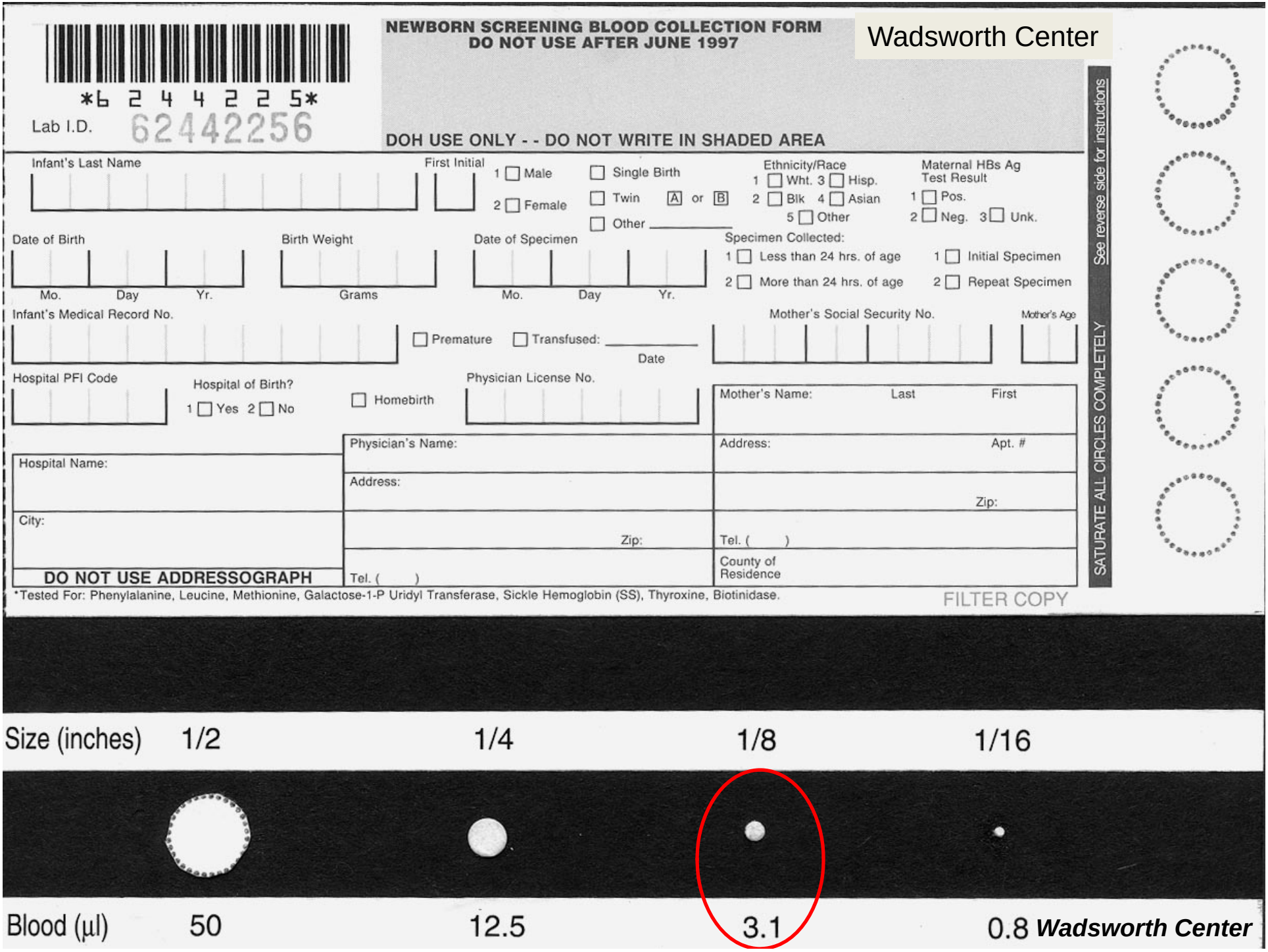
Application of NextGen Sequencing (Ion Torrent) for Second Tier SCID screening in New York State

Carlos A. Saavedra-Matiz, M.D.
Newborn Screening Program
Wadsworth Center
New York State Department of Health

Feliz cumpleaños Tamizaje Neonatal!

