Implementation of a CyBio Integrated System to Aliquot Amplified DNA and Dispense DNA Sequencing Chemistry

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Overview

Two CyBio CyBi-Well Vario Pipettor & CyBi-Drop 3D Dispenser integrated systems have been purchased to aliquot amplified DNA and dispense sequencing chemistry, handling 900 plates per day.

Introduction

The CyBio, CyBi-Well Vario pipettor and two integrated CyBi-Drop dispensers are being implemented into the JGI production sequencing line to replace two ageing Hydra-Twister instruments and Cavro dispensers. The Vario disposable tip 25uL head is used to aliquot low volume amplified DNA samples from an Axygen PCR source plate and dry dispense 1-4uL into two new pre-barcoded destination plates. The source plate is scanned to confirm database consistency and the destination plate is scanned "on the fly" to record forward or reverse primer sequencing chemistry reagent dispensed (2-4uL) using the CyBi-Drop 3D. Throughput is at least twice as fast as our current Hydra-Twister & Cavro instruments.

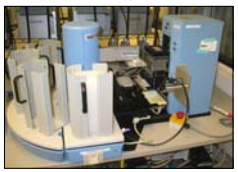
Current System

•Throughput

- •Hydra-Twister; 18 source plates to 36 destination plates; 1 hour
- •Cavro; 18 Fwd chem dispense, 18 Rev chem dispense; 30 min

•Total Dead Volume ~22mL (11mL / sequence chemistry primer)

•Difficult to obtain parts



Hydra-Twister System

Cavro Dispenser System

Acceptance Criteria with Results

The following acceptance criteria were used to specify the performance requirements to be met by the system. The results are shown next to the specific requirement in **red**.

•Plate Types

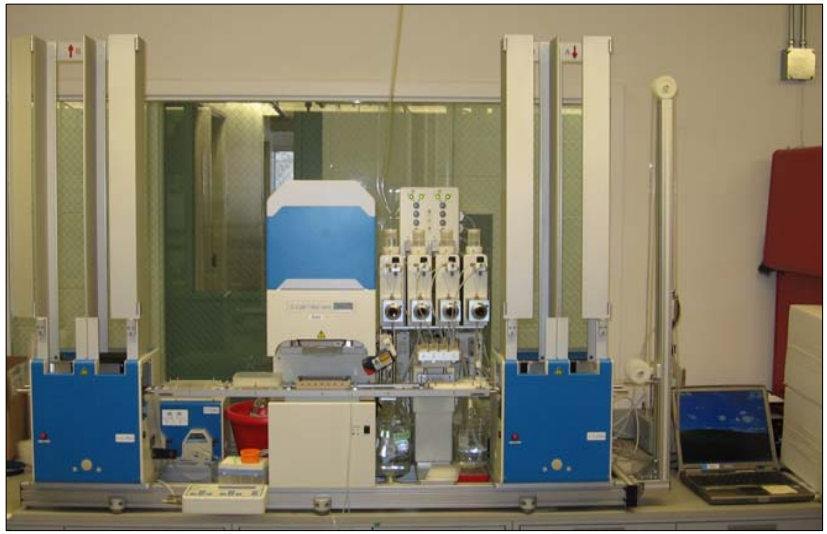
- •Source; Axygen PCR warped due to heat sealing on PlateLoc twice, thermal cycled 95°C for 5 min; incubated 30°C for 20hrs
- •Destination; new Axygen PCR

•Error Rate Definition

- •Major Error [allowable 0-3/yr depending on usage] failure or crash that destroys samples, requires manufacturer call, new parts, major repair or reprogramming
- •Minor Error [1-2/week depending on usage] failure or crash that can be easily recovered from by the operator or occasionally with help from instrumentation support

•Stacker Testing

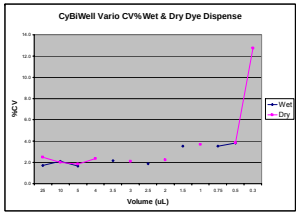
- •1000 plate load/unload cycles without minor error. OK



