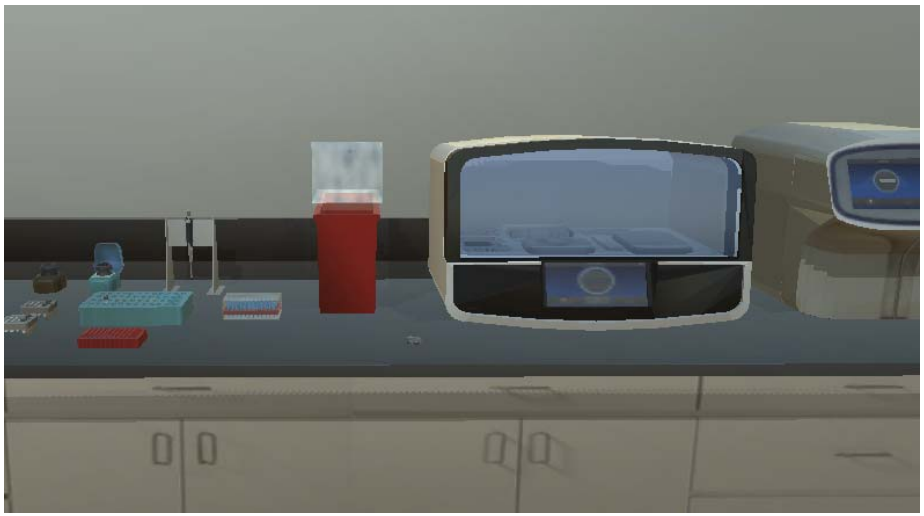
Moving Implementation Mountains: Experiencing the Forensic Laboratory NGS and Bioinformatics Workflow through Simulation



Cooperative Agreement: 2011-DN-BX-K564

NIJ | National Institute of Justice
STRENGTHEN SCIENCE. ADVANCE JUSTICE.

Goals of the Workshop

- Provide practicing forensic DNA analysts with:
  - an immersive and interactive virtual simulation tool that guides the end-user through the three commercially available NGS forensic lab processes on two massively parallel sequencing instrument
  - Provide an overview of the bioinformatics of forensic NGS analysis
  - Provide lessons learned from implementation of NGS into a forensic laboratory

What you will experience

- A complete virtual laboratory, complete with pipettes, vortex, centrifuges, plates, racks, and instruments
- Abbreviated workflows and short vignettes linking the benchwork to the scientific principles of the chemistries
  - Illumina ForenSeq DNA Signature STR, Y-STR, and SNP kit (MiSeq)
  - The Promega PowerSeq STR kit (MiSeq)
  - Life Technologies AmpliSeq mtDNA kit (Ion S5/ Ion Chef)
- Disclaimer – This is not an endorsement of any particular product. There are pros and cons to each, and there continues to be research and development on all workflows. We encourage you to seek out each vendor for detailed information and training. Additionally, we encourage you to stay up to date on past and current research funded by the NIJ and other funding organizations.

Workshop Leaders

Chair:

Donia Slack, M.S. (RTI International)

Presenters:

Bruce Budowle, Ph.D. (University of North Texas Health Science Center)

Jennifer Churchill, Ph.D. (University of North Texas Health Science Center)

Seth Faith, Ph.D. (North Carolina State University)

Walther Parson Ph.D. (Institute of Legal Medicine, Innsbruck Medical University)

Frank Wendt, Ph.D. Candidate (University of North Texas Health Science Center)

NGS Simulation Technical Lead:

Sarah Norsworthy, M.S. (RTI International)

Supported in part by the NIJ's Forensic Technology Center of Excellence

RTI INTERNATIONAL
UNT HEALTH SCIENCE CENTER
DUQUESNE UNIVERSITY
VCU VIRGINIA COMMONWEALTH UNIVERSITY
WestVirginiaUniversity
UCF
NIST FORENSIC SCIENCES
MFRC Midwest Forensics Resource Center

Schedule

Topic	Presenter	Time
General introduction	Donia Slack	8:30-8:40
Introduction of NGS for forensic applications	Bruce Budowle	8:40-8:55
How to use the virtual environment and simulation of Illumina's ForenSeq technology	Donia Slack	8:55-10:15
BREAK	**REFRESHMENTS**	**10:15-10:30**
Simulation of the ThermoFisher Ion S5/Ion Chef workflow for mtDNA	Jennifer Churchill	10:30-11:30
Simulation of the Promega PowerSeq technology and begin Bioinformatics lesson	Seth Faith	11:30-12:00
LUNCH	**NORTHSTAR BALLROOM**	**12:00-1:00**
Continuation of post-analysis Bioinformatics exercises	Seth Faith	1:00-3:00
BREAK	**REFRESHMENTS**	**3:00-3:15**
Post-analysis through STRait Razor	Frank Wendt	3:15-3:45
Implementing Mitochondrial DNA Massively Parallel Sequencing into Forensic Casework	Walther Parsons	3:45-4:30
Round Table / Q&A	All Presenters	4:30 – 5:00

Introduction of NGS for forensic Applications
Dr. Bruce Budowle

ENTER THE LAB……

www.tarheeltechnologies.com/MPS/MPS

Simulation Tool Overview

- **PLEASE READ THE PAPER PROTOCOL WHILE GOING THROUGH THE SIMULATION!**
- Viewpoint – set to an optimal location for the work you are about to perform
- Moving your mouse cursor to the edges of the screen will rotate the camera in that direction
- You can move around using the ARROW KEYS and the letters A, D, W, and S
- UP and DOWN ARROW KEYS = FORWARD and BACK
- LEFT and RIGHT ARROW KEYS = TURN LEFT and RIGHT
- A and D = STEPPING to LEFT and RIGHT
- W and S = LOOK UP and DOWN

Simulation Tool Overview

- A hovering cursor will let you see what consumable or piece of equipment you are about to use
- Read text at the bottom of the screen, and look at the top of the screen for green text
- Move tubes from the master rack to the working rack in the same order
- The bottom of the pipette is the hotspot for picking up tips and pipetting
- **Use your cheat sheet!**

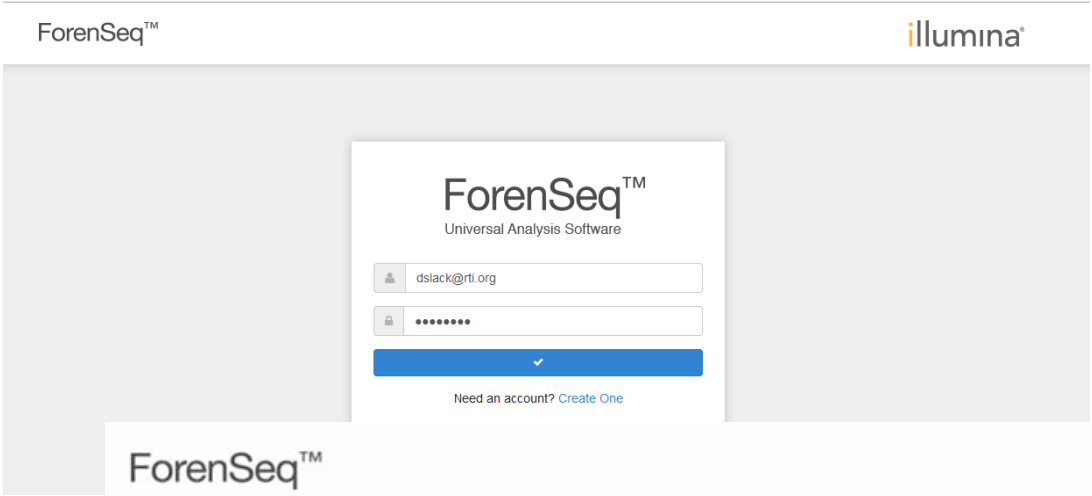
Illumina ForenSeq DNA Signature Prep Kit

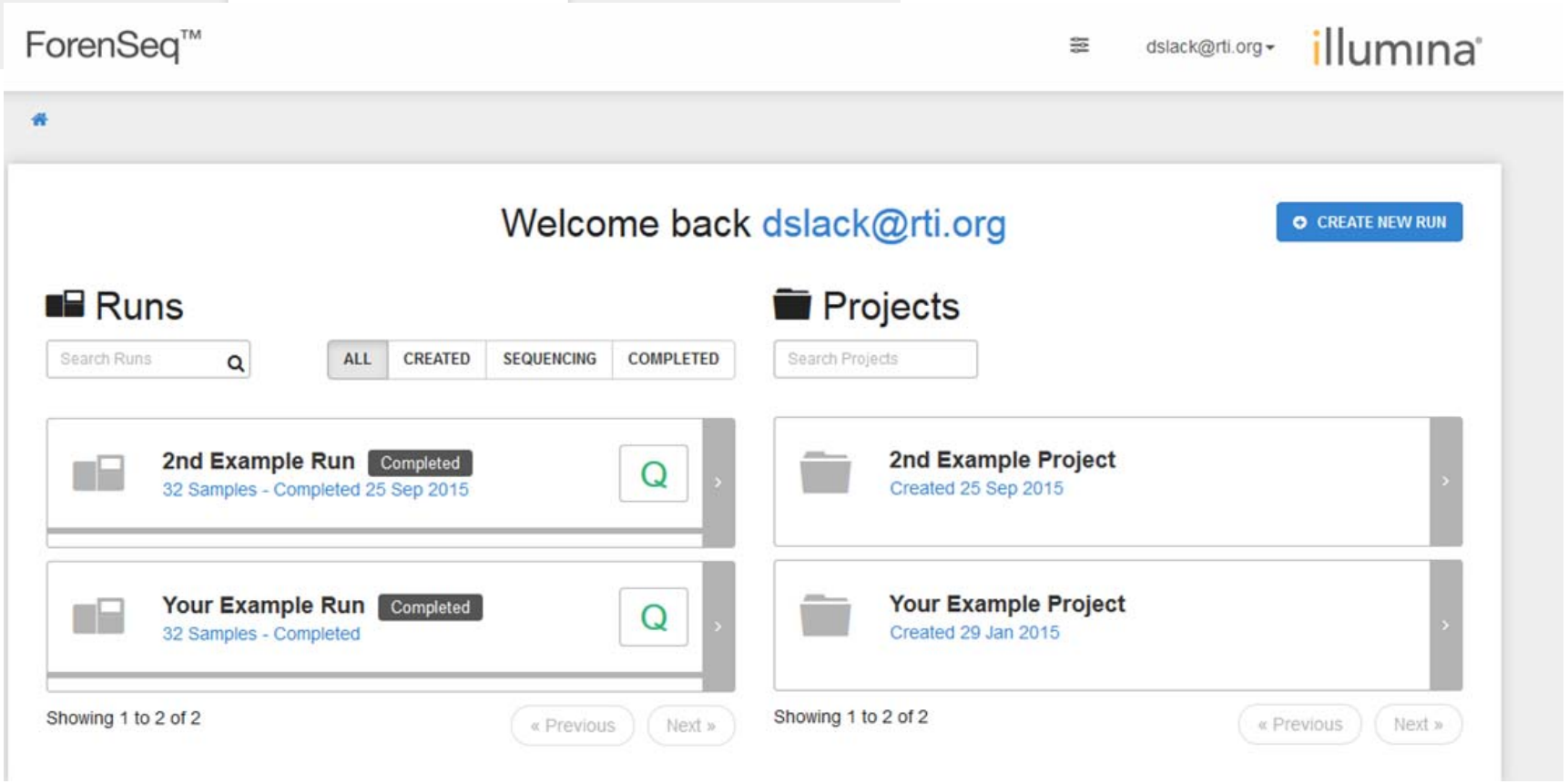
Illumina ForenSeq DNA Signature Prep Kit

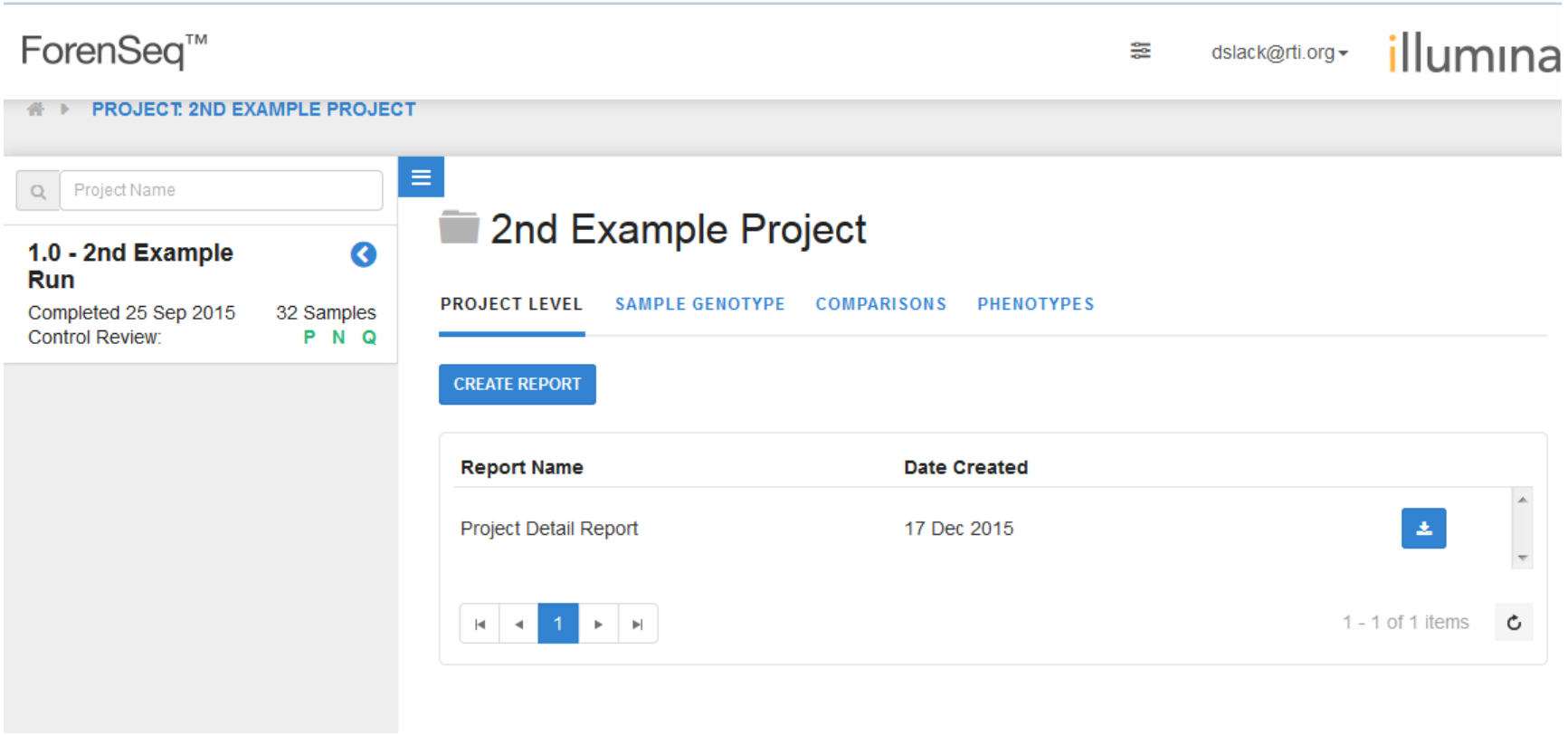
- DNA Primer Mix A:
  - primer pairs for 58 STRs (including 27 autosomal STRs, 7 X STRs and 24 Y-STRs) + 94 identity-informative SNPs
- DNA Primer Mix B:
  - Contains all markers in DNA Primer Mix A, plus primer pairs for 56 ancestry-informative SNPs and 22 phenotypic-informative SNPs (2 SNPs used for both)
- Library generation to allow for preparation of 96 samples to generate libraries of PCR products within a single plate (for the purpose of this simulation, only one sample is being processed)
- Sample recommendations:
  - 1ng of single source DNA
  - Processing of great than 8 samples at one time

