# Development of Tools by EPA to Determine the Effectiveness of Green Infrastructure-Based Approaches to Mitigate Stormwater

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- Acknowledgement of the team:
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  - Eric Villegas
  - Brian Zimmerman
  - Daniel Divelbiss

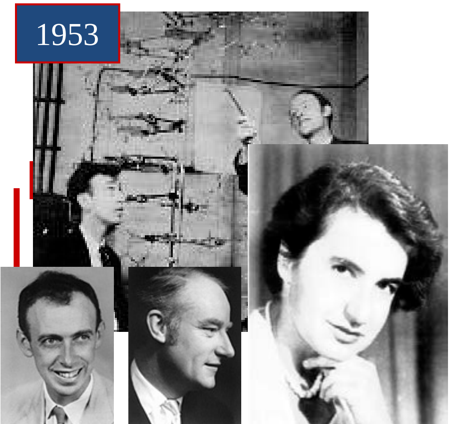
  - All from the Microbiological and Chemical Exposure Assessment Research Division (MCEARD)

# Overview of Talk

- Molecular Context of Tool Development
- General Approach for assessing microbial risks associated with water reuse
  - Performance assessment of treatment via spiking
  - Validate indicators by comparison with pathogens in mixed spiked cocktails
  - Goals
    - Controlled testing to define best management practices
    - Potentially develop real time or near real time monitoring (perhaps)
      - Where appropriate (source, use effects)
      - Pathogens are a difficult target
      - Biologically-based indicators are less difficult but still emerging
      - On-line process performance measures linked to BMP definition
  - Future of green infrastructure

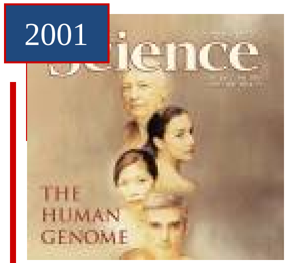
# DNA → PCR → Genomes

1953



1998


2001




2001: *C. parvum* genome

2004: *C. hominis* genome

2008: *C. muris* genome

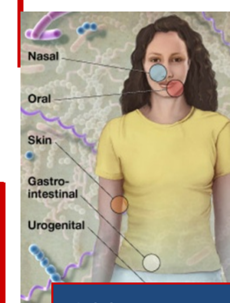
1983/1988: PCR/commercialization

1992: Real-time PCR


1995: Microarray

2007

2005: Pyrosequencing (“Next-gen” sequencing)



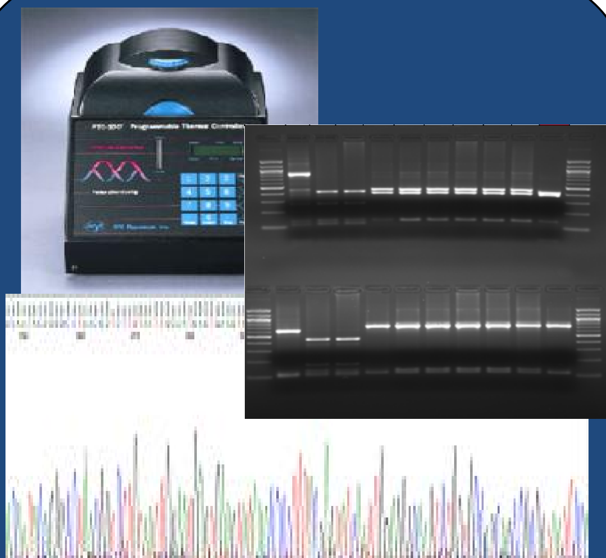

2007 Human microbiome

**Current status:**

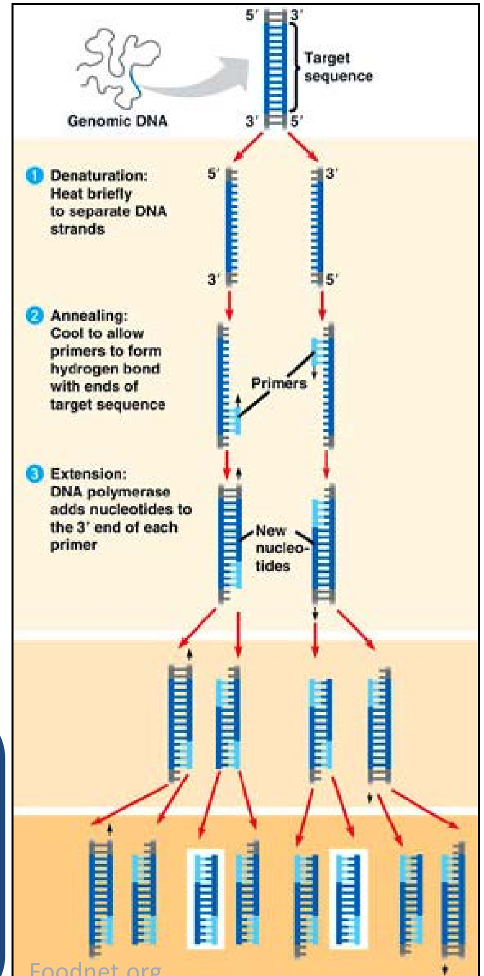
- Personal Genome Project /Knome
- Personal genome service (“know your DNA” $100)
- >10,000 Genomes submitted to NCBI
- >300 Metagenome projects (>70% Environmental)

