Benefits

• Support unique adaptive evolution applications
• Accelerate new therapeutics discovery against drug-resistant microbial pathogens
• Identify unique biology with user-adjustable software parameters
• Select high value clones faster with a reliable, automated solution

Introduction

To meet the growing needs of new therapeutic development against drug-resistant microbial pathogens, competition-based adaptive evolution approaches have been developed. A challenging aspect of these approaches is to identify high value strains from a heterogeneous population of microbial colonies, and to select antibiotic producing strains that represent the best candidates for downstream drug development.

For a library-based approach to screen and select antibiotic producing organisms, the agar plate-based zone of inhibition or clearing zone detection is the method of choice. Colonies providing antimicrobial activity are distinguished from the rest of the population by secreting clearing zones where bacterial growth is inhibited due to secretion of antimicrobial compounds. The diameter of the clearing zone is typically proportional to the amount of antimicrobial compound produced. Thus, high value strains are those that generate the largest clearing zones. These colonies are then selected for further characterization and development of antimicrobial entities.

Manual selection and picking colonies producing zones of inhibition can be a slow, tedious and error-prone process. To address these challenges, Molecular Devices’ QPix™ 400 Series microbial colony pickers offers high-throughput automated colony selection and picking with unmatched productivity and convenience. We describe herein the objective screening and selection of high value colonies producing clearing zones using the system and its software package.

Detection of colonies generating zones of inhibition

Streptomyces clavuligerus produces over 20 secondary bioactive metabolites, including many beta-lactam antibiotics such as cephamycin C, cephalosporin C, and clavulanic acid. *Streptomyces clavuligerus* (ATCC 27064) was used as a model antibiotic producing organism to demonstrate zone of inhibition detection against the target bacteria, *E.coli* (Migula, Castellani and Chalmers ATCC 47076). Both microbial strains were obtained from ATCC and cultured in Trypticase Soy Broth (TSB) medium. Liquid cultures of both organisms were grown aerobically in a shaker incubator for 1-3 days; *S. clavuligerus* at 30°C and *E.coli* at 37°C respectively. After visible growth, the *S. clavuligerus* culture suspension was diluted such that its optical density at 600 nm (OD600) was between 0.10–0.13. Two microliters of *S. clavuligerus* culture suspension was spotted onto 100 mm TSA agar plates and incubated at 28°C for 3-5 days. Following growth of *S. clavuligerus* on the TSA plates, diluted liquid culture of *E.coli* was spread evenly on the

![Figure 1. The growth of E.coli lawn of bacteria is inhibited in the areas where S. clavuligerus colonies produce antimicrobial compounds. This is noted by the appearance of clearing areas or zones of inhibition surrounding S. clavuligerus colonies.](image_url)
plates, ensuring that the *E. coli* inoculum completely surrounds and contacts each *S. clavuligerus* colony. The plates were incubated again at 28°C for 1-2 days and monitored daily. Colonies of *S. clavuligerus* generating zones of inhibition around *E. coli* lawn growth were observed as shown in Figure 1.

White light imaging on QPix 400 Series with QPix Software Zone of Inhibition Detection module was performed to detect colonies exhibiting clearing zones as demonstrated in Figure 2. Advanced software algorithms were used to select and pick colonies producing zones of inhibition based on user-defined selection parameters such as colony size, zone of inhibition diameter, colony diameter, axis ratio and colony compactness (Figure 2).

The selected colonies producing zones of inhibition were subsequently presented in a gallery format. Colonies can be ranked in a variety of orders such as total clearing zone, colony size, axis ratio, compactness, etc (Figure 3). Qualitative details of each colony producing zone of inhibition are listed for easy access to the user.

**Library-based screening and selection of colonies generating zones of inhibition**

To further demonstrate the utility of automated zone of inhibition detection for library-based screening or adaptive evolution studies, Qtrays spotted with a library of microbial cultures were screened and selected. From the 48 colonies grown on Qtrays, three colonies producing zones of inhibition were reliably detected by the software algorithm (Figure 4), selected and picked. This approach is amenable to high-throughput implementation aimed to screen, identify, and pick colonies producing clearing zones from a large microbial library.

**Figure 2.** The Zone of Inhibition Detection module in QPix Software 2.0 or higher can identify the size of each colony and corresponding clearing zones. Colonies producing clearing zones are selected based on user-defined selection criteria.

**Figure 3.** Data on each colony and clearing zone are displayed in a gallery format based on the ranking order defined by the user.
Conclusion
There is a growing need to develop novel antibiotics capable of treating infections caused by antibiotic resistant bacteria. Many efforts are underway to discover and fine tune the regulation of antibiotic biosynthesis. Library based screening and competition-based adaptive laboratory evolution can provide a robust platform to create strains that overproduce broad-spectrum antibiotics.

With the QPix 400 Series of microbial colony pickers and the QPix Zone of Inhibition Detection software module, high-throughput screening of colonies producing clearing zones is enabled with ease and reliability. This automated workflow will increase the ability of finding novel antimicrobial molecules designed to combat existing and emerging antibiotic-resistant microbial pathogens.

Figure 4: Library based high-throughput colony screening of zone of inhibition producers for antibiotics discovery. The Zone of Inhibition Detection module in QPix Software 2.0 or higher enables reliable detection and selection of colonies producing clearing zones. Selected colonies producing corresponding clearing zones are highlighted yellow.