**Engineering *Saccharomyces cerevisiae* for fast vitamin-independent aerobic growth**

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Chemically defined media for cultivation of *Saccharomyces cerevisiae* strains are commonly supplemented with a mixture of multiple Class-B vitamins, whose omission leads to strongly reduced growth rates. Fast growth without vitamin supplementation is interesting for industrial applications, as it reduces costs and complexity of medium preparation and may decrease susceptibility to contamination by auxotrophic microbes. In this study, suboptimal growth rates of *S. cerevisiae* CEN.PK113-7D in the absence of pantothenic acid, *para*-aminobenzoic acid (*p*ABA), pyridoxine, inositol and/or biotin were corrected by single or combined overexpression of *ScFMS1*, *ScABZ1*/*ScABZ2*, *ScSNZ1*/*ScSNO1*, S*cINO1* and *Cyberlindnera fabianii BIO1*, respectively. Several strategies were explored to improve growth of *S. cerevisiae* CEN.PK113-7D in thiamine-free medium. Overexpression of *ScTHI4* and/or *ScTHI5* enabled thiamine-independent growth at 83% of the maximum specific growth rate of the reference strain in vitamin-supplemented medium. Combined overexpression of seven native *S. cerevisiae* genes and *CfBIO1* enabled a maximum specific growth rate of 0.33 ± 0.01 h-1 in vitamin-free synthetic medium. This specific growth rate was only 18 % lower than that of a congenic reference strain in vitamin-supplemented medium. Physiological parameters of the engineered vitamin-independent strain in aerobic glucose-limited chemostat cultures (dilution rate 0.10 h-1) grown on vitamin-free synthetic medium were similar to those of similar cultures of the parental strain grown on vitamin-supplemented medium. Transcriptome analysis revealed only few differences in gene expression between these cultures, which primarily involved genes with roles in Class-B vitamin metabolism. These results pave the way for development of fast-growing vitamin-independent industrial strains of *S. cerevisiae*.

Data Figure 2.xlsx: **File Data supporting Figure 2 | Alleviating single vitamin dependency for fast-growth of *S. cerevisiae.*** The file includes the calculated specific growth rates of the non-engineered *S. cerevisiae* strain CEN.PK113-7D and engineered derivatives in synthetic medium with (+) or without (-) pyridoxine (worksheet Figure 2**A**), *p*ABA (worksheet Figure 2**B**), inositol (worksheet Figure 2**C**) and pantothenate (worksheet Figure 2**D**). Yeast strains were grown in 500 mL shake flasks containing 100 mL medium and incubated at 30 °C, 200 rpm.

Data Figure 3.xlsx: **File Data supporting Figure 3 |** **Alleviating thiamin dependency for fast-growth of *S. cerevisiae.*** The file includes the calculatedspecific growth rates of the non-engineered *S. cerevisiae* CEN.PK113-7D strain and engineered derivatives in synthetic medium with (+) or without

Data Figure 5.xlsx: **Flie Data supporting Figure 5 |Aerobic growth in medium with and without class-B vitamins**. The file includes the calculated specific growth rates of the non-engineered S. cerevisiae strain CEN.PK113-7D and the engineered strain IMX2816 in synthetic medium with (+) or without (-) vitamins and on media to which a single vitamin was added to the vitamin-free medium (biotin, SMDΔvitamins+bio; thiamine, SMDΔvitamins+thi). Yeast strains were grown in 500-mL shake flasks containing 100 mL medium and incubated at 30 °C, 200 rpm.

Data Figure 6.xlsx: File Data supporting Figure 6  **|**

RNA seq data CENPK113-7D IMX2816.xlsx: **File Data supporting Figure 7 | Differentially up-regulated genes in absence of class-B vitamins in aerobic glucose-limited chemostat cultures.** The files contains multiple work sheets (all data, IMX2816(-V)vsCENPK(+V) UP (86), IMX2816(-V)vsCENPK(+V) DWN (54), IMX2816(-V)vsIMX2816(+V) UP (48), IMX2816(-V)vsIMX2816(+V) DWN (7), IMX2816(+V)vsCENPK(+V) up (25), IMX2816(+V)vsCENPK(+V) DWN (36). The details of each worksheets is given below.

