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[1].

In this study, green fluorescent protein (GFP) reporter-labeled *Escherichia coli* strains were created using the DNA2.0 IP-Free© synthetic 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

ATCC® No.	Strain Description	Serotype	Genotype
35150GFP™	*E. coli* O157:H7-GFP	O157:H7	*stx*1+, *stx*2+, *eae*A+
51657GFP™	*E. coli* O157:H7-GFP	O157:H7	*stx*1+, *stx*2+, *eae*A+
BAA-2196GFP™	*E. coli* O26:H11-GFP	O26:H11	*stx*1+, *stx*2+, *eae*A+
BAA-2215GFP™	*E. coli* O103:H11-GFP	O103:H11	*stx*1+, *stx*2-, *eae*A+
BAA-2209GFP™	*E. coli* O111-GFP	O111	*stx*1+, *stx*2+, *eae*A+
BAA-2219GFP™	*E. coli* O121:H19-GFP	O121:H19	*stx*1-, *stx*2+, *eae*A+

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).

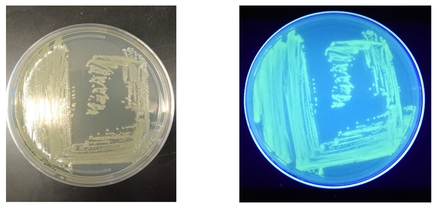

Figure 1: Visualization of the reporter-labeled strains. Toxinogenic ATCC® BAA-2209™ transformed with a plasmid bearing *gfp* (ATCC® BAA-2209GFP™) were grown at 37ºC on Tryptic Soy Agar (TSA). Visualization was realized by exposing the ATCC® BAA-2209GFP™ plate to UV light (302 nm).

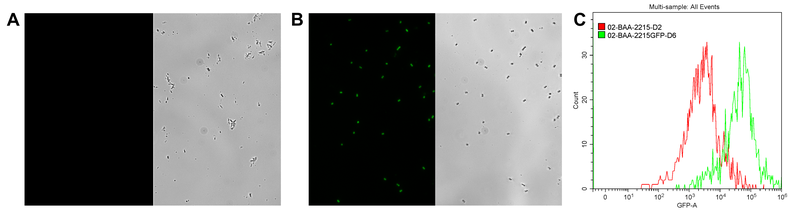

Figure 2: Visualization of the reporter-labeled strains. Microscopy and flow cytometry analyses from representative reporter-labeled and progenitor strains. Using a 40X objective with a 488 nm filter and phase contrast, (A) progenitor ATCC® BAA-2219™ cells did not show any fluorescence, while (B) transformed ATCC® BAA-2219GFP™ 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 (Biolog). Color differences between reporter-labeled and progenitor strains were minimal (Figure 3).

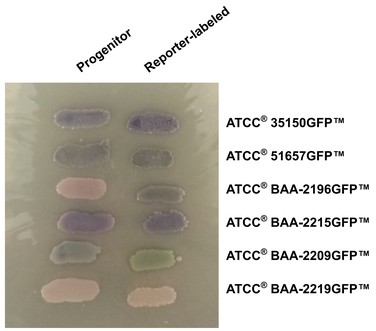

Figure 3: Chromogenic phenotype on rainbow agar. Rainbow Agar was prepared according to manufacturer specifications. The progenitor strains (left) and the reporter-labeled strains (right) were streaked on a plate and incubated for 16h at 37ºC. The chromogenic properties of progenitor strains were compared to those of reporter-labeled strains.

Growth Effects

To determine whether *gfp* affects the growth and viability of the reporter-labeled *E. coli*, growth studies were performed (Figure 4). The growth constant (*k*) varied from 2.3±0.5 to 3.6±1.2 generations/hour in the absence of the plasmid and from 1.6±1.1 to 1.9±0.9 generations/hour in the presence of the plasmid. The maximum decrease in the growth constant was a 45.8% drop for ATCC® BAA-2219GFP™ and ATCC® BAA-2196GFP™. All changes were within an acceptable range as these strains were intended for qualitative assays that simply require visible growth after overnight culturing on plates.

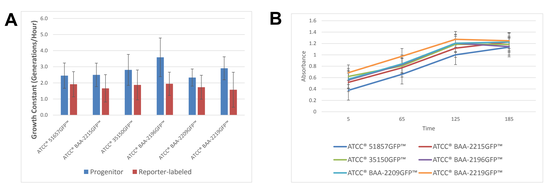

Figure 4: Growth rate of progenitor and reporter-labeled strains. Growth curves were performed in triplicate to determine the (A) growth constant (*k*) and (B) growth curve. Using the Bioscreen C MBR (Oy Growth Curves Ab Ltd.), 200 µL cultures were prepared with a 1:100 inoculum from an overnight culture and were incubated at 37ºC 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 42ºC. The percentage of GFP positive colonies varied depending on the strain, ranging from 87-100% of the population after two days (Figure 4). This level of plasmid stability is within an acceptable range for the intended qualitative testing workflow.

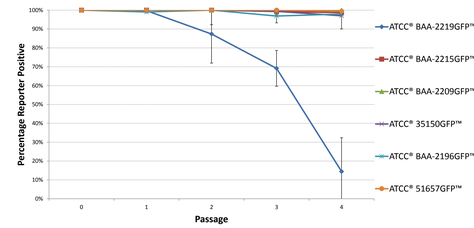

Figure 5: Plasmid stability. Reporter-labeled strains were grown in 5 mL of TSB at 42ºC and passaged 1:100 into fresh TSB once every 24h. A serial dilution was performed to obtain a countable number of colonies. 100 µL of an appropriate dilution was plated on TSA and incubated overnight at 37ºC. The 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 ≥2 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

1. U.S. Food and Drug Administration. "Background on the FDA Food Safety Modernization Act (FSMA)." http://www.fda.gov/food/guidanceregulation/fsma/ucm239907.htm (accessed July 7, 2016).

Disclaimers