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Creating Custom Software for the Genetix QPixII Colony Picker using the Genetix Developer's Toolkit SDK

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Why Custom Software?

The DOE's Joint Genome Institute (http://www.jgi.doe.gov/) is a high throughput sequencing center that is currently averaging 2.5 billion bases of sequence per month. Each well of sequence spends part of its life as a colony on a 22cmx22cm bioassay tray. Each colony is then picked using a Genetix colony picker (QPixII or QPixII XT) and transferred into a 384-well plate.

The traditional Genetix software was used from JGI's inception until September 2004 when the "Custom QSoft" software was released into production. The "Custom QSoft" package was developed to address a number of issues that were specific to JGI's workflow and software needs. These changes included a custom calibration procedure, a sterility procedure, a more straight forward user interface and background correction.

Maintaining many of the features of and the current functionality of the Genetix software were a top priority, so the modifications were written on top of a software package that had many of the features that were deemed necessary for production use. These features included the image results screen, a status bar with an Estimated Time of Completion, ability to carry over pin & well settings and ability to start picking on a plate other than #1.

The custom software has improved the JGI workflow and learning the Developer's Toolkit has given the JGI more control and freedom to do what is necessary to improve the day-to-day experiences of the QPixII Operators.

Training

Training for the Developer's Toolkit took place in the UK over a 3 day period. The training session and programming yielded the first version of the software JGI would need. This primitive version of the program could image an entire bioassay tray, perform a camera alignment and many other functions. What it did not have was pick a partial plate.

Departure from New Milton also left the programmers with knowledge of how to break-up the imaging process so that the captured image could be corrected by an external program and then analyzed by the Genetix software for colony location, a custom calibration routine and a sterility test routine.

Initial Modifications (Beta Version 1)

- Sterility procedure (Figure 1)
- Image only procedure (Figure 1)
- Calibration procedure (Figure 1 & 3)
- Display colonies counts for current plate and previous plate (Figure 1)
- Custom logging

JGI Additions

The **sterility procedure** that JGI uses is to alternate between inoculating quadrants with growth and without growth. Previously this was done by imaging a bioassay tray, picking and inoculating 96 colonies (quadrant A1), sterilizing the picking pins, removing the bioassay tray, picking 96 colonies of air and inoculating (quadrant A2), sterilizing the picking pins & repeating the procedure for quadrants B1 and B2. This will result in a destination plate that has alternating columns of growth and non-growth.

The new sterility routine (Figure 1) removes the manual intervention of removing/replacing the bioassay tray on the deck. Colony picker operators can now put a bioassay tray on the deck, image it and come back a few minutes later when 192 colonies have been picked and 192 colonies have been inoculated immediately after sterilization. The new procedure also removes the requirement of putting the bioassay tray back into the instrument in the same orientation as it came out. Putting the plate in at a rotated orientation can lead to many holes appearing in the sterility plate, which can be initially interpreted as instrument failure.

The **image only routine** (Figure 1) is used to count colonies without picking plates. This is useful to the JGI for tracking the plating efficiency and transformation efficiency upstream in the process. In the past, plates would have been discarded without counting colonies or operators would have had to stop the picking routine before the instrument could get to the sterilization tray.

The **calibration routine** that is used by the standard software images 1 region and uses that value to pick throughout all 35 image regions. This routine did not attain a value that represented all 35 image regions with the pre-poured bioassay trays the JGI uses. Instead of moving to in-house pouring (a huge process shift), the JGI decided to use a calibration routine that worked better in production. 5 image regions are calibrated and the results are averaged on a "Calibration Results" screen which is shown to the operators at the end of the routine (Figure 3). The operator can then deselect checkboxes for regions that have 'bad' calibration values. The remaining values will be averaged and the operator can use that average or re-calibrate. This calibration routine has made the number of holes per plate stabilize more than it used to be (Figure 4) and has drastically reduced the number of work requests based on bad calibration/imaging.

Bioassay tray colony counts are tracked at JGI as metrics for plating, competent cell and transformation efficiency. **Displaying colony counts** (Figure 1) on the Run tab of the software interface eliminated the necessity of the operators to check the QPix picking log for the colony counts.

JGI was also able to log whatever information that was desired. JGI **custom logs** track colony counts, the **number** of pins that were used to inoculate a plate, and criteria settings.

A calibration routine for each tray

Image Bioassay routine allows users to image without picking

Sterility routine removes manual intervention of removing and replacing plates

Colony counts are displayed after each imaging cycle (show for each tray on XT) and for each previous tray(s)