# End-point vs. real-time PCR

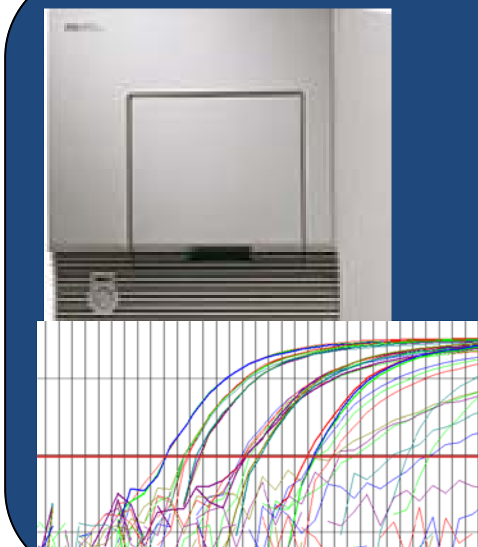
## End-point PCR



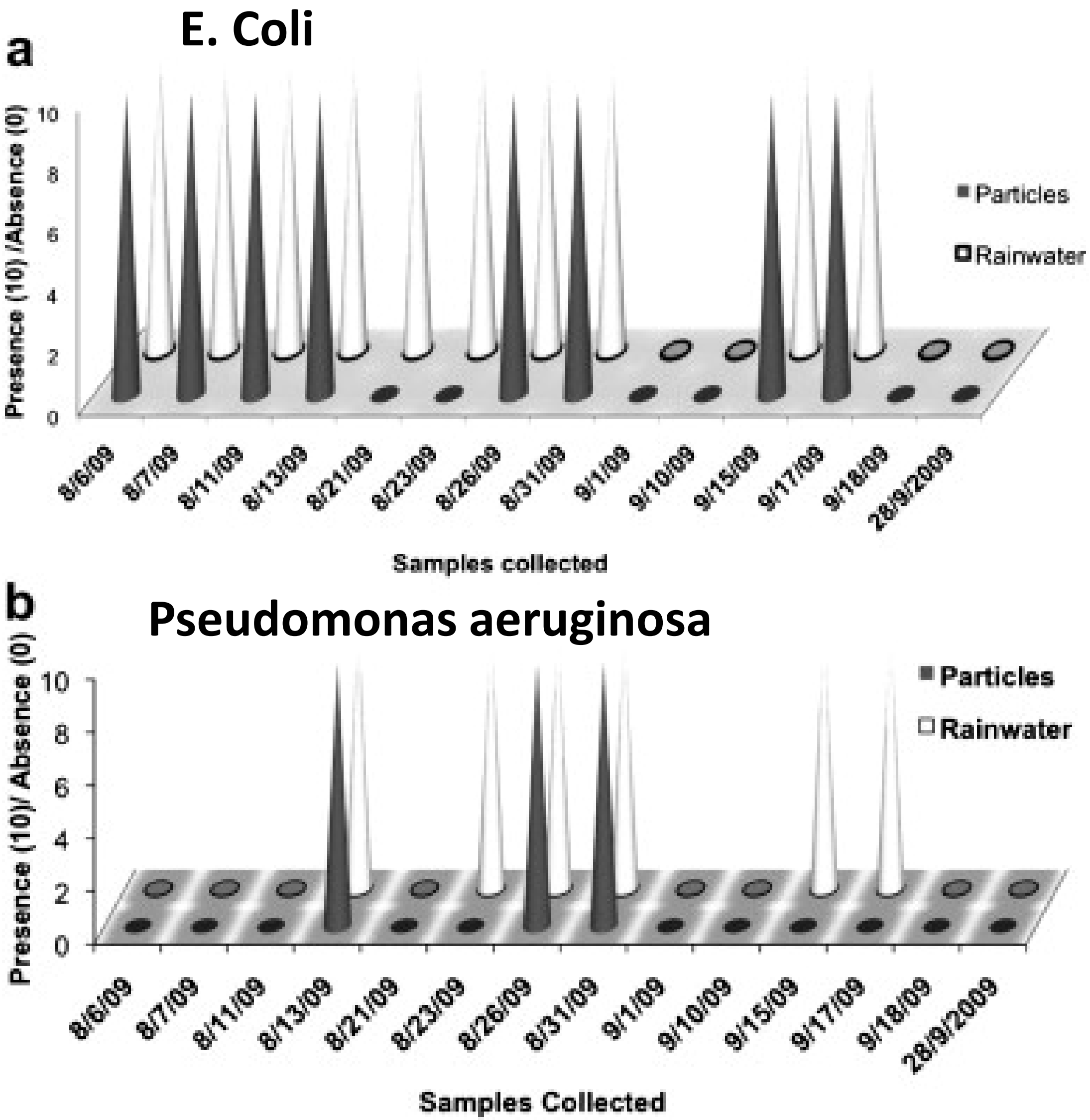
- Semi-quantitative (densitometry)
- Can amplify longer sequences
- Very specific
- Sequencing compatible




## Real-time PCR



- Quantitative/standard curve
- Fluorescent probe
- Short PCR product (amplicon)
- Very specific




**Assessment of bacterial pathogens in fresh rainwater and airborne particulate matter using Real-Time PCR**

**New tools expand sampling possibilities**

**Data still "noisy"**

**event driven**

Kaushik & Balasubramanian
Atmospheric Environment 46:131

# Correlations between fecal indicators and pathogens in rainwater tanks in Australia

Poor correlation

New Indicators and new approach needed!

| Fecal indicator bacteria | *P* value for correlation to the indicated pathogen | | | | | |
|---|---|---|---|---|---|---|
| | *A. hydrophila lip* gene | *C. coli ceuE* gene | *C. jejuni mapA* gene | *L. pneumophila mip* gene | *Salmonella invA* gene | *G. lamblia* β-giardin gene |
| *E. coli* | 0.250 | 0.611 | 0.466 | 0.969 | 0.306 | 0.406 |
| Enterococci | 0.020[b] | 0.142 | 0.552 | 0.878 | 0.986 | 0.873 |
| *C. perfringens* | 0.759 | 0.752 | 0.909 | 0.469 | 0.107 | 0.316 |

