SEEING IS BELIEVING - REPORTER LABELED MICROBIAL CONTROL STRAINS

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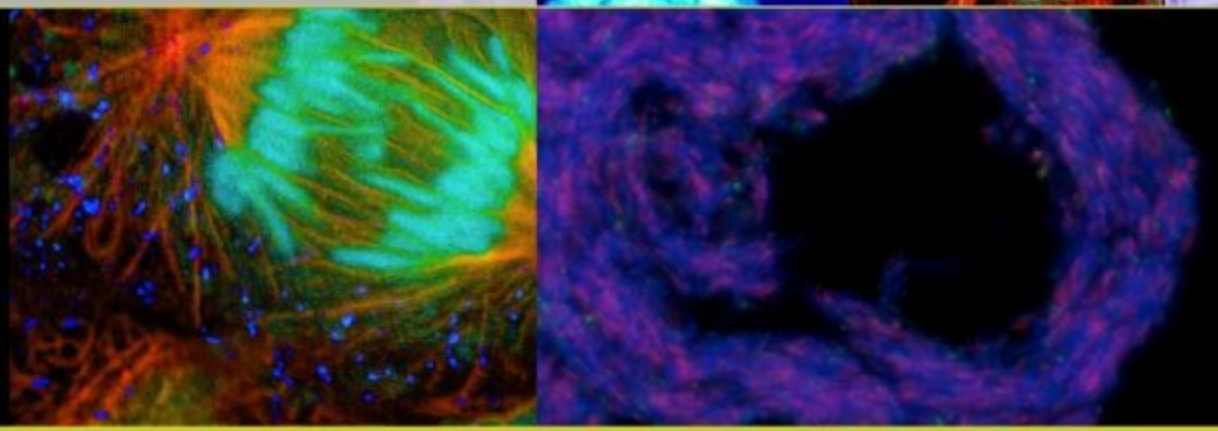
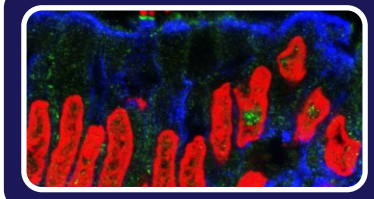
Lead Biologist, ATCC
May 21, 2015



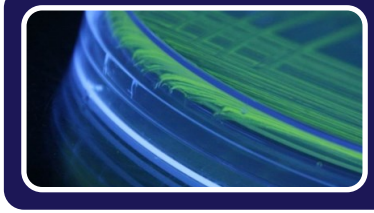
ATCC® THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

Today's discussion

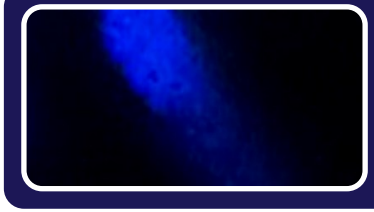
About ATCC



Reporter labels and their use



GFP-labeled strains



NanoLuc®-labeled Shiga toxin-producing *Escherichia coli* strains

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Photo credit: S. Schuller

About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Strong team of 400+ employees; over one third with advanced degrees
- Broad range of biomaterials
  - Cell lines, iPSCs, primary cells, and hTERT immortalized cells
  - Bacteria, yeasts, protists, and viruses
  - Tumor cell panels
  - Media, sera, and reagents

Established partner to global researchers and scientists

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Why label bacteria?

- Visualize bacteria under experimental conditions
- Differentiate between sample contamination and false positives from control cross-contamination
  - Saves money, time, and worry

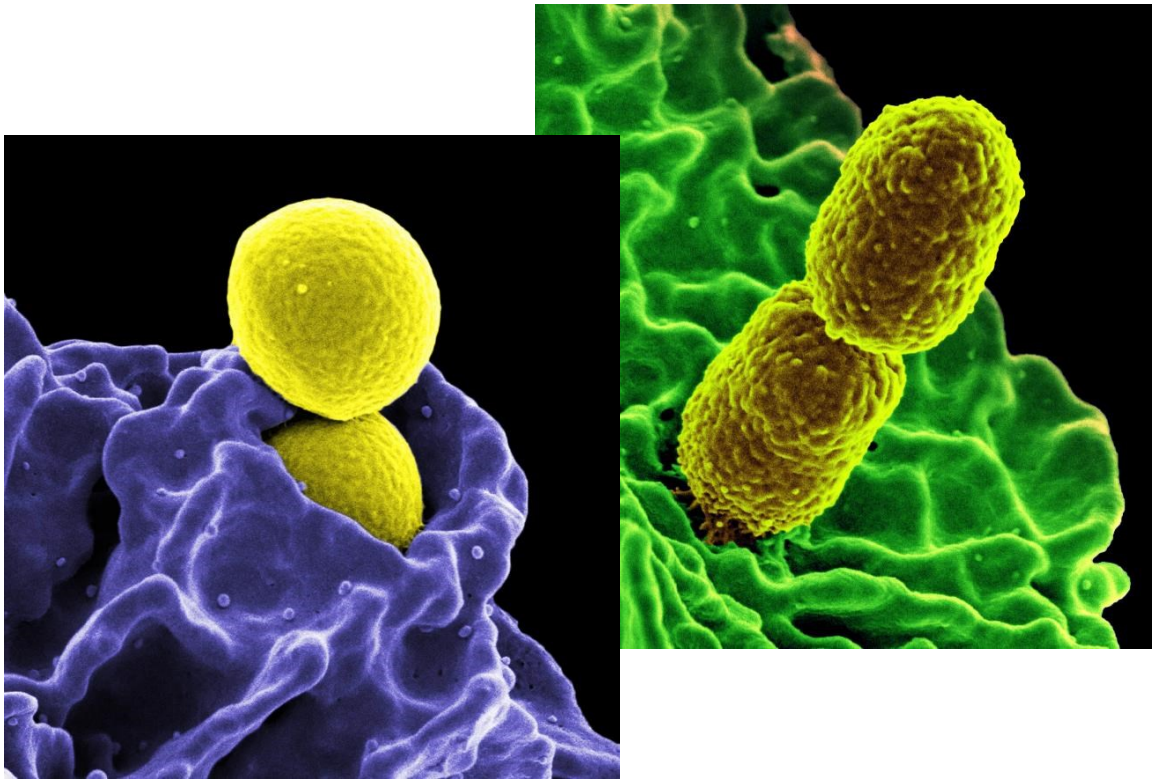

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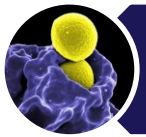
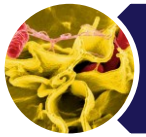
Photo credit: NIAID

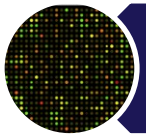
Why label bacteria?

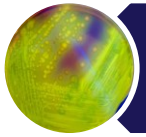

Microbial quantification and detection

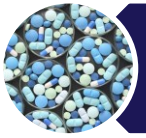

Host-pathogen interactions


Drug discovery


Food testing


Gene expression


Biochemical interactions


Novel therapeutics


Quality control assays

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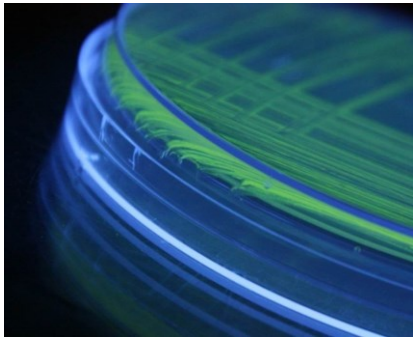
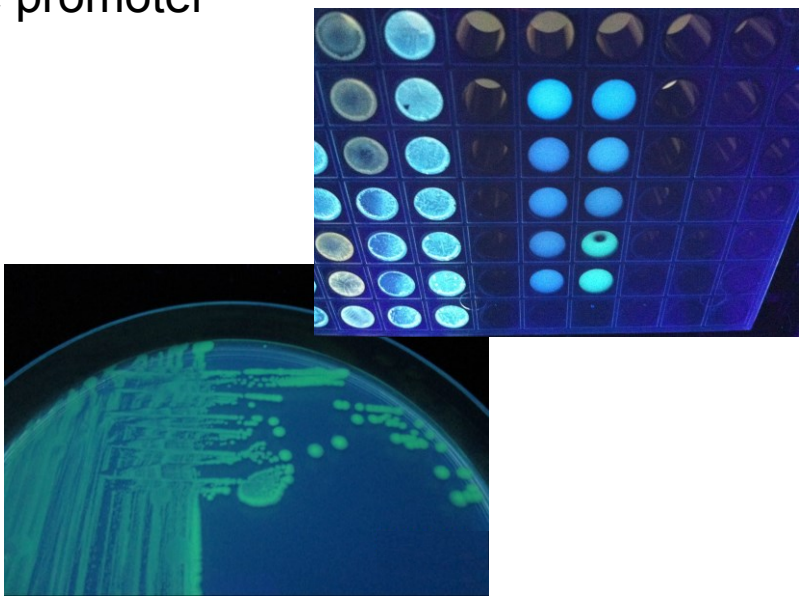
Reporter characteristics