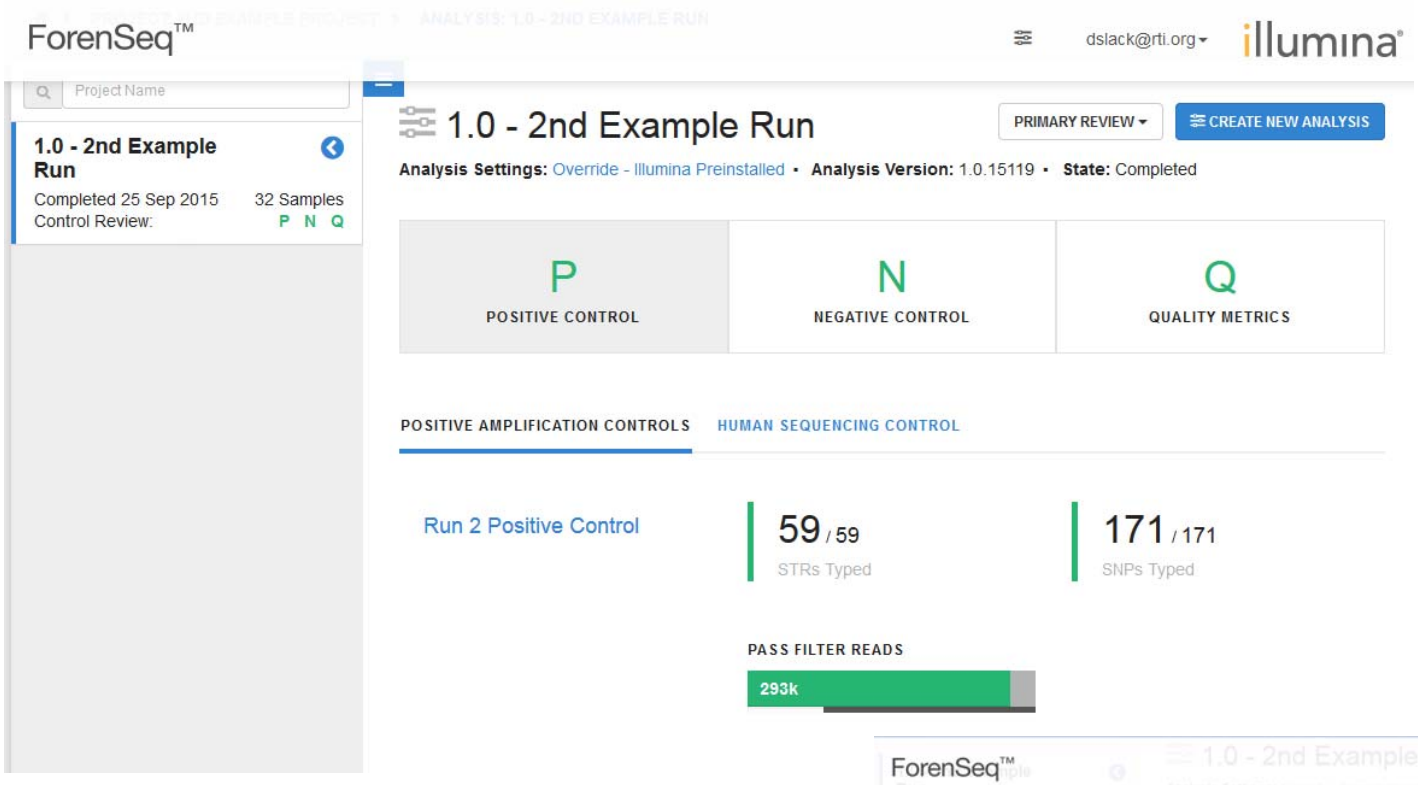
Forenseq Sample Analysis

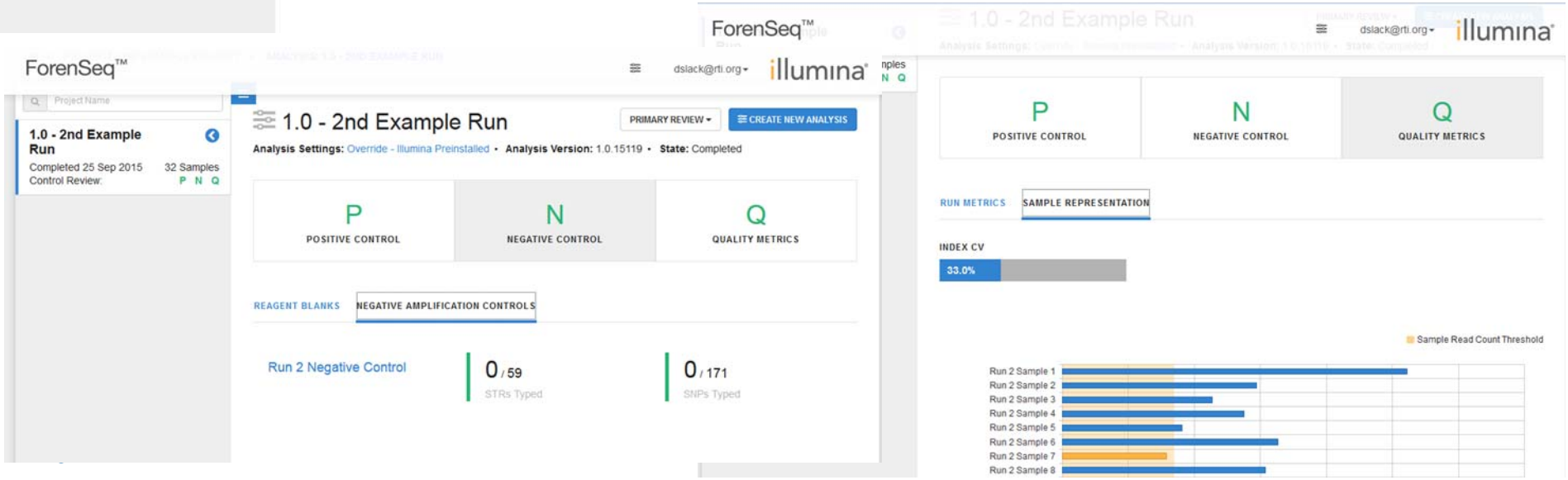

FTCOE

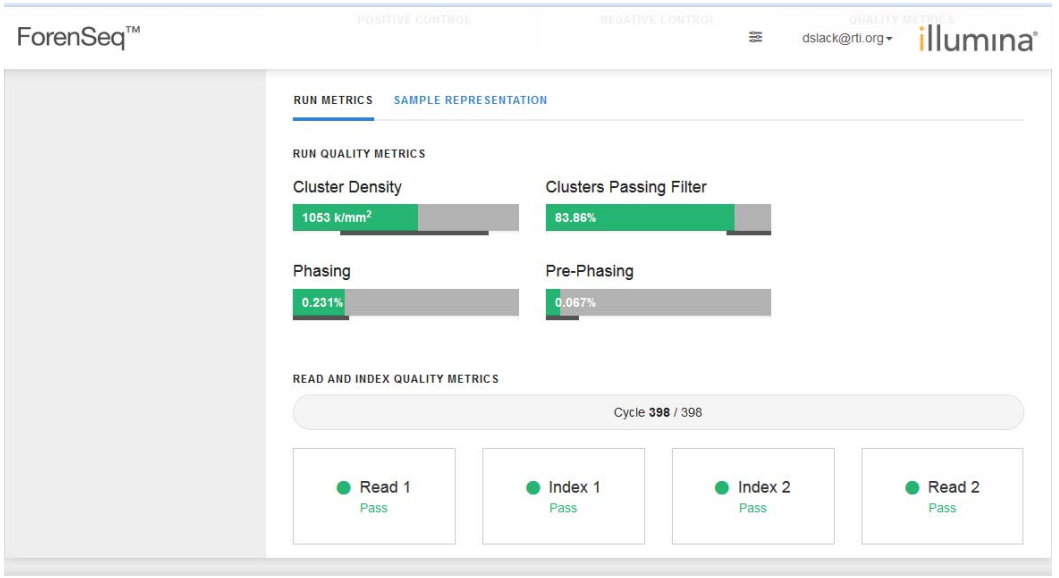

ForenSeq™
illumina
ForenSeq™
Universal Analysis Software
dslack@rti.org
Need an account? Create One

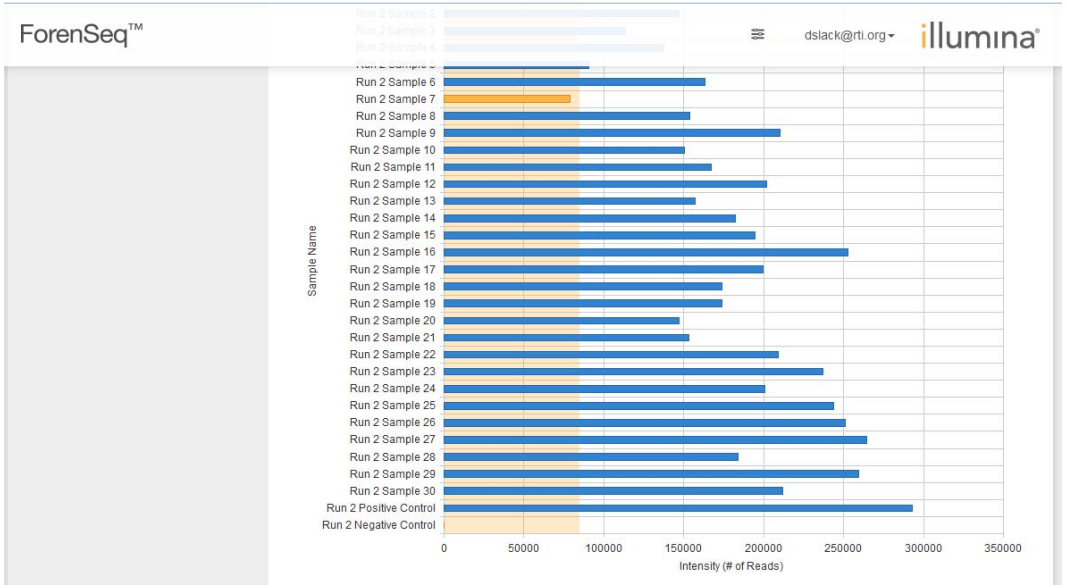

ForenSeq™
dslack@rti.org
illumina
Welcome back dslack@rti.org
CREATE NEW RUN
Runs
Search Runs
ALL
CREATED
SEQUENCING
COMPLETED
2nd Example Run
Completed
32 Samples - Completed 25 Sep 2015
Your Example Run
Completed
32 Samples - Completed
Showing 1 to 2 of 2
« Previous
Next »
Projects
Search Projects
2nd Example Project
Created 25 Sep 2015
Your Example Project
Created 29 Jan 2015
Showing 1 to 2 of 2
« Previous
Next »

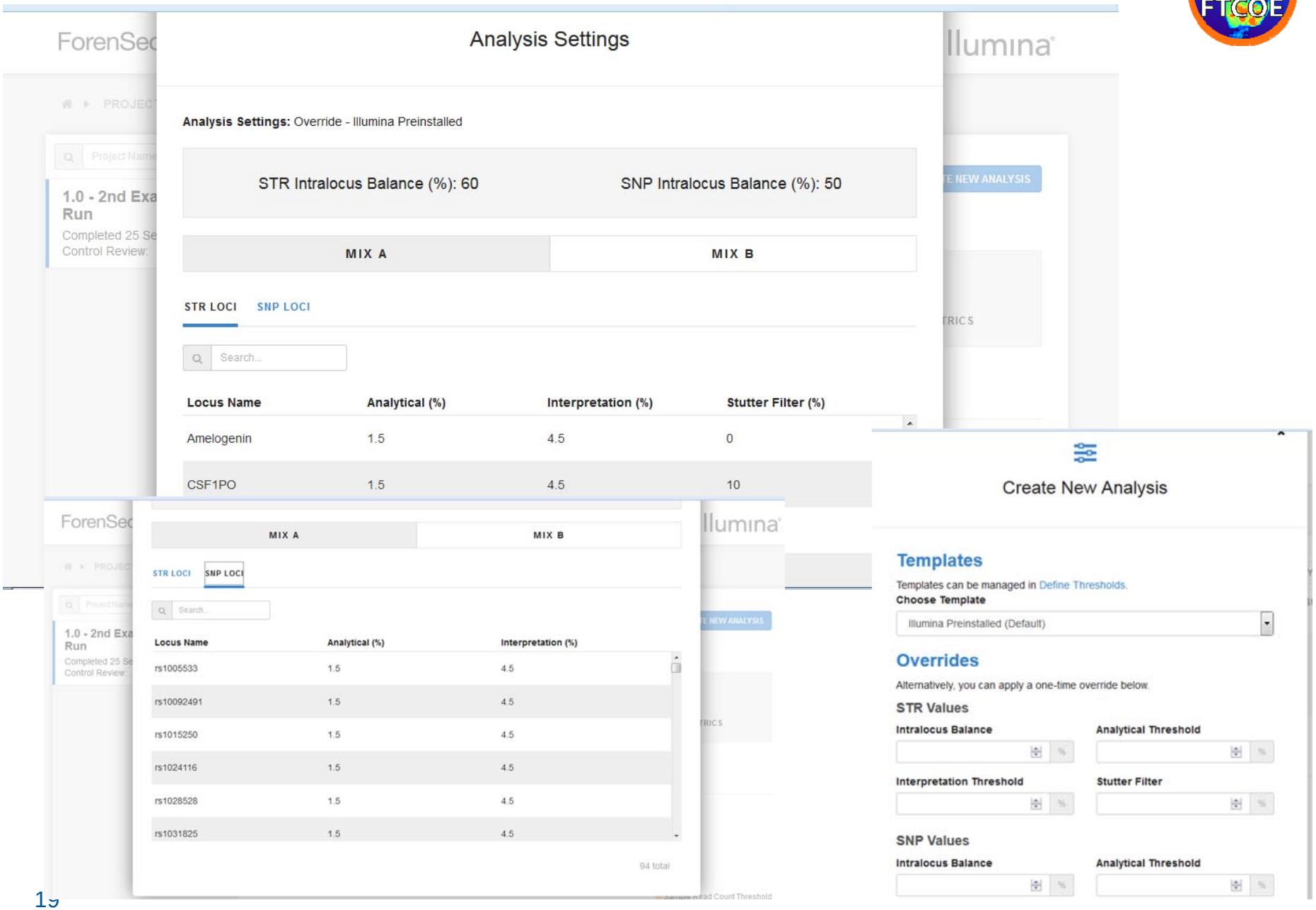

ForenSeq™
dslack@rti.org
illumina
PROJECT: 2ND EXAMPLE PROJECT
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
2nd Example Project
PROJECT LEVEL
SAMPLE GENOTYPE
COMPARISONS
PHENOTYPES
CREATE REPORT
Report Name
Date Created
Project Detail Report
17 Dec 2015
1
1 - 1 of 1 items

