APPLICATION NOTE

Automated colorimetric colony selection: Blue-white colony screening with QPix 400 Series

Benefits

- **Effective monitoring of transformation efficiency using white light**
- **Accurate and robust color differentiation of white, blue or powder-blue colonies**
- **Intelligent colony selection software module with both automated or user-defined features**

Introduction

Screening of bacterial transformants that contain recombinant plasmids with cloned gene inserts is an essential step in molecular cloning. A colorimetric reporter method called "blue-white screening" allows convenient identification of recombinant and non-recombinant colonies based on color. Although blue-white screening provides a visual identification of recombinant transformed colonies, many manual steps in the colony-picking process are subjective, slow, and error-prone.

Molecular Devices' QPix™ 400 Series of microbial colony pickers offers an automated solution especially designed for accurate blue-white colorimetric screening using white light imaging and for effective monitoring of transformation efficiency. The solution incorporates a QPix™ Software 2.0 Blue-White Picking module, optical QPix™ Chroma Filters, and adjustable source-plate holders. This application highlight describes results of a proof-of-concept, colorimetric colony screening experiment utilizing the QPix™ 420 System. Accuracy of the picked colonies was further verified by DNA sequencing.

Principles of blue-white screening

The molecular mechanism of blue-white colony screening relies on the *LacZ* reporter gene. In this technique, non-recombinant colonies with a functional *LacZ* gene encoding for ß-galactosidase turn blue in the presence of culture media containing X-gal. This is in contrast to how recombinant colonies with a disrupted *LacZ* gene due to successful gene insertion turn white (Figure 1).

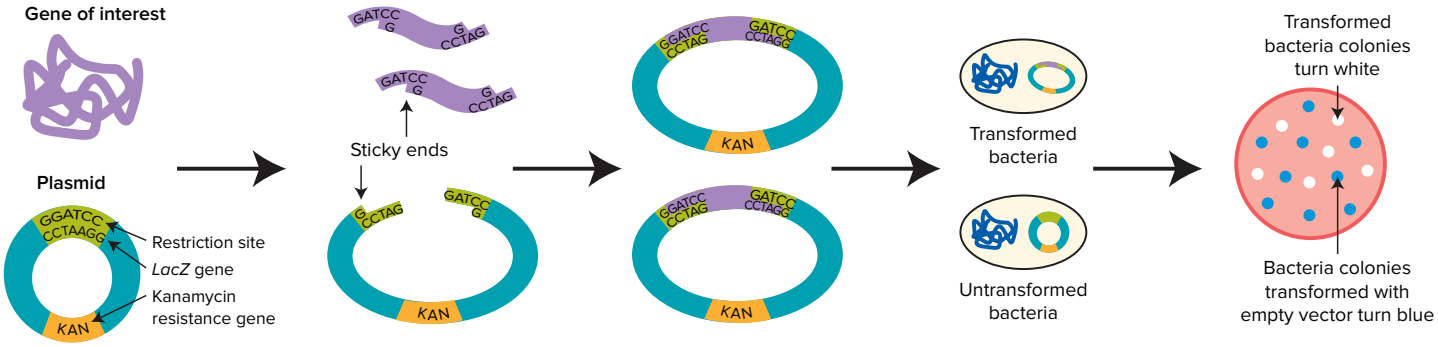

Figure 1. Molecular cloning. Schematic representation of a typical blue-white screening procedure. Blue-white screening of bacterial colonies involves cloning of gene inserted into a plasmid vector with an antibiotic resistance and *LacZ* reporter gene. The ligation of the insert into the multiple cloning site of the vector inactivates the *LacZ* gene. The transformation of competent *E. coli* with the ligated mixture in the presence of X-gal in culture media results in the formation of blue and white colonies.

Use of molecular cloning to transfect colonies with the gene of interest

To create a model construct, human insulin-like growth factor (IGF) gene was cloned into the multiple cloning site (MCS) of pUC57-Kan cloning plasmid vector containing kanamycin resistance and *LacZ* gene (Figure 2A). Chemically competent *E.coli* (One Shot® TOP10, Life Technologies) were transformed with the ligated vector and gene (Human IGF) mixture. Transformed bacteria were then plated onto LB media containing kanamycin, IPTG, and X-gal and incubated overnight at 37°C. Colonies containing vectors with and without the inserted gene were identified by white or blue color, respectively, with the QPix 420 System (Figure 2B).

Adjustable source plate holders holding petri-plates containing bacterial colonies were positioned on the imaging bed with a QPix Chroma Filter (Figure 3A). The petri-plates were held securely by adjustable latches on the plate holders (Figure 3B) and imaged using white light (Figure 3C).

Automatic selection of blue or white colonies

White light images taken on the QPix 420 System were analyzed using the easy-to- use QPix Software 2.0. Blue (Figure 4A) or white (Figure 4B) colonies were automatically identified and selected separately using the built-in Auto Select feature (Figure 4C). The user may adjust the histogram threshold and define colony selection criteria, such as compactness, axis ratio, diameter and proximity manually to optimize selection. Selected blue and white colonies were picked robotically using the fully pneumatic, 96-pin picking head on the QPix 420 System.