Allison Young, Beth Vogel,
Derek Symula, Michele Caggana
NYSDOH

May 8, 2013
NBS&GTS&ISNS
APHL/ISNS/CDC/GSPHL


NEWBORN SCREENING BLOOD COLLECTION FORM
DO NOT USE AFTER JUNE 1997
Wadsworth Center
6 2 4 4 2 2 5
Lab I.D. 62442256
DOH USE ONLY - - DO NOT WRITE IN SHADED AREA
Infant's Last Name
First Initial
1 Male
2 Female
Single Birth
Twin A or B
Other
Ethnicity/Race
1 Wht. 3 Hisp.
2 Blk 4 Asian
5 Other
Maternal HBs Ag Test Result
1 Pos.
2 Neg. 3 Unk.
Date of Birth
Mo. Day Yr.
Birth Weight
Grams
Date of Specimen
Mo. Day Yr.
Specimen Collected:
1 Less than 24 hrs. of age
2 More than 24 hrs. of age
1 Initial Specimen
2 Repeat Specimen
Infant's Medical Record No.
Premature
Transfused:
Date
Mother's Social Security No.
Mother's Age
Hospital PFI Code
Hospital of Birth?
1 Yes 2 No
Homebirth
Physician License No.
Mother's Name: Last First
Hospital Name:
Physician's Name:
Address:
Apt. #
Zip:
City:
Tel. ( )
DO NOT USE ADDRESSOGRAPH
County of Residence
*Tested For: Phenylalanine, Leucine, Methionine, Galactose-1-P Uridyl Transferase, Sickle Hemoglobin (SS), Thyroxine, Biotinidase.
FILTER COPY
See reverse side for instructions
SATURATE ALL CIRCLES COMPLETELY
Size (inches) 1/2 1/4 1/8 1/16
Blood (µl) 50 12.5 3.1 0.8 Wadsworth Center

Clin Chem 59:7 (2013) Mar 18. [Epub ahead of print] doi: 10.1373/clinchem.2012.198945

Cost-Effective and Scalable DNA Extraction Method from Dried Blood Spots.

Saavedra-Matiz CA, Isabelle JT, Biski CK, Duva SJ, Sweeney ML, Parker AL, Young AJ, Diantonio LL, Krein LM, Nichols MJ, Caggana M.

Source

Newborn Screening Program, Division of Genetics, Wadsworth Center, New York State Department of Health, Albany, NY.

Abstract

BACKGROUND: Dried blood spot (DBS) samples have been widely used in newborn screening (NBS) for the early identification of disease to facilitate the presymptomatic treatment of congenital diseases in newborns. As molecular genetics knowledge and technology progresses, there is an increased demand on NBS programs for molecular testing and a need to establish reliable, low-cost methods to perform those analyses. Here we report a flexible, cost-efficient, high-throughput DNA extraction method from DBS adaptable to small- and large-scale screening settings.METHODS: Genomic DNA (g.DNA) was extracted from single 3-mm diameter DBS by the sequential use of red cell lysis, detergent-alkaline, and acid-neutralizing buffers routinely used in whole blood and plant tissue DNA extractions. We performed PCR amplification of several genomic regions using standard PCR conditions and detection methods (agarose gel, melting-curve analysis, TaqMan-based assays). Amplicons were confirmed by BigDye® Terminator cycle sequencing and compared with reference sequences.RESULTS: High-quality g.DNA was extracted from hundreds of DBS, as proven by mutation detection of several human genes on multiple platforms. Manual and automated extraction protocols were validated. Quantification of g.DNA by Oligreen® fluorescent nucleic acid stain demonstrated a normal population distribution closely corresponding with white blood cell counts detected in newborn populations.CONCLUSIONS: High-quality, amplifiable g.DNA is extractable from DBSs. Our method is adaptable, reliable, and scalable to low- and high-throughput NBS at low cost ($0.10/sample). This method is routinely used for molecular testing in the New York State NBS program.

Clin Chem 59:7 (2013) Mar 25. [Epub ahead of print]doi:1373/clinchem.2013.205864 **Editorials**

Newborn Screening by Sequence and the Road Ahead.

