-ERP5-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and ERP5 as the SP; Zeo <sup>R</sup>	This study

pPICZαA-BA75_04668T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_04668T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PLB3-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PLB3 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_00184T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_00184T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_01365T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_01365T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-YKE4-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and YKE4 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_04506T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_04506T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-ERO1-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and ERO1 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-EMP70-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and EMP70 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_02926T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_02926T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_01328T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_01328T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_02520T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_02520T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-BA75_05051T0-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and BA75_05051T0 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-PPlase-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and PPlase as the SP; Zeo <sup>R</sup>	This study
pPICZαA-GAS2-LegH	pPICZαA harboring one copy of the <i>LegH</i> gene, and GAS2 as the SP; Zeo <sup>R</sup>	This study
pPICZαA-αF-bHBα	pPICZαA harboring one copy of the <i>bHBα</i> gene, and α-Factor as the SP; Zeo <sup>R</sup>	This study
pPICZαA-αF-bHBβ	pPICZαA harboring one copy of the <i>bHBβ</i> gene, and α-Factor as the SP; Zeo <sup>R</sup>	This study
pPICZαA-αF-hHBα	pPICZαA harboring one copy of the <i>hHBα</i> gene, and α-Factor as the SP; Zeo <sup>R</sup>	This study
pPICZαA-αF-hHBβ	pPICZαA harboring one copy of the <i>hHBβ</i> gene, and α-Factor as the SP; Zeo <sup>R</sup>	This study
pPICZαA-αF-pHBα	pPICZαA harboring one copy of the <i>pHBα</i> gene, and α-Factor as the SP; Zeo <sup>R</sup>	This study
pPICZαA-αF-pHBβ	pPICZαA harboring one copy of the <i>pHBβ</i> gene, and α-Factor as the SP; Zeo <sup>R</sup>	This study
pGAPZαA - bAHSP	pGAPZαA harboring one copy of the <i>bAHSP</i> gene; Zeo <sup>R</sup>	This study

pGAPZαA - hAHSP	pGAPZαA harboring one copy of the <i>hAHSP</i> gene; Zeo <sup>R</sup>	This study
pGAPZαA - pAHSP	pGAPZαA harboring one copy of the <i>pAHSP</i> gene; Zeo <sup>R</sup>	This study

### CRISPR/Cas9 plasmids & donor fragments

pPIC3.5K- <i>ku70</i> - gRNA1	gRNA-Cas9 all-in-one plasmid containing gRNA targeted <i>ku70</i> ; Zeo <sup>R</sup> ; Amp <sup>R</sup>	[1]
pPIC3.5K- P <sub>FLD1</sub> UP- gRNA1	Replacement of the gRNA in pPIC3.5K- <i>Ku70</i> - gRNA1 with P <sub>FLD1</sub> UP-gRNA1; Zeo <sup>R</sup> ; Amp <sup>R</sup>	[1]
pPIC3.5K- P <sub>TEFI</sub> UP- gRNA1	Replacement of the gRNA in pPIC3.5K- <i>Ku70</i> - gRNA1 with P <sub>TEFI</sub> UP-gRNA1; Zeo <sup>R</sup> ; Amp <sup>R</sup>	[1]
pPIC3.5K- P <sub>GAP</sub> UP-gRNA2	Replacement of the gRNA in pPIC3.5K- <i>Ku70</i> - gRNA1 with P <sub>GAP</sub> UP-gRNA2; Zeo <sup>R</sup> ; Amp <sup>R</sup>	[1]
pPIC3.5K- <i>VPS10</i> -gRNA	Replacement of the gRNA in pPIC3.5K- <i>Ku70</i> - gRNA1 with <i>VPS10</i> -gRNA; Zeo <sup>R</sup> ; Amp <sup>R</sup>	This study
pPIC3.5K- <i>VTH1</i> -gRNA	Replacement of the gRNA in pPIC3.5K- <i>Ku70</i> - gRNA1 with <i>VTH1</i> -gRNA; Zeo <sup>R</sup> ; Amp <sup>R</sup>	This study
pPIC3.5K- <i>PEP5</i> -gRNA	Replacement of the gRNA in pPIC3.5K- <i>Ku70</i> - gRNA1 with <i>PEP5</i> -gRNA; Zeo <sup>R</sup> ; Amp <sup>R</sup>	This study
pMD18-T	A dedicated vector for efficient cloning of PCR products; Amp <sup>R</sup>	Takara
pMD18T-Fg1	pMD18T harboring the gene cassette UpFg1- DoFg1; Amp <sup>R</sup>	Lab stock
pMD18T-Tg1	pMD18T harboring the gene cassette UpTg1- DoTg1; Amp <sup>R</sup>	Lab stock
pMD18T-Gg2	pMD18T harboring the gene cassette UpGg2- DoGg2; Amp <sup>R</sup>	Lab stock
pMD18T-Fg1- αF-bHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (αF-bHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study
pMD18T-Fg1- DSE4-bHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (DSE4-bHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study
pMD18T-Fg1- SUN4-bHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (SUN4-bHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study
pMD18T-Fg1- αF-hHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (αF-hHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study
pMD18T-Fg1- DSE4-hHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (DSE4-hHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study
pMD18T-Fg1- SUN4-hHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (SUN4-hHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study
pMD18T-Fg1- αF-pHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (αF-pHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study
pMD18T-Fg1- DSE4-pHBα	pMD18T harboring the gene cassette UpFg1-P <sub>AOX1</sub> - (DSE4-pHBα)- <i>AOX1TT</i> - DoFg1; Amp <sup>R</sup>	This study



pMD18T-Fg1-SUN4-pHB $\alpha$	pMD18T harboring the gene cassette UpFg1- $P_{AOXI}$ -(SUN4-pHB $\alpha$ )- <i>AOX1TT</i> -DoFg1; Amp <sup>R</sup>	This study
pMD18T-Tg1- $\alpha$ F-bHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -( $\alpha$ F-bHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1-DSE4-bHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -(DSE4-bHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1-SUN4-bHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -(SUN4-bHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1- $\alpha$ F-hHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -( $\alpha$ F-hHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1-DSE4-hHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -(DSE4-hHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1-SUN4-hHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -(SUN4-hHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1- $\alpha$ F-pHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -( $\alpha$ F-pHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1-DSE4-pHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -(DSE4-pHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Tg1-SUN4-pHB $\beta$	pMD18T harboring the gene cassette UpTg1- $P_{AOXI}$ -(SUN4-pHB $\beta$ )- <i>AOX1TT</i> -DoTg1; Amp <sup>R</sup>	This study
pMD18T-Gg2- $\alpha$ F-bHB $\beta$	pMD18T harboring the gene cassette UpGg2- $P_{AOXI}$ -( $\alpha$ F-bHB $\beta$ )- <i>AOX1TT</i> -DoGg2; Amp <sup>R</sup>	This study
pMD18T-Gg2- $\alpha$ F-hHB $\beta$	pMD18T harboring the gene cassette UpGg2- $P_{AOXI}$ -( $\alpha$ F-hHB $\beta$ )- <i>AOX1TT</i> -DoGg2; Amp <sup>R</sup>	This study
pMD18T-Gg2- $\alpha$ F-pHB $\beta$	pMD18T harboring the gene cassette UpGg2- $P_{AOXI}$ -( $\alpha$ F-pHB $\beta$ )- <i>AOX1TT</i> -DoGg2; Amp <sup>R</sup>	This study
pMD18T-VPS10	pMD18T harboring the gene cassette UpVPS10-DoVPS10; Amp <sup>R</sup>	This study
pMD18T-VTH1	pMD18T harboring the gene cassette UpVTH1-DoVTH1; Amp <sup>R</sup>	This study
pMD18T-PEP5	pMD18T harboring the gene cassette UpPEP5-UpPEP5; Amp <sup>R</sup>	This study

## Genes

<i>LegH</i>	Codon optimization of the leghemoglobin gene (NP_001235248.2)	This study
<i>bHB<math>\alpha</math></i>	Codon optimization of the bovine hemoglobin $\alpha$ subunit gene (NP_001070890.2)	This study
<i>bHB<math>\beta</math></i>	Codon optimization of the bovine hemoglobin $\beta$ subunit gene (NP_776342.1)	This study
<i>hHB<math>\alpha</math></i>	Codon optimization of the human hemoglobin $\alpha$ subunit gene (NP_000508.1)	This study
<i>hHB<math>\beta</math></i>	Codon optimization of the human hemoglobin $\beta$ subunit gene (NP_000509.1)	This study

<i>pHB<math>\alpha</math></i>	Codon optimization of the porcine hemoglobin $\alpha$ subunit gene (XP_020942625.1)	This study
<i>pHB<math>\beta</math></i>	Codon optimization of the porcine hemoglobin $\beta$ subunit gene (NP_001138313.1)	This study
<i>bAHSP</i>	Codon optimization of the bovine alpha-hemoglobin stabilizing protein bAHSP gene (NP_803488.1)	This study
<i>hAHSP</i>	Codon optimization of the human alpha-hemoglobin stabilizing protein hAHSP gene (NP_001305150.1)	This study
<i>pAHSP</i>	Codon optimization of the porcine alpha-hemoglobin stabilizing protein pAHSP gene (XP_020942031.1)	This study
<i>VPS10</i>	Vacuolar protein sorting/targeting protein 10 [ <i>Komagataella phaffii</i> (strain GS115 / ATCC 20864), <i>PAS_chr2-1_0625</i> ; <i>K. phaffii</i> (strain ATCC 76273 / CBS PP7435 / CECT 11047 / NRRL Y-11430 / Wegner 21-1), <i>PP7435_Chr2-0657</i> ]	This study
<i>VTH1</i>	Type I transmembrane sorting receptor for multiple vacuolar hydrolases [ <i>K. phaffii</i> (strain GS115 / ATCC 20864), <i>PAS_chr3_0653</i> ; <i>K. phaffii</i> (strain ATCC 76273 / CBS PP7435 / CECT 11047 / NRRL Y-11430 / Wegner 21-1), <i>PP7435_Chr3-0547</i> ]	This study
<i>PEP5</i>	E3 ubiquitin-protein ligase [ <i>K. phaffii</i> (strain GS115 / ATCC 20864), <i>PAS_chr1-3_0306</i> ; <i>K. phaffii</i> (strain ATCC 76273 / CBS PP7435 / CECT 11047 / NRRL Y-11430 / Wegner 21-1), <i>PP7435_Chr1-0237</i> ]	This study
<b>Promoters</b>		
<i>P<sub>AOXI</sub></i>	Methanol-inducible promoter	Invitrogen
<i>P<sub>GAP</sub></i>	Constitutive promoter	Invitrogen

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Zeo<sup>R</sup>, Zeocin resistance; Amp<sup>R</sup>, Ampicillin resistance

**Table S2 Strains used in this study**

Strains	Description	Source
DH5α	<i>E. coli</i> str. K-12 F <sup>-</sup> <i>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20</i> φ80 <i>dlacZ</i> ΔM15	Lab stock
P1	Δ ( <i>lacZYA-argF</i> ) U169 <i>hsdR17</i> ( <i>rK<sup>-</sup>mK<sup>+</sup></i> ) λ <sup>-</sup> X33 with the knockout of <i>ku70</i> and <i>ypsI-1</i> genes deleted, and the <i>MitI</i> gene integrated into the <i>P<sub>AOXI</sub></i> UP-gRNA2 locus.	Lab stock[2]
P1H9	P1H9 engineered strain was described in the reference[2].	Lab stock[2]
P1- αF-LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -αF-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PpPIR2-LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> - PpPIR2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BGL2 -LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -BGL2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_03304T0-LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -BA75_03304T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- MSB2 -LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -MSB2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PP7435_Chr3-1225 -LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -PP7435_Chr3-1225-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- FPR2 -LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -FPR2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- CTS1 -LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -CTS1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PP7435_Chr2-0752 -LegH	Plasmid pPICZαA- <i>P<sub>AOXI</sub></i> -PP7435_Chr2-0752-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study

Strains	Description	Source
P1- FLO5-1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -FLO5-1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- SUN4-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -SUN4-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- RCE3-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -RCE3-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- SCW10-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -SCW10-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- GAS3-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -GAS3-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- FLO11-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -FLO11-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_04148T0- LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -BA75_04148T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- CDA2-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -CDA2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- GAS1-1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -GAS1-1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- EXG1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -EXG1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- UTH1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -UTH1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- KAR2-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -KAR2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_00070T0- LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -BA75_00070T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PRY2-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -PRY2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PDI1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -PDI1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study

Strains	Description	Source
P1- PpPIR1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -PpPIR1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_04419T0- LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -BA75_04419T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- YPS1-1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -YPS1-1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_02630T0- LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -BA75_02630T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PP7435_Chr3- 1213-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -PP7435_Chr3-1213-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PEP4-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -PEP4-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- SCW11-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -SCW11-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PP7435_Chr4- 0690-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -PP7435_Chr4-0690-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_0002T0- LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -BA75_0002T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- DSE4-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -DSE4-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- EPX1-LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -EPX1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_04741T0- LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -BA75_04741T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_04680T0- LegH	Plasmid pPICZαA-P <sub>AOXI</sub> -BA75_04680T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study

Strains	Description	Source
P1- TOS1-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -TOS1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_02525T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_02525T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- YMR321C- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -YMR321C-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- MPD1-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -MPD1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- CPR5-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -CPR5-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_00206T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_00206T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- NCR1-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -NCR1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_01378T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_01378T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_04539T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_04539T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_01990T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_01990T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_02958T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_02958T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_02022T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_02022T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study

Strains	Description	Source
P1- BA75_01121T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_01121T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- ADP1-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -ADP1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- UTR2-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -UTR2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- YBR139W- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -YBR139W-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- OST1-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -OST1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- ERP5-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -ERP5-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_04668T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_04668T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PLB3-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -PLB3-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_00184T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_00184T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_01365T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_01365T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- YKE4-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -YKE4-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_04506T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_04506T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- ERO1-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -ERO1-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study