	Green Fluorescent Protein (GFP)	NanoLuc® Luciferase
Live-cell assays	Yes	No
Well-characterized	Yes	No
Highly stable	Yes	Yes
Substrate required	No	Yes
Glow response	Yes	Yes
Use *in vivo*	Yes	No

GFP-labeled strains

- Labeled Gram-negative organisms with GFPmut3a
  - *Escherichia coli*
  - *Salmonella enterica* subsp. *enterica* serovar Typhimurium
  - *Shigella flexneri*
  - *Pseudomonas aeruginosa*
- *gfp* expressed behind the P_{lac} constitutive promoter
- Bright, consistent label can be used for:
  - Microbial quantification and detection
  - Host-pathogen interactions
  - Drug discovery
  - Food testing



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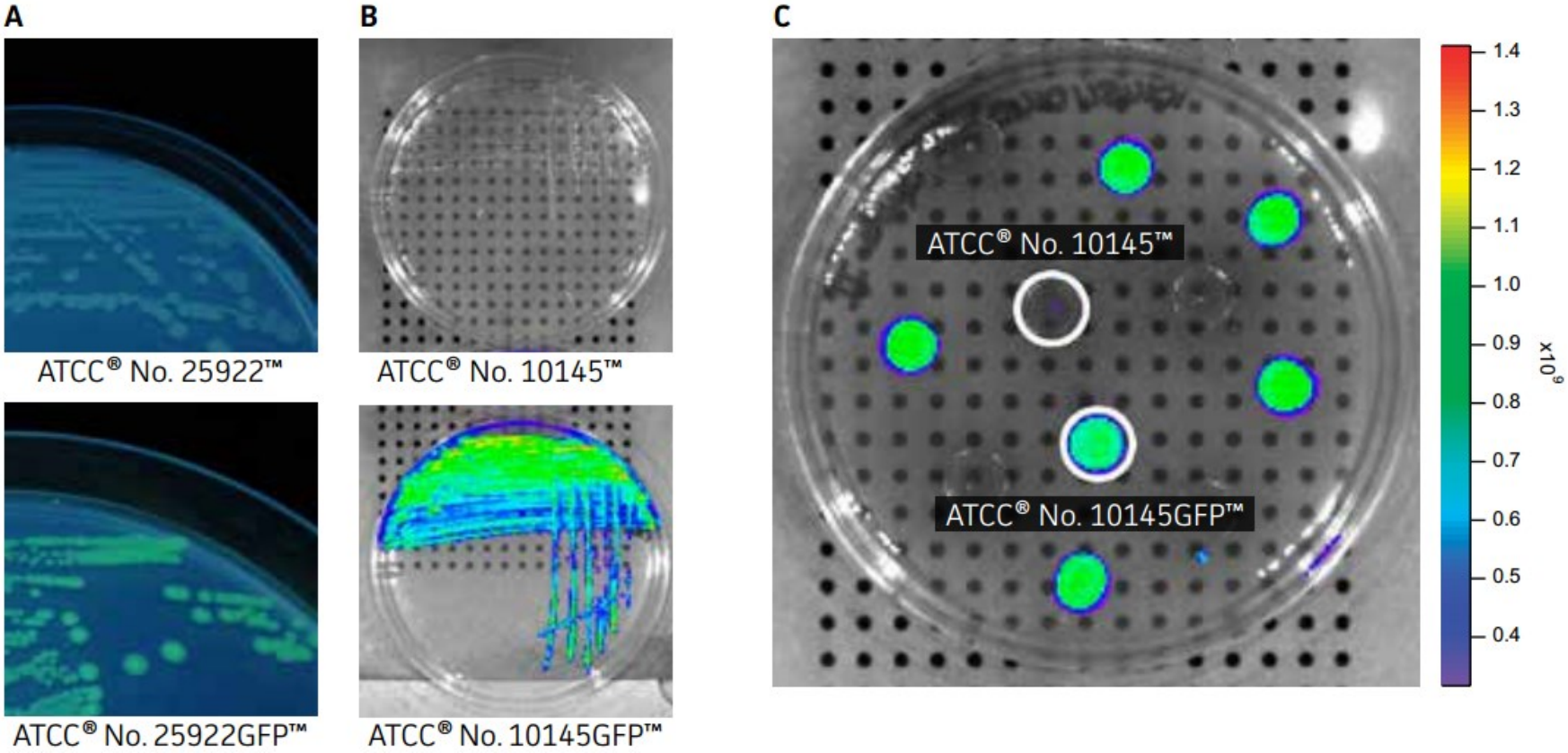
Visual detection of GFP-labeled organisms



The expression of a bright GFP variant on a high-copy number plasmid facilitates visual identification when exposed to UV light (A) or imaged using a detection system (B &C)

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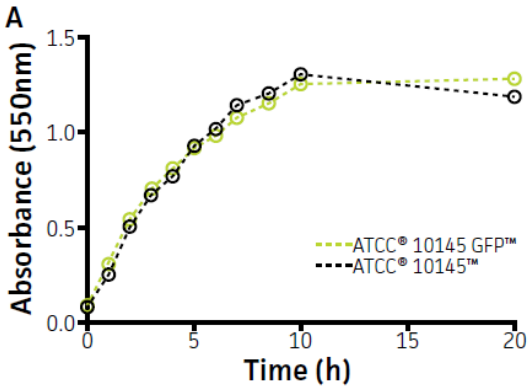
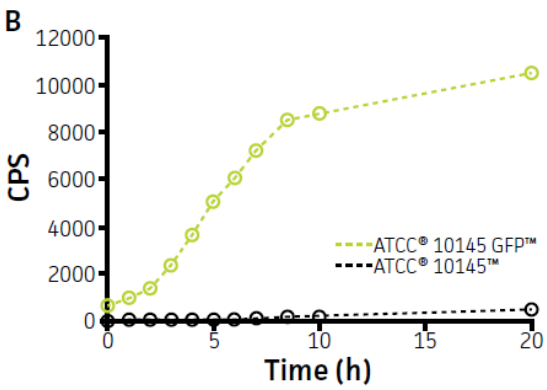
Bacterial fitness and quantification

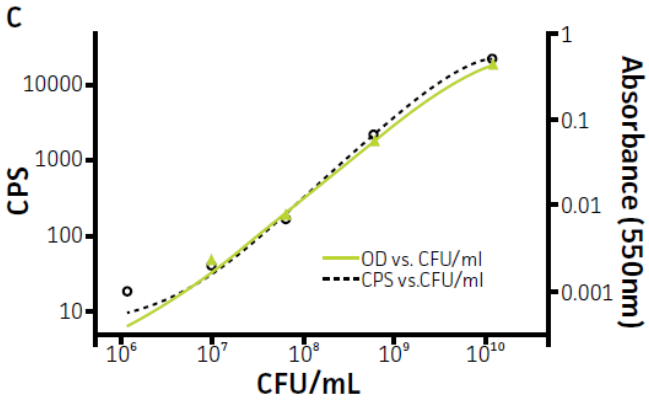
Absorbance



Fluorescence



Quantification



- GFPmut3a is not detrimental to bacterial fitness
- Fluorescence can be used for bacterial quantification

