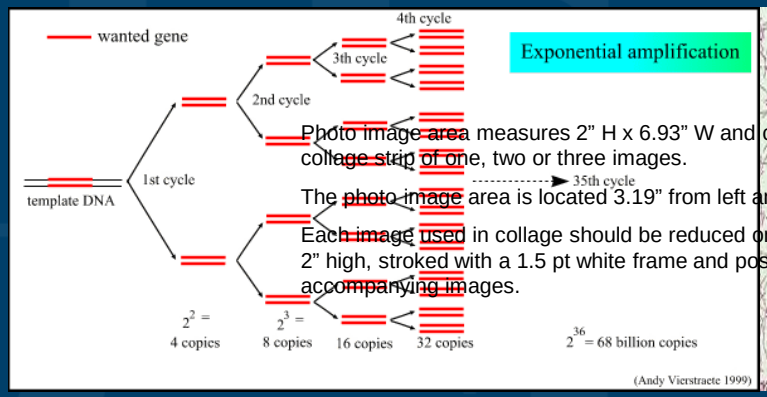
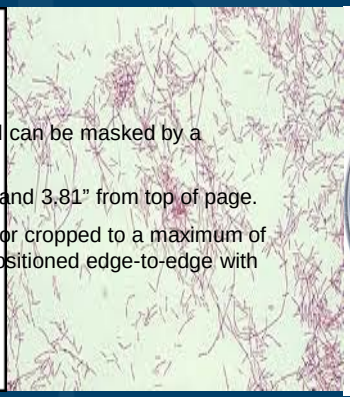
United States Environmental Protection Agency

# *Molecular Analytical Options for the Detection of Legionella Bacteria in Water*

*Mark Rodgers, EPA-Cincinnati*



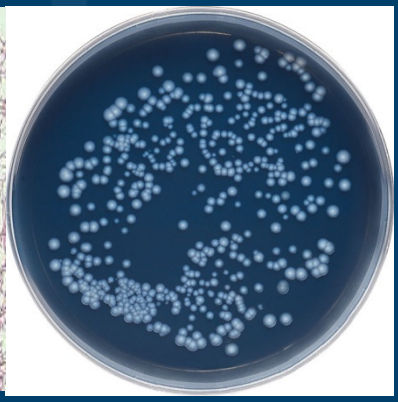


Photo image area measures 2” H x 6.93” W and can be masked by a collage strip of one, two or three images.

The photo image area is located 3.19” from left and 3.81” from top of page.

Each image used in collage should be reduced or cropped to a maximum of 2” high, stroked with a 1.5 pt white frame and positioned edge-to-edge with accompanying images.

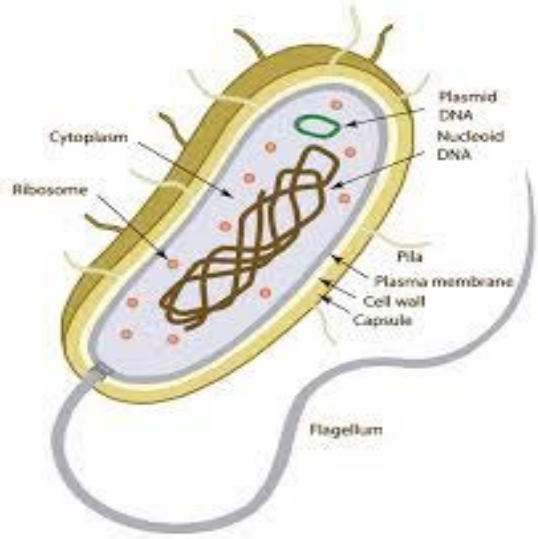
United States Environmental Protection Agency

# Bacteria detection: culture and molecular methods

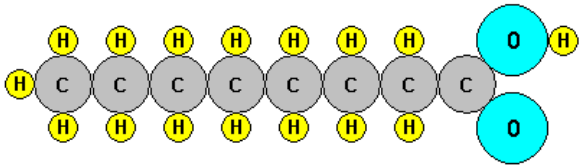
Culture method detects living (viable) **culturable** cells