Strains	Description	Source
P1- EMP70-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -EMP70-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_02926T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_02926T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_01328T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_01328T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_02520T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_02520T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- BA75_05051T0- LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -BA75_05051T0-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- PPase-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -PPase-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1- GAS2-LegH	Plasmid pPICZ $\alpha$ A-P <sub>AOXI</sub> -GAS2-LegH integrated into the 5' <i>AOXI</i> locus of P1; Zeo <sup>R</sup>	This study
P1H9- $\alpha$ F-bHB	P1H9 (X33- <i>Aku70-Mit1-Ayps1</i> -HEME-9) derived strain, gene UpFg1-P <sub>AOXI</sub> -( $\alpha$ F-bHB $\alpha$ )- <i>AOX1TT</i> -DoFg1 integrated into the P <sub>FLDI</sub> UP-gRNA1 locus of P1H9; and gene UpGg2-P <sub>AOXI</sub> -( $\alpha$ F-bHB $\beta$ )- <i>AOX1TT</i> -DoGg2 integrated into the P <sub>GAP</sub> UP-gRNA2 locus of P1H9.	This study
P1H9- $\alpha$ F-hHB	P1H9 (X33- <i>Aku70-Mit1-Ayps1</i> -HEME-9) derived strain, gene UpFg1-P <sub>AOXI</sub> -( $\alpha$ F-hHB $\alpha$ )- <i>AOX1TT</i> -DoFg1 integrated into the P <sub>FLDI</sub> UP-gRNA1 locus of P1H9; and gene UpGg2-P <sub>AOXI</sub> -( $\alpha$ F-hHB $\beta$ )- <i>AOX1TT</i> -DoGg2 integrated into the P <sub>GAP</sub> UP-gRNA2 locus of P1H9.	This study



Strains	Description	Source
P1H9- $\alpha$ F-pHB	P1H9 (X33- <i>Δku70-Mit1-Δyps1</i> -HEME-9) derived strain, gene UpFg1- $P_{AOXI}$ -( $\alpha$ F-pHB $\alpha$ )- <i>AOX1TT</i> -DoFg1 integrated into the $P_{FLDI}$ UP-gRNA1 locus of P1H9; and gene UpGg2- $P_{AOXI}$ - ( $\alpha$ F-pHB $\beta$ )- <i>AOX1TT</i> - DoGg2 integrated into the $P_{GAP}$ UP-gRNA2 locus of P1H9.	This study
P1- $\alpha$ F-bHB (Named P1b)	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, gene UpFg1- $P_{AOXI}$ -( $\alpha$ F-bHB $\alpha$ )- <i>AOX1TT</i> - DoFg1 integrated into the $P_{FLDI}$ UP-gRNA1 locus of P1; and gene UpTg1- $P_{AOXI}$ - ( $\alpha$ F-bHB $\beta$ )- <i>AOX1TT</i> -DoTg1 integrated into the $P_{TEFI}$ UP-gRNA1 locus of P1.	This study
P1-DSE4-bHB	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, the gene UpFg1- $P_{AOXI}$ -(DSE4-bHB $\alpha$ )- <i>AOX1TT</i> -DoFg1 integrated into the $P_{FLDI}$ UP-gRNA1 locus of P1; and the gene UpTg1- $P_{AOXI}$ - (DSE4-bHB $\beta$ )- <i>AOX1TT</i> - DoTg1 integrated into the $P_{TEFI}$ UP-gRNA1 locus of P1.	This study
P1-SUN4-bHB	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, the gene UpFg1- $P_{AOXI}$ -(SUN4-bHB $\alpha$ )- <i>AOX1TT</i> -DoFg1 integrated into the $P_{FLDI}$ UP-gRNA1 locus of P1; and the gene UpTg1- $P_{AOXI}$ - (SUN4-bHB $\beta$ )- <i>AOX1TT</i> - DoTg1 integrated into the $P_{TEFI}$ UP-gRNA1 locus of P1.	This study
P1- $\alpha$ F-hHB (Named P1h)	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, gene UpFg1- $P_{AOXI}$ -( $\alpha$ F-hHB $\alpha$ )- <i>AOX1TT</i> - DoFg1 integrated into the $P_{FLDI}$ UP-gRNA1 locus of P1; and gene UpTg1- $P_{AOXI}$ - ( $\alpha$ F-hHB $\beta$ )- <i>AOX1TT</i> -DoTg1 integrated into the $P_{TEFI}$ UP-gRNA1 locus of P1.	This study

Strains	Description	Source
P1-DSE4-hHB	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, gene UpFg1-P <sub>AOXI</sub> -(DSE4-hHB $\alpha$ )- <i>AOX1TT</i> - DoFg1 integrated into the P <sub>FLDI</sub> UP-gRNA1 locus of P1; and gene UpTg1-P <sub>AOXI</sub> - (DSE4-hHB $\beta$ )- <i>AOX1TT</i> - DoTg1 integrated into the P <sub>TEFI</sub> UP-gRNA1 locus of P1.	This study
P1-SUN4-hHB	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, gene UpFg1-P <sub>AOXI</sub> -( SUN4-hHB $\alpha$ )- <i>AOX1TT</i> - DoFg1 integrated into the P <sub>FLDI</sub> UP-gRNA1 locus of P1; and gene UpTg1-P <sub>AOXI</sub> - (SUN4-hHB $\beta$ )- <i>AOX1TT</i> - DoTg1 integrated into the P <sub>TEFI</sub> UP-gRNA1 locus of P1.	This study
P1- $\alpha$ F -pHB	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, gene UpFg1-P <sub>AOXI</sub> -( $\alpha$ F-pHB $\alpha$ )- <i>AOX1TT</i> - DoFg1 integrated into the P <sub>FLDI</sub> UP-gRNA1 locus of P1; and gene UpTg1-P <sub>AOXI</sub> - ( $\alpha$ F-pHB $\beta$ )- <i>AOX1TT</i> - DoTg1 integrated into the P <sub>TEFI</sub> UP-gRNA1 locus of P1.	This study
P1-DSE4-pHB (Named P1p)	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, gene UpFg1-P <sub>AOXI</sub> -(DSE4-pHB $\alpha$ )- <i>AOX1TT</i> - DoFg1 integrated into the P <sub>FLDI</sub> UP-gRNA1 locus of P1; and gene UpTg1-P <sub>AOXI</sub> - (DSE4-pHB $\beta$ )- <i>AOX1TT</i> - DoTg1 integrated into the P <sub>TEFI</sub> UP-gRNA1 locus of P1.	This study
P1-SUN4-pHB	P1(X33- <i>Δku70-Mit1-Δyps1</i> ) derived strain, gene UpFg1-P <sub>AOXI</sub> -( SUN4-pHB $\alpha$ )- <i>AOX1TT</i> - DoFg1 integrated into the P <sub>FLDI</sub> UP-gRNA1 locus of P1; and gene UpTg1-P <sub>AOXI</sub> - (SUN4-pHB $\beta$ )- <i>AOX1TT</i> - DoTg1 integrated into the P <sub>TEFI</sub> UP-gRNA1 locus of P1.	This study

Strains	Description	Source
P1- <i>bAHSP</i> - $\alpha$ F-bHB (Named P1b- <i>bAHSP</i> )	Plasmid pGAPZA- <i>bAHSP</i> integrated into the <i>5'GAP</i> locus of P1b; Zeo <sup>R</sup>	This study
P1- <i>hAHSP</i> - $\alpha$ F-hHB (Named P1h- <i>hAHSP</i> )	Plasmid pGAPZA- <i>hAHSP</i> integrated into the <i>5'GAP</i> locus of P1h; Zeo <sup>R</sup>	This study
P1- <i>pAHSP</i> -DSE4-pHB (Named P1p- <i>pAHSP</i> )	Plasmid pGAPZA- <i>pAHSP</i> integrated into the <i>5'GAP</i> locus of P1p; Zeo <sup>R</sup>	This study
P1b- $\Delta$ <i>VPS10</i>	P1b derived strain with the <i>VPS10</i> gene deleted	This study
P1b- $\Delta$ <i>VTH1</i>	P1b derived strain with the <i>VTH1</i> gene deleted	This study
P1b- $\Delta$ <i>PEP5</i>	P1b derived strain with the <i>PEP5</i> gene deleted	This study
P1h- $\Delta$ <i>VPS10</i>	P1h derived strain with the <i>VPS10</i> gene deleted	This study
P1h- $\Delta$ <i>VTH1</i>	P1h derived strain with the <i>VTH1</i> gene deleted	This study
P1h- $\Delta$ <i>PEP5</i>	P1h derived strain with the <i>PEP5</i> gene deleted	This study
P1p- $\Delta$ <i>VPS10</i>	P1p derived strain with the <i>VPS10</i> gene deleted	This study
P1p- $\Delta$ <i>VTH1</i>	P1p derived strain with the <i>VTH1</i> gene deleted	This study
P1p- $\Delta$ <i>PEP5</i>	P1p derived strain with the <i>PEP5</i> gene deleted	This study
P1- $\Delta$ <i>Vth1</i> - <i>bAHSP</i> - $\alpha$ F-bHB (Named P1b- $\Delta$ <i>Vth1</i> - <i>bAHSP</i> )	Plasmid pGAPZA- <i>bAHSP</i> integrated into the <i>5'GAP</i> locus of P1b- $\Delta$ <i>Vth1</i> ; Zeo <sup>R</sup>	This study
P1- $\Delta$ <i>Vth1</i> - <i>hAHSP</i> - $\alpha$ F-hHB (Named P1h- $\Delta$ <i>Vth1</i> - <i>hAHSP</i> )	Plasmid pGAPZA- <i>hAHSP</i> integrated into the <i>5'GAP</i> locus of P1h- $\Delta$ <i>Vth1</i> ; Zeo <sup>R</sup>	This study
P1- $\Delta$ <i>Vth1</i> - <i>pAHSP</i> -DSE4-pHB (Named P1p- $\Delta$ <i>Vth1</i> - <i>pAHSP</i> )	Plasmid pGAPZA- <i>pAHSP</i> integrated into the <i>5'GAP</i> locus of P1p- $\Delta$ <i>Vth1</i> ; Zeo <sup>R</sup>	This study

**Table S3 Gene knock-in/out cassettes used in this study**

<b>Description</b>	<b>Cassettes</b>	<b>Locus</b>
Expression of <i>bHBα</i>	UpFg1- <i>P<sub>AOXI</sub></i> -(αF- <i>bHBα</i> )- <i>AOX1TT</i> - DoFg1	
	UpFg1- <i>P<sub>AOXI</sub></i> -(DSE4- <i>bHBα</i> )- <i>AOX1TT</i> - DoFg1	
	UpFg1- <i>P<sub>AOXI</sub></i> -(SUN4- <i>bHBα</i> )- <i>AOX1TT</i> - DoFg1	
	UpFg1- <i>P<sub>AOXI</sub></i> -(αF- <i>hHBα</i> )- <i>AOX1TT</i> - DoFg1	
Expression of <i>hHBα</i>	UpFg1- <i>P<sub>AOXI</sub></i> -(DSE4- <i>hHBα</i> )- <i>AOX1TT</i> - DoFg1	<i>P<sub>FLD1</sub></i> UP-gRNA1 (P1&P1H9)
	UpFg1- <i>P<sub>AOXI</sub></i> -(SUN4- <i>hHBα</i> )- <i>AOX1TT</i> - DoFg1	
	UpFg1- <i>P<sub>AOXI</sub></i> -(αF- <i>pHBα</i> )- <i>AOX1TT</i> - DoFg1	
	UpFg1- <i>P<sub>AOXI</sub></i> -( DSE4- <i>pHBα</i> )- <i>AOX1TT</i> - DoFg1	
Expression of <i>pHBα</i>	UpFg1- <i>P<sub>AOXI</sub></i> -( SUN4- <i>pHBα</i> )- <i>AOX1TT</i> - DoFg1	
	UpTg1- <i>P<sub>AOXI</sub></i> - (αF - <i>bHBβ</i> )- <i>AOX1TT</i> - DoTg1	
	UpTg1- <i>P<sub>AOXI</sub></i> - (DSE4 - <i>bHBβ</i> )- <i>AOX1TT</i> - DoTg1	
	UpTg1- <i>P<sub>AOXI</sub></i> - (SUN4 - <i>bHBβ</i> )- <i>AOX1TT</i> - DoTg1	
Expression of <i>bHBβ</i>	UpTg1- <i>P<sub>AOXI</sub></i> - (αF - <i>hHBβ</i> )- <i>AOX1TT</i> - DoTg1	<i>P<sub>TEF1</sub></i> UP-gRNA1 (P1)
	UpTg1- <i>P<sub>AOXI</sub></i> - (DSE4 - <i>hHBβ</i> )- <i>AOX1TT</i> - DoTg1	
	UpTg1- <i>P<sub>AOXI</sub></i> - (SUN4 - <i>hHBβ</i> )- <i>AOX1TT</i> - DoTg1	
	UpTg1- <i>P<sub>AOXI</sub></i> - (αF - <i>pHBβ</i> )- <i>AOX1TT</i> - DoTg1	
Expression of <i>hHBβ</i>	UpTg1- <i>P<sub>AOXI</sub></i> - (DSE4 - <i>pHBβ</i> )- <i>AOX1TT</i> - DoTg1	
	UpTg1- <i>P<sub>AOXI</sub></i> - (SUN4 - <i>pHBβ</i> )- <i>AOX1TT</i> - DoTg1	
	UpGg2- <i>P<sub>AOXI</sub></i> - (αF- <i>bHBβ</i> )- <i>AOX1TT</i> - DoGg2	
	UpGg2- <i>P<sub>AOXI</sub></i> - (αF- <i>hHBβ</i> )- <i>AOX1TT</i> - DoGg2	
Expression of <i>pHBβ</i>	UpGg2- <i>P<sub>AOXI</sub></i> - (αF- <i>hHBβ</i> )- <i>AOX1TT</i> - DoGg2	<i>P<sub>GAP</sub></i> UP-gRNA2 (PIH9)
Knockout of <i>VPS10</i>	UVPS10-DoVPS10	<i>VPS10</i> -gRNA
Knockout of <i>VTH1</i>	UpVTH1-DoVTH1	<i>VTH1</i> -gRNA
Knockout of <i>PEP5</i>	UpPEP5-DoPEP5	<i>PEP5</i> -gRNA

**Table S4 sgRNA sequences used in this study**

<b>Target sites</b>	<b>Guide sequence (5'-3') <sup>a</sup></b>
P <sub>FLD1</sub> UP-gRNA1	GCGGCAGTAATTGATATCGT <u>AGG</u>
P <sub>TEF1</sub> UP-gRNA1	GCAAGATGGTTAAAAGGTGA <u>AGG</u>
P <sub>GAP</sub> UP-gRNA2	TTTAAAGATTTCATCTTGAT <u>GG</u>
<i>VPS10</i> -gRNA	GTGTACGTGTCTCATAACGG <u>GGG</u>
<i>VTH1</i> -gRNA	GTACCCAGTATTCAACAGGAC <u>G</u> G
<i>PEP5</i> -gRNA	ATTAACCGAAACCTCAACGT <u>CGG</u>

<sup>a</sup> PAMs are underlined.