Ahmed et al. 2008 AEM 74:5490

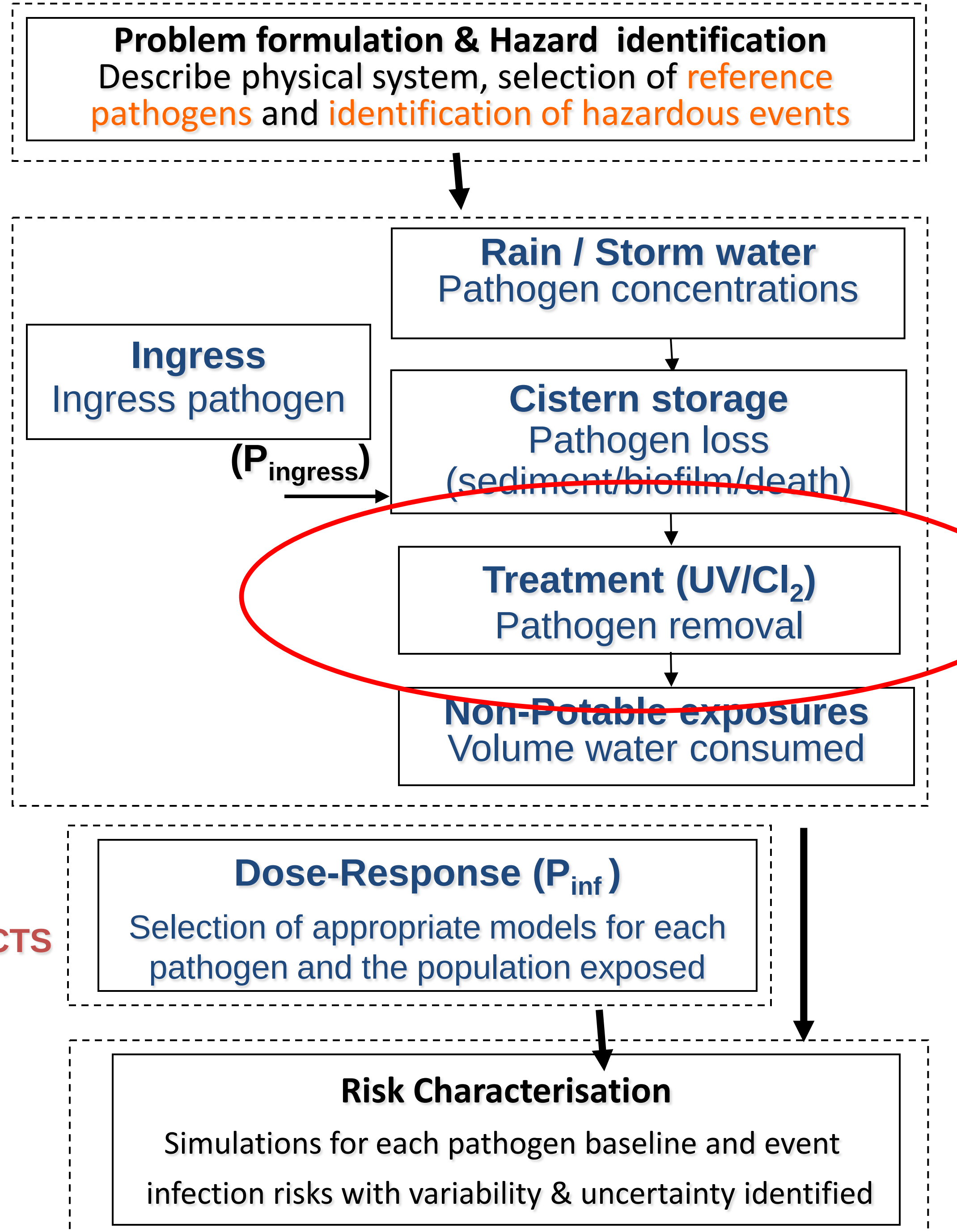
# Quantitative microbial risk assessment (QMRA)

STEP 1
SETTING

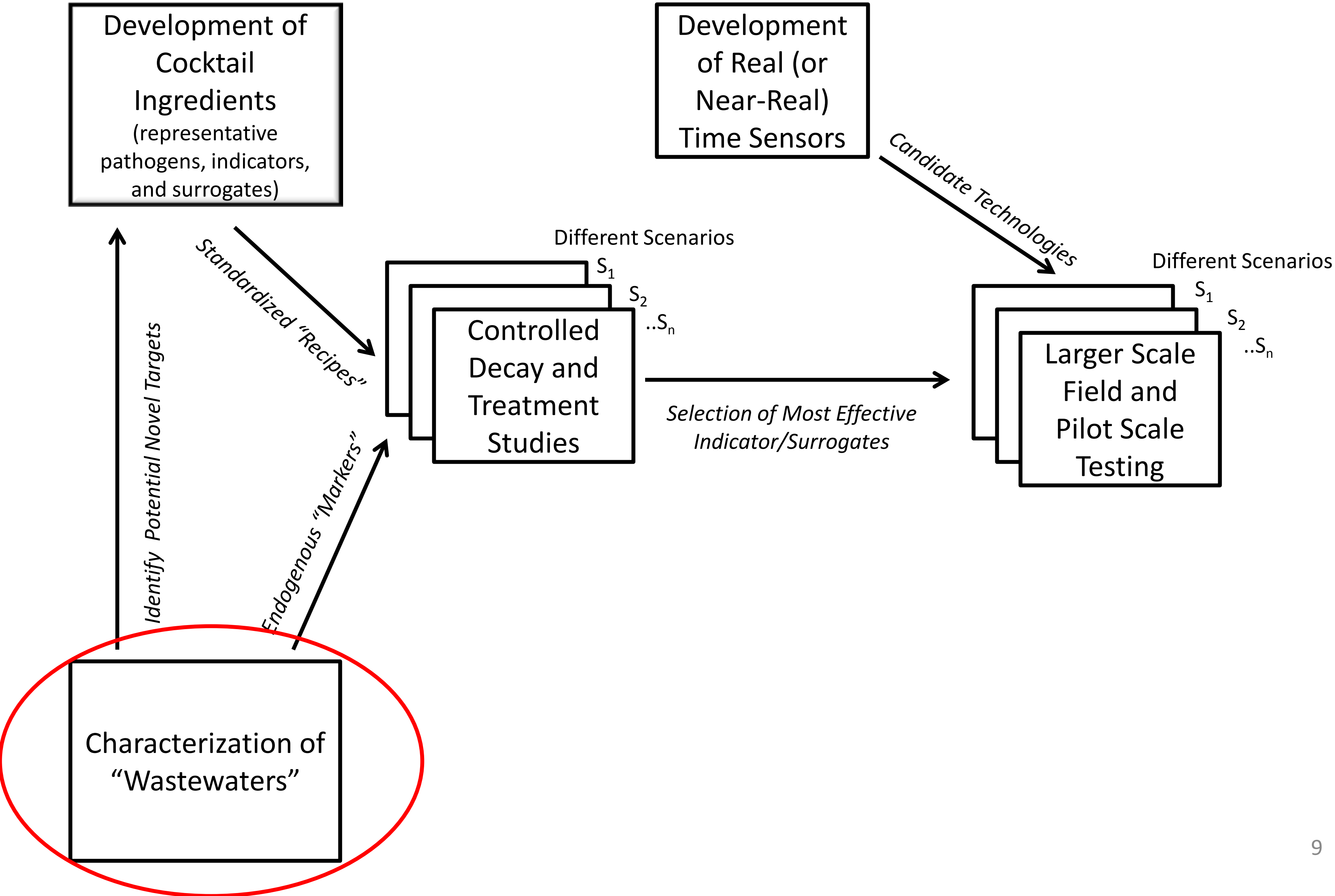
STEP 2
EXPOSURE

STEP 3
HEALTH EFFECTS

STEP 4
RISK



# Overall Research Plan for Developing Tools for Assessing Efficacy of Water Reuse Approaches



# Summary

- 248,000 sequences from 12 GW samples
- Range of sequences per sample
  - 13,173 to 37,592
- Range of genera detected per sample
  - 53 to 122
- 97% of the GW sequences were classified as
  - *Proteobacteria* (209,000 sequences)
  - *Bacteroidetes* (26,000)
  - *Firmicutes* (6,300)