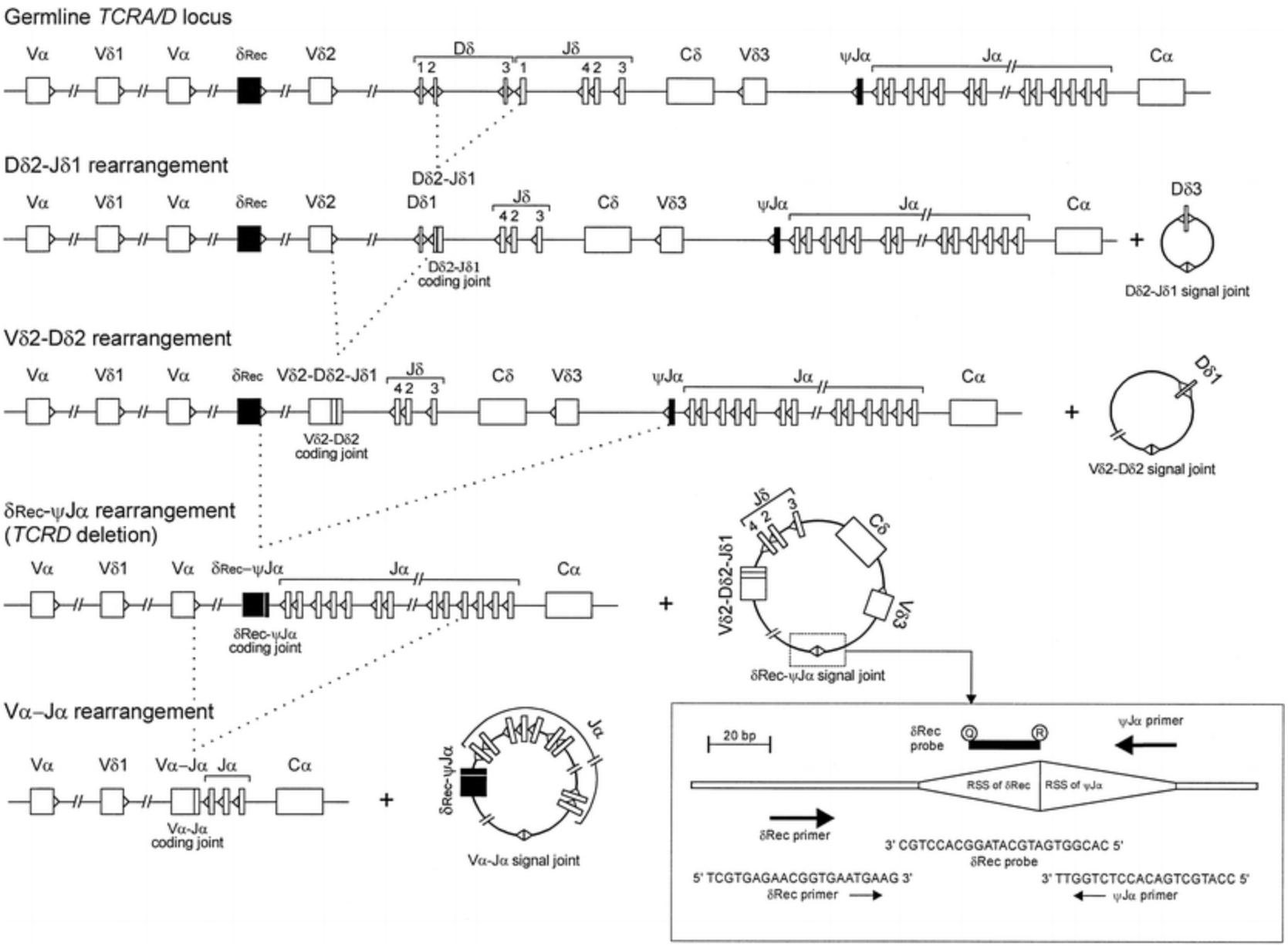
Sondheimer N.

Source

Department of Pediatrics, University of Pennsylvania, and Section of Biochemical Genetics, Children's Hospital Philadelphia, Philadelphia, PA.

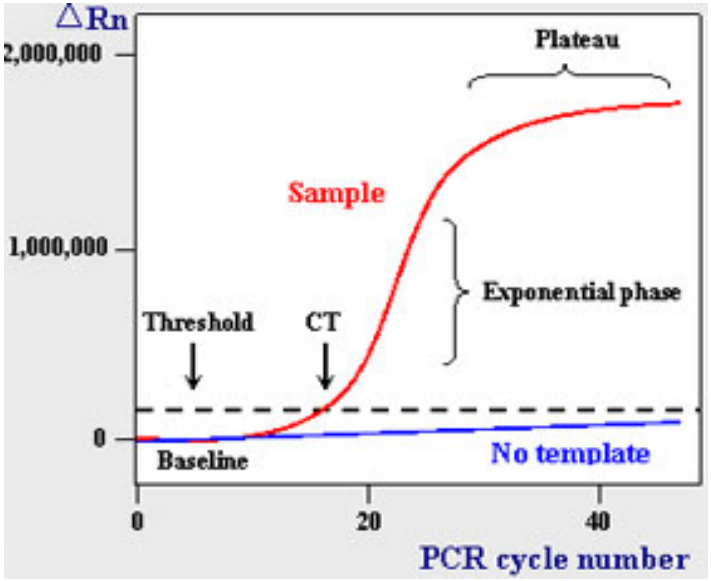
SCID: Severe **C**ombined **I**mmuno**d**eficiency

- Heterogeneous group of genetic disorders
- Defects of cellular and humoral immune responses
- Absence or very low T-cell counts
- Incidence : 1 / 40,000-75,000 newborns
- Clinical symptoms: first months of life (median age at Dx 4-7 months)
- Multiple severe viral, bacterial and fungal infections after birth
- Often fatal within first year of life
- Survival rate is 94% when treated with HSCT by 3.5 months of age
- TREC (T-cell Receptor Excision Circles) are DNA by-products of T-cell maturation in the thymus
- Low or absent TRECs in DBS likely indicates T-cell deficiency

