A W R I

The Australian Wine Research Institute

Construction of a Wine Yeast Genome Deletion Library (WYGDL)

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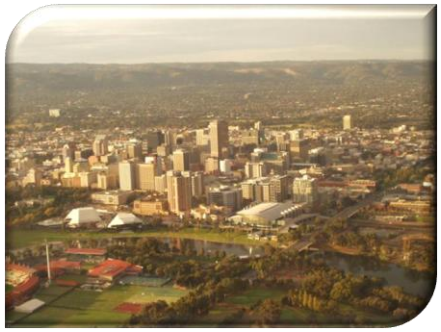
Australian Wine Industry

The Australian Wine Research Institute

- Australia has about 60 wine regions.
- 170,000 hectares under vine
- Australia was ranked 6th in the list of world wine producers in 2005 producing 1.4 billion L of wine
- Australia is the 4th largest exporter after France, Italy and Spain.
- Our wines are drunk in more than 100 countries



AWRI

Established
26th April 1955

Location
Adelaide,
Waite campus

Main funding
Australian Grapegrowers + Winemakers & Australian Gov

Research
Microbiology
Chemistry
Sensory

What is a WYGDL?

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- In winemaking *Saccharomyces cerevisiae* is the species of yeast most commonly used for fermentation.

- *S. cerevisiae* has about 5000 genes.
- We are creating a collection of strains derived from a single *S. cerevisiae* wine strain, in which each strain has a different, single gene deleted.
- Eventually we will have a collection of up to 5000 strains (1 per gene).
- In collaboration with Charlie Boone's lab (University of Toronto).

What is a deletion library good for?

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AWRI

- At the AWRI we are interested in understanding the basis of wine characteristics (flavour, aroma etc) and fermentation processes (robust, predictable etc).

The Deletion Library

- Provides a collection of mutant yeast strains.
  - No need to do gene knockouts, simply go to the library
- Mutant screens.
  - No need to do mutagenesis, all the mutants are there with tags to aid in their identification
- Enables parallel screening using the barcode sequences.

Laboratory Strain Deletion Library

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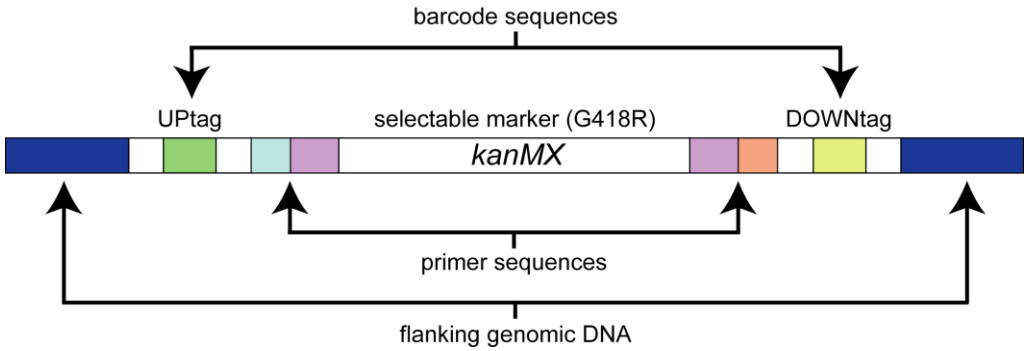
- A Genome Deletion Library is already available in a laboratory strain of *S. cerevisiae, S288c*.
- It was created by a consortium of 13 labs over several years from 1997-2002.
- 96% of all ORFs >100 codons deleted
  - Replaced with kanMX marker gene
  - Two unique 20 bp “barcodes”



Why make a deletion library in a wine yeast?

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- The lab strain yeast performs poorly under winemaking conditions.
- To more fully understand the contribution of yeast genetics to flavour, aroma and grape-juice fermentation characteristics we require a strain that is suitable for both molecular biology and winemaking.
  - Haploid, stable genome
  - Reliable fermentation performance without the production of off-flavours
- We selected a wine strain (N96) from our collection.
- N96 is a robust fermenter with neutral sensory characteristics.