**Table S5 Primers used in this study**

<b>Primer</b>	<b>Sequence (5'-3')</b>
LegH-F1	CGAGAAAAGAGAGGGCTGAAGCTCATCACCATCACCATCA CATGGGTGCTTTTACTG
common-XhoI-F2	GCTAAAGAAGAAGGGGTATCTCTCGAGAAAAGAGAGGGCT GAAGCTCATC
LegH-R	TCTAGAAAGCTGGCGGCCGCCGCGGTTAAAAAGCTTTCTT AATAGCTGCAGCTAATTCA
bHB $\alpha$ -F1	CGAGAAAAGAGAGGGCTGAAGCTCATCACCATCACCATCA CGTTTTATCTGCTGCAG
bHB $\alpha$ -R	TCTAGAAAGCTGGCGGCCGCCGCGGTTATCTATATTTTGAT GTCAAAACAGTAGAAACG
bHB $\beta$ -F	TCTCGAGAAAAGAGAGGGCTGAAGCTCATCACCATCACCA TCACTTAACTGCAGAAG
bHB $\beta$ -R	TTCTAGAAAGCTGGCGGCCGCCGCGGTTAATGGTATCTAT GAGCTAATGCATTAGCAAC
hHB $\alpha$ -F	CGAGAAAAGAGAGGGCTGAAGCTCATCACCATCACCATCA CGTTTTGTCTCCAGCTG
hHB $\alpha$ -R	TCTAGAAAGCTGGCGGCCGCCGCGGTTATCTGTACTTAGA TGTCAAAACAGTTGAAAC
hHB $\beta$ -F1	CGAGAAAAGAGAGGGCTGAAGCTCATCACCATCACCATCA CGTTCATTTGACTCCAG
hHB $\beta$ -R	TCTAGAAAGCTGGCGGCCGCCGCGGTTAATGGTATTTATG AGCTAATGCATTAGCAAC
pHB $\alpha$ -F1	CGAGAAAAGAGAGGGCTGAAGCTCATCACCATCACCATCA CGTTTTATCTGCTGCAG
pHB $\alpha$ -R	TCTAGAAAGCTGGCGGCCGCCGCGGTTATCTGTACTTTGA TGTCAAAACAGTAGAAACG
pHB $\beta$ -F1	CTCGAGAAAAGAGAGGGCTGAAGCTCATCACCATCACCAT CACGTTCAATTTGTCTGC
pHB $\beta$ -R	TCTAGAAAGCTGGCGGCCGCCGCGGTTAATGGTATTTATG AGCTAATGCATTAGCAAC

Primer	Sequence (5'-3')
BA75_00002T0 -F	TTGGAGCGTTAGCCTTACTAAACTCGTCTTGGAATGGGT GCTTTTACTGAAAAAC
BA75_00002T0 -R	GTAAGGCTAACGCTCCAAGCTAGCAACAATAAAAAATTCA TCGTTTTCGAATAATTAGT
BA75_01931T0 /SUN4-F	AGCCTGCGCTGTTACTCTAGCTGGTCTTGCAATTGCTATGG GTGCTTTTACTGAAAAAC
BA75_01931T0 /SUN4-R	CTAGAGTAACAGCGCAGGCTGTAAGAGCGGATATCTTCAT CGTTTCGAATAATTAGTTG
PP7435_CHR2- 0752-F	TTCCGCTTTGCTTTCAGTTTTGTCCGTCGCCTTGGCCATGG GTGCTTTTACTGAAAAAC
PP7435_CHR2- 0752-R	CAAAACTGAAAGCAAAGCGGAAATTTTCAAGTAAGACAT CGTTTCGAATAATTAGTTGT
CTS1-F	GCGGGGTTTCATATCTCTGTTACAGCTGATATTCGCCATGGG TGCTTTTACTGAAAAAC
CTS1-R	TAACAGAGATATGAACCCCGCAAAGTAAAAAAATTTTCATC GTTTCGAATAATTAGTTGT
DSE4-F1	TGTTGACCAATATAGTCAGTGGAATGGGTGCTTTTACTGA AAAACAAGAAGC
DSE4-F2	TCCAACGTGCCACAACCTTTTCTTGTTGTTGGTTCTGTTGA CCAATATAGTCAGTGGAAT
DSE4-R	GAAAAGTTGTGGCACGTTGGAAGAGAATGACATCGTTTC GAATAATTAGTTGTTTTTTG
EXG1-F	CGGTGGATGGCTGGTTTTGGAGCCCTATATCACCATGGGT GCTTTTACTGAAAAACAAG
EXG1-R	CAAAACCAGCCATCCACCGAGGTTGACACCCATCGTTTCG AATAATTAGTTGTTTTTTG
FLO5-1-F1	ACTGCAGCTATTTTGTGTCCTTGGTGTACATGGAATGGGT GCTTTTACTGAAAAACAAG
FLO5-1-F2	AAGAGTAAATTTGTGGTTTCGTTTCTGCTTTTACTGCAGCT ATTTTGTGTCCTTGGTGT
FLO5-1-R	AAACGAAACCACAAATTTACTCTTCTCAAACATCGTTTCG AATAATTAGTTGTTTTTTG

Primer	Sequence (5'-3')
BA75_03304T0	TGCTTAGCACAGTGAATGTGCTGGCTATGGGTGCTTTTAC
-F1	TGAAAAACAAGAAGC
BA75_03304T0	GTTACAGCTGCAATCTTTCATATTTTCTTGCTTAGCACAG
-F2	TGAATGTGCTGGCT
BA75_03304T0	AAAATATGAAAGATTGCAGCTGTAACCTCATCGTTTCGAA
-R	TAATTAGTTGTTTTTTGAT
BGL2-F	GGCTGCGGTTGCAATCTCCATTTCACAAGTGTCTGCAATG GGTGCTTTTACTGAAAAAC
BGL2-R	GATTGCAACCGCAGCCAGTGTTTTAAGATTAAAGATCATC GTTTCGAATAATTAGTTGT
EPX1-F1	CAGCAGCTTCCGCCGTTGTCTCAGCTATGGGTGCTTTTAC TGAAAAACAAGAAGC
EPX1-F2	CTCTCCACCAATTTGATTCTAGCTATTGCAGCAGCTTCCGC CGTTGT
EPX1-R	TAGCTAGAATCAAATTGGTGGAGAGCTTCATCGTTTCGAA TAATTAGTTGTTTTTTG
GAS1-1-F	GTTAATAGGATCCTGCCTATTGAGTTCAGTCTTGGCAATGG GTGCTTTTACTGAAAAAC
GAS1-1-R	AGGCAGGATCCTATTAACATGCACAGAGATTAAACATCG TTTCGAATAATTAGTTGT
KAR2-F1	GTAGTGCCATTTGCTAAACCTGTTAGAGCTATGGGTGCTTT TACTGAAAAACAAG
KAR2-F2	GCATTAATGTATGCCATGCTATTGGTCGTAGTGCCATTTGC TAAACCTGTTAG
KAR2-R1	AAGTCAGCCAAGATGGTTTTAACGACAGCATCGTTTCGAA TAATTAGTTGTTTTTTGAT
KAR2-R2	CAATAGCATGGCATACTTAATGCCGCCAAAGTCAGCCAA GATGGTTTTAACGAC
PDI1-F1	ATTTTGTCCGCTCTCAGCTAGCACAAGCAATGGGTGCTT TACTGAAAAACAAG
PDI1-F2	AACTGGAATATTAATACTGTGGCAAGTATTTGTCCGCTC TCACACTAGCACAAG



Primer	Sequence (5'-3')
PDI1-R	TGCCACAGTTTTTAATATTCCAGTTGAATTGCATCGTTTCGA ATAATTAGTTGTTTTTTG
PEP4-F1	TTTGCTCTCTACTCTAGGTATTGGTGCTGAAGCCATGGGTG CTTTTACTGAAAAACAAG
PEP4-F2	TTGACGGTACTACGATGTCAATTGCCATTGGTTTGCTCTCT ACTCTAGGTATTGGTGCT
PEP4-R	CAATTGACATCGTAGTACCGTCAAATATCATCGTTTCGAAT AATTAGTTGTTTTTTGAT
PpPIR1-F	AATAATTGCTACTGCCCTTACTTGCGGTGCATACAGTATGG GTGCTTTTACTGAAAAAC
PpPIR1-R	AGTAAGGGCAGTAGCAATTATTAAGTTCCTGTACATCATCG TTTCGAATAATTAGTTGT
PRY2-F1	TCAGTATTGACCAAGGCCAACGCTATGGGTGCTTTTACTG AAAAACAAGAAGC
PRY2-F2	TTCACATTTTCATTGCTATCAATTATCTCAGTATTGACCAAG GCCAACGCT
PRY2-R	AATTGATAGCAATGAAATGTGAAGTAATCTCATCGTTTCGA ATAATTAGTTGTTTTTTG
PP7435_CHR3- 1213-F	CGAAAAATATCAAGATCGATTGATTCTTGGAGATGATATGG GTGCTTTTACTGAAAAAC
PP7435_CHR3- 1213-R	CAATCGATCTTGATATTTTTCGGCGTATTGCTTTCCATCGT TTCGAATAATTAGTTGT
RCE3-F1	GCTTCAAAATTCAGCTCTTGCTATGGGTGCTTTTACTGAA AAACAAGAAG
RCE3-F2	CTGACATTGCTGGCTGTGCTGTTGTCGCTTCAAAATTCAG CTCTTGCT
RCE3-R	ACAGCACAGCCAGCAATGTCAGGGTGCTCATCGTTTCGA ATAATTAGTTGTTTTTTGAT
SCW10-F1	CATGTTTCGTTGGCCGTGGCCATGGGTGCTTTTACTGAAAA ACAAGAAG
SCW10-F2	GTAAATCTATCGTTAACCTACTGTTGGCATGTTTCGTTGGC CGTGGCCAT

Primer	Sequence (5'-3')
SCW10-R	ACAGTAGGTTAACGATAGATTAACTTGCATCGTTTCGAAT AATTAGTTGTTTTTTGAT
SCW11-F1	GCTCTTCATACAGGCATCCCTACAGATGGGTGCTTTTACTG AAAAACAAG
SCW11-F2	GCTATCAACTATCTTAAATATCTTTATCCTGTTGCTCTTCAT ACAGGCATCCCT
SCW11-R	CAGGATAAAGATATTTAAGATAGTTGATAGCATCGTTTCGA ATAATTAGTTGTTTTTTG
TOS1-F1	TTCACTTCCATCCAGTCTGCCTACGCTATGGGTGCTTTTAC TGAAAAACAAGAAG
TOS1-F2	AGTTATCAGCAACCTTACTGCTCTCCGTTTTCACTTCCATC CAGTCTGCCTACG
TOS1-R	CGGAGAGCAGTAAGGTTGCTGATAACTTCATCGTTTCGAA TAATTAGTTGTTTTTTGAT
UTH1-F	ACTTATCTTTATGGCTCTTGCCTCTCTGGTGGCCTCCATGG GTGCTTTTACTGAAAAAC
UTH1-R	GGCAAGAGCCATAAAGATAAGTTGAGATTTCATCGTTTCG AATAATTAGTTGTTTTTTG
YPS1-1-F1	ACCGGTTTCCGGCGTGATGGCAATGGGTGCTTTTACTGAA AAACAAG
YPS1-1-F2	TTGCTTTGATAGCGAGCGTACCGGTTTCCGGCGTGATG TAACAAGAACTGATCCTTCAACATCGTTTCGAATAATTAGT
YPS1-1-R1	TGTTTTTTGAT
YPS1-1-R2	ACGCTCGCTATCAAAGCAACCCATAACAAGAACTGATCCT TCAACAT
BA75_02525T0 -F	GTCATCCTTTCACTATTCTGCTACTCATTGGCTATGGGTGC TTTTACTGAAAAAC
BA75_02525T0 -R	GCAGAATAGTGAAAGGATGACAAATATTTTCATCGTTTCG AATAATTAGTTGTTTTTTG
YMR321C-F	GAACCTGTTACTGCTATTGGCTCCCACCATAAGTGCTATGG GTGCTTTTACTGAAAAAC

Primer	Sequence (5'-3')
YMR321C-R	CAATAGCAGTAACAGGTTCAATGGCAAGAGCATCGTTTCG AATAATTAGTTGTTTTTTG
MPD1-F	TATCCTTGGCACTCCTCGTGTCTTTGGTTACTGCGATGGGT GCTTTTACTGAAAAAC
MPD1-R	GACACGAGGAGTGCCAAGGATAGTAACTTCATCGTTTCGA ATAATTAGTTGTTTTTTG
BA75_01272T0 /CPR5-F1	GTTCGGATTATTATCCAGTTCCGTGTTTGCAATGGGTGCTT TTACTGAAAAACAAGAAG
BA75_01272T0 /CPR5-F2	TGAACTTTCTGCTTAGCTTTGTAAGTCTGTTCGGATTATTAT CCAGTTCCG
BA75_01272T0 /CPR5-R	CAAAGCTAAGCAGAAAGTTCAACAATTCATCGTTTCGAA TAATTAGTTGTTTTTTGAT
BA75_00206T0 -F1	TATTGCTGCCTCTGCTTCTGCCATGGGTGCTTTTACTGAAA AACAAGAAGC
BA75_00206T0 -F2	TTGCTCAATATCGCAGCTCTTTTGGCTATTGCTGCCTCTGC TTCTG
BA75_00206T0 -R	AAAAGAGCTGCGATATTGAGCAATTCATCGTTTCGAATA ATTAGTTGTTTTTTGAT
NCR1-F1	GTTTGTGGCCGGTCTGGTACAGGCTATGGGTGCTTTTACT GAAAAACAAGAAGCTTTG
NCR1-F2	TGAAAACCCTGCTCCCCCTACTCTTGCTGTTTGTGGCCGG TCTGGTACAGGCTAT
NCR1-R	AGCAAGAGTAGGGGGAGCAGGGTTTTTCATCGTTTCGAAT AATTAGTTGTTTTTTGAT
BA75_01378T0 -F	AATCTCAGTGCTTCTTCTTTTACATTGGCCTTTGCTATGG GTGCTTTTACTGAAAAAC
BA75_01378T0 -R	AAAAAGAAGAAGCACTGAGATTAATAATTCATCGTTTCG AATAATTAGTTGTTTTTTG
BA75_04539T0 -F1	TTCTGTCAAACCGTCTTTGGTATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
BA75_04539T0 -F2	AAGTCTCTACTGCTACTTCTTTTGGCTTTCTGTCAAACCGT CTTTGGTAT

