

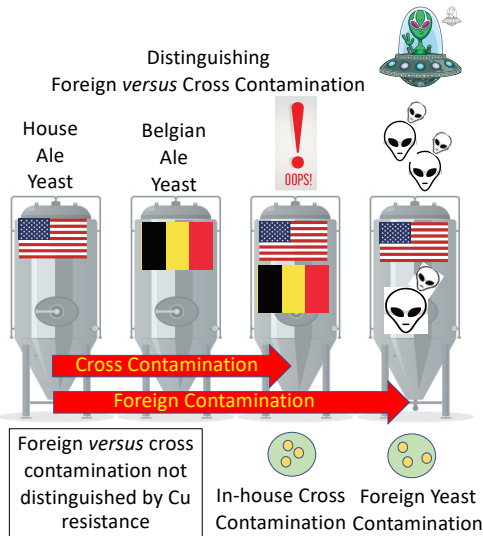
A-42

Matthew T. Cottrell

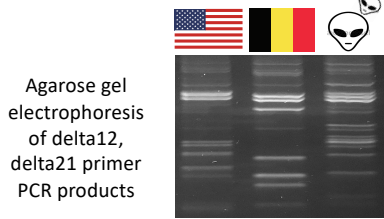
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Graphical Abstract



1- Yeast transposon long terminal repeat (LTR) PCR product fragments distinguishing between yeast strains



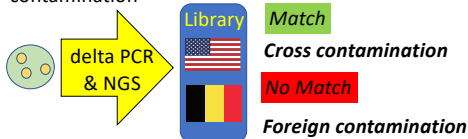
2- Next generation sequencing (NGS) of PCR products generates strain-identifying fingerprints



3- Library of LTR fingerprints identifying in-house yeast strains

- > **House Ale Yeast**
CATCTTAACCCGTATATGATAATATATTGATTGTAG
AGCATGTGGATTTTGATGTAATTGTTGGATTCCAT
- > **Belgian Ale Yeast**
TCAACAATGGAATCCCAACAATTATCTCAAATCCA
CATTCTCAACATTTCTGTGCATCATATAATCTTAGG

4- Identify cross contamination versus foreign contamination



5- Apply appropriate corrective action for cross versus foreign yeast contamination

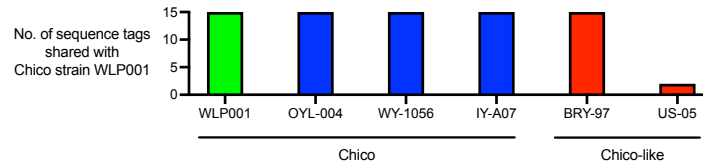
The Full Story

Reproducible Ty1 sequence tags readily distinguish yeast strains

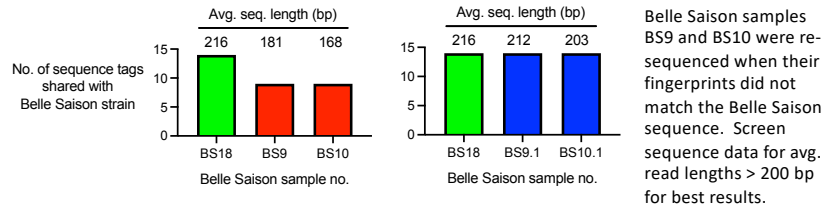
No. of shared sequence tags (n=15 total tags)

Yeast strain	Within strain	Between strains	No. of samples
Chico Ale	15	1	3
Belle Saison	14-15	1	5

Chico strains differentiated from Chico-like strains

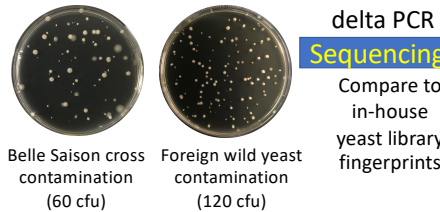


Low quality sequence data identified by average read length

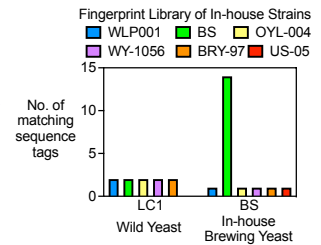


Identifying cross contamination versus foreign contamination

Chico ale yeast sample (1E7 cells) plated on Cu-containing medium (FOWY)

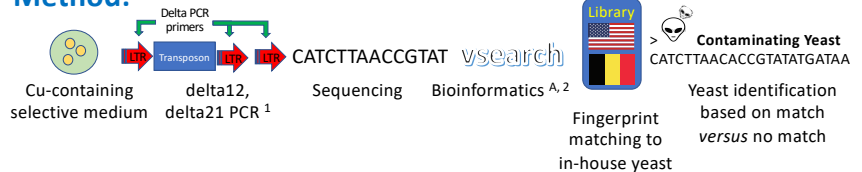


delta PCR Sequencing → Compare to in-house yeast library fingerprints



Corrective actions: Cross contamination – Improve containment of yeasts used in production
Foreign contamination – Improve isolation of fermenting beer from brewery environment

Method:



Summary:

- 1- Identifying the source of yeast contamination is essential for designing a corrective action
- 2- Transposons of yeast (Ty1) amplified by delta PCR provide fingerprints distinguishing among yeast strains
- 3- DNA sequencing of delta PCR products for in-house strains provides a reference library
- 4- Cross contamination versus foreign contamination distinguished by matching to in-house reference library

