Automation of the Pillar Biosciences oncoReveal Solid Tumor 22 gene panel (ORST22) on the Biomek NGeniuS Next Generation Library Prep System

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ABSTRACT

Introduction: Fully automated NGS library preparation workflows are highly desirable for clinical settings. In this study, we used the Biomek NGeniuS instrument (Beckman Coulter) as our platform because it has all necessary components on-deck including cold storage blocks, a magnet position, and a thermal cycler. We developed a fully automated process for our research-use-only (RUO) oncoReveal™ 22-Gene Solid Tumor assay (ORST22) on the Biomek NGeniuS to prepare 4 to 24 libraries requiring only ~1 hour upfront hands-on time and 6 hours of walkaway time from input DNAs to final libraries. In this study, we evaluated the performance of the automated process and assessed the NGS results against the results from the manual workflow.

Method: Two DNA samples with various replicates were used for the study: NA12878 DNA (genome-in-a-bottle) at ~10ng and the moderately formalin-compromised positive DNA (ModfcDNA, Horizon Discovery) at ~20ng. One to three replicates of no-template control (NTC) were also processed in each run. To evaluate the run-to-run precision, three automation runs were performed and sequenced on different days to prepare 8, 16 and 24 libraries, respectively. To assess the NGS results against those from the manual process, the same reagents and the same input DNAs were used for library preparation by both workflows with 24 libraries for automation and 8 for manual prep. Subsequently, the libraries were sequenced on the same flow cells using Illumina NextSeq 550. The secondary analysis metrics and the variants reported by Pillar's PiVAT bioinformatic software were assessed and compared.

Results: No cross-contamination was observed in any NTC replicates in any of the runs. All runs generated high-quality results with consistent >90% effective on-target rates and ~100% of bases covered at ≥ 0.2x mean coverage. Compared with the manual NGS libraries, the automated libraries showed slightly better uniformity with higher percentages of bases covered at ≥0.4x to 1x of the mean coverage. 20 known variants from each positive ModfcDNA with expected VAF from 1-30% were highly concordant between runs with within runs. No false positive variants were detected in any of the samples.

Conclusion: The automated Pillar ORST22 assay on Biomek NGeniuS system produced high quality and robust results with minimal hands-on time and human intervention. Together with the easy-to-use setup of the NGeniuS, and Pillar's single day library preparation workflow, these combined technologies could substantially reduce the lab operation burden.

APPLICATION DESIGN

	Manual	NGeniuS
Sample Count	Up to 24 per batch	4-24 per batch
Input Mass	10-80ng	
Supported DNA Types	Genomic, FFPE	
Estimated hands-on time	3 - 4 hours	0.5 - 1 hour
Estimated hands-off time	3 hours	5-6 hours

Table 1 –Features of ORST22

ORST22 Panel Info										
AKT1	*BRAF*	*DDR2*	*ERBB2*	*FBXW7*	*FGFR2*	*KRAS*	*MET*	*NRAS*	*PTEN*	*STK11*
ALK	*CTNNB1*	*EGFR*	*ERBB4*	*FGFR1*	*FGFR3*	*MAP2K1*	*NOTCH1*	*PIK3CA*	*SMAD4*	*TP53*

Table 2 – The 22 genes covered by the ORST22.

App Setting	Description
Mix beads during Gene-Specific PCR	Mixes AMPure XP beads during gene-specific PCR to prevent them from settling. 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 5-10.

Table 3 – ORST22 Gene Application settings, configured before each library prep run.

Gene	Variant ID	Gene	Variant ID
EGFR	L858R	PIK3CA	N345I; N345T; N345K
	Exon 19 In-Frame Deletions		E542Q; E542K; E542V
	T790M		E545K; E545Q; E545A; E545G; E545D
	G719A; G719C; G719D; G719S		H1047Y; H1047L; H1047R
	Exon 20 In-frame Insertions		R88Q; R88L
KRAS	G12X	FGFR3	R248C
	G13X		S249C
	A59E; A59G; A59T; A59S		G370C
	Q61E; Q61H; Q61K; Q61L;Q61R		Y373C
	K117N	PTEN	R130Q; R130L; R130P; R130G; R130*
	A146T; A146P; A146V		T319del
AKT1	E17K		S249C
BRAF	V600E; V600K		R248C

Table 4 – Examples of commonly mutated, clinically significant, variants in *EGFR, KRAS, PTEN, PIK3CA, FGFR3, AKT1*, and *BRAF* that are targeted in ORST22. Variants in green were used in evaluation during NGeniuS application development.