Primer	Sequence (5'-3')
BA75_04539T0	AGCCAAAAGAAGTAGCAGTAGAGACTTCATCGTTTCGAA
-R	TAATTAGTTGTTTTTTGATC
BA75_01990T0	GTCTCTGGGTGCTTCTGTTTTTGCAATGGGTGCTTTTACTG
-F1	AAAAACAAGAAGCTTTG
BA75_01990T0	ATTGTTCCACATACTAGCGGCTTTGTCTCTGGGTGCTTCTG
-F2	TTTTTGC
BA75_01990T0	AAAGCCGCTAGTATGTGGAACAATTCATCGTTTCGAATA
-R	ATTAGTTGTTTTTTGAT
BA75_02958T0	TTGTGGTTTATGGTGTCTGCAATGGGTGCTTTTACTGAAA
-F1	AACAAGAAGCTTTGGTTTC
BA75_02958T0	GTTTCAGGATTGTTTGGCTGGTGTTTTTGTGGTTTATGGTG
-F2	TCTGCAAT
BA75_02958T0	AAACACCAGCCAAACAATCCTGAAACTCATCGTTTCGAAT
-R	AATTAGTTGTTTTTTGATC
BA75_02022T0	TGCCCTGGCTGTCACAGCTCTGGGAATGGGTGCTTTTACT
-F1	GAAAAACAAGAAGCTTTG
BA75_02022T0	GTTTGGAAGGTTCTATTTGCTATTTCTGCCCTGGCTGTCA
-F2	CAGCTCTGGGAAT
BA75_02022T0	ATAGCAAATAGAACCTTTCCAAACTGCATCGTTTCGAATA
-R	ATTAGTTGTTTTTTGAT
BA75_01121T0	TGCCTGGTAGCAATTTGTCAGGGGATGGGTGCTTTTACTG
-F1	AAAAACAAGAAGCTTTG
BA75_01121T0	GAGATTATCACCGTTGACAGCTTTAATATGCCTGGTAGCAA
-F2	TTTGTCAAGGGGAT
BA75_01121T0	ATTAAAGCTGTCAACGGTGATAATCTCATCGTTTCGAATAA
-R	TTAGTTGTTTTTTGAT
ADP1-F	TCTGGCCCTAACATTGGCTCTTCGATTAGTGCAGGCAATG GGTGCTTTTACTGAAAAAC
ADP1-R	AAGAGCCAATGTTAGGGCCAGAACCGCAAGCATCGTTTC GAATAATTAGTTGTTTTTTG
PPIase-F1	AGCCTTTTGCTTGGTGTGGAGTATGGGTGCTTTTACTGA AAAACAAGAAGCTTTG

Primer	Sequence (5'-3')
PPlase-F2	TGAATTAACTTTGATCTTGACACTAATTAGCCTTTTGCTT GGTGTGGAGTAT
PPlase-R	ATTAGTGTCAAGATCAAAGTTAAATTCATCGTTTCGAATAA TTAGTTGTTTTTTGAT
UTR2-F1	CTGGCTTCTTCGGTACTCGCTATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
UTR2-F2	AGACCAGTACTTTCGTTACTTTTGCTACTGGCTTCTTCGGT ACTCGCTAT
UTR2-R	TAGCAAAAGTAACGAAAGTACTGGTCTCATCGTTTCGAAT AATTAGTTGTTTTTTGATC
YBR139W-F1	TTGCTAGCATTGTGCGCAGGCCATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
YBR139W-F2	AAATCGGTTATTTGGAGCCTTCTATCTTTGCTAGCATTGTC GCAGGCCAT
YBR139W-R	AGATAGAAGGCTCCAAATAACCGATTCATCGTTTCGAAT AATTAGTTGTTTTTTGATC
OST1-F	CTCAATTCTGTTCTTCTTATAGGCAGTGTATTTGGTATGG GTGCTTTTACTGAAAAAC
OST1-R	ATAAGAAGGAACAGAATTGAGATAAATTCATCGTTTCGA ATAATTAGTTGTTTTTTG
ERP5-F1	TTGGTAGGAATCACCAACGCCATGGGTGCTTTTACTGAAA AACAAGAAGC
ERP5-F2	GTCTAAATTGTTGACTTTGTTGTTCTTATTGGTAGGAATCA CCAACGCCAT
ERP5-R	TAGGAACAACAAAGTCAACAATTTAGACATCGTTTCGAAT AATTAGTTGTTTTTTGATC
BA75_04668T0 -F1	CAGCTCTAGTATCACTTGCTGCCGGAATGGGTGCTTTTACT GAAAAACAAGAAGC
BA75_04668T0 -F2	GCTGAAAAGAAGGTTGTGTCGTTGATATTTTCAGCTCTAG TATCACTTGCTGCCGGAAT
BA75_04668T0 -R	TATCAACGACACAACCTTCTTTTCAGCCATCGTTTCGAATA ATTAGTTGTTTTTTGATC

Primer	Sequence (5'-3')
PLB3-F1	CTCTTGGCTTGGAATTTGTTTCTGCAATGGGTGCTTTTACT GAAAAACAAGAAGC
PLB3-F2	CCCTTCCTATTGTGGCAACTTTACTCATAGCTCTTGGCTTG GAATTTGTTTCTGCAAT
PLB3-R	GAGTAAAGTTGCCACAATAGGAAGGGCCATCGTTTCGAAT AATTAGTTGTTTTTTGATC
BA75_00184T0 -F1	CTCGTGCATGTGGTCCTTGGCATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
BA75_00184T0 -F2	TATATTCGGTGACAATTCTTTTGCTTCTCGTGCATGTGGTC CTTGGCAT
BA75_00184T0 -R	AAGCAAAAGAATTGTCACCGAATATAGCATCGTTTCGAAT AATTAGTTGTTTTTTGATC
BA75_01365T0 -F1	TTGTTCTGCACGGCAGAAGCTATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
BA75_01365T0 -F2	AGGCCAGGCTGGATATTCGTGGCGTTAGGAGCTTTGTTCT GCACGGCAGAAGCTAT
BA75_01365T0 -R	TAACGCCACGAATATCCAGCCTGGCCTCATCGTTTCGAAT AATTAGTTGTTTTTTGATC
YKE4-F1	TGTGTAGGAAGCGTGTTTGCTATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
YKE4-F2	GAAACTATCAACTTCGATTCTCCTATTCTTCTGTGTAGGAA GCGTGTTTGCTAT
YKE4-R	GAATAGGAGAATCGAAGTTGATAGTTTCATCGTTTCGAAT AATTAGTTGTTTTTTGATC
BA75_04506T0 -F1	TTTTGTTGTTTGAATGTGTGTACGGTATGGGTGCTTTTACT GAAAAACAAGAAGC
BA75_04506T0 -F2	GCACCTCTAATCAGATATGTAACAACGTTTGTTTTGTTGTT TGAATGTGTGTACGGTAT
BA75_04506T0 -R	CGTTGTTACATATCTGATTAGAGGTGCCATCGTTTCGAATA ATTAGTTGTTTTTTGATC
ERO1-F1	CCTGTTATTGTATAACAGCGTTAGCAATGGGTGCTTTTACT GAAAAACAAGAAGC

Primer	Sequence (5'-3')
ERO1-F2	AGGATAGTAAGGAGCCTAGCTGTTACAATAACCTGTTATT GTATAACAGCGTTAGCAAT
ERO1-R	TGTAACAGCTAGGCTCCTTACTATCCTCATCGTTTCGAATA ATTAGTTGTTTTTTGATC
EMP70-F1	TCGATAAAGATAGTCGAAGGCATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
EMP70-F2	CGATTGTTATTATGGATATTGCTTCTTTCGATAAAGATAGTC GAAGGCAT
EMP70-R	AAGAAGCAATATCCATAATAACAATCGCATCGTTTCGAATA ATTAGTTGTTTTTTGATC
BA75_02926T0 -F1	TTCAGTGTTCAAGTCTTCGCTATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
BA75_02926T0 -F2	TATAGAAAAGCTGCTCCCTCTTGCTTTATTCAGTGTTCAAGT CTTCGCTAT
BA75_02926T0 -R	TAAAGCAAGAGGGAGCAGTTTTCTATACATCGTTTCGAAT AATTAGTTGTTTTTTGATC
BA75_01328T0 -F1	CTGCCATCGGCCATCTACGCTATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
BA75_01328T0 -F2	ATGAAGTTTAAACACATTAATCACATTAGTTGCCCTGCCATC GGCCATCTACGCTAT
BA75_01328T0 -R	AACTAATGTGATTAATGTGTAAACTTCATCGTTTCGAATA ATTAGTTGTTTTTTGATC
BA75_02520T0 -F1	CTAATAGACTTGACGTATGCCATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
BA75_02520T0 -F2	TCTTTTTGAAGGTATGGTTGATATGGTTACTAATAGACTTG ACGTATGCCATGGGT
BA75_02520T0 -R	CCATATCAACCATACCTTCAAAAAGATCATCGTTTCGAATA ATTAGTTGTTTTTTGATC
BA75_05051T0 -F1	GCATTAAGCGTTGTAAAGGCTATGGGTGCTTTTACTGAAA AACAAGAAGCTTTGGTTTC
BA75_05051T0 -F2	CAATTCAAACGTTTGATCGGTGCAATTTTCGCATTAAGCG TTGTAAAGGCTATGGGT

Primer	Sequence (5'-3')
BA75_05051T0	AATTGCACCGATCAAACGTTTGAATTGCATCGTTTCGAAT
-R	AATTAGTTGTTTTTTGATC
GAS2-F1	TTATTGATCCTCTTTGGTACTGCATTGCGCATGGGTGCTTT TACTGAAAAACAAGAAGC
GAS2-F2	TGCTGAGTTTCAAAGGTCTTTTTGTCTTATTGATCCTCTTT GGTACTGCATTGCGCAT
GAS2-R	GACAAAAAGACCTTTGAAACTCAGCATCGTTTCGAATAAT TAGTTGTTTTTTGATCTTC
PpPIR2-F	CTCCACTATTGCATTAATACTATTTTACCCGTTGCCTTGGCTAT GGGTGCTTTTACTGAAAAAC
PpPIR2-R	AAATAGTTAATGCAATAGTGGAGAGTGCAGCGAGCTTCAT CGTTTCGAATAATTAGTTGT
MSB2-F	TCTTATACTTACAGTAACACTGTTATCTCCAGCTTTGGCAA TGGGTGCTTTTACTGAAAAAC
MSB2-R	ACAGTGTTACTGTAAGTATAAGAAAGGAGTTTAAATTAAT CATCGTTTCGAATAATTAGTTGT
PP7435_CHR3- 1225-F	AGCTTGGGGTTTCCTTTATGTTCCCCTTGTAAGTGGGAATGG GTGCTTTTACTGAAAAAC
PP7435_CHR3- 1225-R	ATAAAGGAAACCCCAAGCTAAGATTGTAAAGAGGTTTCATC GTTTCGAATAATTAGTTGT
FPR2-F	TCTGGCTGTGTTCTTATTAGTTAGACTCGTTTGCGCTATGG GTGCTTTTACTGAAAAAC
FPR2-R	CTAATAAGAACACAGCCAGAAATTTGGTCGTAGAACTTT CATCGTTTCGAATAATTAGTTG
GAS3-F	TTGACACTCGTTGCTGTTGCTAAGGCCATGGGTGCTTTTA CTGAAAAAC
GAS3-R	CAGCAACGAGTGTCAATAATGGTAAGAGTAGTTTCATCGT TTCGAATAATTAGTTG
FLO11-F1	CTGCAGGTCTGACAAGAGCTCATGGAATGGGTGCTTTTAC TGAAAAACAAGAAGCTTTG
FLO11-F2	ATCGATATTCACCTCTTCTATCTTAGCTGCAGGTCTGACAA GAGCTC



Primer	Sequence (5'-3')
FLO11-R	CTAAGATAGAAGAGGTGAATATCGATCGCAGTGAAACCAT CGTTTCGAATAATTAGTTG
BA75_04148T0 -F1	ACTGTTTCTACTGCAGCTATTTTGTGTCCTTGGTGTACAAG GAATGGGTGCTTTTACTG
BA75_04148T0 -F2	CGTGTATGTTTATAAGCAATAAAGTTTCGGTCTCGCTACTG TTTCTACTGCAGCTATTTTG
BA75_04148T0 -R	CTTTATTGCTTATAAACATACACGATATAGCACTAGGCATC GTTTCGAATAATTAGTTG
CDA2-F	GTATATAATCATTTTGTGACTCTGATAGTTTCAGCTTTCTC CATGGGTGCTTTTACTG
CDA2-R	CAGAGTCAACAAAATGATTATATACTGTGTCTTTTGAGTCA TCGTTTCGAATAATTAGT
BA75_00070T0 -F	TTTGATCTTAGCTATTGCAGCAGCATCCACTGTTGTCTCAG CTATGGGTGCTTTTACTG
BA75_00070T0 -R	ATGCTGCTGCAATAGCTAAGATCAAATTGGTAGAGAACTT CATCGTTTCGAATAATTAG
BA75_04419T0 -F	GTTGGTGCTATTATTGCTTCTCGTGTTGGGAAGTGGCATGG GTGCTTTTACTGAAAAAC
BA75_04419T0 -R	CCAACACGAGAAGCAATAATAGCACCAACAATAGTAACAT CGTTTCGAATAATTAGTTG
BA75_02630T0 -F	TTCTGCTTTGCTTTCAGTTCTGTCCGTCGTTTTGGCCATGG GTGCTTTTACTGAAAAAC
BA75_02630T0 -R	ACAGAACTGAAAGCAAAGCAGAAATTTTCAAGTAAGACA TCGTTTCGAATAATTAGTTG
PP7435_CHR4- 0690-F1	TTGCTGACTCTAGCCAGTGTCTCAATGGGAATGGGTGCTT TACTGAAAAACAAGAAGC
PP7435_CHR4- 0690-F2	AATTCAACAGTGTCGTCATCAGCCAACCTTTTGCTGACTCT AGCCAGTGTCT
PP7435_CHR4- 0690-R	GTTGGCTGATGACGACACTGTTGAATTGCATCGTTTCGAA TAATTAGTTGTTTTTTGAT
BA75_04741T0 -F1	CGAGCGTACCGGTTTCCAGCGTGTTGGCAATGGGTGCTTT TACTGAAAAACAAG

