Evaluation of the Pillar Biosciences oncoReveal™ Solid Tumor v2 (oRSTv2) Application on the Beckman Coulter Biomek NGeniuS System

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ABSTRACT

Introduction: The manual preparation of libraries for next-generation sequencing (NGS) can lead to high variance in library yield, quality, and turnaround time due to individual differences in pipetting technique and speed. To address this, we developed an application on Beckman Coulter Life Sciences Biomek NGeniuS Next Generation Library Prep System that automates the oncoReveal™ Solid Tumor v2 (oRSTv2) research use only (RUO) panel library preparation workflow from DNA through indexed libraries. The oRSTv2 application enables the preparation of between 4 to 24 samples from input DNAs to final libraries with ~1 to 1.5 hours upfront hands-on time and ~5.5 to 7.5 hours of walkaway time. In this study, we evaluated the laboratory performance of the oRSTv2 application on the Biomek NGeniuS system.

Methods: Twenty (20) replicates of NA12878 (Coriell Institute) DNA samples and 22 moderately formalin-compromised positive DNA (ModfcDNA, Horizon Discovery) samples with an input of 19.35ng across three oRSTv2 application runs. Six (6) No-Template Controls (NTC) were also processed. Three (3) application runs performed on non-consecutive days, and contained 24, 16, and 8 libraries, respectively. The first run (24 libraries) was sequenced on an Illumina NextSeq™ 550. Subsequent runs (16 and 8 libraries) were sequenced on an Illumina MiSeq™. Sequencing metrics and variants reported by PiVAT® (Pillar Variant Analysis Toolkit) software were analyzed.

Results: Total hands-on setup time ranged from 57 minutes to 1 hour 22 minutes. Total hands-off time ranged from 5 hours 44 minutes to 7 hours 27 minutes. Library yields averaged 50.67 nM and 35.95 nM for NA12878 and ModfcDNA samples, respectively. Effective on-target rate was above 90% for all replicates, with an average of 98.25% and 97.52% for NA12878 and ModfcDNA samples, respectively. Additionally, mapping rate was above 95% for all replicates, with an average of 99.45% and 99.02% for NA12878 and ModfcDNA samples, respectively. Overall, all NA12878 and ModfcDNA libraries had their expected variants called at the expected VAF. Cross-contamination was not observed in NTC replicates.

Conclusion: The Pillar oRSTv2 application on the Biomek NGeniuS Next Generation Library Prep System is capable of producing consistent high-quality libraries with minimal hands-on time and manual intervention. This system and our application will now provide clinical laboratories with low to moderate sample volume an opportunity to automate library preparation and expedite the delivery of NGS results.

APPLICATION DESIGN

	Manual	oRSTv2 Application
Sample Count	4-24 per batch	4-24 per batch
Supported DNA Types & Input Mass	gDNA: 20-60ng FFPE: 20-80ng Severely degraded FFPE: >=40ng	
Estimated hands-on time	2 – 3.5 hours	1 – 1.5 hours
Estimated hands-off time	3 hours	5.5 – 7.5 hours

Table 1 –Features of the oRSTv2 application.

App Setting	Description
Mix beads during Exo digestion	Mixes AMPure XP beads during Exonuclease I digestion to reduce bead settling and sample processing time. If not selected, mixing will occur directly before gene-specific product purification.
IndexPlate	Allows the operator to enter in a name for the index plate being used in the batch.
Indexing PCR Cycles	Allows the operator to set the number of indexing PCR cycles performed within a range of 6-10.

Table 3 – oRSTv2 Application settings, configured before each library prep run.

oRSTv2 Panel Info							
AKT1	*CYSLTR2*	*FBXW7*	*GNAS*	*KEAP1*	*NTRK1*	*PTEN**	*SMAD4*
ALK	*DDR2*	*FGFR1*	*H3F3A (H3-3A)*	*KIT*	*PDGFRA*	*PTPN11*	*SRSF2*
ARAF	*EGFR*	*FGFR2*	*HIST1H3B (H3C2)*	*KRAS*	*PIK3CA*	*RAC1*	*STK11*
BRAF	*EIF1AX*	*FGFR3*	*HRAS*	*MAP2K1*	*PLCB4*	*RAF1*	*TERT*
CDKN2A	*ERBB2*	*GNA11*	*IDH1*	*MET*	*POLD1*	*RET*	*TP53*
CTNNB1	*ERBB4*	*GNAQ*	*IDH2*	*NRAS*	*POLE*	*SF3B1*	*TSHR*

