

# Supplemental Materials

*Molecular Biology of the Cell*

Lahtvee et al.

## Supplementary Information

### Table legends

Supplementary Information Table 1. Measured consumption patterns together with carbon and reduction balance calculations for the reference and 9 different stress conditions from chemostats at constant specific growth rate of  $0.1 \text{ h}^{-1}$ . Values are given as an average of at least three biological replicates together with the standard deviation (STDEV).

Supplementary Information Table 2. Normalized mRNA intensities from the RNAseq for each biological replicate. Additionally, calculated covariance and conservation scores are given. Conservation scores are calculated based on data provided by Siepel et al., 2005.

Supplementary Information Table 3. Genes showing significant differential expression ( $p < 0.001$ ) compared to the reference condition under high ethanol, high osmotic stress and high temperature conditions.

Supplementary Information Table 4. Fatty acid composition in the cells under studied environmental conditions in chemostats at specific growth rate  $0.1 \text{ h}^{-1}$ .

Supplementary Information Table 5. Hierarchical clustering (ward) of oxidoreductase activity genes (GO:0016491) for studied osmotic stress conditions (0.2, 0.4 and 0.6 M NaCl) in chemostats at specific growth rate  $0.1 \text{ h}^{-1}$ .

Supplementary Information Table 6. List of genes showing potential transcriptional regulation based on comparison of transcriptional and flux changes between 9 different stress conditions.

### Figure legends

**Supplementary Information Figure 1.** Condition-dependent genome scale metabolic model reduction summary based on transcriptome data. Genome scale metabolic model iTO977 was reduced separately for each condition and biological replicate based on assumption that transcripts with a low expression levels at studied condition will not be translated. Black dots represent genes which produce enzymes for reactions that are taken into account in each model.

**Supplementary Information Figure 2.** Hierarchical clustering of transcriptome results for studied chemostat conditions at specific growth rate  $0.1 \text{ h}^{-1}$ .

**Supplementary Information Figure 3.** Overview of significantly ( $p < 0.001$ ) differentially expressed genes at studied stress conditions. Bar chart indicates the number of differentially expressed genes compared to reference conditions at all studied stress conditions in chemostats at specific growth rate  $0.1 \text{ h}^{-1}$ .

**Supplementary Information Figure 4.** Intracellular ergosterol levels for studied stress conditions in chemostats at specific growth rate  $0.1 \text{ h}^{-1}$ .

**Supplementary Information Figure 5.** Schematic representation of regulation in ergosterol biosynthetic pathway.

**Supplementary Information Figure 6.** Clustering of 284 genes which were significantly differentially expressed ( $p < 0.001$ ) under high ethanol, high osmotic and high temperature stress conditions.

**Supplementary Information Figure 7.** Clustering of environmental stress response genes defined by Gasch et al 2000 for 94 stress conditions reported by Gasch et al and 9 stress conditions from the current chemostat study.

**Supplementary Information Figure 8.** Clustering of oxidoreductase activity genes (according to GO: 0016491) for currently studied 10 environmental conditions in chemostats at specific growth rate  $0.1 \text{ h}^{-1}$ .

**Supplementary Information Figure 9.** Clustering of oxidoreductase activity genes (according to GO: 0016491) for currently reported osmotic conditions under chemostats at specific growth rate  $0.1 \text{ h}^{-1}$  and previously published dynamic osmoregulation data by (Gasch *et al.*, 2000; Lee *et al.*, 2011).

**Supplementary Information Figure 10.** Clustering of oxidoreductase activity genes (according to GO: 0016491) for currently reported osmotic conditions under chemostats at specific growth rate  $0.1 \text{ h}^{-1}$ .

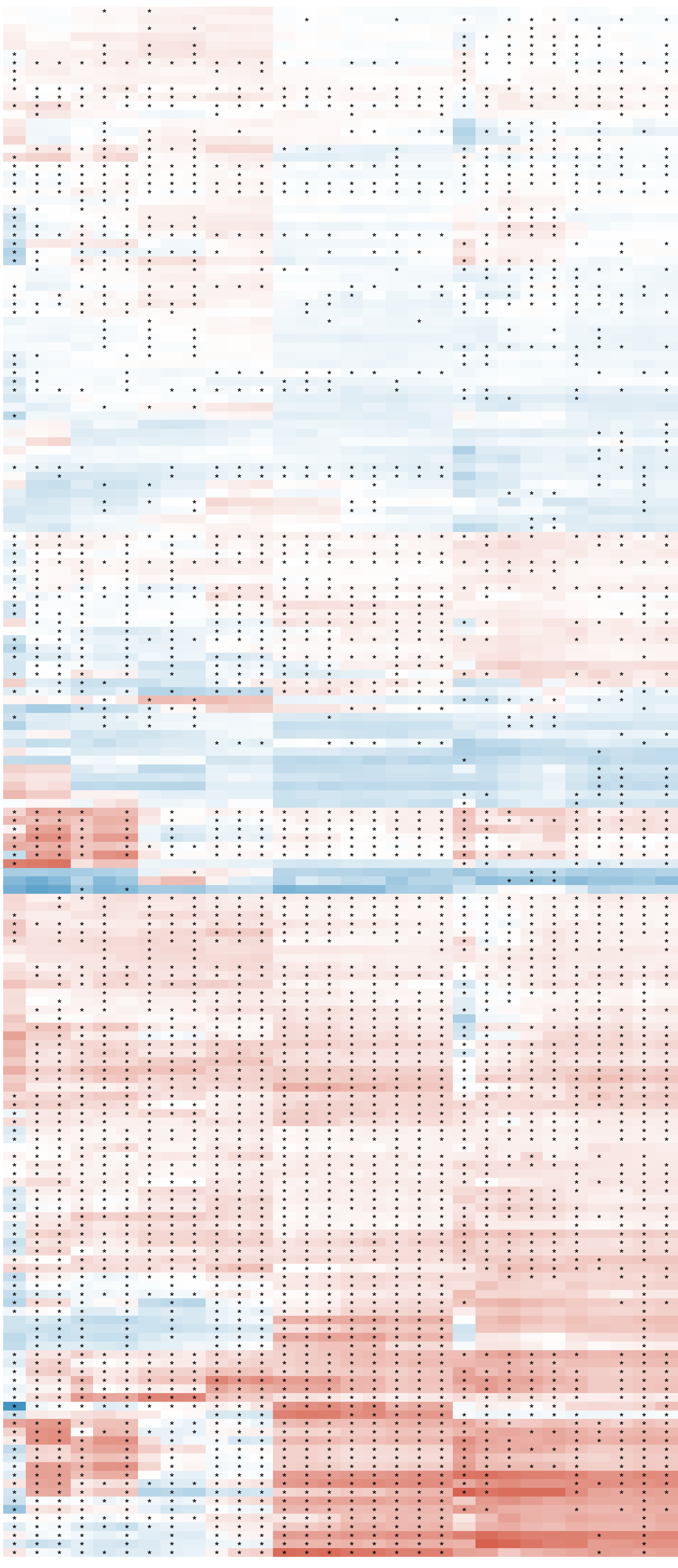
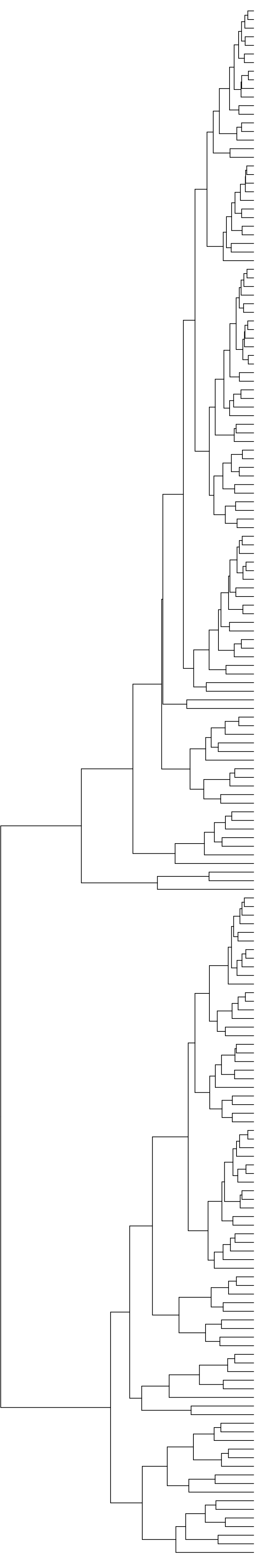
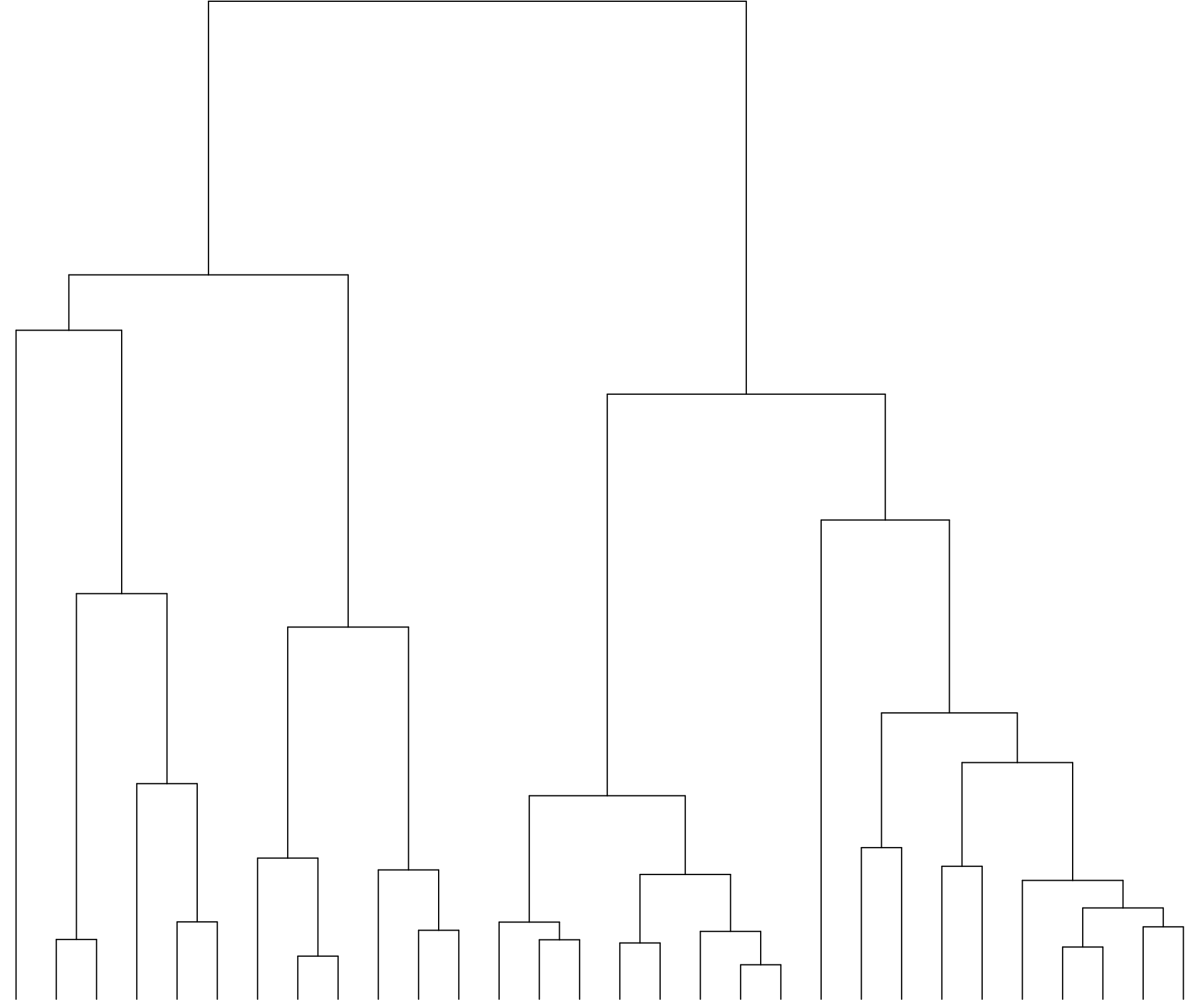
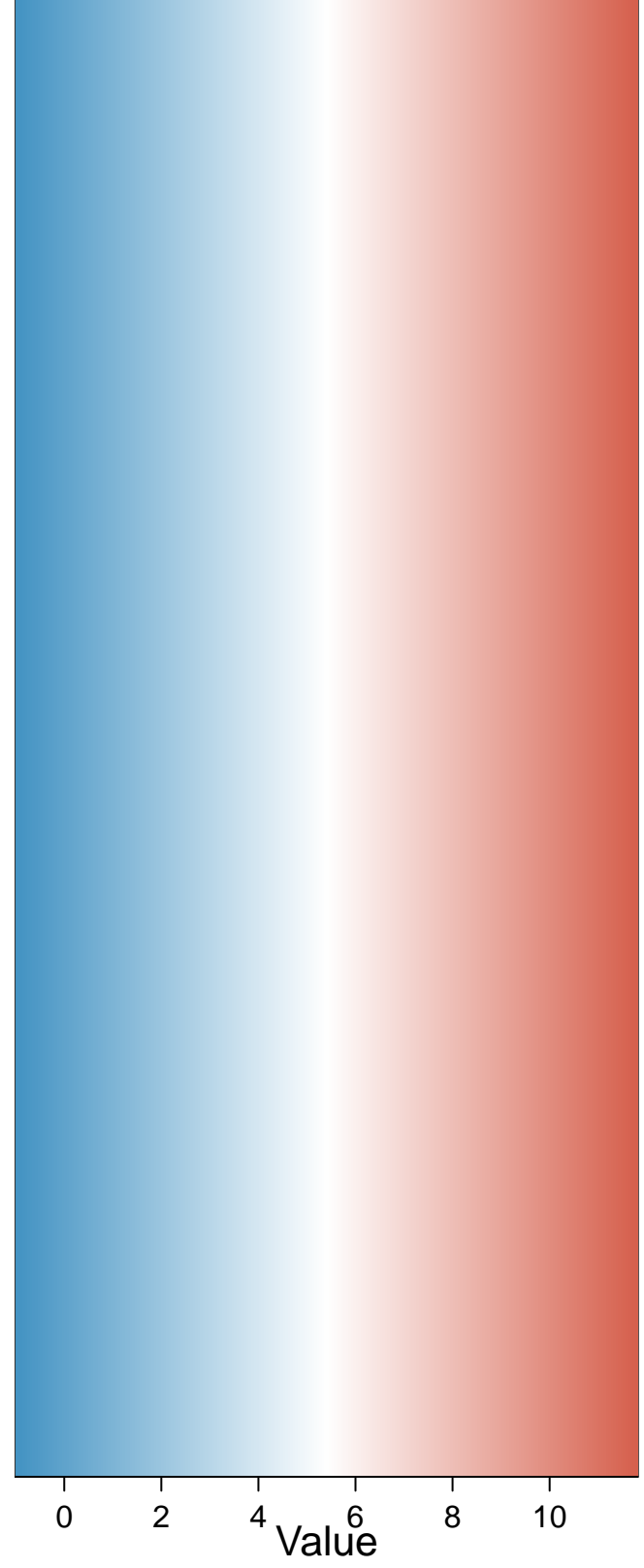
**Supplementary Information Figure 11.** Clustering of significantly different intracellular fluxes ( $p < 0.01$ ) calculated using condition-dependent metabolic models for 10 studied environmental conditions in triplicates and flux balance analysis, where ATP drain function was optimized.

