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Supplemental information

GwAAP: A genome-wide amino acid coding-decoding quantitative proteomics system

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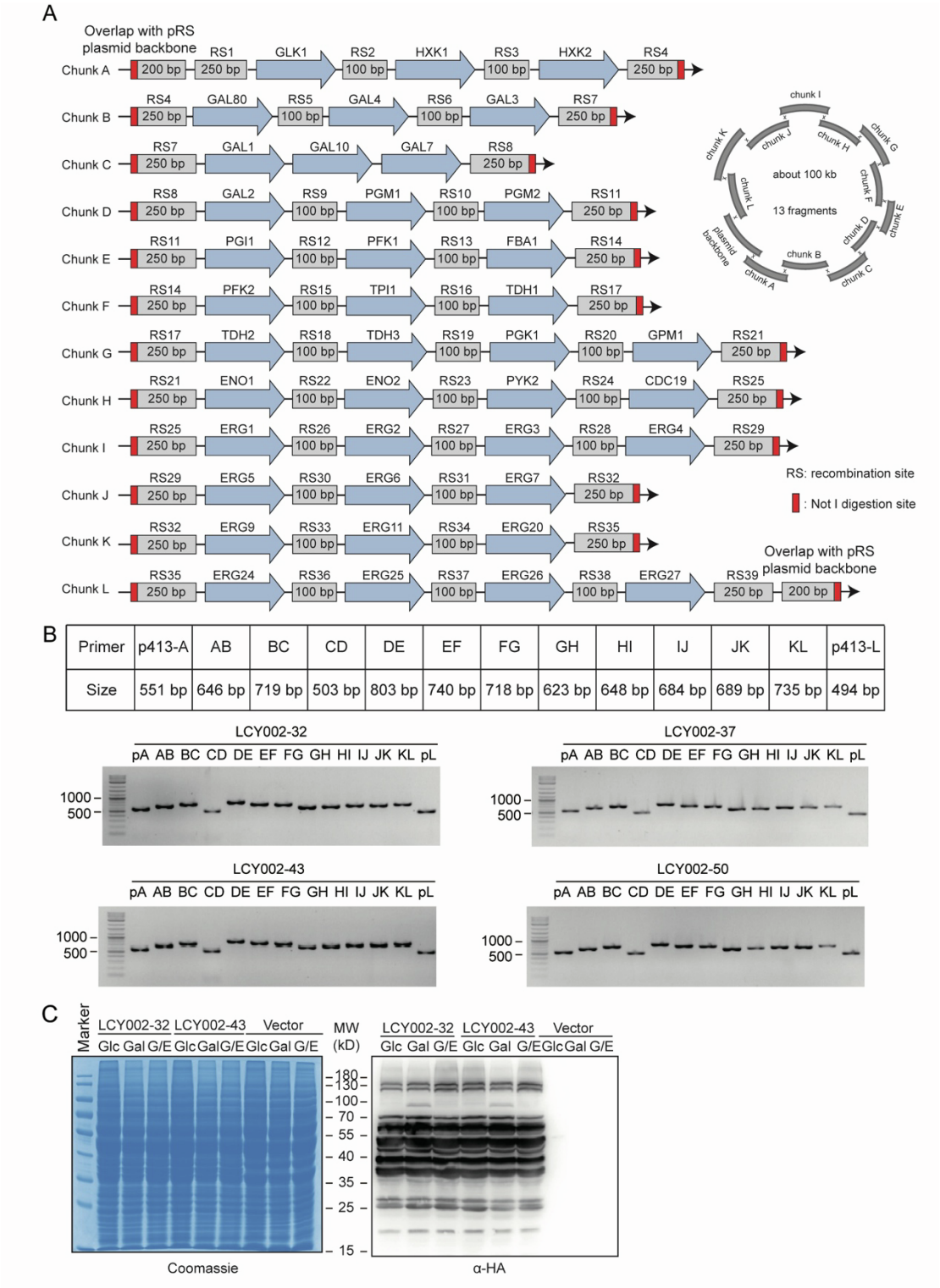


