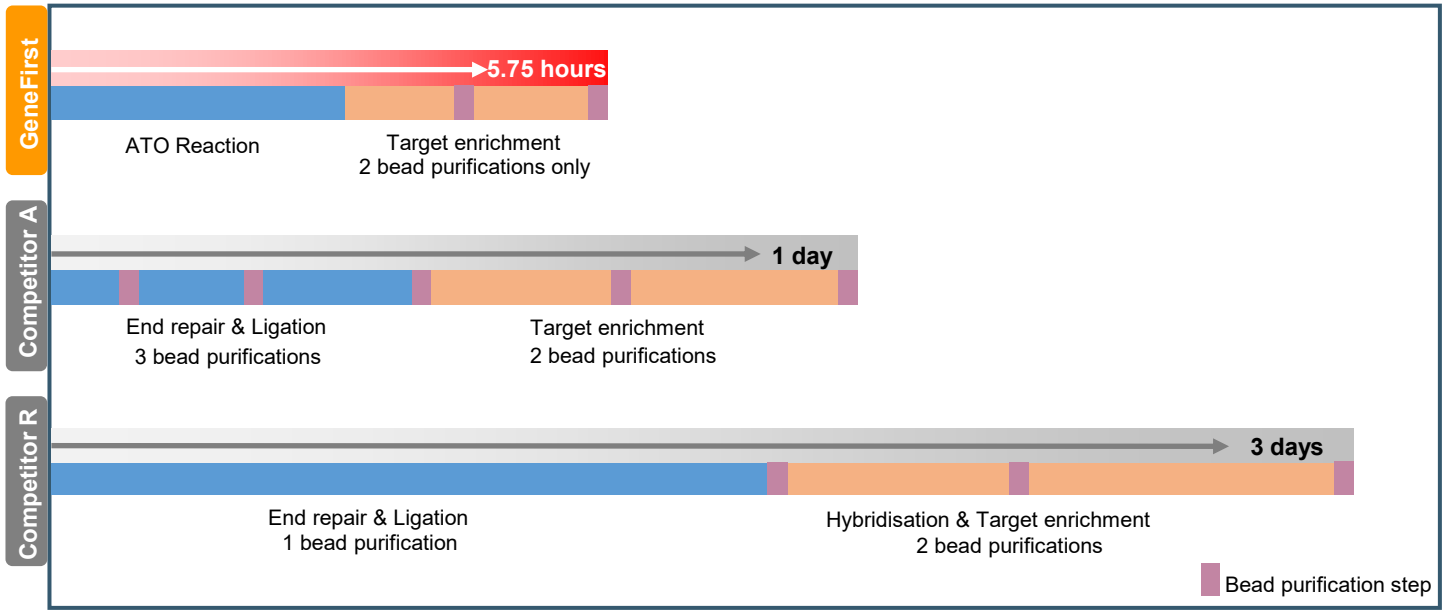
XCeloSeq Pan Cancer Kit Summary

XCeloSeq protocols have many technological advantages. Protocols are not just quicker and easier to complete than competitors but contain unique steps increasing the quality of the final libraries. ATOM-Seq protocols capture a greater proportion of DNA from a sample due to inherent advantages including capturing all single- and double-strand DNA molecules down to 20bp in length.



	Amplicon	Ligation+ Amplicon	Ligation+ Capture	ATOM-Seq
Capture Single Strand DNA	✓	✗	✗	✓
Capture Any DNA Break Point	✗	✓	–	✓
Simple whole exon coverage	✗	✓	✓	✓
Capture ssDNA Down to 20 bp	✗	✗	✗	✓
Sequence DNA Strands Independently	–	–	–	✓
Protocol based enhancements for sensitivity and specificity	✗	✗	✗	✓
Low Sample Loss	✓	✗	✗	✓
Simple Workflow	✓	–	✗	✓
Rapid Protocol	✓	✗	✗	✓
Known Fusion Detection	✓	✓	✓	✓
Unknown Fusion Detection	✗	✓	–	✓

Green ticks indicate aspects the technologies can do.
Green bars indicates aspects of the technologies which are inefficient.
Red crosses indicate aspects the technologies can not do.

