#### UNT HEALTH Science center

Moving Implementation Mountains: Experiencing the Forensic Laboratory NGS and Bioinformatics Workflow Through Simulation

ISHI 2016 Workshop

#### UNT HEALTH Science center

# An Introduction to the Ion S<sup>5TM</sup> and Ion Chef<sup>TM</sup> Technologies

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Department of Molecular and Medical Genetics Institute of Applied Genetics University of North Texas Health Science Center

#### ADVANCEMENTS AND ADOPTION OF THE TECHNOLOGY



# Advancements and Adoption of the Technology

Validation of the Ion Torrent PGM sequencing for the prospective routine molecular diagnostic of colorectal cancer

Francesca Belardinilli <sup>a</sup>, Carlo Capalbo <sup>a</sup>, Amelia Buffone <sup>a</sup>, Marialaura Petroni <sup>a</sup>, Valeria Colicchia <sup>a</sup>, Sergio Ferraro <sup>a</sup>, Massimo Zani <sup>a</sup>, Arianna Nicolussi <sup>b</sup>, Sonia D'Inzeo <sup>b</sup>, Anna Coppa <sup>b</sup>, Isabella Screpanti <sup>a</sup>, Alberto Gulino <sup>a</sup>, Giuseppe Giannini <sup>a,\*</sup>

<sup>a</sup> Department of Molecular Medicine, University La Sapienza, V.le R. Elena, 291, 00161 Rome, Italy
<sup>b</sup> Department of Experimental Medicine, University La Sapienza, V.le R. Elena, 324, 00161 Rome, Italy

#### A Next Generation Semiconductor Based Sequencing Approach for the Identification of Meat Species in DNA Mixtures

Francesca Bertolini<sup>1</sup>, Marco Ciro Ghionda<sup>1,2</sup>, Enrico D'Alessandro<sup>2</sup>, Claudia Geraci<sup>1</sup>, Vincenzo Chiofalo<sup>2,3</sup>, Luca Fontanesi<sup>1</sup>\*

1 Department of Agricultural and Food Sciences, Division of Animal Sciences, University of Bologna, Viale Fanin 46, 40127, Bologna, Italy, 2 Department of Veterinary Sciences, Animal Production Unit, University of Messina, Polo Universitario dell'Annunziata, 98168, Messina, Italy, 3 Meat Research Consortium, Polo Universitario dell'Annunziata, 98168, Messina, Italy

#### A Method for Amplicon Deep Sequencing of Drug Resistance Genes in *Plasmodium falciparum* Clinical Isolates from India

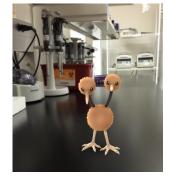
Pavitra N. Rao,<sup>a</sup> Swapna Uplekar,<sup>a</sup> Sriti Kayal,<sup>b</sup> Prashant K. Mallick,<sup>c</sup> Nabamita Bandyopadhyay,<sup>c</sup> Sonal Kale,<sup>c</sup> Om P. Singh,<sup>c</sup> Akshaya Mohanty,<sup>d</sup> Sanjib Mohanty,<sup>e</sup> Samuel C. Wassmer,<sup>f\*</sup> Jane M. Carlton<sup>a</sup>

Center for Genomics and Systems Biology, Department of Biology, New York University, New York, New York, USA\*, National Institute of Technology, Raurkela, Odisha, India<sup>15</sup>, National Institute of Malaria Research, Indian Council of Medical Research, Dwarka, New Delhi, India<sup>5</sup>, Institute of Life Sciences, Bhubaneswar, Odisha, India<sup>4</sup>, Ispat General Hospital, Raurkela, Odisha, India<sup>6</sup>, Department of Microbiology, Division of Parasitology, New York University School of Medicine, New York, New York, USA<sup>4</sup>

#### The complete mitochondrial genome of the *Anabas testudineus* (Perciformes, Anabantidae) and its comparison with other related fish species

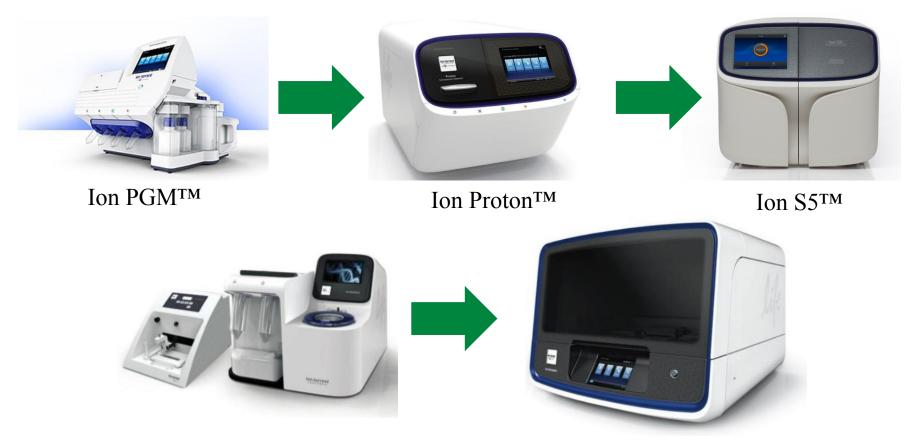
Bijay Kumar Behera 💌 Vishwamitra Singh Baisvar, Kavita Kumari, Ajaya Kumar Rout, Sudip Pakrashi, Prasenjet Paria, A. R. Rao & Anil Rai







# Advancements and Adoption of the Technology



Ion OneTouch<sup>TM</sup> 2 and Ion OneTouch<sup>TM</sup> ES

Ion Chef<sup>TM</sup>

# Advancements and Adoption of the Technology

- Available Panels:
  - Precision ID Ancestry Panel
  - Precision ID Identity Panel
  - Precision ID mtDNA Whole Genome Panel
  - Precision ID mtDNA Control Region Panel
  - Precision ID GlobalFiler NGS STR Panel