Preparing the wine strain

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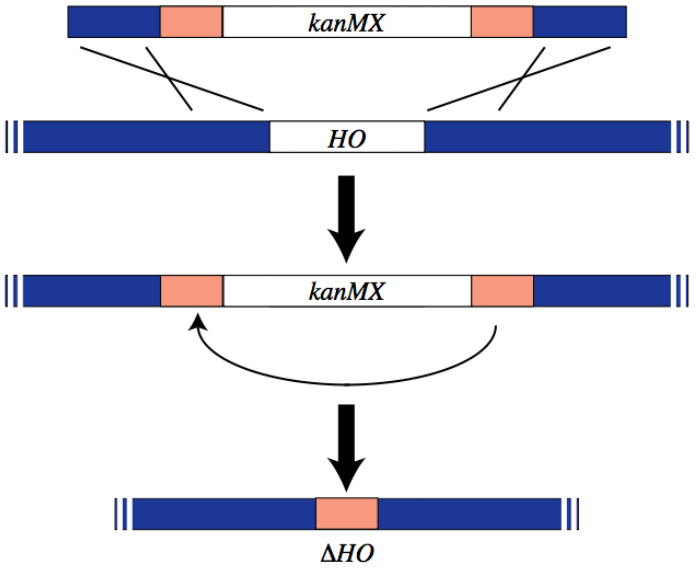
- Most wine-yeast strains, including N96, are diploid.
- Haploid yeasts revert to a diploid state via mating type switching followed by mating between opposite mating types.
- *HO* gene is a key requirement for mating type switching
- *HO* was therefore deleted from the N96 diploid progeny using *HO*::*kanMX* from Walker et al., 2003



Sensory comparisons

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- We obtained several haploid progeny which displayed fermentation rates and metabolite profiles (glycerol, acetic acid, ethanol) which were comparable to the diploid parent.
- Sensory trials determined that the haploids produced wine with similar characteristics to the diploid parent.
- We selected one AWRI1631 to use as the deletion library strain.

Introducing barcoded deletions into *S. cerevisiae*

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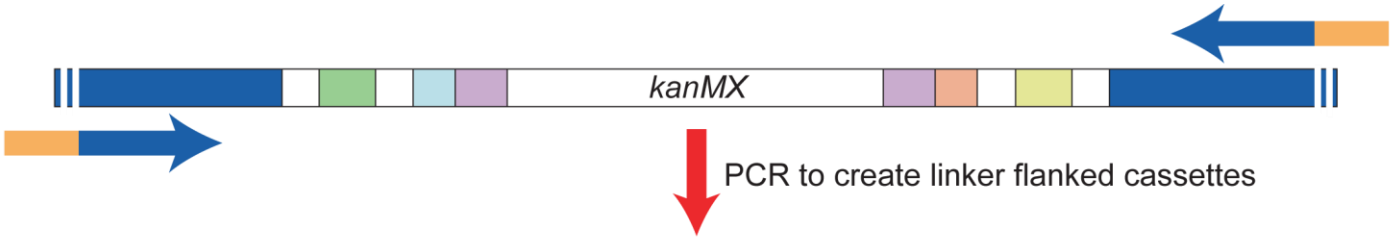

Transformation and confirmation

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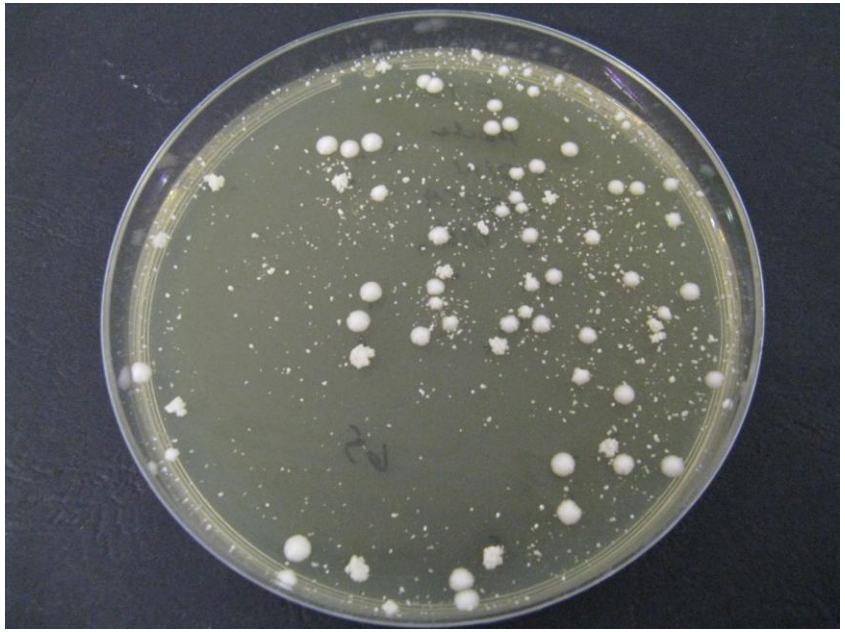
- Transformations are underway using a standard heat shock transformation protocol.
- Strains are selected on YPG agar + G418