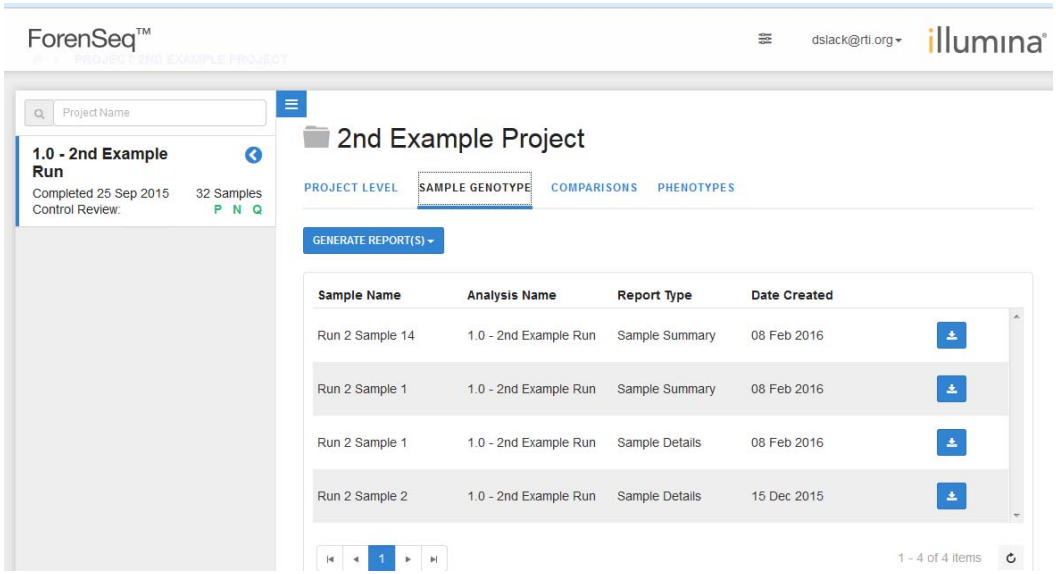

ForenSeq™
dslack@rti.org
illumina
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
1.0 - 2nd Example Run
PRIMARY REVIEW
CREATE NEW ANALYSIS
Analysis Settings: Override - Illumina Preinstalled • Analysis Version: 1.0.15119 • State: Completed
P
POSITIVE CONTROL
N
NEGATIVE CONTROL
Q
QUALITY METRICS
POSITIVE AMPLIFICATION CONTROLS
HUMAN SEQUENCING CONTROL
Run 2 Positive Control
59 / 59
STRs Typed
171 / 171
SNPs Typed
PASS FILTER READS
293k

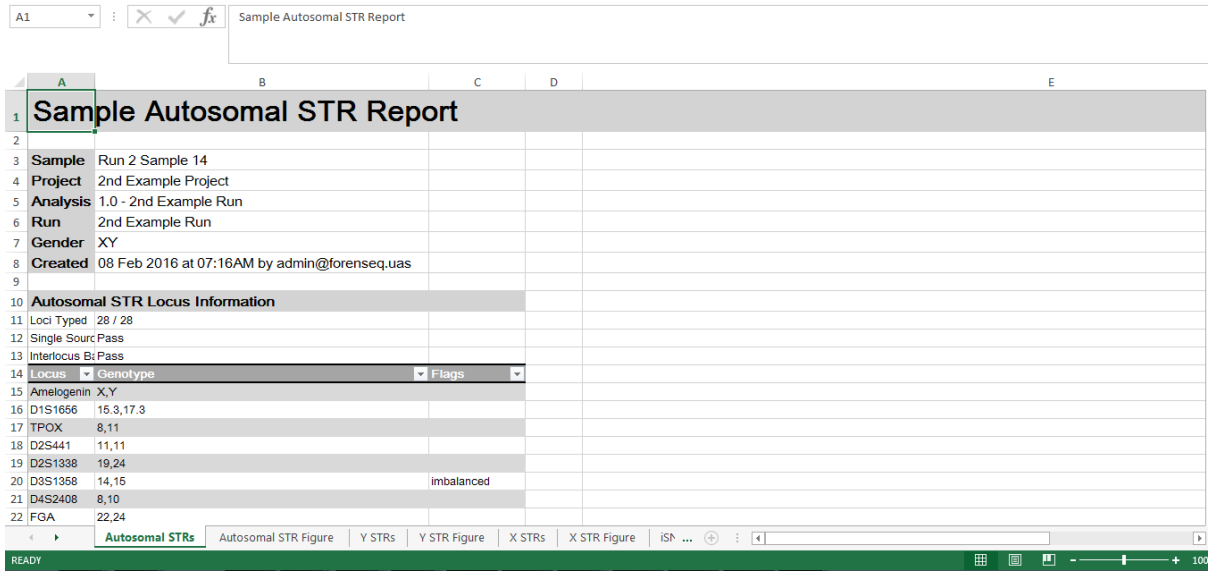

ForenSeq™
dslack@rti.org
illumina
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
1.0 - 2nd Example Run
PRIMARY REVIEW
CREATE NEW ANALYSIS
Analysis Settings: Override - Illumina Preinstalled • Analysis Version: 1.0.15119 • State: Completed
P
POSITIVE CONTROL
N
NEGATIVE CONTROL
Q
QUALITY METRICS
REAGENT BLANKS
NEGATIVE AMPLIFICATION CONTROLS
Run 2 Negative Control
0 / 59
STRs Typed
0 / 171
SNPs Typed
RUN METRICS
SAMPLE REPRESENTATION
INDEX CV
33.0%
Sample Read Count Threshold
Run 2 Sample 1
Run 2 Sample 2
Run 2 Sample 3
Run 2 Sample 4
Run 2 Sample 5
Run 2 Sample 6
Run 2 Sample 7
Run 2 Sample 8


ForenSeq™
dslack@rti.org
illumina
RUN METRICS
SAMPLE REPRESENTATION
RUN QUALITY METRICS
Cluster Density
1053 k/mm²
Clusters Passing Filter
83.86%
Phasing
0.231%
Pre-Phasing
0.067%
READ AND INDEX QUALITY METRICS
Cycle 398 / 398
Read 1
Pass
Index 1
Pass
Index 2
Pass
Read 2
Pass


ForenSeq™
dslack@rti.org
illumina
Run 2 Sample 6
Run 2 Sample 7
Run 2 Sample 8
Run 2 Sample 9
Run 2 Sample 10
Run 2 Sample 11
Run 2 Sample 12
Run 2 Sample 13
Run 2 Sample 14
Run 2 Sample 15
Run 2 Sample 16
Run 2 Sample 17
Run 2 Sample 18
Run 2 Sample 19
Run 2 Sample 20
Run 2 Sample 21
Run 2 Sample 22
Run 2 Sample 23
Run 2 Sample 24
Run 2 Sample 25
Run 2 Sample 26
Run 2 Sample 27
Run 2 Sample 28
Run 2 Sample 29
Run 2 Sample 30
Run 2 Positive Control
Run 2 Negative Control
Sample Name
0
50000
100000
150000
200000
250000
300000
350000
Intensity (# of Reads)


Analysis Settings
Analysis Settings: Override - Illumina Preinstalled
STR Intralocus Balance (%): 60
SNP Intralocus Balance (%): 50
MIX A
MIX B
STR LOCI
SNP LOCI
Search...
Locus Name
Analytical (%)
Interpretation (%)
Stutter Filter (%)
Amelogenin 1.5 4.5 0
CSF1PO 1.5 4.5 10
rs1005533 1.5 4.5
rs10092491 1.5 4.5
rs1015250 1.5 4.5
rs1024116 1.5 4.5
rs1028528 1.5 4.5
rs1031825 1.5 4.5
94 total
Create New Analysis
Templates
Templates can be managed in Define Thresholds.
Choose Template
Illumina Preinstalled (Default)
Overrides
Alternatively, you can apply a one-time override below.
STR Values
Intralocus Balance
Analytical Threshold
Interpretation Threshold
Stutter Filter
SNP Values
Intralocus Balance
Analytical Threshold


ForenSeq™
dslack@rti.org
illumina
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
Control Review:
32 Samples
P N Q
2nd Example Project
PROJECT LEVEL
SAMPLE GENOTYPE
COMPARISONS
PHENOTYPES
GENERATE REPORT(S)
Sample Name
Analysis Name
Report Type
Date Created
Run 2 Sample 14
1.0 - 2nd Example Run
Sample Summary
08 Feb 2016
Run 2 Sample 1
1.0 - 2nd Example Run
Sample Summary
08 Feb 2016
Run 2 Sample 1
1.0 - 2nd Example Run
Sample Details
08 Feb 2016
Run 2 Sample 2
1.0 - 2nd Example Run
Sample Details
15 Dec 2015
1
1 - 4 of 4 items



A1 | Sample Autosomal STR Report

Sample Autosomal STR Report

Sample	Run 2 Sample 14
Project	2nd Example Project
Analysis	1.0 - 2nd Example Run
Run	2nd Example Run
Gender	XY
Created	08 Feb 2016 at 07:16AM by admin@forenseq.uas

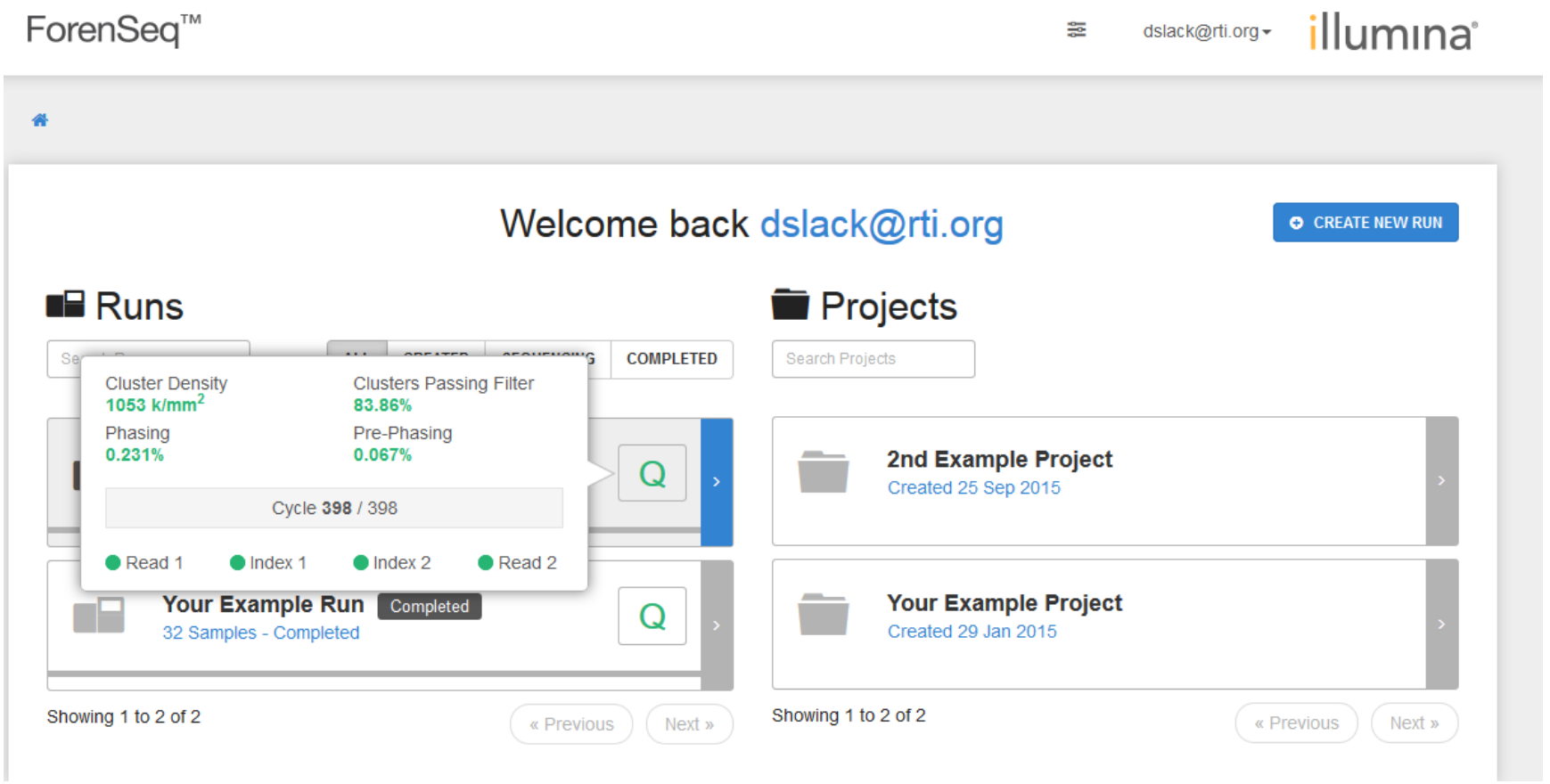
Autosomal STR Locus Information

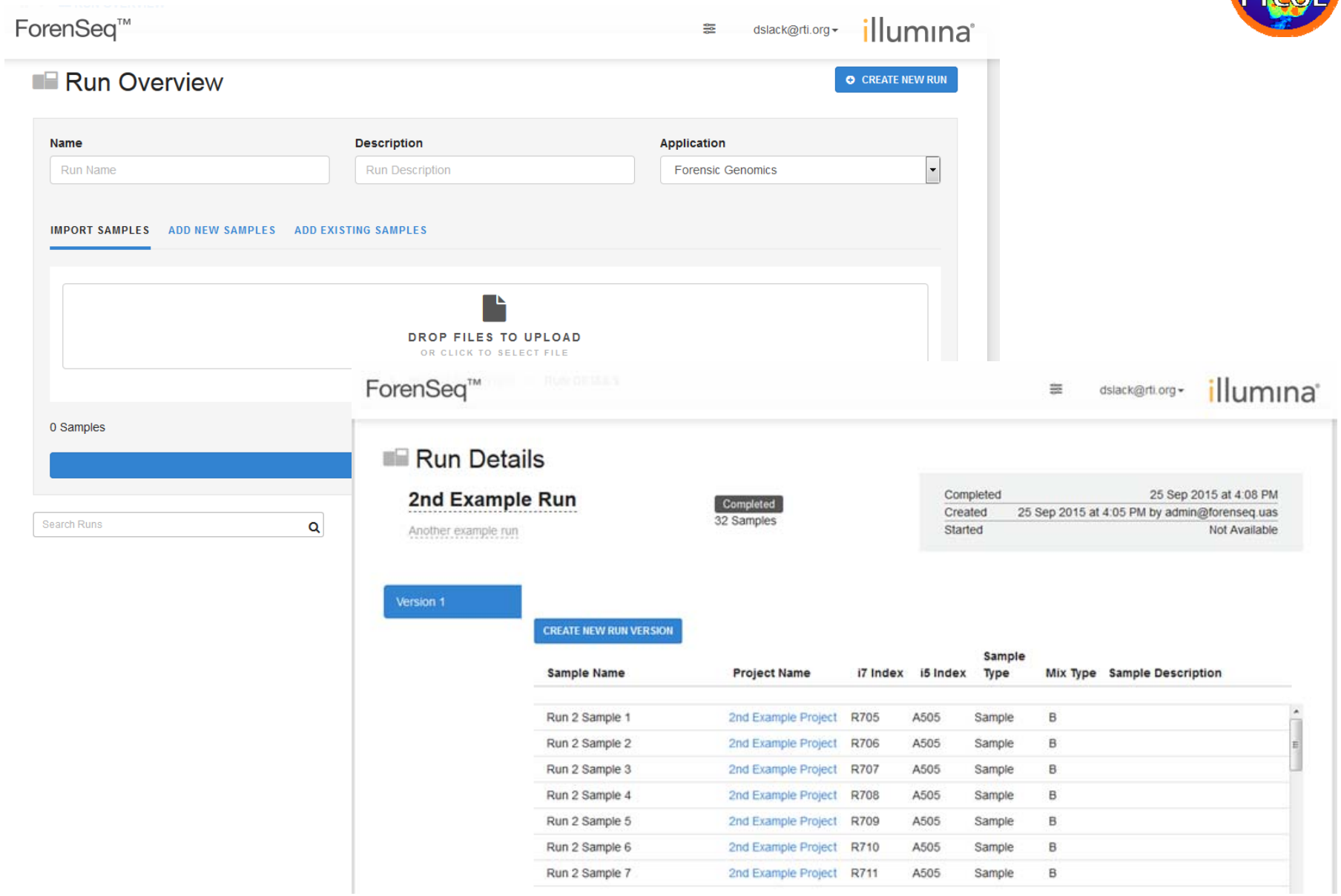
Loci Typed	28 / 28
Single Sourc	Pass
Interlocus Bi	Pass