# Ion Torrent MPS Technologies

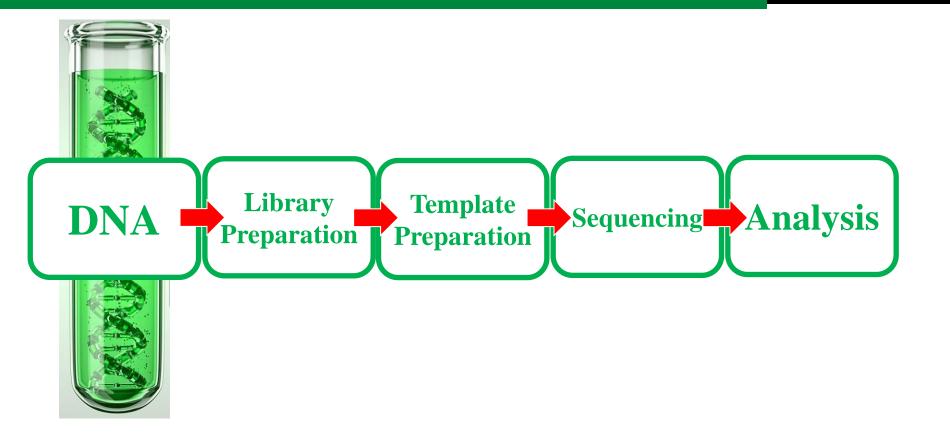
- Increasingly user-friendly
- Highly automated workflow
- Compatible with numerous genetic marker types
- Available data analysis options
- Accurate, reliable data