***Legionella* bacteria growing in lab**

Molecular methods do not detect whole cells- rather these methods detect **parts of cells** which are **specific** to that organism

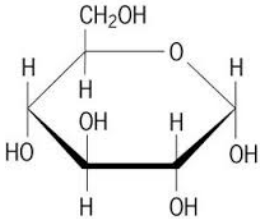


United States Environmental Protection Agency

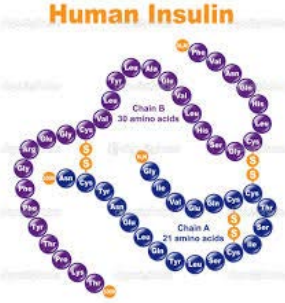


## What do we mean by molecular detection?

All cells have 4 types of molecules:



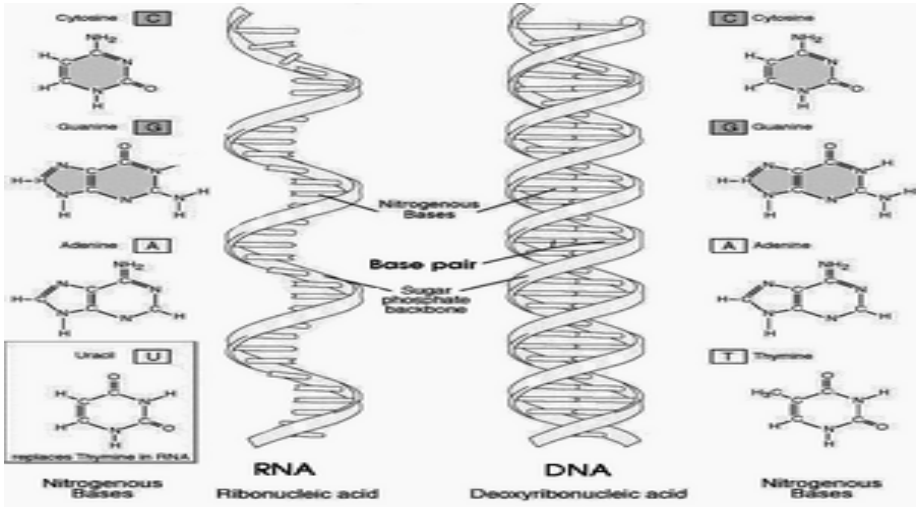

- **Lipids**- long chains of carbon
- **Sugars**- complex molecules, good antigens




- **Proteins**- unique sequence of amino acids
- **Nucleic acids**- most unique molecule in a cell

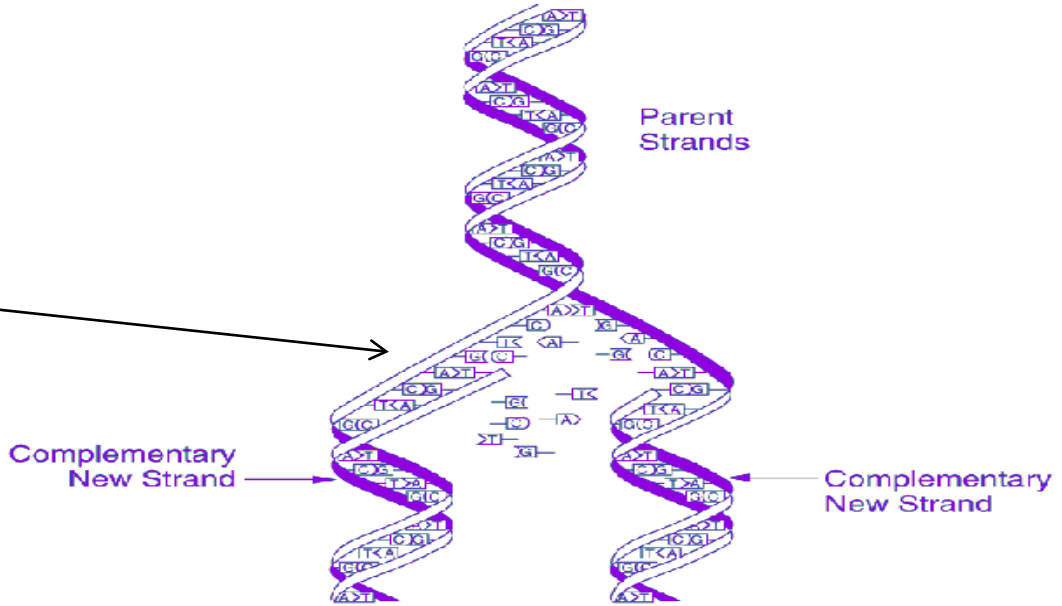
Analytical assays have been developed based on all of these molecules, but DNA/RNA-based methods are common.