**Tab all data**

**Column 1**: Genes: Unique gene identifier corresponding according gff annotation file from CEN.PK113-7D (file….).

**Column 2**: Chr: Assembly contig of the *Saccharomyces cerevisiae* strain CEN.PK113-7D

**Column 3**: Start: coordinate of the start of the ORF on the corresponding contig.

**Column 4**: End: coordinate of the end of the ORF on the corresponding contig.

**Column 5**: Strand: strand on which the ORF is located; + Watson strand, - Crick strand.

**Column 6**: length: if the ORF does not contains intron the length is (end\_coordinate – start\_coordinate+1)

**Column 7**: synname: Systematic ORF identifier. Systematic names for nuclear-encoded ORFs begin with the letter 'Y' (for 'Yeast'); the second letter denotes the chromosome number ('A' is chr I, 'B' is chr II, etc.); the third letter is either 'L' or 'R' for left or right chromosome arm; next is a three digit number indicating the order of the ORFs on that arm of a chromosome starting from the centromere, irrespective of strand; finally, there is an additional letter indicating the strand, either 'W' for Watson (the strand with 5' end at the left telomere) or 'C' for Crick (the complement strand, 5' end is at the right telomere).

**Column 8**: stdname: Gene names, also referred to as genetic names are conferred upon genes by a researchers on the basis of genetic, biochemical, or molecular characterization. Most genes having Gene Names are ORFs, but tRNAs and other non-protein coding RNAs have also received Gene Names. The official name of an S. cerevisiae gene is referred to as the Standard Name on an SGD locus page, and generally becomes the standard name based on its publication in a peer-reviewed paper describing characterization of that gene.. Any alternative Gene Name is referred to as an Alias.

**Column 9 to 11**: (A6\_1; A7\_1; A8\_1) normalized read counts of samples from the *S. cerevisiae* strain CEN.PK113-7D grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 12 and 13**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 14 and 15**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMDvitamins in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 16 to 19**: (3 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMDvitamins relative to the CPM value of the CEN.PK113-7D on SMD.

**Column 20 to 23**: (2 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMD relative to the CPM value of the CEN.PK113-7D on SMD.

**Column 24 to 27**: (3 vs 2) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMDvitamins relative to the CPM value of the IMX2816 on SMD.

**Tab IMX2816(-V)vsCENPK(+V) UP (86)**

Set of 86 genes upregulated in IMX2816 grown in SMDvitamins relative to CEN.PK113-7D on SMD.

**Column 1**: Genes: Unique gene identifier corresponding according gff annotation file from CEN.PK113-7D (file….).

**Column 2**: Chr: Assembly contig of the Saccharomyces cerevisiae strain CEN.PK113-7D (Salazar et al., 2017)

**Column 3**: Start: coordinate of the start of the ORF on the corresponding contig.

**Column 4**: End: coordinate of the end of the ORF on the corresponding contig.

**Column 5**: Strand: strand on which the ORF is located; + Watson strand, - Crick strand.

**Column 6**: length: if the ORF does not contains intron the length is (end\_coordinate – start\_coordinate+1)

**Column 7**: synname: Systematic ORF identifier.

**Column 8**: stdname: Gene names,

**Columns 9 to 12**: (3 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMDvitamins relative to the CPM value of the CEN.PK113-7D on SMD.

**Columns 13 to 15**: (A6\_1; A7\_1; A8\_1) normalized read counts of samples from the *S. cerevisiae* strain CEN.PK113-7D grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 16 and 17**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 18 and 19**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMDvitamins in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 20**: Max normalized read counts of samples A6\_1, A7\_1, A8\_1, A1\_1, A5\_1, A2\_2, A9.

**Columns 21 to 23**: Average, standard deviation and coefficient of variation of the read counts of the replicates of CEN.PK113-7D grown on SMD.

**Columns 24 to 26**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMD.

**Columns 27 to 29**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMDvitamins.

**Tab IMX2816(-V)vsCENPK(+V) DWN (54)**

Set of 54 genes downregulated in IMX2816 grown in SMDvitamins relative to CEN.PK113-7D on SMD.

