A Demonstration of the AVITI Element Sequencer and the NimaGen OmniSTR kit

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0-60
IN 11 MINUTES
ANTIQUE AUTO
56555
NORTH CAROLINA

279mi	155mph	3.5s
Range (EPA est.)	Top Speed	0-60 mph

Why ask a new question?

- A first demonstration of the combination of two NEW technologies: 1) reverse complement PCR used in the OmniSTR kit, and 2) Polony sequencing used in the AVITI Element.

- This is a first look at the combination of these two technologies
- These are NOT optimized data and a first run of the two together
- This is a peek over the horizon?

Element AVITI™ FIT

Empowering More Researchers with Flexible Genomic Solutions

AVITI

Unparalleled performance with throughput from 100 million to 2 billion reads

AVITI LT

Lower barrier to entry with access to industry-leading accuracy

AVITI LT

Cloudbreak™ Flow Cells Enable Any Application at Any Scale

Scale sample batching, extend read length, or run targeted panels

	Low	Medium	High
2 x 75		500M reads $880	1B reads $1,080
2 x 150	250M reads $880	500M reads $1,080	1B reads $1,680
2 x 300		100M reads $1,680	300M reads $2,440

Compatible with AVITI System

Compatible with AVITI System LT

- A **Phred quality score** is a measure of the quality of the identification of the nucleobases generated by automated DNA sequencing
- $Q = -10\log_{10}(\text{Probability of error})$ (base call being wrong)
- The HIGHER the Q scores indicate smaller probability of error

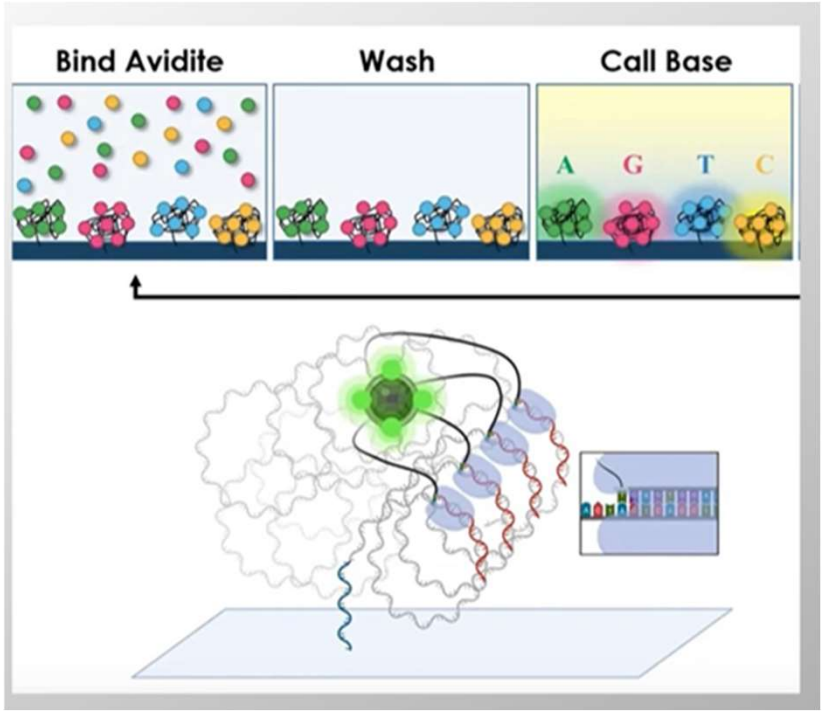
Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

Bode Technology Utilizing AVITI for FIGG

- **Accredited to ISO 17025 standards**
  - Operational in casework for WGS/FIGG
- High-quality data capable of achieving an unprecedented >90% Q30 data quality on 2 X 150 base pair runs
  - Routinely see Q-scores >Q40 (99.99% accuracy) and **pushing Q50**
- Amenable to the wide variety of library prep kits commercially available
- Overcomes high DI values and low template amounts
- Instrument cost and maintenance lower than other WGS platforms
  - Two parallel runs or independent operation with multiplex capabilities