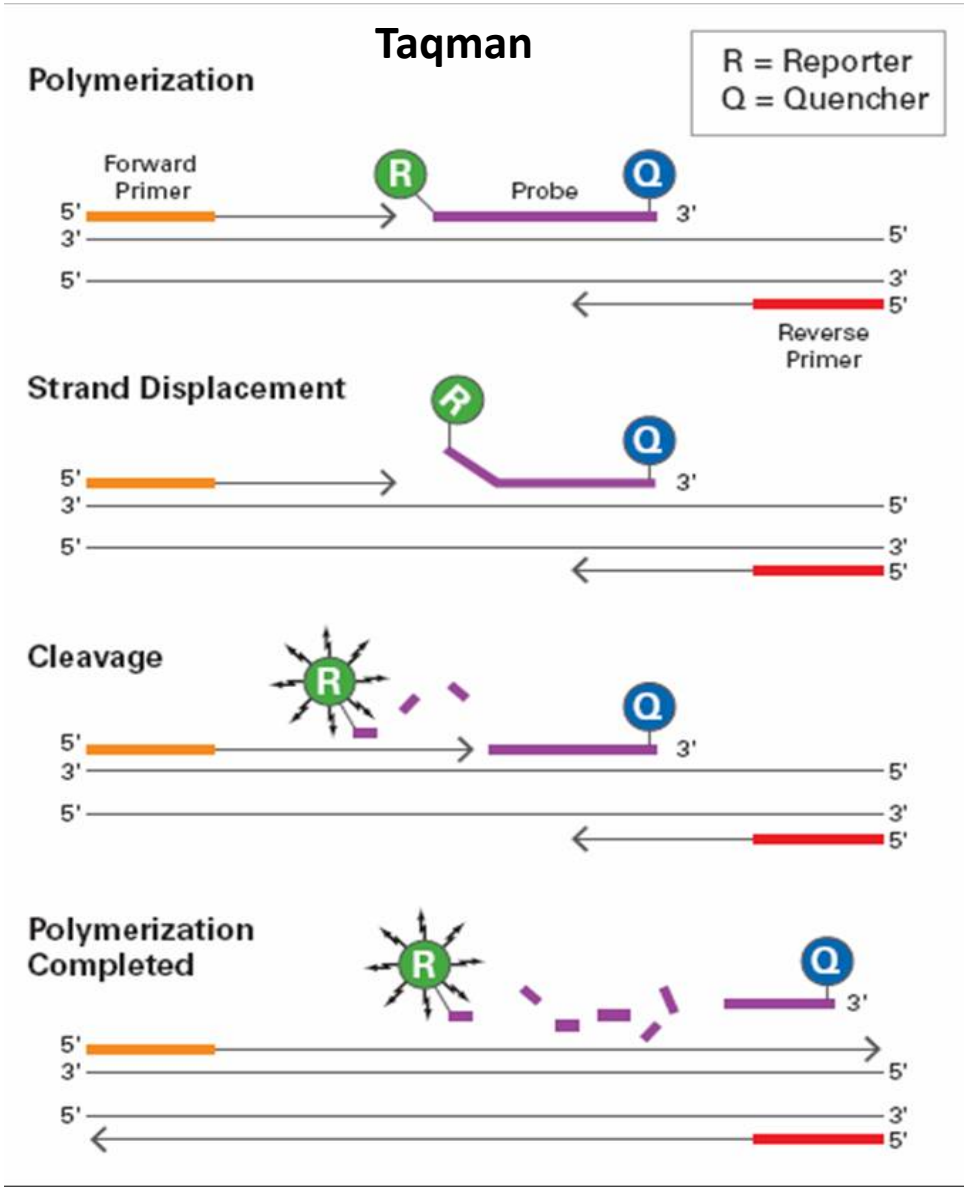


Hazenberg et al. J Mol Med (2001)79:631-40

Real Time PCR

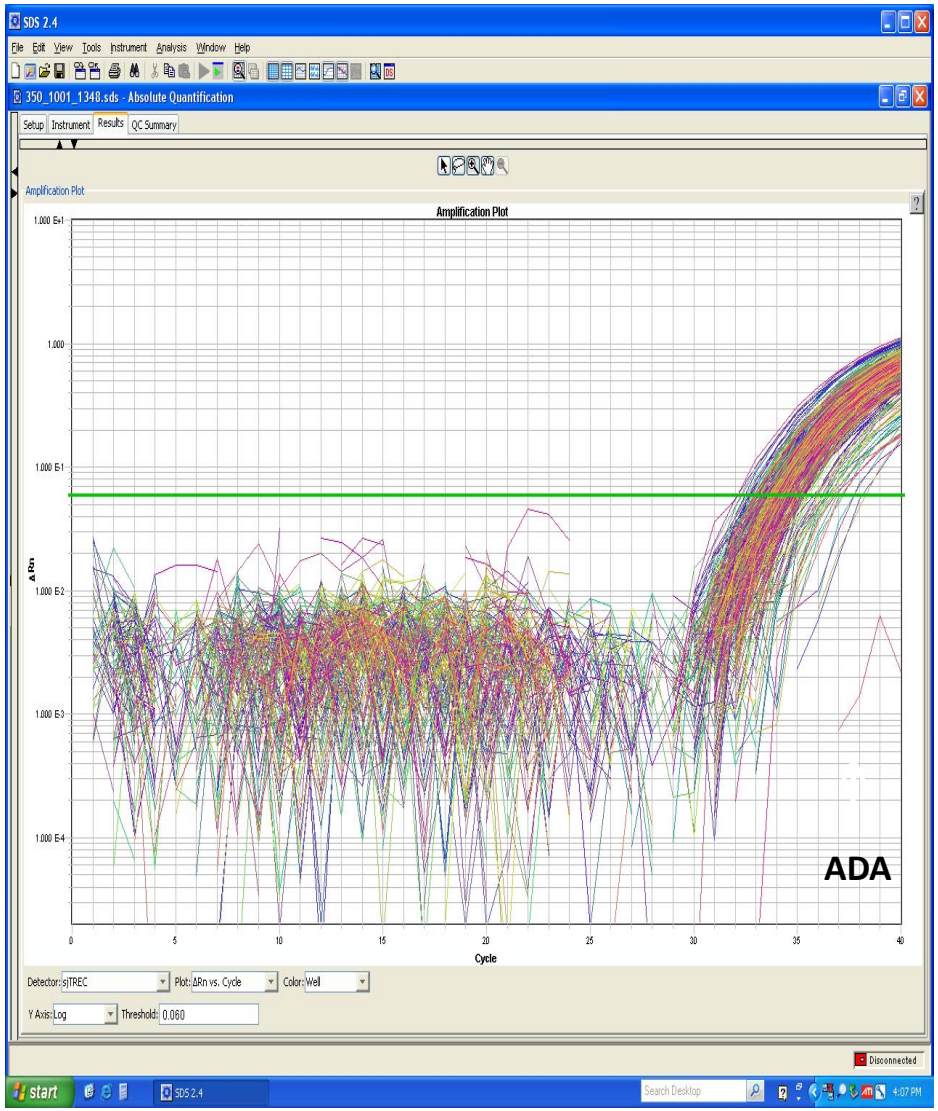


- Reporter/Quencher
- 5’ Nuclease Activity
- Probe Cleavage
- Sequence Specific
- Multiplex Capability

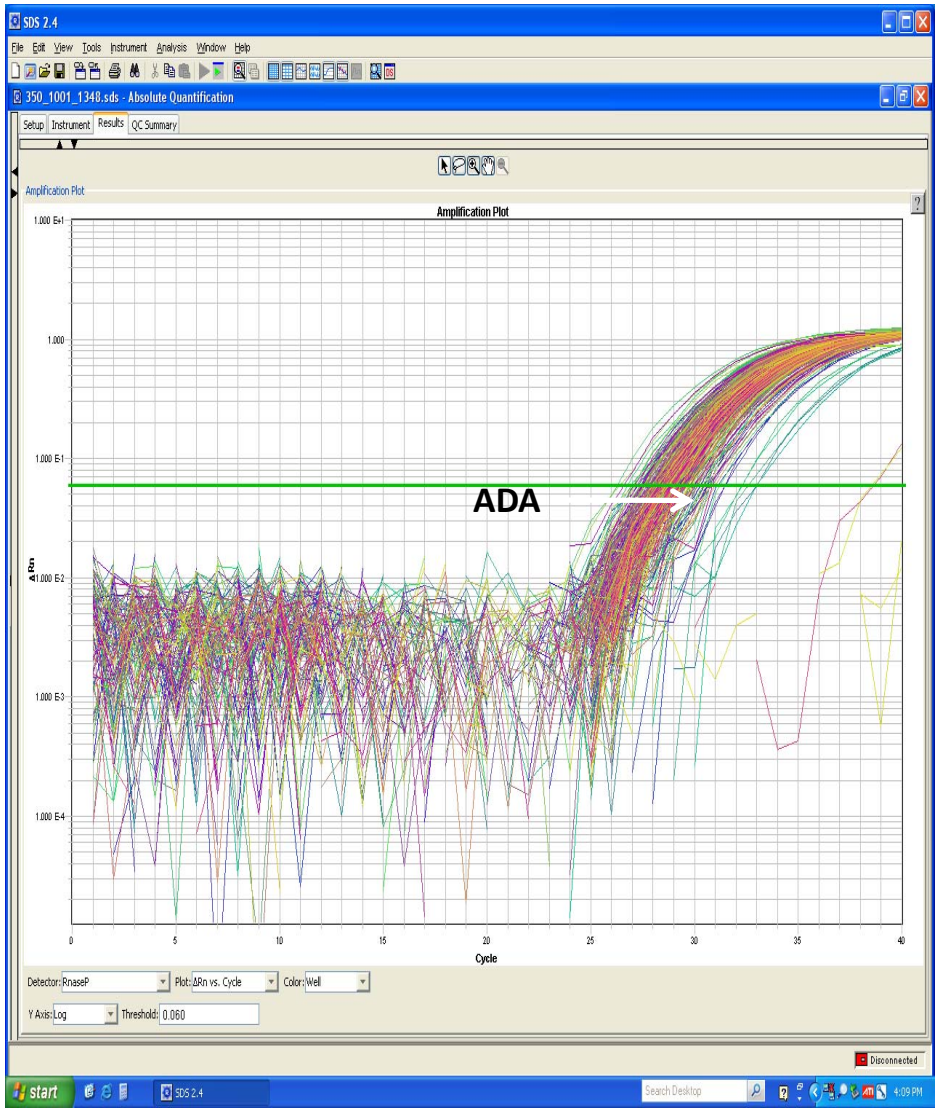


Duplex Amplification Plots

TREC Amplification plot



Rnase p amplification plot



NYS has been performing NBS for SCID since September 2010 using the TREC assay

- More than 500,000 samples screened
- > 500 referred for diagnostic evaluation
- > 90 babies with clinically significant laboratory abnormality
    - Classic SCID
    - Idiopathic T-cell lymphopenia
    - Syndrome with T-cell impairment
    - Secondary T-cell lymphopenia

