APPLICATION NOTE

Fluorescent Bacterial Colony Selection Using QPix 400 Systems

Introduction

Screening bacterial transformants that harbor a plasmid ligated with the gene of interest has become convenient by the use of vectors with fluorescent reporter genes. Fluorescence imaging and screening reveal unique information about individual colonies when studying protein folding or secretion, enzyme evolution or protein localization. In addition, it is a direct method of choice to screen for mutations or to identify transformation markers.

Traditional manual picking of microbial colonies is time-consuming and tedious. The QPix™ 400 Series of microbial colony pickers can pick more than 3,000 colonies in an hour, whereas a skilled technician can manually pick 600 colonies in an hour. Thus, in comparison to manual colony picking which can be slow and error-prone, automated colony picking is at least 5 times faster and is more precise with >98% picking efficiency. The QPix 400 Series with fluorescence imaging module significantly reduces downstream processing time by enabling phenotypic selection of unique colonies based on the levels of fluorescent protein expression. It is a leading microbial colony picker in the market that offers multiple fluorescent filters compatible with a broad range of fluorescent cloning vectors. Fluorescence-based recombinant colony screening method facilitates selection for a gene fusion product by detecting intrinsic levels of fluorescence expression that offers higher screening fidelity for selecting recombinant colonies.

Here, we demonstrate fluorescence-based detection and colony selection capabilities of the QPix 400 Series using a model organism, *Escherichia coli,* harboring recombinant plasmids expressing either Green Fluorescent Protein (GFP) or Cyan Fluorescent Protein (CFP).

Materials and methods

The pFluoroGreen plasmid carrying ampicillin resistance and GFP gene was used to transform chemically competent *E. coli*. The transformed *E. coli* cultures were plated on LB media containing ampicillin and cultured overnight at 37 degrees.

The pCyanFP plasmid carrying the kanamycin resistance and CYP gene was used to transform chemically competent *E. coli*. The transformed *E. coli* cultures were plated on plates containing LB media with kanamycin plus IPTG and cultured overnight at 37°C. The pFluroGreen plasmid vector map is illustrated in Figure 1A and pCyanFP vector map in Figure 1B.

Benefits

- **Quantitative fluorescent screening enables efficient and objective selection of unique clones**
- **A variety of fluorescent filter sets increases experimental flexibility**
- **Easy-to-use software ensures the right colony is picked every time based on the defined experimental parameters**

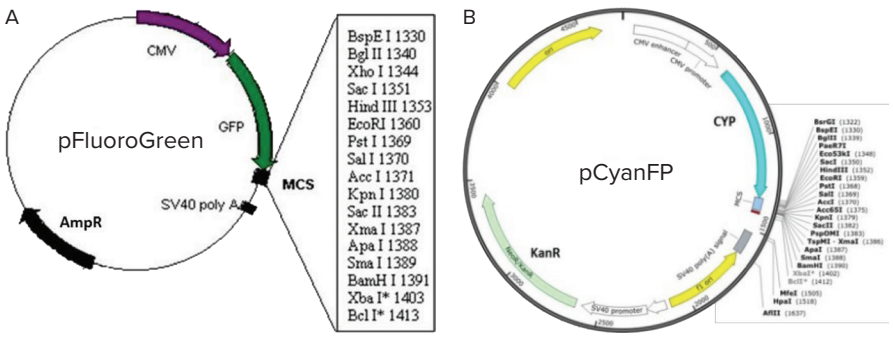

Figure 1. (A) pFluoroGreen plasmid vector map with ampicillin resistance and GFP gene. (B) pCyanFP plasmid with kanamycin resistance and CYP gene.

Colony selection utilizing white light and fluorescent imaging

GFP-transformed bacterial colonies were imaged and screened under white light (Figure 2A) and fluorescence (Figure 2B) on the QPix™ 420 System. Fluorescence-based selection of GFP-expressing bacteria was performed using the blue filter pair (Ex/Em filter: 457/536 nm) option.