Transformation using the Robot

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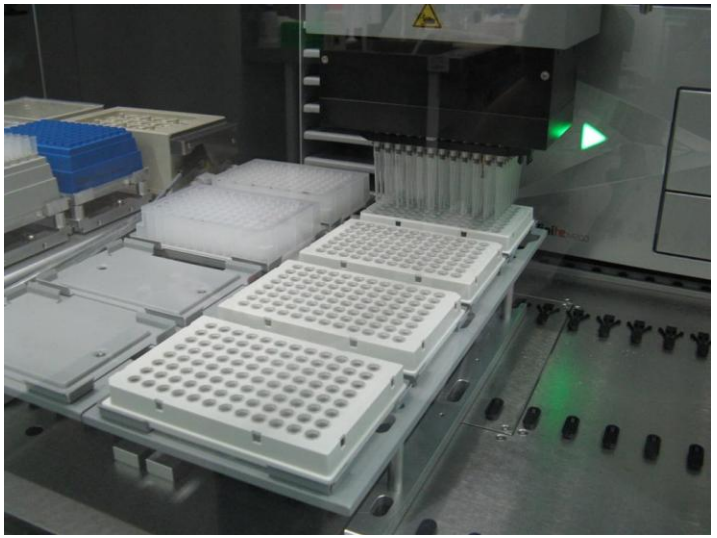
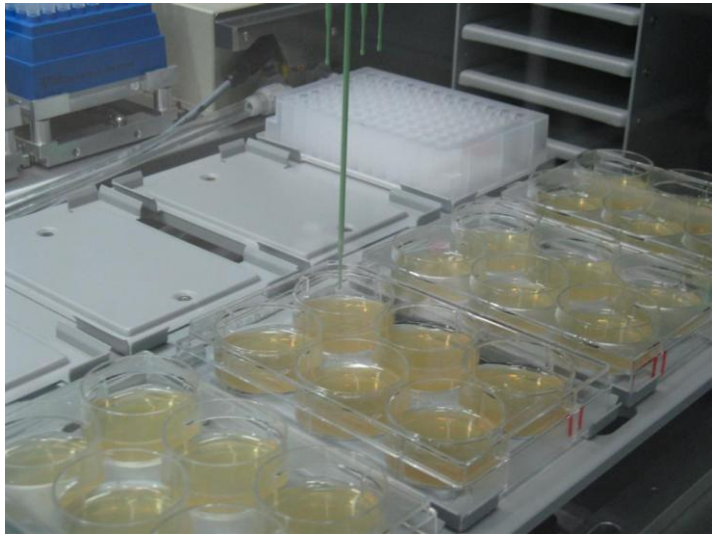
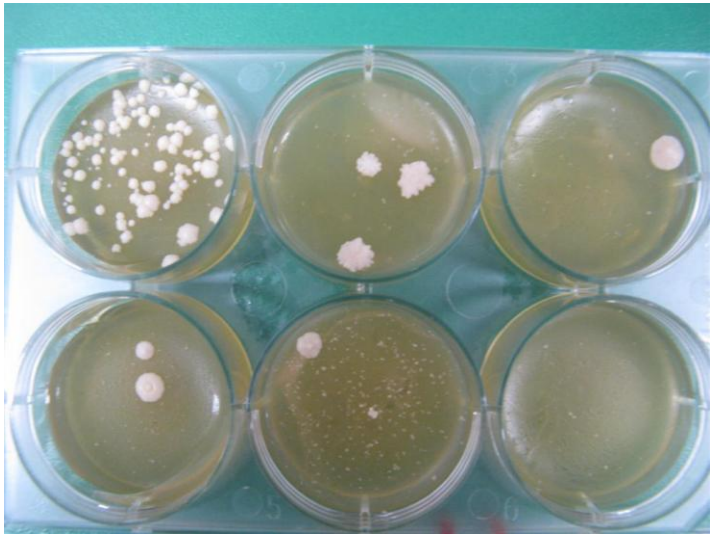
- We are now using a robotic liquid handling system to assist with transformations and for high throughput screening