196 genera

| Table 2. List of Genera Classified from Graywater | | | |
|---|---|---|---|
| Abiotrophia | Cloacibacterium | Lactobacillus | Pseudonocardiaceae |
| Achromobacter | Clostridium | Laribacter | Pseudorhodoferax |
| Acidaminobacter | Coenonia | Leclercia | Pseudoxanthomonas |
| Acidovorax | Comamonas | Legionella | Quatrionicoccus |
| Acinetobacter | Corynebacterium | Leucobacter | Ralstonia |
| Actinomyces | Cupriavidus | Levilinea | Raoultella |
| Aeromonas | Curvibacter | Luteibacter | Rhizobium |
| Alicycliphilus | Daeguia | Lysobacter | Rhodococcus |
| Alkanindiges | Dechloromonas | Magnetospirillum | Rhodocyclus |
| Alteromonadales | Deefgea | Massilia | Rhodoplanes |
| Amaricoccus | Defluviicoccus | Methylobacterium | Riemerella |
| Aminobacterium | Deinococcus | Methylocella | Roseomonas |
| Aminomonas | Delftia | Methylocystis | Rothia |
| Anaerobacter | Derxia | Methyloversatilis | Rugamonas |
| Anaerococcus | Desulfobulbus | Microbacterium | Schlegelella |
| Anaerofilum | Desulfocurvus | Microvirga | Serpens |
| Anaerovorax | Desulforegula | Microvirgula | Simplicispira |
| Ancylobacter | Desulfovibrio | Mitsuaria | Sinobacteraceae |
| Aquabacterium | Diaphorobacter | Mycobacterium | Sinorhizobium |
| Aquaspirillum | Dokdonella | Nakamurella | Solimonas |
| Aquincola | Duganella | Neisseria | Soonwooa |
| Aurantimonas | Dyella | Nocardioidaceae | Spartobacteria |
| Azomonas | Dysgonomonas | Novispirillum | Sphingobacterium |
| Azonexus | Elizabethkingia | Novosphingobium | Sphingobium |
| Azorhizobium | Elusimicrobium | Nubsella | Sphingomonas |
| Azospira | Enhydrobacter | Oceanospirillales | Sphingopyxis |
| Azospirillum | Enterobacter | Olsenella | Sphingosinicella |
| Azotobacter | Enterococcus | Opitutus | Spirochaeta |
| Azovibrio | Epilithonimonas | Oribacterium | Staphylococcus |
| Bacteriovorax | Eubacterium | Ottowia | Stenotrophomonas |
| Bacteroides | Ferribacterium | Paludibacterium | Streptobacillus |
| Bdellovibrio | Filimonas | Parabacteroides | Streptococcus |
| Beijerinckia | Finegoldia | Paracoccus | Sulfuricurvum |
| Bellilinea | Flavobacterium | Parvimonas | Sulfurospirillum |
| Bilophila | Formivibrio | Pedobacter | Telmatospirillum |
| Blastomonas | Fusibacter | Pelomonas | Tessaracoccus |
| Bosea | Fusobacterium | Peptoniphilus | Thermomonas |
| Brachymonas | Gemella | Peptostreptococcus | Tolumonas |
| Bradyrhizobium | Geobacter | Perlucidibaca | Trabulsiella |
| Brevundimonas | Geothrix | Phenylobacterium | Treponema |
| Brooklawnia | Granulicatella | Phyllobacteriaceae | Uliginosibacterium |
| Burkholderia | Haemophilus | Planctomycetaceae | Uruburuella |
| Butyrivibrio | Heliothrix | Pleomorphomonas | Variovorax |
| Capnocytophaga | Herbaspirillum | Porphyromonas | Victivallis |
| Caulobacter | Holophaga | Prevotella | Vogesella |
| Chitinimonas | Inquilinus | Prolixibacter | Xanthobacter |
| Chryseobacterium | Janthinobacterium | Propionibacterium | Yokenella |
| Citrobacter | Klebsiella | Propionivibrio | Zobellella |
| Cloacibacillus | Kluyvera | Pseudomonas | Zoogloea |

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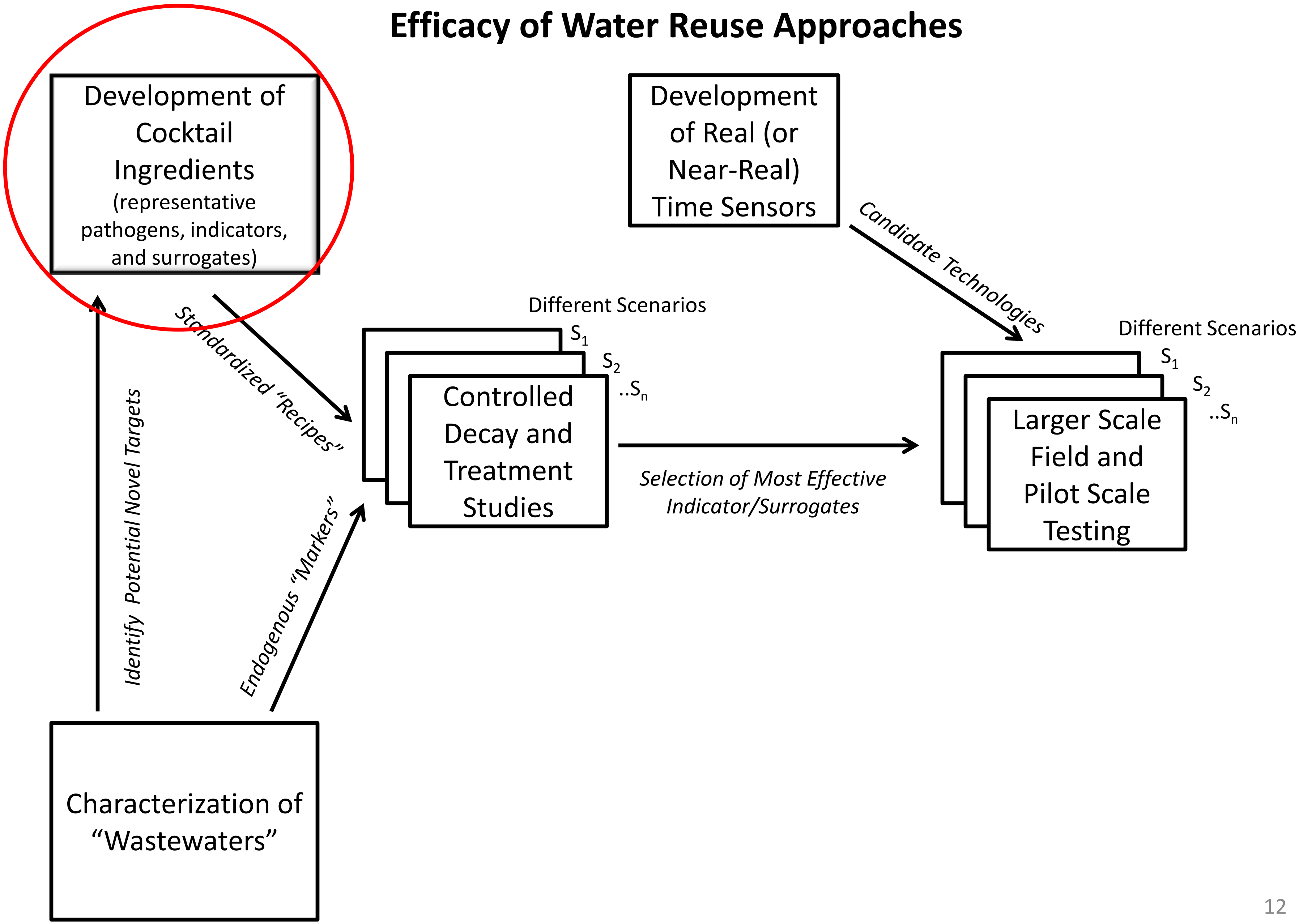