- Screening does not provide information on the molecular defect
- Gene testing is requested by providers based on CBC and cellular phenotype (flow cytometry)
- Slow process (individual genes) & More than 20 known SCID associated genes

NEWBORN SCREENING PROGRAM
New York State Department of Health
Wadsworth Center, Biggs Laboratory, P.O. Box 509
Albany, NY 12201-0509
Phone: (518)473-7552 Fax: (518) 474-0405
E-mail: nbsinfo@health.state.ny.us
Website: http://www.wadsworth.org/newborn/

PRIMARY CARE PROVIDER
<<phyfname>> <<phylname>>
<<phyadd1>>
<<phyadd2>>
<<phycity>>, <<phystate>> <<phyzip>>

<<date>>

NEWBORN INFORMATION
Name: <<last>>, <<sex>> <<twinstat>>
AKA: <<casaka>>
Date of Birth: <<birthdt>>
Birth Weight: <<weight>>
Mother: <<mfirst>> <<mlast>>
Tel.: <<phone>>
Initial Accession : <<patnum>>
Hospital: <<hname>>
Medical Record No.: <<medrecno>>

SEVERE COMBINED IMMUNODEFICIENCY DIAGNOSTIC FORM

Please complete this form in its entirety and return it to the Newborn Screening Program as soon as possible. Your response is required, as specified in Title 10 New York Code of Rules and Regulations subpart 69-1.5e.

Note: Newborn Screening results do not constitute a diagnosis. Confirmatory testing is required.

PLEASE INDICATE A DIAGNOSIS:

- ❑ No Evidence of immune dysfunction
- ❑ Severe combined immunodeficiency, specify gene and mutations, if Available_____
- ❑ Idiopathic T cell lymphopenia
- ❑ DiGeorge syndrome
- ❑ Other, specify_____

❑ **PLEASE COMPLETE OR CHECK BOX IF CONFIRMATORY TESTING IS ATTACHED**

CBC WITH DIFFERENTIAL DATE:

	RESULTS	NORMAL RANGE
WBC		
RBC		
HgB		
Hct		
Platelet Ct.		
ABS Neutrophils		
ABS Lymphocytes		
ABS Monocytes		
ABS Eosinophils		
ABS Basophils		

FLOW CYTOMETRY DATE:

	RESULTS	NORMAL RANGE
% T cells(CD3)		
% B cells(CD19)		
% NK cells(CD16CD56)		
% Helper cells(CD4)		
% Suppressor cells(CD8)		
Total lymphocytes(CD45)		
ABS T cells(CD3)		
ABS B cells(CD19)		
ABS NK cells(CD16CD56)		
ABS Helper cells(CD4)		
ABS Suppressor cells(CD8)		

Was the patient referred for transplant evaluation? □Yes □No
Where were they referred? _____

MITOGENS DATE: _____ □Normal □Abnormal
PHA:□S.I.□%NC Results _____Normal Range_____
ConA:□S.I.□%NC Results _____Normal Range_____
PWM:□S.I.□%NC Results _____Normal Range_____

Comments: _____

Physician signature: _____ **Date:** _____

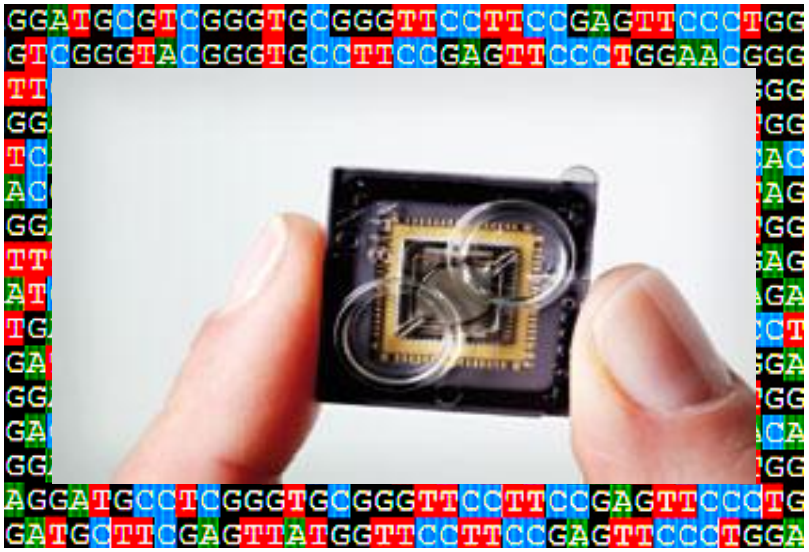
Print Name_____ **Facility/practice:** _____

SPECIALTY CARE CENTER
<<scidcfname>> <<scidclname>>
<<scidcdesc1>>
<<scidcdesc2>>
<<scidcstreet>>
<<scidccity>>, <<scidcstate>> <<scidczip>>

HOSPITAL OF BIRTH DESIGNEE
<<dsgimdname>>
<<dsgimdtle>>
<<hname>>
<<hstreet>>
<<hcity>>, <<hstate>> <<hzip>>