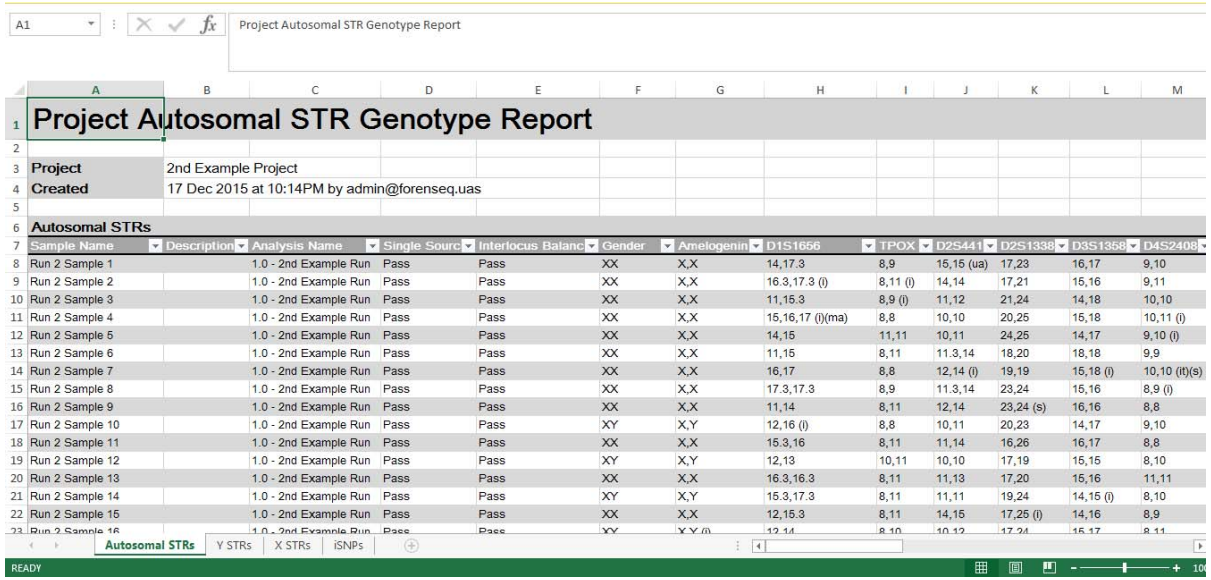
Locus	Genotype	Flags
Amelogenin	X,Y	
D1S1656	15.3,17.3	
TPOX	8,11	
D2S441	11,11	
D2S1338	19,24	
D3S1358	14,15	imbalanced
D4S2408	8,10	
FGA	22,24	

Autosomal STRs | Autosomal STR Figure | Y STRs | Y STR Figure | X STRs | X STR Figure | iSN ...

READY


ForenSeq™
dslack@rti.org
illumina
Welcome back dslack@rti.org
CREATE NEW RUN
Runs
Projects
COMPLETED
Search Projects
Cluster Density
1053 k/mm2
Clusters Passing Filter
83.86%
Phasing
0.231%
Pre-Phasing
0.067%
Cycle 398 / 398
Read 1
Index 1
Index 2
Read 2
Your Example Run
Completed
32 Samples - Completed
2nd Example Project
Created 25 Sep 2015
Your Example Project
Created 29 Jan 2015
Showing 1 to 2 of 2
« Previous
Next »
Showing 1 to 2 of 2
« Previous
Next »


ForenSeq™
dslack@rti.org
illumina
Run Overview
CREATE NEW RUN
Name
Run Name
Description
Run Description
Application
Forensic Genomics
IMPORT SAMPLES
ADD NEW SAMPLES
ADD EXISTING SAMPLES
DROP FILES TO UPLOAD
OR CLICK TO SELECT FILE
0 Samples
Search Runs
ForenSeq™
dslack@rti.org
illumina
Run Details
2nd Example Run
Another example run
Completed
32 Samples
Completed 25 Sep 2015 at 4:08 PM
Created 25 Sep 2015 at 4:05 PM by admin@forenseq.uas
Started Not Available
Version 1
CREATE NEW RUN VERSION
Sample Name | Project Name | i7 Index | i5 Index | Sample Type | Mix Type | Sample Description
Run 2 Sample 1 | 2nd Example Project | R705 | A505 | Sample | B
Run 2 Sample 2 | 2nd Example Project | R706 | A505 | Sample | B
Run 2 Sample 3 | 2nd Example Project | R707 | A505 | Sample | B
Run 2 Sample 4 | 2nd Example Project | R708 | A505 | Sample | B
Run 2 Sample 5 | 2nd Example Project | R709 | A505 | Sample | B
Run 2 Sample 6 | 2nd Example Project | R710 | A505 | Sample | B
Run 2 Sample 7 | 2nd Example Project | R711 | A505 | Sample | B



A1 | Project Autosomal STR Genotype Report

Project Autosomal STR Genotype Report

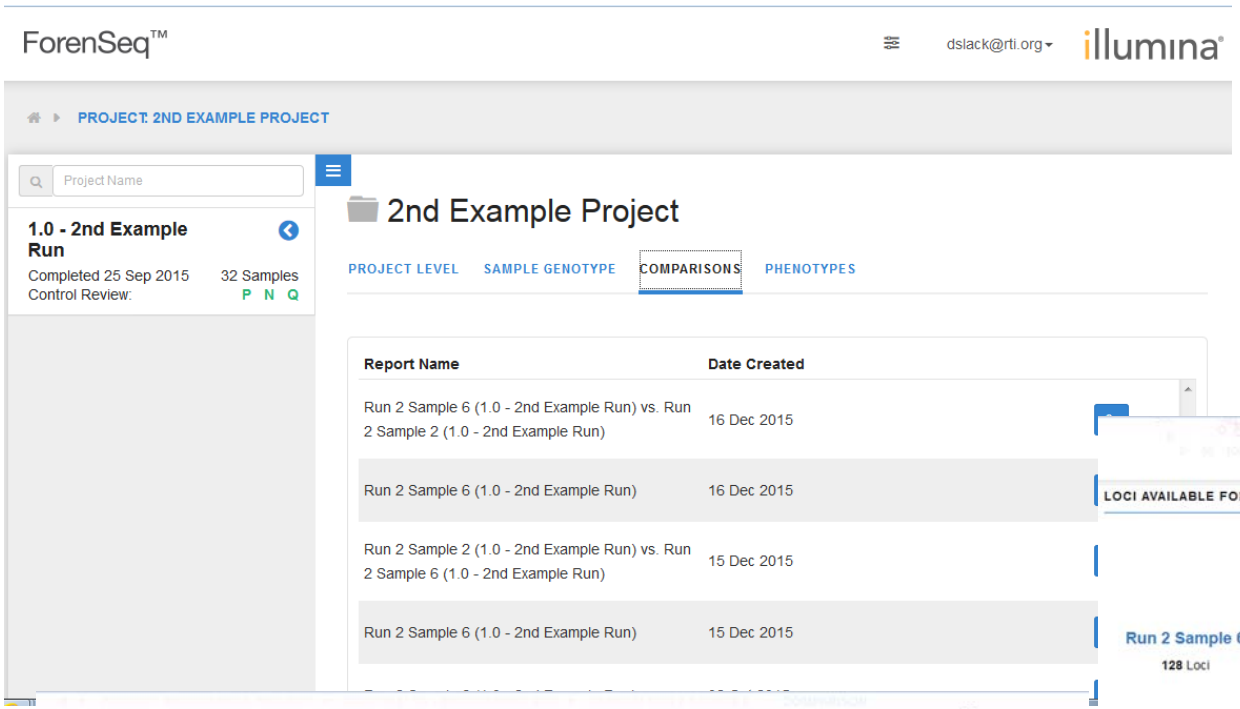
Project	2nd Example Project
Created	17 Dec 2015 at 10:14PM by admin@forenseq.uas

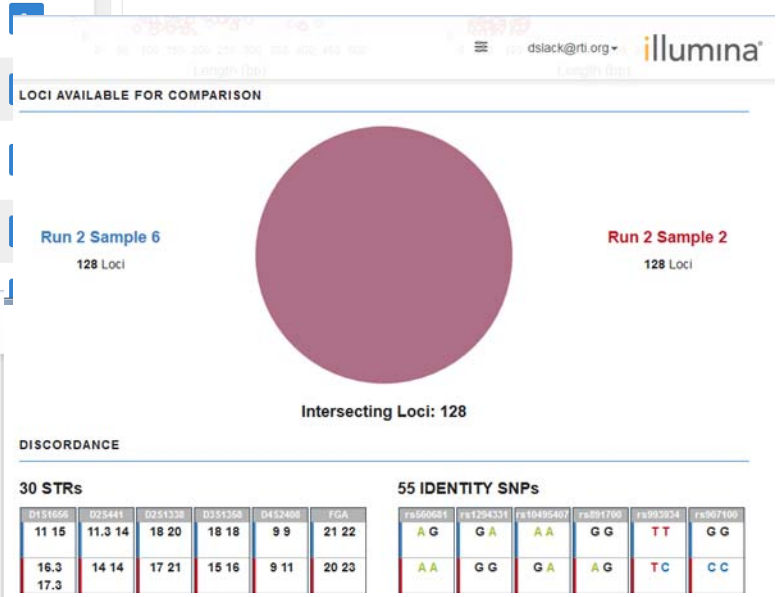
Autosomal STRs

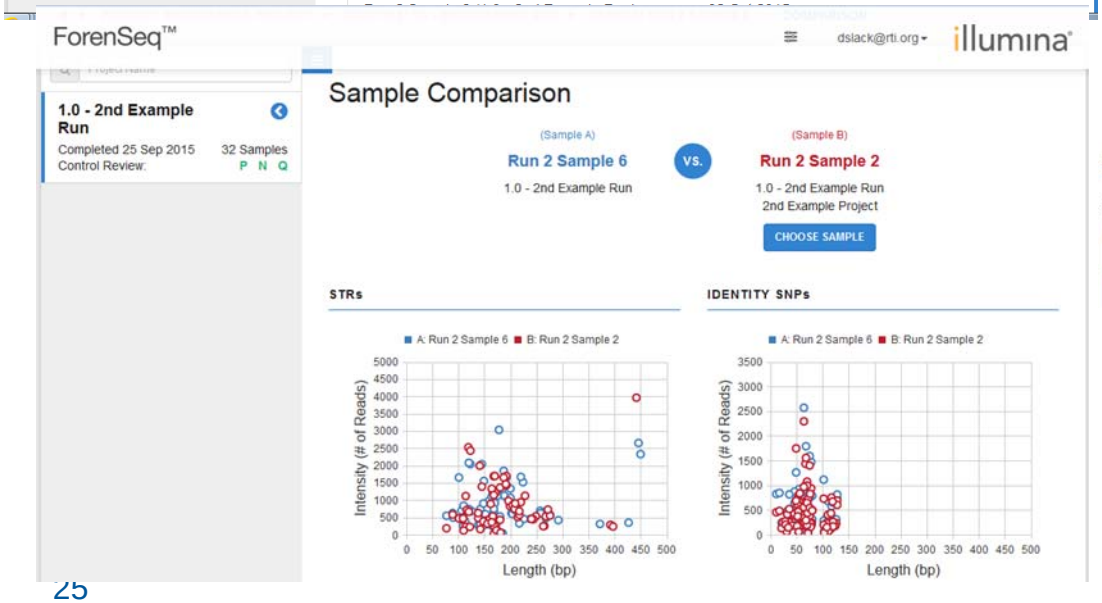
Sample Name	Description	Analysis Name	Single Source	Interlocus Balance	Gender	Amelogenin	D1S1656	TPOX	D2S441	D2S1338	D3S1358	D4S2408
Run 2 Sample 1		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	14,17.3	8,9	15,15 (ua)	17,23	16,17	9,10
Run 2 Sample 2		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	16.3,17.3 (i)	8,11 (i)	14,14	17,21	15,16	9,11
Run 2 Sample 3		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	11,15.3	8,9 (i)	11,12	21,24	14,18	10,10
Run 2 Sample 4		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	15,16,17 (i)(ma)	8,8	10,10	20,25	15,18	10,11 (i)
Run 2 Sample 5		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	14,15	11,11	10,11	24,25	14,17	9,10 (i)
Run 2 Sample 6		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	11,15	8,11	11.3,14	18,20	18,18	9,9
Run 2 Sample 7		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	16,17	8,8	12,14 (i)	19,19	15,18 (i)	10,10 (it)(s)
Run 2 Sample 8		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	17.3,17.3	8,9	11.3,14	23,24	15,16	8,9 (i)
Run 2 Sample 9		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	11,14	8,11	12,14	23,24 (s)	16,16	8,8
Run 2 Sample 10		1.0 - 2nd Example Run	Pass	Pass	XY	X,Y	12,16 (i)	8,8	10,11	20,23	14,17	9,10
Run 2 Sample 11		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	15.3,16	8,11	11,14	16,26	16,17	8,8
Run 2 Sample 12		1.0 - 2nd Example Run	Pass	Pass	XY	X,Y	12,13	10,11	10,10	17,19	15,15	8,10
Run 2 Sample 13		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	16.3,16.3	8,11	11,13	17,20	15,16	11,11
Run 2 Sample 14		1.0 - 2nd Example Run	Pass	Pass	XY	X,Y	15.3,17.3	8,11	11,11	19,24	14,15 (i)	8,10
Run 2 Sample 15		1.0 - 2nd Example Run	Pass	Pass	XX	X,X	12,15.3	8,11	14,15	17,25 (i)	14,16	8,9
Run 2 Sample 16		1.0 - 2nd Example Run	Pass	Pass	XX	X,X (i)	12,14	8,10	10,12	17,24	15,17	8,11

Autosomal STRs | Y STRs | X STRs | iSNPs

READY


ForenSeq™
dslack@rti.org
illumina
PROJECT: 2ND EXAMPLE PROJECT
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
2nd Example Project
PROJECT LEVEL
SAMPLE GENOTYPE
COMPARISONS
PHENOTYPES
Report Name
Date Created
Run 2 Sample 6 (1.0 - 2nd Example Run) vs. Run 2 Sample 2 (1.0 - 2nd Example Run)
16 Dec 2015
Run 2 Sample 6 (1.0 - 2nd Example Run)
16 Dec 2015
Run 2 Sample 2 (1.0 - 2nd Example Run) vs. Run 2 Sample 6 (1.0 - 2nd Example Run)
15 Dec 2015
Run 2 Sample 6 (1.0 - 2nd Example Run)
15 Dec 2015


dslack@rti.org
illumina
LOCI AVAILABLE FOR COMPARISON
Run 2 Sample 6
128 Loci
Run 2 Sample 2
128 Loci
Intersecting Loci: 128
DISCORDANCE
30 STRs
55 IDENTITY SNPs