**Column 1**: Genes: Unique gene identifier corresponding according gff annotation file from CEN.PK113-7D (file….).

**Column 2**: Chr: Assembly contig of the Saccharomyces cerevisiae strain CEN.PK113-7D (Salazar et al., 2017)

**Column 3**: Start: coordinate of the start of the ORF on the corresponding contig.

**Column 4**: End: coordinate of the end of the ORF on the corresponding contig.

**Column 5**: Strand: strand on which the ORF is located; + Watson strand, - Crick strand.

**Column 6**: length: if the ORF does not contains intron the length is (end\_coordinate – start\_coordinate+1)

**Column 7**: synname: Systematic ORF identifier.

**Column 8**: stdname: Gene names,

**Columns 9 to 12**: (3 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMDvitamins relative to the CPM value of the CEN.PK113-7D on SMD.

**Columns 13 to 15**: (A6\_1; A7\_1; A8\_1) normalized read counts of samples from the *S. cerevisiae* strain CEN.PK113-7D grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 16 and 17**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 18 and 19**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMDvitamins in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 20**: Max normalized read counts of samples A6\_1, A7\_1, A8\_1, A1\_1, A5\_1, A2\_2, A9.

**Columns 21 to 23**: Average, standard deviation and coefficient of variation of the read counts of the replicates of CEN.PK113-7D grown on SMD.

**Columns 24 to 26**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMD.

**Columns 27 to 29**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMDvitamins.

**Tab IMX2816(-V)vsIMX2816(+V) UP (48)**

Set of48 genes upregulated in IMX2816 grown in SMDvitamins relative to IMX2816 on SMD.

**Column 1**: Genes: Unique gene identifier corresponding according gff annotation file from CEN.PK113-7D (file….).

**Column 2**: Chr: Assembly contig of the Saccharomyces cerevisiae strain CEN.PK113-7D (Salazar et al., 2017)

**Column 3**: Start: coordinate of the start of the ORF on the corresponding contig.

**Column 4**: End: coordinate of the end of the ORF on the corresponding contig.

**Column 5**: Strand: strand on which the ORF is located; + Watson strand, - Crick strand.

**Column 6**: length: if the ORF does not contains intron the length is (end\_coordinate – start\_coordinate+1)

**Column 7**: synname: Systematic ORF identifier.

**Column 8**: stdname: Gene names,

**Columns 9 to 12**: (3 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMDvitamins relative to the CPM value of the IMX2816 on SMD.

**Columns 13 to 15**: (A6\_1; A7\_1; A8\_1) normalized read counts of samples from the *S. cerevisiae* strain CEN.PK113-7D grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 16 and 17**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 18 and 19**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMDvitamins in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 20**: Max normalized read counts of samples A6\_1, A7\_1, A8\_1, A1\_1, A5\_1, A2\_2, A9.

**Columns 21 to 23**: Average, standard deviation and coefficient of variation of the read counts of the replicates of CEN.PK113-7D grown on SMD.

**Columns 24 to 26**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMD.

**Columns 27 to 29**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMDvitamins.

Salazar, A. N., Gorter de Vries, A. R., van den Broek, M., Wijsman, M., de la Torre Cortes, P., Brickwedde, A., Brouwers, N., Daran, J. G., Abeel, T., 2017. Nanopore sequencing enables near-complete de novo assembly of *Saccharomyces cerevisiae* reference strain CEN.PK113-7D. FEMS Yeast Res. 17.

**Tab IMX2816(-V)vsIMX2816(+V) DWN (7)**

Set of7 genes downregulated in IMX2816 grown in SMDvitamins relative to IMX2816 on SMD.

**Column 1**: Genes: Unique gene identifier corresponding according gff annotation file from CEN.PK113-7D (file….).

**Column 2**: Chr: Assembly contig of the Saccharomyces cerevisiae strain CEN.PK113-7D (Salazar et al., 2017)

**Column 3**: Start: coordinate of the start of the ORF on the corresponding contig.

**Column 4**: End: coordinate of the end of the ORF on the corresponding contig.

**Column 5**: Strand: strand on which the ORF is located; + Watson strand, - Crick strand.

**Column 6**: length: if the ORF does not contains intron the length is (end\_coordinate – start\_coordinate+1)

**Column 7**: synname: Systematic ORF identifier.

**Column 8**: stdname: Gene names,

**Columns 9 to 12**: (3 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMDvitamins relative to the CPM value of the IMX2816 on SMD.