LESC30

An integrated semiconductor device enabling non-optical genome sequencing



JM Rothberg *et al. Nature* **475**, 348-352 (2011) doi:10.1038/nature10242

nature

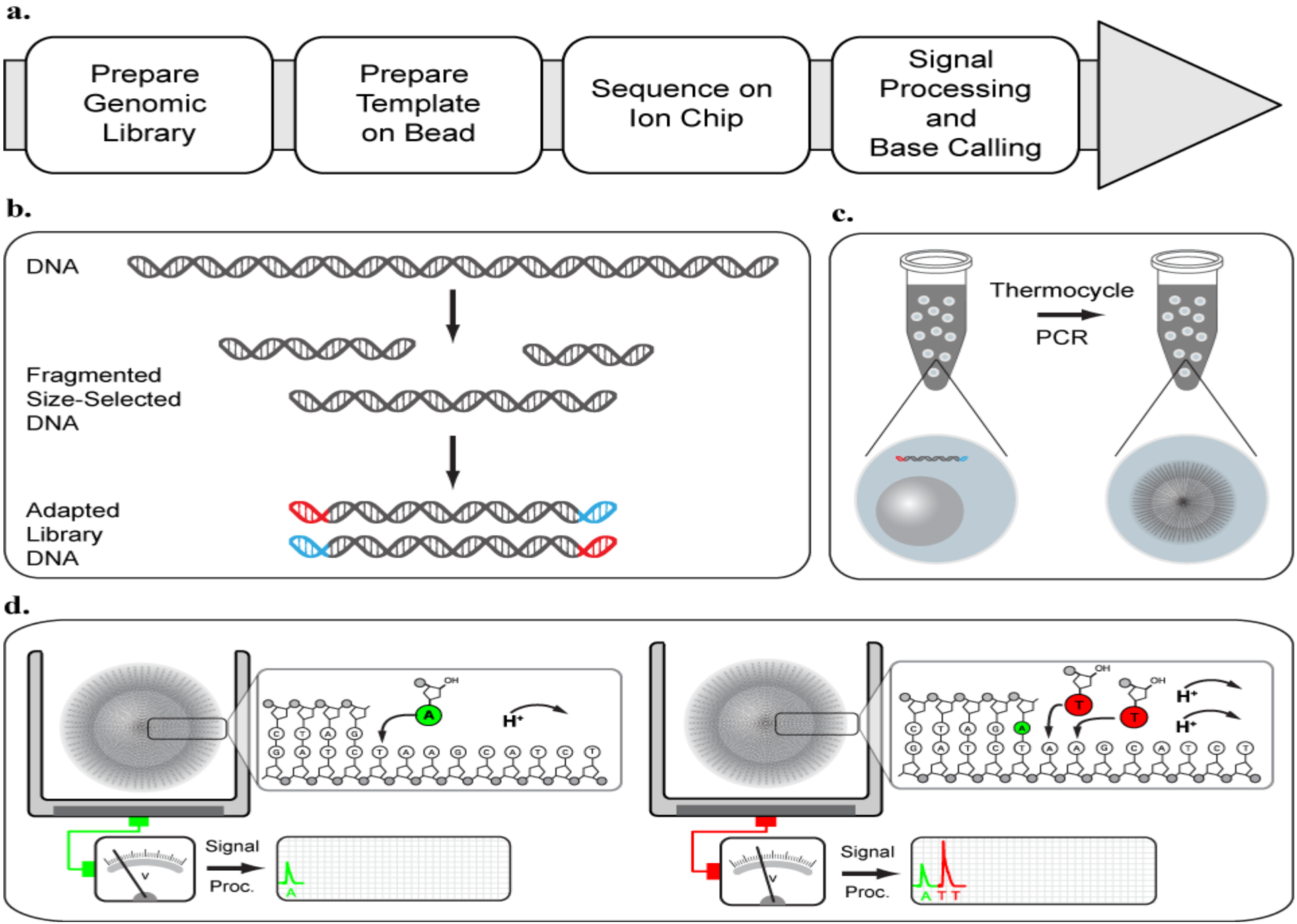


Figure S1 Process overview

The Ion Torrent Personal Genome Machine (PGM™) sequencer enables researchers to obtain highly accurate sequence in record time.

Ion Semiconductor Sequencing Chip	Output	Read Length		Total Sequencing Time
314	> 10Mb	2011	2012	< 2 hours
316	> 100Mb	> 200bp	> 400bp	
318	> 1Gb			
Accuracy:	>99.99% consensus accuracy and >99.5% raw accuracy.			