**Supplementary Information Figure 12.** Overview of transcription factor analysis under studied stress conditions. (A) Overlap of TFs significantly regulated at studied stress conditions ( $p < 0.001$ ). Circos plots illustrate TFs and main gene ontology groups they are regulating for high ethanol (B), salt (C) and temperature (D) conditions.

**Supplementary Information Figure 13.** Summary of significantly regulated gene sets at studied stress conditions ( $p < 0.001$ ). Red colour represents up, blue down, white both directional regulation and grey represents no significant change.

**Supplementary Information Figure 14.** Overview of preliminary batch (for ethanol and salt stress) and turbidostat (for temperature stress) results which were used to determine the highest stress conditions to be used in chemostat experiments at chosen specific growth rate of  $0.1 \text{ h}^{-1}$ .

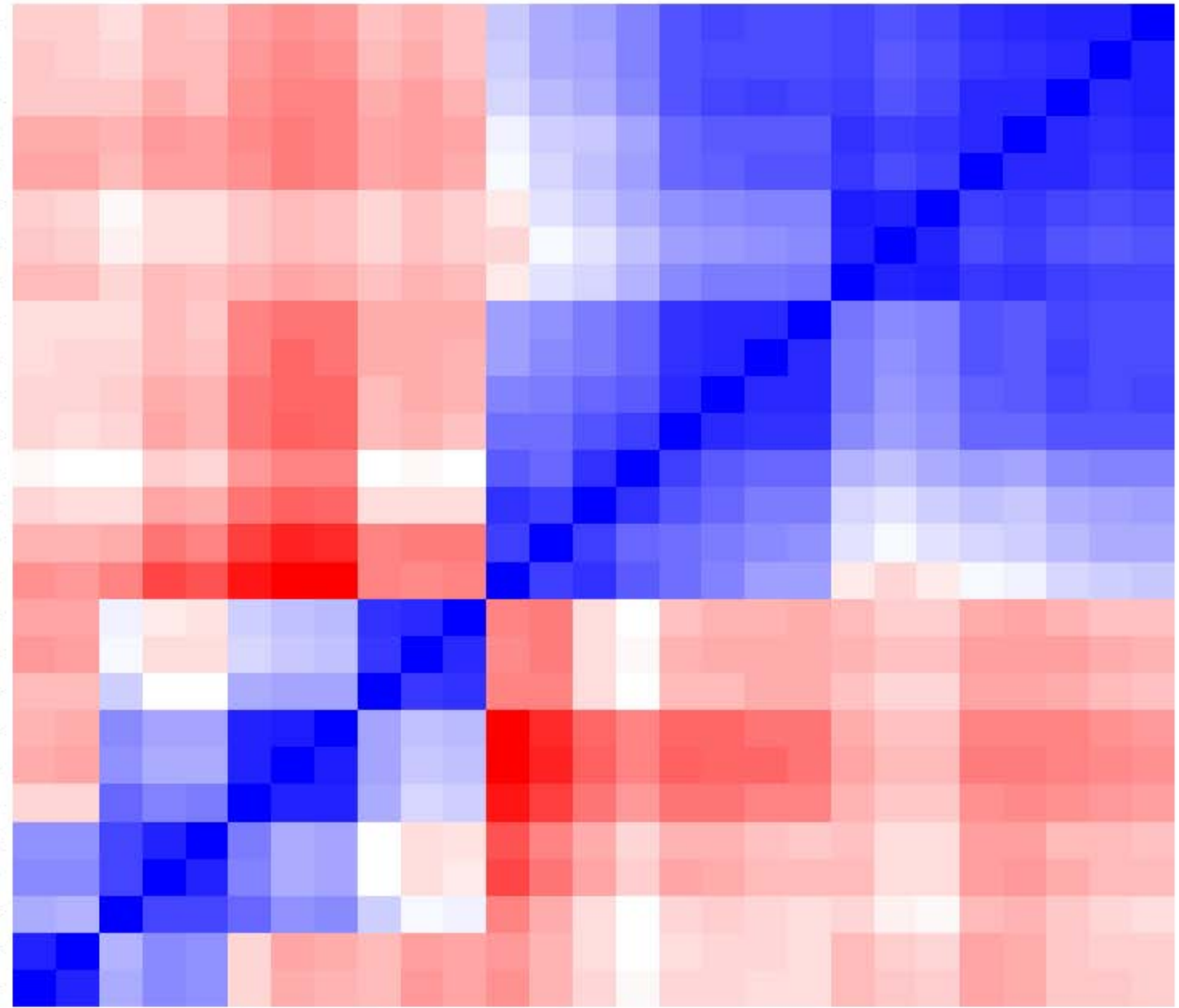
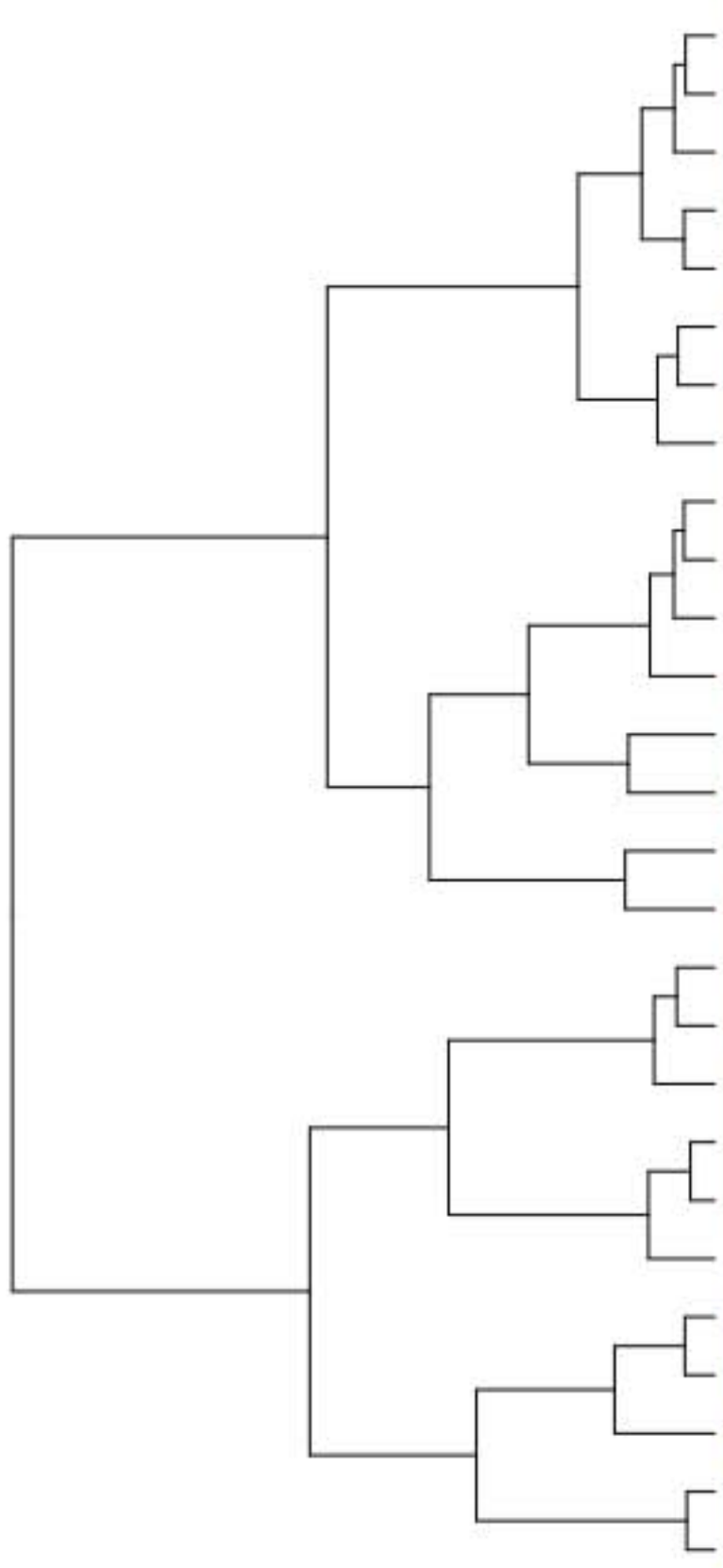
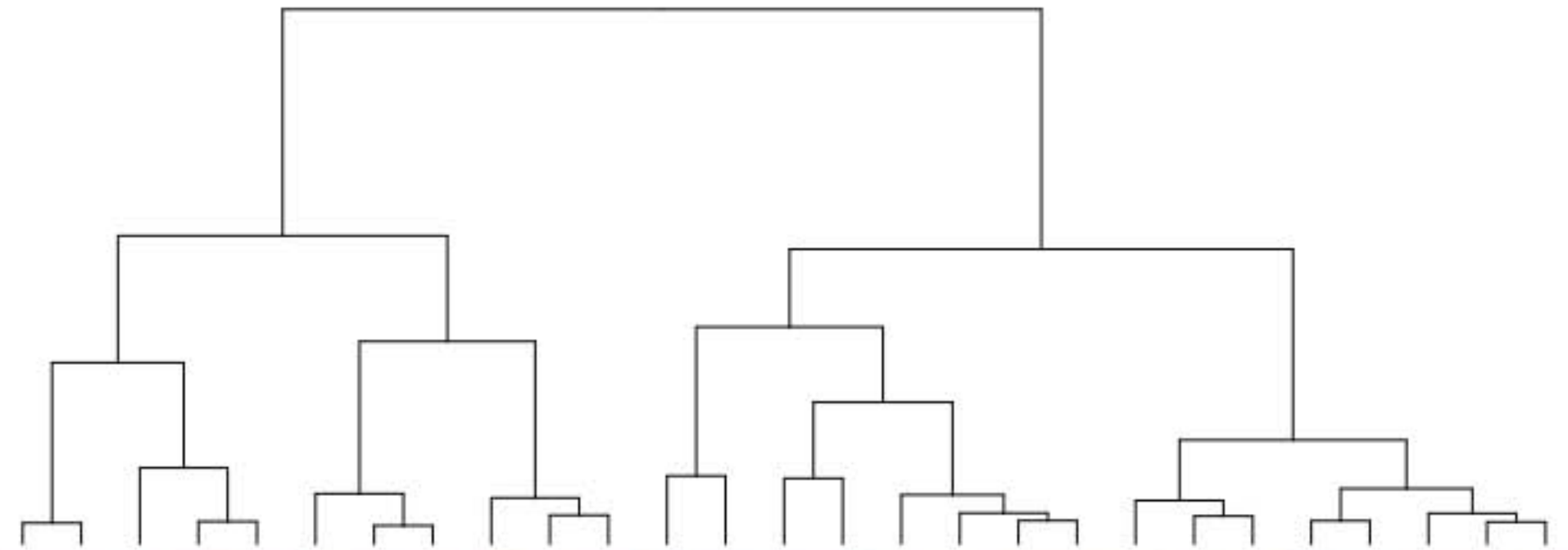
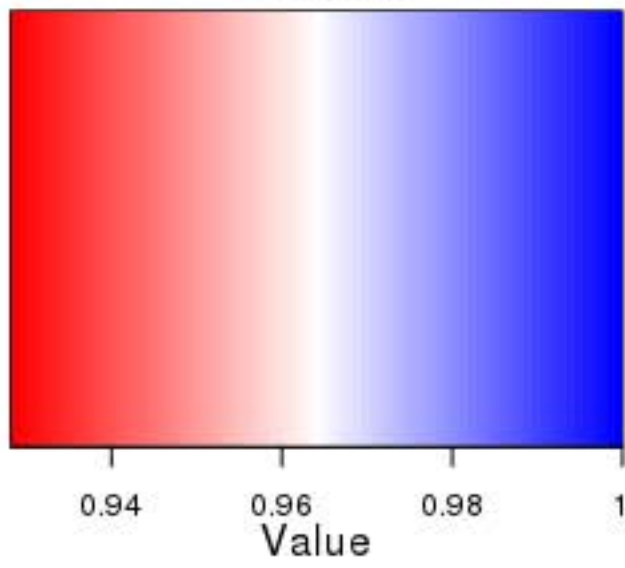
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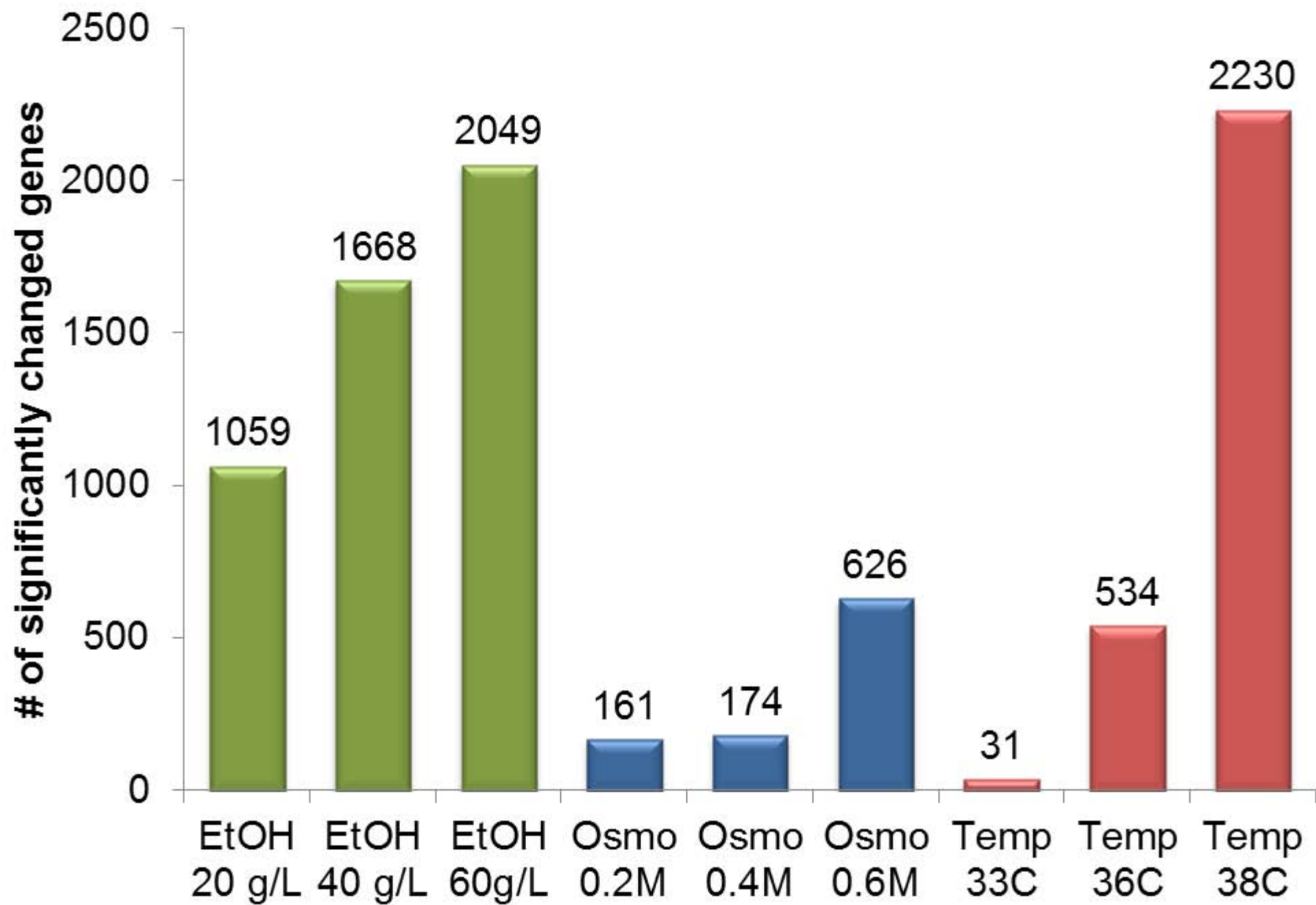
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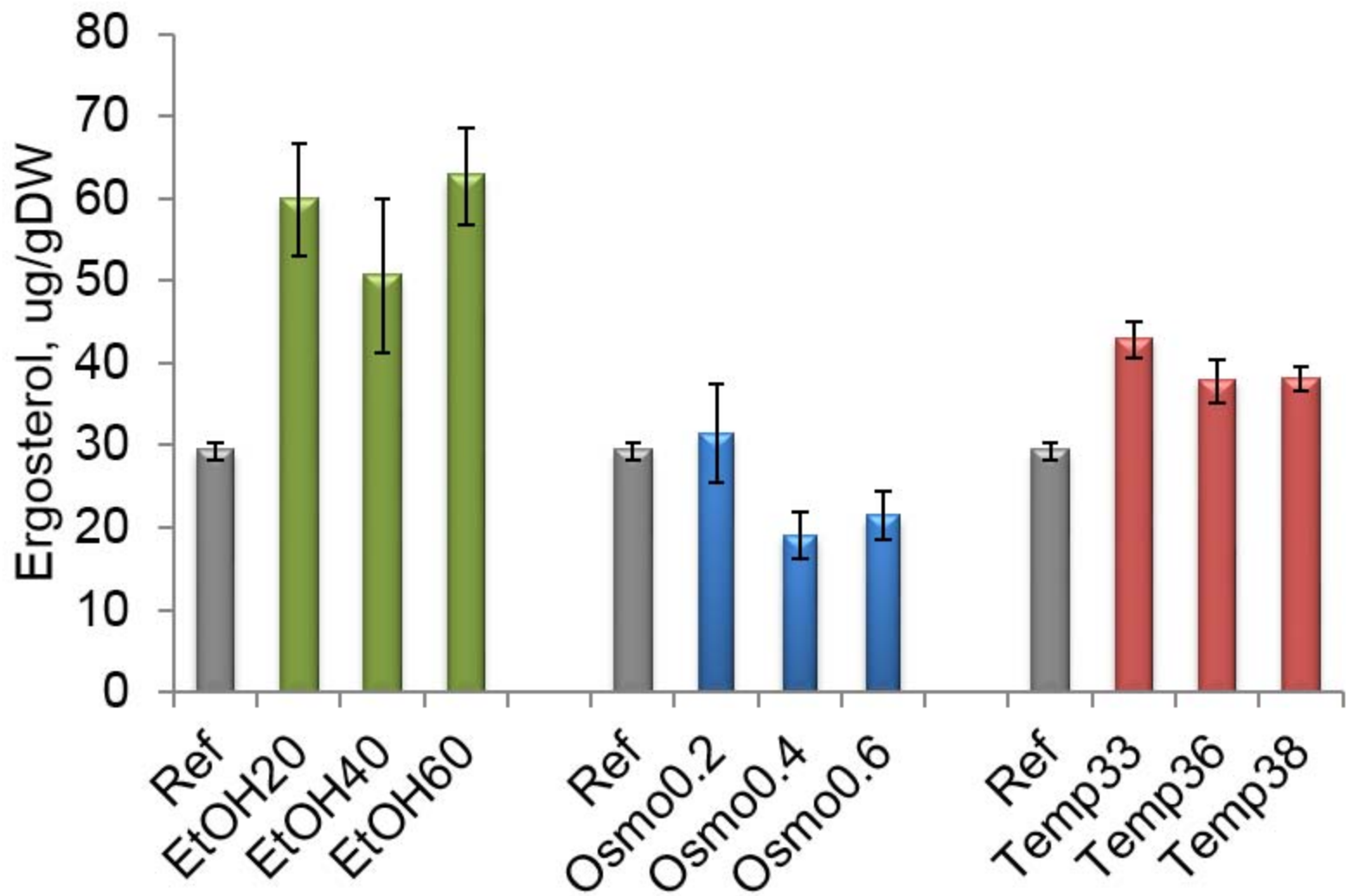
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Color Key