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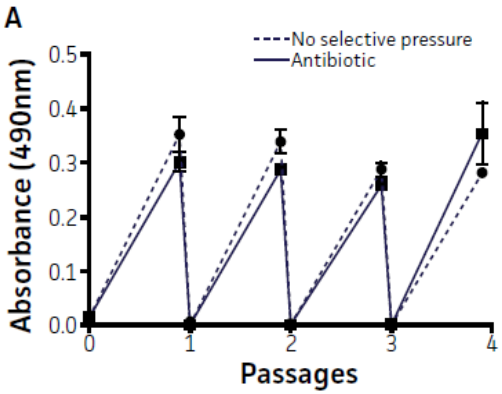
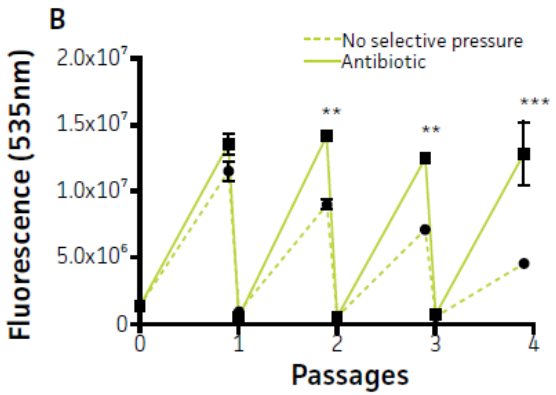
Plasmid stability

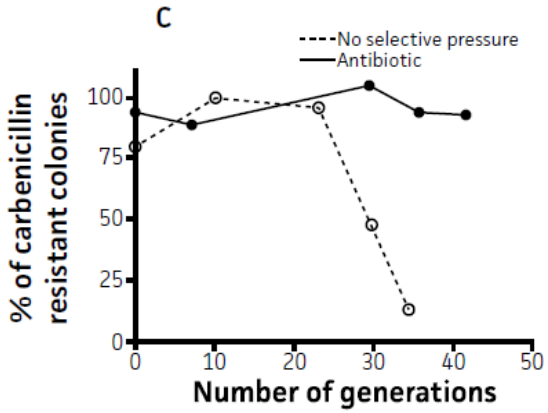
Absorbance



Fluorescence



Stability



Plasmid is stable for at least 20 generations at 37°C in the absence of antibiotic pressure

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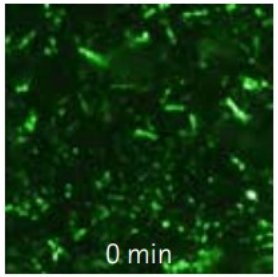
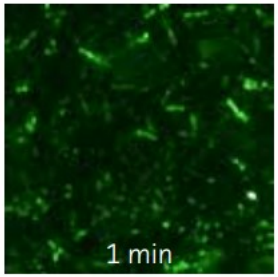
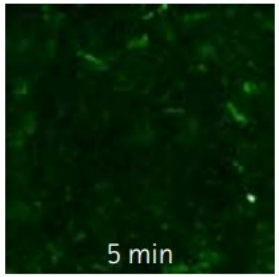
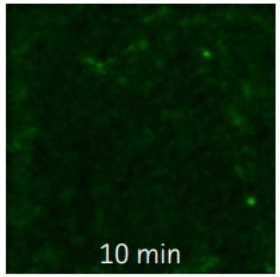
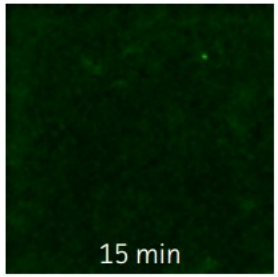
Photo bleaching



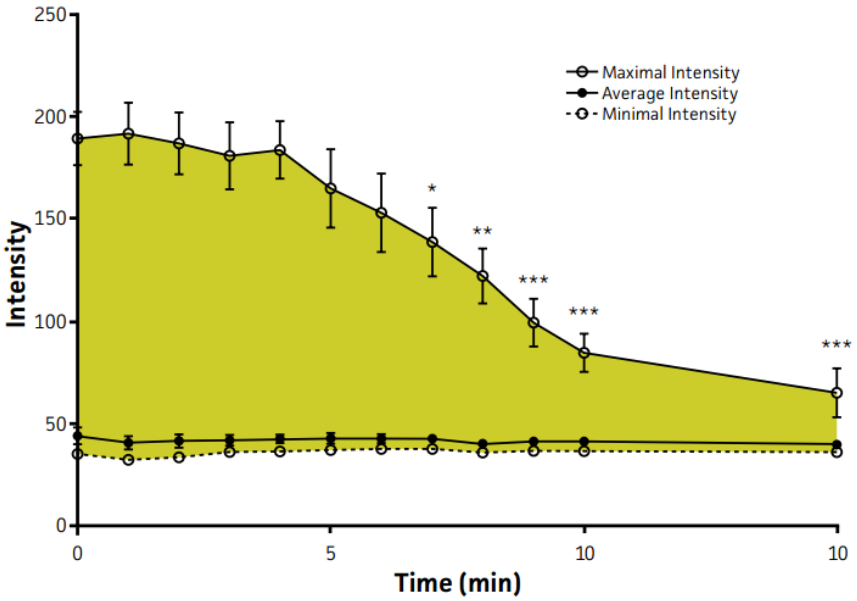








- *P. aeruginosa*-GFP (ATCC® 10145GFP™) was continuously exposed to UV light to determine resistance to photo bleaching
- Fluorescence remained stable during the first 5 minutes
- Significant loss of signal after 7 minutes of exposure



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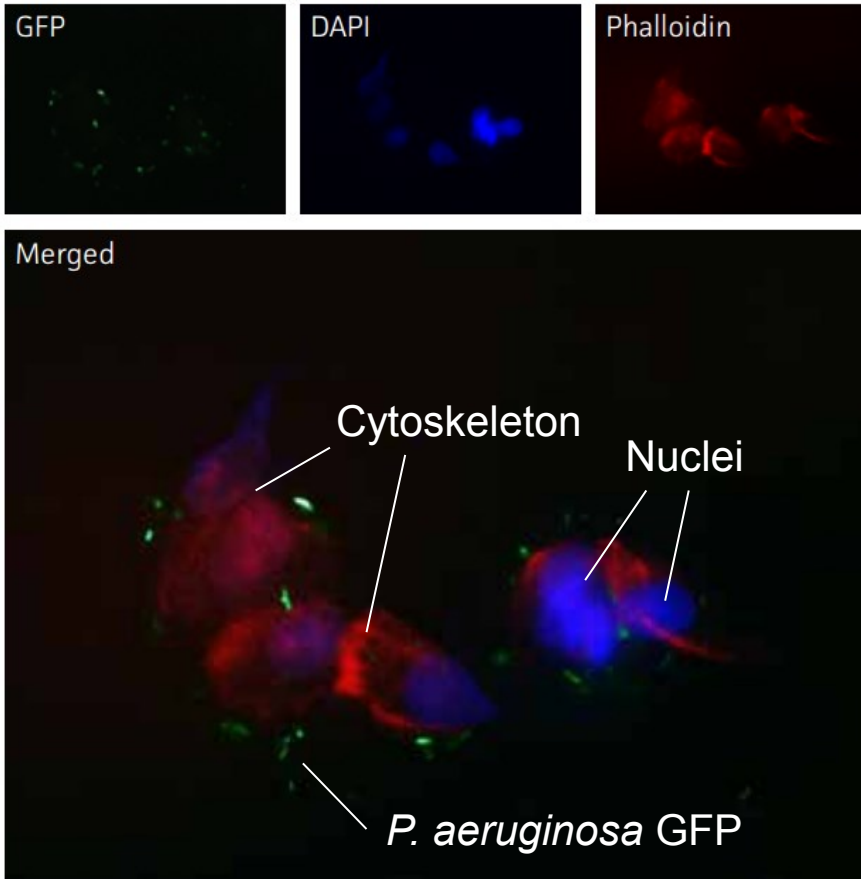
Fluorescent microscopy detection of pathogen-host interactions