Primer	Sequence (5'-3')
BA75_04741T0 -F2	TTATGGGTCGCTTTGATAGCGAGCGTACCGGTTTCCAGC
BA75_04741T0 -R	CTATCAAAGCGACCCATAACAAGAACTGATCCTTCAACAT CGTTTCGAATAATTAGTTG
BA75_04680T0 -F	TGCGCTATTGTCGCTCCAAAATTTAGCTCTTGCTATGGGTG CTTTTACTGAAAAACAAG
BA75_04680T0 -R	GGAGCGACAATAGCGCAGCCAGCAGTGCCAGAGTGCTCA TCGTTTCGAATAATTAGTTG
bAHSP-F	ACAACTATTTCGAAACGCGGCCGCGCATGGCTTTGATTCAAA CTAATAAGGATTTGATTTC
bAHSP-R	TAGAAAGCTGGCGGCCGCGCGGAGATCTTTATGAAGATT TCAAGAAATTTCTG
hAHSP-F	CAACTATTTTCGAAACGGGATCCATGGCTTTGTTGAAGGCA AATAAGGATTTGATCTC
hAHSP-R	TAGAAAGCTGGCGGCCGCGCGGTTATGAAGATGGTGGT GGATGTGATG
pAHSP-F	GAACAACTATTTTCGAAACGCGGCCGCGCATGGCTTTGTTGCC AACTAACCAAGATTTGATC
pAHSP-R	TGTTCTAGAAAGCTGGCGGCCGCGCGGAGATCTTTATAA AGATTTCAAGAAATTTCTG
pGAP-F	AGATCTCCGCGGCCGCGGCCAGCTTTCTAGAACAAAAAC TCATCTCAGAAG
pGAP-R	CATGCGGCCGCGTTTTTCGAAATAGTTGTTCAATTGATTGAA ATAGGGACAAAT
VPS10g1-F1	TGAGGACGAAACGAGTAAGCTCGTCGTGTACGTGTCTCAT AACGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC TAGTCCG
VPS10g1-R2	CGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGGTGTA CTTTGATTTGTTTAGGTAAGTTGAACTGGATGTATTAGTTT GGTGAC

Primer	Sequence (5'-3')
VTH1g2-F1	TGAGGACGAAACGAGTAAGCTCGTCGTACCCAGTATTCA ACAGGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG GCTAGTCCG
VTH1g2-R2	CGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGGTACC CTTTGATTTGTTTAGGTAAGTTGAACTGGATGTATTAGTTT GGTGAC
PEP5g1-F1	TGAGGACGAAACGAGTAAGCTCGTCATTAACCGAAACCT CAACGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGG CTAGTCCG
PEP5g1-R2	CGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGATTAA CTTTGATTTGTTTAGGTAAGTTGAACTGGATGTATTAGTTT GGTGAC
Cas9-backbone- R1	TGTTTGCCGGATCAAGAGCTACCAACTCT
Cas9-backbone- F2	AGAGTTGGTAGCTCTTGATCCGGCAAAC
UPVPS-F	GGTTTGACTGAACAAGAGTTCAAAAGTAAATACAAAGAA GAATACCAG
UPVPS-R	CCGTGGGTGAGCTATTTCCAAAAGCAATTGGAAACAGTT G
DoVPS-F	CAATTGCTTTTGGAATAGCTCACCCACGGTCACACAC
DoVPS-R	TCCATGGGTGGAGTTCTGCCTGGAG
Up-Do -VPS-F	TGCAGGTCGACGATGGTTTGACTGAACAAGAGTTCAAAA GTAAATAC
Up-DO -VPS-R	GATCCTCTAGAGATTCCATGGGTGGAGTTCTGCCTGGAG
T-VPS-F	ACTCCACCCATGGAATCTCTAGAGGATCCCCGGGTACCGA G
T-VPS-R	G TTCAGTCAAACCATCGTCGACCTGCAGGCATGCAAG
UpVTH1-F	TCGGATTTTTTAGAAAGCCTTTGTACAAATAAGTATGCTCG AAACATTC

Primer	Sequence (5'-3')
UpVTH1-R	TATTTTCTTTCCTTTGATCACCAGATCTTTTAACTCGTTGTA TATATTTATTAGACATG
DoVTH1-F	ACGAGTTAAAAGATCTGGTGATCAAAGGAAAGAAAATAA AGAGTTTCTATGGATTG
DoVTH1-R	TACCCATGAAACCGTCAATATGAAGAATAACACTAACCAG
Up-Do -VTH1-F	TGCAGGTCGACGATTCGGATTTTTTAGAAAGCCTTTGTAC AAATAAG
Up-DO -VTH1-R	GATCCTCTAGAGATTACCCATGAAACCGTCAATATGAAGA ATAACAC
T-VTH1-F	CGGTTTCATGGGTAATCTCTAGAGGATCCCCGGGTACCGA G
T-VTH1-R	CTAAAAAATCCGAATCGTCGACCTGCAGGCATGCAAG
UpPEP5-F	TTGTTGTCAAATGGTGTGGATGCAAGCCATTGTATTTTT CACACCTA
UpPEP5-R	GGGTAAAGTAAGCACAGATTATTAGTTTGGAGGGTGTATT TCAGCCATTTGATGATAAT
DoPEP5-F	TACACCCTCCAACTAATAATCTGTGCTTACTTAACCCTGA CATCACCCCATATAGC
DoPEP5-R	GTGTGTTTTTCAGAGCAAATAGTATCAATAACGTCAGTGGG TGGAGATC
Up-Do -PEP5-F	TGCAGGTCGACGATTTGTTGTCAAATGGTGTGGATGCA AGCCATT
Up-DO -PEP5-R	GATCCTCTAGAGATGTGTGTTTTTCAGAGCAAATAGTATCA ATAACGTCAG
T-PEP5-F	CTCTGAAAACACACATCTCTAGAGGATCCCCGGGTACCGA G
T-PEP5-R	ATTTTGACAACAAATCGTCGACCTGCAGGCATGCAAG
Fg1-HB $\alpha$ -F	AGTTACCCAACTCTGTTATCTCACTAATTCAGATCTAACAT CCAAAGACGAAAGGTTG
Fg1-HB $\alpha$ -R	ATTATTAAGCACATCCGCTTGTATTCTAGGATCCGCACAAA CGAAGGTCTCACTTAATC
Fg1pMD18T-F	TAGAATACAAGCGGATGTGCTTAATAAT

Primer	Sequence (5'-3')
FglpMD18T-R	GAATTAGTGAGATAACAGAGTTGGGTAAC
bHB $\alpha$ -DSE4-F1	TGTTGACCAATATAGTCAGTGGACATCACCATCACCATCA CGTTTTATCTGCTGCAGAT
All-DSE4-F2	TTCCAACGTGCCACAACCTTTCTTGTTGTTGGTTCTGTTG ACCAATATAGTCAGTGGAC
All-DSE4-R	GAAAAGTTGTGGCACGTTGGAAGAGAATGACATCGTTTC GAATAATTAGTTGTTTTTTG
hHB $\alpha$ -DSE4-F1	TGTTGACCAATATAGTCAGTGGACATCACCATCACCATCA CGTTTTGTCTCCAGCTGAT
pHB $\alpha$ -DSE4-F1	TGTTGACCAATATAGTCAGTGGACATCACCATCACCATCA CGTTTTATCTGCTGCAG
bHB $\alpha$ -SUN4-F1	TAGCTGGTCTTGCAATTGCACATCACCATCACCATCACGTT TTATCTGCTGCAGATAAG
All-SUN4-F2	TTACAGCCTGCGCTGTTACTCTAGCTGGTCTTGCAATTGC ACATCACCATCACCATCAC
All-SUN4-R	AACAGCGCAGGCTGTAAGAGCGGATATCTTCATCGTTTCG AATAATTAGTTGTTTTTTG
hHB $\alpha$ -SUN4-F1	GCTGGTCTTGCAATTGCACATCACCATCACCATCACGTTTT GTCTCCAGCTGATAAAAC
pHB $\alpha$ -SUN4-F1	AGCTGGTCTTGCAATTGCACATCACCATCACCATCACGTT TTATCTGCTGCAGATAAAG
Tg1-HB $\beta$ -F	ATTCCAATCCTGTCTGGCCTCCTAGATCTAACATCCAAAGA CGAAAGGTTGAATG
Tg1-HB $\beta$ -R	CGCAATCTGACACGAGTTGGAATGGGGATCCGCACAAAC GAAGGTCTCACTTAATCTTC
Tg1pMD18T-F	CCATTCCAACCTCGTGTGTCAGATTGCGT
Tg1pMD18T-R	AGGAGGCCAGACAGGATTG
Gg2-HB $\beta$ -F	ATTTTAAGATTTCAATCTTGAAGATCTAACATCCAAAGACG AAAGGTTGAATGAAACCT
Gg2-HB $\beta$ -R	ACATGCGACTATTATCGATCAATGAAATGGATCCGCACAA ACGAAGGTCTCACTTAATC

Primer	Sequence (5'-3')
Gg2pMD18T-F	ATTCATTGATCGATAATAGTCGCATGTGAGGCTGAAATGT GC
Gg2pMD18T-R	TCAAGATTGAAATCTTAAAATTGCCCCTTTCAGTTGACAG GAT
bHB $\beta$ -DSE4-F1	TGTTGACCAATATAGTCAGTGGACATCACCATCACCATCA CTTAACTGCAGAAGAAAAAGCT
hHB $\beta$ -DSE4-F1	TGTTGACCAATATAGTCAGTGGACATCACCATCACCATCA CGTTCATTTGACTCCAGAAG
pHB $\beta$ -DSE4-F1	TCTGTTGACCAATATAGTCAGTGGACATCACCATCACCATC ACGTTTCATTTGTCTGCAGAAG
bHB $\beta$ -SUN4-F1	AGCTGGTCTTGCAATTGCACATCACCATCACCATCACTTA ACTGCAGAAGAAAAAGCTG
hHB $\beta$ -SUN4-F1	GGTCTTGCAATTGCACATCACCATCACCATCACGTTTCATT GACTCCAGAAGAAAAATC
pHB $\beta$ -SUN4-F1	TGGTCTTGCAATTGCACATCACCATCACCATCACGTTTCATT TGTCTGCAGAAGAAAAAG
Donor-Fg1-F	ACAGACATGTCTTGTCCTTAGGAG
Donor-Fg1-R	TCTCCCCTAGTTCACGGTTTATTAG
Donor-Tg1-F	TTCAACTTCAATTTTCGGCTCTGTCTG
Donor-Tg1-R	TCTCCTCGTATCTCTTTTGAGACCATTG
Donor-Gg2-F	CAAACAGTTGGTAGTACAAGAAGCATTG
Donor-Gg2-R	GATTATGGTACATCCCTTAAATGCAGCG
DonorVPS10-F	GGTTTGACTGAACAAGAGTTCAAAAGTAAATACAAAG
DonorVPS10-R	TCCATGGGTGGAGTTCTGCCT
DonorVTH1-F	TCGGATTTTTTAGAAAGCCTTTGTACAAATAAGTATGCTC
DonorVPS10-R	TACCCATGAAACCGTCAATATGAAGAATAACACTAAC
DonorPEP5-F	TTGTTGTCAAATGGTGTGGATGCAAG
DonorPEP5-R	GTGTGTTTTTCAGAGCAAATAGTATCAATAACGTCAGTG

**Table S6 Endogenous signal peptides used in this study**

<b>GenBank Accession</b>	<b>Ordered locus name / Gene name</b>	<b>Signal peptides Sequence (5'-3')</b>
<b>The first 40 endogenous signal peptides predicted by signalP6.0 in <i>P. pastoris</i></b>		
ANZ73100.1	BA75_01736T0 / MPD1	ATGAAGTTACTATCCTTGGCACTCCTCGTG TCTTTGGTTACTGCG
ANZ75146.1	BA75_02525T0 / EMP24	ATGAAAATATTTGTCATCCTTTCCTATTTC TGCTACTCATTGGCT
ANZ76017.1	BA75_01931T0	ATGAAGATATCCGCTCTTACAGCCTGCGCT GTTACTCTAGCTGGTCTTGCAATTGCA
ANZ76280.1	BA75_03275T0 / YMR321C	ATGCTCTTGCCATTGAACCTGTTACTGCTA TTGGCTCCCACCATAAGTGCT
ANZ73842.1	BA75_01272T0	ATGAAATTGTTGAACCTTCTGCTTAGCTTT GTAACCTCTGTTTCGGATTATTATCCAGTTCC GTGTTTGCA
ANZ77874.1	BA75_04419T0	ATGTTACTATTGTTGGTGCTATTATTGCTT CTCGTGTTGGGAAGTGGC
ANZ74063.1	BA75_00206T0	ATGAAATTGCTCAATATCGCAGCTCTTTTG GCTATTGCTGCCTCTGCTTCTGCC
ANZ73700.1	BA75_01371T0 / NCR1	ATGAAAACCCTGCTCCCCCTACTCTTGCTG TTTGTGGCCGGTCTGGTACAGGCT
ANZ74444.1	BA75_01378T0	ATGAAATTATTAATCTCAGTGCTTCTTCTT TTTACATTGGCCTTTGCT
ANZ73364.1	BA75_00070T0 / PRY2	ATGAAGTTCTCTACCAATTTGATCTTAGCT ATTGCAGCAGCATCCACTGTTGTCTCAGCT
ANZ77614.1	BA75_04539T0	ATGAAGTCTCTACTGCTACTTCTTTTGGCT TTCTGTCAAACCGTCTTTGGT
ANZ76231.1	BA75_01990T0	ATGAAATTGTTCCACATACTAGCGGCTTTG TCTCTGGGTGCTTCTGTTTTTGCA
ANZ76279.1	BA75_02958T0	ATGAGTTTCAGGATTGTTTGGCTGGTGTTT TTGTGGTTTATGGTGTCTGCA
ANZ75129.1	BA75_02022T0	ATGCAGTTTGGAAAGGTTCTATTTGCTATT TCTGCCCTGGCTGTCACAGCTCTGGGA
ANZ74354.1	BA75_01121T0	ATGAGATTATCACCGTTGACAGCTTTAATA TGCCTGGTAGCAATTTGTCAGGGG
ANZ76406.1	BA75_03570T0 / ADP1	ATGCTTGCGGTTCTGGCCCTAACATTGGCT CTTCGATTAGTGCAGGCA
ANZ77943.1	BA75_04786T0 / PPIase	ATGAATTTAACTTTGATCTTGACACTAATT AGCCTTTTGCTTGGTGTTTGGAGT
ANZ74747.1	BA75_01243T0 / UTR2	ATGAGACCAGTACTTTCGTTACTTTTGCTA CTGGCTTCTTCGGTACTCGCT
ANZ76773.1	BA75_03915T0 / YBR139W	ATGAAATCGGTTATTTGGAGCCTTCTATCT TTGCTAGCATTGTCGCAGGCC
ANZ76351.1	BA75_03798T0 / OST1	ATGAAATTTATCTCAATTCTGTTTCCTTCTT ATAGGCAGTGTATTTGGT