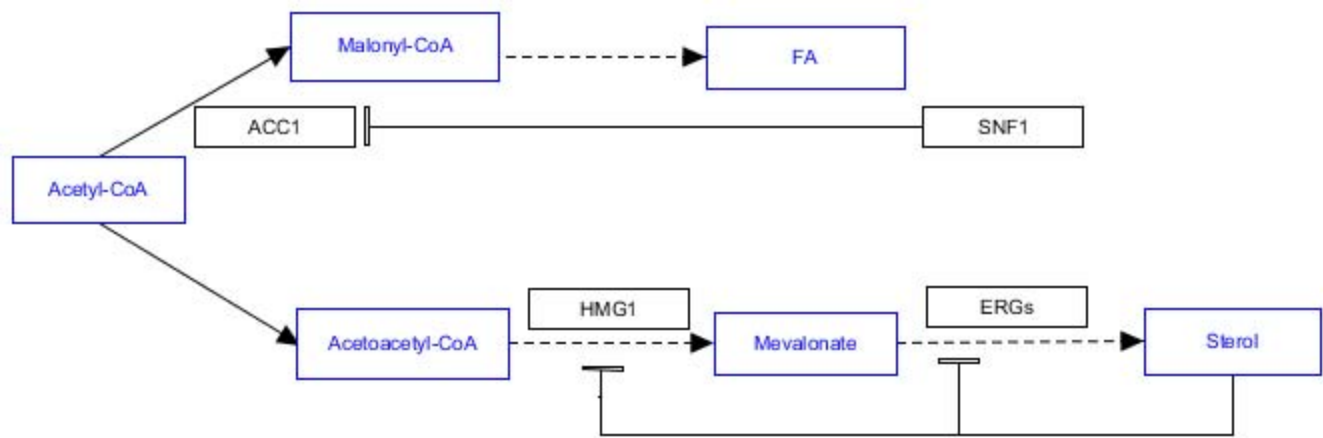


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ref\_2  
ref\_1  
ref\_3  
temp33\_3  
temp36\_1  
temp36\_3  
temp33\_2  
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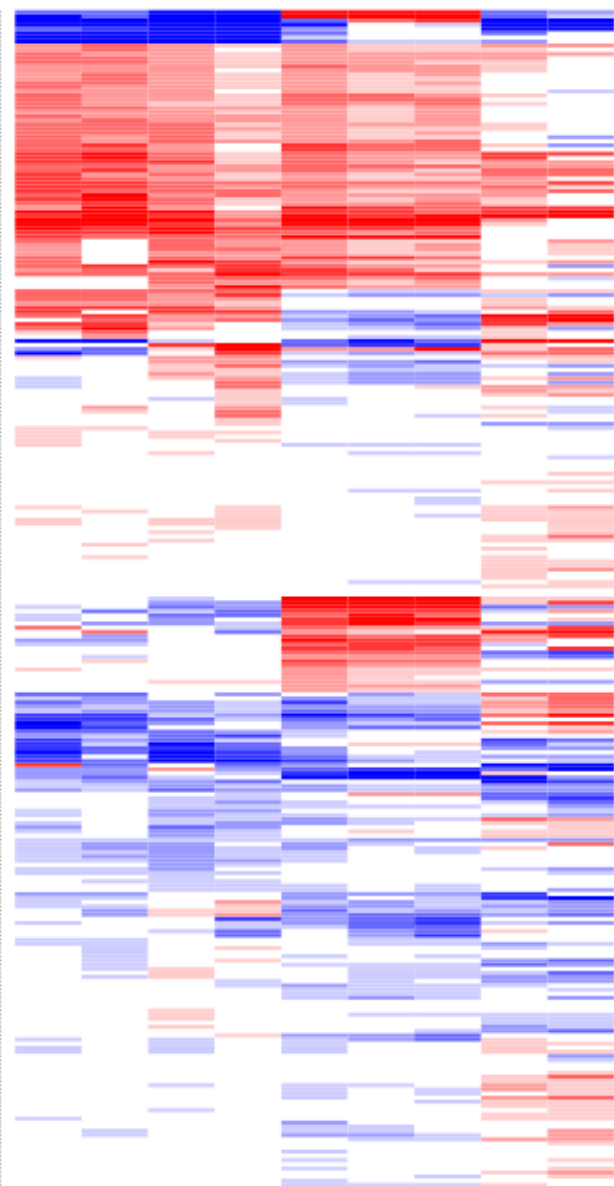
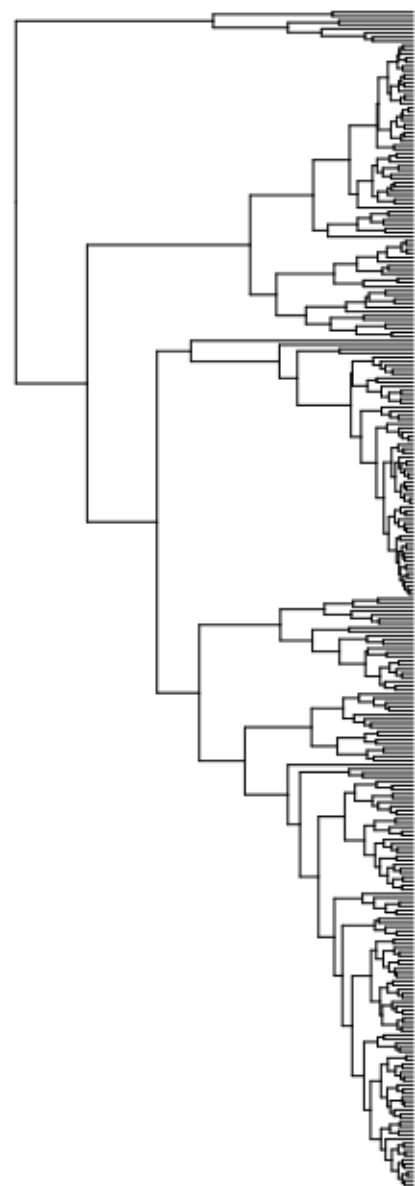
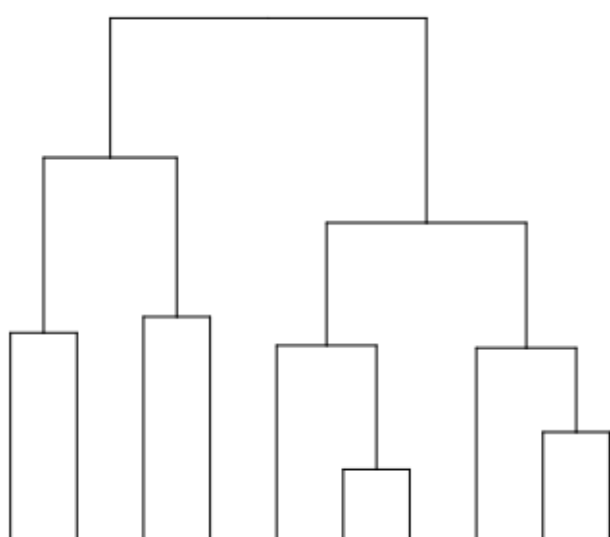
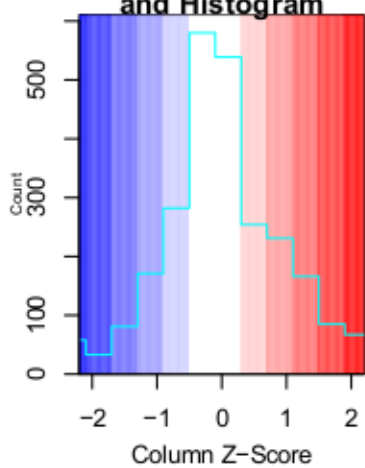