# What's the Best Way to Make a Microbial Cocktail?

"Fresh" Sewage

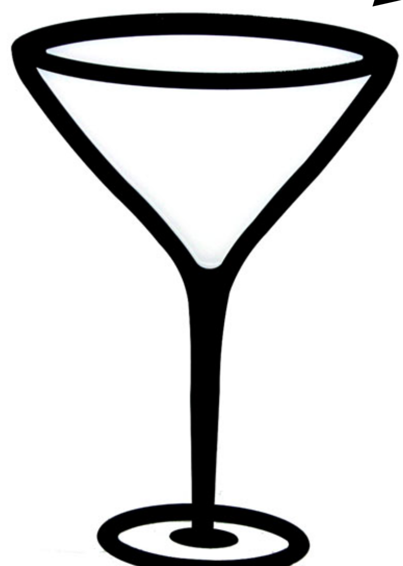
Adsorption (Celite),
Precipitation (pH),
Filtration

Specific Pathogens
e.g., protozoan cysts

Cultures of Indicators

Pooled, Frozen
Stock of Viral
Pathogens

Direct Addition
Indicators
Human Source Marker Bacteria,
Human Source Marker Phage,
Overall Phage
Activated Sludge Organisms



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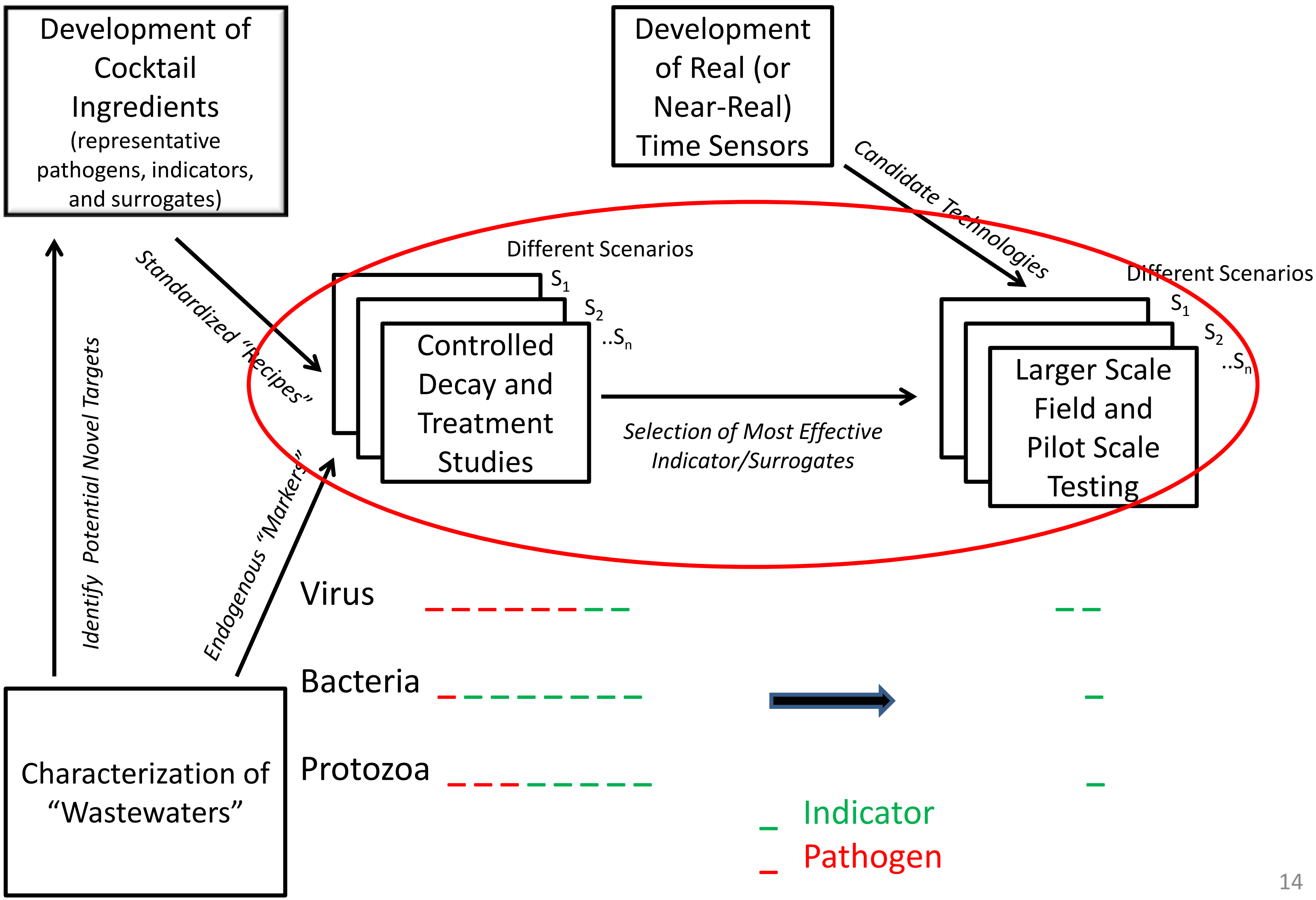

