

## Supplemental Material

*Enhanced wort fermentation with de novo lager hybrids adapted to high ethanol environments*

Running title: Adaptive evolution of *de novo* lager yeast hybrids

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**Table S1** – Unique SNPs, Indels and SVs that were detected in the eight variant strains.

Table S1 can be found as a separate Microsoft Excel spreadsheet among the Supplemental material.

**Table S2** – The parameters of the 2L-scale wort fermentations with the wild-type and variant strains (also see Figure 5 in the main text). Values are means from two independent fermentations (standard deviation in parentheses), and asterisks depict a significant difference in the variant compared to the wild-type as determined by two-tailed Student's t-test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).

<b>Yeast strain</b>	<b>Maximum fermentation rate (% alcohol hr<sup>-1</sup>)</b>	<b>Mean fermentation rate (% alcohol hr<sup>-1</sup>)</b>	<b>Final alcohol level (% v/v)</b>
Y1	0.037 (±0.0009)	0.021 (±0.0002)	6.64 (±0.06)
Y1_M1	0.045 (±0.0006)*	0.024 (±0.0001)**	7.37 (±0.00)*
Y1_M6	0.036 (±0.0001)	0.023 (±0.0004)*	7.16 (±0.01)*
Y2	0.057 (±0.0015)	0.024 (±0.0002)	7.02 (±0.04)
Y2_M1	0.065 (±0.0009)*	0.031 (±0.0001)**	7.26 (±0.01)*
Y2_M6	0.073 (±0.0012)**	0.031 (±0.0001)**	7.22 (±0.03)*
Y3	0.051 (±0.0006)	0.023 (±0.0004)	7.13 (±0.06)
Y3_M1	0.069 (±0.0009)**	0.027 (±0.0002)*	7.15 (±0.00)
Y3_M6	0.075 (±0.0003)**	0.030 (±0.0000)*	7.08 (±0.00)
Y4	0.044 (±0.0006)	0.020 (±0.0004)	6.35 (±0.13)
Y4_M1	0.045 (±0.0006)	0.021 (±0.0004)	6.54 (±0.06)
Y4_M6	0.045 (±0.0003)	0.022 (±0.0006)	6.60 (±0.01)

**Table S3** – The concentrations of nine yeast-derived aroma compounds (mg/L) in the beers fermented with the wild-type and variant strains (also see Figure 6 in the main text). Values are means from two independent fermentations (standard deviation in parentheses), and asterisks depict a significant difference in the variant compared to the wild-type as determined by two-tailed Student’s t-test (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Me: methyl.

Yeast strain	2-Me-Propanol	2-Me-Butanol	3-Me-Butanol	3-Me-Butylacetate	Ethyl acetate	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate	Diacetyl
Y1	8.49 (±0.21)	8.97 (±0.11)	31.63 (±0.32)	0.63 (±0.05)	30.18 (±1.35)	0.37 (±0.06)	0.18 (±0.09)	0.016 (±0.008)	0.236 (±0.006)
Y1_M1	8.34 (±0.03)	8.81 (±0.04)	32.45 (±0.28)	0.92 (±0.05)*	30.95 (±0.97)	0.54 (±0.07)	0.29 (±0.12)	0.051 (±0.021)	0.156 (±0.011)***
Y1_M6	8.35 (±0.16)	9.09 (±0.13)	32.01 (±1.02)	1.01 (±0.05)**	27.97 (±0.05)	0.33 (±0.04)	0.22 (±0.06)	0.056 (±0.016)	0.089 (±0.003)***
Y2	19.71 (±0.08)	23.42 (±0.14)	57.63 (±0.31)	1.52 (±0.08)	32.37 (±0.82)	0.31 (±0.03)	0.12 (±0.00)	0.06 (±0.003)	0.082 (±0.002)
Y2_M1	18.14 (±0.39)*	19.33 (±0.02)**	62.69 (±0.2)**	1.20 (±0.01)	38.67 (±0.04)*	0.41 (±0.02)*	0.11 (±0.01)	0.061 (±0.01)	0.102 (±0.022)
Y2_M6	13.16 (±0.32)**	13.2 (±0.08)***	50.02 (±0.45)**	1.45 (±0.06)	35.09 (±0.16)	0.59 (±0.08)	0.30 (±0.00)***	0.142 (±0.029)	0.058 (±0.005)***
Y3	18.8 (±0.1)	21.81 (±0.03)	55.44 (±0.95)	0.88 (±0.02)	40.30 (±0.38)	0.28 (±0.00)	0.09 (±0.00)	0.019 (±0.000)	0.239 (±0.027)
Y3_M1	12.34 (±0.3)*	14.67 (±0.17)**	59.64 (±0.79)*	1.51 (±0.03)**	29.47 (±0.17)**	0.29 (±0.01)	0.17 (±0.01)*	0.043 (±0.002)*	0.118 (±0.012)**
Y3_M6	9.23 (±0.29)**	10.20 (±0.15)**	59.93 (±0.12)*	1.90 (±0.04)**	29.06 (±0.14)**	0.58 (±0.02)*	0.39 (±0.03)*	0.081 (±0.002)*	0.100 (±0.007)**
Y4	22.89 (±0.65)	22.66 (±0.56)	54.61 (±0.18)	0.67 (±0.00)	25.84 (±0.01)	0.17 (±0.00)	0.12 (±0.00)	0.029 (±0.003)	0.043 (±0.001)
Y4_M1	17.96 (±0.56)*	19.45 (±0.81)	56.56 (±4.02)	2.06 (±0.33)	36.85 (±5.03)	0.25 (±0.06)	0.21 (±0.01)*	0.049 (±0.005)*	0.046 (±0.013)
Y4_M6	19.98 (±0.81)	20.59 (±0.61)	59.77 (±2.65)	2.22 (±0.13)*	37.05 (±1.95)*	0.27 (±0.02)	0.27 (±0.02)*	0.068 (±0.009)*	0.047 (±0.008)

