

# ASBC Annual Meeting

June 4–7 ■ Fort Myers, Florida

*See what SCIENCE can brew for you*

# Molecular Diagnostics – Going Small for Big Gains

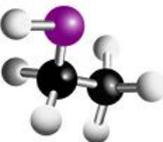
Karen Fortmann, Ph.D.  
Senior Research Scientist  
White Labs, San Diego CA



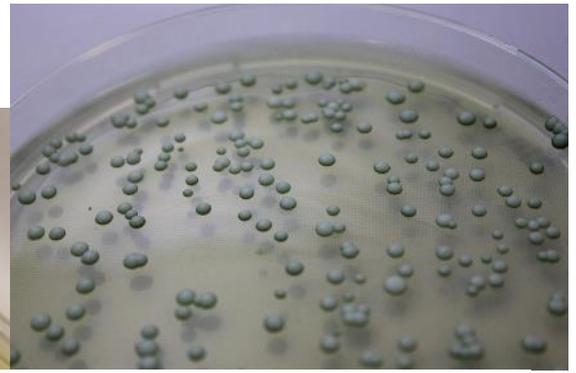
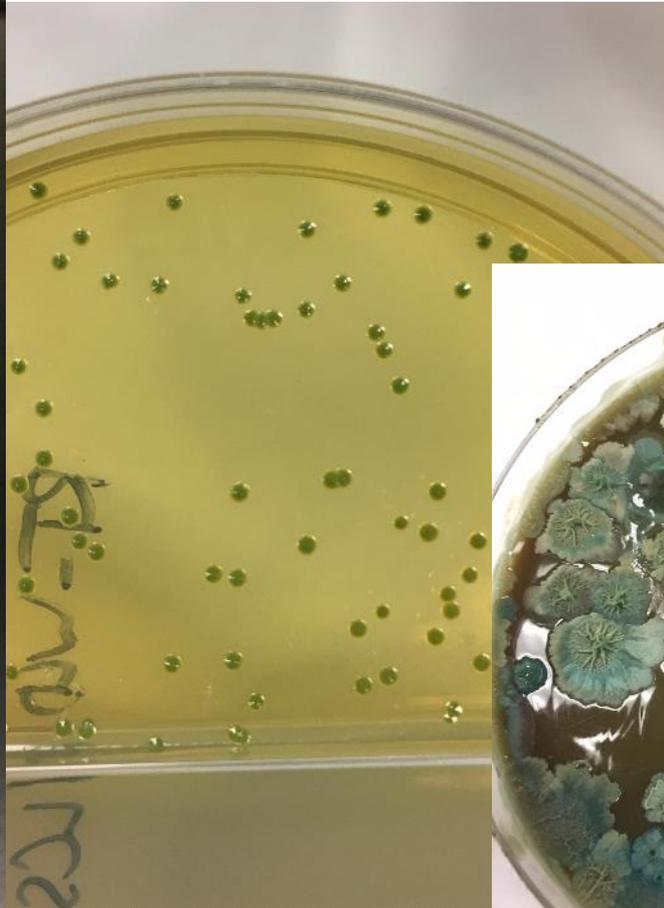
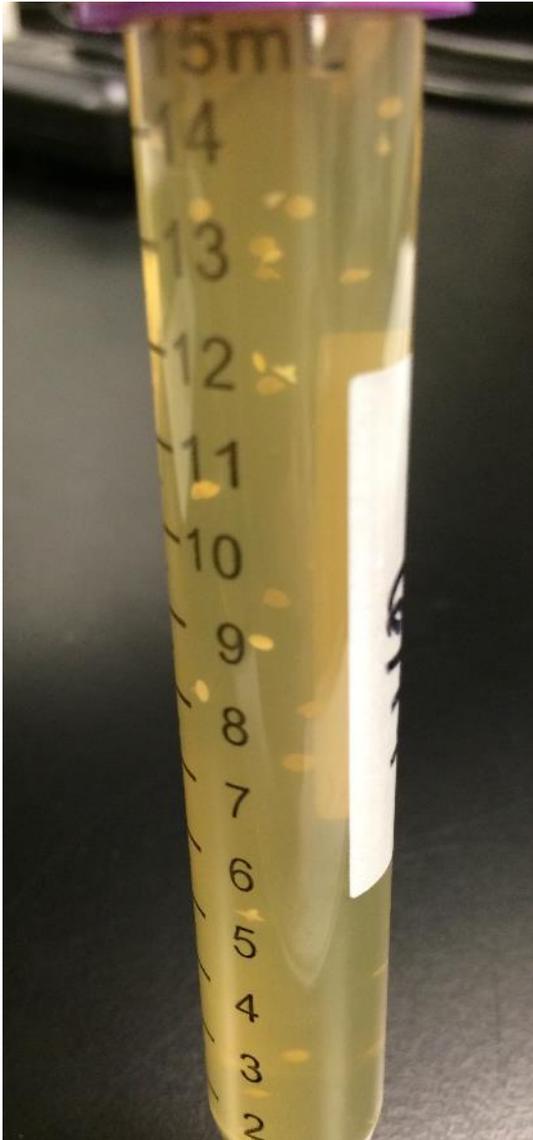


# Outline

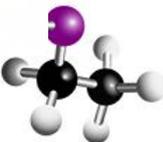
- Current micro techniques and where they are utilized
- Using MT in the brewery
  - End-point PCR
  - Fingerprints
  - qPCR
- NGS potential