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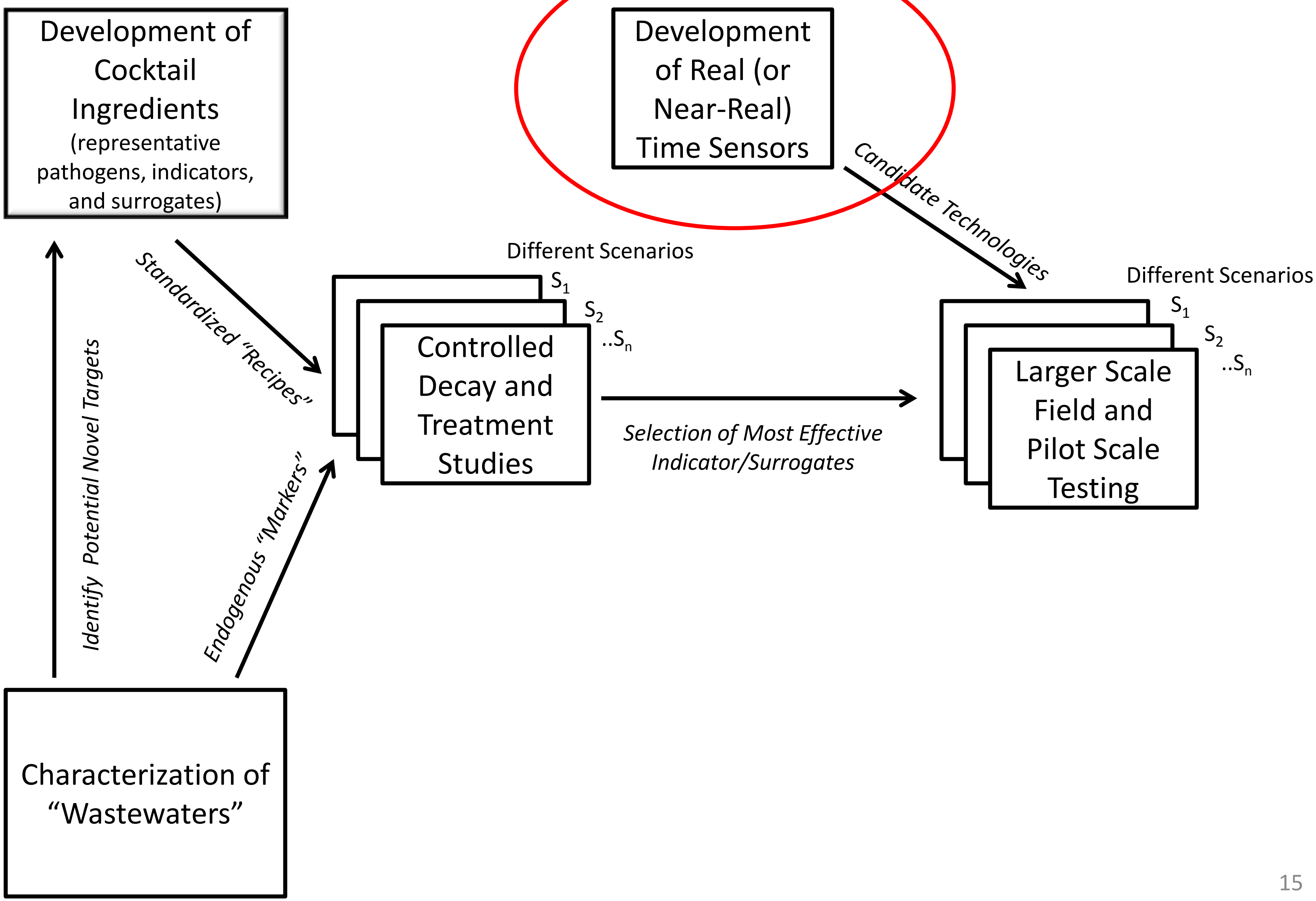
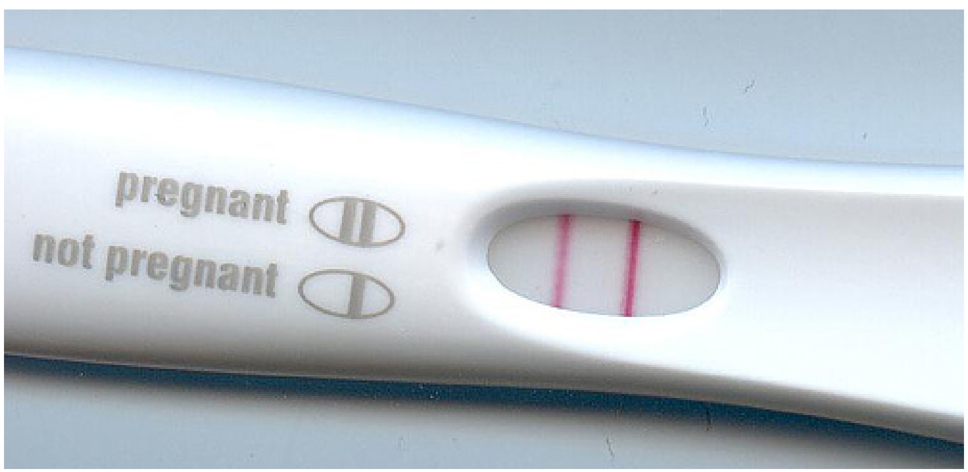
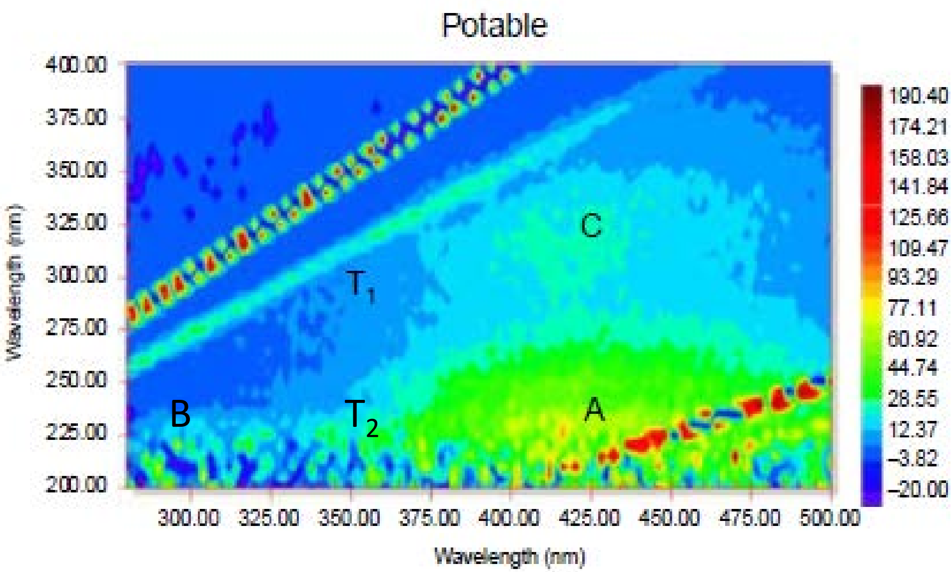
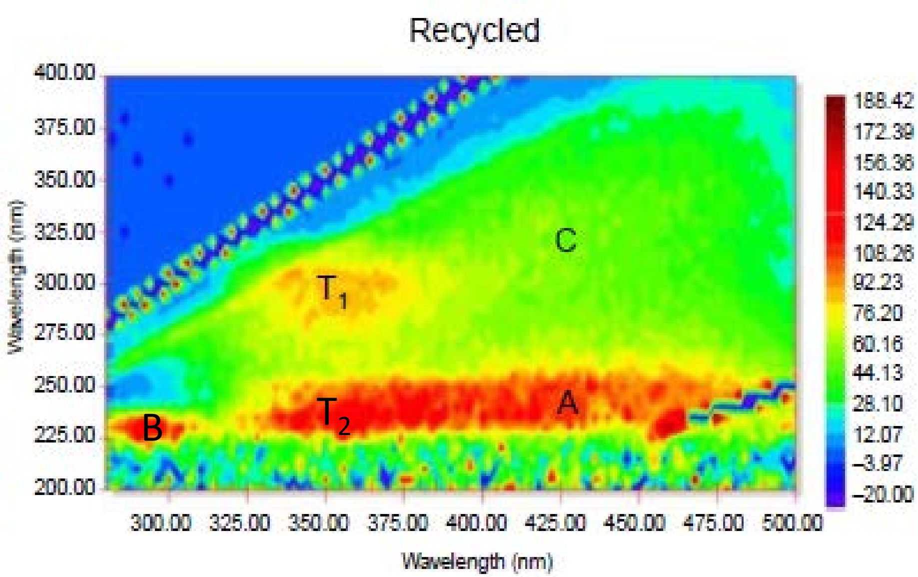