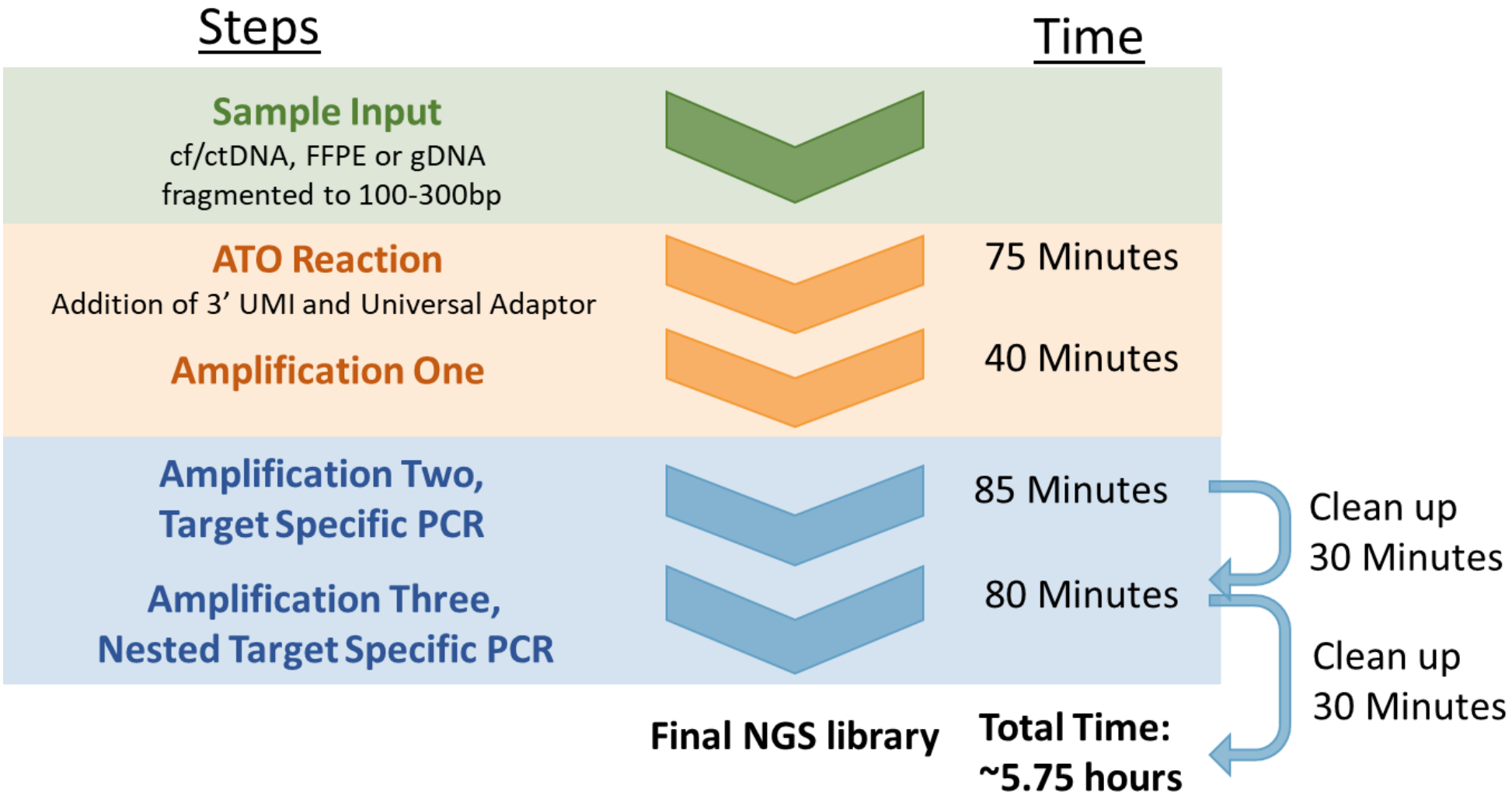
XCeloSeq Pan Cancer Kit

The Pan Cancer Kit has been designed for detection of hot spot mutations in 100 genes by interrogating over 570 target regions