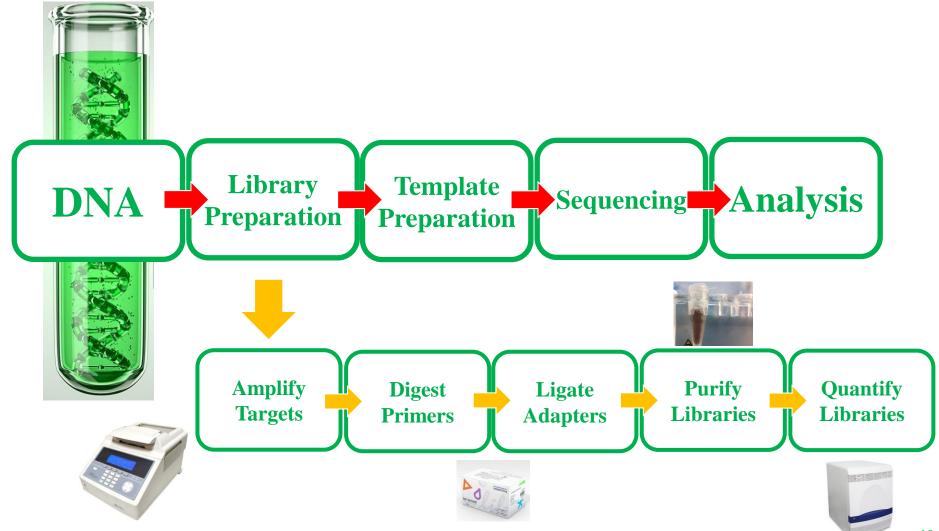
#### **ION TORRENT MPS WORKFLOW**



#### General MPS Workflow



### Library Preparation on the Ion Chef<sup>TM</sup>



### Library Preparation on the Ion Chef<sup>TM</sup>

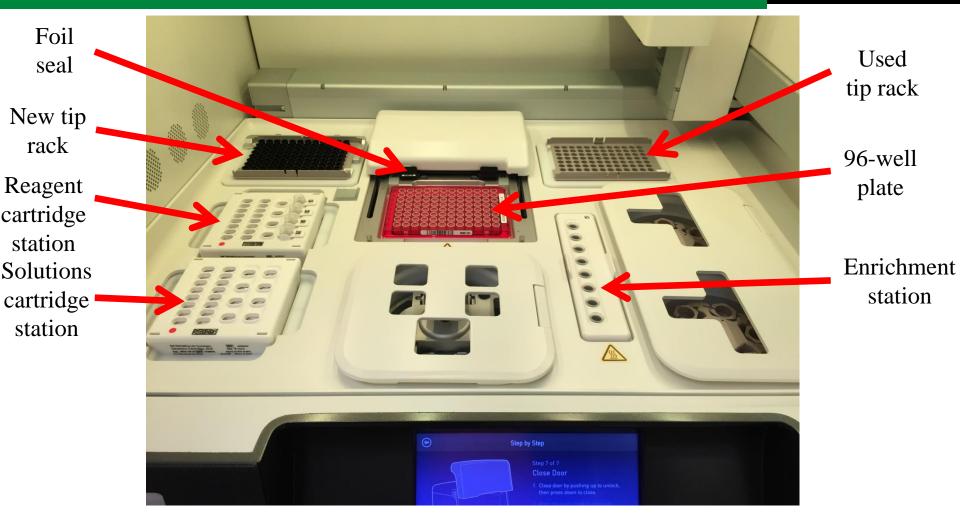


○ Ion Chef<sup>TM</sup> automates the library preparation process

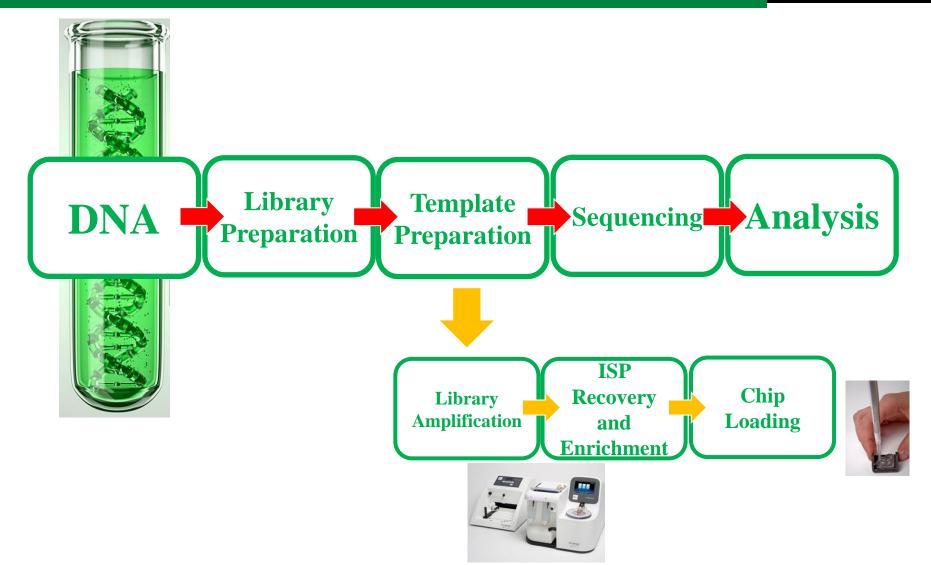


- Hours of hands-on steps Two pipetting steps
- Minimizes user-introduced variability
  - Can work on other steps while Chef is running

#### Library Preparation on the Ion Chef<sup>TM</sup>



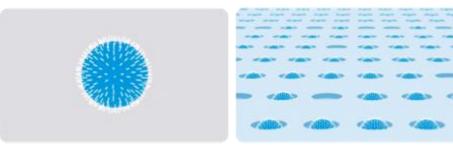
### Template Preparation on the Ion Chef<sup>TM</sup>



#### Ion Torrent Chemistry

#### **Clonal Amplification**

#### Load chip



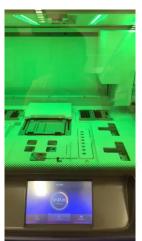
PCR is performed on gDNA, and each fragment (amplicon) is attached to its own bead

The fragment is copied until it covers the bead

This automated process produces millions of beads covered with millions of different fragments The beads are then flowed across the chip, each being deposited into a well

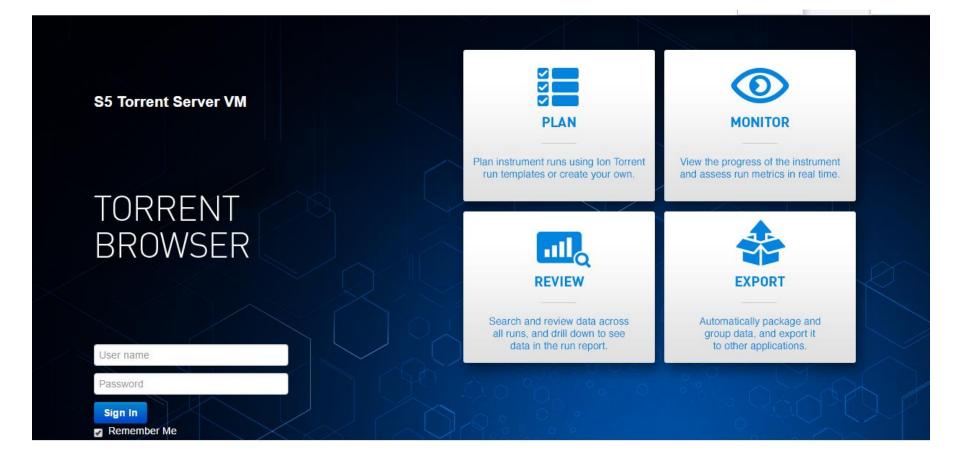
### Template Preparation on the Ion Chef<sup>TM</sup>





- $\circ~$  Ion Chef<sup>TM</sup> automates the template preparation process
- Hours of hands-on steps One pipetting step
- Multiple instruments > One instrument
- Minimizes user-introduced variability

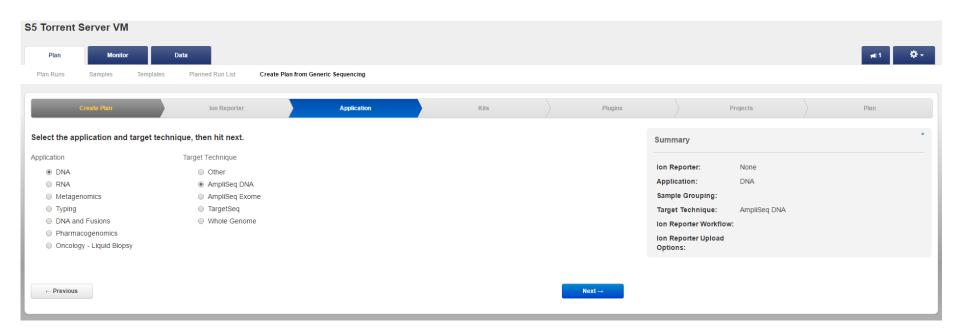






S5 Torrent Server VM				
Plan Monitor Data				rei1 🔅 -
Plan Runs Samples Templates Planned Run	In List Create Plan from Generic Sequencing			
Create Plan Ion F	Reporter Application	Cits Plugins	Projects	Plan
Select an Ion Reporter account, workflow and samp	ble grouping, then hit next.	C Refresh Configure	Summary	×
Ion Reporter Account	Sample Grouping		Ion Reporter: None	
None	<ul> <li>Sample_Control</li> <li>Self</li> <li>Tumor_Normal</li> <li>Trio</li> <li>Other</li> <li>DNA and Fusions</li> </ul>		Application:     DNA       Sample Grouping:     Other       Target Technique:     Other       Ion Reporter Workflow:     Ion Reporter Upload       Octional:     Ion Reporter Upload	
1	Single Fusions		Options:	







