Robotic Enrichment Processing of Roche 454 Titanium Emulsion PCR at the DOE Joint Genome Institute

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Abstract

Enrichment of emulsion PCR product is the most laborious and pipette-intensive step in the 454 Titanium process, posing the biggest obstacle for production-oriented scale up. The Joint Genome Institute has developed a pair of custom-made robots based on the Microlab Star liquid handling deck manufactured by Hamilton to mediate the complexity and ergonomic demands of the 454 enrichment process. The robot includes a custom built centrifuge, magnetic deck positions, as well as heating and cooling elements. At present processing eight emulsion cup samples in a single 2.5 hour run, these robots are capable of processing up to 24 emulsion cup samples. Sample emulsions are broken using the standard 454 breaking process and transferred from a pair of 50ml conical tubes to a single 2ml tube and loaded on the robot. The robot performs the 454 enrichment protocol and produces beads in 2ml tubes ready for counting. The robot follows the Roche 454 enrichment protocol with slight exceptions to the manner in which it resuspends beads via pipette mixing rather than vortexing and a set number of null bead removal washes. The robotic process is broken down in similar discrete steps: First Melt and Neutralization, Enrichment Primer Annealing, Enrichment Bead Incubation, Null Bead Removal, Second Melt and Neutralization and Sequencing Primer Annealing. Data indicating our improvements in enrichment efficiency and total number of bases per run will also be shown.

454 Sample Enrichment Workflow

Emulsion PCR → Emulsion Breaking → Enhancing Fluid Washes → Denature Sample Beads → Neutralization and Enrichment Primer Annealing → Primer Washes → Enrichment Bead Incubation → Null Bead Removal → Denature Sample Beads → Neutralization and Sequencing Primer Annealing → Bead Counting → Sequencing
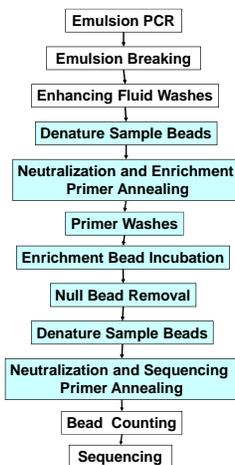
At present a single operator can enrich up to eight samples in four hours using the Enrichment Robot program. The enrichment robot begins the enrichment process immediately following the enhancing fluid washes after breaking, as seen in the flowchart diagram to the left (Robotic Steps in blue). Actual hands-on time comprises less than one hour of the total time and is limited to initiating the program and loading tip racks and reagents before each of the two stages of the program. Robot progress can be monitored from a desktop computer via remote viewing software.

Using the method we have developed for the pair of Hamilton MLStar robots, we can successfully enrich samples to be sequenced on the 454 platform. We are currently optimizing this method to produce the most reliable

Enrichment Robot Deck Layout
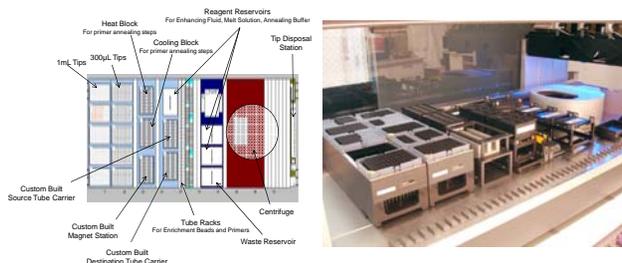
At present the enrichment robot can parallel process up to 8 samples, with changes to it's program the deck layout will support up to 24 samples at a time. Program duration for a double enrichment run is 3.5-4 hours.

Performance of Robot-Enriched Samples

Bases Per Region (2 region runs)
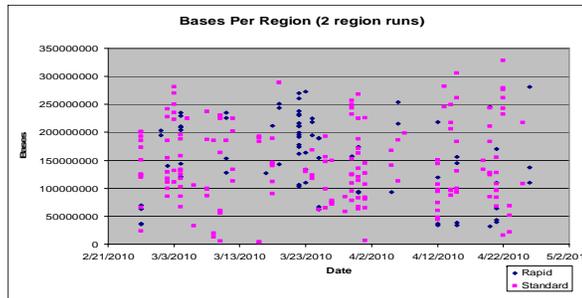
After implementing robotic enrichment in December of 2009 the JGI experienced a rather large spread of total bases achieved in runs of robotically processed samples comprised of both Rapid and Standard library types. The chart above shows approximately three months of data demonstrating the fluctuating numbers of bases between runs.