Table 2 – The 48 genes covered by the oRSTv2 panel. Genes marked in green indicate full CDS coverage.
* Indicates full CDS with exception of exon 9, chr10; 89725157-89725229

Gene	Variant ID	Genomic Position	Gene	Variant ID	Genomic Position
EGFR	p.G719S	chr7:55241707-55241707	NRAS	p.Q61K	chr1:115256530-115256530
	p.L858R	chr7:55259515-55259515	PDGFRA	p.Pro567=	chr4:55141055-55141055
	p.E746_A750del	chr7:55242465-55242479	PDGFRA	p.V824=	chr4:55152040-55152040
	p.T790M	chr7:55249071-55249071	PIK3CA	p.E545K	chr3:178936091-178936091
	p.Q787=	chr7:55249063-55249063	PIK3CA	p.H1047R	chr3:178952085-178952085
	N/A	chr7:55228053-55228053	PTEN	N/A	chr10:89720907-89720907
KRAS	p.G12D	chr12:25398284-25398284	FGFR3	p.T651=	chr4:1807894-1807894
	p.G13D	chr12:25398281-25398281	KEAP1	p.L471=	chr19:10600442-10600442
POLE	p.T1052=	chr12:133236000-133236000	KIT	p.D816V	chr4:55599321-55599321
	p.A31S	chr12:133257837-133257837	BRAF	p.V600E	chr7:140453136-140453136
	N/A	chr12:133250118-133250118	RET	p.L769=	chr10:43613843-43613843
RAC1	N/A	chr7:6426941-6426941	TERT	N/A	chr5:1295243-1295243
	N/A	chr7:6426953-6426953			

Table 4 – Clinically significant variants evaluated in the development of the oRSTv2 application.

Figure 1 – Overview of oncoReveal™ Solid Tumor v2 application sections and approximate hands-on and hands-off time. For a 24 sample run, the on-deck portion of the workflow can be completed in ~7.5 hours with several optional stopping points.

RESULTS AND CONCLUSIONS

Figure 2 - Average final library yield (nM) across run and sample type.

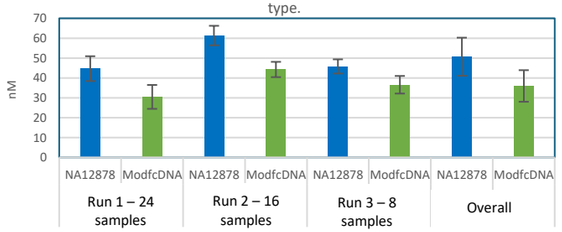

Figure 3 - Average mapping rate (%) and on-target rate (%) across run and DNA type.

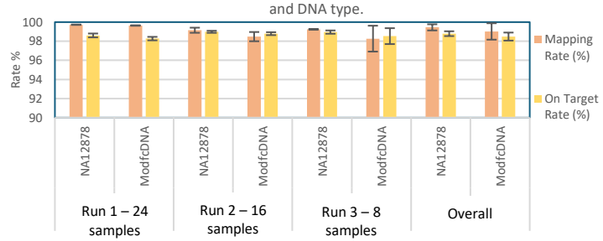

Figure 4 - Average base coverage depth relative to mean coverage at 0.2x, 0.3x, and 0.4x across run and DNA type.

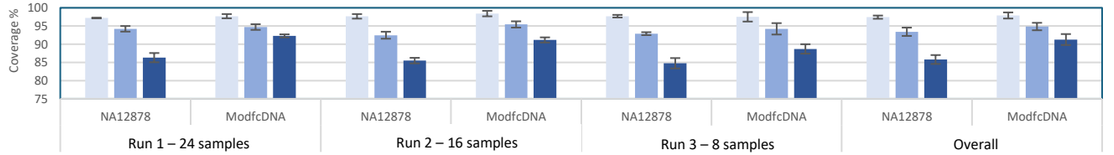

Figure 5 - Hands-on and hands-off time across runs.