GenBank Accession	Ordered locus name / Gene name	Signal peptides Sequence (5'-3')
ANZ77164.1	BA75_04148T0	ATGCCTAGTGCTATATCGTGTATGTTTATA AGCAATAAAGTTTCGGTCTCGCTACTGTTT CTACTGCAGCTATTTTGTGTCTTGGTGTA CAAGGA
ANZ75389.1	BA75_02602T0 / ERP5	ATGTCTAAATTGTTGACTTTGTTGTTTCTA TTGGTAGGAATCACCAACGCC
ANZ75795.1	BA75_02630T0	ATGTCTTACTTGAAAATTTCTGCTTTGCTTT CAGTTCTGTCCGTCGTTTTGGCC
ANZ77919.1	BA75_04668T0	ATGGCTGAAAAGAAGGTTGTGTCTGTTGAT ATTTTCAGCTCTAGTATCACTTGCTGCCGG A
ANZ76251.1	BA75_03304T0	ATGAGGTTACAGCTGCAATCTTTCATATTT TTCTTGCTTAGCACAGTGAATGTGCTGGCT ATGTTGAAGGATCAGTTCTTGTATGGGTC
ANZ77259.1	BA75_04741T0	GCTTTGATAGCGAGCGTACCGGTTTCCAGC GTGTTGGCA
ANZ75299.1	BA75_03164T0 / PLB3	ATGGCCCTTCCTATTGTGGCAACTTTACTC ATAGCTCTTGGCTTGGAATTTGTTTCTGCA
ANZ73777.1	BA75_00184T0	ATGCTATATTCGGTGACAATTCTTTTGCTT CTCGTGCATGTGGTCCTTGGC
ANZ73985.1	BA75_01365T0	ATGAGGCCAGGCTGGATATTCGTGGCGTT AGGAGCTTTGTTCTGCACGGCAGAAGCT
ANZ74717.1	BA75_00578T0 / YKE4	ATGAACTATCAACTTCGATTCTCCTATTC TTCTGTGTAGGAAGCGTGTTTGCT
ANZ77995.1	BA75_04506T0	ATGGCACCTCTAATCAGATATGTAACAAC GTTTGTTTTGTGTTTGAATGTGTGTACGG T
ANZ74048.1	BA75_01546T0 / ERO1	ATGAGGATAGTAAGGAGCCTAGCTGTTAC AATAACCTGTTATTGTATAACAGCGTTAGC A
ANZ75750.1	BA75_02375T0 / EMP70	ATGCGATTGTTATTATGGATATTGCTTCTT TCGATAAAGATAGTCGAAGGC
ANZ76012.1	BA75_02926T0	ATGTATAGAAAAGCTGCTCCCTCTTGCTTTA TTCAGTGTTCAAGTCTTCGCT
ANZ77964.1	BA75_04680T0	ATGAGCACTCTGGCACTGCTGGCTGCGCT ATTGTCGCTCCAAAATTTAGCTCTTGCT
ANZ73481.1	BA75_00002T0	ATGAATTTTTTATTGTTGCTAGCTTGGAGC GTTAGCCTTACTAACTCGTCTTGGGA
ANZ73657.1	BA75_01328T0	ATGAAGTTTAACACATTAATCACATTAGTT GCCCTGCCATCGGCCATCTACGCT
ANZ76075.1	BA75_02520T0	ATGATCTTTTTGAAGGTATGGTTGATATGG TACTAATAGACTTGACGTATGCC
ANZ78090.1	BA75_05051T0	ATGCAATTCAAACGTTTGATCGGTGCAATT TTCGCATTAAGCGTTGTTAAGGCT

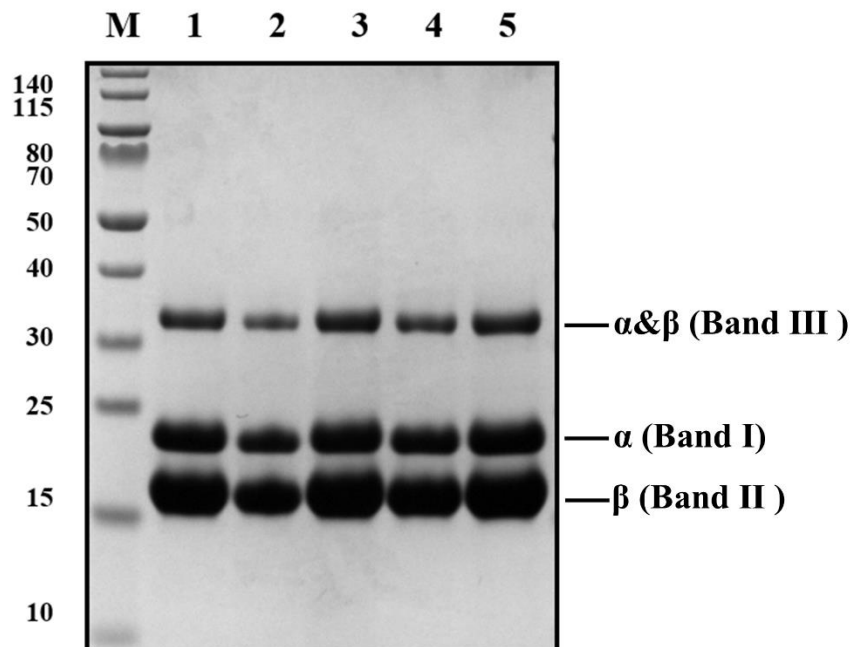


GenBank Accession	Ordered locus name / Gene name	Signal peptides Sequence (5'-3')
ANZ73202.1	BA75_00325T0 / GAS2	ATGCTGAGTTTCAAAGGTCTTTTGTCTTA TTGATCCTCTTTGGTACTGCATTTGGC
<b>Signal peptides obtained by secretome in <i>P. pastoris</i> [3]</b>		
CCA37346.1	PP7435_Ch1- 1218/BGL2	ATGATCTTTAATCTTAAAACACTGGCTGCG GTTGCAATCTCCATTTACAAAGTGTCTGCA
CCA36562.2	PP7435_Ch1- 0405/CDA2	ATGACTCAAAAGACACAGTATATAATCAT TTTGTGACTCTGATAGTTTCAGCTTTCTCC
CCA36730.1	PP7435_Ch1- 0581/CPR5	ATGAAATTGTTGAACCTTTCTGCTTAGCTTT GTAACCTCTGTTTCGGATTATTATCCAGTTCC GTGTTTGCA
CCA39139.1	PP7435_Ch3- 0167/CTS1	ATGAAATTTTTTTACTTTGCGGGGTTTCATA TCTCTGTTACAGCTGATATTTCGCC
CCA36592.1	PP7435_Ch1- 0439/DSE4	ATGTCATTCTCTTCCAACGTGCCACAACCTT TTCTTGTTGTTGGTTCTGTTGACCAATATA GTCAGTGGA
CCA40103.1	PP7435_Ch3- 1160/EPX1	ATGAAGCTCTCCACCAATTTGATTCTAGCT ATTGCAGCAGCTTCCGCCGTTGTCTCAGCT
CCA38524.1	PP7435_Ch2- 0842/EXG1	ATGGGTGTCAACCTCGGTGGATGGCTGGT TTTGGAGCCCTATATCACC
CCA37962.1	PP7435_Ch2- 0267/FLO11	ATGGTTTCACTGCGATCGATATTCACCTCT TCTATCTTAGCTGCAGGTCTGACAAGAGCT CATGGA
CCA37505.1	PP7435_Ch1- 1389/FLO5-1	ATGTTTGAGAAGAGTAAATTTGTGGTTTCG TTTCTGCTTTTACTGCAGCTATTTTGTGTCC TTGGTGTACATGGA
CCA38992.1	PP7435_Ch3- 0018/FPR2	ATGAAAGTTTCTACGACCAAATTTCTGGCT GTGTTCTTATTAGTTAGACTCGTTTGCGCT
CCA36391.1	PP7435_Ch1- 0229/GAS1-1	ATGTTTAAATCTCTGTGCATGTTAATAGGA TCCTGCCTATTGAGTTCAGTCTTGGCA
CCA37670.1	PP7435_Ch1- 1559/GAS3	ATGAACTACTCTTACCATTATTGACACTC GTTGCTGTTGCTAAGGCC
CAH244875 4.1	PP7435_Ch2- 1167/KAR2	ATGCTGTCGTTAAAACCATCTTGGCTGACT TTGGCGGCATTAATGTATGCCATGCTATTG GTCGTAGTGCCATTTGCTAAACCTGTTAGA GCT
CCA36444.1	PP7435_Ch1- 0283/MSB2	ATGATTAATTTAAACTCCTTTCTTATACTT ACAGTAACACTGTTATCTCCAGCTTTGGCA
CCA40283.1	PP7435_Ch4- 0107/PDI1	ATGCAATTCAACTGGAATATTAATACTGT GGCAAGTATTTTGTCCGCTCTCACACTAGC ACAAGCA
CCA39046.1	PP7435_Ch3- 0072/PEP4	ATGATATTTGACGGTACTACGATGTCAATT GCCATTGGTTTGCTCTCTACTCTAGGTATT GGTGCTGAAGCC

<b>GenBank Accession</b>	<b>Ordered locus name / Gene name</b>	<b>Signal peptides Sequence (5'-3')</b>
CCA38189.1	PP7435_Ch2-0501/PIR1	ATGATGTACAGGAACTTAATAATTGCTACT GCCCTTACTTGCGGTGCATACAGT
CCA40849.1	PP7435_Ch4-0692/PIR2	ATGAAGCTCGCTGCACTCTCCACTATTGCA TTAACTATTTTACCCGTTGCCTTGGCT
CCA38437.1	PP7435_Ch2-0752	ATGTCTTACTTGAAAATTTCCGCTTTGCTT TCAGTTTTGTCCGTCGCCTTGGCC
CCA40153.1	PP7435_Ch3-1213	ATGGAAAGCAAATACGCCGAAAAATATCA AGATCGATTGATTCTTGGAGATGAT
CCA40165.1	PP7435_Ch3-1225	ATGAACCTCTTTACAATCTTAGCTTGGGGT TTCCTTTATGTTCCCCTTGTA CTGGGA
CCA40847.1	PP7435_Ch4-0690	ATGCAATTCAACAGTGTCTCATCAGCCA ACTTTTGCTGACTCTAGCCAGTGTCTCAAT GGGA
CCA37069.1	PP7435_Ch1-0934/PRY2	ATGAGATTACTTCACATTTTCATTGCTATCA ATTATCTCAGTATTGACCAAGGCCAACGCT
CCA40496.1	PP7435_Ch4-0326/RCE3	ATGAGCACCTTGACATTGCTGGCTGTGCTG TTGTCGCTTCAA AATTCAGCTCTTGCT
CCA36394.1	PP7435_Ch1-0232/SCW10	ATGCAAGTTAAATCTATCGTTAACCTACTG TTGGCATGTTTCGTTGGCCGTGGCC
CCA38930.1	PP7435_Ch2-1255/SCW11	ATGCTATCAACTATCTTAAATATCTTTATC CTGTTGCTCTTCATACAGGCATCCCTACAG
CCA37765.1	PP7435_Ch2-0068/SUN4	ATGAAGATATCCGCTCTTACAGCCTGCGCT GTTACTCTAGCTGGTCTTGCAATTGCA
CCA37668.1	PP7435_Ch1-1557/TOS1	ATGAAGTTATCAGCAACCTTACTGCTCTCC GTTTTCACTTCCATCCAGTCTGCCTACGCT
CCA41119.1	PP7435_Ch4-0969/UTH1	ATGAAATCTCAACTTATCTTTATGGCTCTT GCCTCTCTGGTGGCCTCC
CCA40555.1	PP7435_Ch4-0387/YPS1-1	ATGTTGAAGGATCAGTTCTTGTTATGGGTT GCTTTGATAGCGAGCGTACCGGTTTCCGGC GTGATGGCA

## Identification results by MALDI TOF mass spectrometry:

### Supplementary Note S1 (Bovine hemoglobin) :



### $\alpha$ subunit (band I) :

#### **Mascot Search Results**

##### Protein View

Match to: HBA\_BOVIN Score: 392  
Hemoglobin subunit alpha OS=Bos taurus GN=HBA PE=1 SV=2  
Found in search of DATA.TXT  
  
Nominal mass ( $M_r$ ): 15175; Calculated pI value: 8.07  
NCBI BLAST search of HBA\_BOVIN against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Bos taurus](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 43%

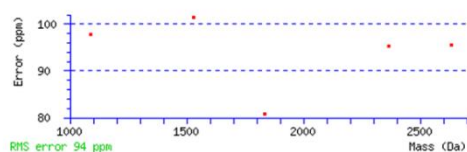
Matched peptides shown in **Bold Red**

1 MVLSAADKGN VKAAMGK**VGG HAAEYGAEL ERMFLSFPTT KTYFPHPDLS**  
51 **HGSAQVK**GHG AKVAALTKA VEHLDDLPGA LSELSDLHAH KLRVDPVNFK  
101 LLSHSLVLTL ASHLPSDFTP AVHASLDKFL ANVSTVLTSK YR