BODE TECHNOLOGY

Courtesy of Bode Technology



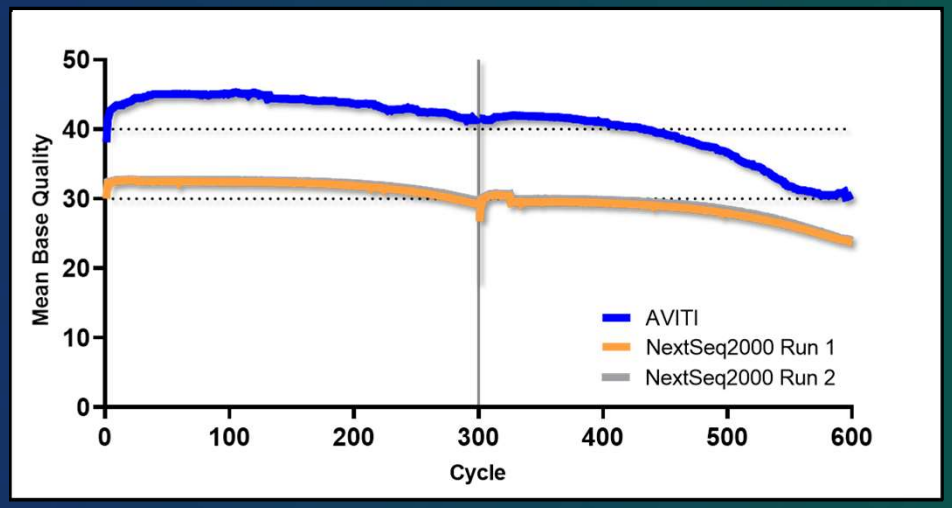
One of the main benefits is reduced requirements

Arslan, S., Garcia, F. J., Guo, M., Kellinger, M. W., Kruglyak, S., LeVieux, J. A., ... & Previte, M. (2024). Sequencing by avidity enables high accuracy with low reagent consumption. *Nature biotechnology*, *42*(1), 132-138.

AVITI 2 x 300 Kit Provides Leading Data Quality

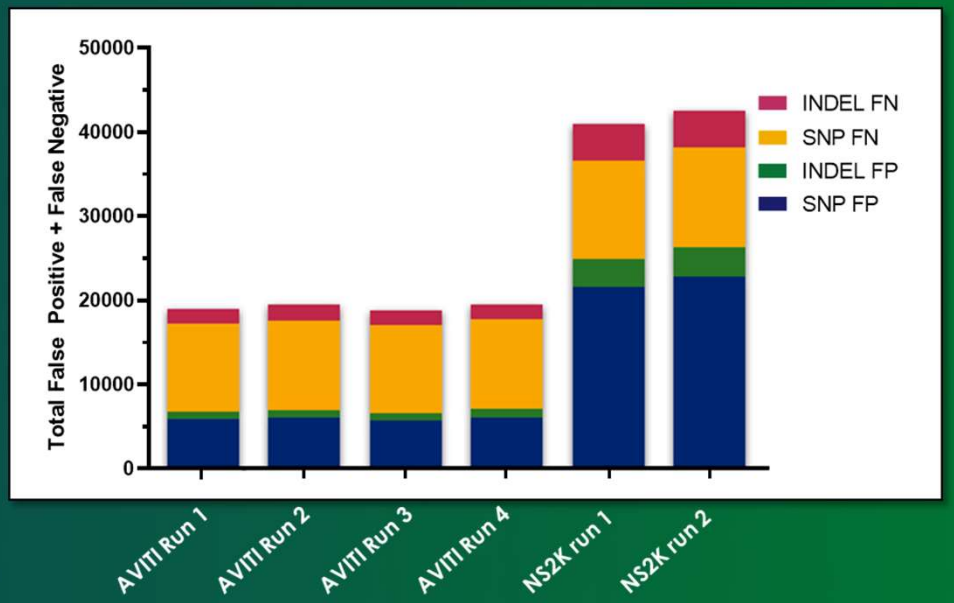
Measuring quality and accuracy: Element Cloudbreak 2 x 300, Illumina NextSeq 2000 600-cycle

End-to-end Read Quality



Superior Q-values throughout the length of reads with a significant increase in base call accuracy

Variant-calling Accuracy



Greater than 50% reduction FP/FN rate with significantly higher SNP accuracy

PCR-free Elevate and Illumina fragment libraries for HG001 human control DNA generated using standard protocols. Elevate library sequenced on four separate runs at Element Biosciences; Illumina library sequenced twice via service provider. QC metrics from Illumina runs passed or exceeded specifications. Q values processed using GATK BaseRecalibrator and mean value across replicates plotted across paired reads for both platforms. Data from both platforms aligned and variant-calling performed using Google DeepVariant pipeline. Total errors plotted and binned by error type.

Newer Technologies are Reducing Costs

Costs Per Casework Samples

Parameter	Kit	
	MiSeq 600 v3	**Element**
Samples/Run	32	96
Flow Cell	$1,720	$2,400
OmniSTR kit and Plate	$3,325	$3,325
Cost/Sample	**$158**	**$60**

Table 1 Maximum number of libraries

Primer Mix	Sample Type	MiSeq FGx Reagent Micro Kit	MiSeq FGx Reagent Kit
DPMA	Database or reference	36	96
	Casework	12	32
DPMB	Database or reference	12	32
	Casework	12	32