Colony detection using white light and fluorescence imaging on the QPix 400 Series enables objective identification and selection of colonies expressing the fluorescent reporter gene of interest. Once images are captured, the QPix Software detects, analyzes feature properties and identifies the location of individual colonies on the culture plate. Flexible software allows the user to define colony selection parameters such as size, compactness and morphology. Quantitative, tunable fluorescence intensity threshold specifies the level of gene or protein expression, recorded as fluorescence intensity measure. Once colonies are selected based on user-defined criteria, they are picked with a high precision, fully pneumatic, 96 pin picking head. For this experiment, colonies expressing GFP with an interior mean fluorescent intensity (MFI) value greater than 40,000 were selected. Colonies included or excluded based on selection criteria are depicted in Figure 3A and 3B respectively.

Similarly, CFP-expressing bacterial colonies were identified and selected using white light (Figure 2C) and fluorescence imaging (Figure 2D) using the blue filter pair (Ex/Em filter: 457/536 nm) option. Fluorescent colonies exhibiting an interior mean fluorescent intensity value greater than 20,000 were selected. The colony selection features are displayed, wherein colonies included (Figure 3C) or excluded (Figure 3D) based on user-defined criteria are highlighted. The ability to manually adjust fluorescent selection criteria within the QPix Software provides the user additional flexibility and control in objective and quantitative pre-screening of bacterial colonies.

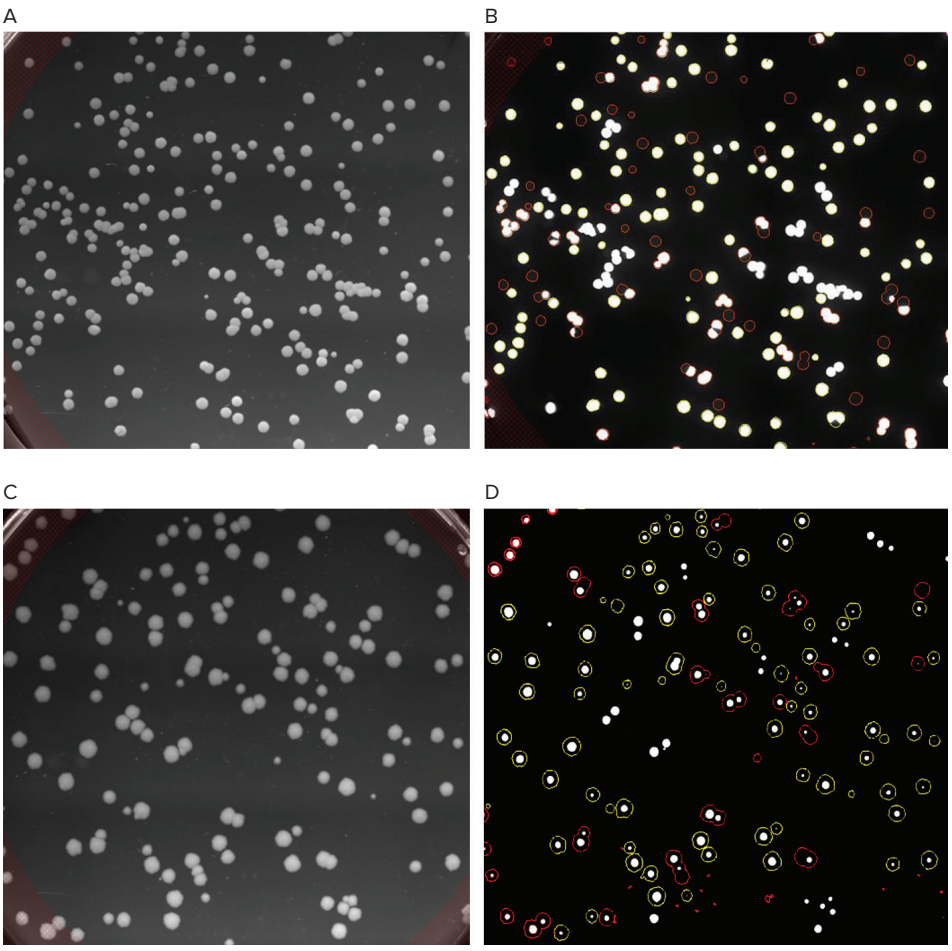

Figure 2. (A) All bacterial colonies that are transformed with pFluoroGreen vector are detected in white light. (B) Colonies that are transformed with pFluoroGreen vector and only those colonies expressing GFP fluorescent gene of interest are identified in fluorescence channel. (C) All bacterial colonies transformed with pCyanFP vector are detected in white light. (D) Colonies that are transformed with pCyanFP vector and only those colonies expressing CFP fluorescent gene of interest are identified in fluorescence channel. This demonstrates the versatility of available fluorescence filter sets that are compatible with multiple fluorescent cloning vectors as demonstrated with GFP and CFP.

