Evaluation of GFP reporter-labeled control strains for Shiga toxin-producing Escherichia coli (STEC) Assays

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Introduction
Growing concern over bacterial food contamination has led to increased examination of food testing protocols in today’s industry. Currently, the use of bacterial strains as positive controls in testing protocols is not widely practiced for fear of cross-contaminating samples. Due to ongoing scrutiny of food testing methodology and growing regulations under the Food and Drug Administration (FDA) Food Safety Modernization Act, it is imperative to have control strains with unique, easily detectable traits that distinguish positive control strains from actual food contaminants, diminishing the fear of cross-contamination and improving current practices.

In this study, green fluorescent protein (GFP) reporter-labeled Escherichia coli strains were created using the plasmid-based GFP construct, which is a plasmid-based expression system. Here, Shiga toxin-producing O157 and four of the “Big Six” non-O157 E. coli strains were developed as reporter-labeled positive controls (Table 1).

Table 1: ATCC reporter-labeled strains

<table>
<thead>
<tr>
<th>ATCC No.</th>
<th>Strain Description</th>
<th>Serotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>35150GFP™</td>
<td>E. coli O157: H7-GFP</td>
<td>O157:H7</td>
<td>αE1+, αE2+, esaaA+</td>
</tr>
<tr>
<td>51657GFP™</td>
<td>E. coli O157: H7</td>
<td>O157:H7</td>
<td>αE1+, αE2+, esaaA+</td>
</tr>
<tr>
<td>BAA-2196GFP™</td>
<td>E. coli O26:H11-GFP</td>
<td>O26:H11</td>
<td>αE1+, αE2+, esaaA+</td>
</tr>
<tr>
<td>BAA-2215GFP™</td>
<td>E. coli O103:H11-GFP</td>
<td>O103:H11</td>
<td>αE1+, αE2+, esaaA+</td>
</tr>
<tr>
<td>BAA-2209GFP™</td>
<td>E. coli O111-GFP</td>
<td>O111</td>
<td>αE1+, αE2+, esaaA+</td>
</tr>
<tr>
<td>BAA-2219GFP™</td>
<td>E. coli O121:H19-GFP</td>
<td>O121:H19</td>
<td>αE1+, αE2+, esaaA+</td>
</tr>
</tbody>
</table>

Results

Reporter Signal Detection
The GFP signal from reporter-labeled strains was detected by exposing colonies to UV light (Figure 1). The fluorescence from individual cells was also confirmed via microscopy and flow cytometry using the appropriate excitation and emission (Figure 2).

Figure 1: Visualization of the reporter-labeled strains. Transexgenic ATCC® BAA-2209GFP™ transformed with a plasmid bearing gfp (ATCC® BAA-2209GFP™) was grown at 37°C on Tryptic Soy Agar (TSA). Visualization was achieved by exposing the ATCC® BAA-2209GFP™ plate to UV light (302 nm).

Figure 2: Visualization of the reporter-labeled strains. Microscopy and flow cytometry analyses from representative reporter-labeled and progenitor strains. Using a 40X objective with a 488 nm filter and phase contrast, (A) progenitor ATCC® BAA-2215GFP™ cells did not show any fluorescence, while (B) transformed ATCC® BAA-2215GFP™ cells showed green fluorescence. GFP presence was also determined by flow cytometry analysis. (C) The overlaid histogram exhibits data from progenitor (red) and reporter-labeled E. coli ATCC® BAA-2215GFP™ cells (green) acquired on a CytoFLEX Cytometer (Beckman Coulter, Inc.) using a 488 nm laser and GFP filter set. The figure demonstrates a clear separation of the reporter-labeled GFP strain and the progenitor strain.

Chromogenic Medium
Chromogenic media may be used to assist in the identification E. coli serotypes; the color of colonies from reporter-labeled strains and their progenitor strains should be in the same color family. GFP reporter-labeled strains were compared with their progenitor strains to identify phenotypic changes on Rainbow™ Agar (Biológ). Color differences between reporter-labeled and progenitor strains were minimal (Figure 3).

Figure 3: Chromogenic phenotype on rainbow agar. Rainbow Agar was prepared according to manufacturer specifications. The progenitor strains (left) and the reporter-labeled strains (right) were streaked on a plate and incubated for 18h at 37°C. The chromogenic properties of progenitor strains were compared to those of reporter-labeled strains.

Figure 4: Growth rate of progenitor and reporter-labeled strains. Growth curves were determined by determining the (A) growth constant (k) and (B) growth curves. Using the Bioscreen C NMR (Oy Growth Curves Ab Ltd.), 200 µL cultures were prepared with a 1:100 inoculum from an overnight culture and incubated at 37°C in Tryptic Soy Broth (TSB) with constant shaking. Error bars represent standard error.

Plasmid Stability
To determine the stability of the GFP plasmid, the engineered reporter-labeled strains were passaged once every 24h under temperature stress at 4°C. The percentage of GFP positive colonies varied depending on the strain, ranging from 87-100% of the population after two days (Figure 5). This level of plasmid stability is within an acceptable range for the intended qualitative testing workflow.

Figure 5: Plasmid stability. Reporter-labeled strains were grown in TSB at 4°C and passaged 1:100 into fresh TSB once every 24h. A serial dilution was performed to obtain a countable number of colonies. 100 µl of an appropriate dilution was plated on TSA and incubated overnight at 37°C. The percentage of colonies expressing the reporter was recorded daily over four days. Error bars represent standard deviation.

Conclusions
In this study, multiple serotypes of E. coli were engineered with GFP reporters. Phenotypic changes between the progenitor and reporter-labeled strains were minimal on chromogenic medium. As expected, growth rate differences between the progenitor and reporter-labeled strains were present in liquid culture, but were acceptable for the qualitative assays for which the strains were designed. The GFP plasmid was stable in bacterial populations for 22 days. These reporter-labeled bacteria strongly emit light and can be detected immediately after exposure to UV light, eliminating uncertainty about cross-contamination. This study demonstrates that GFP reporter-labeled QC strains can be routinely used as positive controls to increase reliability in food testing assays.

References

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