United States
Environmental Protection
Agency



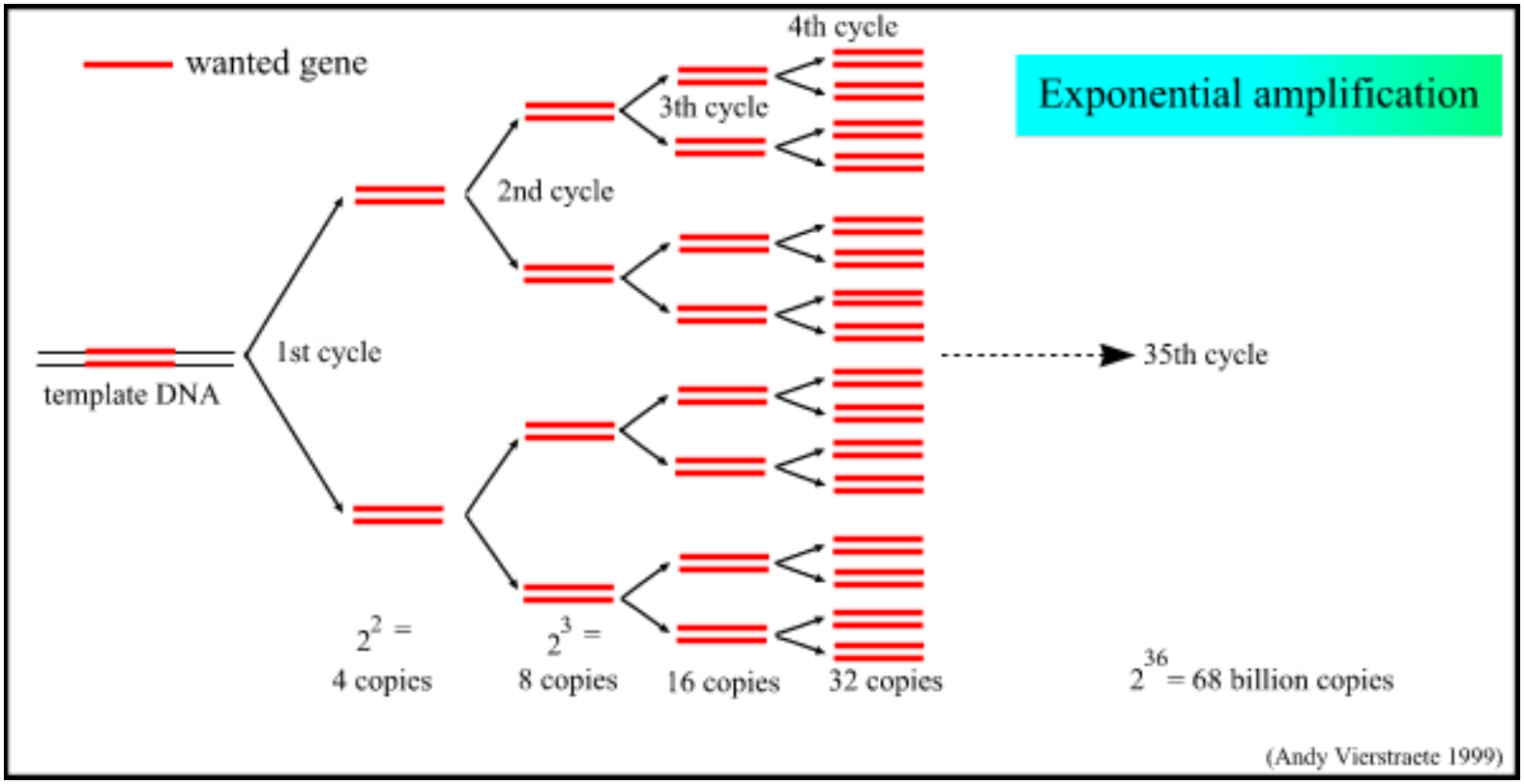

# Nucleic acids: RNA and DNA

DNA replication



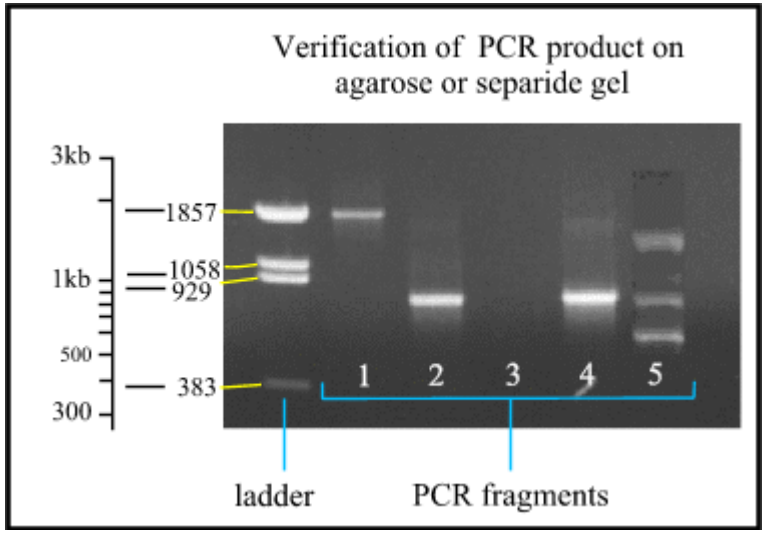
United States Environmental Protection Agency

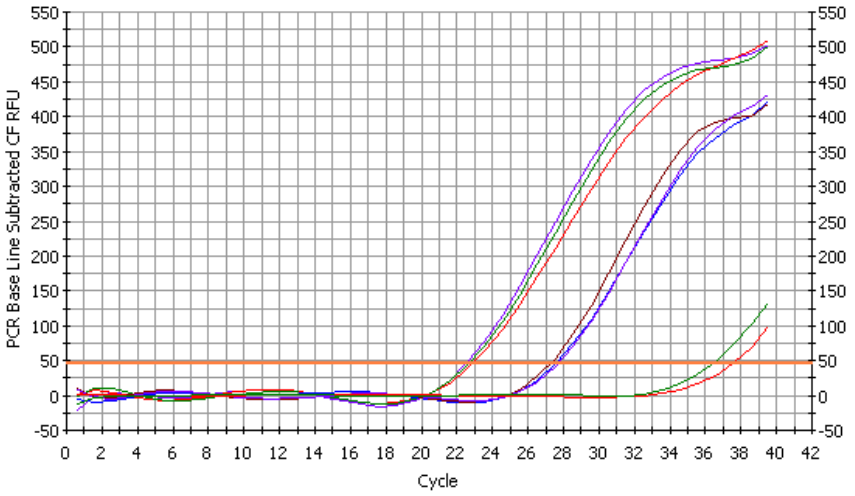
# Exponential Amplification of template DNA




http://users.ugent.be/~avierstr/index.html

# Picture of PCR gel results…….. and qPCR results




*courses.csusm.edu/*

http://users.ugent.be/~avierstr/index.html

United States Environmental Protection Agency

# Benefits to using molecular detection methods

1. Able to detect un-culturable organisms
2. Able to get results fast (hours vs days)
3. Assays can be designed to be highly sensitive and very specific- can detect target organism in a complex mixture of organisms
4. Quality control measurements can ensure sound interpretation of results
5. Confidence of positive results increased by using multiple genetic targets
6. Can be presence/absence or quantitative