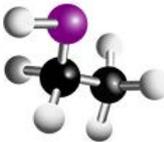
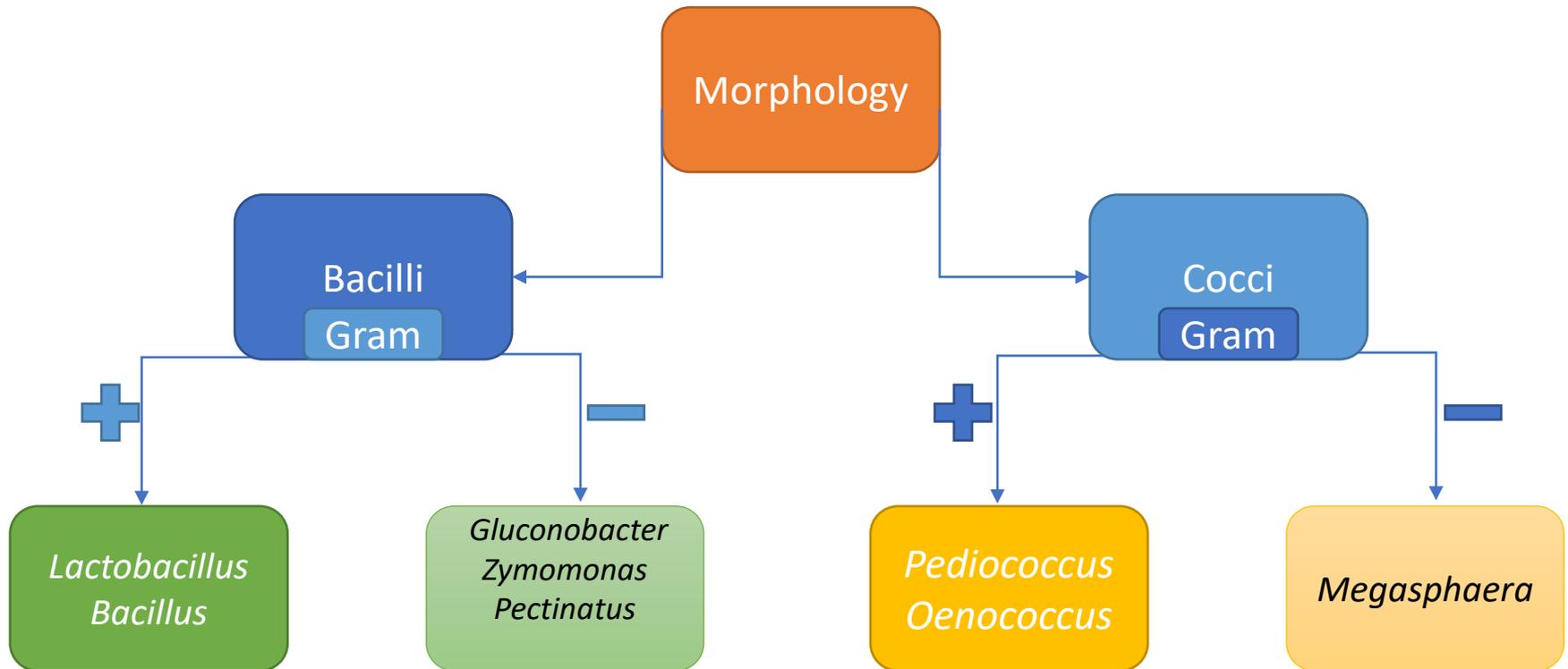
# Beer Spoilers



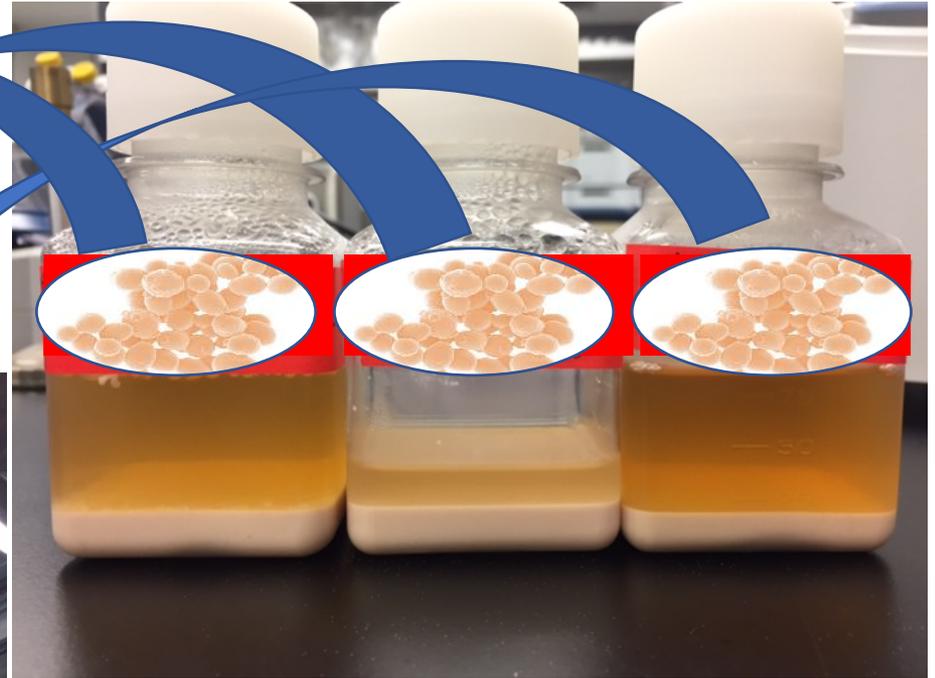
2017 ASBC Meeting



# Traditional Microbiology



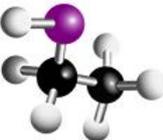
# Yeast



?