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
18 - 32	1529.8895	1528.8822	1528.7270	102	0	K.VGGHAAEYGAELER.M ( <a href="#">Ions score 146</a> )
42 - 57	1834.0401	1833.0328	1832.8846	81	0	K.TYFPHPDLSHGSAQVK.G ( <a href="#">Ions score 137</a> )
70 - 91	2367.4196	2366.4124	2366.1866	95	0	K.AVEHLDDLFGALSELSDLHAHK.L ( <a href="#">Ions score 59</a> )
70 - 93	2636.6311	2635.6239	2635.3718	96	1	K.AVEHLDDLFGALSELSDLHAHKLR.V ( <a href="#">Ions score 36</a> )
92 - 100	1087.7322	1086.7249	1086.6186	98	1	K.LRVDPVNFK.L ( <a href="#">Ions score 16</a> )



β subunit (band II) :

*MATRIX*  
*SCIENCE* Mascot Search Results

Protein View

Match to: HBB\_BOVIN Score: 476  
Hemoglobin subunit beta OS=Bos taurus GN=HBB PE=1 SV=1  
Found in search of DATA.TXT

Nominal mass (M<sub>0</sub>): 15944; Calculated pI value: 7.01  
NCBI BLAST search of HBB\_BOVIN against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Bos taurus](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 55%

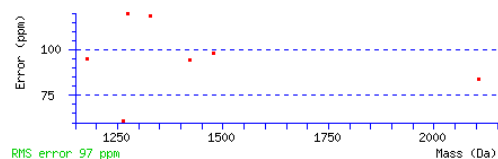
Matched peptides shown in **Bold Red**

1 MLTAEKAAV TAFWGW**KVVD** EVGGEALGRL LVVYPWTQRF FESFGDLSTA  
51 **DAVMNNPK**VK AHGKKVLDSF SNGMKHLDDL KGTFALSEL HCDKLHVDPE  
101 NFK**LLGNVLV** VVLAR**NFGKE** FTFVLQADFQ KVVAGVANAL AHRYH

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
17 - 29	1328.8744	1327.8672	1327.7096	119	1	K.VKVDEVGGEALGR.L ( <a href="#">Ions score 74</a> )
30 - 39	1274.8781	1273.8708	1273.7183	120	0	R.LLVVYPWTQR.F ( <a href="#">Ions score 70</a> )
40 - 58	2106.1250	2105.1177	2104.9412	84	0	R.FFESFGDLSTADAVMNNPK.V Oxidation (M) ( <a href="#">Ions score 125</a> )
104 - 115	1265.9078	1264.9005	1264.8231	61	0	K.LLGNVLVVVLAR.N ( <a href="#">Ions score 15</a> )
120 - 131	1422.8601	1421.8529	1421.7191	94	0	K.EFTFVLQADFQK.V ( <a href="#">Ions score 83</a> )
132 - 143	1177.7919	1176.7846	1176.6727	95	0	K.VVAGVANALAHRY.Y ( <a href="#">Ions score 69</a> )
132 - 145	1477.9467	1476.9395	1476.7950	98	1	K.VVAGVANALAHRYH.- ( <a href="#">Ions score 42</a> )



# $\alpha$ - and $\beta$ -subunit dimers (band III):

## Mascot Search Results

### Protein View

Match to: HBA\_BOVIN Score: 134  
Hemoglobin subunit alpha OS=Bos taurus GN=HBA PE=1 SV=2  
Found in search of DATA.TXT  
  
Nominal mass ( $M_r$ ): 15175; Calculated pI value: 8.07  
NCBI BLAST search of [HBA\\_BOVIN](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Bos taurus](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 26%

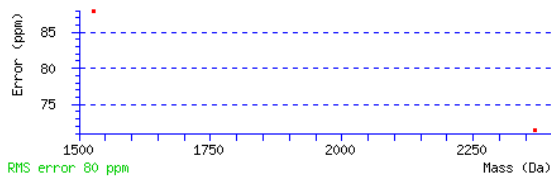
Matched peptides shown in **Bold Red**

1 MVLSAADKGN VKAANGK**VGG HAAEYGAEL ERMFLSFPTI** KTYFPHFDLS  
51 HGSAQVKGHG AKVAAAL**KA VEHLDDLPGA LSELSDLHAH** KLRVDPVNFK  
101 LLSHSLVTL ASHLPSDFTP AVHASLDKFL ANVSTVLTSK YR

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
18 - 32	1529.8687	1528.8614	1528.7270	88	0	K.VGGHAAEYGAELER.M ( <a href="#">Ions score 63</a> )
70 - 91	2367.3631	2366.3558	2366.1866	72	0	K.AVEHLDDLPGALSELSDLHAHK.L ( <a href="#">Ions score 71</a> )



## Mascot Search Results

### Protein View

Match to: HBB\_BOVIN Score: 535  
Hemoglobin subunit beta OS=Bos taurus GN=HBB PE=1 SV=1  
Found in search of DATA.TXT  
  
Nominal mass ( $M_r$ ): 15944; Calculated pI value: 7.01  
NCBI BLAST search of [HBB\\_BOVIN](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Bos taurus](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 49%

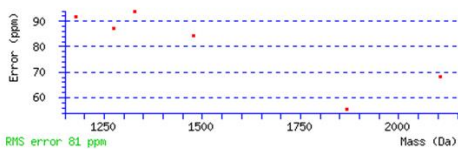
Matched peptides shown in **Bold Red**

1 MLTAEKKAIV TAFWGK**VKVD EVGGEALGR L LVVYPWQRF FESFGDLSTA**  
51 **DAVMNPKVK** AHGKKVLDSE SNGMKHLDDL KGTFALSEL HCDKLHVDPE  
101 NFKLLGNVLV VVLARN**PKKE FTFVLQADFQ KVVAGVANAL AHRYH**

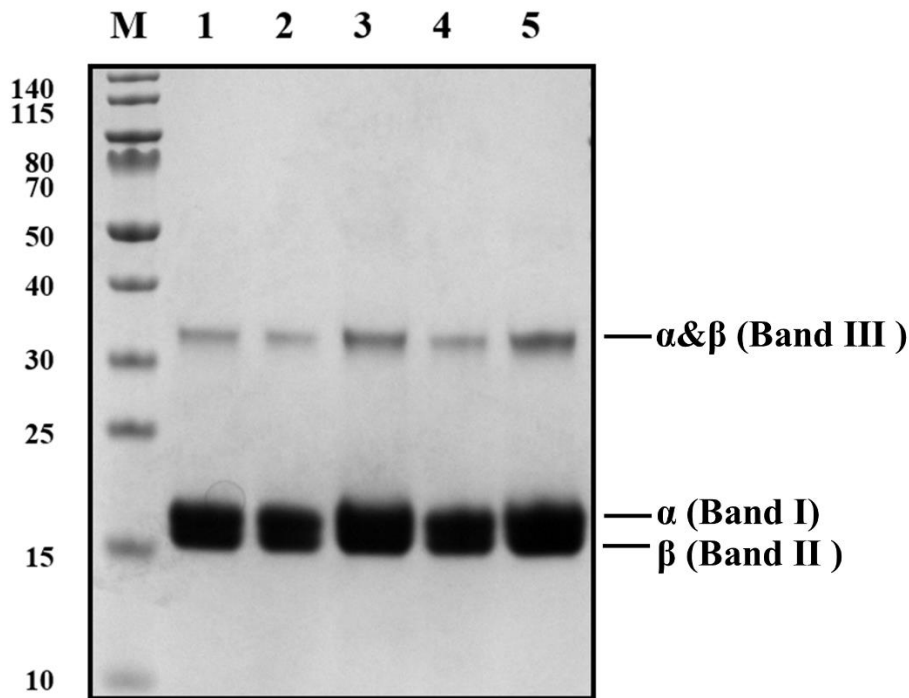
Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
17 - 29	1328.8416	1327.8344	1327.7096	94	1	K.VKVDEVGGEALGR.L ( <a href="#">Ions score 60</a> )
30 - 39	1274.8368	1273.8295	1273.7183	87	0	R.LLVVYEWQRF.F ( <a href="#">Ions score 70</a> )
40 - 58	2106.0916	2105.0844	2104.9412	68	0	R.FFESFGDLSTADAVMNNPK.V Oxidation (M) ( <a href="#">Ions score 150</a> )
116 - 131	1869.0573	1868.0501	1867.9469	55	1	R.NFGKEFTFVLQADFQK.V ( <a href="#">Ions score 146</a> )
132 - 143	1177.7877	1176.7805	1176.6727	92	0	K.VVAGVANALAHRY.Y ( <a href="#">Ions score 72</a> )
132 - 145	1477.9265	1476.9192	1476.7950	84	1	K.VVAGVANALAHRYH.- ( <a href="#">Ions score 39</a> )



Supplementary Note S2 (Human hemoglobin) :



$\alpha$  subunit (band I) :

*MATRIX* SCIENCE Mascot Search Results

Protein View

Match to: HBA\_HUMAN Score: 181  
Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2  
Found in search of DATA.TXT

Nominal mass ( $M_r$ ): 15248; Calculated pI value: 8.72  
NCBI BLAST search of [HBA\\_HUMAN](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 16%

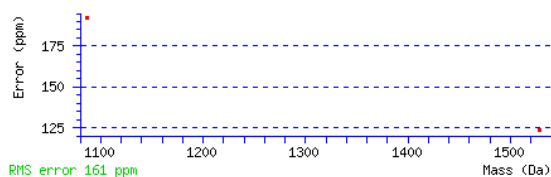
Matched peptides shown in **Bold Red**

1 MVLSPADKTN VKAANGK**VGA HAGEYGAEAL ERMFLSFPTT** KTYFPFHDL  
51 HGSAQVKGHG KKVADALINA VAHVDDMPNA LSALSDLHAH KLRVDPVNF  
101 LLSHCLLVTL AAHLPAEFTP AVHASLDKFL ASVSTVLTSK YR

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
18 - 32	1529.9228	1528.9155	1528.7270	123	0	<b>K.VGAHAGEYGAEALER.M</b> ( <a href="#">Ions score 149</a> )
33 - 41	1087.7585	1086.7512	1086.5420	193	0	<b>R.MFLSFPTTK.T</b> Oxidation (M) ( <a href="#">Ions score 33</a> )



β subunit (band II) :

*MATRIX*  
*SCIENCE* Mascot Search Results

Protein View

Match to: **HBB HUMAN** Score: 337  
Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2  
Found in search of DATA.TXT  
  
Nominal mass (M<sub>r</sub>): 15988; Calculated pI value: 6.75  
NCBI BLAST search of **HBB HUMAN** against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 39%

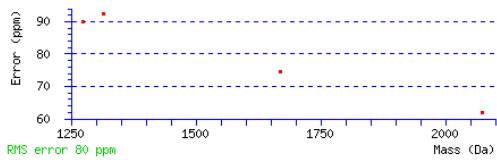
Matched peptides shown in **Bold Red**

1 MVHLIPEERS AVTALWGK**VN VDEVGGEALG RLIVVYPWTQ RPFESFGDLS**  
51 **TPDAVMGNPK** VKAHGKK**VLG AFS**DGLAHL**D NLK**GTIFATLS ELHCDKLHVD  
101 PENFRLLGNV LVCVLAHHFG KEFTPPVQAA YQKVVGAVAN ALAHKYH

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start	End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence
19	31	1314.7860	1313.7788	1313.6575	92	0	K.VNVDEVGGEALGR.L ( <a href="#">Ions score 51</a> )
32	41	1274.8401	1273.8328	1273.7183	90	0	R.LLVVYPWTQR.F ( <a href="#">Ions score 40</a> )
42	60	2075.0712	2074.0640	2073.9354	62	0	R.FFESFGDLSTPDAVMGNPK.V Oxidation (M) ( <a href="#">Ions score 132</a> )
68	83	1670.0154	1669.0082	1668.8835	75	0	K.VLGAFSDGLAHLDNLK.G ( <a href="#">Ions score 114</a> )



$\alpha$ - and  $\beta$ -subunit dimers (band III) :

**Mascot Search Results**

**Protein View**

Match to: **HBA\_HUMAN** Score: 100  
Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2  
Found in search of DATA.TXT

Nominal mass ( $M_r$ ): 15248; Calculated pI value: 8.72  
NCBI BLAST search of **HBA\_HUMAN** against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 10%

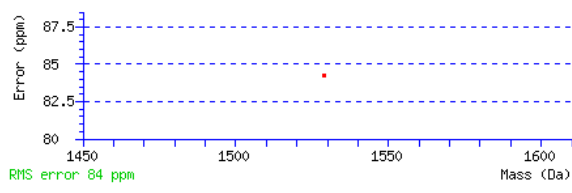
Matched peptides shown in **Bold Red**

1 MVLSPADKTN VKAANGK**VGA HAGEYGAEAL ERMFLSFPTT** KTYFPHFDLS  
51 HGSAQVKGHG KKVADALTNA VAHVDDMPNA LSALS~~DL~~HAH KLRVDPVNFK  
101 LLSHCLLVIL AAHLPAEFTP AVHASL~~DK~~FL ASVSTVLT~~SK~~ YR

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	
18 - 32	1529.8631	1528.8558	1528.7270	84	0	K.VGAHAGEYGAEALER.M	( <a href="#">Ions score 100</a> )



**Mascot Search Results**

**Protein View**

Match to: **HBB\_HUMAN** Score: 299  
Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2  
Found in search of DATA.TXT

Nominal mass ( $M_r$ ): 15988; Calculated pI value: 6.75  
NCBI BLAST search of **HBB\_HUMAN** against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Homo sapiens](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 34%

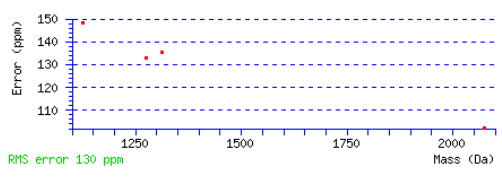
Matched peptides shown in **Bold Red**

1 MVHLIPEEKS AVTALWGK**VN VDEVGGEALG RLIVVYPTQ RPFESFGDLS**  
51 **TPDAVMGNPK** VKAHGKKVLG AFSDGLAHL~~D~~ NLKGTIFATLS ELHCDK~~L~~HVD  
101 **PENFRLLGNV** LVCVLAHHFG KEFTFPVQAA YQKVVGAVAN ALAHKYH