ForenSeq™
dslack@rti.org
illumina
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
Sample Comparison
(Sample A)
Run 2 Sample 6
1.0 - 2nd Example Run
vs.
(Sample B)
Run 2 Sample 2
1.0 - 2nd Example Run
2nd Example Project
CHOOSE SAMPLE
STRs
IDENTITY SNPs
A: Run 2 Sample 6
B: Run 2 Sample 2
Intensity (# of Reads)
Length (bp)

D1S1656	D2S441	D2S1338	D3S1358	D4S2408	FGA
11 15	11.3 14	18 20	18 18	9 9	21 22
16.3 17.3	14 14	17 21	15 16	9 11	20 23

D5S818	CSF1PO	D6S1043	D7S820	D8S1179	D9S1122
12 12	11 12	12 19	8 10	8 13	11 12
9 11	11 11	11 18	9 11	14 14 +1 allele	11 12

D10S1248	TH01	vWA	D12S391	D13S317	PentaE
14 15	9 9.3	17 18	20 21	12 13	7 18
13 16	8.3 9.3	16 19	18 22	11 12	11 12

D16S539	D18S51	D19S433	D20S482	D21S11	PentaD
8 12	14 17	15 17	14 15	29 32	11 13
12 13	13 17	15 16	13 14	32.2 33.2	12 13

D22S1045	DXS10135	DXS8378	DXS7432	DXS10074	DXS10103
11 15	25 29	11 12	13 14	16 17	19 21
15 16	24 25	10 10	14 15	16 18	18 20

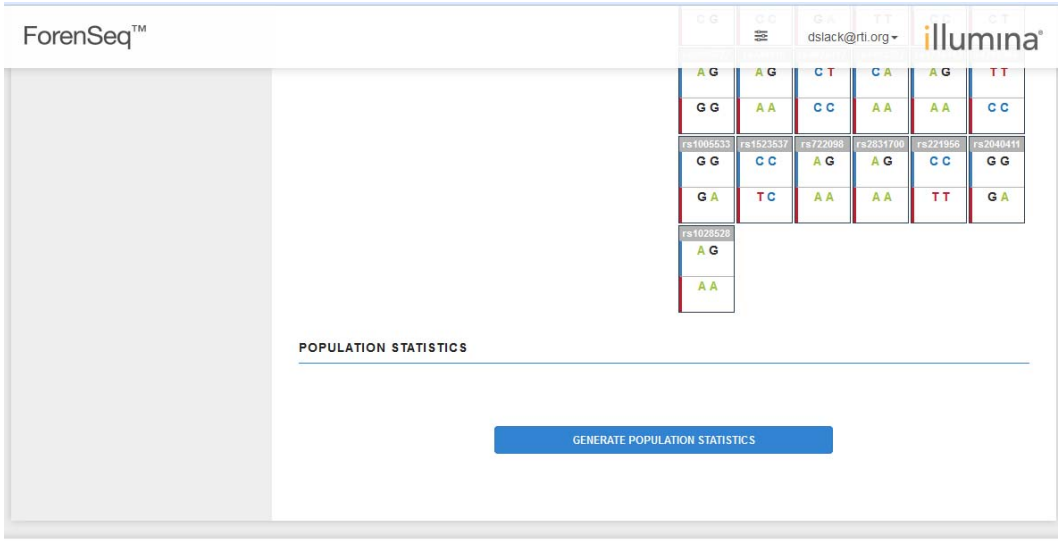
rs560681	rs1294331	rs10495407	rs891700	rs993934	rs907100
A G	G A	A A	G G	T T	G G
A A	G G	G A	A G	T C	C C

rs1357617	rs4364205	rs2399332	rs1355366	rs6444724	rs279844
T A	T T	C C	A A	C C	T T
A A	T G	A C	A G	T C	A A

rs6811238	rs1979255	rs338882	rs13218440	rs1336071	rs917118
T T	G G	C C	G G	G G	C T
G G	G C	C T	A A	A G	C C

rs321198	rs763869	rs10092491	rs4606077	rs7041158	rs1463729
C C	T T	T C	C C	C C	G A
T C	C C	C C	T C	T T	A A

rs1360288	rs10776839	rs735155	rs3780962	rs740598	rs1498553
C T	G G	G G	T T	G A	C T
C C	T T	G A	T C	G G	T T


ForenSeq™
dslack@rti.org
illumina
A G
A G
C T
C A
A G
T T
G G
A A
C C
A A
A A
C C
rs1005533
rs1523537
rs722098
rs2831709
rs221956
rs2040411
G G
C C
A G
A G
C C
G G
G A
T C
A A
A A
T T
G A
rs1028528
A G
A A
POPULATION STATISTICS
GENERATE POPULATION STATISTICS
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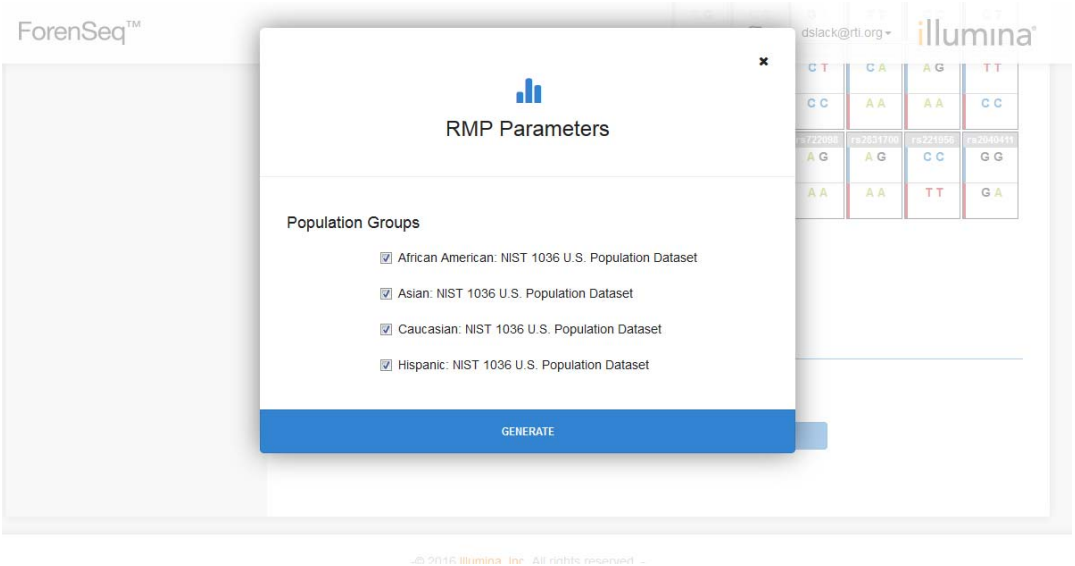
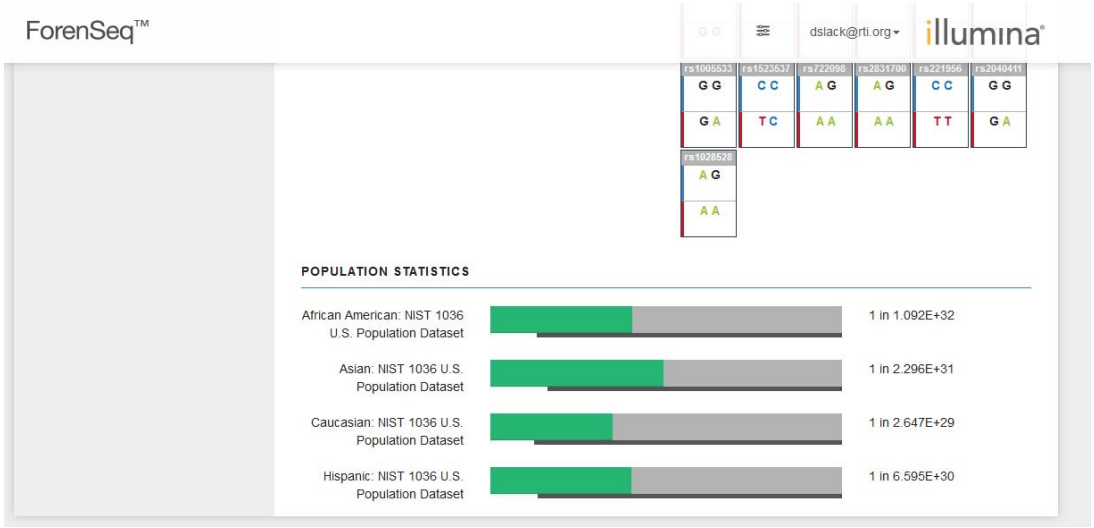
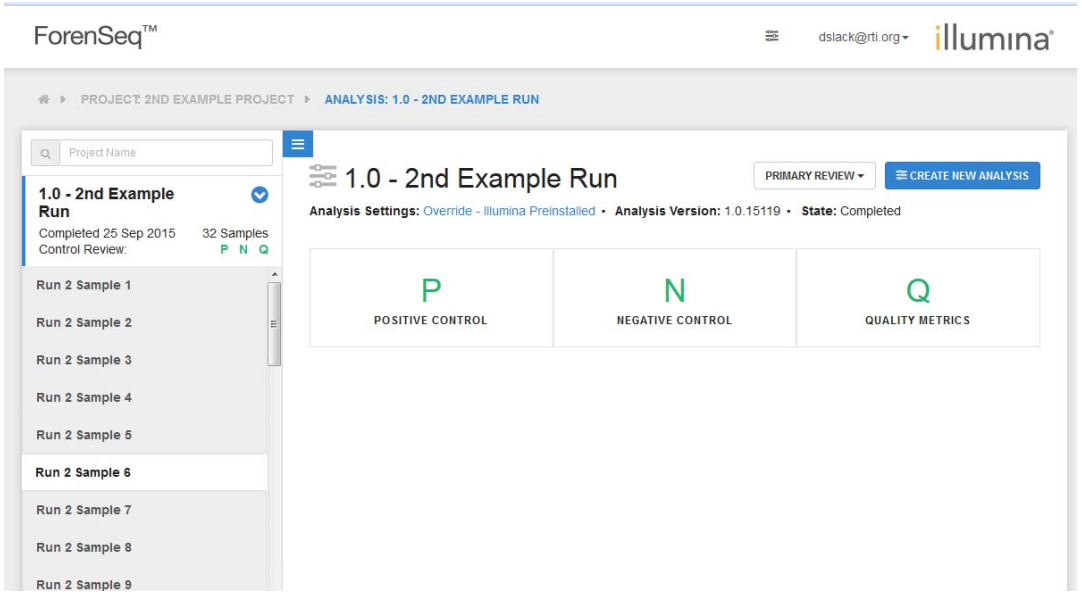
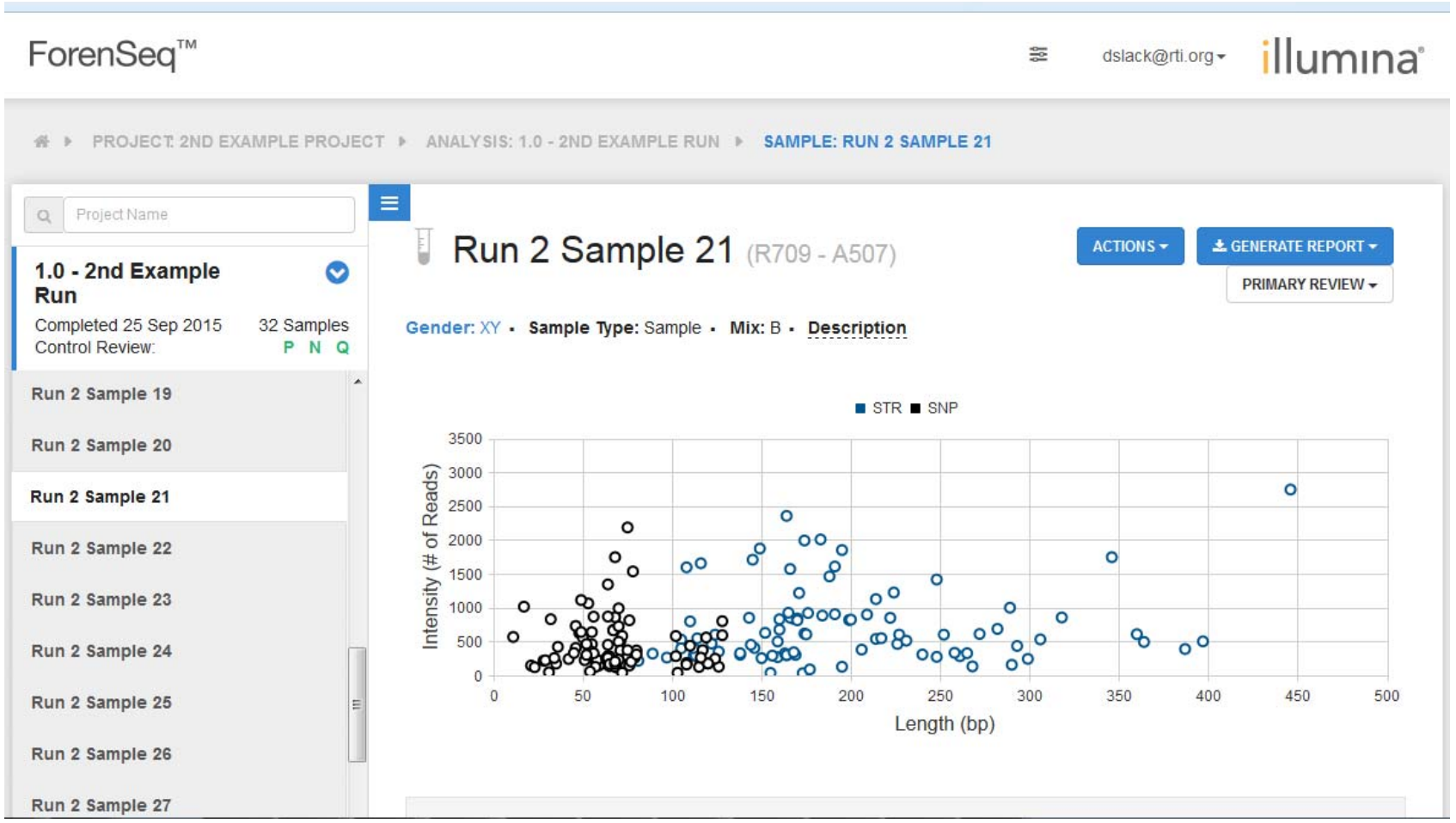
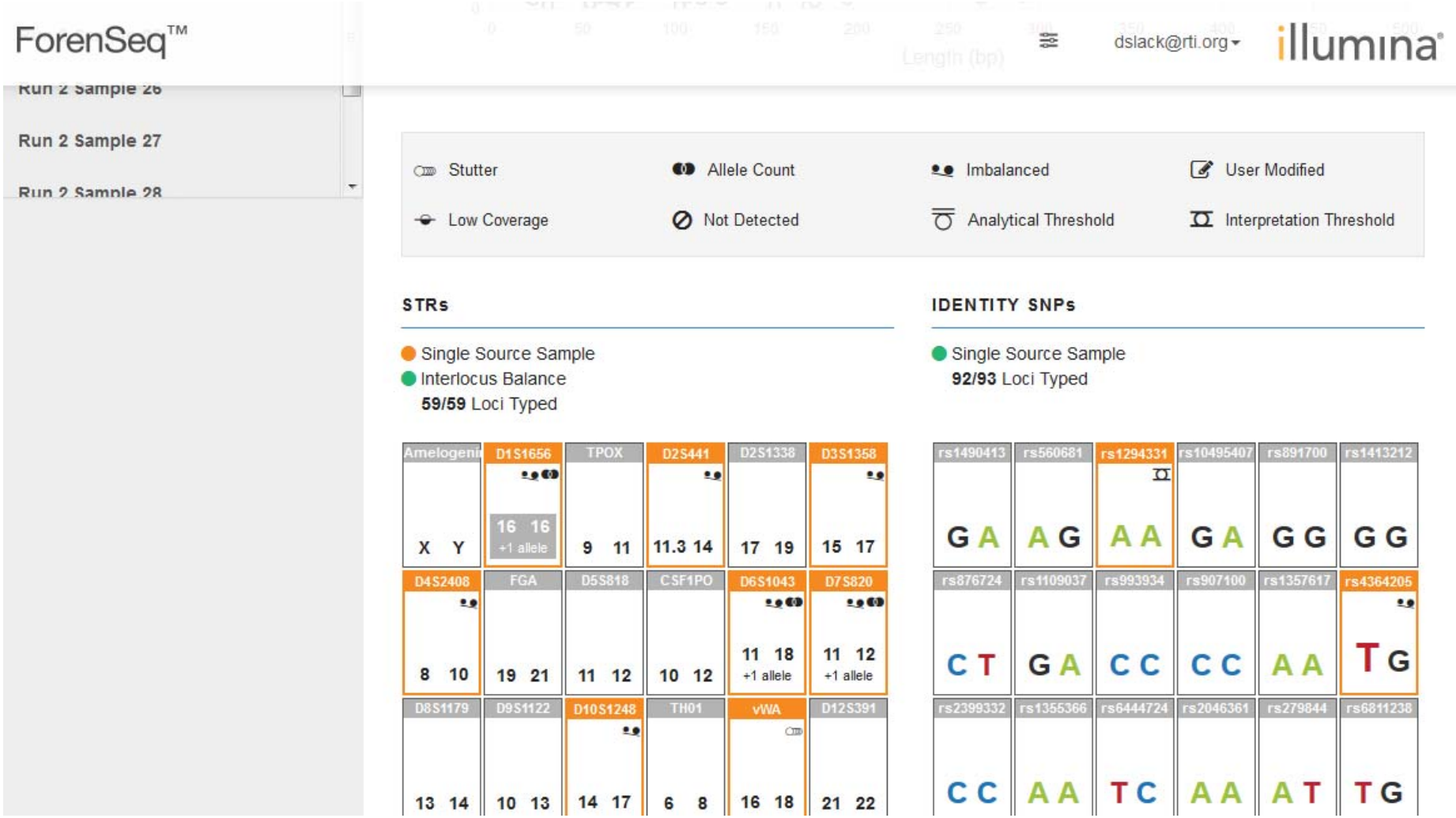
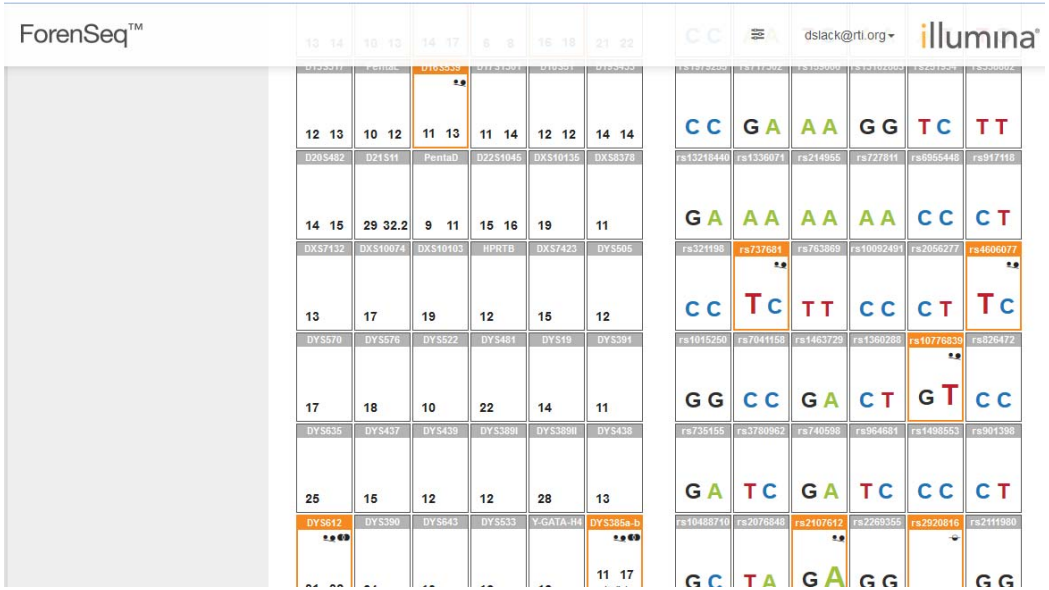