United States Environmental Protection Agency

## Challenges with DNA detection assays

1. Environmental samples can have chemicals that inhibit PCR
   - Controls can be used to detect inhibitory samples
   - New reagents available that minimize inhibition
2. Difficult to distinguish DNA signals from live and dead cells
   - Bacteria can be culturable, completely dead, alive but dormant
   - EPA research indicates DNA detection is likely from viable or recently killed cells
   - Methods exist that can help distinguish live vs dead (PMA)
3. PCR is prone to false positive results via contamination of assay reagents
4. Offers limited options to further analyse sample
   - Having isolates allows serotyping and genetic comparison with other cultured bacteria from same location or from patients

United States Environmental Protection Agency

## What is important to know about molecular DNA assays?

- **Sensitivity of assay-** how many molecules must be present to give a reliable positive result?
  - Is defined using a test sample that is known to be positive
  - Is usually reported in terms of genome units or cell equivalents or copy number per liter (for water samples); per ug protein or square centimeters (for biofilm samples)

- **Specificity of assay-** can it distinguish between genera or species or strains?
  - Examples:
    - less specific assay will identify *Legionella* in a sample
    - more specific assay will identify *L. pneumophila*
    - most specific assay will identify *L. pneumophila* serogroup 1

# Legionella qPCR assays and specificities

| Reference | Genetic target | *Legionella* specificity |
|---|---|---|
| Mérault, N. et al (2011) Appl Environ Microbiol, 77:1708-1717. | *wzm*: gene coding for the transmembrane component of O-antigenic polysaccharide in the outer membrane of *Legionella* | *L. pneumophila* serogroup 1 |
| Donohue, M. et al (2013) submitted to Environmental Science and Technology | 16S rDNA: gene coding for the small subunit of the ribosome | *L. pneumophila* |
| Templeton, K. et al (2003) J Clin Microbiol, 41:4016-4021. | 16S rDNA | *Legionella* species |
| | | |
| | | |

United States Environmental Protection Agency

| | | *Legionella* detection results | | | | |
|---|---|---|---|---|---|---|
| **Reference** | **Gene Target** | **Culture +** | **PCR +** | **Culture -- PCR +** | **Culture + PCR --** | **Culture -- PMA-PCR +** |
| | | | | | | |
| Miyamoto et al, 1997 | 16S | **80%** | **92%** | | | |
| | | | | | | |
| Wellinghausen et al, 2001 | 16S, *mip* | **70%** | **96-99%** | | | |
| | | | | | | |
| Joly et al, 2006 | 16S, *mip* | **43%** | **77-91%** | | | |
| | | | | | | |
| Yaradou et al, 2007 | unspecified | **45%** | **66%** | | | |
| | | | | | | |
| Nazarian et al, 2008 | 23S, *mip* | **69%** | | **29%** | **2%** | |
| | | | | | | |
| Yuanez et al, 2011 | *dotA* | **70%** | | **30%** | **0** | **17%** |

United States Environmental Protection Agency

## Research needs:

- Positive *Legionella* spp results- what is the significance to public health and what is an appropriate management response?
- Monitoring for *Legionella* virulence genes- could this type of information/analysis be a part of a management response?
- What is the best sample for routine analysis- water or biofilm?
- Rapid *Legionella* tests- are they reliable enough for routine monitoring?

United States Environmental Protection Agency

# Thank you for your attention