S5 Torrent Server VM				
Plan Monitor Data				<b>a</b> ∎1 🗳 -
Plan Runs Samples Templates Planned Run List Create Plan from A	mpliSeq DNA			
Create Plan Ion Reporter	Application Kits	Plugins	P	Projects Plan
Select instrument, chip and kits and then hit next.			Summary	×
Instrument :	Chip Type (required) :			
Ion S5™ System ▼	Ion 530 <sup>TM</sup> Chip		Ion Reporter:	None
			Application:	DNA
Sample Preparation Kit (optional) :	Control Sequence (optional) :		Sample Grouping:	
T	×		Target Technique:	AmpliSeq DNA
Library Kit Type Details + :	Barcode Set (optional) :		Ion Reporter Workflow	
Ion AmpliSeq Kit for Chef DL8	IonCode Barcodes 1-32 •		lon Reporter Upload Options:	
Template Kit O OneTouch I IonChef:			Sample Preparation	
Ion 520/530 Kit-Chef 🔹			Library Kit Type:	Ion AmpliSeq Kit for Chef DL8
Read Length:  200 400			Library Key:	Ion TCAG (TCAG)
Sequencing Kit :	Flows :		Template Kit:	Ion 520/530 Kit-Chef
Ion S5 Sequencing Kit	500 \$		Templating Size:	
Base Calibration Mode :	🕄 Mark as Duplicates Reads 📄 :		Sequencing Kit:	Ion S5 Sequencing Kit
Default Calibration	0 Enable Realignment 📃 :		Library Read Length:	200
			Flows:	500
			Control Sequence:	
← Previous		Next→	Chip Type:	Ion 530™ Chip
← Pievious		Next→	3' Adapter:	Ion P1B (ATCACCGACTGCCCATAGAGAGGCTGAGAC)
			Flow Order:	None
			Barcode:	IonCode Barcodes 1-32
			Mark as Duplicate Reads:	False



S5 Torrent Server VM							
Plan Monitor	Data					1 <b>1</b>	۰.
Plan Runs Samples Temp	plates Planned Run List Create Plan from AmpliSeq DNA						
Create Plan	Ion Reporter Application	h Kits	Plugins		Projects	Plan	
Select plugins to execute, then o	click Next.		Select All Clear	Summary			×
ampliSeqRNA	FileExporter	RNASeqAnalysis					
AssemblerSPAdes	FilterDuplicates	RunTransfer		Ion Reporter:	None		
coverageAnalysis Configure	HIDGenotyper	sampleID		Application:	DNA		
DataExport	HID_SNP_Genotyper	variantCaller Configure		Sample Grouping:			
ERCC_Analysis	PGxAnalysis			Target Technique:	AmpliSeq DNA		
				Ion Reporter Workflow	<i>N</i> :		
← Previous			Next→	lon Reporter Upload Options:			
				Sample Preparation Kit:			
				Library Kit Type:	Ion AmpliSeq Kit for Chef DL8		
				Library Key:	Ion TCAG (TCAG)		
				Template Kit:	Ion 520/530 Kit-Chef		
				Templating Size:			
				Sequencing Kit:	Ion S5 Sequencing Kit		



S5 Torrent Server VM						
Plan Monitor Data						e∎1 🗘 +
Plan Runs Samples Templates Planned Run List Create Plan from Gen	eric Sequencing					
Create Plan Ion Reporter	Application	Kits	Plugins		Projects	Plan
Select the project(s) that will receive data from runs planned in this template,	then hit next.			Summary		×
Mito_Panel raptor_test				lon Reporter:	None	
S5_Alpha_Test				Application:	DNA	
				Sample Grouping:		
				Target Technique:	AmpliSeq DNA	
				Ion Reporter Workflow	v:	
Search Add Project				lon Reporter Upload Options:		
				Sample Preparation Kit:		



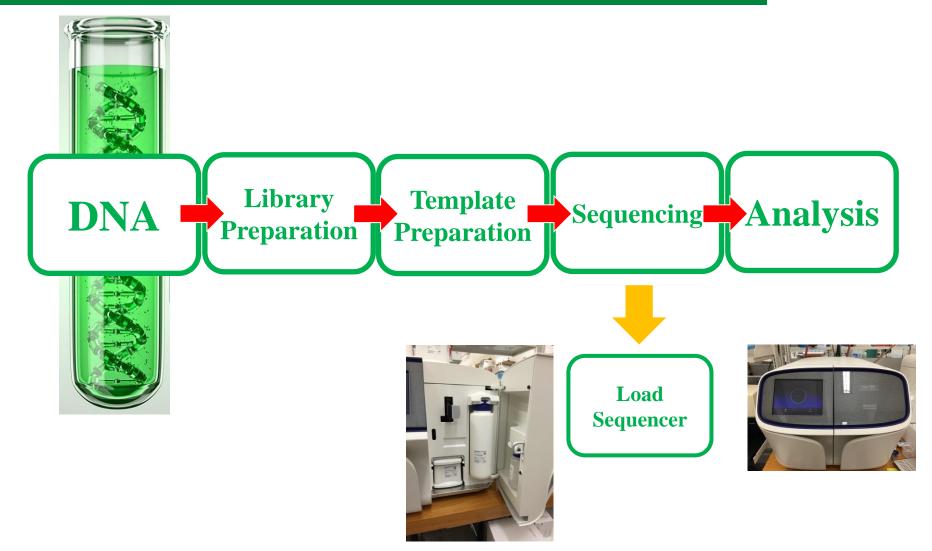
S5 Tori	rent Server VM						, , ,
Pla	n Monitor	Data					at Ø-
Plan Ru	ns Samples Te	emplates Planned Run List Create Plan from Am	pliSeq DNA				
	Create Plan	Ion Reporter	Application	Kits	Plugins	P	rojects Plan
Run Pla	n Name (required) :					Summary	×
JDC_9-2	e-2016_mtDNA						
Analysis	Parameters:					Ion Reporter:	None
Analysis	Default	(Recommended) Custom Details +				Application:	DNA
Defau	It Reference & BED Fil	es			-	Sample Grouping:	
Refer	ence Library :					Target Technique:	AmpliSeq DNA
		RS_plus_80(Extended rCRS for Mito Part				Ion Reporter Workflow: Ion Reporter Upload	
	rci	RS_plus_80nts_mt_targets.bed				Options: Sample Preparation	
Hots	rCF	RS_plus_80nts_mt_hot_spot_v2.bed •				Kit:	
- 11	se same reference & BED					Library Kit Type:	Ion AmpliSeq Kit for Chef DL8
<b>v</b> 0	se same reference & BED	nies for all barcodes				Library Key:	Ion TCAG (TCAG)
						Template Kit:	Ion 520/530 Kit-Chef
Number	of barcodes :	8		Save Samples Table Load Samp	les Table	Templating Size:	
Sample	Tube Label :	A00123456				Sequencing Kit:	Ion S5 Sequencing Kit
Chip ID	:					Library Read Length:	200
- i - i						Flows:	500
Enter a s	sample name for each bar	code used (require at least one sample) 🗍 🛍	•			Control Sequence:	lon 530™ Chip
						Chip Type: 3' Adapter:	Ion 550*** Chip Ion P1B (ATCACCGACTGCCCATAGAGAGGCTGAGAC)
#	Barcode	Sample (required)	Sample Description	Sample ID		Flow Order:	
1	IonCode_0101 (CTAAGGTAAC	)  v Sample 1	you		<b>^</b>	Barcode:	IonCode Barcodes 1-32
2	IonCode_0102 (TAAGGAGAAC	c) v Sample 2	me			Mark as Duplicate	False
3	IonCode_0103 (AAGAGGATTC	) V Sample 3	him			Reads:	
4	IonCode_0104 (TACCAAGATC	) The sample 4	her			Bead Loading (%):	30
	IonCode_0105 (CAGAAGGAA		that other guy			Key Signal (1-100):	30
						Usable Sequence (%):	
	IonCode_0106 (CTGCAAGTTC		her too			Reference Library:	rCRS_plus_80(Extended rCRS for Mito Panel)
7	IonCode_0107 (TTCGTGATTC	) The sample 7	:)			Target Regions:	rCRS_plus_80nts_mt_targets.bed