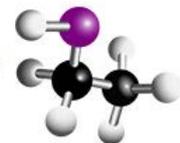


2017 ASBC Meeting



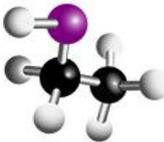
# Molecular Techniques

- DNA work/manipulation usually defines molecular techniques
- With breweries it is usually related to yeast and contamination analysis rather than GM

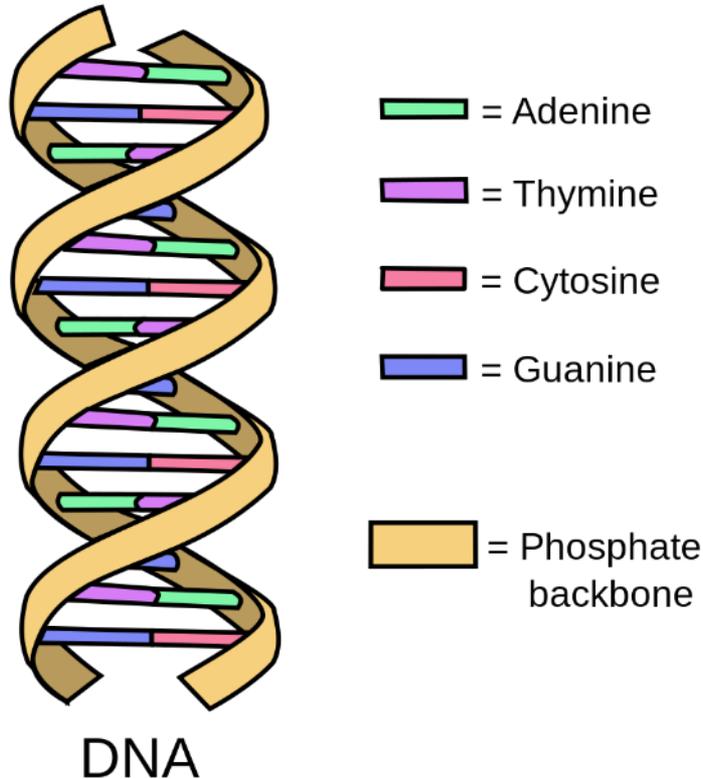


# Brewing Molecular Techniques

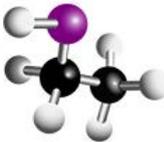
- From the Practical Brewer (1999):
  - Protein fingerprinting by PAGE
  - Immunoanalysis by monoclonal antibodies
  - Chromosome fingerprinting by PFGE
  - PCR
    - Conserved region sequencing
    - RAPD
    - RFLP
- qPCR
- NGS



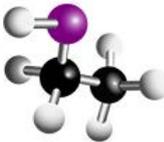
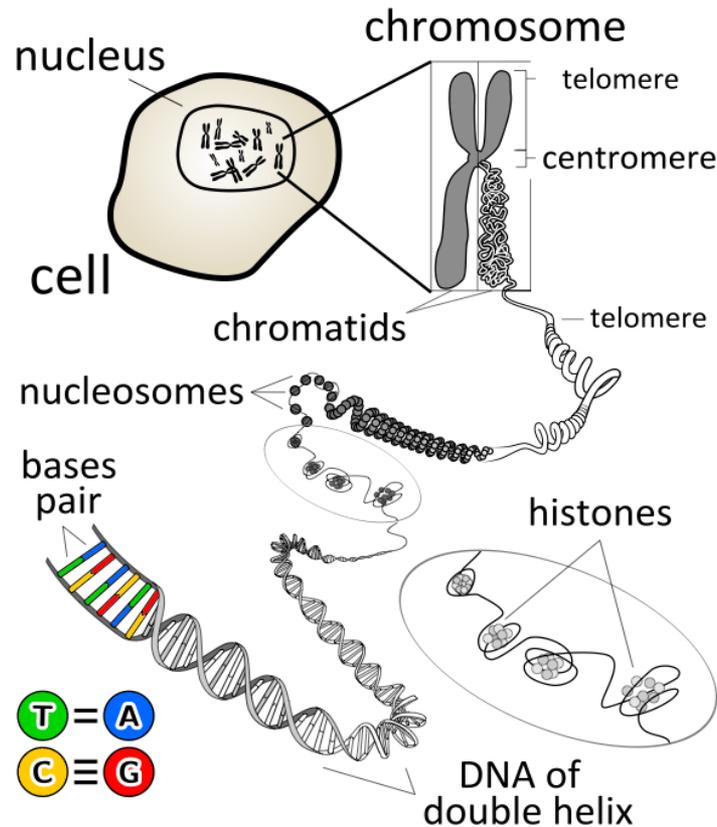
# 5 Second Lesson on What DNA Is



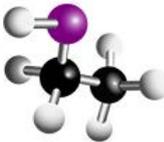
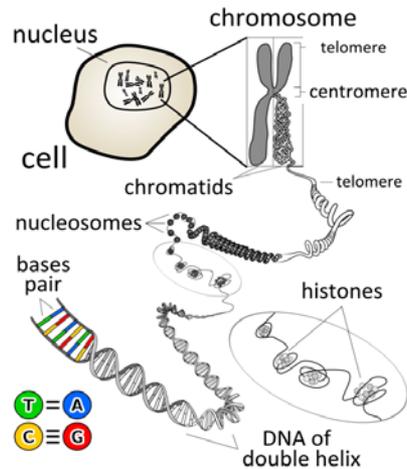
- DNA is made of chemical building blocks called nucleotides.
- These building blocks are made of three parts: a phosphate group, a sugar group and one of four types of nitrogen bases.
- DNA strands are held together by hydrogen bonding