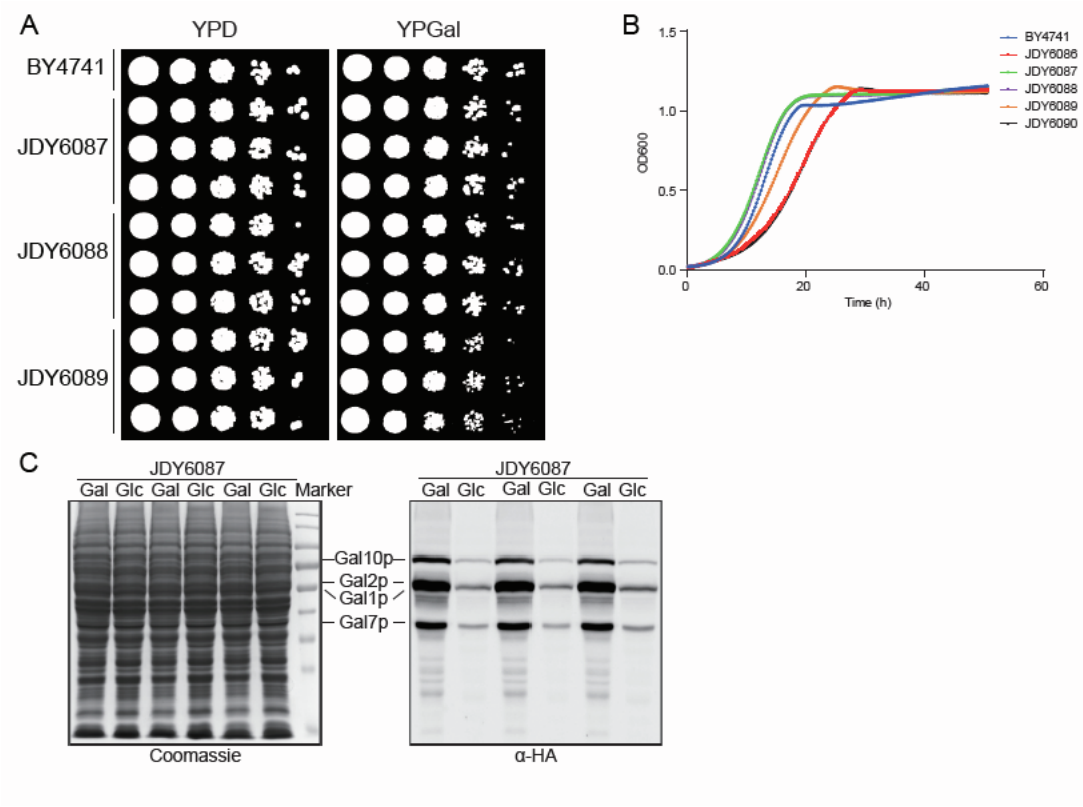
Supplementary Figure 1. Construction of the plasmid system in yeast, related to Figure 2.

A. Recoded genes were obtained by in vitro synthesis. Twelve chunks (A-L) were assembled into one vector.

B. PCR analysis of the four monoclonal strains. The strain plasmid DNA could be

amplified with synthetic primers to assemble chunk A-L and pRS413 plasmid backbone (13 fragments).

C. SDS-PAGE (left panel) and western blotting (right panel) indicated the differential expression of 40 proteins under glucose, galactose and glycerol/ethanol conditions.