Color Key  
and Histogram



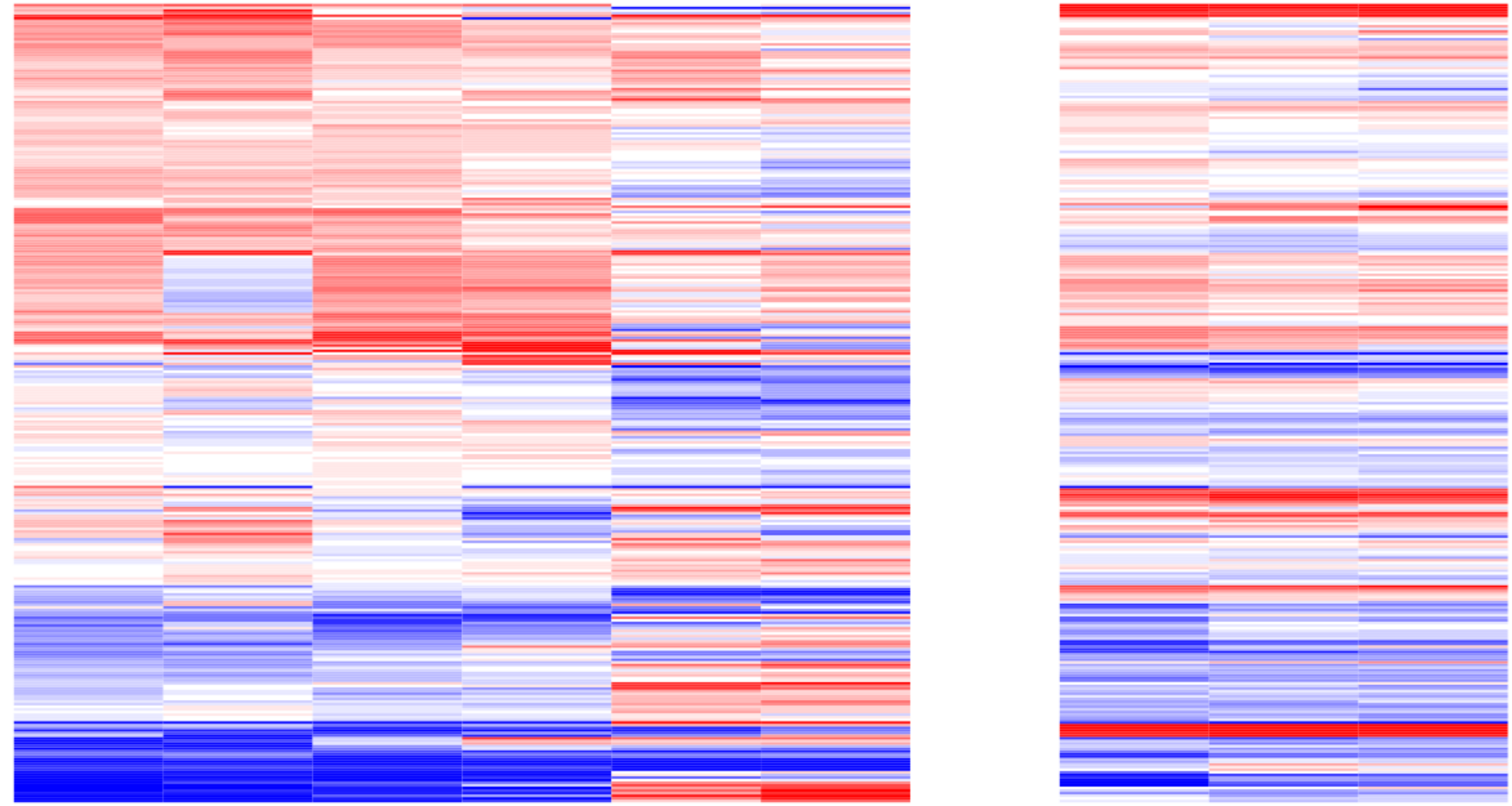
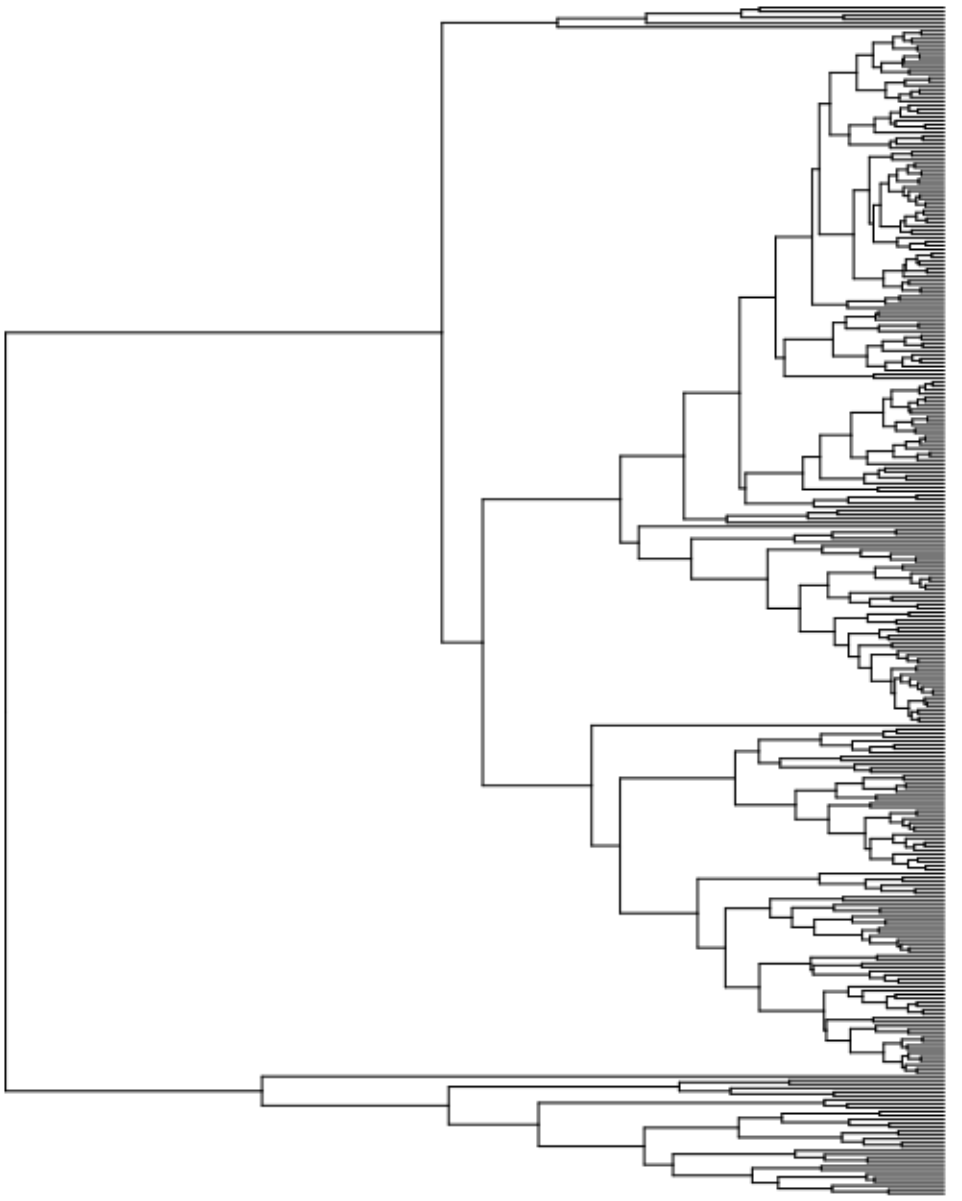
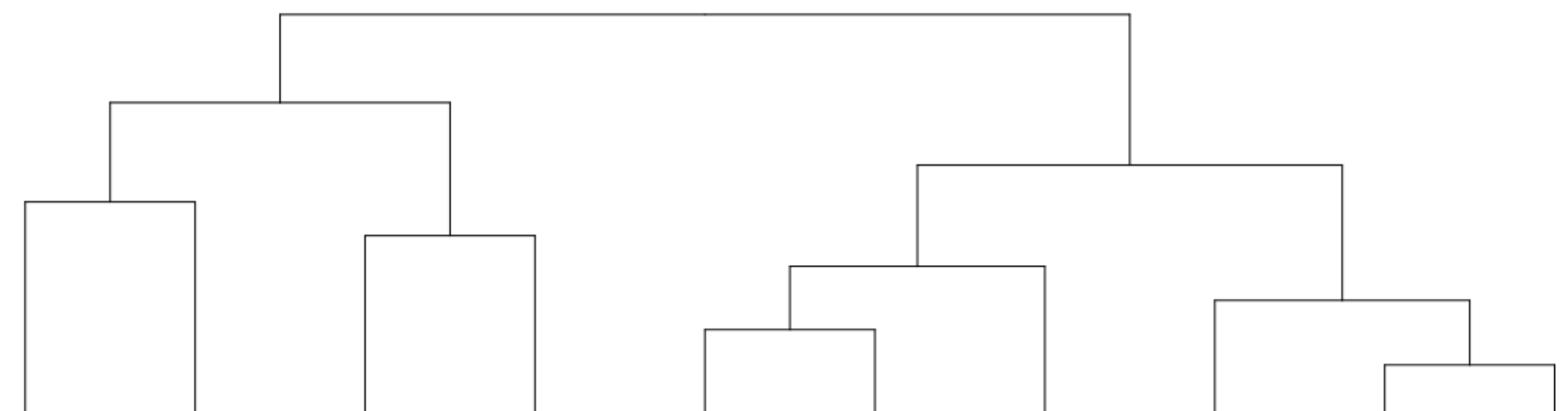
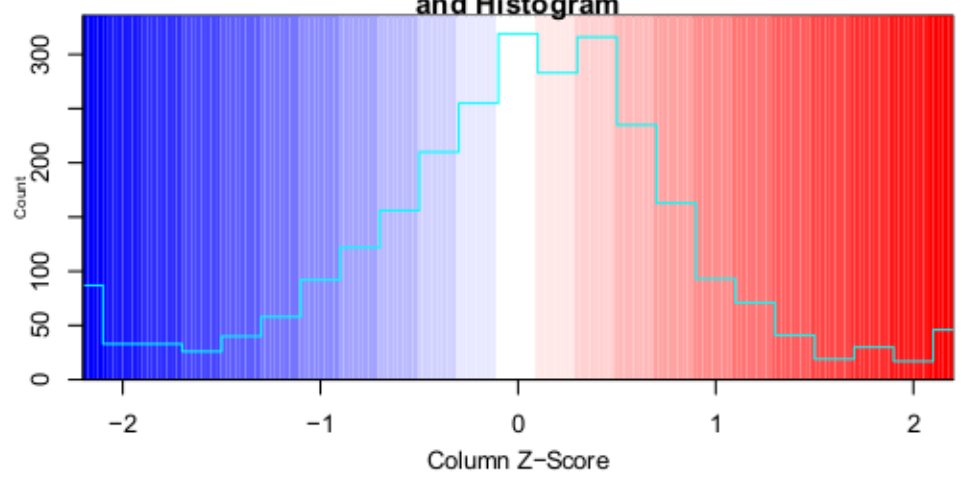
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REF\_chemostats