**Columns 13 to 15**: (A6\_1; A7\_1; A8\_1) normalized read counts of samples from the *S. cerevisiae* strain CEN.PK113-7D grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 16 and 17**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 18 and 19**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMDvitamins in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 20**: Max normalized read counts of samples A6\_1, A7\_1, A8\_1, A1\_1, A5\_1, A2\_2, A9.

**Columns 21 to 23**: Average, standard deviation and coefficient of variation of the read counts of the replicates of CEN.PK113-7D grown on SMD.

**Columns 24 to 26**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMD.

**Columns 27 to 29**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMDvitamins.

**Tab IMX2816(+V)vsCENPK(+V) up (25)**

Set of25 genes upregulated in IMX2816 grown in SMDrelative to CEN.PK113-7D on SMD.

**Column 1**: Genes: Unique gene identifier corresponding according gff annotation file from CEN.PK113-7D (file….).

**Column 2**: Chr: Assembly contig of the Saccharomyces cerevisiae strain CEN.PK113-7D (Salazar et al., 2017)

**Column 3**: Start: coordinate of the start of the ORF on the corresponding contig.

**Column 4**: End: coordinate of the end of the ORF on the corresponding contig.

**Column 5**: Strand: strand on which the ORF is located; + Watson strand, - Crick strand.

**Column 6**: length: if the ORF does not contains intron the length is (end\_coordinate – start\_coordinate+1)

**Column 7**: synname: Systematic ORF identifier.

**Column 8**: stdname: Gene names,

**Columns 9 to 12**: (3 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMDrelative to the CPM value of the CEN.PK113-7D on SMD.

**Columns 13 to 15**: (A6\_1; A7\_1; A8\_1) normalized read counts of samples from the *S. cerevisiae* strain CEN.PK113-7D grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 16 and 17**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 18 and 19**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMDvitamins in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 20**: Max normalized read counts of samples A6\_1, A7\_1, A8\_1, A1\_1, A5\_1, A2\_2, A9.

**Columns 21 to 23**: Average, standard deviation and coefficient of variation of the read counts of the replicates of CEN.PK113-7D grown on SMD.

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**Columns 27 to 29**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMDvitamins.

**Tab IMX2816(+V)vsCENPK(+V) DWN (36)**

Set of 36 genes downregulated in IMX2816 grown in SMDrelative to CENPK on SMD.

**Column 1**: Genes: Unique gene identifier corresponding according gff annotation file from CEN.PK113-7D (file….).

**Column 2**: Chr: Assembly contig of the Saccharomyces cerevisiae strain CEN.PK113-7D (Salazar et al., 2017)

**Column 3**: Start: coordinate of the start of the ORF on the corresponding contig.

**Column 4**: End: coordinate of the end of the ORF on the corresponding contig.

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**Column 6**: length: if the ORF does not contains intron the length is (end\_coordinate – start\_coordinate+1)

**Column 7**: synname: Systematic ORF identifier.

**Column 8**: stdname: Gene names,

**Columns 9 to 12**: (3 vs 1) log2 fold change (logFC), log2 of the count per million (logCPM), PValue and FDR corrected QValue (FDR) of the pairwise comparison of CPM value of the IMX2816 on SMD relative to the CPM value of the CEN.PK113-7D on SMD.

**Columns 13 to 15**: (A6\_1; A7\_1; A8\_1) normalized read counts of samples from the *S. cerevisiae* strain CEN.PK113-7D grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 16 and 17**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMD in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Columns 18 and 19**: (A1\_1; A5\_1) normalized read counts of samples from the *S. cerevisiae* strain IMX2816 grown on SMDvitamins in oxic carbon-limited chemostat culture at a dilution rate of 0.1 h-1.

**Column 20**: Max normalized read counts of samples A6\_1, A7\_1, A8\_1, A1\_1, A5\_1, A2\_2, A9.

**Columns 21 to 23**: Average, standard deviation and coefficient of variation of the read counts of the replicates of CEN.PK113-7D grown on SMD.

**Columns 24 to 26**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMD.

**Columns 27 to 29**: Average, standard deviation and coefficient of variation of the read counts of the replicates of IMX2816 grown on SMDvitamins.