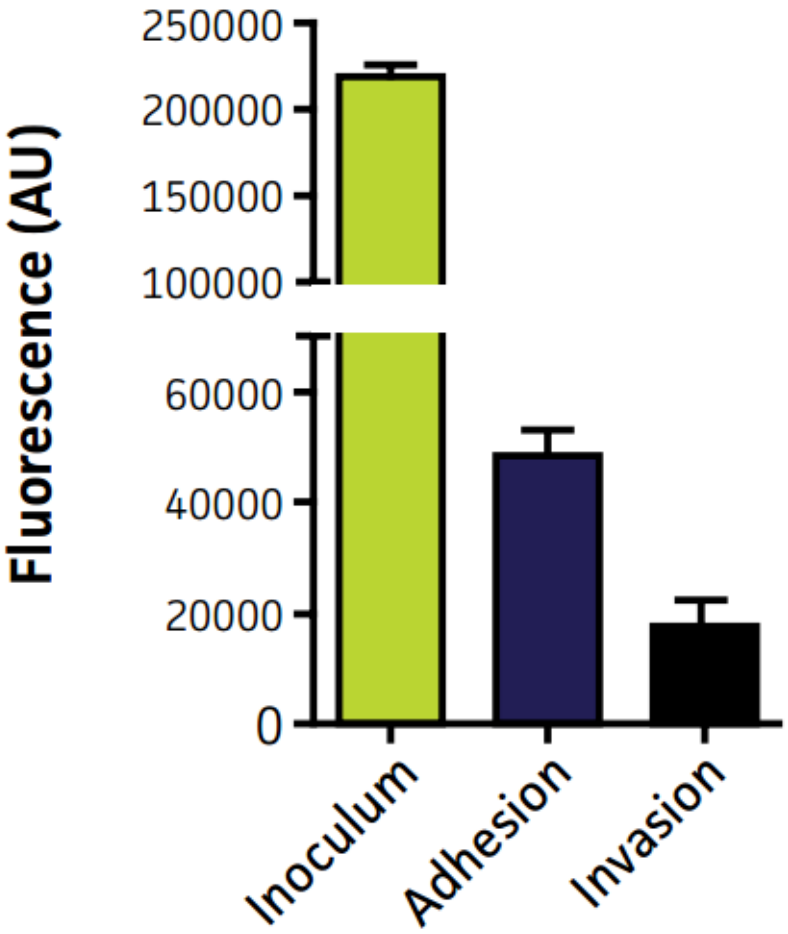
P. aeruginosa GFP (ATCC®10145GFP™) interaction with A549 (ATCC® CCL-185™) airway epithelial cells (100X magnification)

ATCC®

High-throughput detection of pathogen-host interactions

Invasion study

- *P. aeruginosa* GFP (ATCC®10145GFP™) was incubated in the presence of A549 (ATCC® CCL-185™) airway epithelial cell monolayers
- Cells were washed and medium supplemented with 100 µL gentamicin was added to kill extracellular bacteria (Adhesion)
- Cells were washed again (Invasion) and measured on a microplate reader



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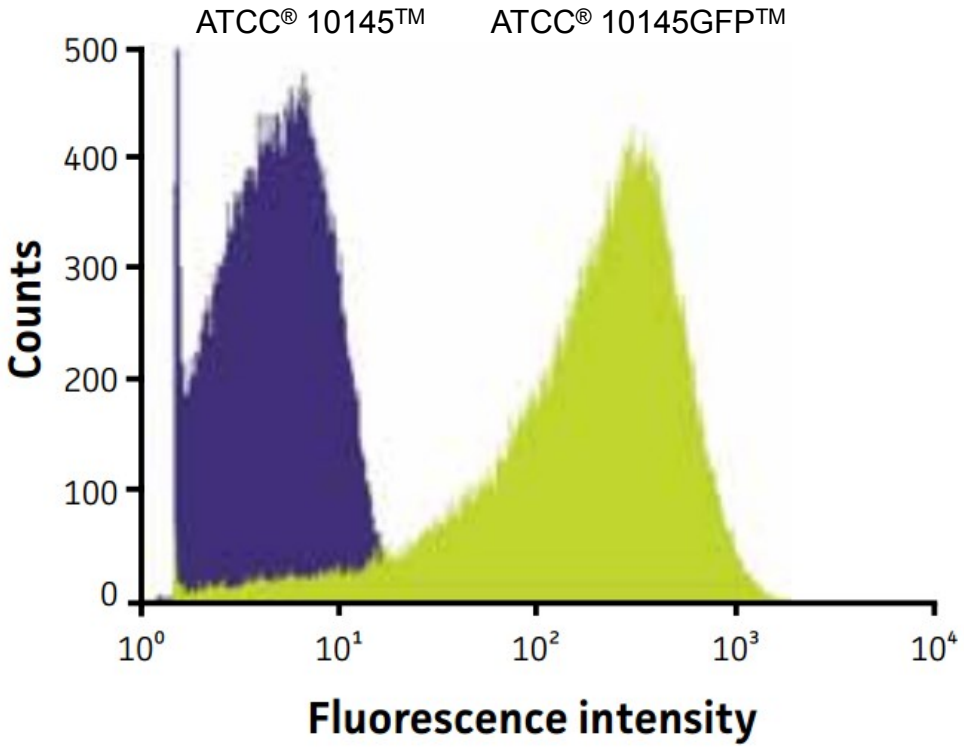
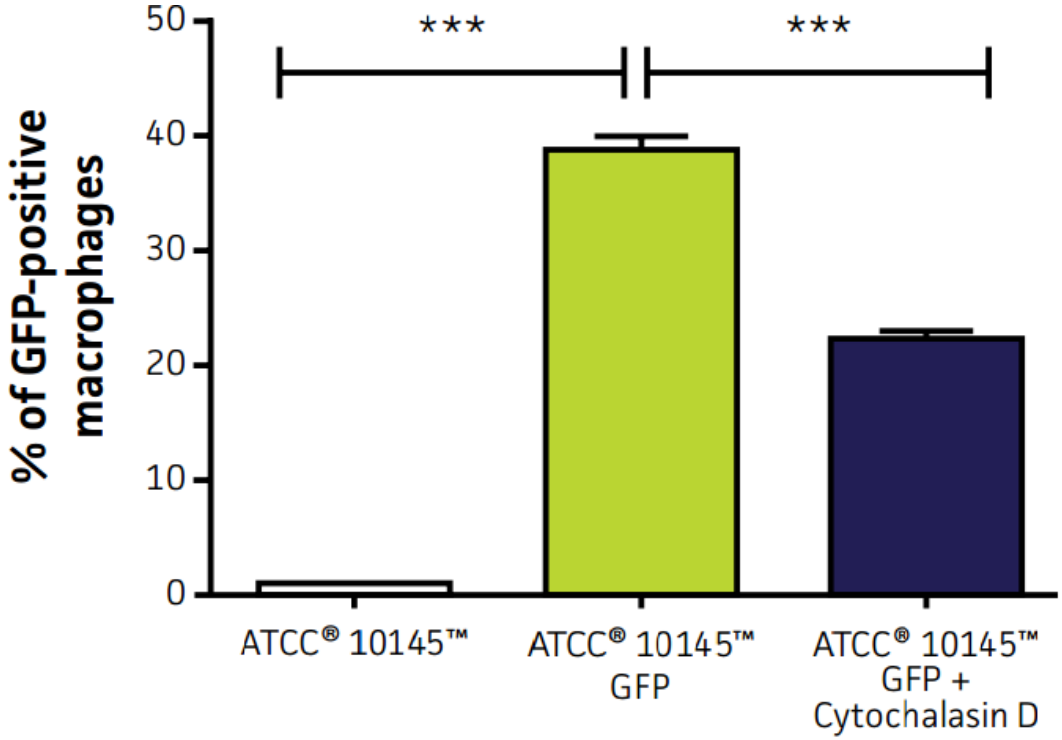
High-throughput detection of pathogen-host interactions



Flow Cytometry

- *P. aeruginosa* (ATCC® 10145™) (purple) and *P. aeruginosa* GFP (ATCC® 10145GFP ™) (green) suspensions were analyzed by flow cytometry