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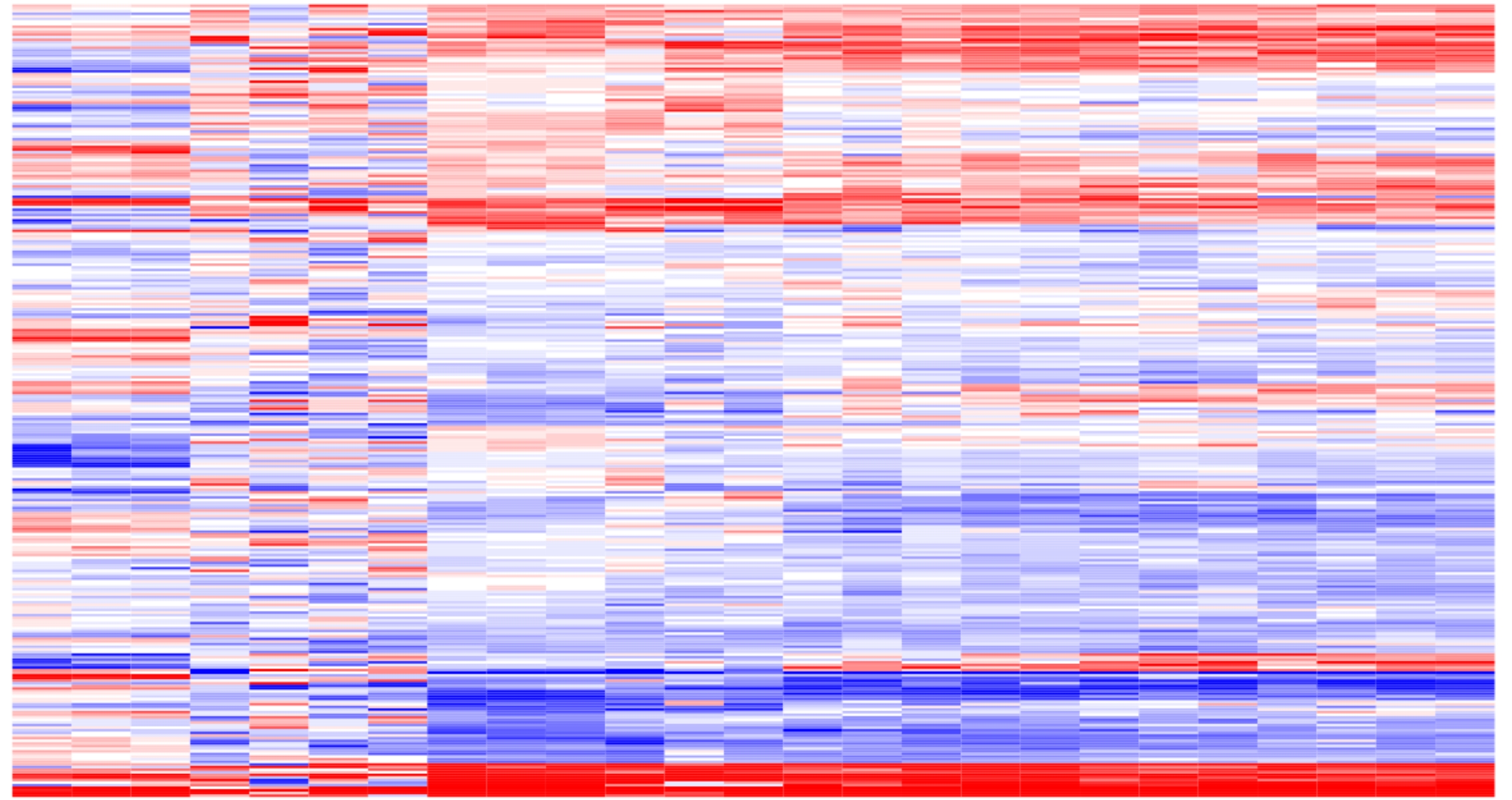
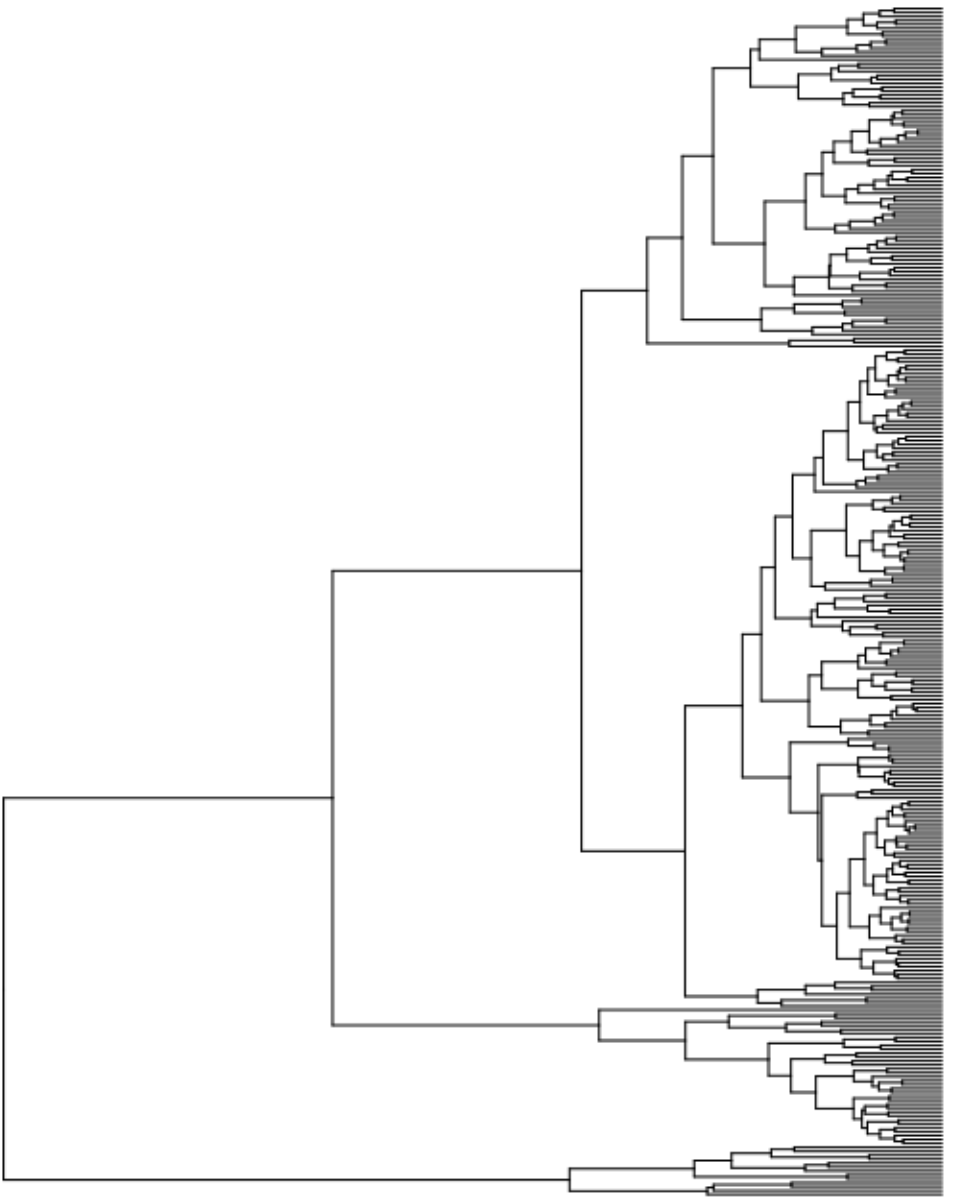
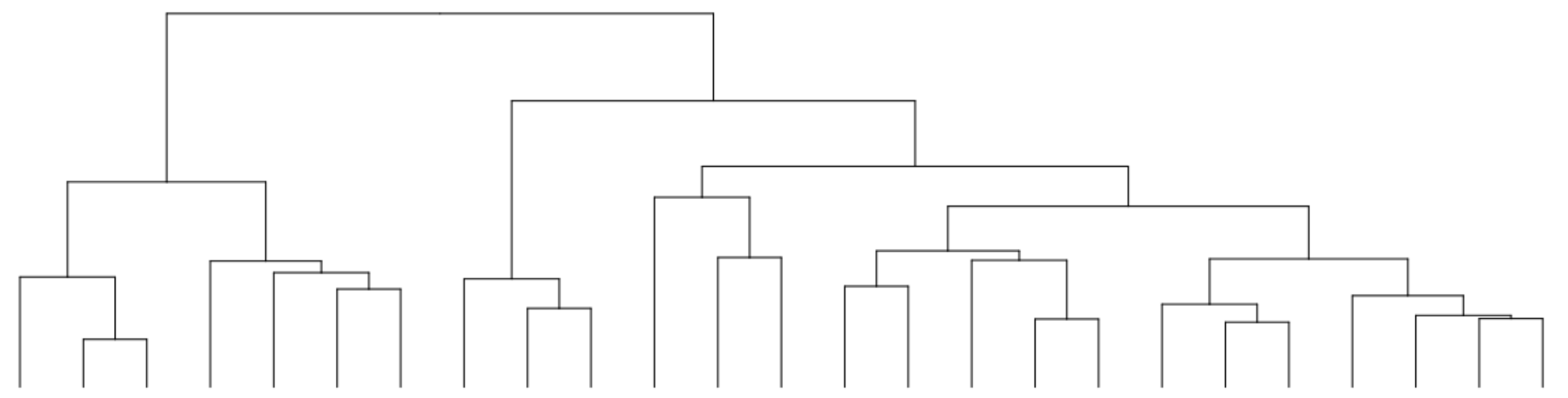
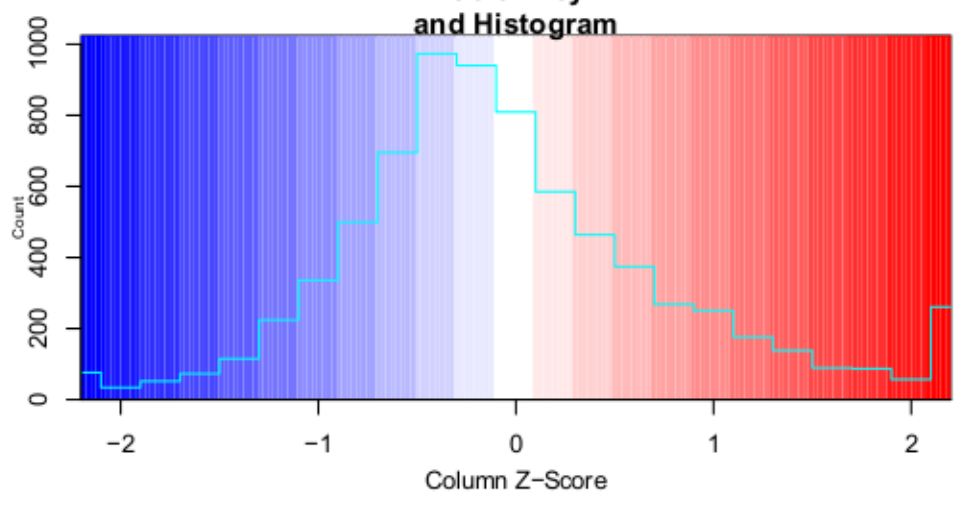


Color Key and Histogram



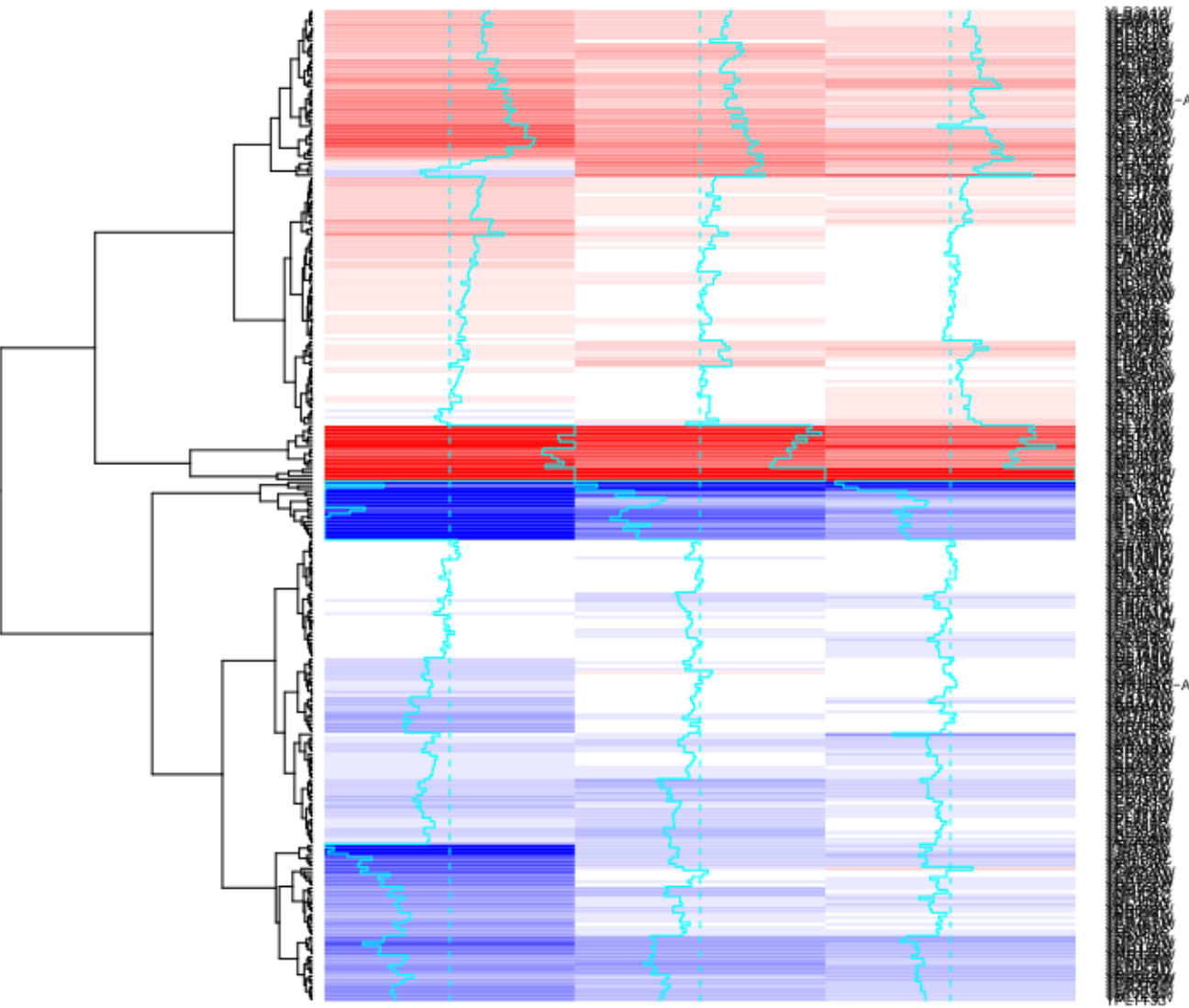
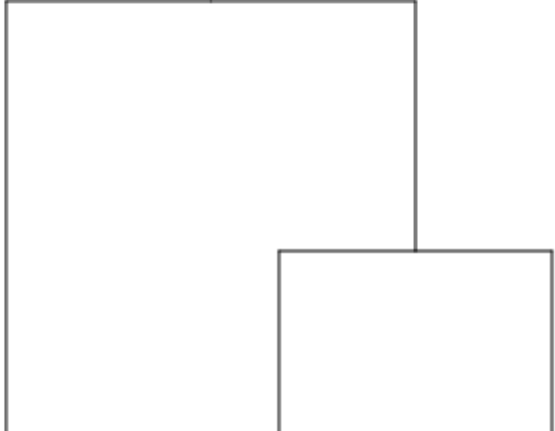
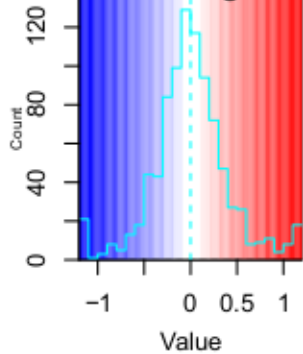
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Color Key and Histogram