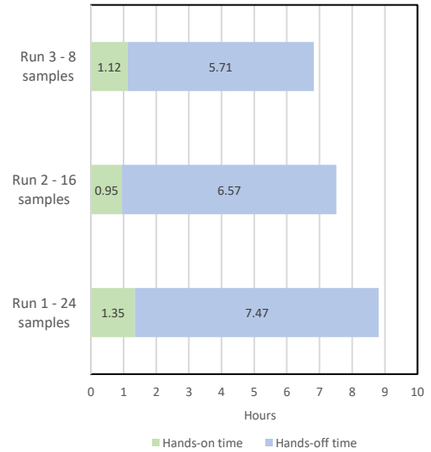

Table 5 – Average variant read frequency % for expected ModfcDNA and NA12878 variants

DNA type	Gene	HGVSC	Genomic Position	Expected VAF (%)	Hit Rate	Average VAF (%)
ModfcDNA	BRAF	c.1799T>A	chr7:140453136-140453136	10.5	22/22	11.94 ± 0.91
	EGFR	c.2155G>A	chr7:55241707-55241707	24.5	22/22	22.46 ± 1.11
	EGFR	c.2573T>G	chr7:55259515-55259515	3	22/22	3.62 ± 0.47
	KIT	c.2447A>T	chr4:55599321-55599321	10	22/22	8.11 ± 0.98
	KRAS	c.35G>A	chr12:25398284-25398284	6	22/22	5.93 ± 0.88
	KRAS	c.38G>A	chr12:25398281-25398281	15	22/22	14.81 ± 0.94
	NRAS	c.181C>A	chr1:115256530-115256530	12.5	22/22	13.08 ± 1.26
	PIK3CA	c.1633G>A	chr3:178936091-178936091	9	22/22	7.34 ± 0.58
	PIK3CA	c.3140A>G	chr3:178952085-178952085	17.5	22/22	17.37 ± 1.33
	EGFR	c.2235_2249del	chr7:55242465-55242479	2	15/22*	2.14 ± 0.42
	EGFR	c.2369C>T	chr7:55249071-55249071	1	11/22*	1.09 ± 0.33
			ModfcDNA overall hit rate		198/198	
NA12878	EGFR	c.2361G>A	chr7:55249063-55249063	49.2	20/20	49.3 ± 1.16
	FGFR3	c.1953G>A	chr4:1807894-1807894	99.5	20/20	98.79 ± 0.15
	KEAP1	c.1413C>G	chr19:10600442-10600442	50	20/20	49.75 ± 1.07
	PDGFRA	c.1701A>G	chr4:55141055-55141055	99.9	20/20	99.5 ± 0.17
	PDGFRA	c.2472C>T	chr4:55152040-55152040	50	20/20	47.92 ± 1.5
	POLE	c.3156G>A	chr12:133236000-133236000	99.7	20/20	99.11 ± 0.12
	POLE	c.91G>T	chr12:133257837-133257837	48.5	20/20	49.73 ± 1.15
	RET	c.2307G>T	chr10:43613843-43613843	99.7	20/20	99.29 ± 0.12
	EGFR	c.1498+22A>T	chr7:55228053-55228053	99.7	20/20	98.47 ± 0.33
	POLE	c.1359+43G>A	chr12:133250118-133250118	99.6	20/20	98.69 ± 0.22
	PTEN	c.1026+32T>G	chr10:89720907-89720907	48.6	20/20	48.33 ± 2
	RAC1	c.107+27C>T	chr7:6426941-6426941	51.5	20/20	49.35 ± 0.8
	RAC1	c.107+39C>T	chr7:6426953-6426953	50.8	20/20	49.26 ± 0.89
	TERT	N/A	chr5:1295243-1295243	56.6	20/20	56.72 ± 3.99
			NA12878 overall hit rate		280/280	
Overall			*Study overall hit rate*		478/478	

* Variant is below VAF cutoff in PiVAT software. Not included in overall hit rate analysis.

Conclusions:

1. Using the oRSTv2 application on the Biomek NGeniuS system, up to 24 libraries can be prepared with ~1 - 1.5 hours hands-on time and ~5.75 - 7.5 hours hands-off time (Figure 5).
2. The Biomek NGeniuS oRSTv2 application can produce high quality libraries (Figures 2-4, Table 5).
3. Resulting variant calls for libraries prepared via the oRSTv2 application were consistent with expected VAF %s (Table 5).
4. Combined with a rapid NGS platform, this automation system & application can potentially accelerate the delivery of next day tumor profiling results.