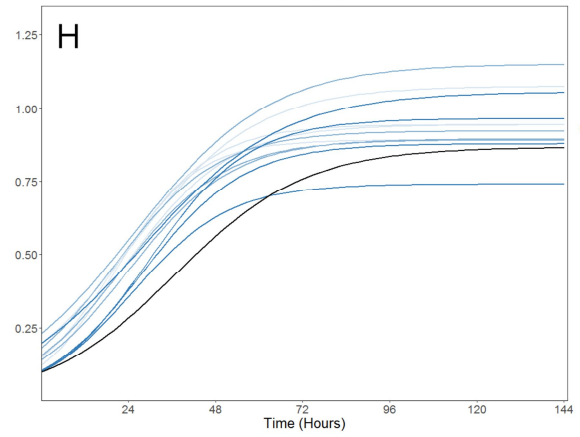
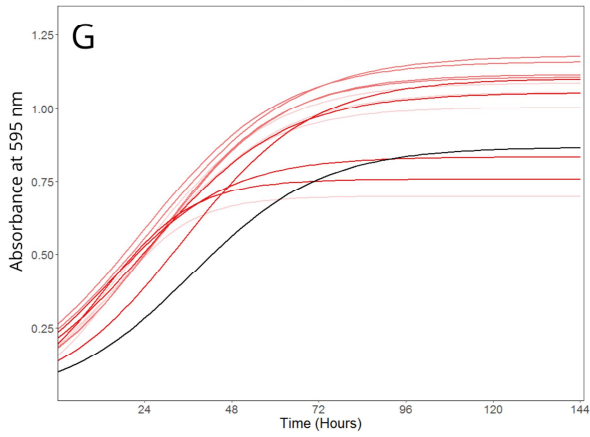
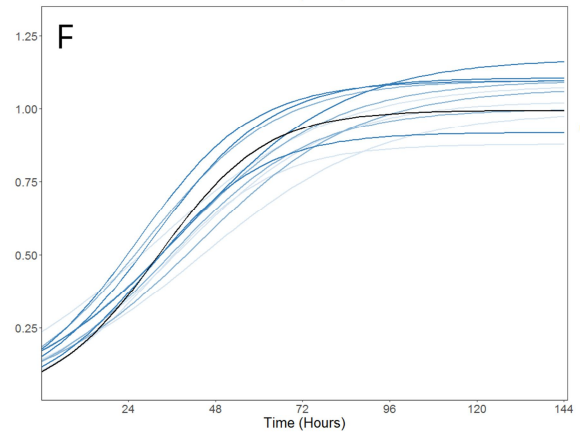
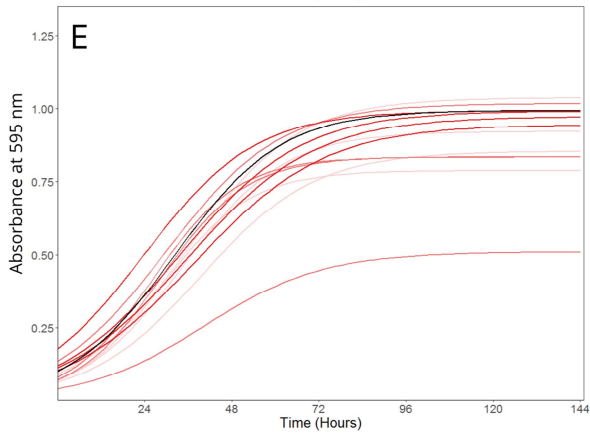
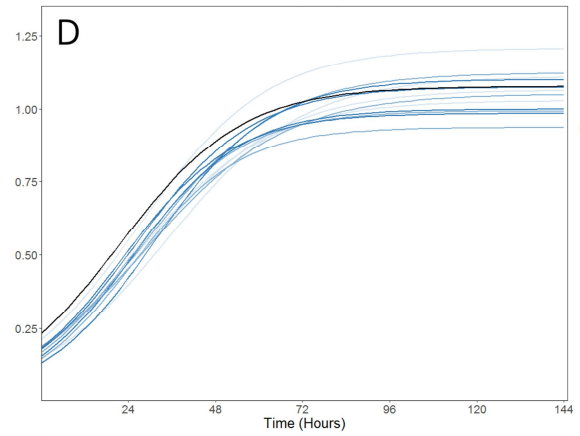
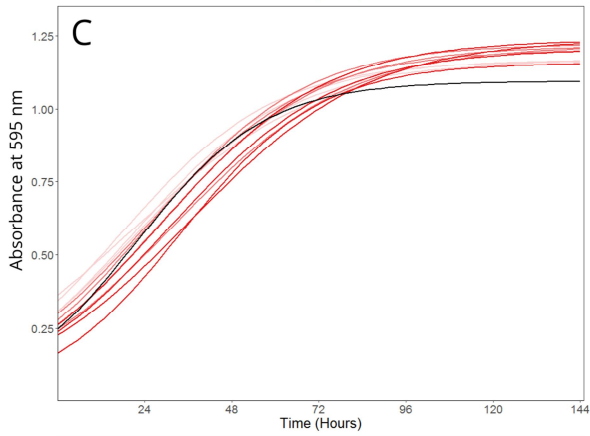
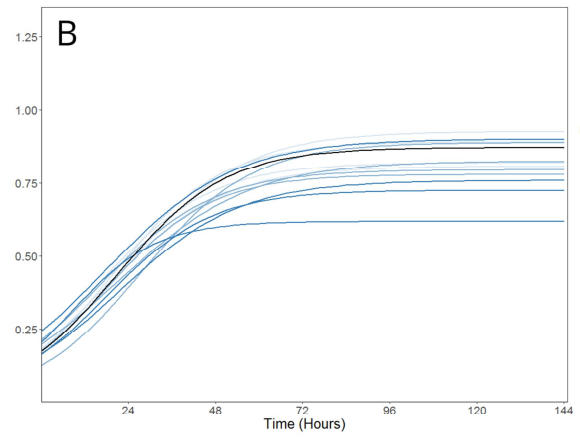
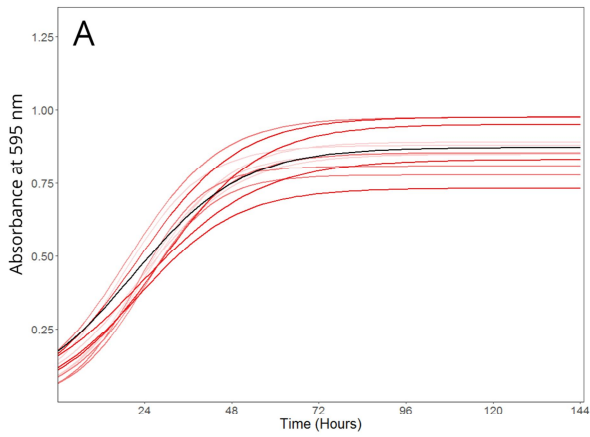
**Table S4** – The chromosome copy numbers of the *S. cerevisiae* (*Scer*)-derived and *S. eubayanus* (*Seub*)-derived chromosomes in the unevolved wild-type strains Y1-Y4. Mt: mitochondria.

Chromosome	Y1		Y2		Y3		Y4	
	<i>Scer</i>	<i>Seub</i>	<i>Scer</i>	<i>Seub</i>	<i>Scer</i>	<i>Seub</i>	<i>Scer</i>	<i>Seub</i>
<b>I</b>	2	-	2	2	2	1	1	0
<b>II</b>	2	-	2	2	2	1	1+1*	1
<b>III</b>	2	-	1	2	1	1	1	1
<b>IV</b>	2	-	2	2	2	1	2	0+1*
<b>V</b>	2	-	2	2	2	1	2	0
<b>VI</b>	2	-	2	2	2	1	2	1
<b>VII</b>	2	-	2	2	2	1	2	0
<b>VIII</b>	2	-	2	2	2	1	2	1
<b>IX</b>	2	-	2	2	2	1	1	1
<b>X</b>	2	-	2	2	2	1	1	1
<b>XI</b>	2	-	2	2	2	1	2	1
<b>XII</b>	2	-	2	2	2	1	1	1
<b>XIII</b>	2	-	2	2	2	1	2	0
<b>XIV</b>	2	-	2	2	2	1	2	0
<b>XV</b>	2	-	2	2	2	1	1	1
<b>XVI</b>	2	-	2	2	2	1	2	0
<b>Mt</b>	Yes	-	-	Yes	-	Yes	-	Yes

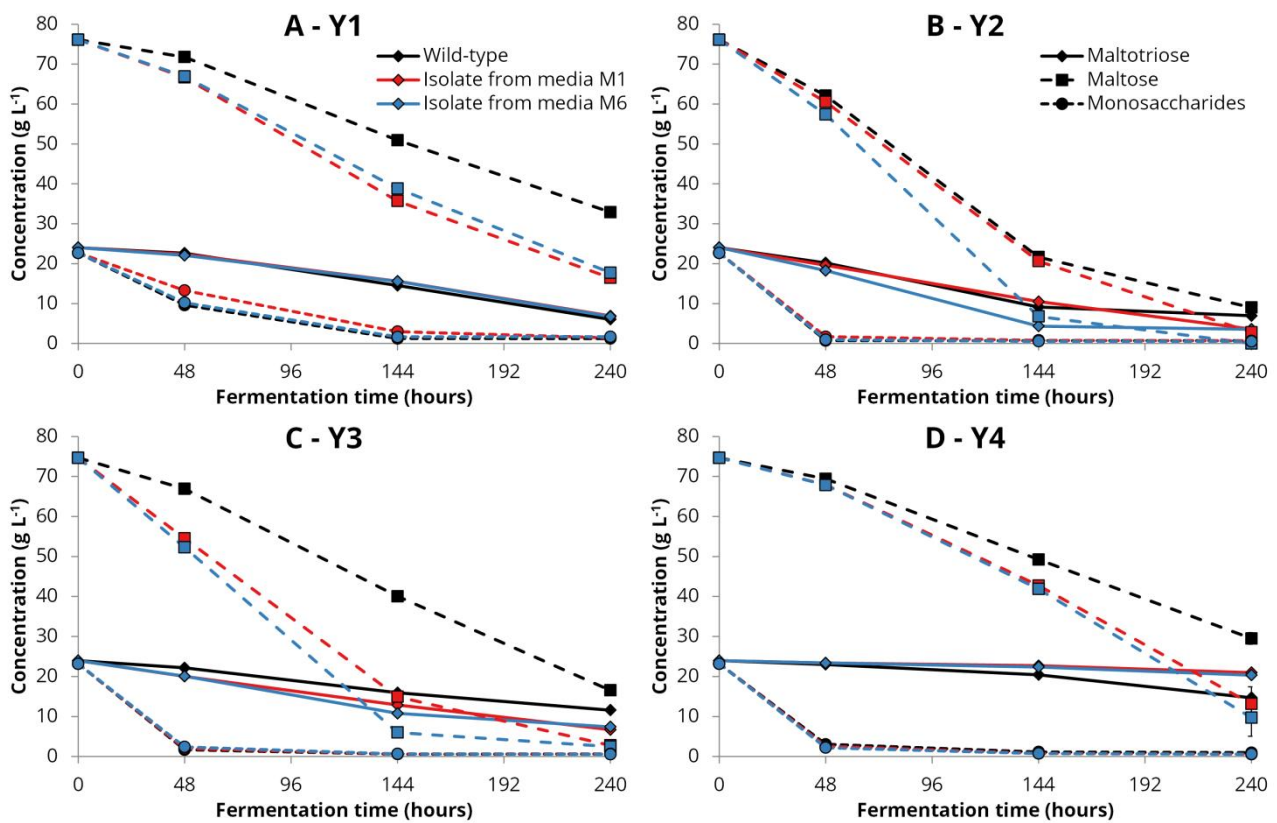
\* Strain Y4 contains a single chimeric chromosome consisting of the left arm of the *Scer*-derived chromosome II and the right arm of the *Seub*-derived chromosome IV.

**Table S5** – The copy numbers of genes related to maltose transport in the wild-type and variant strains. Copy numbers were estimated based on sequencing coverage using CNVkit.