DNA sequence confirmation of picked colonies

The accuracy of colorimetric selection and picking of blue and white colonies was confirmed by DNA sequencing to verify the presence of inserted gene. Rolling circle amplification confirmed 98% of the picked white colonies contained the cloned gene insert, human IGF, in the pUC57-Kan cloning plasmid vector. Additionally, 100% of the picked blue colonies contained empty pUC57-Kan cloning vector

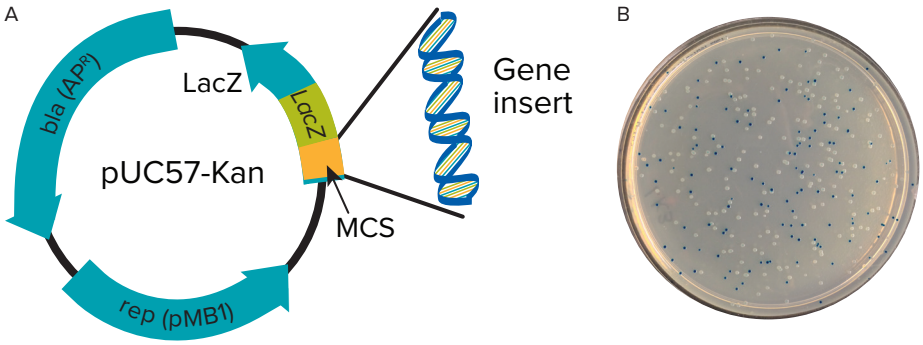

Figure 2. Cloning of human IGF in pUC57-Kan cloning plasmid vector and blue-white colony screening. (A) The gene insert, human IGF, was ligated into the MCS of pUC57-Kan plasmid cloning vector. **(B)** Blue and white colonies appear on the LB media plates containing X-gal as a result of *LacZ* reporter based gene expression used in blue-white colony screening.

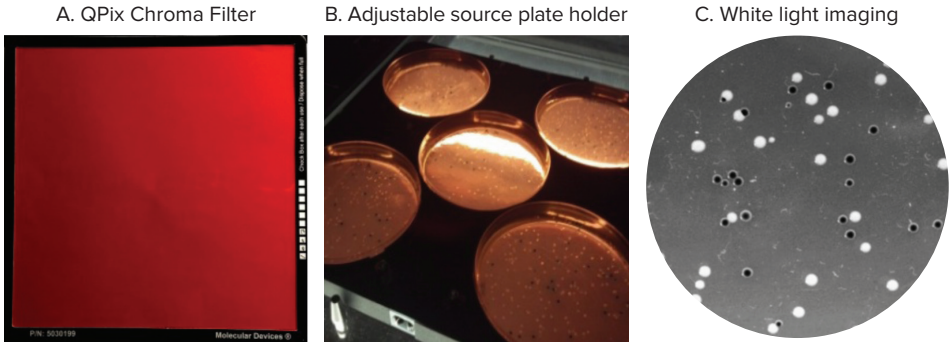

Figure 3. Blue-white colony screening utilizing the QPix Systems employs **(A)** optical QPix Chroma Filters for enhanced color differentiation and **(B)** adjustable source plate holders for improved plastic-ware flexibility. Colonies imaged under white light **(C)** are further analyzed by the software.

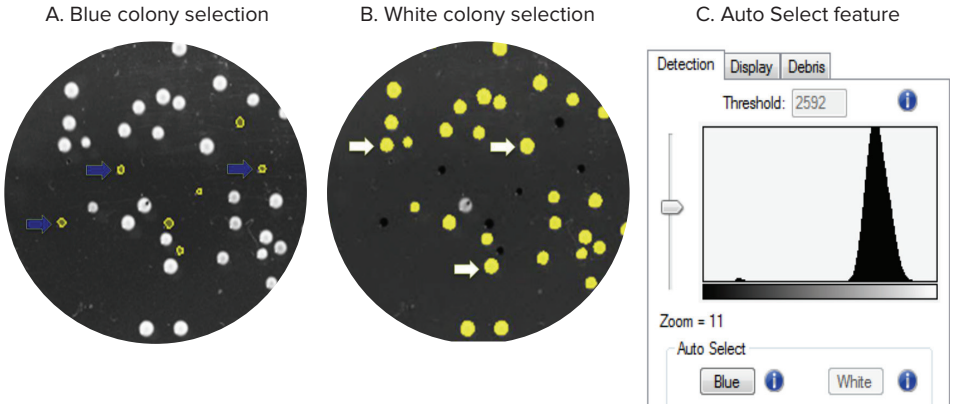

Figure 4. (A) Example blue colonies selected with Auto Select Blue are indicated with blue arrows. **(B)** Example white colonies selected with Auto Select White are indicated with white arrows. **(C)** Flexibility of the software allows manual adjustment of the intensity threshold to optimize results, such as to select for powered blue colonies.

without the inserted gene. Results of this experiment provide compelling confidence in confirming color differentiation between white and blue colony selection using the QPix 400 Series of microbial colony pickers.

Summary

Blue-white colony screening is a colorimetric detection method that allows for convenient distinction of transformed recombinant colonies harboring the gene insert as a result of molecular cloning. Molecular Devices offers an automated solution tailored for blue-white screening on QPix 400 Series of robotic microbial colony pickers. The user-friendly software interface of QPix Software 2.0, along with optical filters, enables accurate and robust color differentiation in white light. Accessories such as plate holders with adjustable latches further improve plasticware compatibility. The QPix 400 Series thus provides a highly efficient and reliable alternative to conventional, manual color-based approaches to colony screening.

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