High-throughput detection of pathogen-host interactions

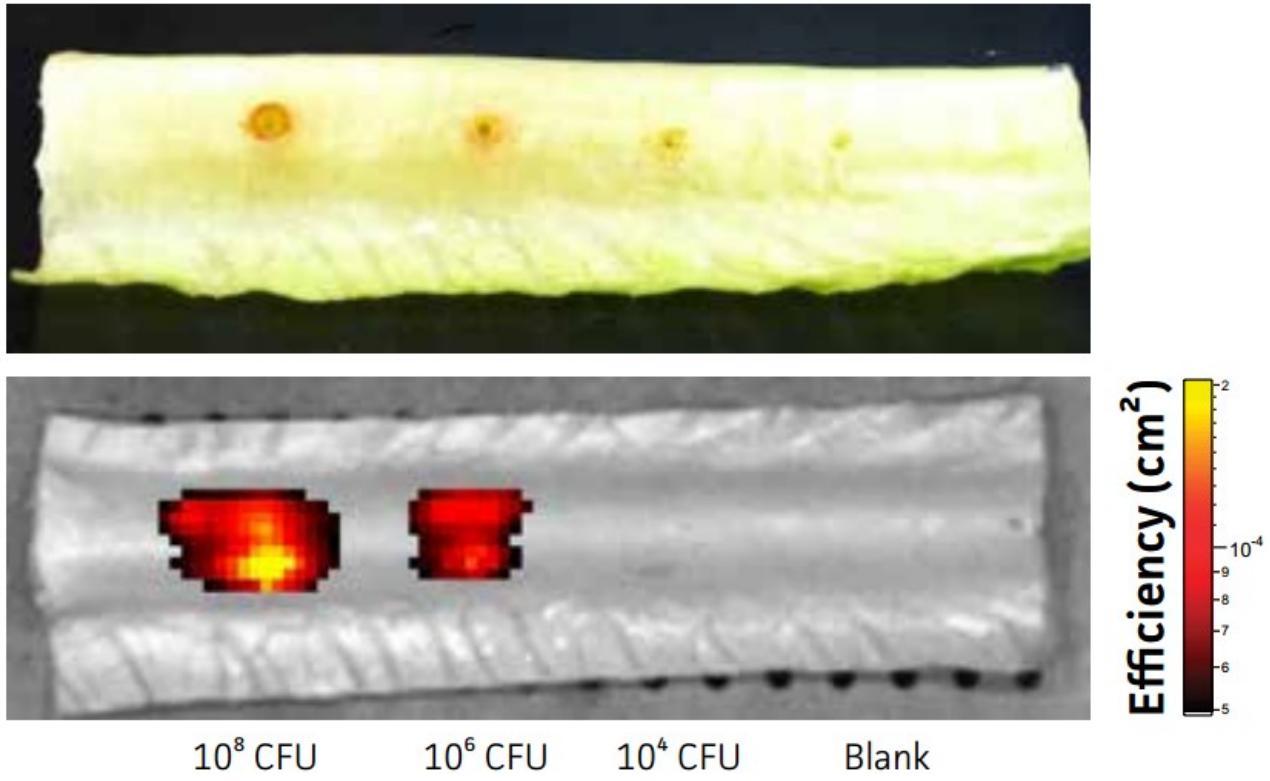


Macrophage uptake of *Pseudomonas aeruginosa*

- *P. aeruginosa (*ATCC® 10145™) and *P. aeruginosa* GFP (ATCC® 10145GFP™) were incubated in the presence or absence of Cytochalasin D and analyzed by flow cytometry

ATCC®

In vivo detection of GFP-labeled *P. aeruginosa*



- Various doses of *P. aeruginosa* GFP (ATCC® 10145GFP™) were injected into the mid-rib of *Lactuca sativa* L. var. *longifolia*
- The bacteria was easily detected at higher concentrations in the plant host, indicating that this vector can be successfully employed to monitor bacterial growth within a plant host

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ATCC GFP-labeled strains

ATCC® No.	Species	Reporter	Parental Strain (ATCC® No.)
25922GFP™	*Escherichia coli*	GFP	25922™
14028GFP™	*Salmonella enterica* subsp. *enterica* serovar Typhimurium	GFP	14028™
12022GFP™	*Shigella flexneri*	GFP	12022™
10145GFP™	*Pseudomonas aeruginosa*	GFP	10145™
15692GFP™	*Pseudomonas aeruginosa*	GFP	15692™

www.atcc.org/reporters

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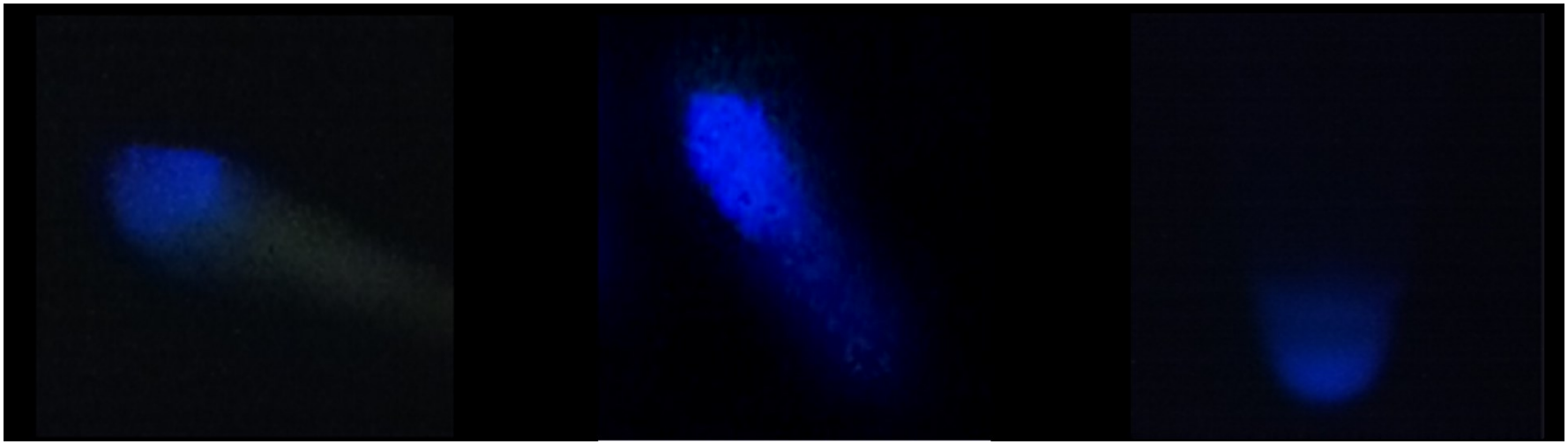
Promega NanoLuc® reporter



Cotton Swab | Filter Paper | Cell Pellet

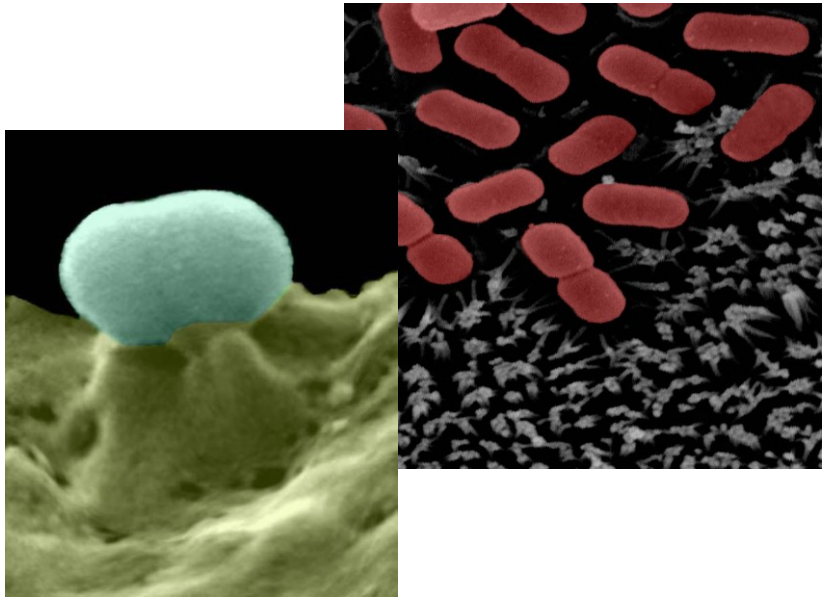
- Intensely bright reporter
- Glow response
- Portable, does not require instrumentation

ATCC®

Shiga toxin-producing *E. coli*

- >265,000 cases of STEC infection in the United States each year
  - *E. coli* O157:H7 accounts for about 36% of STEC infections
  - ~5-10% of diagnosed infections develop into hemolytic uremic syndrome, a life threatening complication which can cause permanent health damage

- Food Safety and Modernization Act calls for expanded testing to include Non-O157 strains
  - O26
  - O45
  - O103
  - O111
  - O121
  - O145



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Source: Centers for Disease Control and Prevention Photo credit: Dr. Paul Dean