Gene	Chromosome	Y1	Y1_M1	Y1_M6	Y2	Y2_M1	Y2_M6	Y3	Y3_M1	Y3_M6	Y4	Y4_M1	Y4_M6
<b><i>S. cerevisiae</i> sub-genome</b>													
<i>MAL11/AGT1</i>	VII	4	4	6	4	6	4	4	6	6	4	4	4
<i>MAL31</i>	VII	2	2	3	2	3	2	2	3	3	4	4	4
<i>MTT1/MTY1</i>	II	2	2	4	2	1	1	2	2	2	0	0	0
<i>MPH2</i>	IV	2	2	4	2	2	2	2	2	2	2	2	2
<b><i>S. eubayanus</i> sub-genome</b>													
<i>MALT1</i>	II	0	0	0	2	2	2	1	1	1	1	1	1
<i>MALT2</i>	V	0	0	0	2	2	2	1	1	0	0	0	0
<i>MALT3</i>	XIII	0	0	0	2	2	2	1	1	1	0	0	0
<i>MALT4</i>	XVI	0	0	0	2	2	2	1	1	1	0	0	0

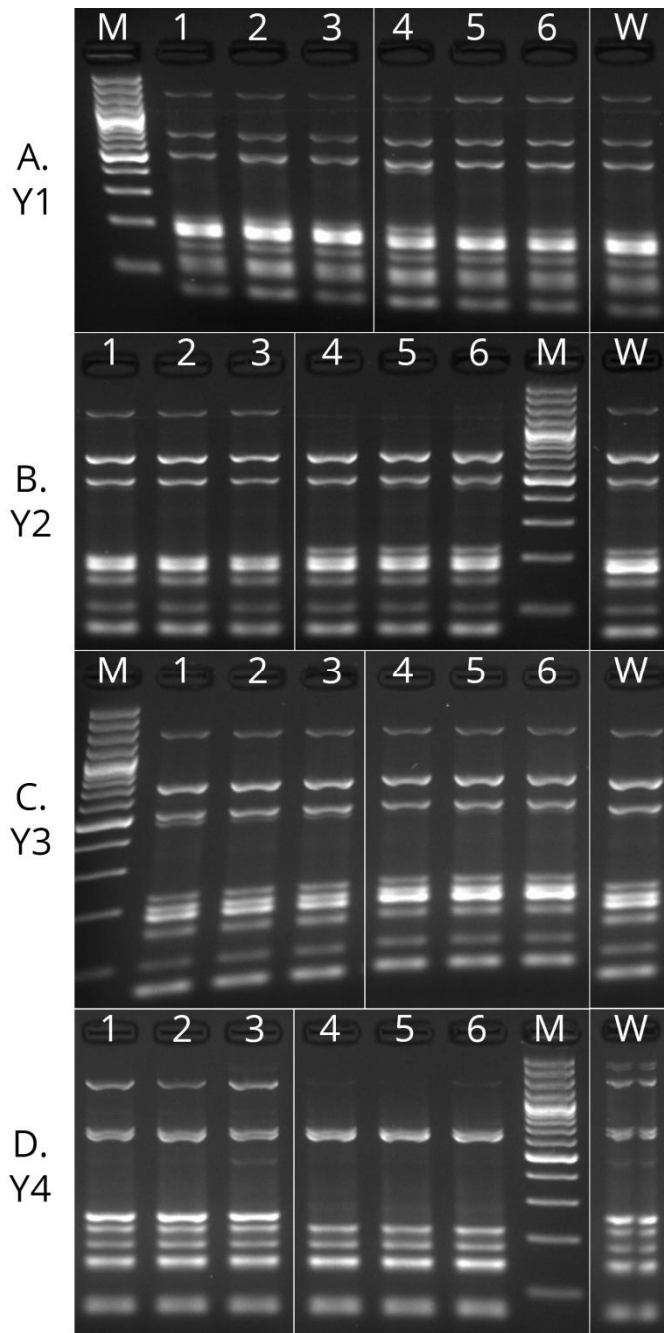


**Figure S1** – The optical densities of the isolates (selected after 10, 20 and 30 consecutive batch fermentations) of the yeast strains **(A, B)** Y1, **(C, D)** Y2, **(E, F)** Y3, and **(G, H)** Y4 in the screening media (6.2% malt extract, 10% sorbitol, 5% ethanol) during high-throughput screening in 96-well plates. The black line depicts the wild-type strain (average calculated from 12-16 replicate fermentations). Red lines to the left **(A, C, E, G)** and blue lines to the right **(B, D, F, H)** depict isolates obtained from adaptation in the media M1 (containing 10% ethanol - 2% maltose) and M6 (10% ethanol - 1% maltose – 1% maltotriose), respectively. For each of the three isolation points (10, 20 and 30 consecutive batch fermentations), four isolates were selected per yeast strain per media (a total of 24 isolates per parent strain). Three replicate fermentations were carried out for each isolate.

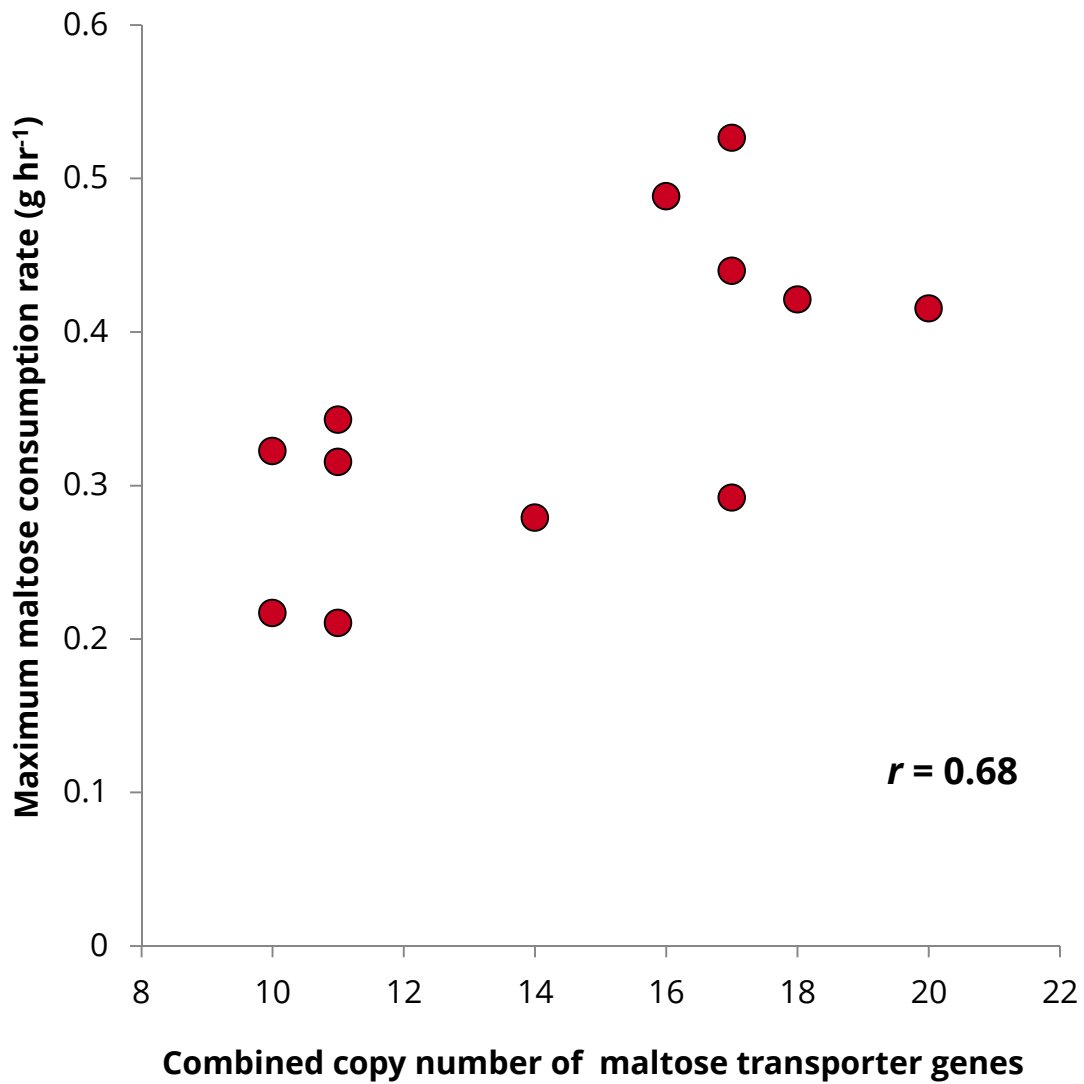


**Figure S2** – The concentrations of maltotriose (diamonds and solid lines), maltose (squares and dashed lines) and monosaccharides (sum of glucose and fructose; circles and dotted lines) in the wort during the 2L-scale fermentations from 15 °P wort at 15 °C with wild-type (black symbols) and variant (red and blue symbols) strains derived from yeast strains **(A)** Y1, **(B)** Y2, **(C)** Y3, and **(D)** Y4. Values are means from two independent fermentations and error bars where visible represent the standard deviation.