#### Template Preparation on the Ion Chef<sup>TM</sup>



# Massively Parallel Sequencing on the Ion S5<sup>TM</sup>



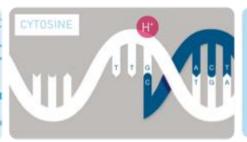
### Ion Torrent Chemistry

#### Load chip

The beads are then flowed across the chip, each being deposited into a well

Then the chip is flooded with one of the four nucleotides

#### Incorporate nucleotide



Whenever a nucleotide is incorporated into a single strand of DNA, a hydrogen ion is released changing the pH of the surrounding solution

#### Detect and call



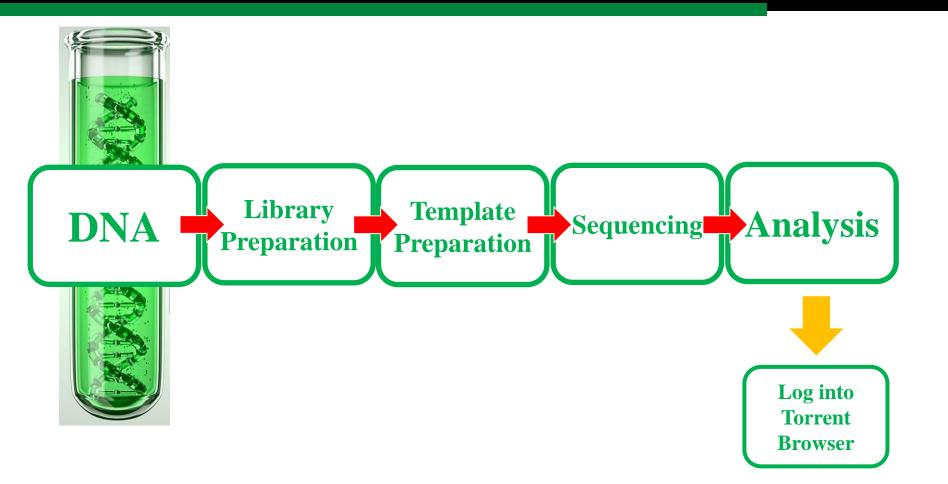
Ion semiconductor sequencing chip is able to detect this change in pH and convert it directly to base calls

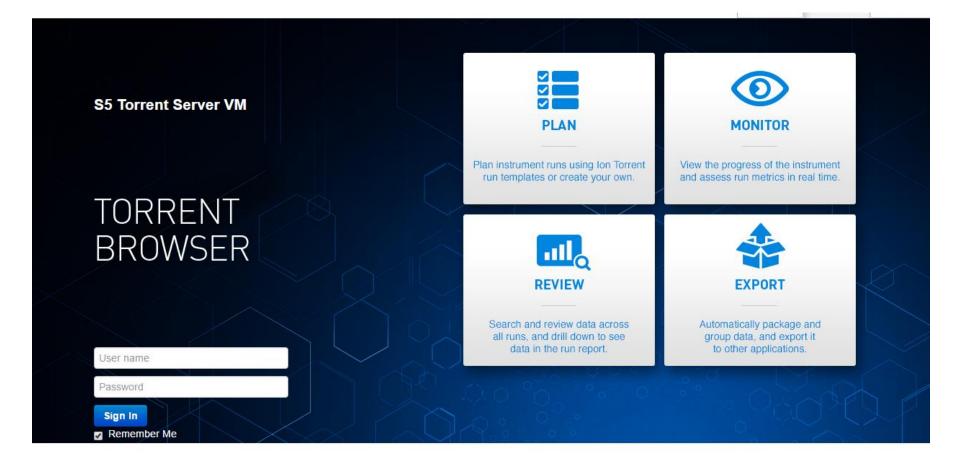
# Massively Parallel Sequencing on the Ion S5<sup>TM</sup>





- Thaw reagents
- Empty waste container
- Load reagents
- Initialize instrument
- Load chip
- Start sequencing run







Torrent Server	Hi, ionadmin Help 👻 Sign Out
Plan Monitor Data	rel 3 🗘 ★
Completed Runs & Results Projects Data Management	
Completed Runs & Results List View   Table View	Page is static until refreshed Auto Update
Run Name Sample Sample Set App. Run Analysis Status Chip Report Name Reference Barcode Sample Tube Flows Total Real	uds Mean Read Q20 Bases <b>Output</b> Len.