Microbial strain authentication

ATCC utilizes both classical and modern techniques

- Phenotypic analysis
- Genotypic & proteotypic analyses
- Functional analysis

No single method of identification is sufficient

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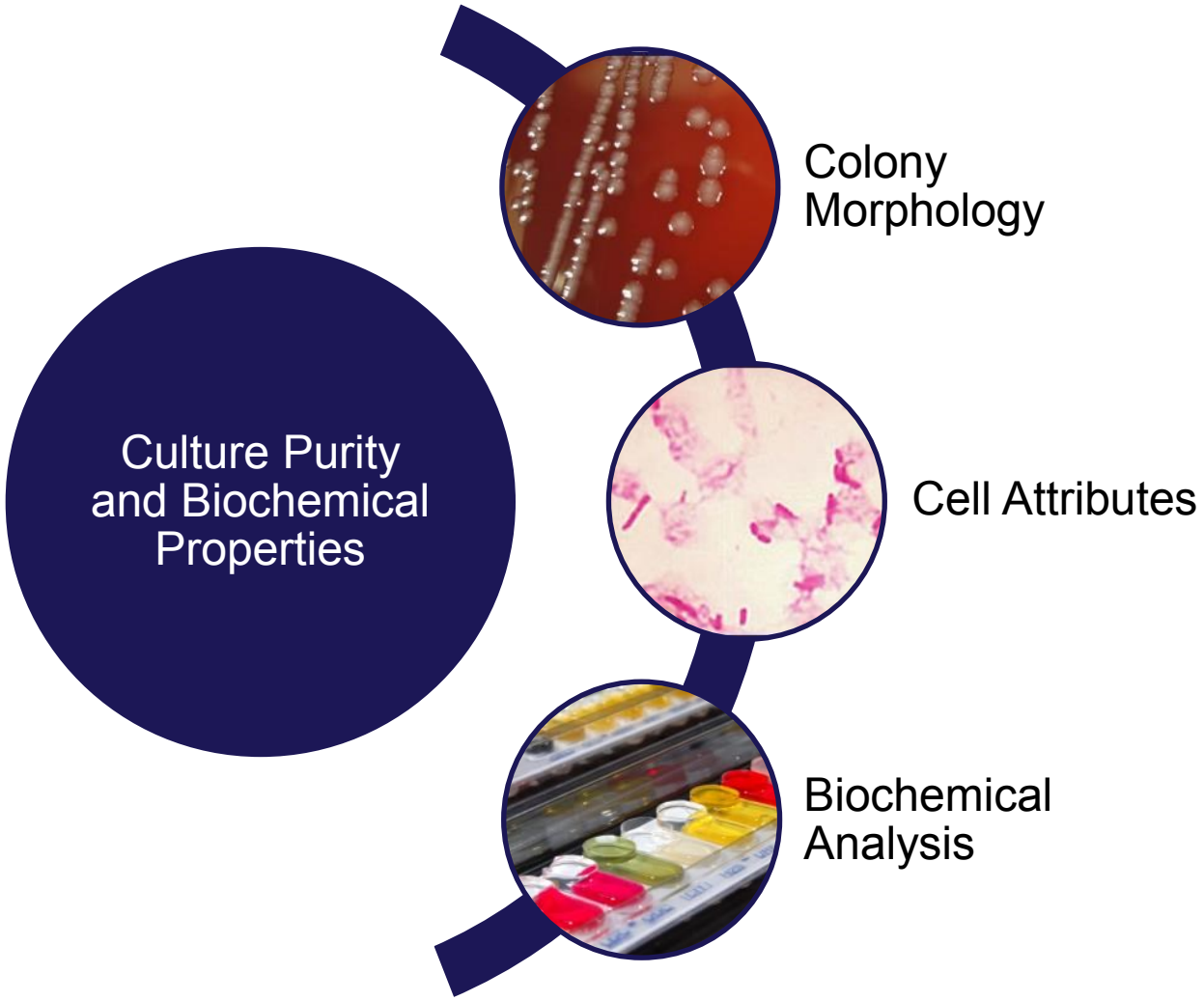
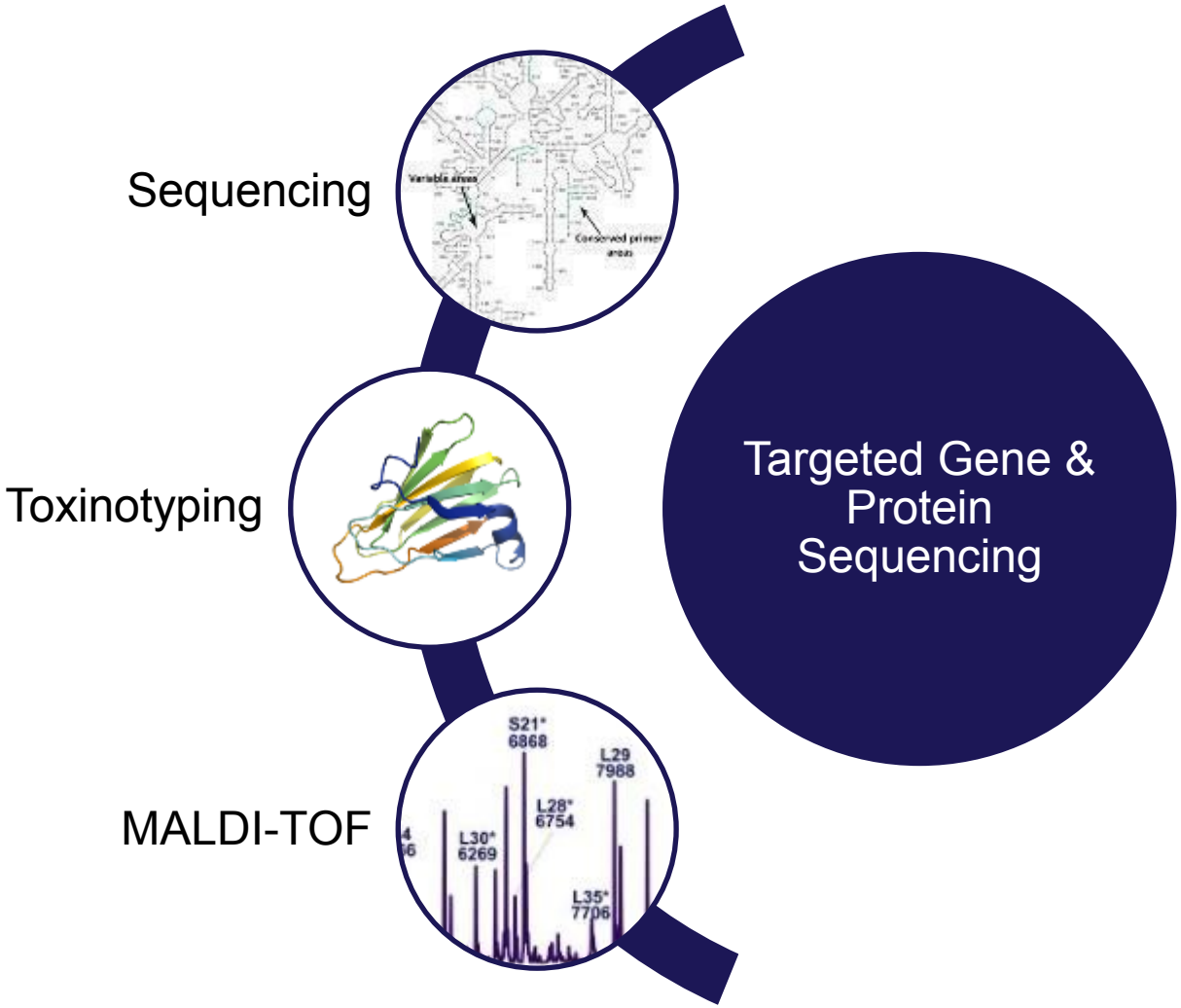
Phenotypic testing