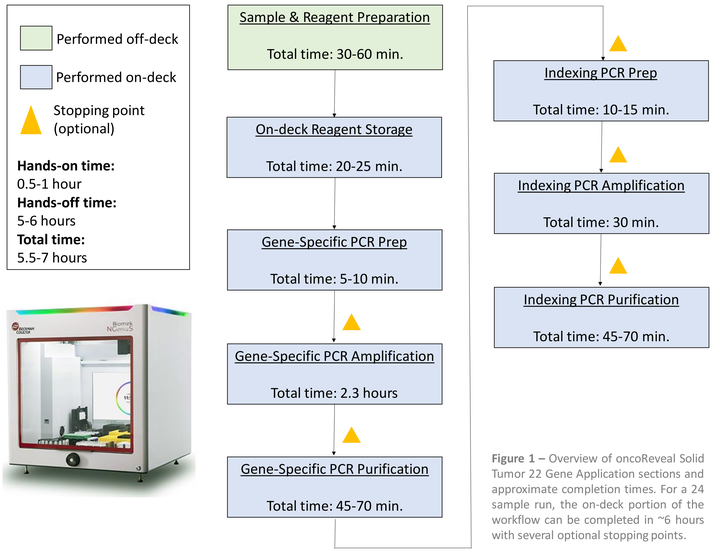

Figure 1 – Overview of oncoReveal Solid Tumor 22 Gene Application sections and approximate completion times. For a 24 sample run, the on-deck portion of the workflow can be completed in ~6 hours with several optional stopping points.

RESULTS AND CONCLUSIONS

Figure 2 – Final library yield (nM) across preparation method and sample type conditions.

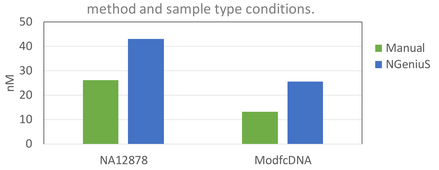

Figure 3 – Average mapping rate (%) and on-target rate (%) across preparation method and DNA type.

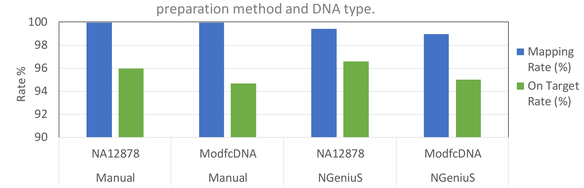

Figure 4 – Average Base coverage depth relative to mean coverage at 0.2x, 0.4x, and 1x across library preparation method and DNA type.

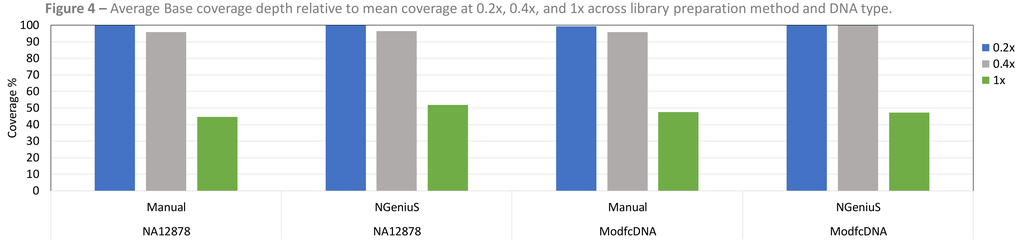

Table 5 – Average variant read frequency % for expected ModfcDNA variants across NGeniuS and manual sample preparation methods. N=2 (manual), 4(NGeniuS).