ForenSeq™
dslack@rti.org
illumina
RMP Parameters
Population Groups
African American: NIST 1036 U.S. Population Dataset
Asian: NIST 1036 U.S. Population Dataset
Caucasian: NIST 1036 U.S. Population Dataset
Hispanic: NIST 1036 U.S. Population Dataset
GENERATE


ForenSeq™
dslack@rti.org
illumina
rs1005533 GG GA
rs1523537 CC TC
rs722098 AG AA
rs2831700 AG AA
rs221956 CC TT
rs2040411 GG GA
rs1028528 AG AA
POPULATION STATISTICS
African American: NIST 1036 U.S. Population Dataset
1 in 1.092E+32
Asian: NIST 1036 U.S. Population Dataset
1 in 2.296E+31
Caucasian: NIST 1036 U.S. Population Dataset
1 in 2.647E+29
Hispanic: NIST 1036 U.S. Population Dataset
1 in 6.595E+30

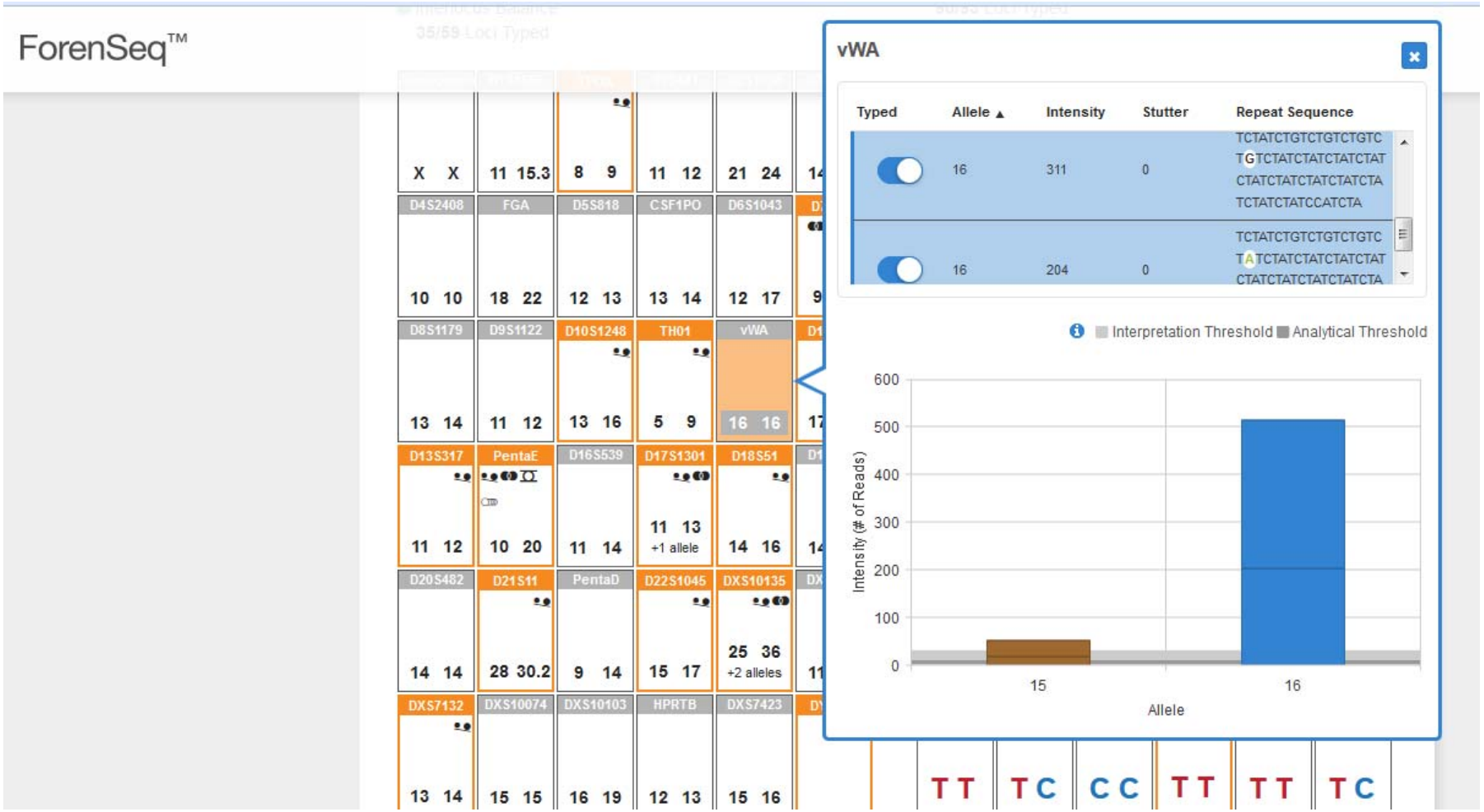

ForenSeq™
dslack@rti.org
illumina
PROJECT: 2ND EXAMPLE PROJECT
ANALYSIS: 1.0 - 2ND EXAMPLE RUN
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
Run 2 Sample 1
Run 2 Sample 2
Run 2 Sample 3
Run 2 Sample 4
Run 2 Sample 5
Run 2 Sample 6
Run 2 Sample 7
Run 2 Sample 8
Run 2 Sample 9
1.0 - 2nd Example Run
PRIMARY REVIEW
CREATE NEW ANALYSIS
Analysis Settings: Override - Illumina Preinstalled • Analysis Version: 1.0.15119 • State: Completed
P
POSITIVE CONTROL
N
NEGATIVE CONTROL
Q
QUALITY METRICS

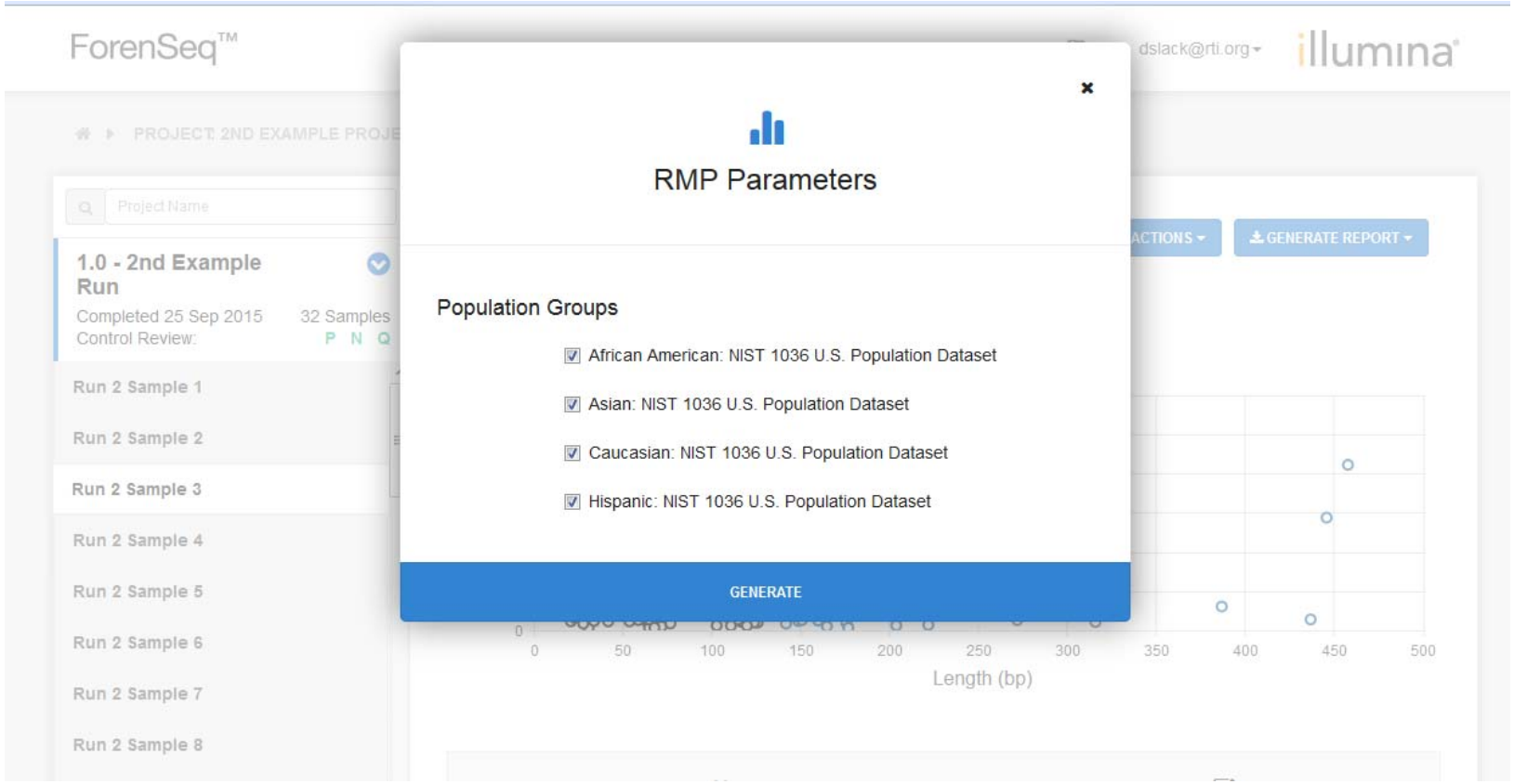

ForenSeq™
dslack@rti.org
illumina
PROJECT: 2ND EXAMPLE PROJECT
ANALYSIS: 1.0 - 2ND EXAMPLE RUN
SAMPLE: RUN 2 SAMPLE 21
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
Control Review:
32 Samples
P N Q
Run 2 Sample 19
Run 2 Sample 20
Run 2 Sample 21
Run 2 Sample 22
Run 2 Sample 23
Run 2 Sample 24
Run 2 Sample 25
Run 2 Sample 26
Run 2 Sample 27
Run 2 Sample 21 (R709 - A507)
ACTIONS
GENERATE REPORT
PRIMARY REVIEW
Gender: XY • Sample Type: Sample • Mix: B • Description
STR SNP
Intensity (# of Reads)
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
250
300
350
400
450
500
Length (bp)