Genotypic & proteotypic testing



Functional testing

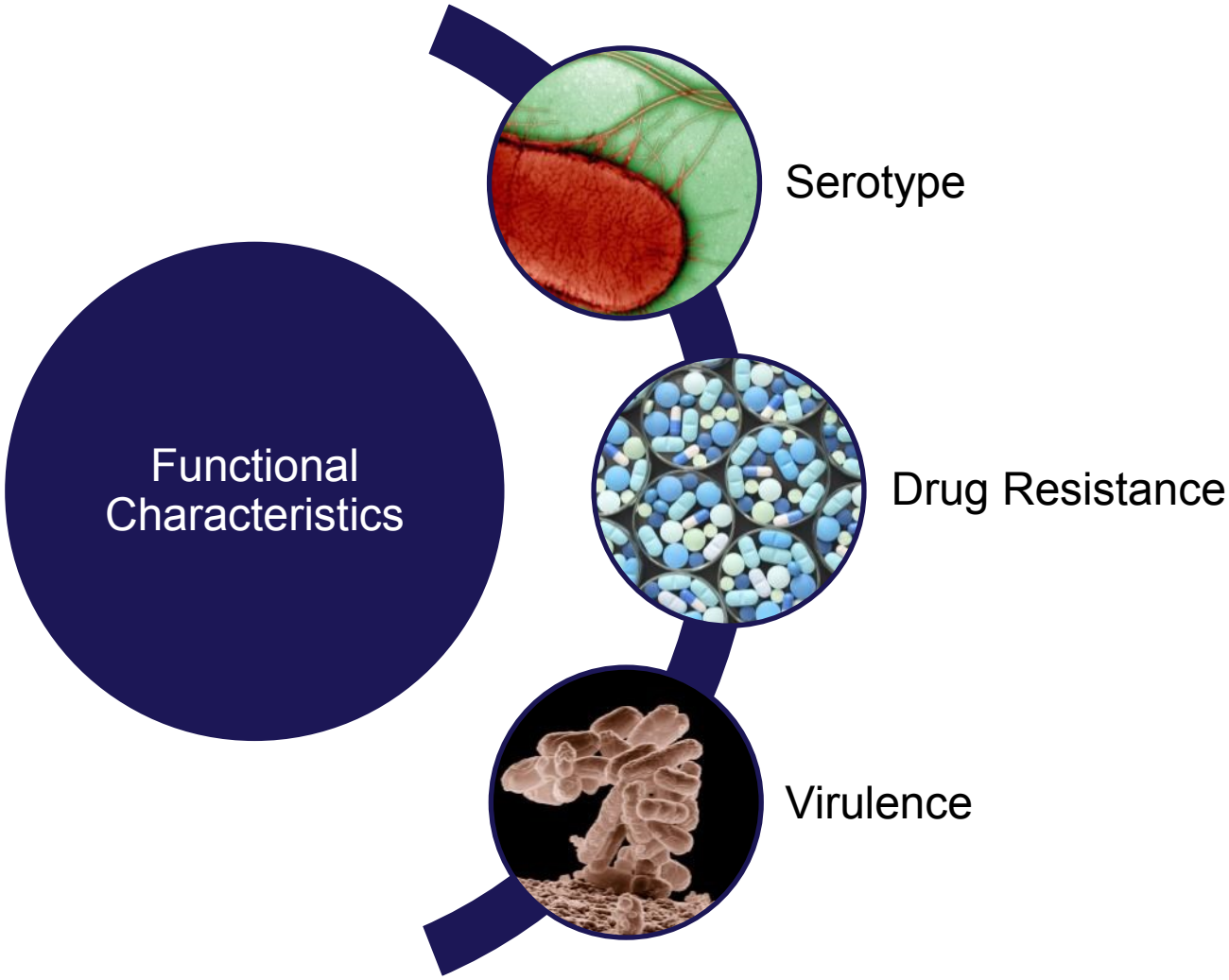


Photo credit: David Gregory & Debbie Marshall, Eric Erbe & Christopher Pooley (USDA ARS)

STEC Testing

- Bacterial identification
  - VITEK® 2, VITEK® MS, API® Strips, 16S rRNA sequencing
- Molecular characterization to assess the presence of the *stx*1, *stx*2, and *eae*A genes
  - PCR
- Serogroup identification
  - Immunoprecipitation assay

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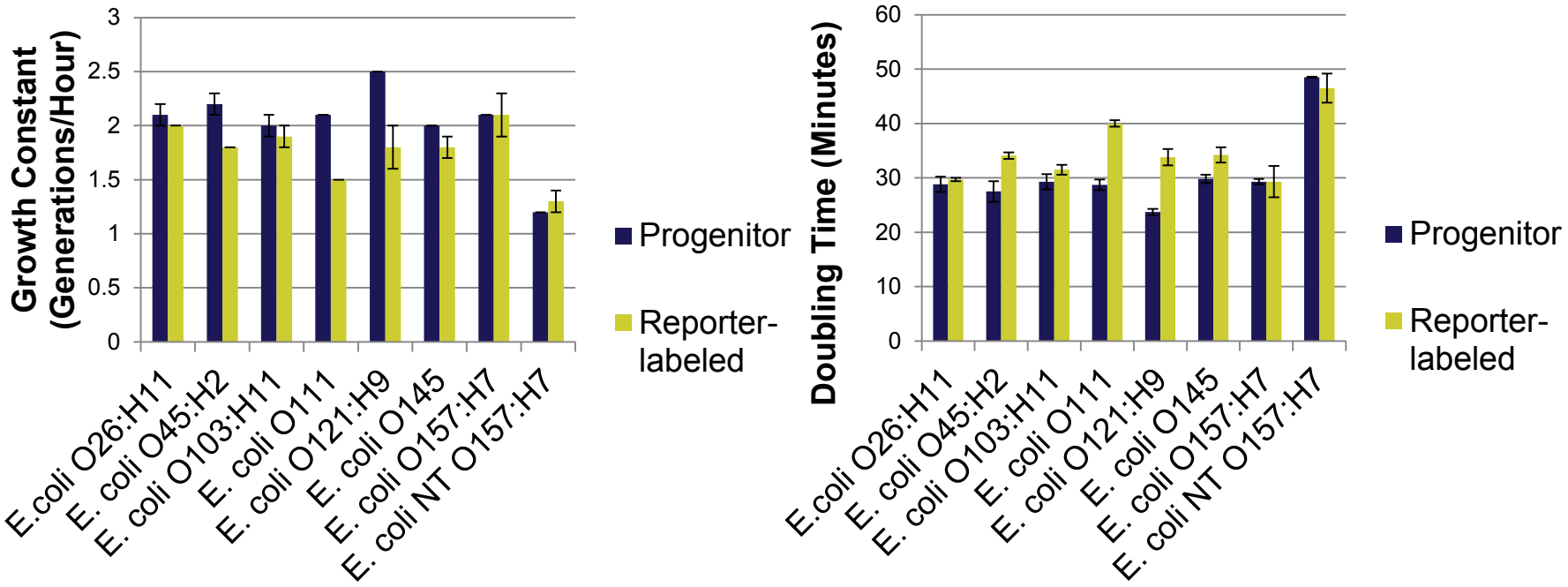
Minimal reporter effects on bacterial fitness



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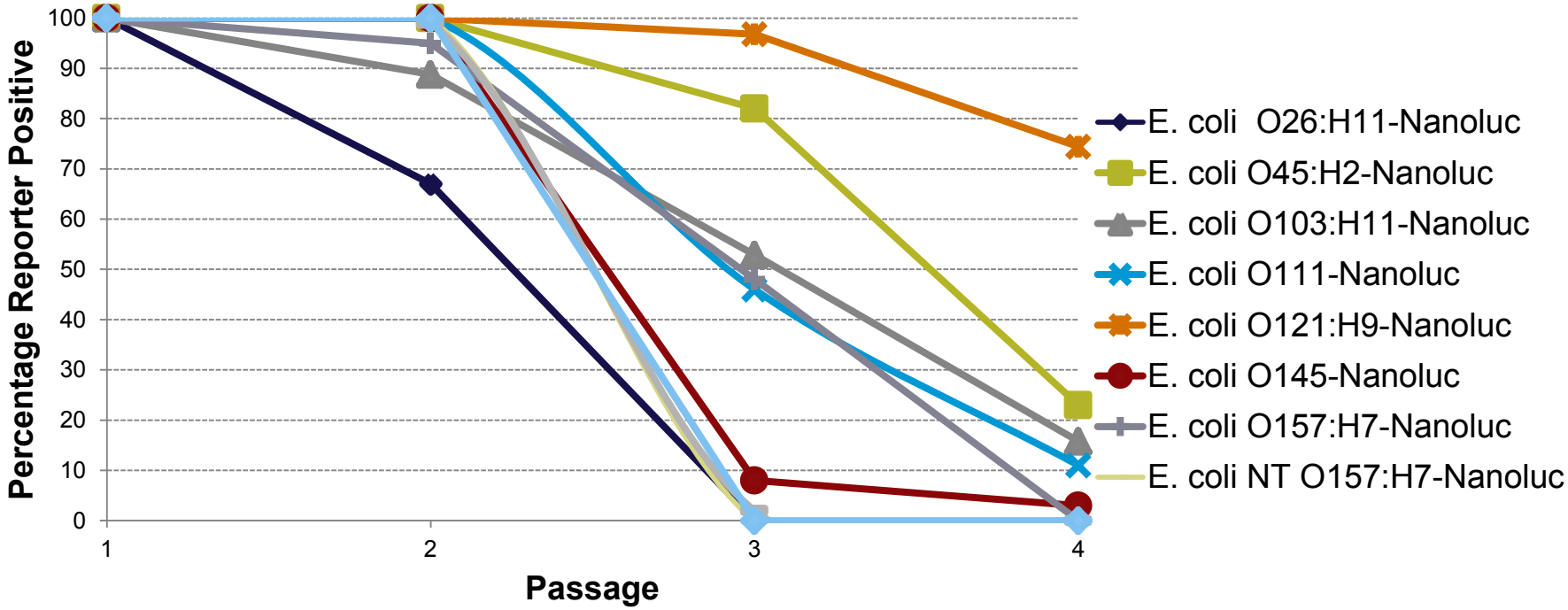
Plasmid stable for ≥2 days