More Capacity Means Better Detection of Low-Level Contributors

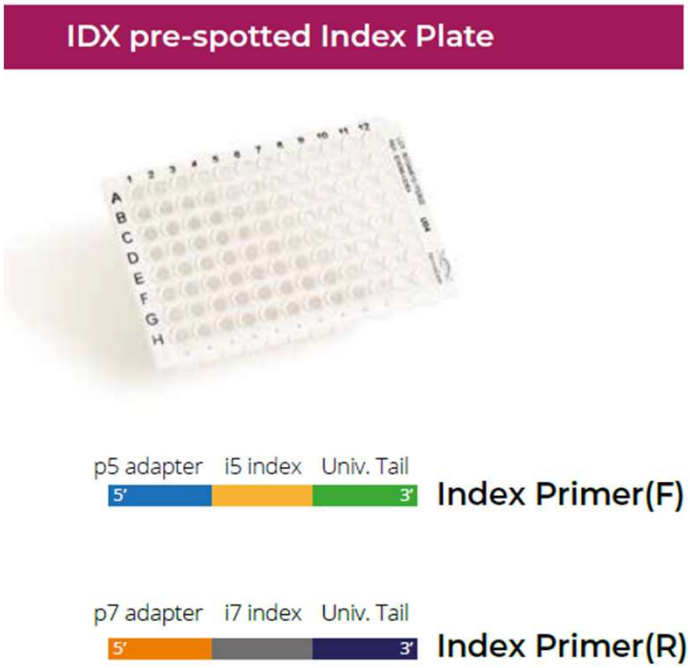
Parameter	Expected Number of Reads	
	MiSeq	Element
Flow Cell Capacity	25,000,000	100,000,000
Samples/Run	96	96
Reads/Sample	**260,417**	**1,041,667**
Markers/Kit	30	30
Reads/Sample/Marker	**8,681**	**34,722**
Alleles/Marker	2	2
Reads/Allele (NOC=1)	**4,340**	**17,361**
NOC/Sample	2	2
Reads/Contributor/ Allele	2,170	8,681
Lowest Locus As a Pct. Of Mean Reads	0.5	0.5
Reads per Lowest Allele	**1,085**	**4,340**
Contribution Ratio	10:1	10:1
Reads/Lowest Locus in Lowest Contributor	**217**	**868**
Verogen Recommended Minimum Thresholds	**650 Reads/Locus 1.5% of Reads/Allele (10 Reads)**	

IDseek® MPS Library Prep by RC-PCR: How does it work?

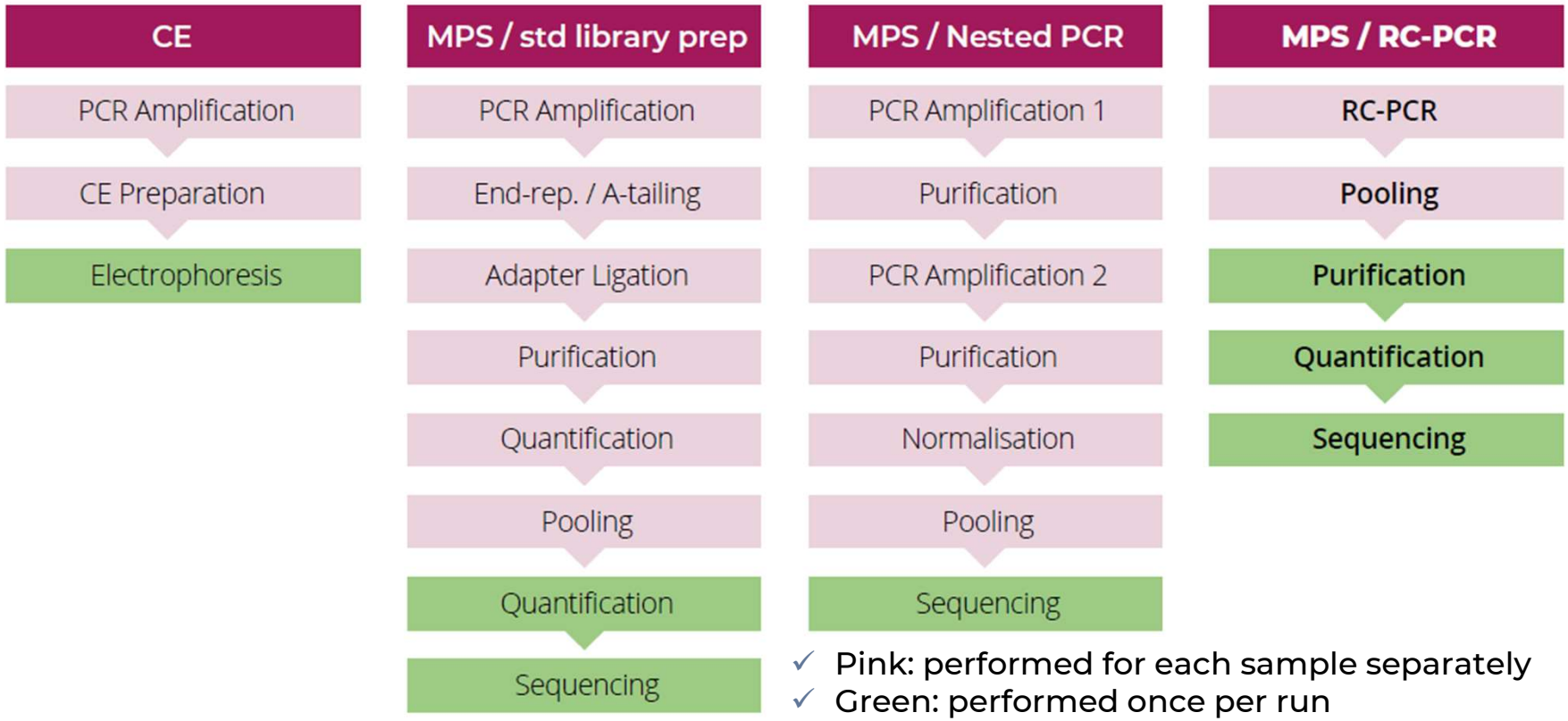
NimaGen.