# Verifying Quality in Sequential Batches




- Rapid “real-time” detection
  - Enzymatic
  - Signal amplification



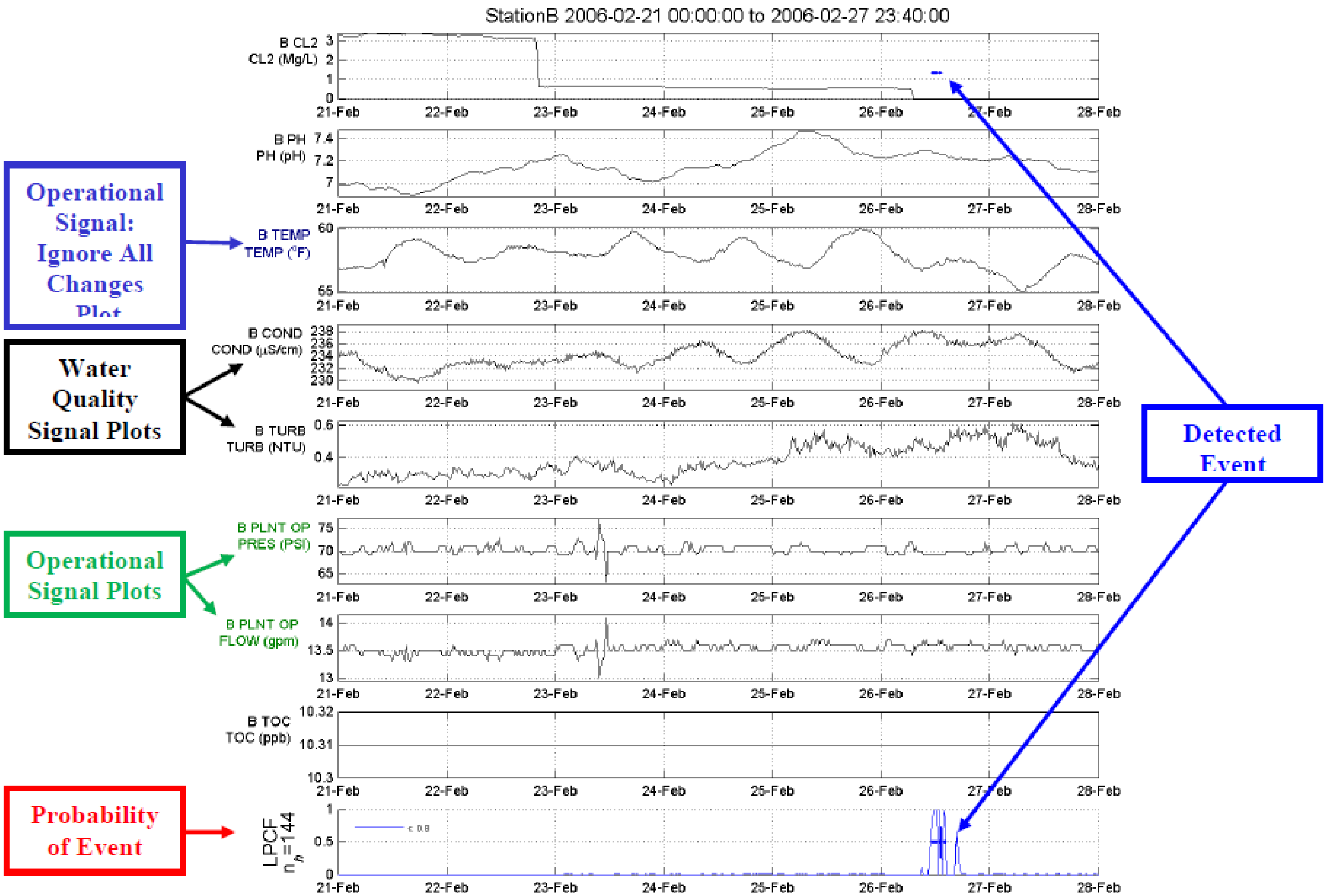




Peak definitions (Henderson, 2009)

- A: Humic-like
- B: Tryosine-like
- C: Humic-like
- T1: Tryptophan-like
- T2: Tryptophan-like

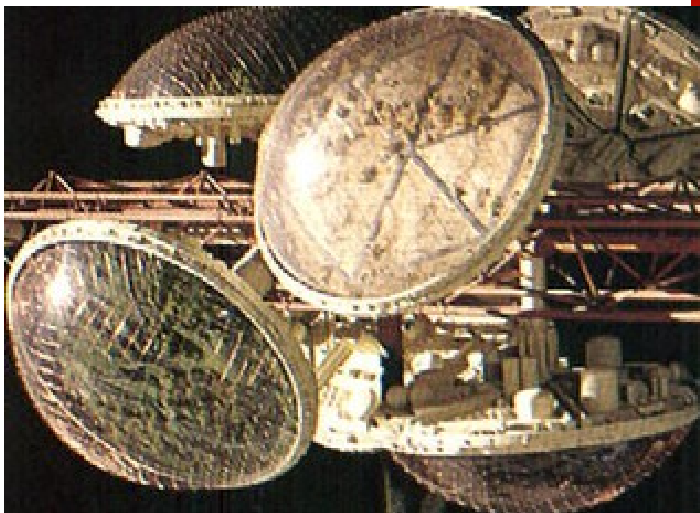
- Extracellular proteins are mainly excreted by microorganisms. Tryptophan fluorescence is the dominant part of the protein fluorescence, which has a fluorescence maximum at Peak T1 and T2 (Ni, 2009)
- Peak T2 fluorescence correlates with HB, TC, E. Coli ($R^2$ values of .81, .78, .72, respectively) from diluted river water and sewage works final effluent (Cumberland, 2012)