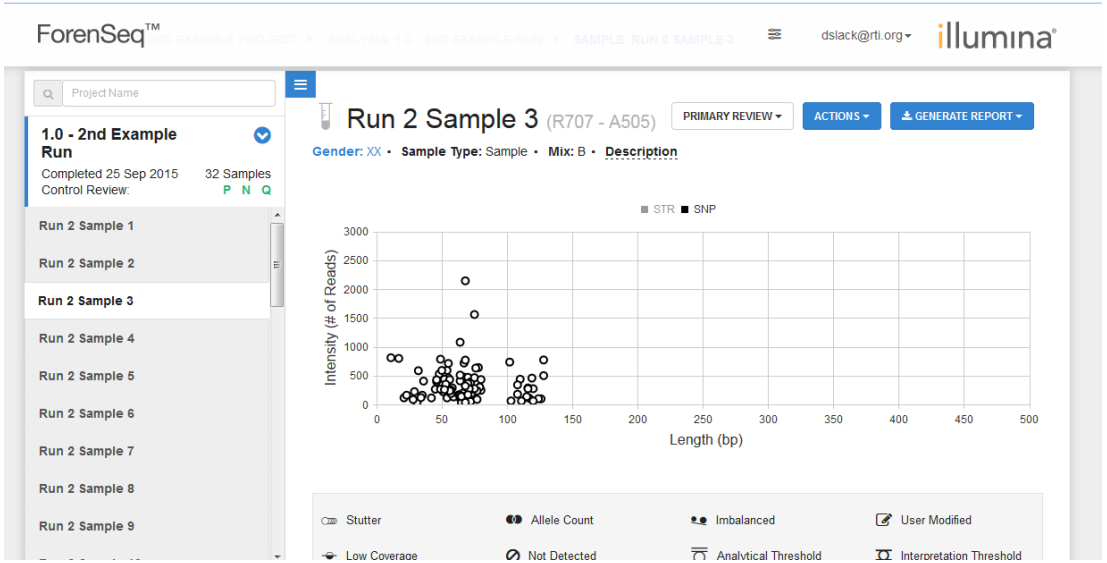

ForenSeq™
dslack@rti.org
illumina
Run 2 Sample 26
Run 2 Sample 27
Run 2 Sample 28
Stutter
Allele Count
Imbalanced
User Modified
Low Coverage
Not Detected
Analytical Threshold
Interpretation Threshold
STRs
Single Source Sample
Interlocus Balance
59/59 Loci Typed
Amelogeni X Y
D1S1656 16 16 +1 allele
TPOX 9 11
D2S441 11.3 14
D2S1338 17 19
D3S1358 15 17
D4S2408 8 10
FGA 19 21
D5S818 11 12
CSF1PO 10 12
D6S1043 11 18 +1 allele
D7S820 11 12 +1 allele
D8S1179 13 14
D9S1122 10 13
D10S1248 14 17
TH01 6 8
vWA 16 18
D12S391 21 22
IDENTITY SNPs
Single Source Sample
92/93 Loci Typed
rs1490413 G A
rs560681 A G
rs1294331 A A
rs10495407 G A
rs891700 G G
rs1413212 G G
rs876724 C T
rs1109037 G A
rs993934 C C
rs907100 C C
rs1357617 A A
rs4364205 T G
rs2399332 C C
rs1355366 A A
rs6444724 T C
rs2046361 A A
rs279844 A T
rs6811238 T G

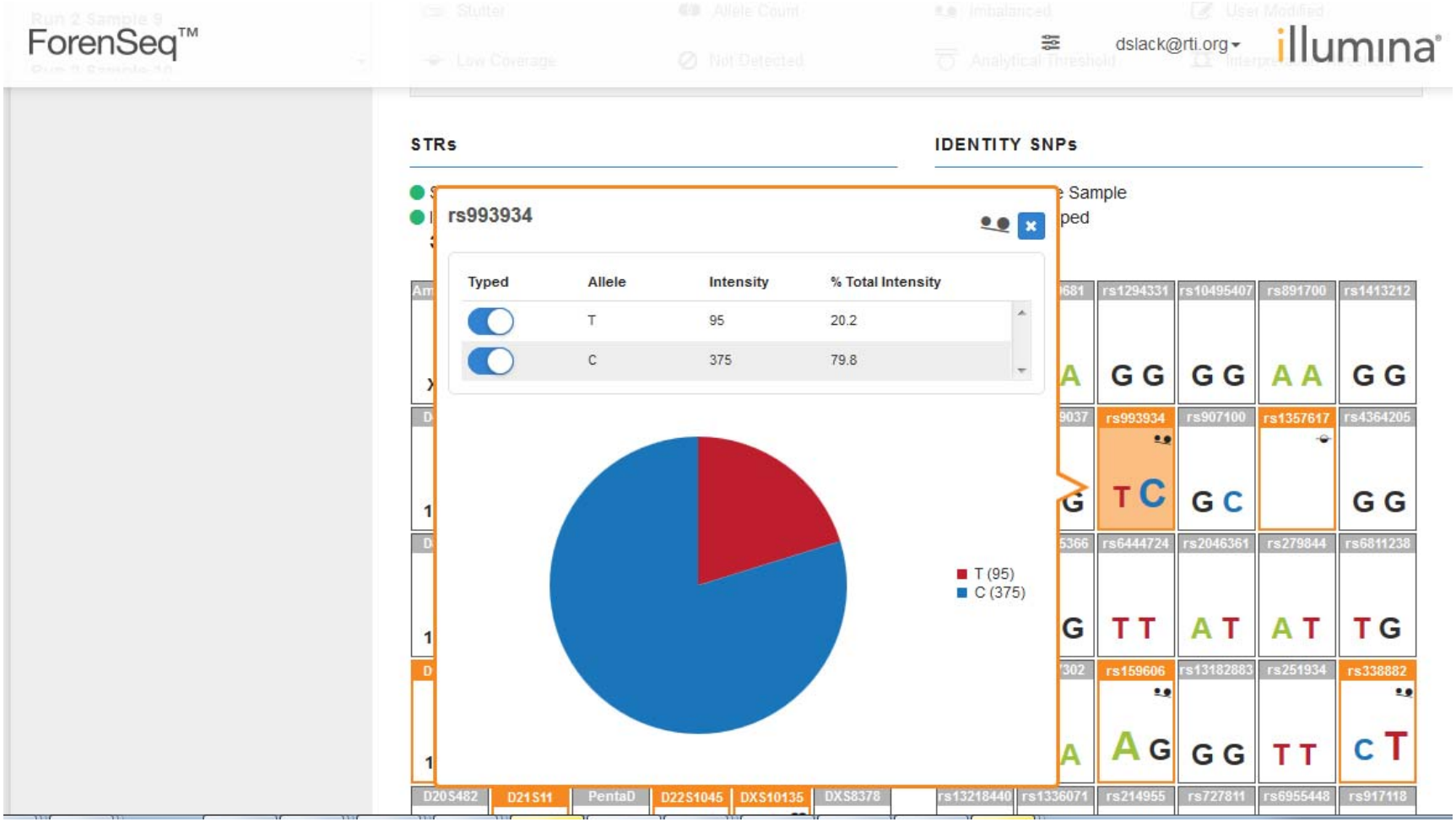

ForenSeq™
dslack@rti.org
illumina
12 13
10 12
11 13
11 14
12 12
14 14
D20S482
D21S11
PentaD
D22S1045
DXS10135
DXS8378
14 15
29 32.2
9 11
15 16
19
11
DXS7132
DXS10074
DXS10103
HPRTB
DXS7423
DYS505
13
17
19
12
15
12
DYS570
DYS576
DYS522
DYS481
DYS19
DYS391
17
18
10
22
14
11
DYS635
DYS437
DYS439
DYS389I
DYS389II
DYS438
25
15
12
12
28
13
DYS612
DYS390
DYS643
DYS533
Y-GATA-H4
DYS385a-b
11 17
C C
G A
A A
G G
T C
T T
rs13218440
rs1336071
rs214955
rs727811
rs6955448
rs917118
G A
A A
A A
A A
C C
C T
rs321198
rs737681
rs763869
rs10092491
rs2056277
rs4606077
C C
T C
T T
C C
C T
T C
rs1015250
rs7041158
rs1463729
rs1360288
rs10776839
rs826472
G G
C C
G A
C T
G T
C C
rs735155
rs3780962
rs740598
rs964681
rs1498553
rs901398
G A
T C
G A
T C
C C
C T
rs10488710
rs2076848
rs2107612
rs2269355
rs2920816
rs2111980
G C
T A
G A
G G
G G


ForenSeq™
STRs
Single Source Sample
Interlocus Balance
35/59 Loci Typed
Amelogeni X X
D1S1656 11 15.3
TPOX 8 9
D2S441 11 12
D2S1338 21 24
D3S1358 14 18
D4S2408 10 10
FGA 18 22
D5S818 12 13
CSF1PO 13 14
D6S1043 12 17
D7S820 9 12
D8S1179 13 14
D9S1122 11 12
D10S1248 13 16
TH01 5 9
vWA 16 16
D12S391 17 20
D13S317 11 12
PentaE 10 20
D16S539 11 14
D17S1301 11 13 +1 allele
D18S51 14 16
D19S433 14 16
D20S482 14 14
D21S11 28 30.2
PentaD 9 14
D22S1045 15 17
DXS10135 25 36 +2 alleles
DXS8378 11 14
D7S820
Typed
Allele ▲
Intensity
Stutter
Repeat Sequence
9
131
0
GATAGATAGATAGATAG ATAGATAGATAGATAGA TAGACAGATTGATAGTT T
11
23
10.7
GATAGATAGATAGATAG ATAGATAGATAGATAGA
Interpretation Threshold
Analytical Threshold
250
200
150
100
50
0
Intensity (# of Reads)
9
11
12
Allele
G G
G G
G G
A A
C T
C C


ForenSeq™
vWA
Typed
Allele ▲
Intensity
Stutter
Repeat Sequence
16
311
0
TCTATCTGTCTGTCTGTC
TGTCTATCTATCTATCTAT
CTATCTATCTATCTATCTA
TCTATCTATCCATCTA
16
204
0
TCTATCTGTCTGTCTGTC
TATCTATCTATCTATCTAT
CTATCTATCTATCTATCTA
Interpretation Threshold
Analytical Threshold
Intensity (# of Reads)
600
500
400
300
200
100
0
15
16
Allele
D4S2408
FGA
D5S818
CSF1PO
D6S1043
D8S1179
D9S1122
D10S1248
TH01
vWA
D13S317
PentaE
D16S539
D17S1301
D18S51
D20S482
D21S11
PentaD
D22S1045
DXS10135
DXS7132
DXS10074
DXS10103
HPRTB
DXS7423
TT
TC
CC
TT
TT
TC


ForenSeq™
dslack@rti.org
illumina
PROJECT: 2ND EXAMPLE PROJE
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
Run 2 Sample 1
Run 2 Sample 2
Run 2 Sample 3
Run 2 Sample 4
Run 2 Sample 5
Run 2 Sample 6
Run 2 Sample 7
Run 2 Sample 8
ACTIONS
GENERATE REPORT
RMP Parameters
Population Groups
African American: NIST 1036 U.S. Population Dataset
Asian: NIST 1036 U.S. Population Dataset
Caucasian: NIST 1036 U.S. Population Dataset
Hispanic: NIST 1036 U.S. Population Dataset
GENERATE
0
50
100
150
200
250
300
350
400
450
500
Length (bp)


ForenSeq™
dslack@rti.org
illumina
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
Run 2 Sample 1
Run 2 Sample 2
Run 2 Sample 3
Run 2 Sample 4
Run 2 Sample 5
Run 2 Sample 6
Run 2 Sample 7
Run 2 Sample 8
Run 2 Sample 9
Run 2 Sample 3 (R707 - A505)
PRIMARY REVIEW
ACTIONS
GENERATE REPORT
Gender: XX • Sample Type: Sample • Mix: B • Description
STR SNP
Intensity (# of Reads)
3000
2500
2000
1500
1000
500
0
0 50 100 150 200 250 300 350 400 450 500
Length (bp)
Stutter
Allele Count
Imbalanced
User Modified
Low Coverage
Not Detected
Analytical Threshold
Interpretation Threshold


