



CONFIDENTIAL

**Analysis of
Kveik sample
(Ex Tore Hjelle)
D5654 : #12 Hjelle
Received: 06/01/17
Via Lars Marius Garshol
for
Jarle Nupen
Eidsdal
Norway
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National Collection of Yeast Cultures
IFR Extra Ltd.
Quadram Institute Bioscience
www.ncyc.co.uk

**Yeast Genetic Fingerprinting
National Collection of Yeast Cultures
Quadram Institute Bioscience
Norwich Research Park
Colney
Norwich
NR4 7UA UK**

Contacts: Dr. Ian N Roberts - Senior Scientist
Chris Bond - Collection Manager
Dr Stephen A. James - Molecular Biologist
Jeanette Newman - Research Scientist
Carmen Nueno-Palop - Business Manager

Telephone: National 01603 255274
International +44 (0) 1603 255274

Fax: National 01603 458414
International +44 (0) 1603 458414

Email: ncyc@ncyc.co.uk

Internet Web Site: www.ncyc.co.uk

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 Kveik sample (Ex Tore Hjelle) D5654 : #12 Hjelle

A sample of Hjelle kveik yeast as dried yeast 'chips' was received from Lars Marius Garshol for analysis and deposit into the NCYC collection. This was assigned the temporary NCYC number D5654. A sample from this was rehydrated for 10 minutes in YM broth and then diluted in sterile water and streaked onto standard YM plates to provide separate colonies for analysis.

No bacterial contamination was detected on the agar plates of the Hjelle 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 plate of the yeast were used for the analysis.

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

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

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

14. Standard Marker

Results:

Eight different pattern types were produced by the 12 individual colonies

Each pattern type was assigned a number.

Colonies 1 and 5 produced the same pattern as each other. This was designated Type 1.

Colony 2 produced a unique pattern. This was designated Type 2.

Colonies 3 and 10 produced the same pattern as each other. This was designated Type 3.

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

Colonies 6 and 7 produced the same pattern as each other. This was designated Type 5.

Colonies 8 and 9 produced the same pattern as each other. This was designated Type 6

Colony 11 produced a unique pattern. This was designated Type 7.

Colony 12 produced a unique pattern. This was designated Type 8.

The patterns produced are complex and difficult to group. However, Types 1, 2, 4, 5, 7 & 8 show some similarities and may be related.

Type 3 shows more significant differences in the pattern to the above Types and may be an unrelated strain.

Similarly, Type 6 may be a strain unrelated to the others.

Conclusions:

- 1) 8 different pattern Types were given by the 12 individual colonies tested. The Hjelle kveik sample is therefore composed of a mixture of at least 8 strains.
- 2) No contaminants, either bacterial or non- *Saccharomyces* were found.
- 3) 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 one parent strain type, which gave rise to the Type 1, 2, 4, 5, 7 & 8 variants, and two other unrelated strains (Types 3 & 6).

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 probably related.

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

Further work for deposit in the NCYC open collection:

The D5654 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.

D5654 Type 1 = D5713

D5654 Type 2 = D5714

D5654 Type 3 = D5715

D5654 Type 4 = D5716

D5654 Type 5 = D5717

D5654 Type 6 = D5718

D5654 Type 7 = D5719

D5654 Type 8 = D5720