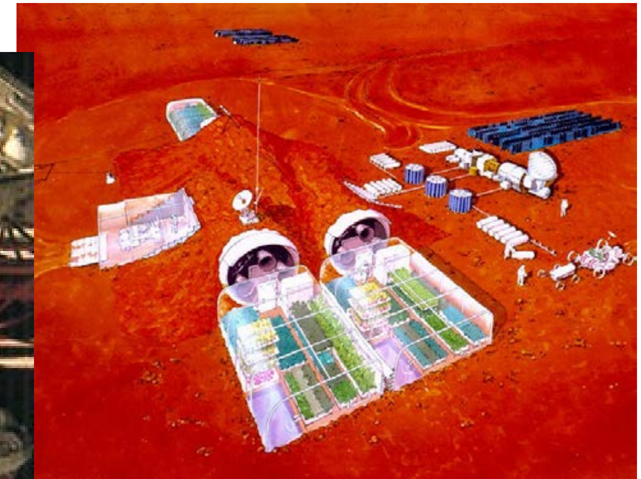
# Multivariate Online Monitoring with Event Detection

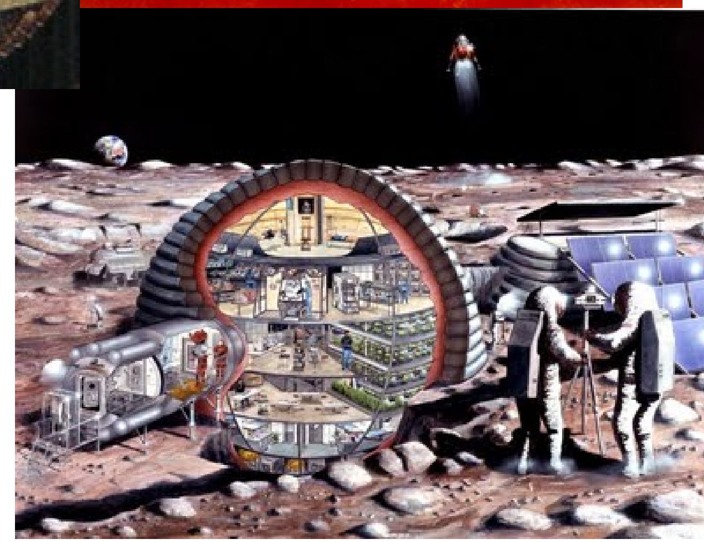



CANARY

# *The Need for Eco-Effective Designs for Long Term Human Missions in Space*



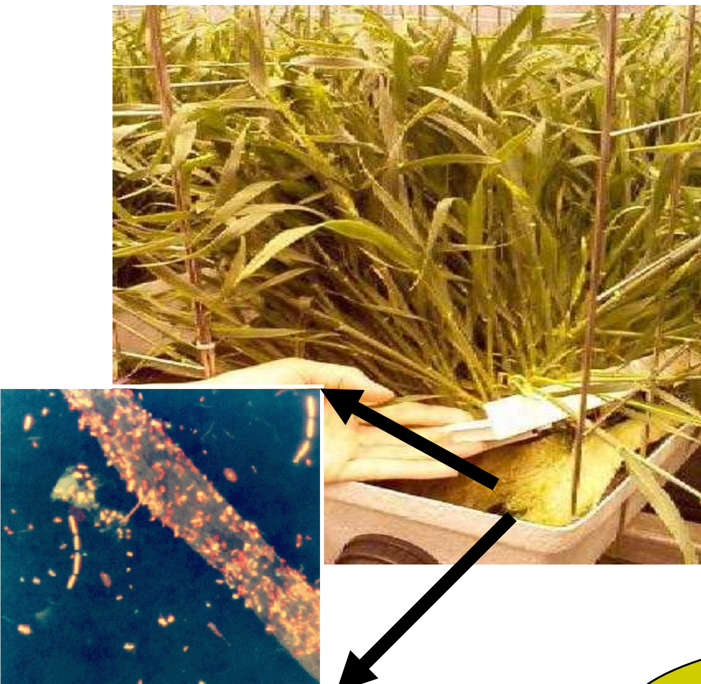




"If we knew how to live on Mars, we'd know how to reduce our footprint on Earth. Space colonization is the Rosetta stone for earthly sustainability because it's entirely about living in the absence of ecosystem services. The Moon, Mars and the asteroids are a great experimental laboratory that we're ignoring at our own peril."

*Karl Schroeder*

# Direct Graywater Processing Scheme



Transpiration

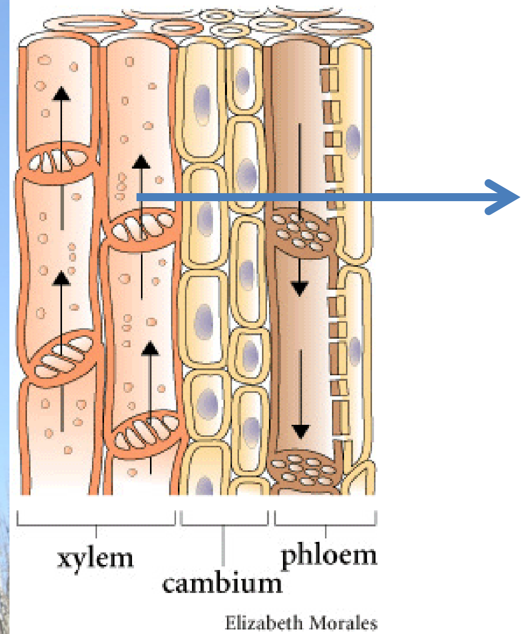
Condensate

Surfactant Degradation
in the Rhizosphere

Graywater addition as
liquid level replenishment

Maintenance of water quality

*Water Research (2004) 38:1952*
*Water Research (2000) 34:3075*




Tap “free”
Membrane purifed
Solar pumped water

Linking biophillia and low environmental impact design to a new paradigm of sustainable development, referred to as ‘restorative environmental design’

*Stephen Kellert*