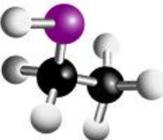
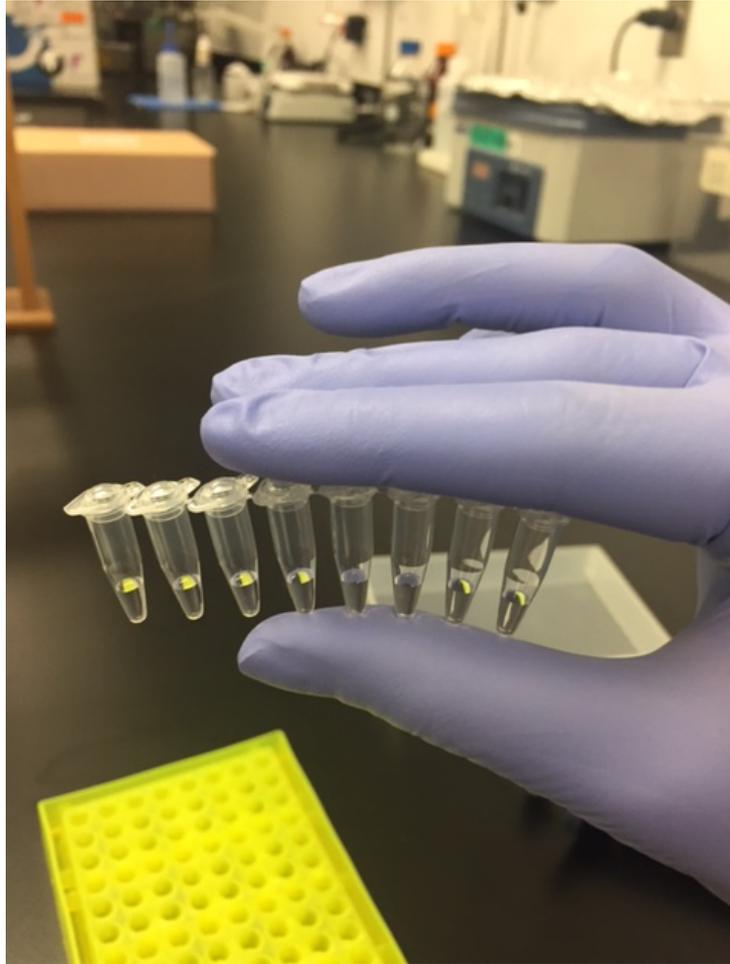
# 5 Seconds on Where DNA is Found...