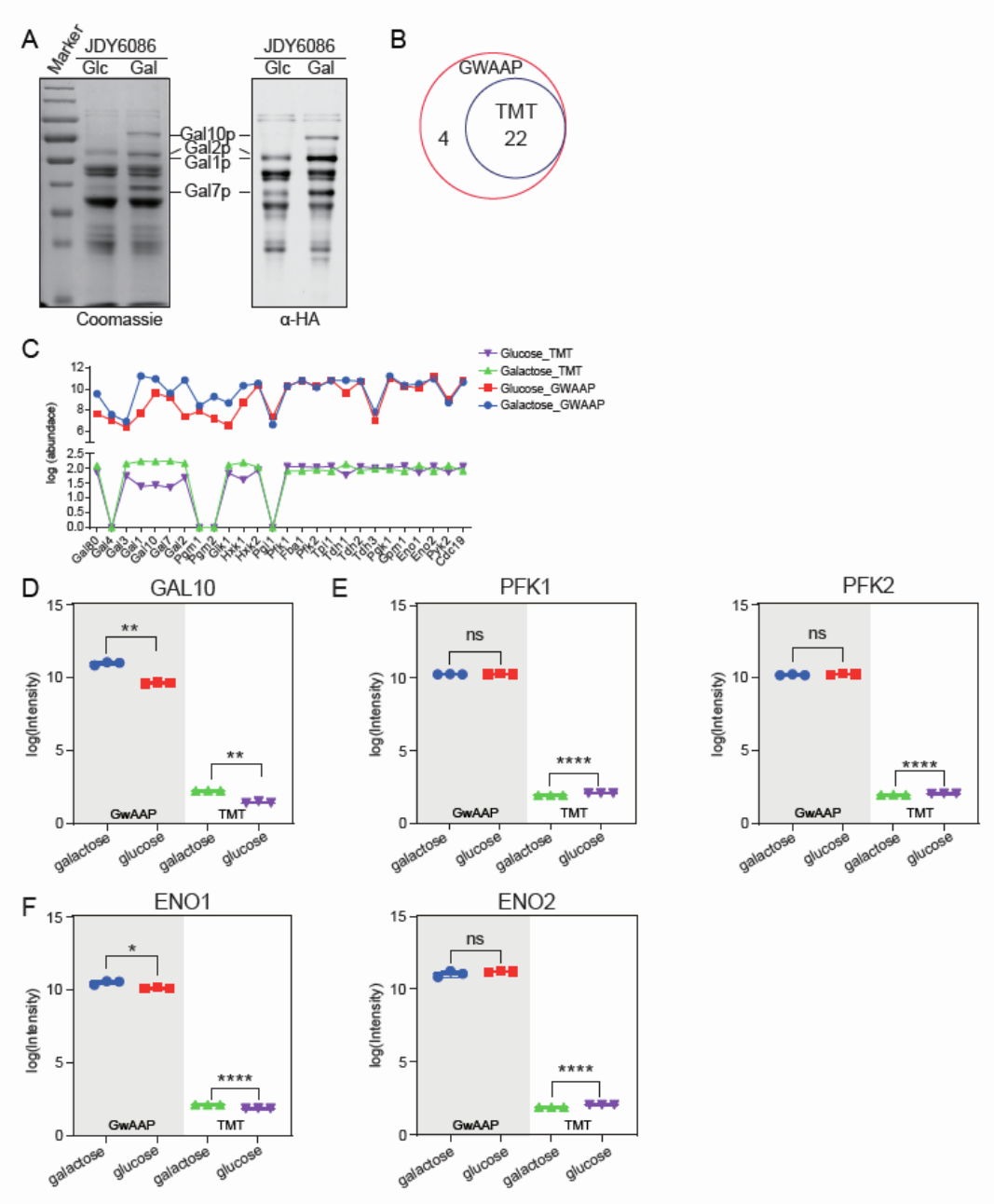


Supplementary Figure 2. Construction of yeast strains with HA codes knock-in *in vivo*, related to Figure 4.

A. Fitness analysis of BY4741 with or without HA code knock-in on glucose (YPD) and galactose (YPGal) media. All the photos were taken at 48 h. JDY6087, strains with 7 HA codes knock-in, 9 galactose metabolic genes except *PGM1* and *PGM2*; JDY6088, strains with 8 HA codes knock-in, 9 galactose metabolic genes except *PGM2*; JDY6089, strains with 8 HA codes knock-in, 9 galactose metabolic genes except *PGM1*.

B. Growth curves of BY4741 and strains with different HA codes knock-in when they were cultured in YPGal media.

C. SDS-PAGE (left panel) and western blotting (right panel) indicated the differential expression of 7 proteins under glucose and galactose conditions.



Supplementary Figure 3. Quantification of 26 proteins from glycolysis and galactose metabolic pathway using TMT and GwAAP methods, related to Figure 5.

A. SDS-PAGE (left panel) and western blotting (right panel) analysis of the GwAAP output using anti-HA antibodies as indicated. Gal10p, Gal2p, Gal1p, and Gal7p were overexpressed under galactose condition.

B. The venn diagram showed the proteins detected by GWAAP (left) and TMT (right), respectively.

C. The intensities of 26 proteins cultured in galactose and glucose media detected by GWAAP and TMT were represented by line charts.

D. The plot of GAL10 in galactose metabolic pathway detected by GWAAP and TMT. The student's test was used, and the p values less than 0.05, 0.01 and 0.001 were marked with *, ** and ***, respectively.

E. The plot of PFK1 and PFK2 in galactose metabolic pathway detected by GWAAP and TMT.

F. The plot of ENO1 and ENO2 in galactose metabolic pathway detected by GWAAP and TMT.

Supplementary Table 1. The plasmid information, related to Figure 2 to Figure 5.

Supplementary Table 2. The transition list of the 40 code sequences, related to Figure 2 and Table 1.

Supplementary Table 3. Method repeatability and the intensities cultured in glucose and glucose media quantified by GwAAP and TMT methods, related to Figure 3 to Figure 5.

Table S3A. The forty tagged protein expression in three biological repeats of trypsin digestion experiments, related to Figure 3. The samples were digested by trypsin and detected directly by PRM to assess the enzymatic efficiency. The abundance of code peptide represented corresponding protein abundances.

Table S3B. The forty tagged protein expression in three independent experiments of peptide enrichment, related to Figure 3. The samples were digested by trypsin, enriched by HA antibody and detected by PRM to assess the enrichment efficiency. The abundance of code peptide represented corresponding protein abundances.

Table S3C. The protein expression levels by profiling between BY4741 and JDY6086, related to Figure 4.

Table S3D. Twenty-six proteins of galactose and glycolysis pathway in JPY6086 yeast cultured in glucose and glucose medium using TMT methods, related to Figure 5. The data was processed in normalization and scaling mode on PD 2.3 software.

Table S3E. The intensities of twenty-six proteins in JPY6086 yeast cultured in glucose and glucose media quantified by GWAAP methods, related to Figure 5. The abundance of code peptide represented corresponding protein abundances.

Supplementary Table 4. AUC of 40 code sequences and the comparison of the linearity range of dilution curves with the GwAAP and Script-Map approaches, related to Figure 3.

Table S4A. The total area fragments of forty tagged protein in the serially diluted yeast lysates.

Table S4B. The curves of the best response peptides for 12 proteins detected by SCRIPT-MAP.

Supplementary Table 5. The primers used in this study, related to Figure 4.

Supplementary Table 6. The strain information, relate to Figure 2 to Figure 5.