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 by the Joint Genome Institute (JGI) to aliquot amplified DNA and dispense sequencing chemistry, intended throughput is 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 two 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 & manually loaded Cavro instruments.
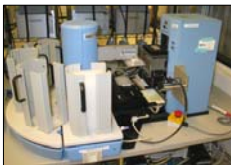
Replacing Current Hydra-Twister & Cavro System

Throughput

• Hydra-Twister; 18 source plates to 36 destination plates; 1 hour

• Cavro; 18 Fwd chem dispense, 18 Rev chem dispense; 30 min

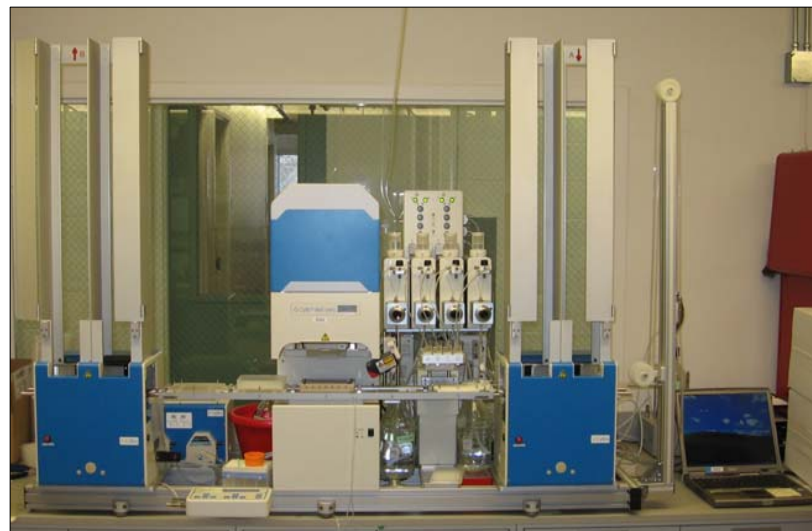### Total Dead Volume ~22mL (11mL / sequence chemistry primer)

• Difficult to obtain parts.
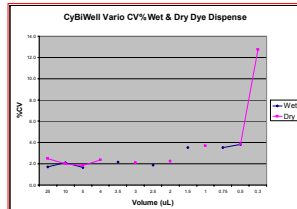
• Both systems >5yrs old.

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.

1) 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 pre-barcoded

2) Error Rate Definition & Frequency

• 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

3) Stacker Testing

• 1000 plate load/unload cycles without minor error. **Conditioned, warped plates & real production plates tested successfully.**

Acceptance Criteria with Results Cont'd

4) Pipettor Testing

• Precision 5-25uL <2%CV, 0.5-5uL <5%CV

CyBiWell Vario CV% Wet & Dry Dye Dispense

• Accuracy ±5% of target volume

Plate	25.0	10.0	5.0	3.5	2.5	1.5	0.8	0.5	0.3
					Volume (ul)				
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	0.280	0.208
AVG	9.687	3.928	2.021	1.456	1.070	0.691	0.385	0.280	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%
Expected	9.600	3.840	1.920	1.344	0.960	0.576	0.288	0.192	0.115
% Diff	1.01%	1.02%	1.05%	1.08%	1.11%	1.20%	1.34%	1.51%	1.81%

5) Plate Positioner

• 1000 cycles, raise/lower, 4 direction tip touch. **Successful.**

6) Dispenser Testing

• Precision 0.5uL <10%CV, ≥1-5uL <5%CV, >5uL <4%CV

CyBi-Drop CV% Wet Dye Dispense
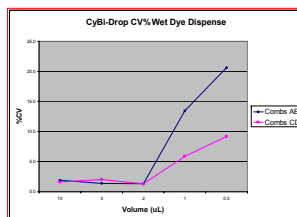
Acceptance Criteria with Results Cont'd

• Accuracy ±5% of target volume

Plate	25.0	10.0	5.0	2.0	1.0	0.70	0.50
				Volume (ul)			
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%
Expected	9.600	3.840	1.920	0.768	0.384	0.269	0.192
% Diff	1.00%	1.00%	1.00%	0.91%	0.91%	0.84%	0.78%

• Compatibility with Sequence chemistry, repeat plate dispense/pause 1min, test plate weight every 100 plates.

Sequence Chemistry Plate Weights (2.5uL)
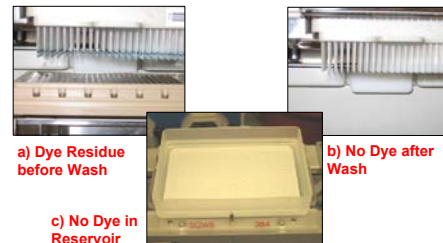48 dispenses at 1min intervals, 100 times (~2hrs between plate weights)
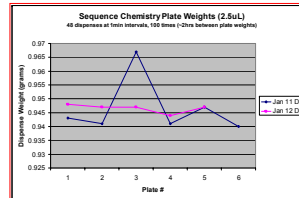
7) Barcode Reader

• >99.5% efficiency, error handling. **Pre-barcoded & JGI barcodes, OK, working on exceptions.**

8) Wash Bath

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

a) Dye Residue before Wash

b) No Dye after Wash

c) No Dye in Reservoir

Acceptance Criteria with Results Cont'd

9) Dead Volume

• Better than Cavro systems 22mL. **3.5mL per pump/comb combination → 7mL/seq chem, 14mL Total**

10) Throughput

• 42 source/batch, 84 destination, 7 batch/day, goal <2min 30sec per source plate. **Measured at 3min/plate before optimization.**

11) Redundancy Built-In

• 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 including Error Handling
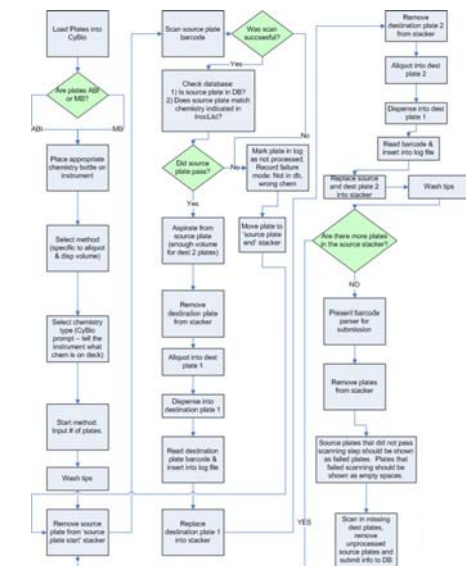
Conclusions

The DNA sequencing production line at the JGI is characterized by modular machine stations with batches of microtiter plates moving between them. JGI is currently producing 3 Gigabases 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 instrument workstations at the JGI.

Advantages:

• More consistent plate handling.

• Allows operator walk-away time (90min).

• Eliminates two manual barcode scanning steps.

• Eliminates hand application of barcode labels on destination plates.

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.

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