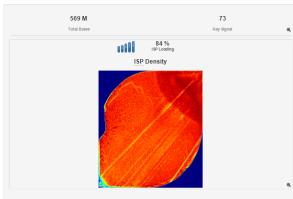


#### Run Summary: R\_2015\_04\_08\_08\_42\_13\_user\_UNT-83-JC\_2-9-2016\_MiniMito\_PopRun\_AFRplate1

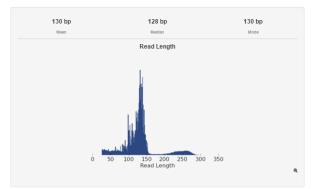
#### Reports : Auto\_user\_UNT-83-JC\_2-9-2016\_MiniMito\_PopRun\_AFRplate1\_188 (162)

\*

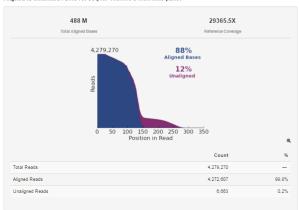
#### Read Summary: Unaligned

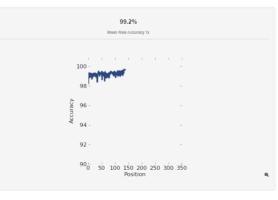






#### Aligned to extended rCRS ref seq for Thermo's mini mito panel





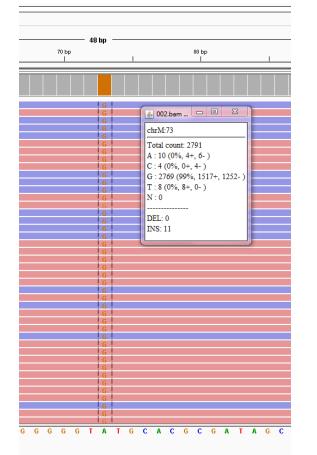
	456 M		
	AQ17 Total Bases		
	Alignment Quality		
	AQ17	AQ20	Perfect
Fotal Number of Bases [bp]	456 M	416 M	346 M
Mean Length [bp]	113	108	94
ongest Alignment [bp]	328	328	315
Mean Coverage Depth [x]	27427.1	25014.2	20839.6

#### **Output Files**

File Type				Unaligned Read	ds		Aligned Reads		
Library				BAM			BAM BAI		
Barcode Name	Sample	Bases	>=Q20 Bases	Reads	Mean Read Length	Read Length Histogram		Files	
No barcode	None	11,517,965	10,105,088	94,385	122 bp	ó 50 100 10 200 250 300		UBAM BAM BAI	
IonXpress_001	Sample 1	16,893,928	15,285,674	128,744	131 bp	0 50 100 50 200 250 300		UBAM BAM BAI	
IonXpress_002	Sample 2	3,014,982	2,766,875	25,581	118 bp	0 50 100 150 200 250 300		UBAM BAM BAI	
IonXpress_003	Sample 3	18,332,884	16,820,335	143,031	128 bp	0 50 100 50 200 250 300		UBAM BAM BAI	
IonXpress_004	Sample 4	24,395,902	22,336,498	197,626	123 bp	0 50 100 50 200 250 300		UBAM BAM BAI	
IonXpress_005	Sample 5	19,632,779	17,886,823	156,661	125 bp	0 50 100 50 200 250 300		UBAM BAM BAI	
IonXpress_006	Sample 6	14,823,074	13,545,588	115,761	128 bp	0 50 100 50 200 250 300		UBAM BAM BAI	
IonXpress_007	Sample 7	25,286,219	23,066,993	189,657	133 bp	0 50 100 50 200 250 300		UBAM BAM BAI	
IonXpress_008	Sample 8	23,946,517	21,841,092	188,694	127 bp	0 50 100 50 200 250 300		UBAM BAM BAI	
IonXpress_009	Sample 9	30,914,275	28,035,367	226,825	136 bp	0 50 100 150 200 250 300		UBAM BAM BAI	
	10	* items per	ir page						1 - 10 of 28 items



File Genomes View Tr rCRS_plus80nts.fasta	racks Regions Tools	GenomeSpace Help	Go	< ► @ 🛙 X	<b>P</b>			
	db	2 kb 4 kl	b 64b			12 kb	14 kb	16 kb
002.bam Coverage								





#### variantCaller (v4.6.0.7) variantCaller.html

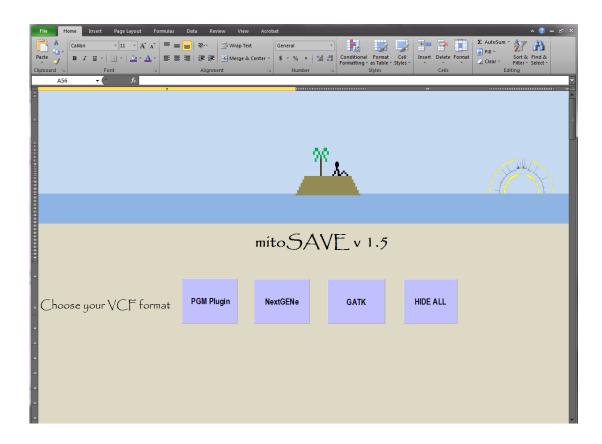
Output Directory: variantCaller_out.56 Download all barcodes: VCF.ZIP XLS.Z	eneric - PGM - Germ Line - Low Stringency 4	targeted regions matching those specified above	/e
Barcode Name	Sample Name	Variants	Download Links
IonXpress_001	Sample 1	39	VCF.GZ VCF.GZ.TBI XLS
IonXpress_002	Sample 2	241	VCF.GZ VCF.GZ.TBI XLS
lonXpress_003	Sample 3	60	VCF.GZ VCF.GZ,TBI XLS
IonXpress_004	Sample 4	41	VCF.GZ VCF.GZ.TBI XLS
IonXpress_005	Sample 5	54	VCF.GZ VCF.GZ.TBI XLS
IonXpress_006	Sample 6	58	VCF.GZ VCF.GZ.TBI XLS
IonXpress_007	Sample 7	58	VCF.GZ VCF.GZ.TBI XLS
IonXpress_008	Sample 8	48	VCF.GZ VCF.GZ.TBI XLS
IonXpress_009	Sample 9	82	VCF.GZ VCF.GZ.TBI XLS
IonXpress_010	Sample 10	42	VCF.GZ VCF.GZ.TBI XLS
IonXpress_011	Sample 11	96	VCF.GZ VCF.GZ.TBI XLS
IonXpress_012	Sample 12	86	VCF.GZ VCF.GZ.TBI XLS
IonXpress_013	Sample 13	51	VCF.GZ VCF.GZ.TBI XLS
IonXpress_014	Sample 14	32	VCF.GZ VCF.GZ.TBI XLS
lonXpress_015	Sample 15	64	VCF.GZ VCF.GZ.TBI XLS