Show predicted peptides also

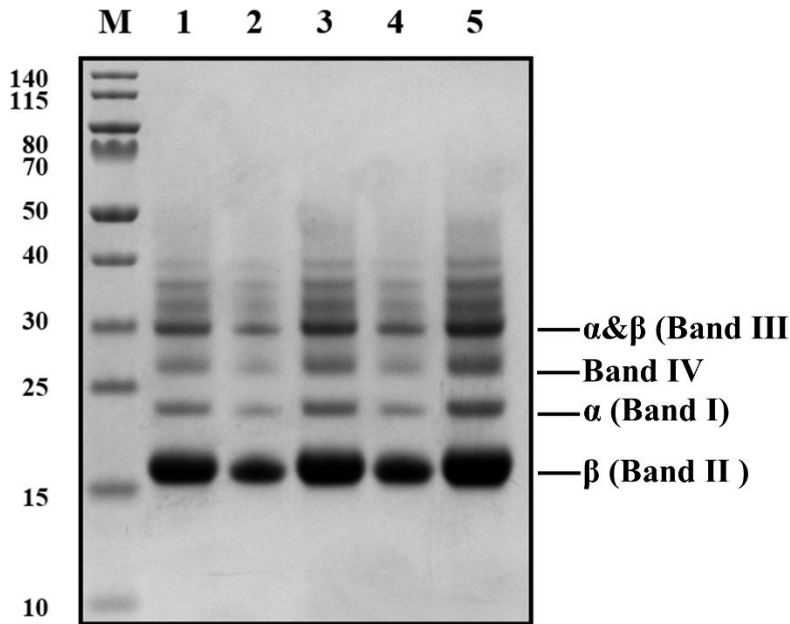
Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	
19 - 31	1314.8430	1313.8358	1313.6575	136	0	K.VNVDEVGGEALGR.L	( <a href="#">Ions score 107</a> )
32 - 41	1274.8947	1273.8874	1273.7183	133	0	R.LLIVVYPTQR.F	( <a href="#">Ions score 27</a> )
42 - 60	2075.1549	2074.1476	2073.9354	102	0	R.PFESFGDLSTPDAVMGNPK.V	Oxidation (M) ( <a href="#">Ions score 144</a> )
97 - 105	1126.7309	1125.7237	1125.5567	148	0	K.LHVDPENFR.L	( <a href="#">Ions score 22</a> )





Supplementary Note S3 (Porcine hemoglobin) :



$\alpha$  subunit (band I) :

**Mascot Search Results**

Protein View

Match to: HBA\_PIG Score: 117  
Hemoglobin subunit alpha OS=Sus scrofa GN=HBA PE=1 SV=1  
Found in search of DATA.TXT  
  
Nominal mass ( $M_r$ ): 15030; Calculated pI value: 8.76  
NCBI BLAST search of HBA\_PIG against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Sus scrofa](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 21%

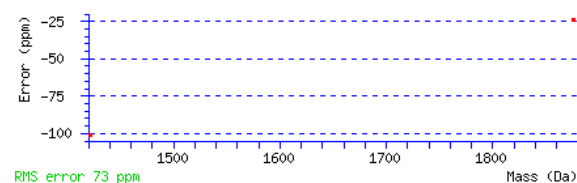
Matched peptides shown in **Bold Red**

1 VLSAADKANV KAANGK**VGGQ** AGAHGAEALE RMFLGFPTIK **TYFPHFNL**SH  
51 **GSDQVK**AHGQ KVADALTKAV GHLDLPGAL SALSDLHAHK LRVDPVNFKL  
101 LSHCLLVTLA AHPDDFNPS VHASLDKFLA NVSTVLTSKY R

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start	End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence	
17	31	1422.5649	1421.5576	1421.7011	-101	0	K.VGGQAGAHGAEALER.M	( <a href="#">Ions score 6</a> )
41	56	1876.8530	1875.8457	1875.8904	-24	0	K.TYFPHFNLHSGSDQVK.A	( <a href="#">Ions score 111</a> )



β subunit (band II) :

*MATRIX* Mascot Search Results

Protein View

Match to: HBB\_PIG Score: 519  
Hemoglobin subunit beta OS=Sus scrofa GN=HBB PE=1 SV=3  
Found in search of DATA.TXT

Nominal mass (M<sub>0</sub>): 16155; Calculated pI value: 7.10  
NCBI BLAST search of HBB\_PIG against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Sus scrofa](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 53%

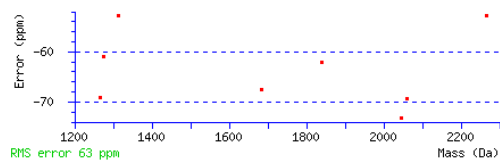
Matched peptides shown in **Bold Red**

1 MVHLSAEKE **AVLGLWGRVN VDEVGGEALG RLLVVYPWTQ RFFESPGDLS**  
51 **NADAVMGMPK** VKAHGKKVLQ SFSDGLKHLN NLKGTFAKLS ELHCDQLHVD  
101 PENFR**LLGNV IVVVLARRLG HDFNPNVQAA FQKVVAGVAN** ALAHKYH

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start	End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
10	31	2268.0784	2267.0711	2267.1910	-53	1	K.EAVLGLWGRKVVNDEVGGEALGR.L ( <a href="#">Ions score 187</a> )
19	31	1314.5954	1313.5881	1313.6575	-53	0	K.VNVDEVGGEALGR.L ( <a href="#">Ions score 26</a> )
32	41	1274.6478	1273.6405	1273.7183	-61	0	R.LLVVYPWTQR.F ( <a href="#">Ions score 44</a> )
42	60	2045.7775	2044.7702	2044.9200	-73	0	R.RFFESPGDLSNADAVMGMPK.V ( <a href="#">Ions score 77</a> )
42	60	2061.7795	2060.7722	2060.9150	-69	0	R.RFFESPGDLSNADAVMGMPK.V Oxidation (M) ( <a href="#">Ions score 109</a> )
106	117	1265.7430	1264.7358	1264.8231	-69	0	R.LLGNVIVVVLAR.R ( <a href="#">Ions score 14</a> )
118	133	1841.8260	1840.8188	1840.9333	-62	1	R.RLGHDFNPNVQAAFQK.V ( <a href="#">Ions score 52</a> )
119	133	1685.7258	1684.7186	1684.8322	-67	0	R.RLGHDFNPNVQAAFQK.V ( <a href="#">Ions score 117</a> )



# $\alpha$ - and $\beta$ -subunit dimers (band III) :

## *{MATRIX}* *{SCIENCE}* Mascot Search Results

### Protein View

Match to: HBA\_PIG Score: 179  
Hemoglobin subunit alpha OS=Sus scrofa GN=HBA PE=1 SV=1  
Found in search of DATA.TXT

Nominal mass ( $M_r$ ): 15030; Calculated pI value: 8.76  
NCBI BLAST search of [HBA\\_PIG](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Sus scrofa](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 26%

Matched peptides shown in **Bold Red**

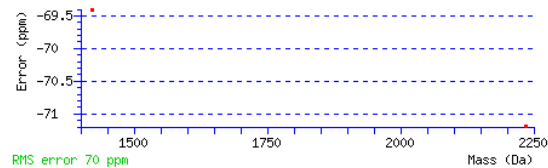
1 VLSAADKANV KAANGK**VGGQ** AGAHGAEALE RMFLGFPTIK TYFPFNLISH  
51 GSDQVKAHGQ KVADALTK**AV** GHLLDLPAL SALSDLHAHK LRVDPVNFKL  
101 LSHCLLVTLA AHHPDDFNPS VHASLDKFLA NVSTVLISKY R

Show predicted peptides also

Sort Peptides By

☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
17 - 31	1422.6097	1421.6025	1421.7011	-69	0	K.VGGQAGAHGAEALER.M ( <a href="#">Ions score 55</a> )
69 - 90	2237.0081	2236.0008	2236.1600	-71	0	K.AVGHLLDLPALGALSALSDLHAHK.L ( <a href="#">Ions score 125</a> )



## *{MATRIX}* *{SCIENCE}* Mascot Search Results

### Protein View

Match to: HBB\_PIG Score: 444  
Hemoglobin subunit beta OS=Sus scrofa GN=HBB PE=1 SV=3  
Found in search of DATA.TXT

Nominal mass ( $M_r$ ): 16155; Calculated pI value: 7.10  
NCBI BLAST search of [HBB\\_PIG](#) against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Sus scrofa](#)

Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 45%

Matched peptides shown in **Bold Red**

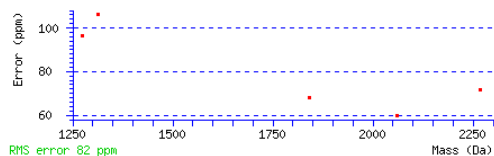
1 MVHLSAEKE AVLGWLGRVN VDEVGGEALG RLLVVYPWTQ RFFESFGDLS  
51 **NADAVMGNPK** VKAHGKKVLQ SFSDGLKHLN NLKGIFAKLS ELHCDQLHVD  
101 PENFRLLGNV IVVVLAR**RLG** **HDFNPNVQAA** **FQK**VVAGVAN ALAHKYH

Show predicted peptides also

Sort Peptides By

☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr (expt)	Mr (calc)	ppm	Miss	Sequence
10 - 31	2268.3602	2267.3529	2267.1910	71	1	K.EAVLGLWGRVNVDEVGGEALGR.L ( <a href="#">Ions score 141</a> )
19 - 31	1314.8043	1313.7970	1313.6575	106	0	K.VNVDEVGGEALGR.L ( <a href="#">Ions score 40</a> )
32 - 41	1274.8482	1273.8410	1273.7183	96	0	R.LLVVYPWTQR.F ( <a href="#">Ions score 75</a> )
42 - 60	2062.0455	2061.0382	2060.9150	60	0	R.FFESFGDLSNADAVMGNPK.V Oxidation (M) ( <a href="#">Ions score 94</a> )
118 - 133	1842.0662	1841.0589	1840.9333	68	1	R.RLGHDFFNPNVQAAPQK.V ( <a href="#">Ions score 94</a> )



band IV:

**Mascot Search Results**

Protein View

Match to: **HBA\_PIG** Score: 45  
Hemoglobin subunit alpha OS=Sus scrofa GN=HBA PE=1 SV=1  
Found in search of DATA.TXT  
  
Nominal mass (M<sub>r</sub>): 15030; Calculated pI value: 8.76  
NCBI BLAST search of **HBA\_PIG** against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Sus scrofa](#)  
  
Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 10%

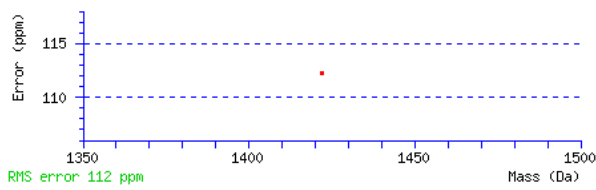
Matched peptides shown in **Bold Red**

1 VLSAADKANV KAAWG**KVGGQ AGAHGAEALE** RMFLGFPTTK TYFPHFNLSH  
51 GSDQVKAHGQ KVADALTKAV GHLDLPGAL SALSDLHAHK LRVDPVNFKL  
101 LSHCLLVTLA AHHPDDFNPS VHASLDKFLA NVSTVLTSKY R

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	
17 - 31	1422.8680	1421.8607	1421.7011	112	0	K.VGGQAGAHGAEALER.M	( <a href="#">Ions score 45</a> )



**Mascot Search Results**

Protein View

Match to: **HBB\_PIG** Score: 160  
Hemoglobin subunit beta OS=Sus scrofa GN=HBB PE=1 SV=3  
Found in search of DATA.TXT  
  
Nominal mass (M<sub>r</sub>): 16155; Calculated pI value: 7.10  
NCBI BLAST search of **HBB\_PIG** against nr  
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Sus scrofa](#)  
  
Variable modifications: Oxidation (M)  
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P  
Sequence Coverage: 23%

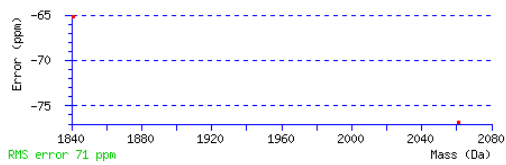
Matched peptides shown in **Bold Red**

1 MVHLSAEEKE AVLGWLWGKVN VDEVGGEALG RLLVVPWTQ **RFFESFGDLS**  
51 **NADAVMGNPK** VKAHGKKVLQ SFSDGLKHLD NLKGTFAKLS ELHCDQLHVD  
101 PENFRLLGNV IVVVLAR**RLG HDPNPNVQAA FQK**VVAGVAN ALAHKYH

Show predicted peptides also

Sort Peptides By ☒ Residue Number ☐ Increasing Mass ☐ Decreasing Mass

Start - End	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Sequence	
42 - 60	2061.7639	2060.7567	2060.9150	-77	0	R.FFESFGDLSNADAVMGNPK.V	Oxidation (M) ( <a href="#">Ions score 123</a> )
118 - 133	1841.8206	1840.8134	1840.9333	-65	1	R.RLGHDNPNVQAAFQK.V	( <a href="#">Ions score 37</a> )



## Supplementary References

1. Liu, Q.; Shi, X.; Song, L.; Liu, H.; Zhou, X.; Wang, Q.; Zhang, Y.; Cai, M. CRISPR–Cas9-mediated genomic multiloci integration in *Pichia pastoris*. *Microb. Cell. Fact.* **2019**, *18*, 144.
2. Yu, F.; Zhao, X.; Zhou, J.; Lu, W.; Li, J.; Chen, J.; Du, G. Biosynthesis of high-active hemoproteins by the efficient heme-supply *Pichia pastoris* chassis. *Adv. Sci.* **2023**, *10*, 2302826.
3. Burgard, J.; Grünwald-Gruber, C.; Altmann, F.; Zanghellini, J.; Valli, M.; Mattanovich, D.; Gasser, B. The secretome of *Pichia pastoris* in fed-batch cultivations is largely independent of the carbon source but changes quantitatively over cultivation time. *Microb. Biotechnol.* **2020**, *13*, 479-494.