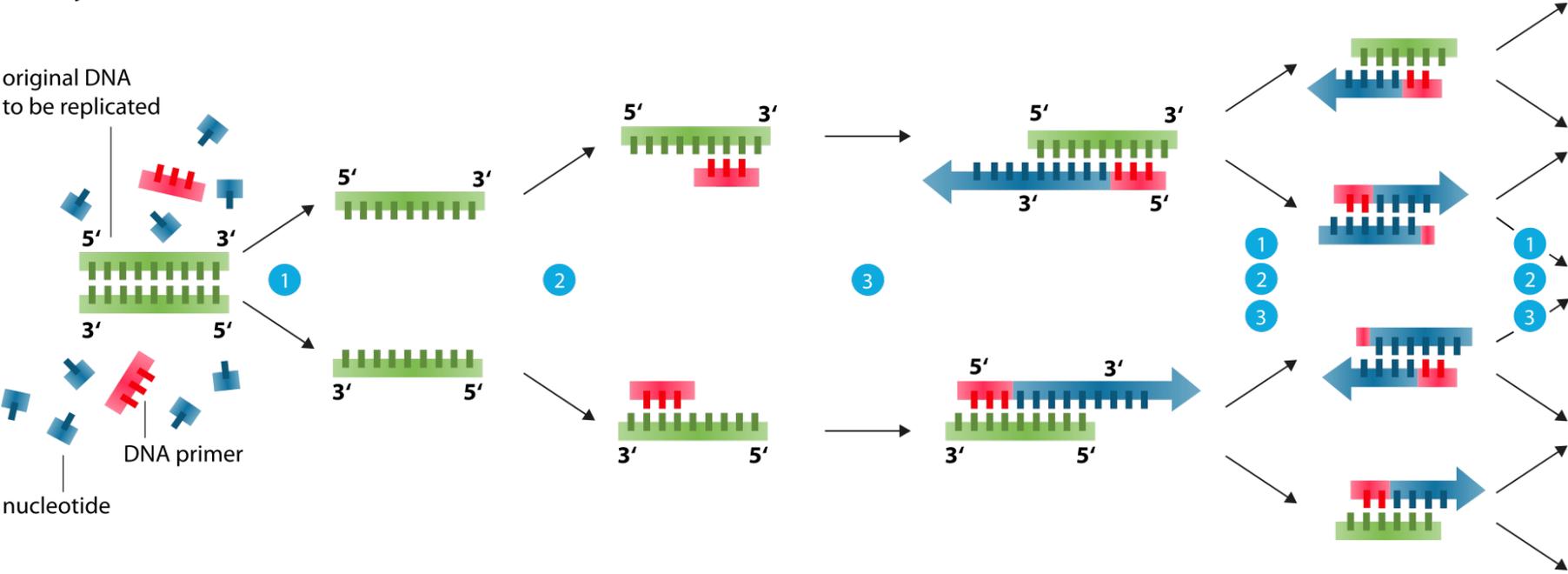
# The Yeast Cell



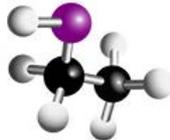
# Polymerase Chain Reaction



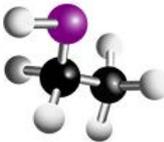
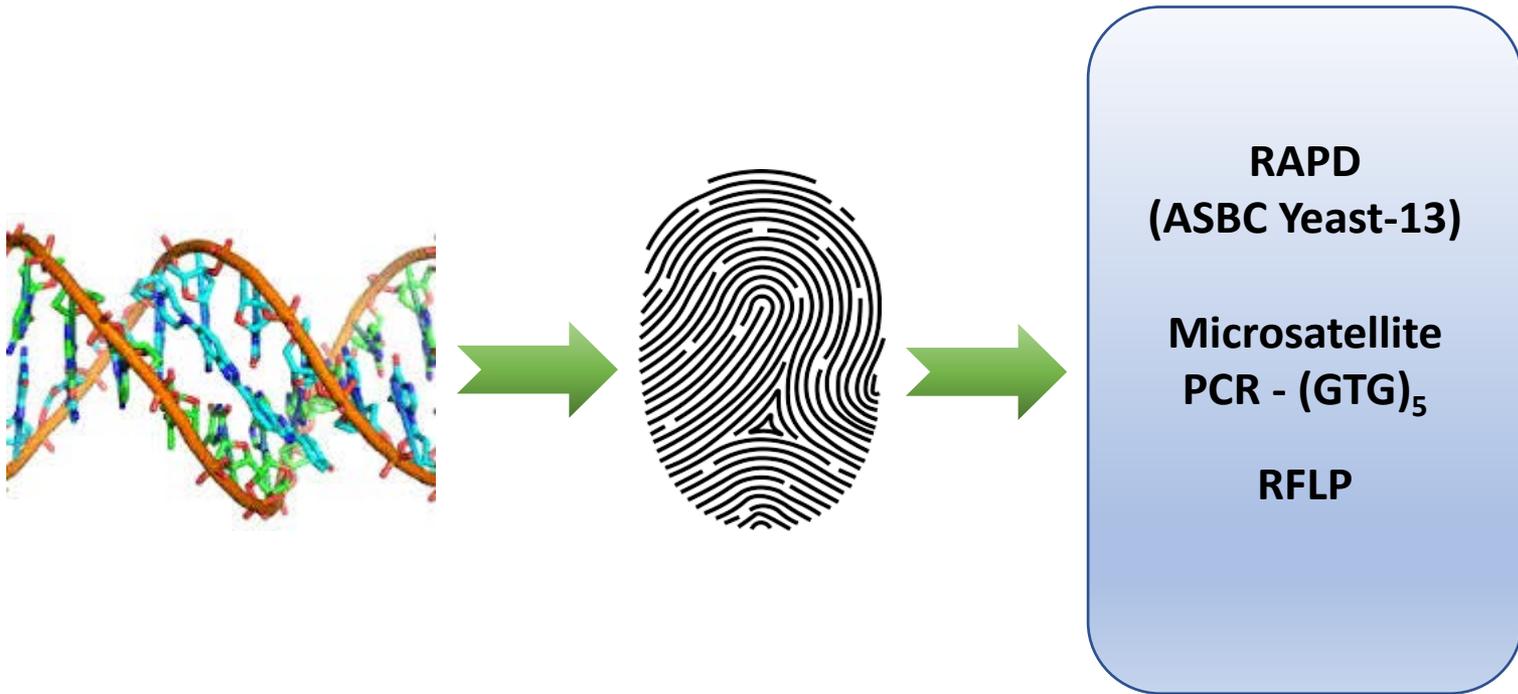
# Polymerase chain reaction - PCR



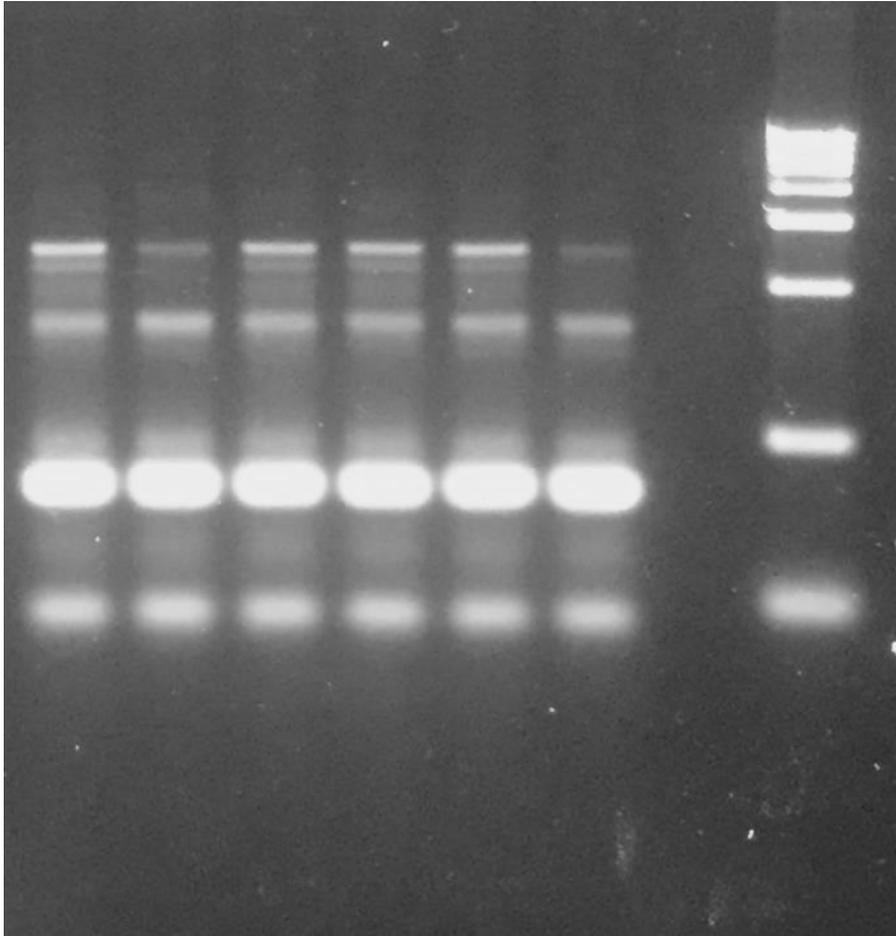
- 1 Denaturation at 94-96°C
- 2 Annealing at ~68°C
- 3 Elongation at ca. 72 °C