- Reverse complement PCR GREATLY reduces the amount of labor involved with library preparation
- All reagents come in a 96 well plate (can be separated apart for less samples processed) IT MAKES IT OWN PRIMERS AND LIBRARY ALL ON THE PLATE.
- The kit is better balanced than previous kits.
- Sensitive: 100% of markers called down to 60pg DNA.
- Marker balance optimized for 1ng with limited impacts on low inputs down to 60pg.
- High on-target read percentage, even with low input, reducing sequencing costs.
- Robust and inhibitor tolerant, tested for humic acid, tannic acid, indigo, hematin and bacterial DNA.

Inter Locus Balance As Measured by Kullback-Leibler Divergence From an Ideal Balance



Comparing MPS workflows versus CE



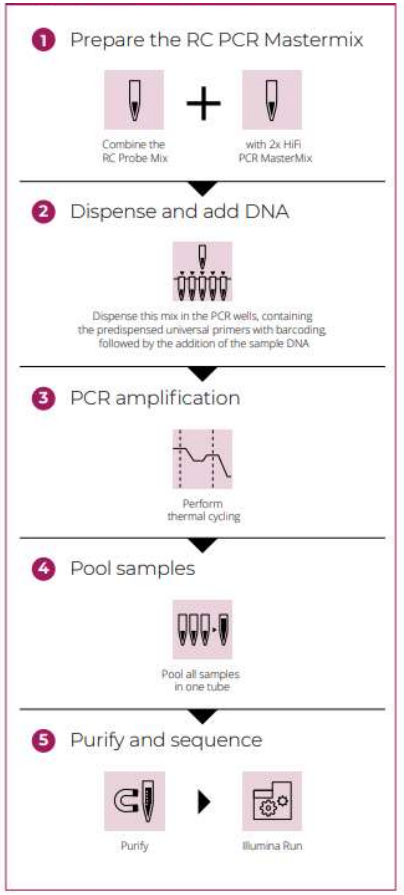
- ✓ Pink: performed for each sample separately
- ✓ Green: performed once per run

IDseek® Multiplex STR Kits for MPS

Powered by Reverse Complement PCR

RC-PCR based MPS library prep with its single reaction, closed-tube workflow adds:

- ✓ Reduced hands-on time
- ✓ Minimized risk of sample swapping, PCR contamination and pipetting errors
- ✓ Reliable results, safer and easier
- ✓ Unique Dual Indexing in the very first step of the library prep (Early Indexing)
- ✓ High sensitivity and specificity due to the specific reaction kinetics of RC-PCR



Proof of Principle Testing

Experimental Design

- Samples were prepared at NimaGen (Nijmegen, Netherlands). A custom library preparation method was used to incorporate AVITI Element barcodes.
- Sequencing was performed at VIB Nucleomics Core (Leuven, Belgium).
- Data analysis was performed at NicheVision (Akron, Ohio). AVITI Element barcodes were trimmed, and analysis was performed using MixtureAce software.

Samples Used in Proof of Principle Testing			
Code	**Sample**	**Description**	**Source**
A	NA12877	Female	1000G/CEPH
B	NA12878	Female	1000G/CEPH
C	NA24631	Male	NIGMS
D	2800M	Male	Promega

	Samples in Triplicate			NTC	Total Samples
	Single Source	**3:1 mixture**	**10:1 mixture**		
	A	A:B	A:B		
	B	B:C	B:C		
	C	D:B	D:B		
	D				
Total	**12**	**9**	**9**	**1**	**31**

Mix BC 10:1 (NA12878:NA24631)
LOD = 3%; 325 reads/locus

Allele from Minor Contributor