Completed •

Variant Caller											
Plan Monitor	Data										0-
← Go back to Auto_user_UNT-83-JC_2	2-9-2016_MiniMito_PopRun_AFRplate1_188										
Run - R_2015_04_08_0	08_42_13_user_UNT-83-JC	C_2-9-2016_MiniMit	o_PopRun_AFRplate1								
Barcode	IonXpress_001				Марр	ed Reads	BAM BAI				
Sample Name	Sample 1				TVC-F	rocessed Reads	BAM BAI				
Reference Genome	rCRS_plus80nts				Varia	nt Calls	VCF.GZ VCF.GZ.TBI XLS				
Library Type	AmpliSeq				View	/ariant Calls in IGV	IGV				
Read trimming	Enabled					cated Features	Classic				
Targeted Regions	rCRS_plus_80_mtVlonv2	tamoto RED									
	None	_targets_BED			Ion Co	ommunity	Torrent Variant Caller documentation				
Hotspot Regions											
Effective Regions	rCRS_plus_80_mtVlonv2										
Parameter Settings	Custom based on Generic custom, TS version: 4.6	c - PGM - Germ Line - Low St	Parameters File								
Variant Caller Version	tvc 4.6-11 (0c0ef91)										
Chrom   Positio Total Cov ≥	on to A	Allele Name	Gene ID		Region Name	Allele Source	туре				
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chrM:73	Ref Variant A G C T	Allele Call Homozygous Homozygous	Frequency C 100.0 % 100.0 %	uality 6137.4 9428.5	Coverage Coverage 639 993	+ Coverage - 383 565	Allele Cov 278 428	Allele Cov + 639 993			0.600
chrM:73	A G	Homazygous	100.0 %	6137.4	639	363	276	639	Allele Cov - 363	Strand Bias	
chrMt73 chrMt150	A G C T	Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 %	8137.4 9428.5 9492.2 9491.8	839 003 094 099	363 665 548 544	278 428 448 455	639 993 994 999	Allele Cov - 363 565 548 544	278 428 448 455	0.500 0.500 0.500
chrM.73     chrM.150     chrM.189     chrM.200     chrM.263	A G C T A G A G A G	Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9428.5 9492.2 9491.8 0679.4	639 093 994 099 695	983 685 548 544 353	276 428 448 455 342	839 993 994 999 895	Allele Cov - 383 685 548 544 383	Strand Bias 276 428 440 455 342	0.500 0.500 0.500 0.500 0.500
chrM.73           chrM.150           chrM.189           chrM.200           chrM.201           chrM.211	A G C T A G A G A G - C	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9428.5 9492.2 9491.6 0679.4 3888.2	839 993 994 999 899 895 458	383 685 648 544 383 4	278 428 446 455 342 454	639 993 994 999 695 458	Allele Cov - 303 505 548 544 303 4	Strand Blas           278           428           448           455           342           454	0.500 0.500 0.500 0.500 0.500 0.500 0.500
chrik 73           chrik 150           chrik 150           chrik 200           chrik 200           chrik 311           chrik 750	A G C T A G A G A G - C A G	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9428.5 9492.2 9491.6 6679.4 3886.2 6839.3	639 093 094 099 695 495 723	383 685 644 383 4 340	278 428 448 455 342 454 454	639 993 994 999 695 458 723	Allele Cov - 303 865 548 544 353 4 340	Strand Bias           276           428           440           455           342           454           383	0.500 0.500 0.500 0.500 0.500 0.500 0.500
chrik73 chrik73 chrik150 chrik200 chrik200 chrik203 chrik311 chrik750 chrik750	A 0 C T A 0 A 0 A 0 - C A 0 A 0 A 0 A 0 A 0	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9428.5 9492.2 9491.8 0679.4 3886.2 0639.3 2117.9	639 063 064 066 665 455 723 245	983 865 848 844 953 4 340 138	278 428 446 445 942 424 838 107	839 993 994 909 895 458 723 238	Alleie Cov - 383 565 548 544 353 4 340 134	Strand Blas           278           428           449           455           342           454           383           102	0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500
<ul> <li>chrik 73</li> <li>chrik 73</li> <li>chrik 150</li> <li>chrik 203</li> <li>chrik 203</li> <li>chrik 203</li> <li>chrik 203</li> <li>chrik 131</li> <li>chrik 14750</li> <li>chrik 1438</li> <li>chrik 1438</li> <li>chrik 1352</li> </ul>	A G C T A G A G A G - C A G	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9428.5 9492.2 9491.6 8670.4 93896.2 8389.3 2117.9 13108.4	639 003 004 600 600 468 723 245 1.375	983 665 648 644 953 4 340 138 601	278 428 448 455 342 454 383 107 874	639 093 094 099 695 488 723 238 1,375	Alleie Cov - 363 565 548 544 353 4 340 134 501	Strand Bias           278           428           446           455           342           454           383           102           874	0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500
chrik73 chrik73 chrik150 chrik200 chrik200 chrik203 chrik311 chrik750 chrik750	A         G           C         T           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9428.5 9492.2 9491.8 0679.4 3886.2 0639.3 2117.9	639 063 064 066 665 455 723 245	983 865 848 844 953 4 340 138	278 428 446 445 942 424 838 107	839 993 994 909 895 458 723 238	Alleie Cov - 383 565 548 544 353 4 340 134	Strand Blas           278           428           449           455           342           454           383           102	0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500