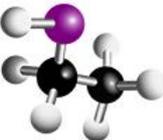
# DNA Fingerprint



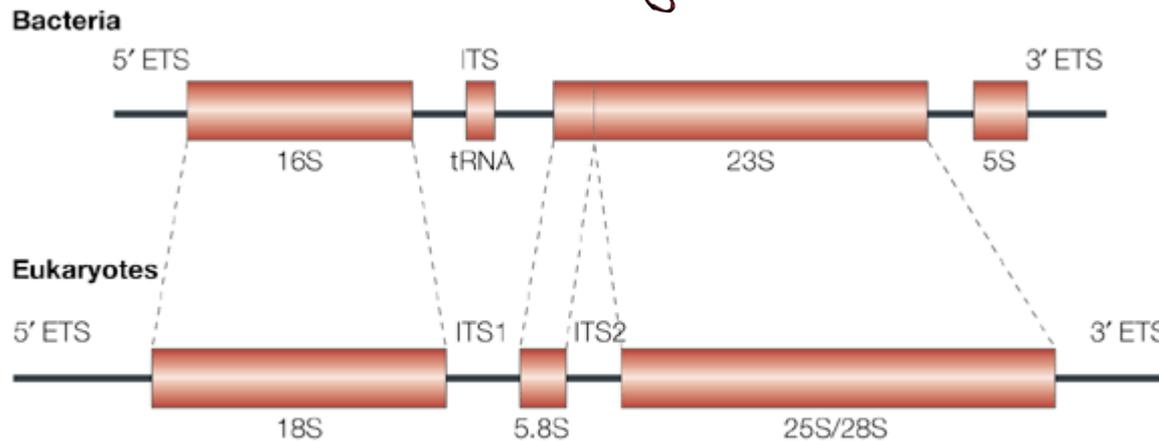
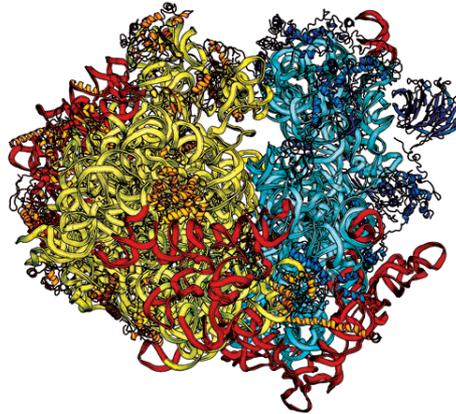
# Uses for DNA Fingerprinting



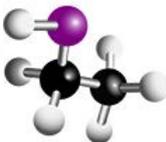
- Differentiation of strains
  - Not just brewing strains
- Cross contamination
  - Multiple strains in brewery
- Genetic stability
  - Only RFLP
  - Serial repitching



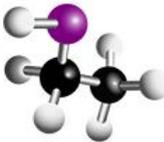
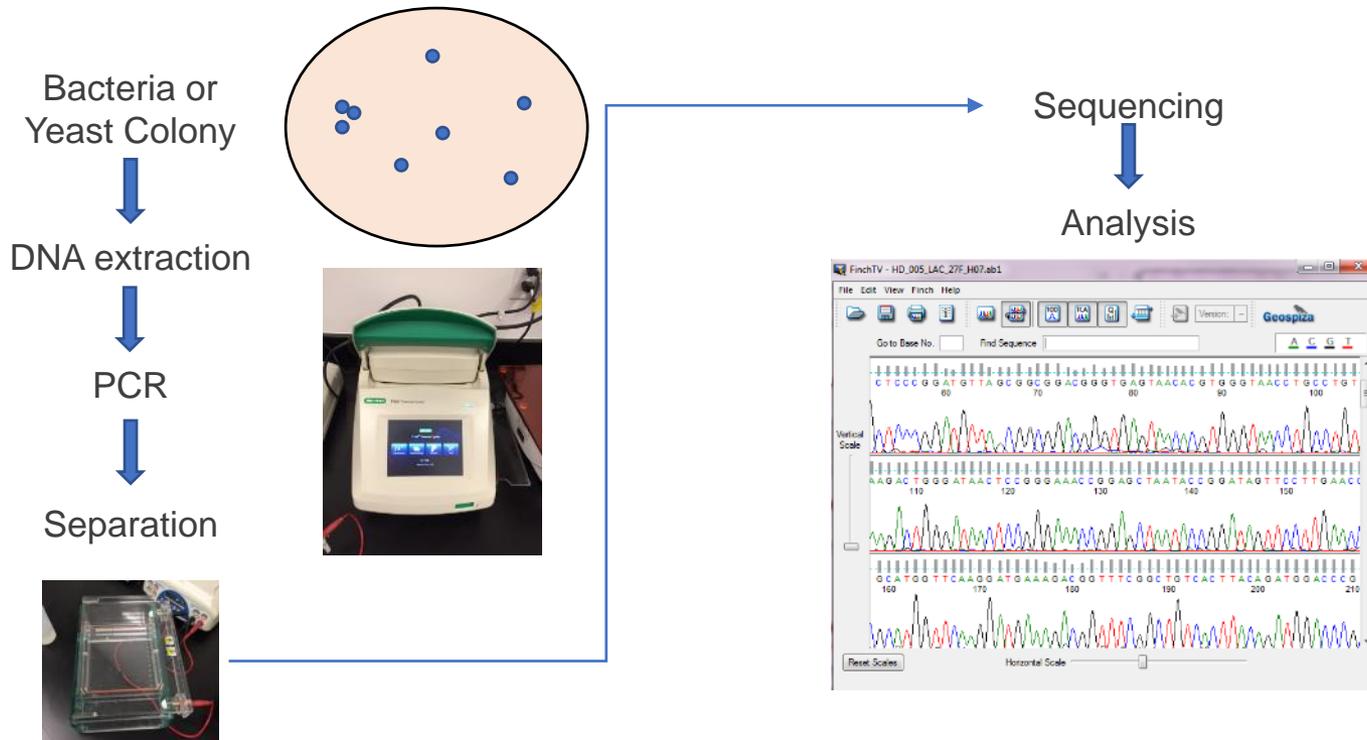
# Differentiating Microorganisms With PCR



