



CONFIDENTIAL

**Analysis of
Stein Langlo #1 yeast isolate
NCYC 4021
for
Lars Marius Garshol
RÆLINGEN
Norway
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**Yeast Genetic Fingerprinting
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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 Stein Langlo #1 yeast isolate :

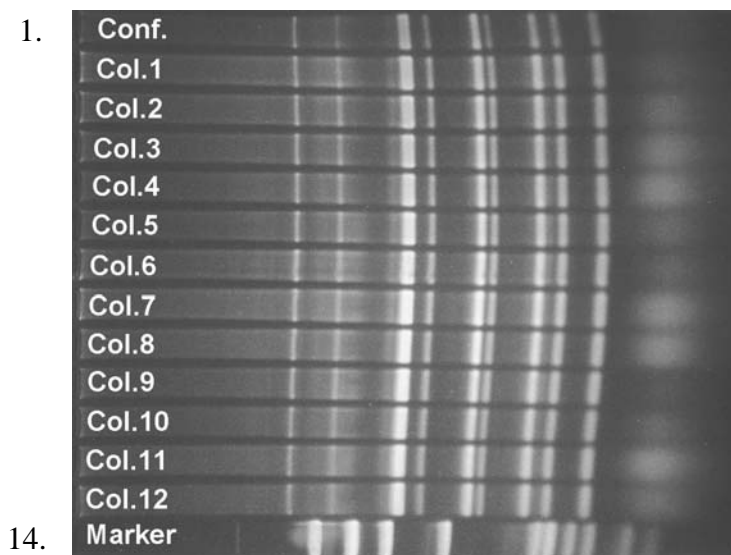
A sample of Stein Langlo yeast #1 was received in August 2014. Although the viability of the sample was found to be very low, live yeast was eventually isolated from the sample after continued incubation at room temperature in YM broth on an orbital shaker.

Since only a small proportion of the original yeast was recovered it was expected that the recovered sample may only contain a single strain type even if the original sample had been composed of a mixture of closely related strains (as has been seen in previous Kveik samples). A loop from the recovered yeast was therefore spread on a YM agar plate to provide confluent growth and individual colonies in order to check if this was the case.

After 3 days incubation at 25°C confluent growth and colonies on the plate had grown sufficiently for analysis.

No bacterial contamination was detected in the sample.

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



1. Confluent growth from Langlo Yeast #1
2. Single colony 1 from Langlo Yeast #1
3. Single colony 2 from Langlo Yeast #1
4. Single colony 3 from Langlo Yeast #1
5. Single colony 4 from Langlo Yeast #1
6. Single colony 5 from Langlo Yeast #1
7. Single colony 6 from Langlo Yeast #1
8. Single colony 7 from Langlo Yeast #1
9. Single colony 8 from Langlo Yeast #1
10. Single colony 9 from Langlo Yeast #1
11. Single colony 10 from Langlo Yeast #1
12. Single colony 11 from Langlo Yeast #1
13. Single colony 12 from Langlo Yeast #1
14. Standard Marker

Results:

All of the individual colonies of the Langlo Yeast #1 sample gave the same fingerprint pattern as the

confluent growth of the Langlo Yeast #1. No variations in pattern type were seen.

Conclusions:

1) The individual colonies of the Langlo Yeast #1 isolate produced an identical fingerprint pattern to the confluent growth of the isolate. It is therefore highly likely to be composed of a single strain.

2) No bacteria, non-*Saccharomyces* or *Saccharomyces* unrelated to the Langlo Yeast #1 isolate were detected.

Overall Conclusions:

Based on the analysis of the sample:

1) The sample is free of any contaminants either bacterial, non-*Saccharomyces* or *Saccharomyces* unrelated to Langlo Yeast #1.

2) The Langlo Yeast #1 isolate is highly likely to be composed of a single strain of yeast. This may be a consequence of having isolated only a small proportion of the original Langlo Yeast #1 sample, due to its low viability. The original may therefore have been either composed of a single strain or may have been composed of a mixture of strains with the current isolate (accessioned into the NCYC as **NCYC 4021**) representing only one of the strains present in the original.

Note: Comparison to other Kveik cultures received from Norway:

The pattern of the Langlo Yeast #1 isolate shows a number of differences to the Kveik samples received in 2009 from Haken Hveem (NCYC 3545 to 3552), the Voss samples and isolates (NCYC 3995 to 3998) and the Muri Farm sample and isolates. It is therefore most likely to be a strain unrelated to the other Norwegian isolates.

Future work will include comparison of all of these on a single gel.

THE RAPID FINGERPRINTING OF YEAST STRAINS

CONDITIONS OF ANALYSIS

The National Collection of Yeast Cultures (NCYC) will analyse yeast samples under the following conditions:

1. Price £275 per strain/gel (unless included in Premium Safe Deposit package).

Payment is net 30 days following receipt of notice.

2. Delivery

The Customer will deliver the samples carriage paid by any method of transport at the Customer's option; all samples will be at the Customer's risk during transit.

3. Performance

3.1

The NCYC will use its best endeavours to carry out the Analysis but in the event of it becoming impossible of performance or otherwise frustrated then the NCYC shall inform the Customer and shall discuss with the Customer whether to abandon or otherwise redirect the Analysis.

3.2

While the NCYC will use its best endeavours to ensure accuracy, the Customer understands and accepts that the results are subject to normal experimental error and are based on the evidence and methods and techniques known at the time.

4. Endorsement

The Customer shall not use the results supplied by the NCYC in such a way as to suggest or imply any recommendation by the NCYC of the Customer's products or services or any connection between the NCYC and the Customer. The Customer agrees to submit for the NCYC's prior written approval any advertising or similar material in which the Customer refers to the NCYC.

5. Confidentiality

All communications from the Customer to the NCYC and all information and other material supplied to or received by the NCYC from the Customer which is by its nature intended to be exclusively by the knowledge of the Customer alone shall unless otherwise agreed to be kept confidential by the NCYC.

6. Limitation of Liability

The total liability of the NCYC to the Customer for any and all losses and damages arising from any cause whatsoever shall in no circumstances exceed the greater of the Contract price or the full economic cost actually paid by the Customer to the NCYC.

7. Health and Safety

The Customer shall disclose fully and fairly to the NCYC any risk or hazard to the health of humans, animals or plants known or reasonably thought by the Customer to be inherent to any item or associated with its testing in accordance with the Contract.

8. Arbitration

If after discussion concerning any difference, dispute or question arising under this Contract parties are unable to reach agreement, the matter shall be resolved according to the provisions of the Arbitration Act 1950 or any statutory modification or reenactment thereof by any independent body or person nominated by the parties and in the absence of agreement to such nomination by a body or person nominated by the President of the Law Society.

10 Jurisdiction

This Contract shall be subject to English Law.