Acceptance Criteria with Results

Pipettor Testing

•Precision 5-25uL <1%CV, 0.5-5uL <5%CV



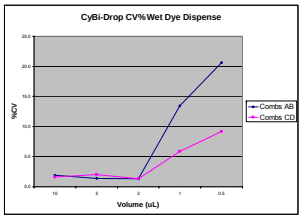
•Accuracy ±5% of target volume

	Volume (ul)							
Plate	25.0	10.0	5.0	3.5	2.5	1.5	0.8	0.5	0.3
1	9.640	3.918	2.014	1.458	1.065	0.692	0.383	0.290	0.211
2	9.707	3.930	2.024	1.463	1.072	0.691	0.389	0.288	0.208
3	9.715	3.937	2.025	1.447	1.072	0.690	0.383	0.289	0.208
AVG	9.687	3.928	2.021	1.456	1.070	0.691	0.385	0.289	0.209
SD	0.0412	0.0096	0.0061	0.0082	0.0040	0.0010	0.0035	0.0010	0.0017
%CV	0.43%	0.24%	0.30%	0.56%	0.38%	0.14%	0.90%	0.35%	0.83%

Plate Positioner 1000 cycles, raise/lower, 4 dirn tip touch. **OK**

Dispenser Testing

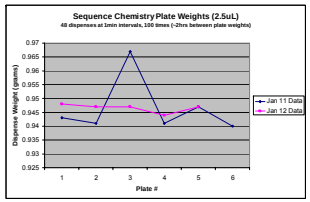
•Precision ≥0.5-5uL <5%CV, >5uL <4%CV



•Accuracy ±5% of target volume

	Volume (ul)						
Plate	25.0	10.0	5.0	2.0	1.0	0.70	0.50
1	9.646	3.852	1.924	0.700	0.347	0.234	0.149
2	9.641	3.856	1.924	0.703	0.349	0.223	0.149
3	9.637	3.854	1.922	0.705	0.347	0.224	0.149
AVG	9.641	3.854	1.923	0.703	0.348	0.227	0.149
SD	0.0045	0.0020	0.0012	0.0025	0.0012	0.0061	0.0000
%CV	0.05%	0.05%	0.06%	0.36%	0.33%	2.68%	0.00%

•Compatibility with Sequence chemistry, repeat dispense/pause test, weigh plates



•Barcode Reader >99.5% efficiency, error handling. OK

•Wash Bath 10uL blue dye mix cycle, regular wash then test for wash off residue

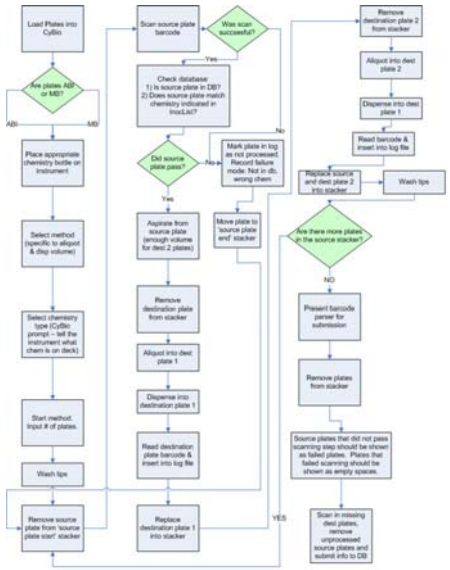
•Dead Volume better than Cavro system 22mL. 3.5mL pump/comb combination → 7mL/seq chem, 14mL Total

•Throughput = 42 source/batch, 84 destination, 7 batch/day, < 2min 30sec per source plate

•Redundancy

- •Can use pipettor or dispenser separately, routinely use two dispense combs/primer (single pass), option to use single comb if one fails before replacement can be installed (double pass)

Process Flow Chart



The process flowchart above shows the logical progression of plate interactions and handling with the system including error handling.

Conclusions

The DNA sequencing production line at the Joint Genome Institute (JGI) is characterized by modular machine stations with batches of microtiter plates moving between them. The DNA sequencers determine the throughput for the production line. JGI is currently producing 3 Gb of sequence per month.

The production instrumentation engineering goals focus on increasing the quality and reliability at each process step and allowing for maximum operator efficiency. These instruments integrate what has historically been two independent process steps at the JGI.

These instruments in the next 3-4 months should become a key part of the automation required to setup the cycle sequencing reaction in every plate processed at the JGI.

LBNL-58812 Poster

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