Confirmation of deletion constructs

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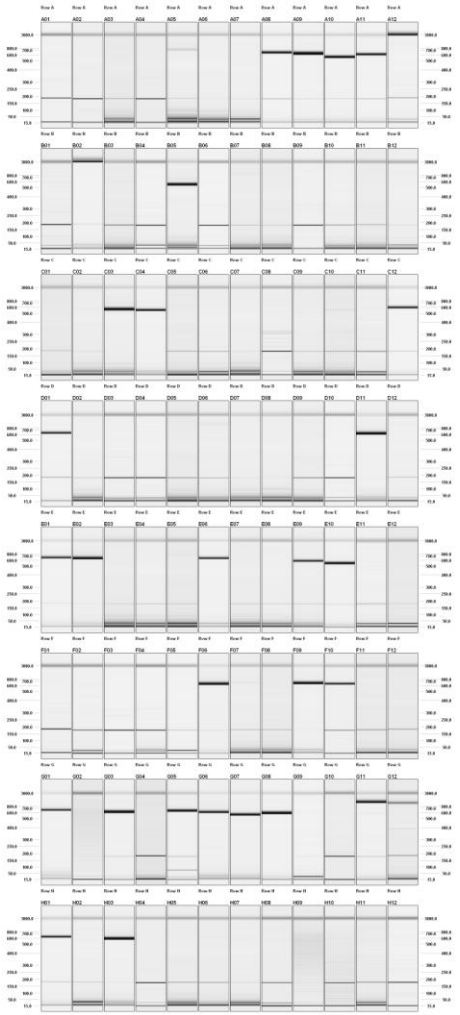
- Insertion of the deletion cassette at the correct location is confirmed by PCR.
- We have >2000 confirmed deletion strains.



What are we doing with the deletion strains?

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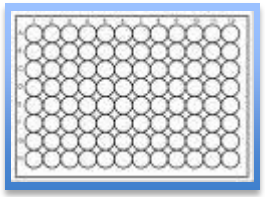
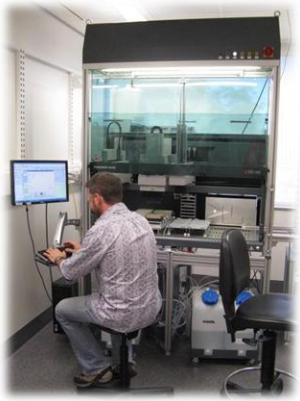
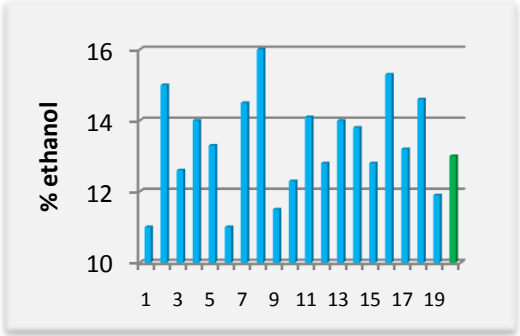
Understanding the *S. cerevisiae* genome

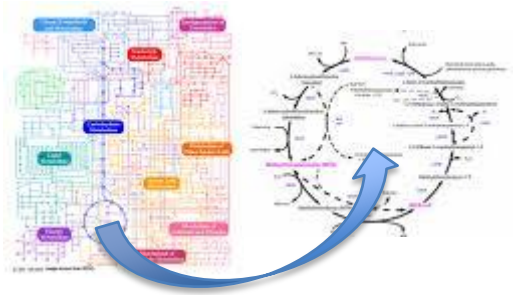


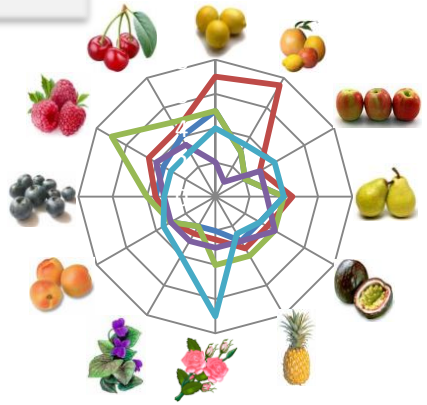


- 96 well master plates for screening
  - ‘live’ cultures
  - DNA



- *Saccharomyces cerevisiae*
  - Grape fermentation parameters
    - Alcohol %
    - Rate of fermentation
    - Aroma & flavour profiles
  - Genetics
    - Function of gene(s)





In conclusion …

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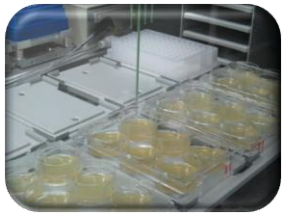
- Constructed a deletion library in a winemaking *S. cerevisiae* strain
  - AWRI1631 (haploid)

- > 2000 single gene deletion strains

- Robotic high throughput screening developed



- *Next* …
  - Screening for fermentation ↑ & ↓
  - Aroma & flavour characteristics
  - Specific gene function

Acknowledgements

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GWRDC GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

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