Gene	HGVSC	HGVSP	Genomic Position	Expected VAF (%)	Manual		NGeniuS	
					Hit Rate	VAF (%)	Hit Rate	VAF (%)
STK11	c.816C>T	p.Tyr272=	chr19:1221293-1221293	9.0	2/2	8.01 ± 1.01	6/6	8.55 ± 1.14
TP53	c.376-7C>T	N/A	chr17:7578561-7578561	26.0	2/2	29.37 ± 0.71	6/6	27.42 ± 1.38
KRAS	c.38G>A	p.Gly13Asp	chr12:25398281-25398281	15.0	2/2	14.28 ± 1.51	6/6	14.78 ± 0.9
KRAS	c.35G>A	p.Gly12Asp	chr12:25398284-25398284	6.0	2/2	6.81 ± 0.52	6/6	6.94 ± 0.61
ERBB2	c.2411G>A	p.Gly804Asp	chr17:37881082-37881082	5	2/2	4.55 ± 0.62	6/6	4.78 ± 0.66
CTNNB1	c.98C>A	p.Ser33Tyr	chr3:41266101-41266101	30	2/2	32.17 ± 0.51	6/6	31.01 ± 1.75
CTNNB1	c.133_135del	p.Ser45del	chr3:41266134-41266136	10	2/2	9.92 ± 1.24	6/6	9.37 ± 1.38
EGFR	c.1839C>T	p.Ala613=	chr7:55233089-55233089	8	2/2	6.94 ± 0.21	6/6	7.17 ± 0.47
EGFR	c.2155G>A	p.Gly719Ser	chr7:55241707-55241707	24.5	2/2	24.79 ± 0.41	6/6	24.43 ± 0.99
EGFR	c.2235_2249del	p.Glu746_Ala750del	chr7:55242465-55242479	2	2/2	1.96 ± 0.06	6/6	1.86 ± 0.31
EGFR	c.2369C>T	p.Thr790Met	chr7:55249071-55249071	1	2/2	1.28 ± 0.39	4/6*	0.81 ± 0.64
EGFR	c.2573T>G	p.Leu858Arg	chr7:55259515-55259515	3	2/2	3.36 ± 0.93	6/6	3.85 ± 0.47
MAP2K1	c.167A>C	p.Gln56Pro	chr15:66727451-66727451	30	2/2	32.29 ± 0.71	6/6	31.47 ± 2.19
NRAS	c.181C>A	p.Gln61Lys	chr1:115256530115256530	12.5	2/2	11.35 ± 0.08	6/6	11.51 ± 0.69
BRAF	c.1799T>A	p.Val600Glu	chr7:140453136140453136	10.5	2/2	12.12 ± 1.33	6/6	13.25 ± 0.95
DDR2	c.351G>A	p.Met117Ile	chr1:162724579162724579	10	2/2	9.79 ± 1.82	6/6	9.93 ± 0.92
PIK3CA	c.1173A>G	p.Ile391Met	chr3:178927410178927410	9	2/2	8.56 ± 0.86	6/6	8.53 ± 0.56
PIK3CA	c.1633G>A	p.Glu545Lys	chr3:178936091178936091	9	2/2	9.11 ± 0.45	6/6	8.43 ± 0.87
PIK3CA	c.3140A>G	p.His1047Arg	chr3:178952085178952085	17.5	2/2	18.39 ± 0.96	6/6	17.66 ± 0.75
FGFR3	c.1953G>A	p.Thr651=	chr4:1807894-1807894	100	2/2	98.83 ± 0.09	6/6	99.00 ± 0.28
EGFR	c.2361G>A	p.Gln787=	chr7:55249063-55249063	15	2/2	14.13 ± 0.98	6/6	13.83 ± 1.85

*Variant is below VAF cutoff in PiVAT software.

Conclusions:

1. Using the NGeniuS ORST22 application, 24 libraries can be prepared in approximately 7 hours with ~1 hour hands-on time and ~6 hours hands-off time (Figure 1).
2. The NGeniuS automated platform can produce high quality libraries using Pillar Biosciences oncoReveal Solid Tumor 22 Gene panel (Figures 2-4).
3. Resulting variant calls for libraries prepared on the NGeniuS platform were as expected and comparable to manual preparation (Table 5).