ABL1	BRCA1	CHEK2	ESR1	GNAQ	KEAP1	MSH2	NTRK3	RHOA	SMO
AKT1	BRCA2	CSF1R	EZH2	GNAS	KIT	MSH6	PDGFRA	RIT1	SRC
ALK	CASP8	CTNNB1	FBXW7	HNF1A	KLF5	MTOR	PIK3CA	RNF43	STK11
AMER1	CCND1	DDR2	FGFR1	HRAS	KRAS	MYC	PTCH1	ROS1	TCF7L2
APC	CCND2	DMD	FGFR2	IDH1	MAP2K1	NF1	PTEN	SETD2	TP53*
AR	CCND3	EGFR	FGFR3	IDH2	MAP2K2	NFE2L2	PTPN11	SF3B1	TSC1
ARAF	CDH1	EP300	FGFR4	JAK2	MET	NOTCH1	RAF1	SMAD2	TSC2
ARID1A	CDK4	ERBB2	FLT3	JAK3	MGA	NPM1	RB1	SMAD4	UA2F1
ATM	CDK6	ERBB3	GATA3	KDM6A	MLH1	NRAS	RBM10	SMARCA4	VHL
BRAF	CDKN2A	ERBB4	GNA11	KDR	MPL	NTRK1	RET	SMARCB1	ZFP36L2

* Whole Coding Region

XCeloSeq Pan Cancer Protocol Summary



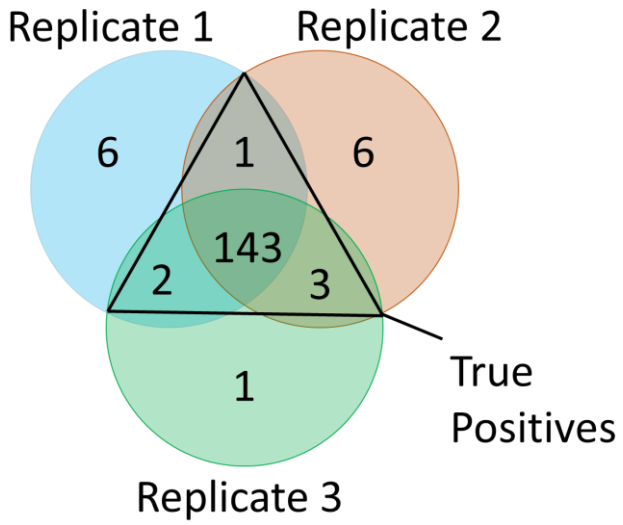
XCeloSeq Pan Cancer Kit Protocol: Representative Data

	AF	5%		1.30%		0.10%
	Input (ng)	0.5	1	1	5	20
Average Depth Per Primer	Sense DNA	395	807	870	2193	9361
	Antisense DNA	426	973	1147	2448	9759
	Combined	820	1780	2017	4640	19119
Average Primer Uniformity	Sense DNA	95.7%	96.1%	96.4%	96.8%	96.5%
	Antisense DNA	94.0%	94.3%	94.7%	94.3%	95.1%
	Combined	94.9%	95.2%	95.6%	95.5%	95.8%
Average Primer On Target Rate	Sense DNA	84.5%	85.6%	84.4%	83.9%	84.4%
	Antisense DNA	90.8%	90.6%	91.1%	90.5%	89.6%
	Combined	87.7%	88.1%	87.7%	87.2%	87.0%
Reference Mutations Detected Across 3 Replicates		**100%**	**100%**	**90.2%**	**100%**	**94.0%**

Reference material from Horizon Discovery was used to assess sensitivity. Using 0.5ng or 1.0ng of a reference DNA material containing 5% allele frequency mutations, 100% of mutations were found. With 1.0ng of a reference DNA material containing 1.3% allele frequency mutations 90% were identified, this increase to 100% when using 5.0ng of DNA. Using 20ng of DNA we were able to identify 94% of mutations of a 0.13% reference material, generated by diluting the 1.3% material 1:10 in wild type DNA.