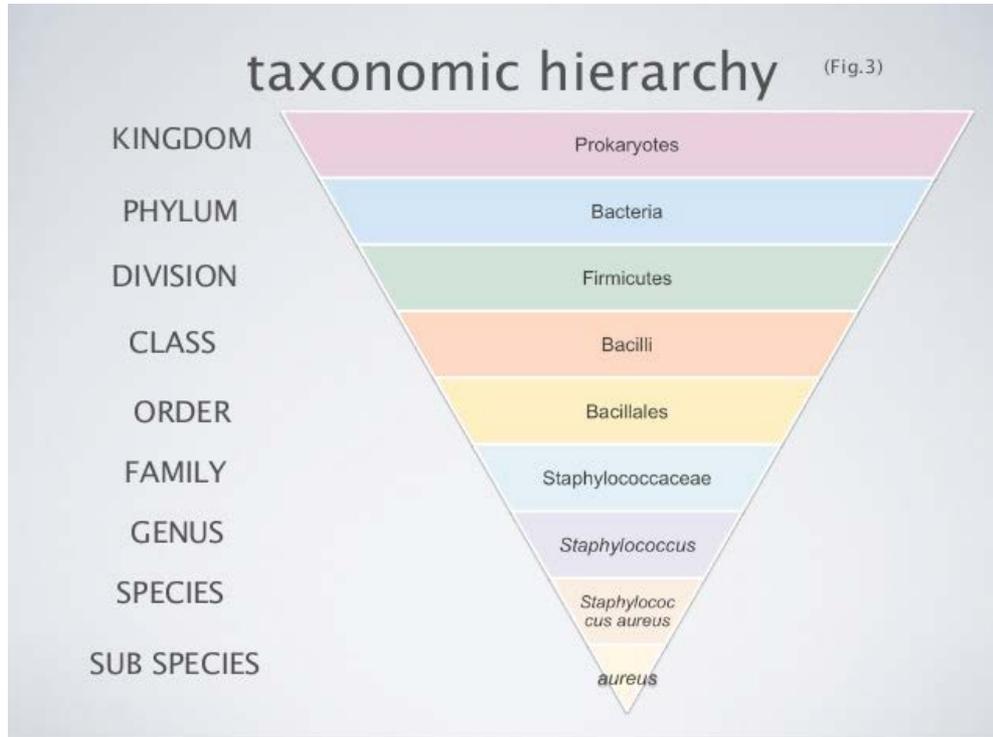
Nature Reviews | Molecular Cell Biology



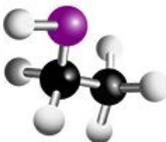
# Methodology



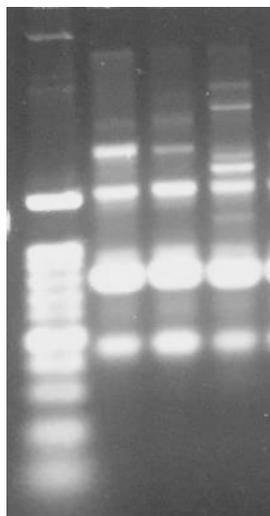
# Uses



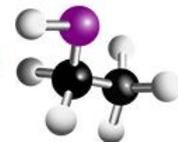
- Kit based systems look for specific targets
- rDNA sequencing accurate for any target
- Great for trouble shooting
- More often than not cannot confirm species



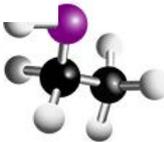
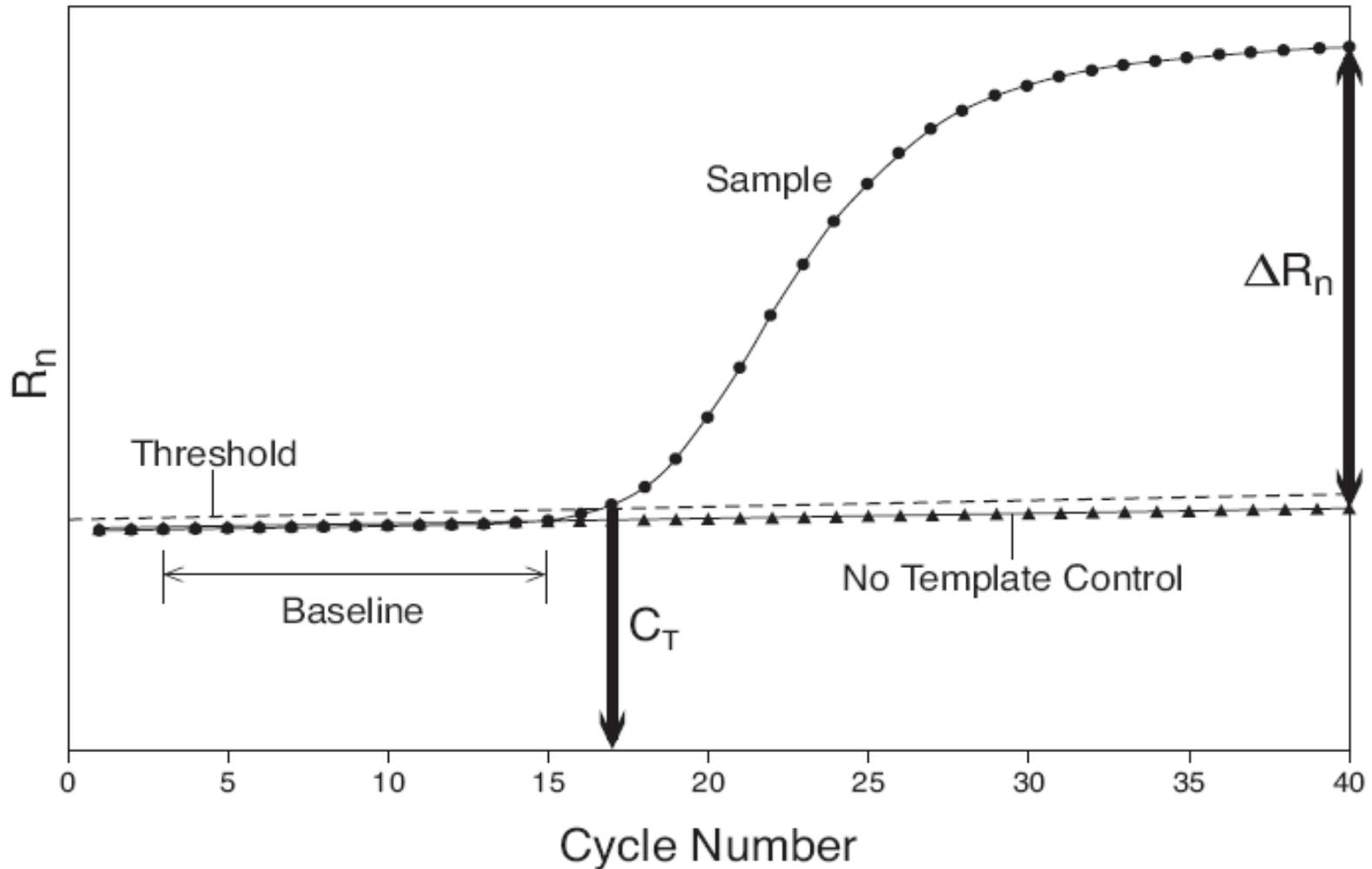
# With the Good Comes the Bad