Preset criteria values allow operators to look at bioassay and choose correct values (see Table 1 below)
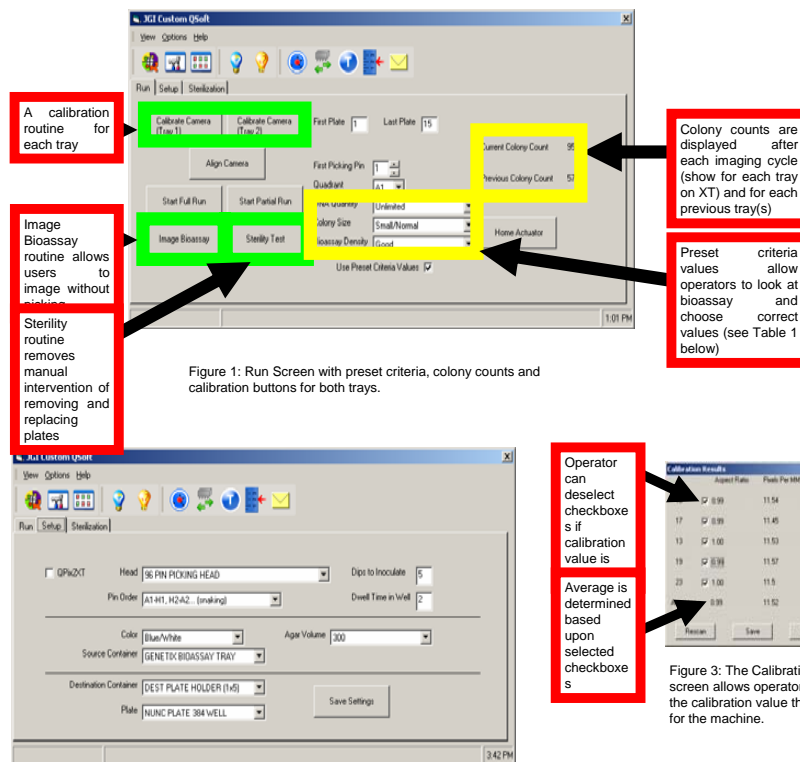
Figure 1: Run Screen with preset criteria, colony counts and calibration buttons for both trays.
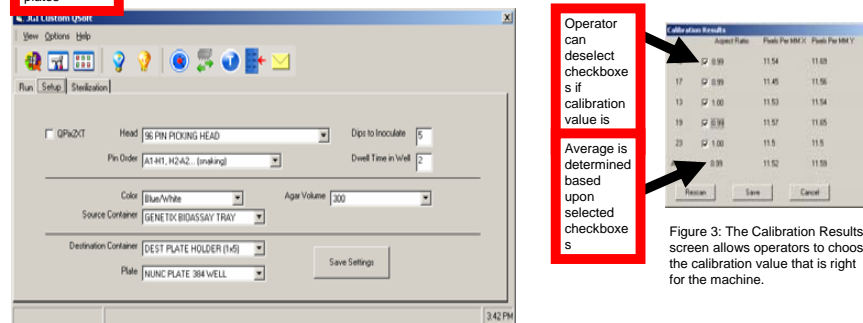
Figure 2: Setup screen is on separate tab from Run screen.
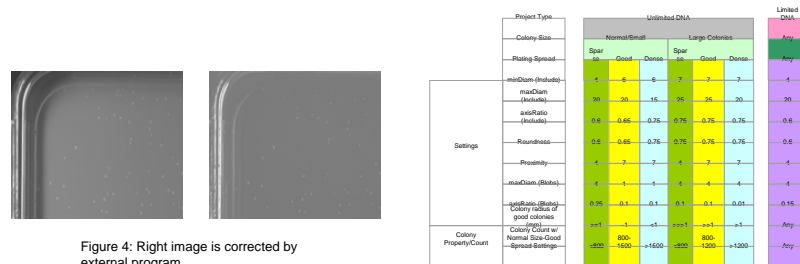

Operator can deselect checkboxes if calibration value is

Average is determined based upon selected checkboxes

Figure 3: The Calibration Results screen allows operators to choose the calibration value that is right for the machine.

Figure 4: Right image is corrected by external program.

Table 1: Preset criteria values that are used in picking.

Beta Testing & Rollout (QPixII)

JGI Custom QSoft software was rolled-out in late-May 2004 for testing on 1 QPixII instrument (out of 4). The QPix operators and QC/QA group assisted in testing the software for bugs and determining if other features would be necessary to make the software production ready.

Most bugs found by the operators were those that caused the light tray to not home or for the light tray to turn off after imaging. The most notorious bug found was one that caused B2 quadrants to be inoculated multiple times. This was found quickly and was able to be tracked in the picking logs.

During Beta testing, it was determined that some colony criteria were not being set correctly – this would result in colonies being picked even though they were too close together. To address this problem a set of values (Figure 1 & Table 1) were created that would be valid for bioassay trays of differing colony count densities, colony sizes and project sizes.

Operators requested various modifications including rearranging the graphical user interface so it flowed better, leaving the preset criteria values set to those that were selected in the previous run (so they would not have to change the values for each run), a checkbox that would allow operators to use manually selected values if they chose to (these values would be tracked in the log regardless).

Modifications for XT

Plate presentation after selecting 'Carry over pin & well settings' was a key reason JGI went with custom software on the XT. The development build of the QSoft engine that JGI uses had a bug that replaced all plates at the beginning of a run. This did not suit JGI's needs as these partially picked plates would not be passed through our production line unless they were filled (by hand) with good samples.

Displaying colony counts for both bioassay trays (after imaging and on the 'Run' tab) was a requirement so that the operators would not have to scour the log files for information on each tray.

Calibration values for each tray help the operators pick more accurately on each tray. Since each pre-poured bioassay is unique, it is helpful to have calibration values for each tray – especially if a bad tray must be picked.

Future Considerations

- Background Correction (solving the colorspace issue – Figure 4)
- Alignment for 2 Trays
- Moving Images to database
- Associate bioassay tray with destination plates

Conclusion

The JGI Custom QSoft was finally rolled into production in late-September 2004 for the QPixIIs and mid-November for the XT. By maintaining the functionality of the standard QSoft Software and tuning it specifically to the needs of the client, the JGI Instrumentation Group has made picking colonies even easier for the employees in the Library Support Group.

Advancing Science Through DNA Sequence