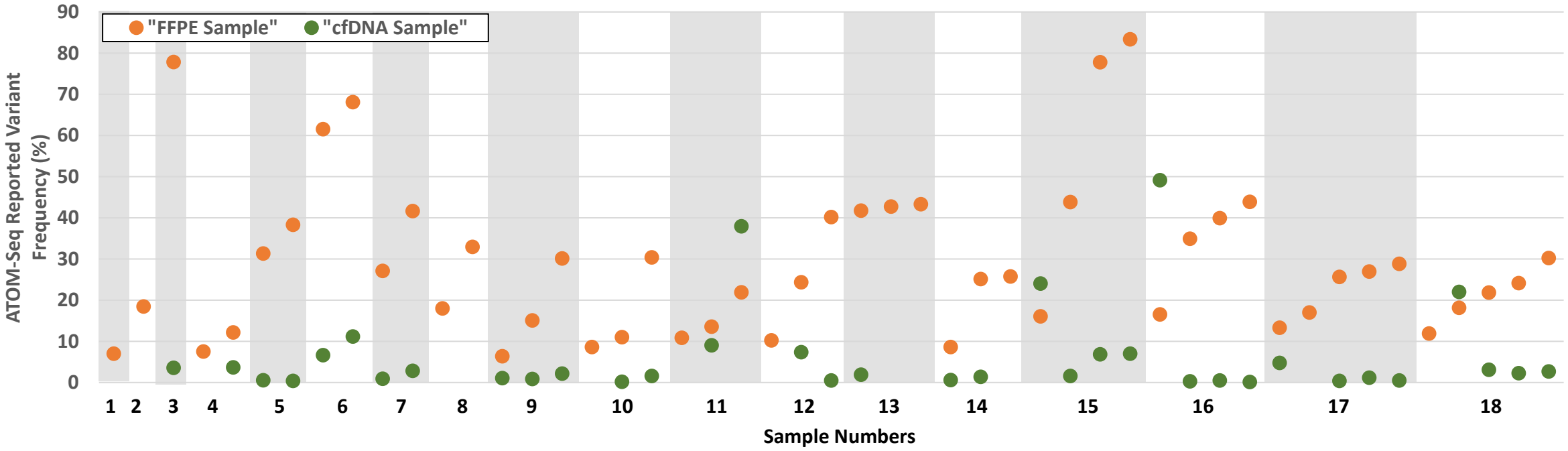
XCeloSeq Pan Cancer Kit Protocol: Representative Data

Replicate	Variant Depth	Total Variants	True Positives Detected (n=149)	True Positive Specificity	True Positive Sensitivity
1	≥3	153	146	96.1%	98.0%
2	≥3	153	147	96.1%	98.7%
3	≥3	149	148	99.3%	99.3%
Average		151	147	97.2%	98.7%



The sensitivity and specificity of the ATOM-Seq protocol was examined with a semi-blind approach.
We approximated the 'ground truth' by combining the three reference material replicates generated for 5 ng of starting material with 1.3% AF. With these combined samples we identified all variants which were identified with a count ≥3, this generated a list with between 149-152 variants across a total of 575 target regions per sample. Of these, a total of 149 were identified as 'true positives', those variants present in 2 or 3 of the 3 samples. As summarised in the table, we found that true positive specificity was between 96.1-99.3% (average 97.2%) and true positive sensitivity was between 98.0-99.3% (average 98.7%)

XCeloSeq Pan Cancer Kit Protocol: Representative Data



A pilot clinical study of 20 paired lung cancer FFPE and cfDNA samples was done to validate the Pan Cancer Kit. Mutations were found in 18 of the paired samples. A total of 49 mutations were identified in the FFPE samples and of these 37 concordant mutations were identified in a paired cfDNA sample.

The allele frequency of the concordant mutations ranged between 0.13-49.1% for cfDNA and 6.38-83.3% for FFPE samples. All 20 FFPE samples were previously sequenced using an alternative NGS technology. In genomic regions covered by both the ATOM-Seq panel and the previously used gene panels, all but 2 FFPE mutations were confirmed. A total of three of the cfDNA samples were also previously sequenced with only two concordant mutations detected, compared to eight concordant mutations detected using ATOM-Seq (samples 4, 9, and 18).