**Figure S3** - DNA fingerprints with delta12 and delta21 primers of the eight variant strains (derived from **(A)** Y1, **(B)** Y2, **(C)** Y3, and **(D)** Y4) before and after cultivation for over 80 generations. **M**: 100 bp DNA ladder. **W**: Wild-type strain. **1**: Variant from Media M1. **2-3**: Random colonies of **(1)** after 80 generations. **4**: Variant from Media M6. **5-6**: Random colonies of **(4)** after 80 generations.



**Figure S4** – The correlation between the maximum maltose consumption rate (g hr<sup>-1</sup>) observed during the 2L-scale wort fermentations, and the combined copy number of genes related to maltose transport (*MALx1*, *MTT1* and *MPH2*) in the wild-type and variant strains (Table S5). *r* denotes the Pearson’s correlation coefficient between the two datasets.

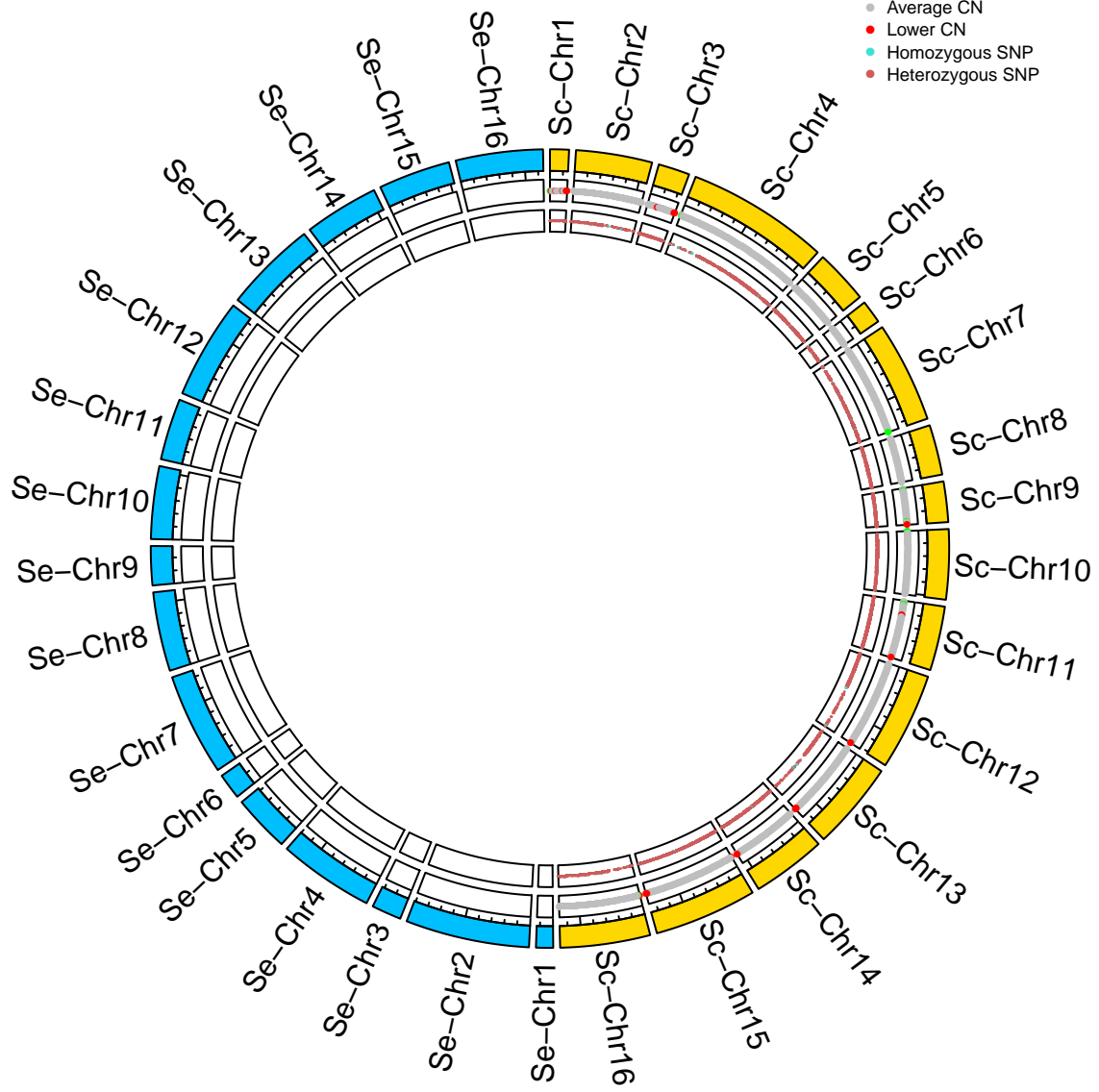
**Figures S5 – S16** – ‘Circos-style’ plots of all the yeast strains used in the study. The outer most track shows the chromosomes of the reference genome to which the sequencing reads were aligned (*S. cerevisiae* chromosomes in blue, *S. eubayanus* chromosomes in yellow). The middle track shows the normalized median coverage in 10000 bp windows (green denotes coverage higher than 1.25 times the mean coverage, red denotes coverage lower than 0.75 times the mean coverage, and grey denotes coverage between 0.75 to 1.25 times the mean coverage). The inner most track shows the SNPs and small Indels detected by FreeBayes (blue denotes a homozygous SNP, while purple denotes a heterozygous SNP). Ribbons between chromosomes show interchromosomal translocations.

<b>Figure #</b>	<b>Yeast strain</b>
Figure S5	Y1
Figure S6	Y1_M1
Figure S7	Y1_M6
Figure S8	Y2
Figure S9	Y2_M1
Figure S10	Y2_M6
Figure S11	Y3
Figure S12	Y3_M1
Figure S13	Y3_M6
Figure S14	Y4
Figure S15	Y4_M1
Figure S16	Y4_M6

Y1

Tracks:

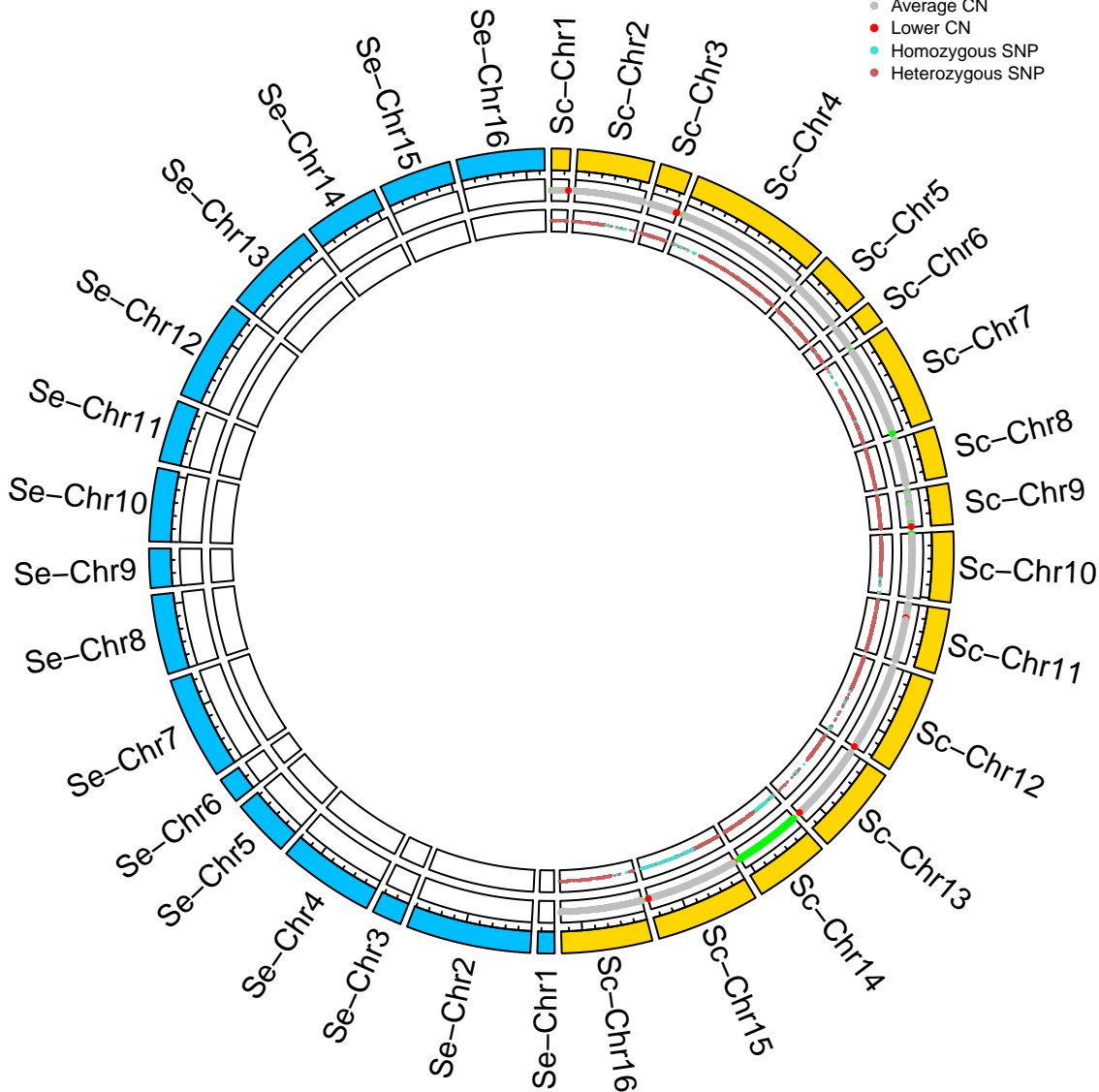
- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP



# Y1\_M1

Tracks:

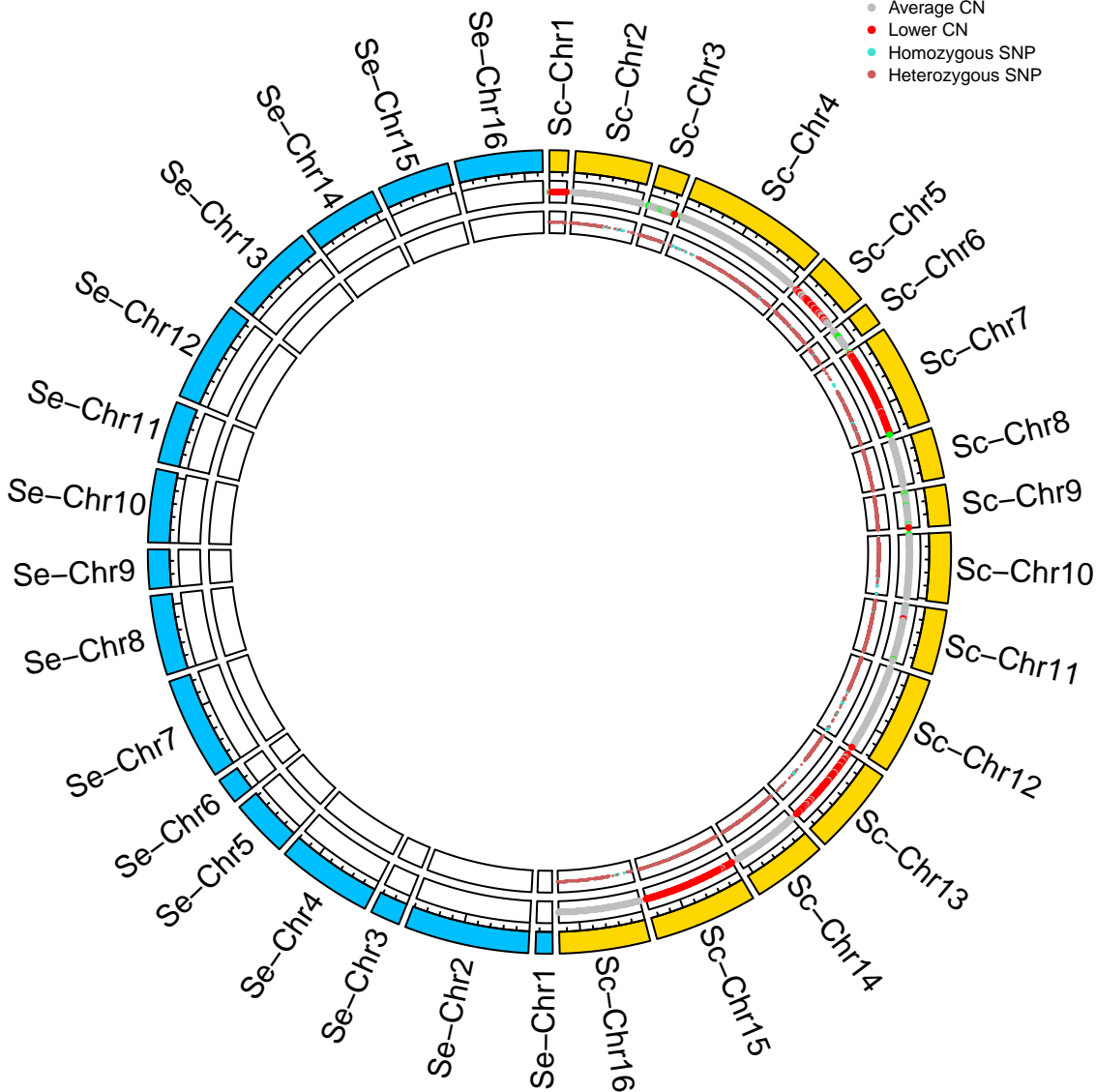
- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP



# Y1\_M6

Tracks:

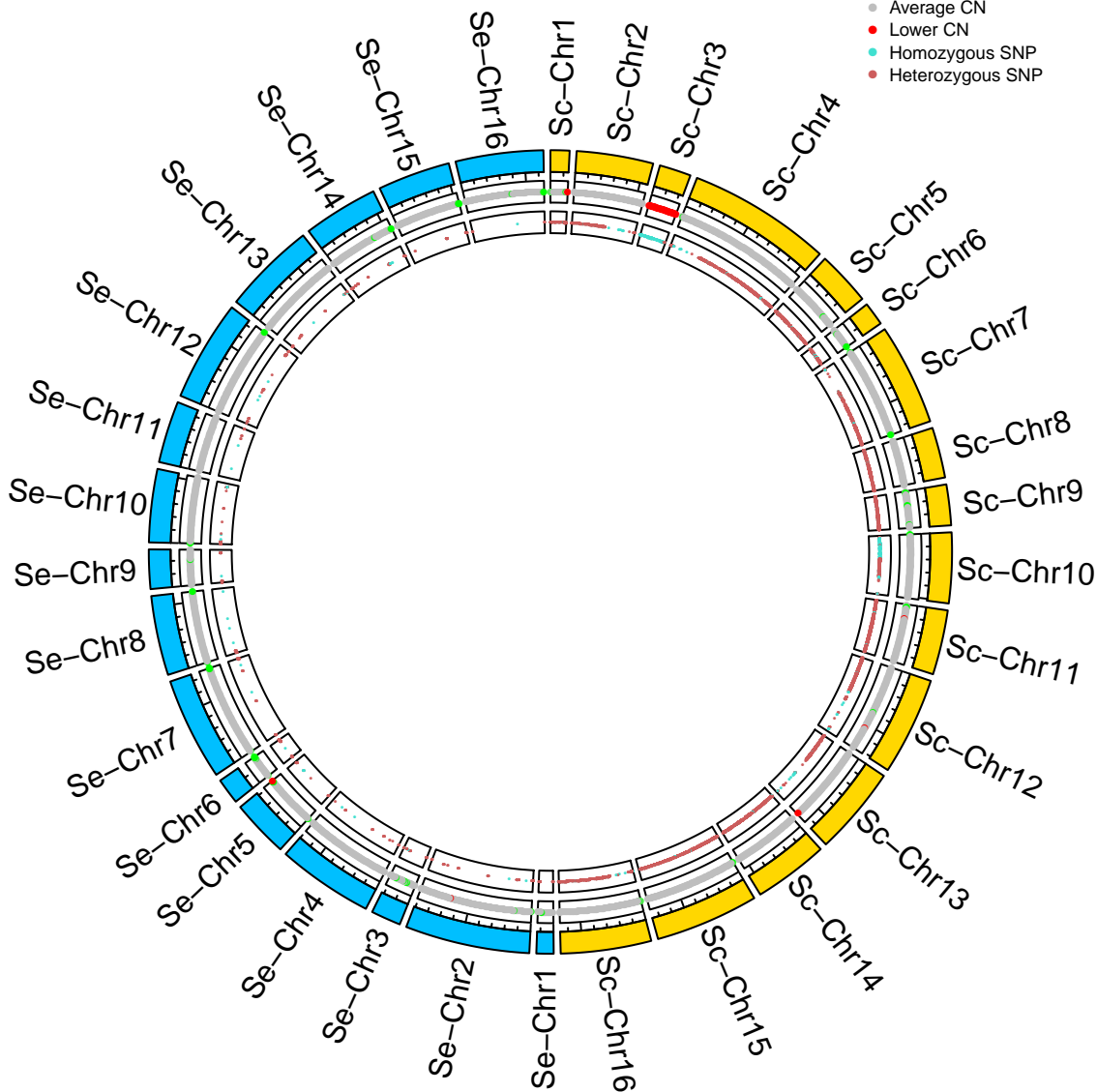
- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP



# Y2

Tracks:

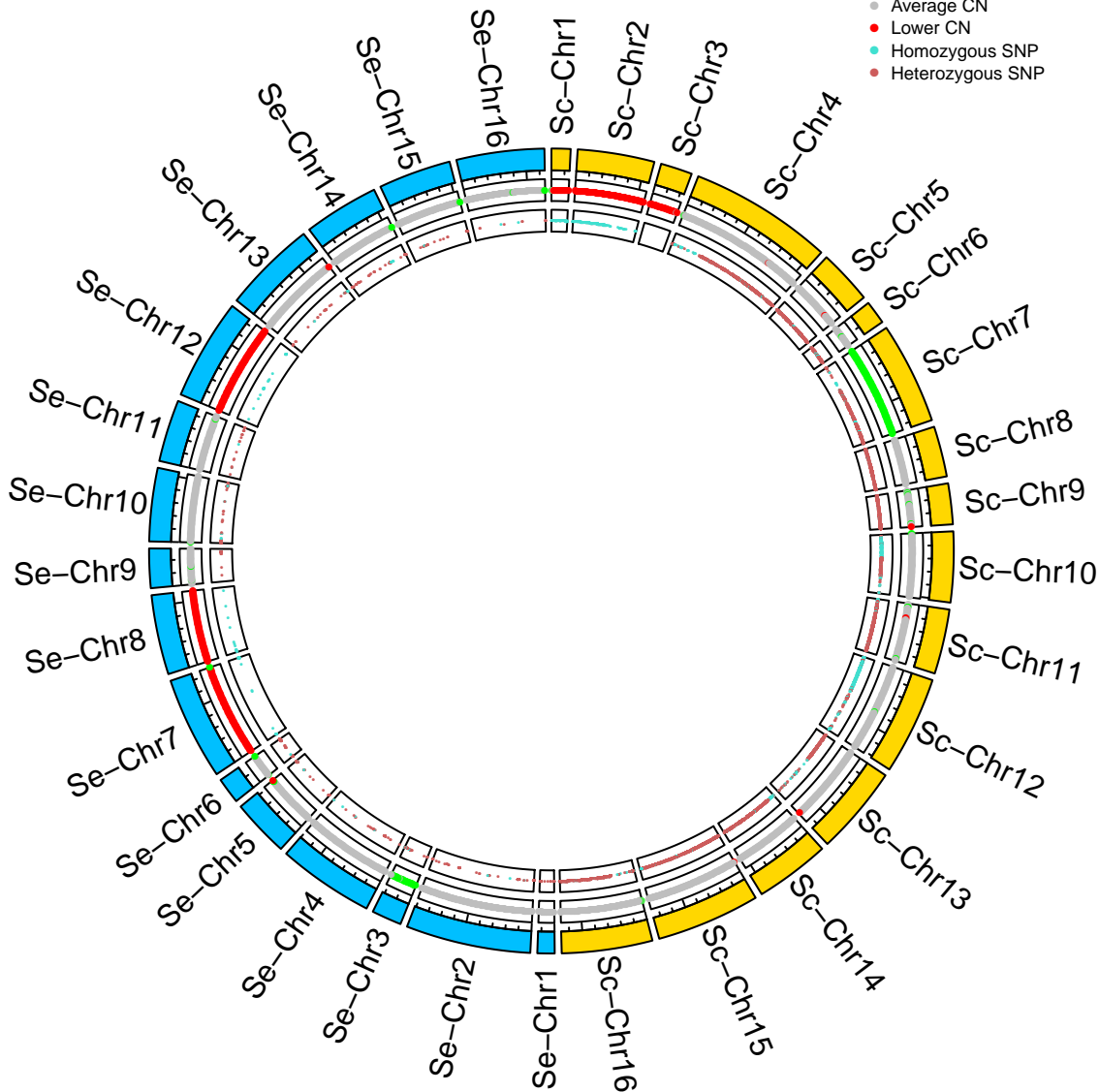
- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP



# Y2\_M1

Tracks:

- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP

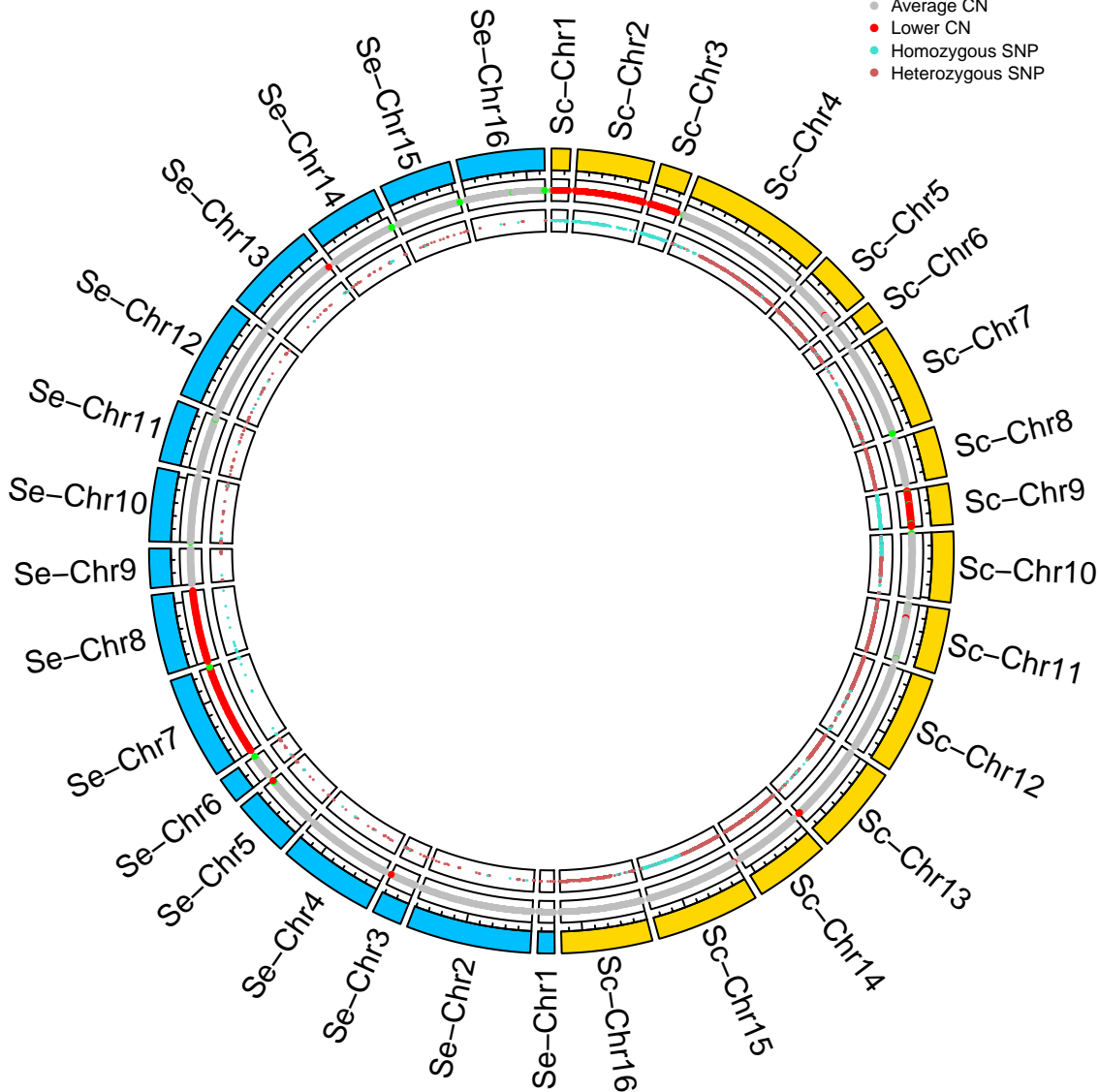




# Y2\_M6

Tracks:

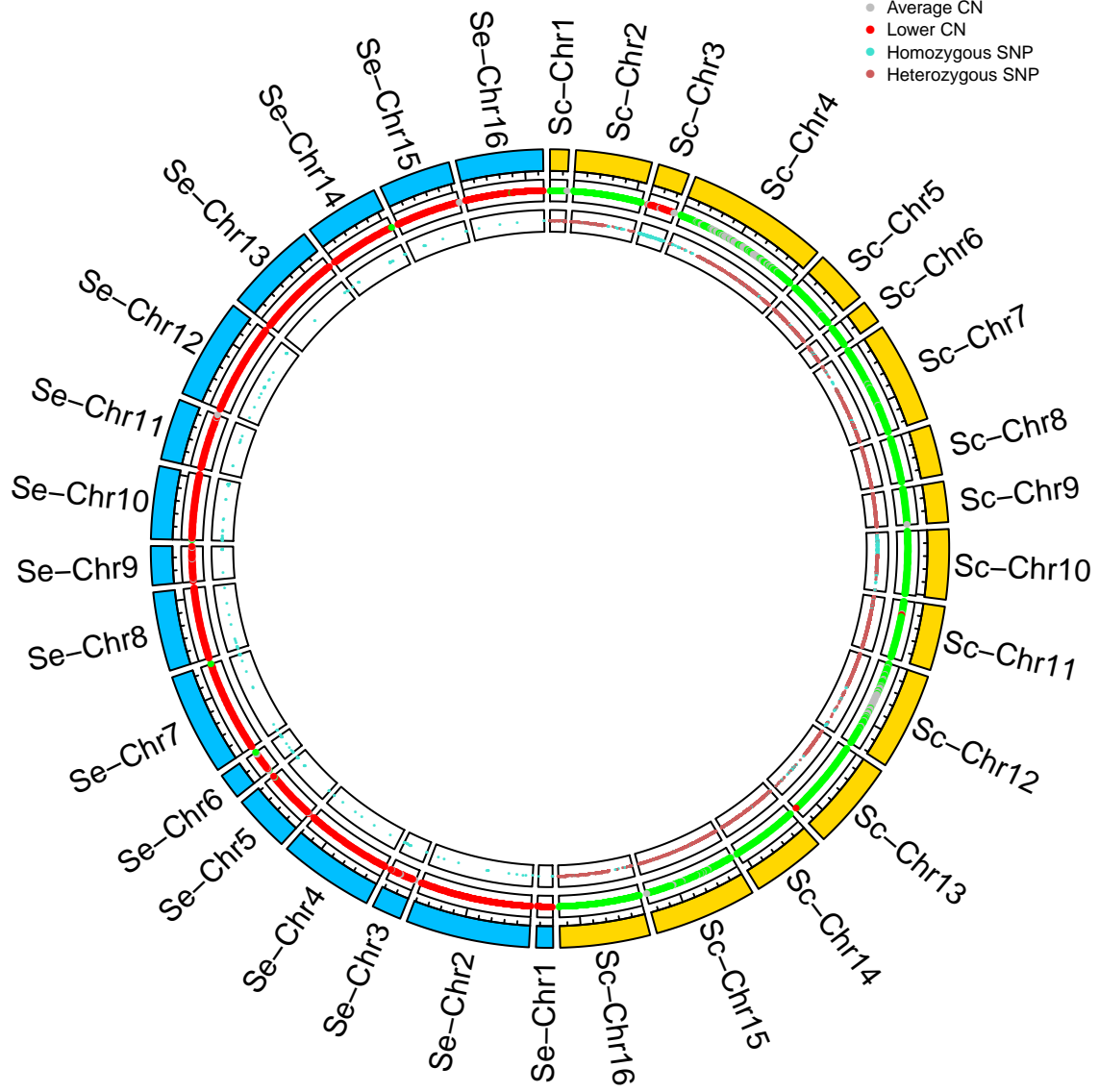
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- Homozygous SNP
- Heterozygous SNP



Y3

Tracks:

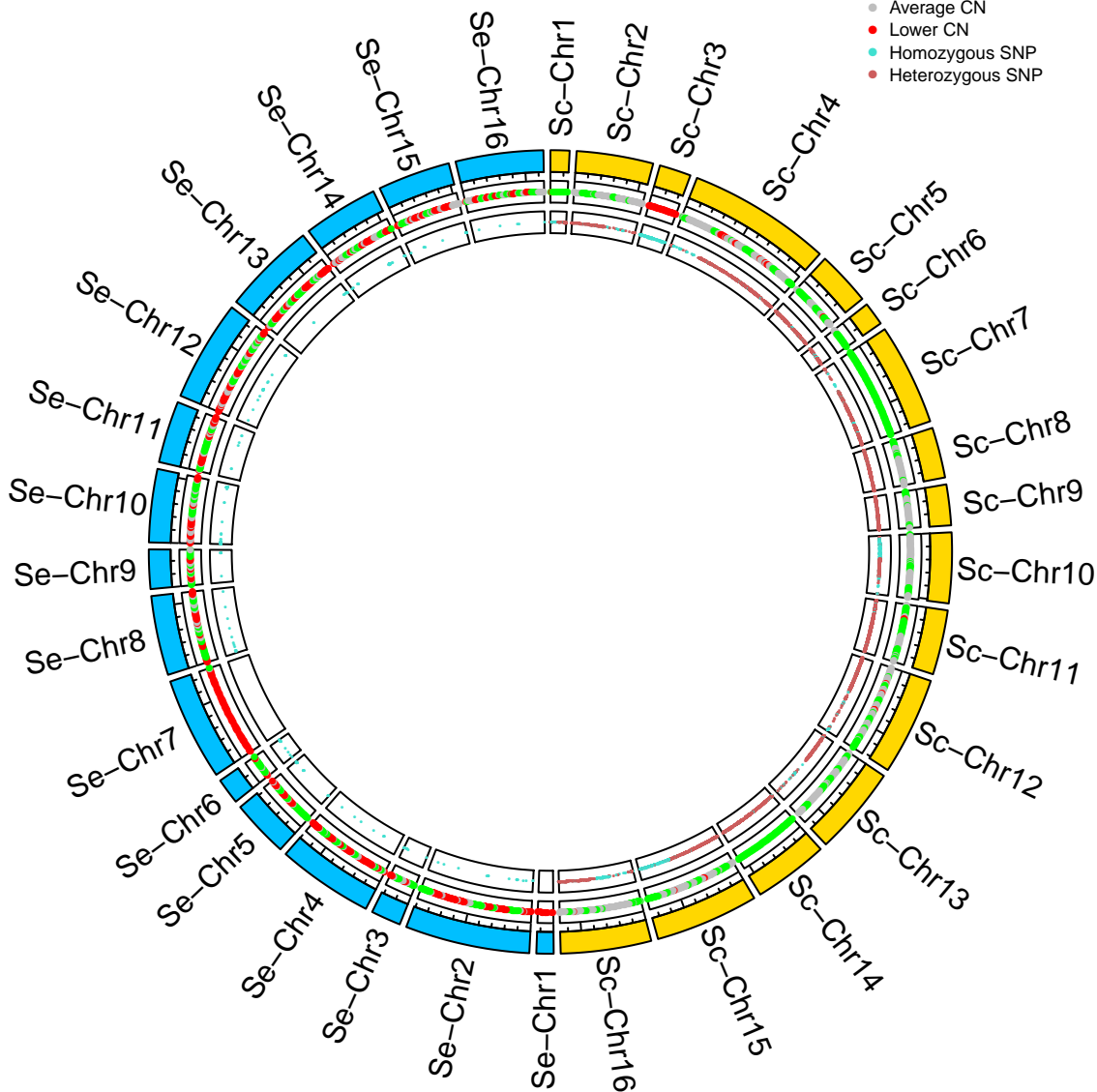
- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP



# Y3\_M1

Tracks:

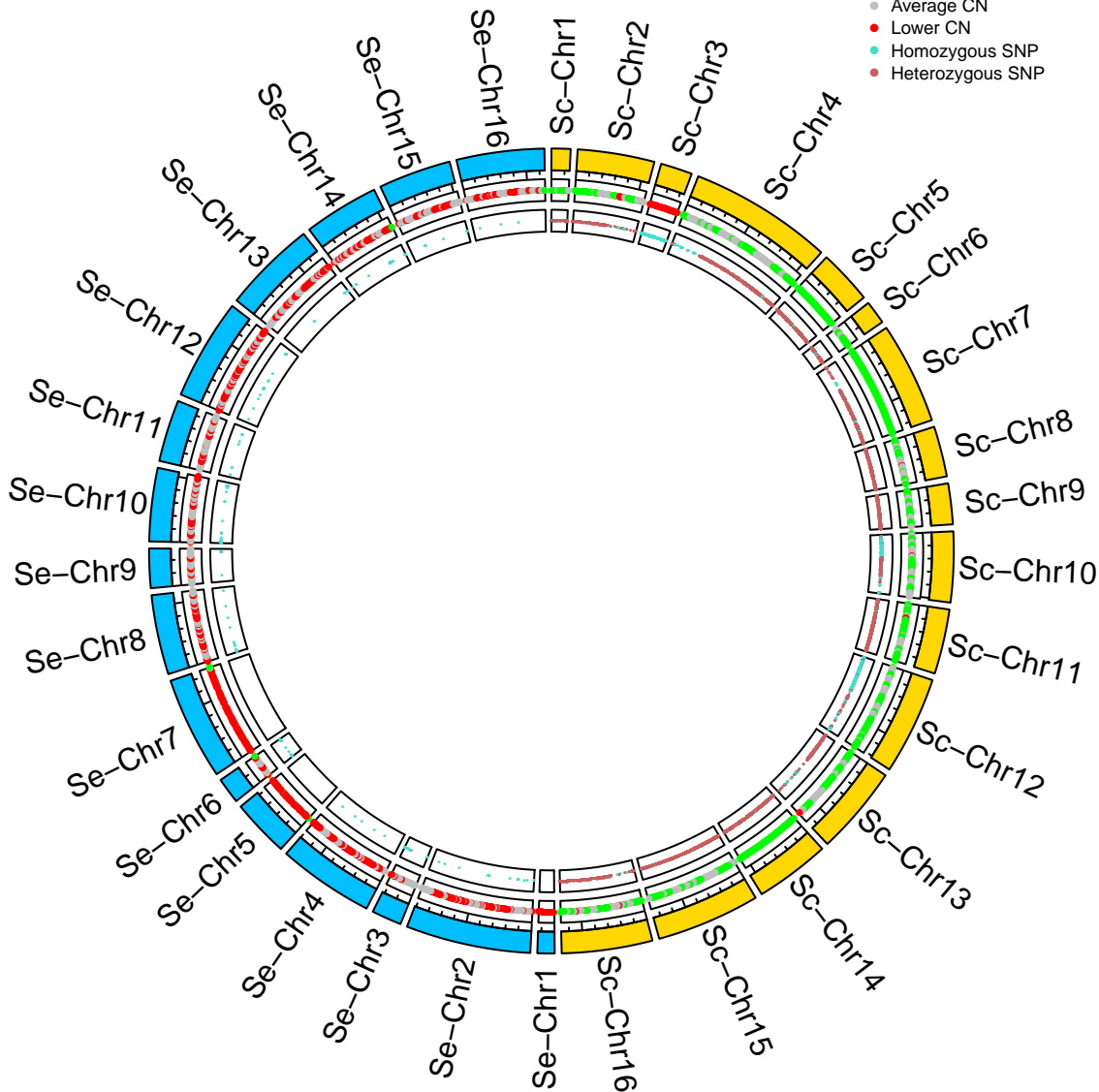
- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP



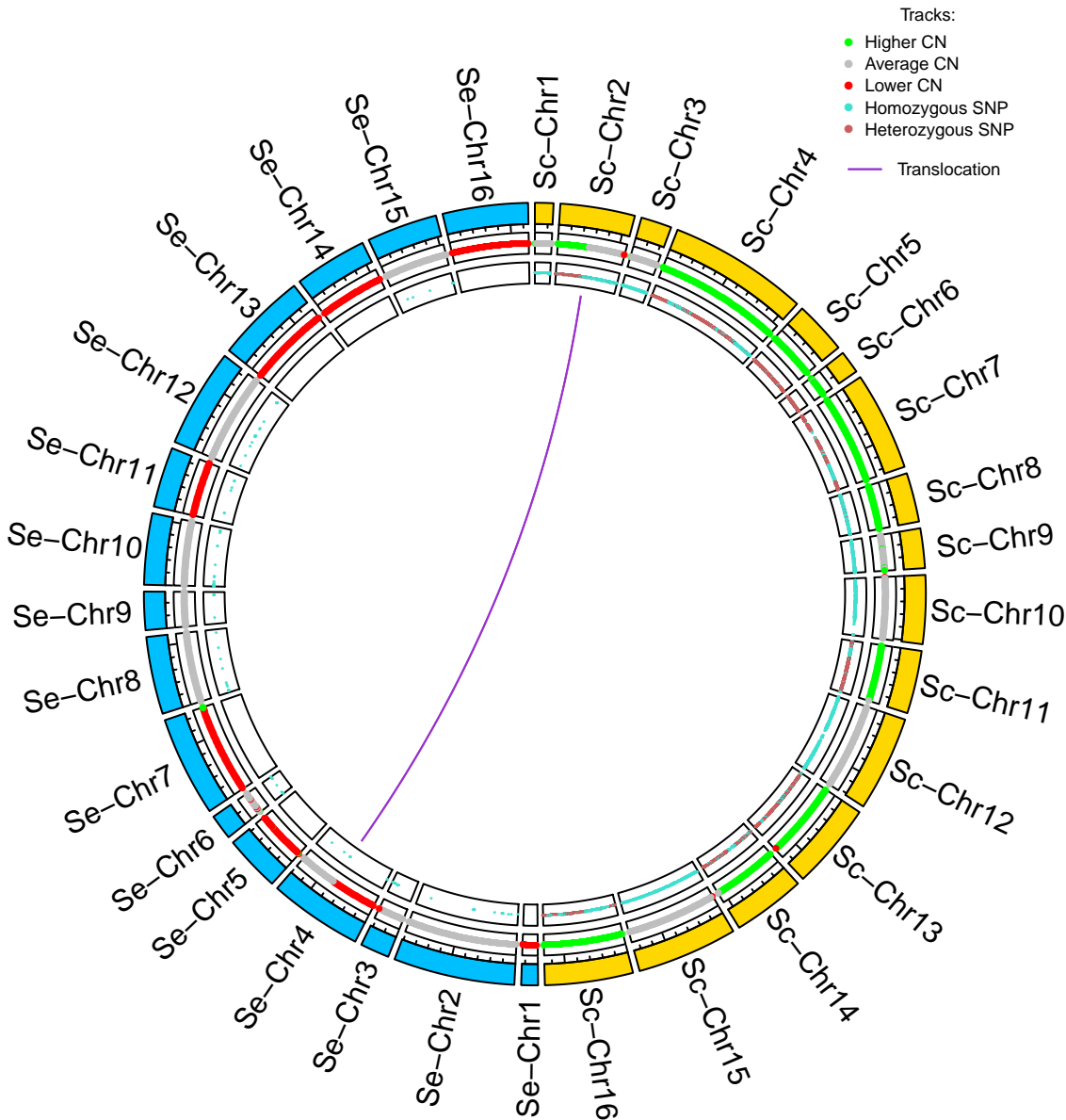
# Y3\_M6

Tracks:

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- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP



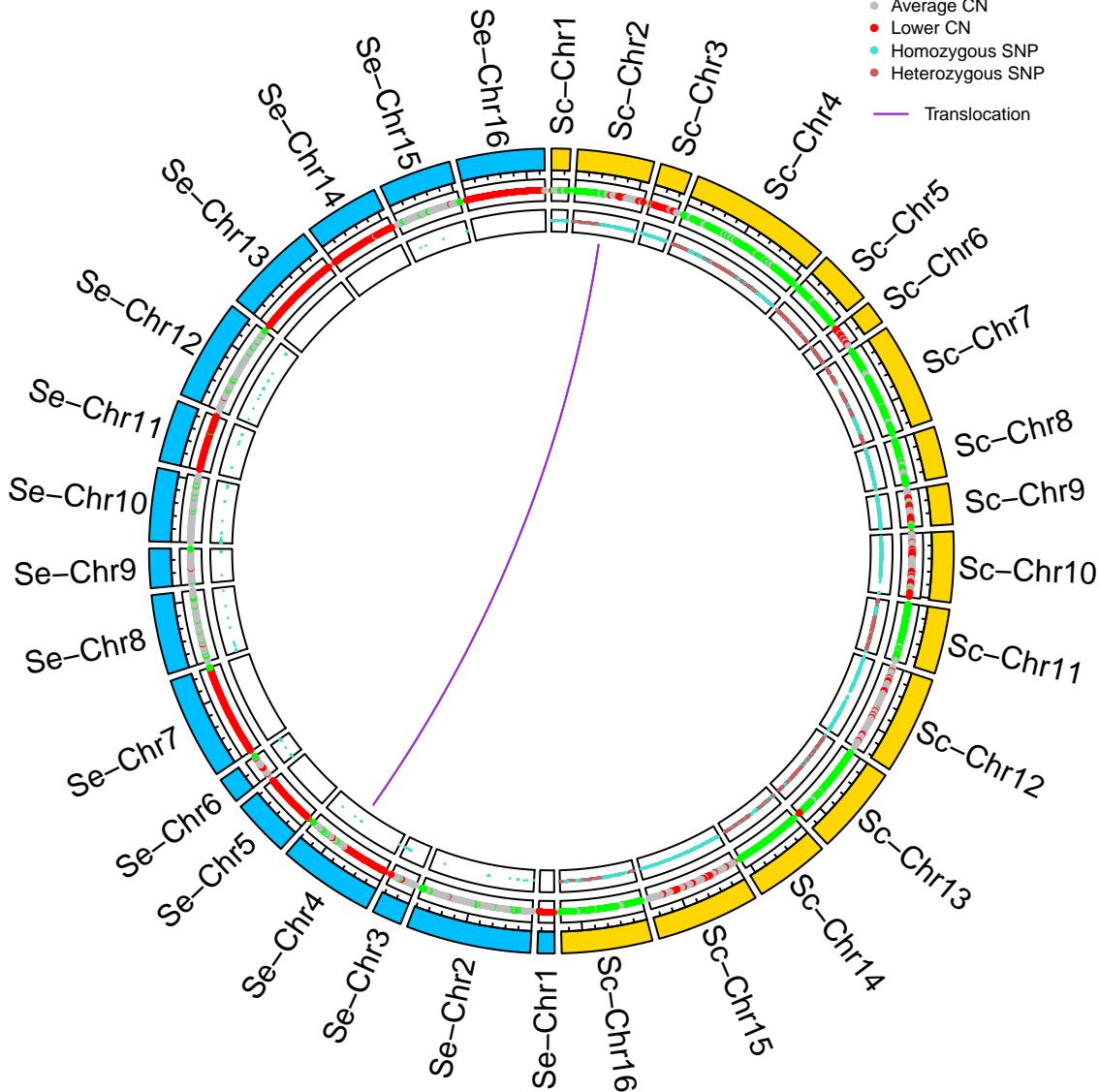
# Y4



# Y4\_M1

Tracks:

- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP
- Translocation



# Y4\_M6

Tracks:

- Higher CN
- Average CN
- Lower CN
- Homozygous SNP
- Heterozygous SNP
- Translocation

