SEEING IS BELIEVING - REPORTER LABELED MICROBIAL CONTROL STRAINS

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Today’s discussion

About ATCC

Reporter labels and their use

GFP-labeled strains

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

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
Why label bacteria?

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

- Microbial quantification and detection
- Host-pathogen interactions
- Drug discovery
- Food testing
- Gene expression
- Biochemical interactions

Resulting in:
- Novel therapeutics
- Quality control assays

Photo credit: NIAID
# Reporter characteristics

<table>
<thead>
<tr>
<th></th>
<th>Green Fluorescent Protein (GFP)</th>
<th>NanoLuc® Luciferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live-cell assays</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Well-characterized</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Highly stable</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Substrate required</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Glow response</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Use <em>in vivo</em></td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
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
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)
Bacterial fitness and quantification

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

Plasmid is stable for at least 20 generations at 37°C in the absence of antibiotic pressure.
• *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
Fluorescent microscopy detection of pathogen-host interactions

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

High-throughput detection of pathogen-host interactions
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.
## ATCC GFP-labeled strains

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Species</th>
<th>Reporter</th>
<th>Parental Strain (ATCC® No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25922GFP™</td>
<td><em>Escherichia coli</em></td>
<td>GFP</td>
<td>25922™</td>
</tr>
<tr>
<td>14028GFP™</td>
<td><em>Salmonella enterica</em> subsp. <em>enterica</em></td>
<td>GFP</td>
<td>14028™</td>
</tr>
<tr>
<td></td>
<td>serovar Typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12022GFP™</td>
<td><em>Shigella flexneri</em></td>
<td>GFP</td>
<td>12022™</td>
</tr>
<tr>
<td>10145GFP™</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>GFP</td>
<td>10145™</td>
</tr>
<tr>
<td>15692GFP™</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>GFP</td>
<td>15692™</td>
</tr>
</tbody>
</table>

[www.atcc.org/reporters]
Promega NanoLuc® reporter

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

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
Phenotypic testing

Culture Purity and Biochemical Properties

Colony Morphology

Cell Attributes

Biochemical Analysis
Genotypic & proteotypic testing

- Sequencing
- Toxinotyping
- MALDI-TOF

Targeted Gene & Protein Sequencing
Functional testing

- Functional Characteristics
- Serotype
- Drug Resistance
- Virulence

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 \( eaeA \) genes
  - PCR
- Serogroup identification
  - Immunoprecipitation assay
Minimal reporter effects on bacterial fitness

Growth Constant (Generations/Hour)

Doubling Time (Minutes)

E. coli O26:H11
E. coli O45:H2
E. coli O103:H11
E. coli O111
E. coli O121:H9
E. coli O145
E. coli O157:H7
E. coli NT O157:H7

Progenitor

Reporter-labeled

Progenitor

Reporter-labeled
Plasmid stable for ≥2 days

Observe reporter in >65% of c.f.u. after 2 days at 42°C
Shiga toxin-producing *E. coli* (STEC) control strains

Labeled controls rule out cross-contamination of samples with the control strain.
NanoLuc®-labeled control detection

External testing confirmed a clear signal after FSIS protocol for ground beef
# NanoLuc®-labeled *Escherichia coli*

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Species</th>
<th>Serotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAA-2580-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O26:H11</td>
<td>stx1+, stx2+, eaeA+</td>
</tr>
<tr>
<td>BAA-2581-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O45:H2</td>
<td>stx1+, stx2-, eaeA+</td>
</tr>
<tr>
<td>BAA-2582-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O103:H11</td>
<td>stx1+, stx2-, eaeA+</td>
</tr>
<tr>
<td>BAA-2583-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O111</td>
<td>stx1+, stx2-, eaeA+</td>
</tr>
<tr>
<td>BAA-2584-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O121:H19</td>
<td>stx1-, stx2+, eaeA+</td>
</tr>
<tr>
<td>BAA-2585-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O145</td>
<td>stx1-, stx2+, eaeA+</td>
</tr>
<tr>
<td>BAA-2586-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O157:H7</td>
<td>stx1+, stx2+, eaeA+</td>
</tr>
<tr>
<td>BAA-2587-PACK™</td>
<td><em>Escherichia coli</em></td>
<td>O157:H7</td>
<td>stx1-, stx2-, eaeA-</td>
</tr>
</tbody>
</table>

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

[www.atcc.org/reporters](http://www.atcc.org/reporters)
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
www.atcc.org/MP

ATCC Food Testing Solutions

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.
Thank you!

Watch recorded ATCC® “Excellence in Research” webinars on demand at www.atcc.org/webinars.


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