drkt73 chrkt190 chrkt190 chrkt190 chrkt200 chrkt200 chrkt210 chrkt311 chrkt311 chrkt311 chrkt232 chrkt232 chrkt220 chrkt220 chrkt220 chrkt220	A         G           C         T           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9428.5 9492.2 9491.6 6679.4 38882.3 8839.3 2117.9 13108.4 117740.9	639 003 004 009 455 723 245 1.375 1.229	383 865 648 644 333 4 340 138 601 837	278 428 440 455 342 454 383 107 874 592	639 003 004 000 005 468 723 238 723 238 1.375 1.229	Attele Cov - 363 565 544 343 4 340 134 601 637	Strand Bias           278           428           449           455           342           454           383           102           874           692	0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500
<ul> <li>chrik 73</li> <li>chrik 73</li> <li>chrik 150</li> <li>chrik 200</li> <li>chrik 200</li> <li>chrik 201</li> <li>chrik 201</li> <li>chrik 750</li> </ul>	A         G           C         T           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 1000.0 % 1000.0 % 1000.0 % 1000.0 % 1000.0 % 000.0 % 1000.0 %	6137.4 9428.5 9422.2 9491.6 0679.4 3886.2 0839.3 2117.9 13108.4 11740.9 9708.8	639 043 044 055 458 723 245 1.375 1.229 1.012	983 865 848 844 353 4 340 138 801 837 858	278 428 445 342 455 342 454 383 107 874 454	639 993 994 999 995 458 723 230 1,375 1,229 1,012	Allete Cov - 383 565 548 544 353 4 340 134 501 637 558	Strand Bias           276           428           440           455           342           454           102           874           692           454	0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500
chrk/73           chrk/73           chrk/150           chrk/189           chrk/200           chrk/201	A         G           C         T           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	6137.4 9438.5 9449.2 9491.6 8679.4 3888.2 8839.3 2117.9 13108.4 11740.9 9778.8 9588.1	639 693 694 695 695 723 245 1.375 1.229 1.012 1.009	983 605 648 544 363 4 340 138 601 837 659 504	278 438 448 455 342 454 383 1077 874 692 454 695	639 993 999 699 695 455 723 236 1.375 1.229 1.012 1.009	Allete Cov - 383 605 648 544 363 4 363 4 340 134 601 637 639 504	Strang Bas           278           428           448           455           342           454           383           102           874           592           454           605	0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500 0.500
<ul> <li>chrk/73</li> <li>chrk/73</li> <li>chrk/150</li> <li>chrk/150</li> <li>chrk/200</li> <li>chrk/201</li> <li>chrk/201</li> <li>chrk/201</li> <li>chrk/201</li> <li>chrk/201</li> <li>chrk/138</li> <li>chrk/202</li> <li>chrk/4750</li> <li>chrk/4769</li> <li>chrk/4769</li> <li>chrk/4702</li> <li>chrk/7028</li> <li>chrk/7028</li> </ul>	A         G           C         T           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           C         T           C         T           A         G	Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous Homozygous	100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 % 100.0 %	61374 94285 94422 94476 66704 38862 06980 21170 131084 947058 96981 947058 96981 47557 06981 22248	639 603 604 605 458 723 245 1,375 1,229 1,012 1,000 611 731 285	983 565 544 353 4 340 138 501 637 655 504 207 398 27	278 438 445 55 342 454 454 454 454 655 454 454 505 244 335 238	639 699 699 699 695 458 723 238 1.375 1.229 1.012 1.009 611 731 285	Attele Cov -  363 565 568 568 544 363 4 340 134 501 637 658 504 207 368 27	Strang Bas           278           428           448           455           342           454           333           102           874           592           454           592           454           353           102           874           952           454           353           234           235	0.500 0.5000 0.5000 0.5000 0.5000 0.500000000
<ul> <li>chrik 73</li> <li>chrik 73</li> <li>chrik 150</li> <li>chrik 169</li> <li>chrik 200</li> <li>chrik 201</li> <li>chrik 201</li> <li>chrik 700</li> <li>chrik 700</li> <li>chrik 700</li> <li>chrik 2052</li> <li>chrik 2052</li> <li>chrik 2052</li> <li>chrik 2052</li> <li>chrik 2052</li> <li>chrik 4769</li> <li>chrik 4597</li> <li>chrik 4597</li> <li>chrik 4708</li> </ul>	A         G           C         T           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           A         G           T         C           A         G           T         C           T         C           T         C           T         C           C         T	Homezygous Homezygous Homezygous Homezygous Homezygous Homezygous Homezygous Homezygous Homezygous Homezygous Homezygous Homezygous	100.0 % 100.0 %	61374 94285 94422 94478 66774 38862 68393 21179 131084 117409 97086 668651 47757 678341	839 003 004 000 605 458 723 245 1.229 1.012 1.012 1.020 611 731	383 685 644 344 340 138 601 637 688 604 207 308	278 428 446 342 455 342 454 333 107 874 692 454 454 505 505 244 454	639 993 994 996 995 995 995 995 723 230 1,375 1,229 1,012 1,009 611 731	Allete Cov - 383 605 648 544 333 4 340 134 601 607 668 664 207 398	Strand Bias           278           448           449           455           342           454           353           102           874           505           605           338           338	0.500 0.5000 0.5000 0.5000 0.500000000







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		G	12257.8 PASS	AF=1;AO= GT:GQ:D	F 1/1:99:134	3:1322:19:0:131	19:1322:1:552	0:1137:5:5:13 :767:8:11:555	829:1139:0 5:767:0:0	0:0												
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#### QUESTIONS?



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