



CONFIDENTIAL

**Analysis of
Sykkylven Kveik sample
D5559**

**Received: 16/03/16
Via Lars Marius Garshol
for
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Tormodgarden

Sykkylven

Norway

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Interpretation of Results

The fingerprinting technique gives rise to DNA fragments of various lengths. The distribution of fragment lengths will vary from strain to strain. To visualise the DNA fragments the mixture is loaded onto a 1.5% agarose gel and electrophoretically separated. The rate of migration through the gel depends on size. The smaller the piece of DNA the further it migrates. Following staining the DNA can be visualised and photographed under UV illumination. These Photograph(s) are included in the report.

Normally there will be between 5-20 bands per track, depending on the strain. The differences between strains are shown as the presence or absence of bands of specific sizes. Closely related strains will have a number of bands in common, while unrelated strains will have totally different banding patterns.

The presence or absence of bands reflect a difference in the DNA, but this need not necessarily manifest itself as a phenotypic difference.

The resolving power of the technique can be used for a variety of different purposes eg. to authenticate the identity of a strain grown by a yeast supply company, or to monitor a strain in a mixed strain fermentation. The technique works on colonies, yeast cake, liquid cultures and spray dried yeast. Patterns can be obtained from very small samples.

Analysis of Sykkylven Kveik sample D5559:

A sample of Sykkylven kveik yeast as a liquid yeast slurry was received from Lars Marius Garshol for analysis and deposit into the NCYC collection. This was assigned the temporary NCYC number D5559. A sample from this was diluted in sterile water and streaked onto standard YM plates in order to provide separate colonies for analysis.

No bacterial contamination was detected on the agar plates of the Sykkylven yeast.

After 3 days growth at 25°C, the colonies on the plates were of sufficient size for analysis. 12 single colonies and a sample of confluent growth from the streak plates of the yeast were used for the analysis.

The fourteen tracks seen on the photograph of the gel are as follows:

1.	Sykkylven Conf.		Type
	Sykkylven Col. 1		1
	Sykkylven Col. 2		2
	Sykkylven Col. 3		2
	Sykkylven Col. 4		3
	Sykkylven Col. 5		4
	Sykkylven Col. 6		5
	Sykkylven Col. 7		6
	Sykkylven Col. 8		7
	Sykkylven Col. 9		8
	Sykkylven Col. 10		6
	Sykkylven Col. 11		9
	Sykkylven Col. 12		10
14.	Marker		

1. Confluent growth from agar plate of Sykkylven kveik.
2. Single colony 1 from agar plate of Sykkylven kveik.
3. Single colony 2 from agar plate of Sykkylven kveik.
4. Single colony 3 from agar plate of Sykkylven kveik.
5. Single colony 4 from agar plate of Sykkylven kveik.
6. Single colony 5 from agar plate of Sykkylven kveik.
7. Single colony 6 from agar plate of Sykkylven kveik.
8. Single colony 7 from agar plate of Sykkylven kveik.
9. Single colony 8 from agar plate of Sykkylven kveik.
10. Single colony 9 from agar plate of Sykkylven kveik.
11. Single colony 10 from agar plate of Sykkylven kveik.
12. Single colony 11 from agar plate of Sykkylven kveik.
13. Single colony 12 from agar plate of Sykkylven kveik.
14. Standard Marker

Results:

Ten different pattern types were produced by the 12 individual colonies

Each pattern type was assigned a number.

Colony 1 produced a unique pattern. This was designated Type 1

Colonies 2 and 3 produced the same pattern as each other. This was designated Type 2

Colony 4 produced a unique pattern. This was designated Type 3

Colony 5 produced a unique pattern. This was designated Type 4

Colony 6 produced a unique pattern. This was designated Type 5

Colonies 7 and 10 produced the same pattern as each other. This was designated Type 6

Colony 8 produced a unique pattern. This was designated Type 7

Colony 9 produced a unique pattern. This was designated Type 8

Colony 11 produced a unique pattern. This was designated Type 9

Colony 12 produced a unique pattern. This was designated Type 10

The patterns produced are complex and difficult to group. However Types 9 and 10 appear to be very closely related. Types 2 and 3 may also be related.

Types 1 and 6 may be related to each other.

Similarly Types 7, 8 and 4 may be related. Type 5 may also be distantly related to this group.

Hence the patterns possible fall into three main groups:

Types 2, 3, 9 and 10.

Types 1 and 6

Types 7, 8, 4 and possibly 5

Conclusions:

- 1) 10 different pattern Types were given by the 12 individual colonies tested. The Sykkylven kveik sample as a whole is therefore composed of a complex mixture of strains.
- 2) Since nearly all the colonies tested gave a unique pattern it is likely that a number of other unique strain types are present in the mixture, which were undetected in the current study.
- 3) No contaminants, either bacterial or non- *Saccharomyces* were found.
- 4) The patterns produced are complex and therefore difficult to compare although, as mentioned above, the patterns may belong to three main groups, possibly denoting the presence of at least three parent/majority strain types which have produced variants.

Overall Conclusions:

Based on the analysis of the supplied sample:

The sample is free of any contaminants, either bacterial, or non-*Saccharomyces*.

The sample is composed of a complex mixture of strain types several of which are likely to be related.

It is likely that the mixed culture contains further unique strains in addition to those already found.

Further work for deposit in the NCYC open collection:

The D5559 sample will have chemo-taxonomic tests carried out on it, be assigned a NCYC collection number and be stored as a mixture, thus preserving all the various strain types present, including those not already isolated.

Each of the individual strain types will be assigned a temporary NCYC D-number and have chemo-taxonomic tests carried out on it. Once these are completed a final NCYC collection number will be assigned and each of the strains stored separately by freeze drying and in liquid nitrogen.

D5559 Type 1 = D5672
D5559 Type 2 = D5673
D5559 Type 3 = D5674
D5559 Type 4 = D5675
D5559 Type 5 = D5676
D5559 Type 6 = D5677
D5559 Type 7 = D5678
D5559 Type 8 = D5679
D5559 Type 9 = D5680
D5559 Type 10 = D5681