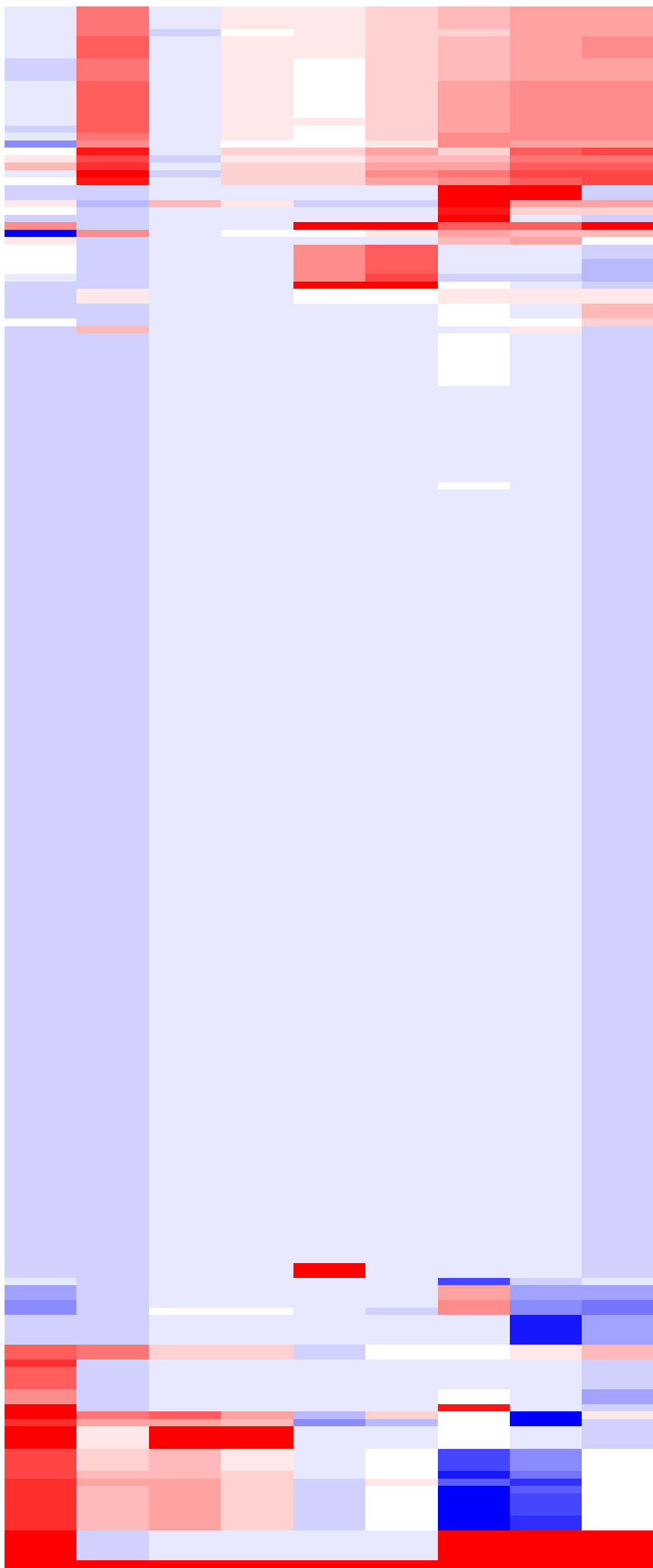
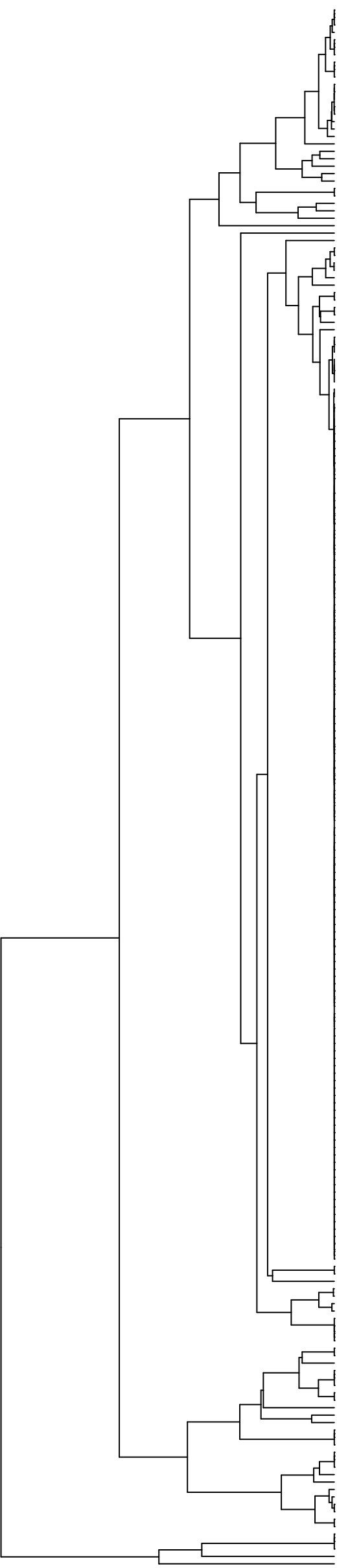
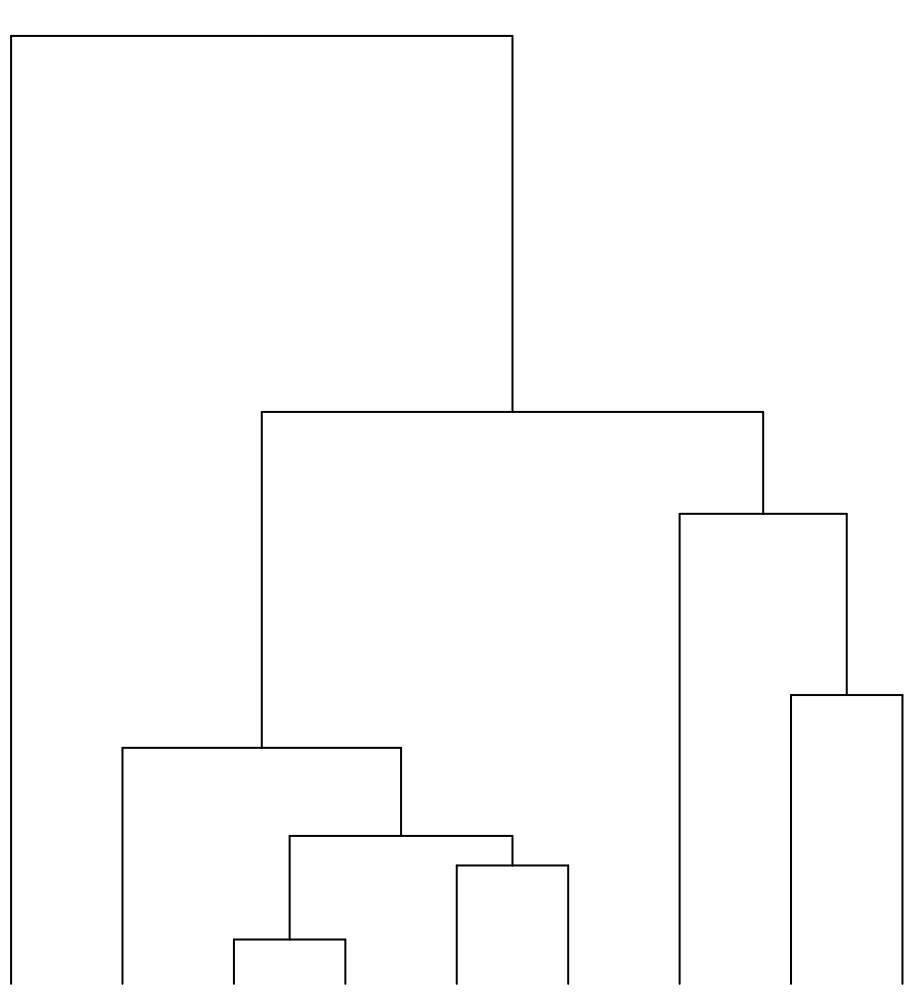
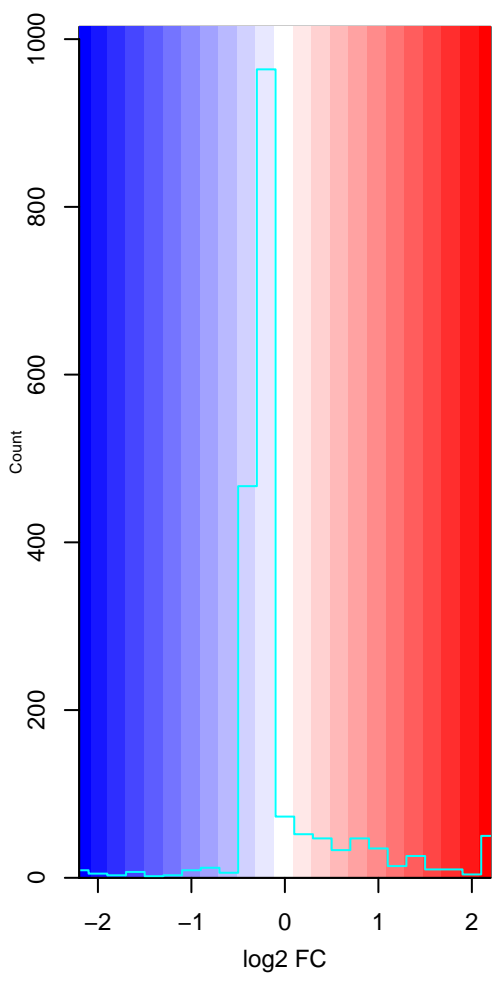


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Lee\_et\_al\_30\_min.1  
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# Color Key and Histogram