Observe reporter in >65% of c.f.u. after 2 days at 42°C

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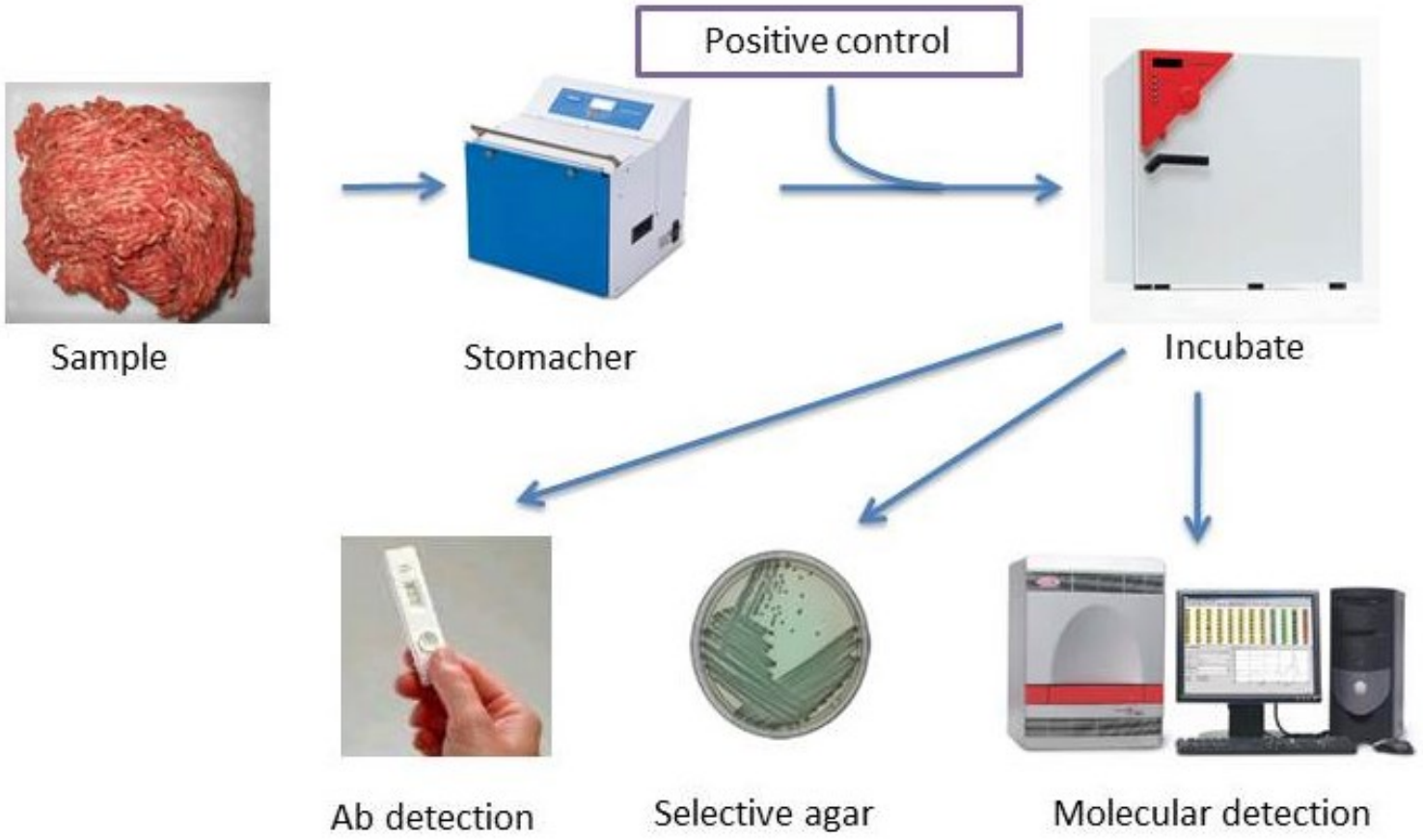
Shiga toxin-producing *E. coli* (STEC) control strains

Labeled controls rule out cross-contamination of samples with the control strain



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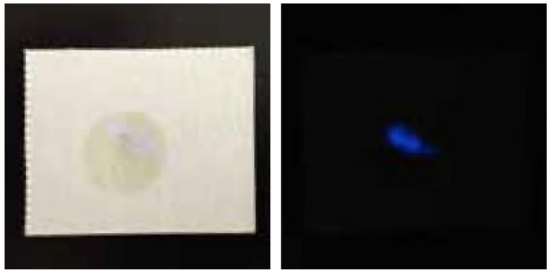
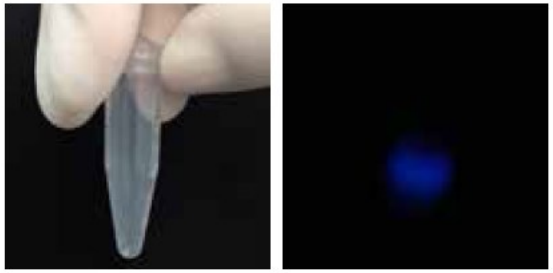
NanoLuc®-labeled control detection

Cotton Swab

Paper



Broth



External testing confirmed a clear signal after FSIS protocol for ground beef

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NanoLuc®-labeled *Escherichia coli*

ATCC® No.	Species	Serotype	Genotype
BAA-2580-PACK™	*Escherichia coli*	O26:H11	*stx*1+, *stx*2+, *eae*A+
BAA-2581-PACK™	*Escherichia coli*	O45:H2	*stx*1+, *stx*2-, *eae*A+
BAA-2582-PACK™	*Escherichia coli*	O103:H11	*stx*1+, *stx*2-, *eae*A+
BAA-2583-PACK™	*Escherichia coli*	O111	*stx*1+, *stx*2-, *eae*A+
BAA-2584-PACK™	*Escherichia coli*	O121:H19	*stx*1-, *stx*2+, *eae*A+
BAA-2585-PACK™	*Escherichia coli*	O145	*stx*1-, *stx*2+, *eae*A+
BAA-2586-PACK™	*Escherichia coli*	O157:H7	*stx*1+, *stx*2+, *eae*A+
BAA-2587-PACK™	*Escherichia coli*	O157:H7	*stx*1-, *stx*2-, *eae*A-

www.atcc.org/reporters

Coming soon – Big-Six *Escherichia coli* NanoLuc® Strains Panel

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Additional testing resources

Food Testing Reference Strains

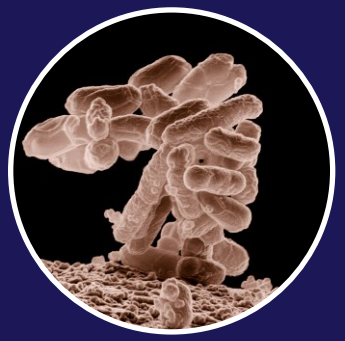
www.atcc.org/Food

Proficiency Testing Programs

www.atcc.org/PTPrograms

ATCC® Minis Quality Control Strains

www.atcc.org/Minis



"Big Six" STEC DNA and Strains Panels

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ATCC Food Testing Solutions

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Photo credit: Eric Erbe and Christopher Pooley (USDA ARS)

Conclusions

- Reporter labels are a flexible research tool
- Using labels for microbial detection enhances safety and reliability in food testing
- ATCC strains provide
  - Authenticated reference standards
  - Low Passage
  - Significant savings in time and effort

Acknowledgements

- Mariette Barbier, Ph.D.

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Thank you!

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Thank you for joining today!
Please send additional questions to tech@atcc.org

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