- Fingerprinting & rDNA amp/seq require expensive equipment and trained personnel
- FP sometimes difficult to interpret
- Kit based kits accurate for specific targets

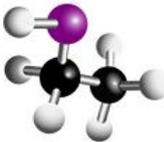


# Real Time PCR (qPCR)



# Commercially Available Kits

- Biotech companies have realized that breweries are a market for kits that don't require much insight into workings of qPCR
  - Big up front cost for the machine, however, consistent results



# Yin with the Yang

## Pros

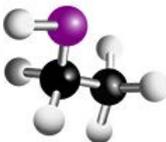
- Extremely sensitive and accurate for specific targets
- A variety of commercially available kits from multiple manufacturers

## PCR Protocol



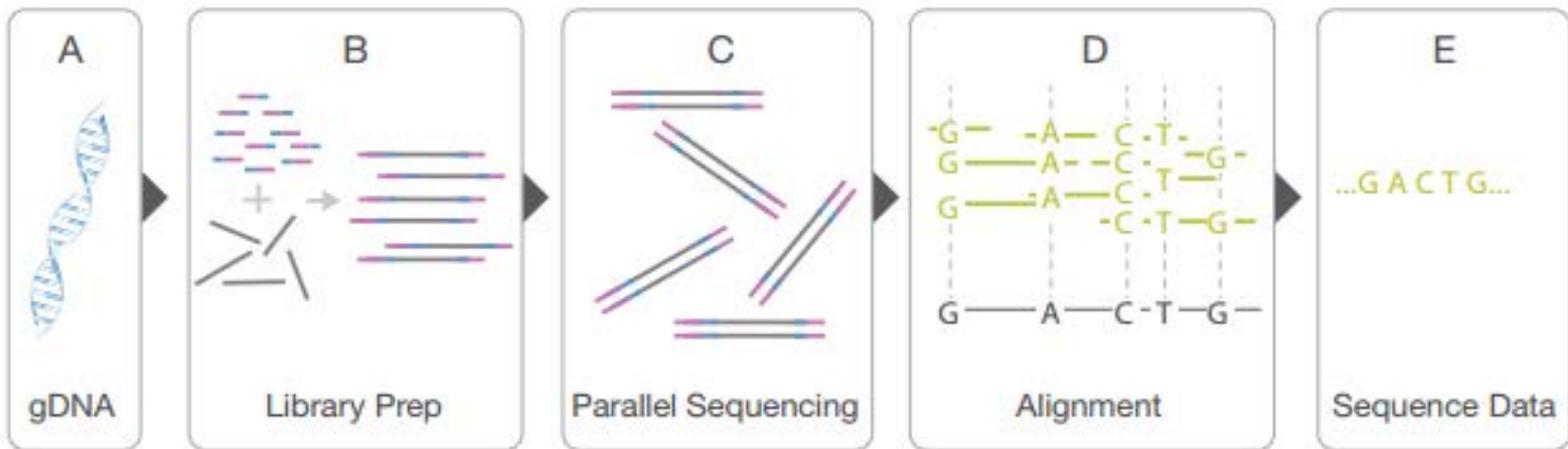
## Cons

- Extremely sensitive and accurate for specific targets
- Enrichment highly recommended
- Can detect dead material
- Proprietary primers from kit makers

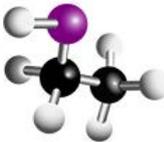


# Next Generation Sequencing (NGS)

Figure 1: Concepts of Next-Generation Sequencing



- A. Extracted gDNA.
- B. Sample preparation fragments genomic DNA and adds adapters to generate a library.
- C. DNA fragments within the library are each sequenced in parallel.
- D. Individual sequence reads are reassembled by aligning to a reference genome.
- E. The whole genome sequence is derived from the consensus of aligned reads.



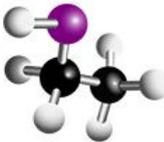
# NGS, The Future (?)

## Pros

- Useful for even “unculturable” organisms
- More data to use to determine if actual “spoiler”
- Can be used for:
  - Differentiation of strains
  - Cross contamination
  - Genetic drift

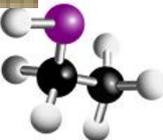
## Cons

- Very expensive and requires trained personnel
- Lots of data to interpret



# Take Away Message

- Molecular techniques are here to stay
- As our industry grows we will start to see a larger adoption of MT in the brewery



# References & Acknowledgements

PCR protocols: a guide to methods and applications

Quantitative Real-Time PCR: Methods and Protocols

ASBC Methods of Analysis – Yeast 13

An Introduction to Illumina NGS Technology for Microbiologist

Liu et al. American Journal of Enology & Viticulture (2014)

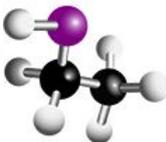
Lafontaine & Tollervey, Nature Reviews Molecular Cell Biology (2001)

Various google searches for DNA pictures and memes 😊



*The Science of Beer*

**2017 ASBC Meeting**



# Thank you for listening!

Questions?

[kfortmann@whitelabs.com](mailto:kfortmann@whitelabs.com)



2017 ASBC Meeting