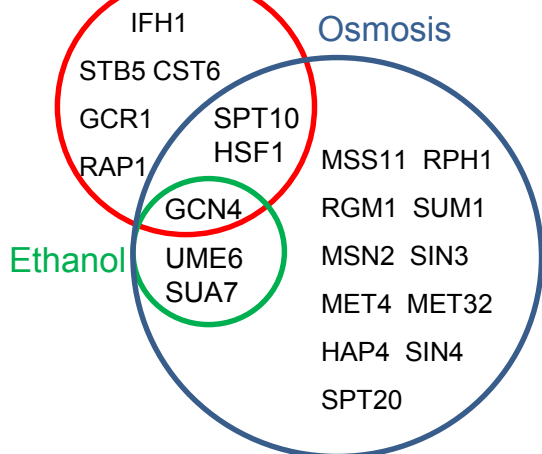
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 Malate dehydrogenase, mitochondrial  
 Succinate dehydrogenase [ubiquinone] cytochrome b subunit, mitochondrial  
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 Succinate dehydrogenase [ubiquinone] cytochrome b subunit, mitochondrial  
 yaf1\_old  
 yaf2\_old  
 Transport of O2, mitochondrial  
 Cytochrome c oxidase subunit 1  
 Cytochrome b-c1 complex subunit Rieske, mitochondrial  
 Uptake of oxygen  
 Transport of oxygen, extracellular  
 ADP/ATP carrier protein 1  
 ATP synthase complex, mitochondrial  
 Transport (ADP/ATP), mitochondrial  
 External NADH-ubiquinone oxidoreductase 1, mitochondrial  
 Succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial  
 NADH-ubiquinone oxidoreductase, mitochondrial  
 Transport of O2, mitochondrial  
 Isocitrate dehydrogenase [NAD] subunit 1, mitochondrial  
 Alpha-ketoglutarate dehydrogenase  
 Excretion of acetate  
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 Malate dehydrogenase, cytoplasmic  
 Malate synthase 2, glyoxysomal  
 Mitochondrial oxaloacetate transport protein  
 Inorganic phosphate transporter  
 Acetaldehyde dehydrogenase (acetylating)  
 Aspartate aminotransferase, cytoplasmic  
 Uptake of NH3  
 Ammonium transporter MEP1  
 3-hydroxy-3-methylglutaryl-coenzyme A reductase 1 (HMG-CoA reductase)  
 NADP-specific glutamate dehydrogenase 1  
 Transport of H2O, extracellular  
 Excretion of H2O  
 Isocitrate dehydrogenase [NADP], cytoplasmic (2)  
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 Adenylyl kinase cytosolic  
 Nucleoside diphosphate kinase (2)  
 Nucleoside diphosphate kinase (1)  
 Cytidylyl kinase  
 Acyl-carrier-protein acetyltransferase (1)  
 Ribonucleoside-triphosphate reductase (CTP)  
 Alanine-glyoxylate aminotransferase 1  
 Aminomethyltransferase, mitochondrial  
 Serine hydroxymethyltransferase, cytosolic  
 Transport of formate, extracellular  
 Excretion of formate  
 Transketolase 1 (1)  
 Ribulose-phosphate 3-epimerase  
 Ribulose-5-phosphate isomerase  
 Phosphoribosylglycinamide formyltransferase  
 Phosphoribosylformylglycinamide synthase  
 Phosphoribosylglycinamide cyto-ligase  
 Phosphoribosylaminoimidazole-succinocarboxamide synthase  
 Phosphoribosylaminoimidazole carboxylase  
 Phosphoribosylamine-glycine ligase  
 Amidophosphoribosyltransferase  
 Adenylosuccinate lyase (2)  
 Dihydrofolate reductase  
 Uptake of phosphate  
 Transport of 2-isopropylmalate, mitochondrial  
 Branched-chain-amino-acid aminotransferase, cytosolic (1)  
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 2-isopropylmalate dehydrogenase  
 Phosphoribosylaminoimidazolecarboxamide formyltransferase  
 IMP cyclohydrolase  
 Methylene-tetrahydrofolate dehydrogenase  
 Methylenetetrahydrofolate cyclohydrolase  
 Thymidylate synthase  
 Methyl-CoA carboxylase  
 Model-specific reaction, involved in the elongation of fatty acids  
 Microsomal beta-oxo-reductase (5)  
 Enoyl reductase TSC13 (5)  
 Elongation of fatty acids protein 1  
 Fatty acid 3-phosphate synthase  
 Inositol monophosphatase 1  
 GDP-diacylglycerol-inositol 3-phosphatidyltransferase  
 Spermidinyl-diphosphate Delta-isomerase  
 Geranyltransferase  
 Dimethylallyltransferase  
 Adenylosuccinate synthetase  
 Adenylosuccinate lyase (1)  
 Enoyl-acyl-carrier-protein reductase [NADH] (5)  
 3-oxoacyl-acyl-carrier-protein synthase (5)  
 3-oxoacyl-acyl-carrier-protein reductase (5)  
 3-hydroxyacyl-acyl-carrier-protein dehydrase (5)  
 Acyl-carrier-protein acetyltransferase (3)  
 Phosphatidylserine decarboxylase proenzyme 2  
 Glutamine synthetase  
 Model-specific reaction, pools acyl-CoAs  
 Dihydrodipicolinate dehydrogenase  
 Ornithine 5-phosphate decarboxylase  
 Ornithine phosphoribosyltransferase 2  
 Dihydrodipicolinate synthase  
 Aspartate carbamoyltransferase  
 Squalene synthetase 2  
 Squalene synthetase  
 Lanosterol synthase  
 Probable inosine-5-monophosphate dehydrogenase IMD5  
 GMP synthase [glutamine-hydrolyzing]  
 S-adenosylmethionine synthetase 1  
 Adenosylhomocysteinase  
 Adenosine kinase  
 Cytochrome P450 51  
 C-14 sterol reductase  
 Glycerol-3-phosphate O-acyltransferase 2  
 Probable 1-acyl-sn-glycerol-3-phosphate acyltransferase  
 Ribose-phosphate pyrophosphokinase 1  
 Uridylate kinase  
 Fumarate hydratase, mitochondrial (1)  
 Nucleoside diphosphate kinase (3)  
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 3-hydroxyacyl-acyl-carrier-protein dehydrase (1)  
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 Chorismate mutase  
 Acyl-carrier-protein malonyltransferase  
 Thioester reductase  
 Tryptophan synthase  
 Sterol 24-C-methyltransferase  
 Serine palmitoyltransferase  
 Ribonucleoside-diphosphate reductase large chain 1  
 Model-specific reaction, used to group lipid formation for biomass  
 Model-specific reaction, involved in the elongation of fatty acids  
 Model-specific reaction, involved in the elongation of fatty acids  
 Model-specific reaction, involved in the elongation of fatty acids  
 Model-specific reaction, involved in the elongation of fatty acids  
 Microsomal beta-keto-reductase (4)  
 Microsomal beta-keto-reductase (1)  
 Microsomal beta-keto-reductase (2)  
 Microsomal beta-keto-reductase (1)  
 Glycerol-3-phosphate synthase  
 Guanylate kinase  
 Growth  
 Excretion of biomass  
 Enoyl reductase TSC13 (4)  
 Enoyl reductase TSC13 (3)  
 Enoyl reductase TSC13 (2)  
 Enoyl reductase TSC13 (1)  
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 Elongation of fatty acids protein 2 (1)  
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 Anthranilate synthase component 1  
 Anthranilate phosphoribosyltransferase  
 5-ketodihydroxytryptophan reductase TSC10  
 Transketolase (2)  
 Transaldolase  
 Diphosphate-fructose-6-phosphate 1-phosphotransferase  
 Tricarboxylate transport protein (2)  
 Tricarboxylate transport protein (1)  
 Isocitrate lyase  
 Mitochondrial dicarboxylate transporter  
 Spontaneous conversion  
 Pyruvate-5-carboxylate reductase  
 Glutamate 5-kinase  
 Gamma-glutamyl phosphate reductase  
 Transport of carbon dioxide, extracellular  
 Excretion of CO2  
 Branched-chain-amino-acid aminotransferase, cytosolic (2)  
 Dihydroxy-acid dehydratase, mitochondrial  
 Acetylacetyl synthase catalytic subunit, mitochondrial  
 Ketol-acid reductoisomerase, mitochondrial  
 Isocitrate dehydrogenase [NADP], mitochondrial (2)  
 Isocitrate dehydrogenase [NADP], mitochondrial (1)  
 Transport of pyruvate, mitochondrial  
 Phosphotransferase 1  
 Pyruvate decarboxylase isozyme 1  
 Excretion of ethanol, extracellular  
 Alcohol dehydrogenase 1  
 Transport of ethanol, extracellular  
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 Fructose-bisphosphate aldolase  
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 Enolase 1  
 Aspartokinase  
 Aspartate-semialdehyde dehydrogenase  
 Homoserine dehydrogenase  
 Aspartate-3-phosphate dehydrogenase [NAD+] 1  
 Amino acid flux for constant maintenance requirements

Temp.38C  
 Osmo.0.6.M  
 Osmo.0.4.M  
 Osmo.0.2.M  
 Temp.33C  
 Temp.36C  
 EtOH.20g/L  
 EtOH.40.g/L  
 EtOH.60.g/L

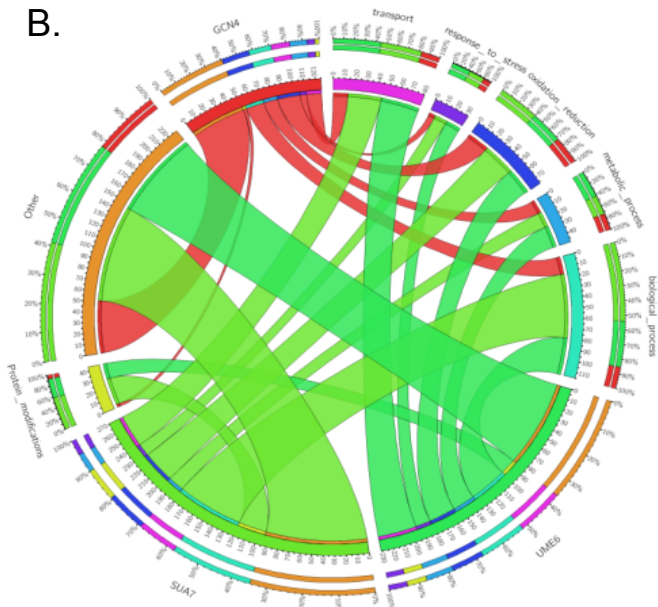
Fluxes

A.

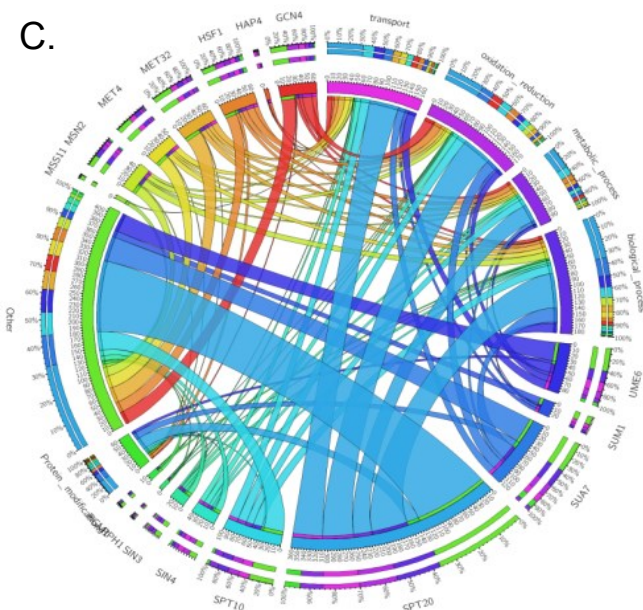
Temperature



B.



C.



D.

