

[16736] In order to confirm this difference is due to neither the difference of hybridization efficiency of each probe set nor cross hybridization between Sc and non-Sc type probe sets, comparative genomic hybridization with the bottom fermenting yeast DNA array was carried out using strain 34/70, a laboratory strain (*S. cerevisiae*) S288C and *S. carlsbergensis* strain IF011023. The preparation of genomic DNA, hybridization to DNA array and detection of the signal intensities were carried out with the method described before. As shown in Table 6, the ratio of signal intensity of non-Sc type to that of Sc type was 1.0 for SSU1 genes and 1.3 for MET14 genes in strain 34/70. This result shows that hybridization efficiencies of Sc and non-Sc probe sets were almost same.

[16737] Furthermore, strain S288C, which does not have non-Sc type genes, showed very low signal intensities to non-Sc type probe sets, and strain IF011023, which does not have Sc type SSU1 gene and Sc type MET14, showed very low signal intensities to Sc type SSU1 and Sc type MET14 probe sets. These results clearly show that cross hybridization did not occur between Sc and non-Sc type probe sets.

[16738] From these results, in strain 34/70, the expressions of non-Sc SSU1 and non-Sc MET14 were significantly higher than those of Sc SSU1 and Sc MET14, respectively. These genes are thought to be candidates which contribute to the high sulfite production ability of bottom fermenting yeast.

[16739] In conclusion, it was revealed that gene expression analysis of brewing yeast strains using the bottom fermenting yeast DNA array was useful for the selection of gene(s) for functional analysis.

TABLE 5

	gene			
	Sc_SSU1	non_Sc_SSU1	Sc_MET14	non_Sc_MET14
	probe set			
	Sc-3594-1 at	Lg-3333-1 at	Sc-2246-1 at	Lg-1564-1 at
signal intensity	145.2	490.4	177.3	1245.8

[16740]

TABLE 6

strains	gene probe set	Sc_SSUI	non_Sc_SSUI	Sc_MET14	non_Sc_MET14
		Sc-3594-1_at	Lg-3333-1_at	Sc-2246-1_at	Lg-1564-1_at
34/70	signal	360.9	356.8	244.2	324.8
S288C	intensity	516.2	6.5	405.3	13.4
<i>S. carlsbergensis</i> IF011023		8.5	746.9	6.8	508.4

Example 20

Classification of Brewing Strains by Comparative Genomic Hybridization with the Bottom Fermenting Yeast DNA Array

[16741] Preparation of yeast genomic DNA and hybridization to the DNA array was carried out as described in (Example 8). Detection of the signal intensity of the DNA array was carried out using a Gene Chip Analysis Basic

System and analysis software (GCOS; GeneChip Operating Software 1.0) manufactured by Affymetrix. The percentage of probes, which the DNA of brewing yeast hybridized was calculated and the identity between strain 34/70 and the tested strain was estimated as shown in Table 7. Strains BH225, BH232 and BH235 hybridized to more than 99% of both Sc type and non-Sc type probes of the bottom fermenting yeast DNA array. It suggests that these strains are very close to strain 34/70, and that this array is useful for the gene expression analysis of these strains. On the other hand, strain BH212 showed relatively low (97.8 and 97.7% for Sc type and non-Sc type probe, respectively) percentage of hybridization, which means this strain is a little bit different from strain 34/70. From these results, relationship among lager brewing strains can be estimated and classification of lager brewing strains can be carried out.

[16742] From the result of analysis of strain BH212, some loci which showed very low signal intensities were found. They may be lost in strain BH212 or their sequences may be different from those of strain 34/70. In contrast, some loci which showed high signal intensities were also found. They may be high in copy number in strain BH212. Such loci can be selected for functional analysis because they may contribute to the difference of fermentation character between strain BH212 and strain 34/70.

TABLE 7

	percentage of hybridized probes				
	Strain No.				
	34/70	BH225	BH232	BH235	BH212
Sc type	99.6	99.8	99.8	99.8	97.8
Non-Sc type	99.5	99.9	99.9	99.6	97.7

Example 21

Detection of Nucleotide Polymorphism

[16743] Furthermore, (single) nucleotide polymorphism was detectable by the analysis of comparative genomic

hybridization. The sets of oligonucleotides for each probe consist of Perfect Match oligonucleotide (PM) which is identical to the sequence of strain 34/70 and MisMatch oligonucleotide (MM) which contains a single base mismatch in the central position of the oligonucleotide. Genomic DNA of a laboratory strain S288C was hybridized to the bottom fermenting yeast DNA array. As shown in Table 8, probes which showed higher (more than 5-fold) signal in MM than in PM had single nucleotide polymorphism.