Percent Pass Filter
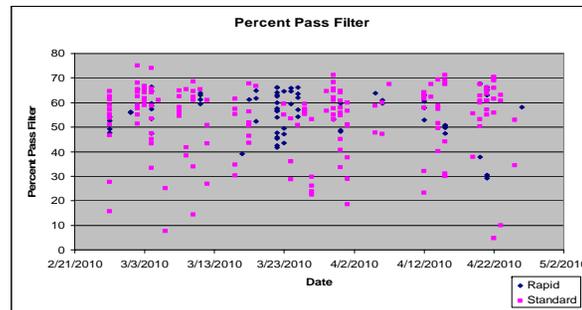
By examining run metrics over the same period we were able to determine that low total number of bases per region was not caused by an overall lower quality of beads coming out of emPCR. The chart above shows the percent of beads passing mixed/dot/tooshortquality/tooshort primer filters averaging between 55-70%.


Raw Wells (2 region runs)
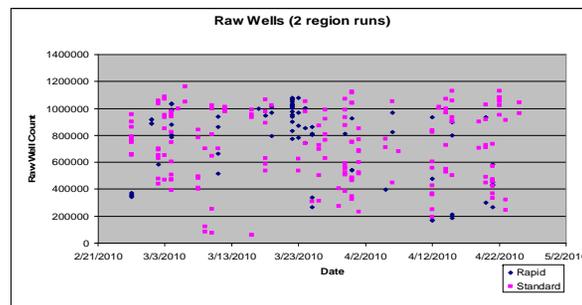
We discovered that robotic processing was not fully removing null beads and they were being loaded into PTPs and were producing runs with low numbers of raw wells as indicated in the chart above.

Improved Robot Method and 2X Enrichment
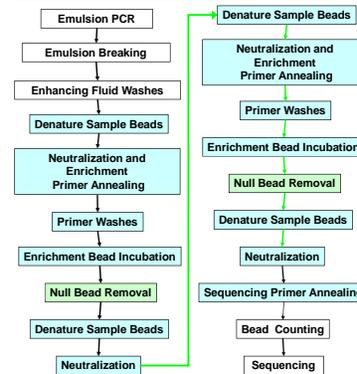
We made two changes to the robotic procedure as detailed in the process flowchart to the left.

1. We improved the pipetting technique used in the Null Bead Removal step to include an additional removal.

2. We also implemented an additional iteration of the enrichment process utilizing diluted enrichment kit reagents for both rounds.

Raw Wells - Auto Enriched Samples
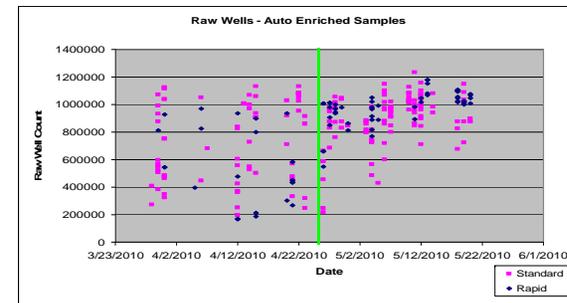
Beginning 4/26/2010 the JGI introduced an improved robotic pipetting method, incorporating additional null bead removals as well as enriching every sample twice using a single enrichment kit's worth of reagents. We saw a dramatic tightening in the range of raw well counts we were seeing in both our Rapid and Standard Library runs.

Bases per Region (2 Region Runs)
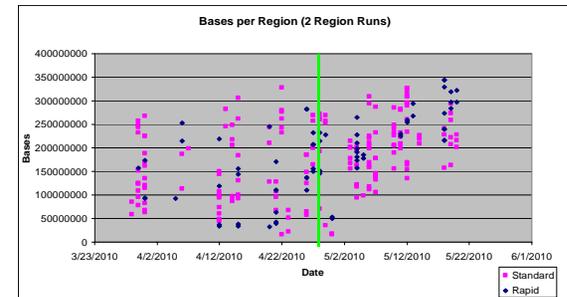
This increase in raw wells was also reflected in our Total Bases per region achieved, as pictured in the chart above.

Conclusions

1. The JGI implemented a robotic method of performing 454 enrichment on up to eight samples in parallel with a cycle time of about 4 hours.

2. Improving robotic pipetting methods during Null Bead Removal and performing two enrichment iterations with a single Roche enrichment kit resolved the issues we were seeing with reduced numbers of raw wells which was affective overall run output.

3. Future development may include alterations in programming to shorten cycle time and increase the number of samples processed in a cycle.

I would like to acknowledge the JGI Production Sequencing Group for sequencing these libraries, and Andrew Allison for working with qPCR and Illumina libraries on the BioMek.