Summary

Fluorescent screening using reporters such as GFP and CFP offers significant advantages over conventional screening methods. This efficient technique has many potential applications, including tracking of protein folding, monitoring protein secretion *in vitro*, and enabling library mutational screening for enzyme evolution. With the aid of fluorescence capabilities on the QPix 400 Series, high-throughput screening and selection of recombinant colonies is straightforward. Various fluorescent reporters are supported by a variety of fluorescent filter sets available. The simple user interface enables researchers to customize and define analysis and colony selection criteria tailored to their unique biology. QPix 400 Series of microbial colony picking systems thus provides a highly productive, reliable alternative to conventional manual approaches that significantly shortens time to obtain recombinant protein expressing colonies of choice.

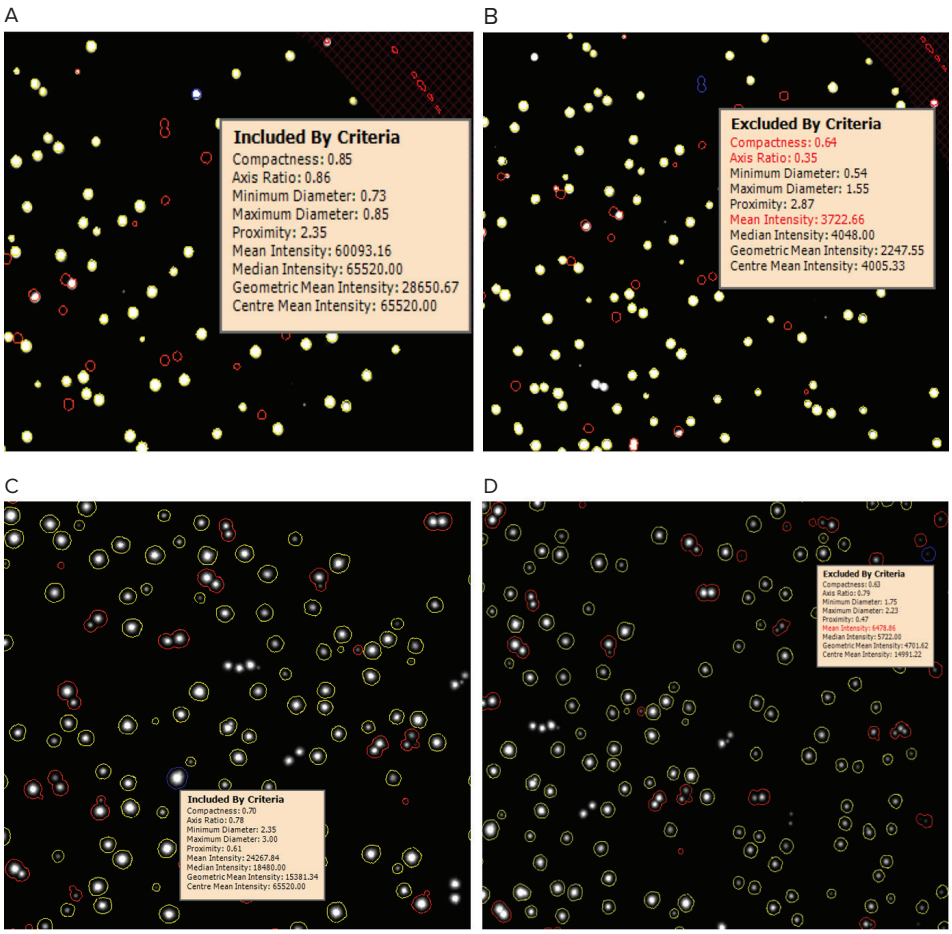

Figure 3. Colonies identified in the GFP (A) and CFP (C) fluorescent channels based on user-defined selection criteria are highlighted in yellow. Those colonies that do not meet user defined selection criteria are excluded from the total population as shown in red (expressing GFP; B and expressing CFP; D).

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