ForenSeq™
dslack@rti.org
illumina
STRs
IDENTITY SNPs
rs993934
Typed
Allele
Intensity
% Total Intensity
T
95
20.2
C
375
79.8
T (95)
C (375)
rs1294331
rs10495407
rs891700
rs1413212
rs993934
rs907100
rs1357617
rs4364205
rs6444724
rs2046361
rs279844
rs6811238
rs159606
rs13182883
rs251934
rs338882
D20S482
D21S11
PentaD
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DXS10135
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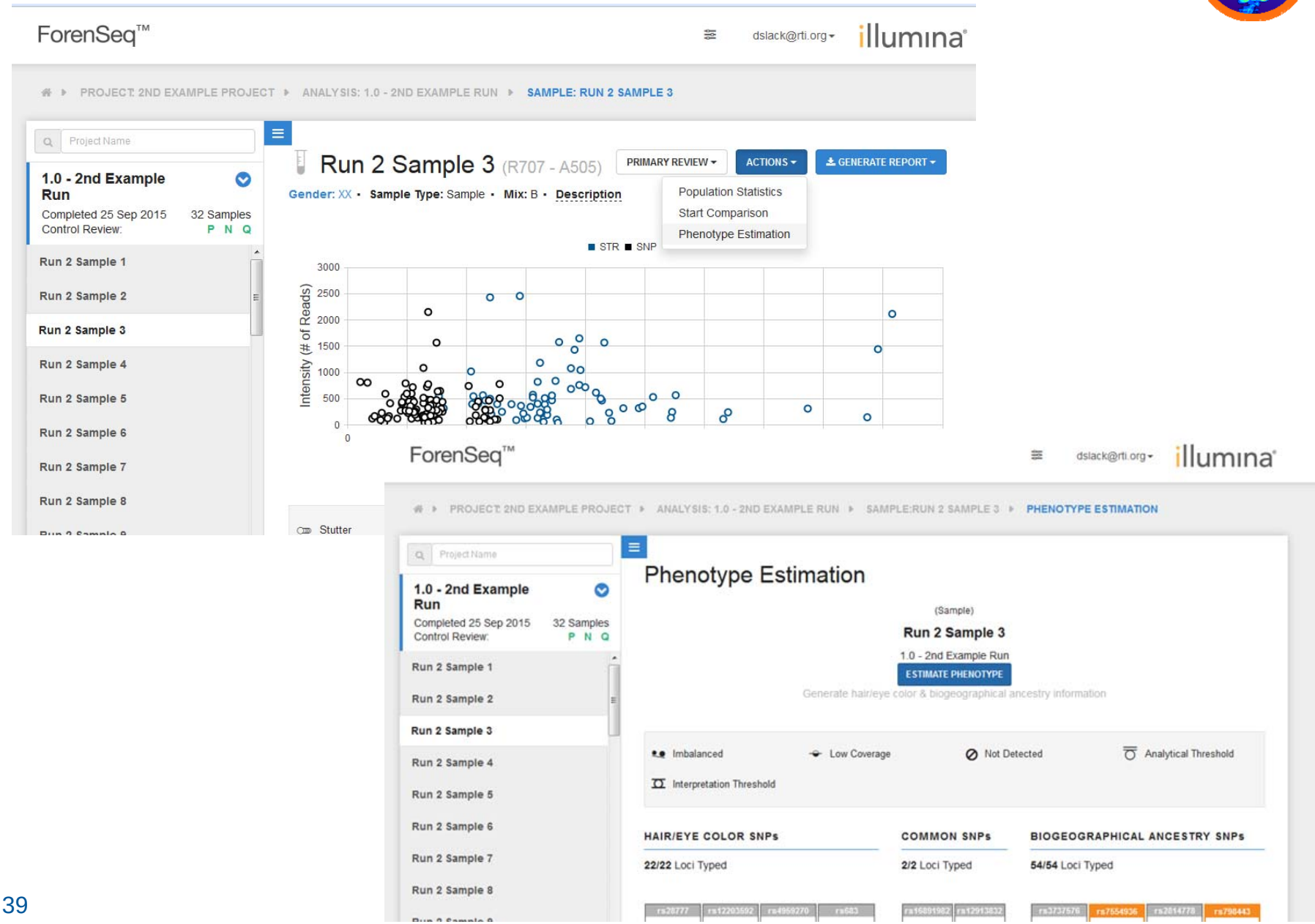
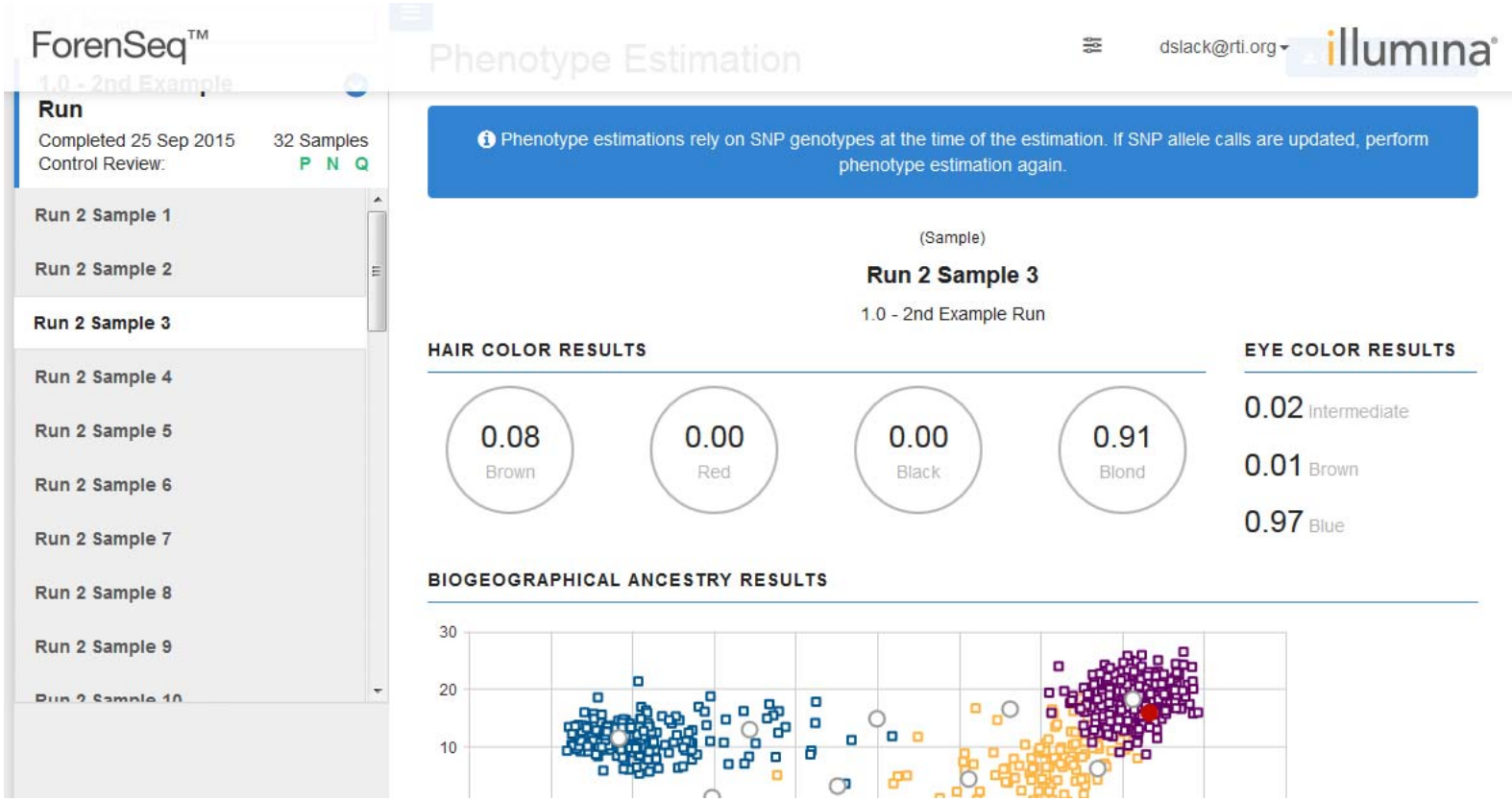
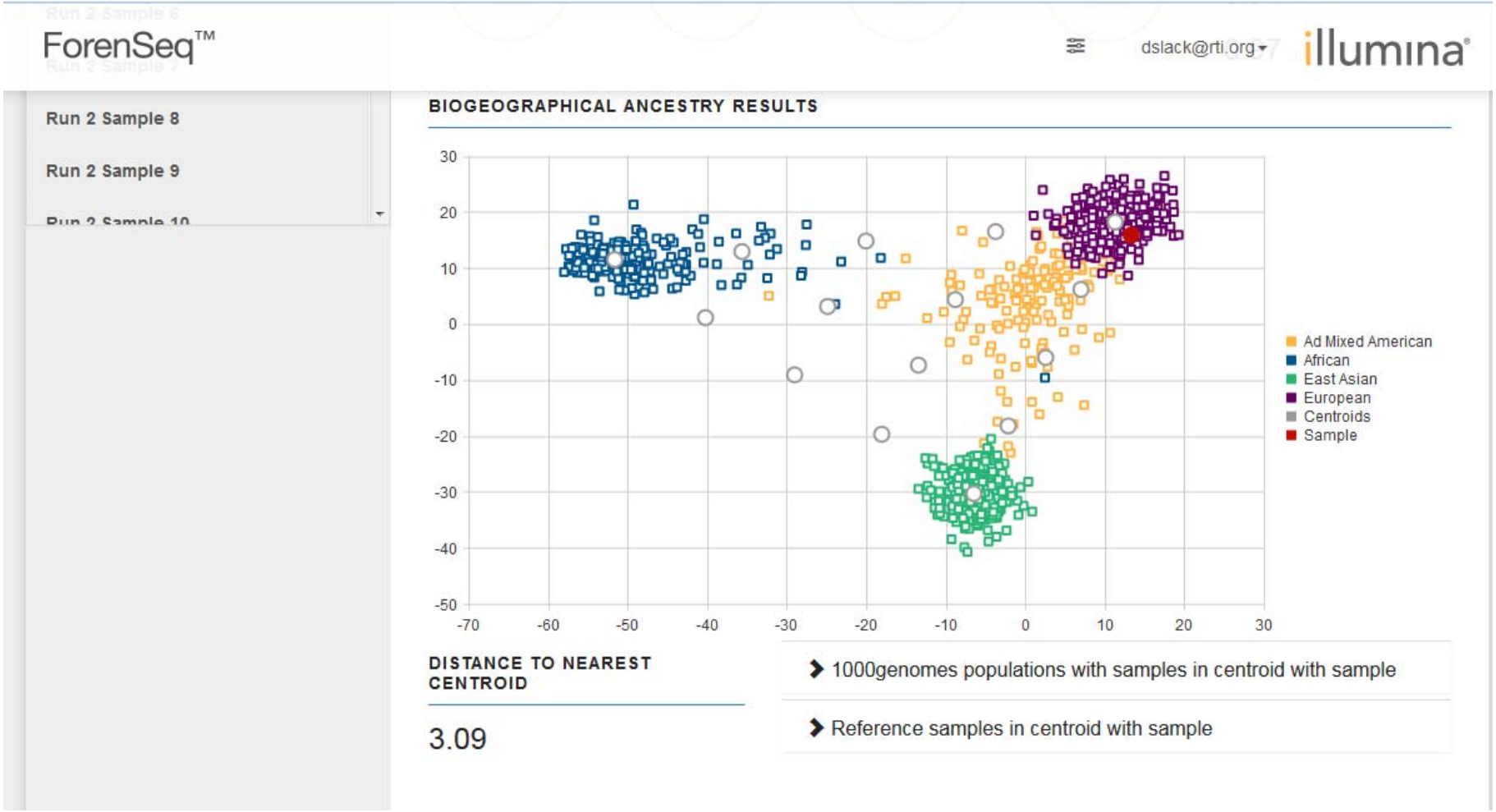
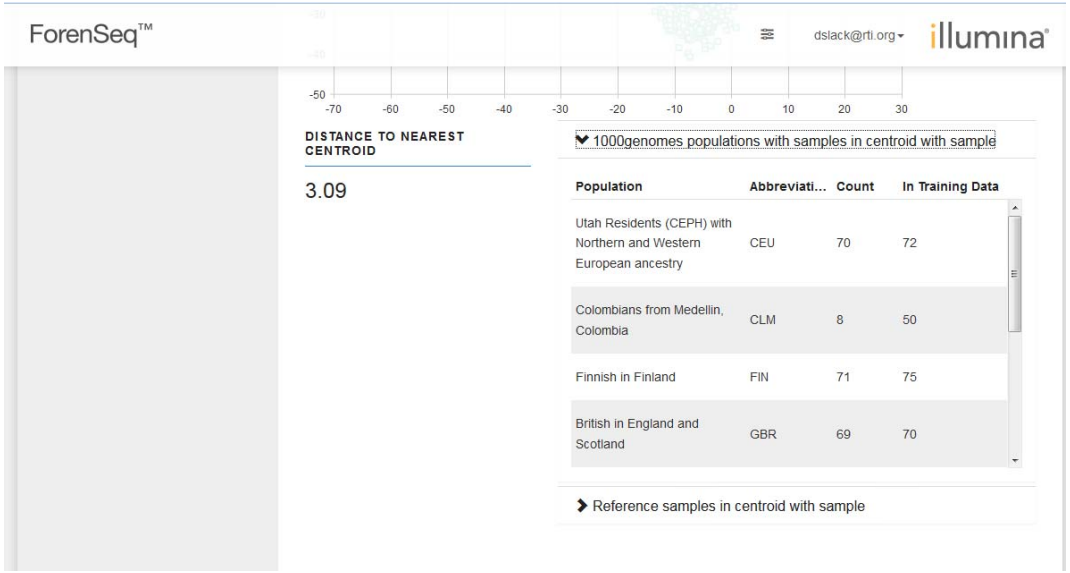
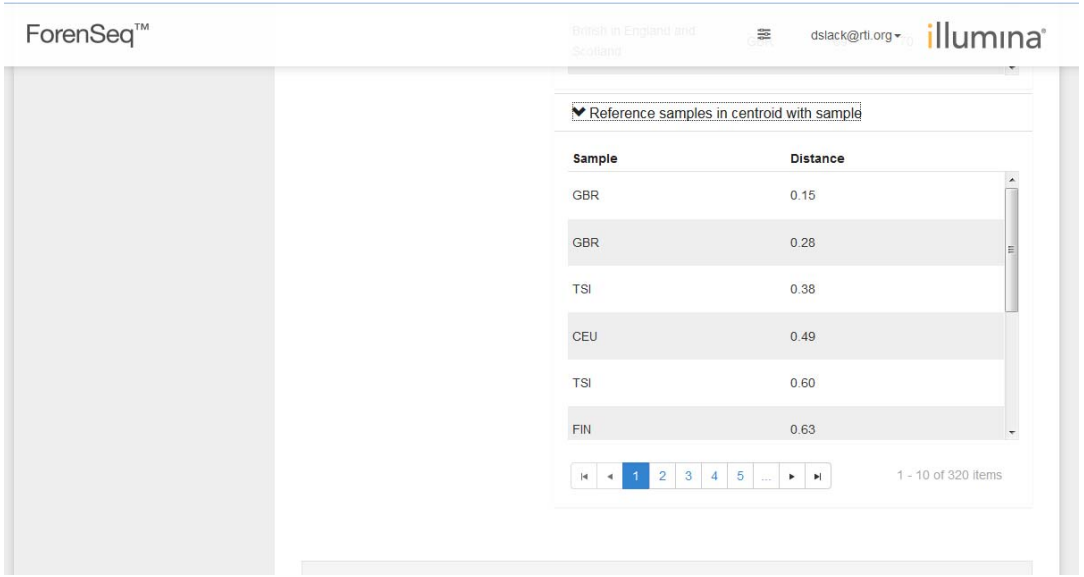
rs1336071
rs214955
rs727811
rs6955448
rs917118


ForenSeq™
dslack@rti.org
illumina
PROJECT: 2ND EXAMPLE PROJECT
ANALYSIS: 1.0 - 2ND EXAMPLE RUN
SAMPLE: RUN 2 SAMPLE 3
Project Name
1.0 - 2nd Example Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
Run 2 Sample 1
Run 2 Sample 2
Run 2 Sample 3
Run 2 Sample 4
Run 2 Sample 5
Run 2 Sample 6
Run 2 Sample 7
Run 2 Sample 8
Run 2 Sample 3 (R707 - A505)
PRIMARY REVIEW
ACTIONS
GENERATE REPORT
Gender: XX • Sample Type: Sample • Mix: B • Description
Population Statistics
Start Comparison
Phenotype Estimation
STR SNP
Intensity (# of Reads)
3000
2500
2000
1500
1000
500
0
Stutter
PROJECT: 2ND EXAMPLE PROJECT
ANALYSIS: 1.0 - 2ND EXAMPLE RUN
SAMPLE:RUN 2 SAMPLE 3
PHENOTYPE ESTIMATION
Phenotype Estimation
(Sample)
Run 2 Sample 3
1.0 - 2nd Example Run
ESTIMATE PHENOTYPE
Generate hair/eye color & biogeographical ancestry information
Imbalanced
Low Coverage
Not Detected
Analytical Threshold
Interpretation Threshold
HAIR/EYE COLOR SNPs
22/22 Loci Typed
COMMON SNPs
2/2 Loci Typed
BIOGEOGRAPHICAL ANCESTRY SNPs
54/54 Loci Typed


ForenSeq™
Phenotype Estimation
dslack@rti.org
illumina
Run
Completed 25 Sep 2015
32 Samples
Control Review:
P N Q
Run 2 Sample 1
Run 2 Sample 2
Run 2 Sample 3
Run 2 Sample 4
Run 2 Sample 5
Run 2 Sample 6
Run 2 Sample 7
Run 2 Sample 8
Run 2 Sample 9
Phenotype estimations rely on SNP genotypes at the time of the estimation. If SNP allele calls are updated, perform phenotype estimation again.
(Sample)
Run 2 Sample 3
1.0 - 2nd Example Run
HAIR COLOR RESULTS
0.08 Brown
0.00 Red
0.00 Black
0.91 Blond
EYE COLOR RESULTS
0.02 Intermediate
0.01 Brown
0.97 Blue
BIOGEOGRAPHICAL ANCESTRY RESULTS
30
20
10


ForenSeq™
dslack@rti.org
illumina
Run 2 Sample 8
Run 2 Sample 9
BIOGEOGRAPHICAL ANCESTRY RESULTS
30
20
10
0
-10
-20
-30
-40
-50
-70
-60
-50
-40
-30
-20
-10
0
10
20
30
Ad Mixed American
African
East Asian
European
Centroids
Sample
DISTANCE TO NEAREST CENTROID
3.09
1000genomes populations with samples in centroid with sample
Reference samples in centroid with sample


ForenSeq™
dslack@rti.org
illumina
-70 -60 -50 -40 -30 -20 -10 0 10 20 30
DISTANCE TO NEAREST CENTROID
3.09
1000genomes populations with samples in centroid with sample
Population
Abbreviati...
Count
In Training Data
Utah Residents (CEPH) with Northern and Western European ancestry
CEU
70
72
Colombians from Medellin, Colombia
CLM
8
50
Finnish in Finland
FIN
71
75
British in England and Scotland
GBR
69
70
Reference samples in centroid with sample


ForenSeq™
dslack@rti.org
illumina
Reference samples in centroid with sample
Sample
Distance
GBR
0.15
GBR
0.28
TSI
0.38
CEU
0.49
TSI
0.60
FIN
0.63
1 2 3 4 5 ...
1 - 10 of 320 items