Gene	Gene c.DNA Pos	Ref Pos	Amplicon Size	Ref	Ion SNP	Freq 1	Freq 2	Counts 1	Counts 2	Ion Coverage	Sanger SNP	% Concor dance	Comments
MCAD	-1375	4056	702	A	A/G	51.9	48.1	329	305	634	A/G	100	
MCAD	-1028	4403	702	T	T/C	51.1	48.9	505	484	989	T/C	100	
MCAD	-985	4446	702	C	G/C	50.5	49.4	516	505	1021	G/C	100	
MCAD	-725	4703	779	C	G	76.6	23.4	441	135	576	G/C	50	
MCAD	-409	5022	779	G	G/A	58.7	41.3	101	71	172	G	50	Polymorphic region
MCAD	-361	5069	779	T	T				0		T/C	50	
MCAD	-257	5174	779	G	A	82.3	17.7	345	74	419	A/G	50	
MCAD	30+67	5527	453	C	G	72.4	27.6	71	27	98	G/C	50	
MCAD	708+79	26637	564	A	A/C	62.0	38.0	88	54	142	A	50	Primer Region
MCAD	708+85	26643	564	T	T/A	60.6	39.4	83	54	137	T	50	Primer Region
MCAD	1161	41980	401	A	A/G	53.6	46.1	207	178	385	A/G	100	p.V387V
MCAD	1184	42003	401	A	A/G	52.3	47.5	232	211	443	A/G	100	p.K395R
GALC	-196	5252	930	T	C/T	58.6	41.4	99	70	169	C/T	100	
GALC	-7	5441	930	G	G/C	52.0	48.0	90	83	173	G/C	100	
GALC	13	5460	312	G	G/C	52.2	47.0	60	54	114	G/C	100	p.A5P
GALC	27	5474	312	C	A/C	62.2	36.7	61	36	97	A/C	100	p.G9G
GALC	148-70	9971	357	G	A/G	55.9	44.1	66	52	118	G	50	Polymorphic region
GALC	148-43	9998	357	C	T	100.0		178		178	C	0	Polymorphic region
GALC	281-35	11927	344	G	G/A	55.4	44.6	128	103	231	G/A	100	
GALC	286	11967	344	A	A/G	50.0	50.0	126	126	252	A/G	100	p.T96A
GALC	301	11982	344	A	G/A	52.5	47.5	124	112	236	G/A	100	p.M101V
GALC	395-51	13980	421	G	A/G	51.2	48.8	22	21	43	A/G	100	
GALC	534+89	14269	421	C	C/T	60.5	39.5	46	30	76	C/T	100	
GALC	694	22196	526	G	A/G	63.6	36.4	7	4	11	A/G	100	p.D232N
GALC	704+56	22262	526	T	T/C	55.6	44.4	5	4	9	T/C	100	
GALC	705-59	30015	564	C	T/C	50.8	49.2	130	126	256	T/C	100	
GALC	1290+23	48742	343	T	C	68.6	31.4	168	77	245	C/T	50	
GALC	1291-61	50625	404	C	C/T	53.4	46.6	127	111	238	C/T	100	
GALC	1572	52961	417	G	G/A	51.7	48.3	138	129	267	G/A	100	P.T524T (Cis-M101V)
GALC	1623-15	56991	416	C	T/C	51.7	48.3	90	84	174	T/C	100	
GALC	1650	57033	416	A	A/T	50.5	49.5	99	97	196	A/T	100	p.V550V
GALC	1786+5	57174	416	C	C/G	58.0	42.0	94	68	162	C/G	100	Cis-M101V
GALC	1873	63695	570	A	G/A	54.9	44.8	197	161	358	G/A	100	p.A625T (Cis-M101V)
VLCAD	3	5154	1560	G	A/G	55.1	44.9	75	61	136	A/G	100	p.M1I
VLCAD	138+99	5463	1560	T	G/T	54.1	45.9	60	51	111	G/T	100	
VLCAD	477+52	6277	1560	A	T	67.9	32.1	55	26	81	A	50	
VLCAD	478-101	6604	1061	A	C	77.5	22.5	79	23	102	C	100	?
VLCAD	623-8	7111	1061	C	T	65.8	34.2	127	66	193	T/C	50	
VLCAD	1600	9555	1040	G	G/A	51.8	48.2	177	165	342	G/A	100	p.E534K
VLCAD	1605+5	9566	1040	T	T/C	51.9	48.1	180	167	347	T/C	100	
PKU	168+19	9831	291	T	C/T	56.4	43.3	774	594	1368	C/T	100	
PKU	353-22	45031	340	C	C/T	50.4	49.6	185	182	367	C/T	100	
PKU	442-48	55892	287	A	G	100.0		211		211	G	100	
PKU	510-54	67217	354	G	A/G	52.2	47.8	565	518	1083	A/G	100	
PKU	696	67457	354	A	A/G	61.4	38.6	784	493	1277	A/G	100	p.Q312Q
PKU	735	69681	423	G	G/A	54.0	46.0	593	505	1098	G/A	100	p.V245V
PKU	1155	78913	264	G	C/G	50.9	49.1	1229	1184	2413	C/G	100	p.L383L
GALT	1-28	5040	413	T	A	99.1		316		316	A	100	
GALT	855	7395	570	G	G/T	53.5	46.1	251	216	467	G/T	100	p.K285N
GALT	959	7827	459	C	C/T	55.3	44.7	198	160	358	C/T	100	p.A320V
GALT	*124	8936	360	A	T	100.0		693		693	T	100	

Designed primers to amplify all exonic and their flanking regions for ADA, IL2RG, IL7RA, RAG1 and RAG2

- PCR for 23 amplicons was standardized to cover promoter and all exonic regions for the 5 genes

- Five different anonymous DBS samples were amplified for all 5 genes

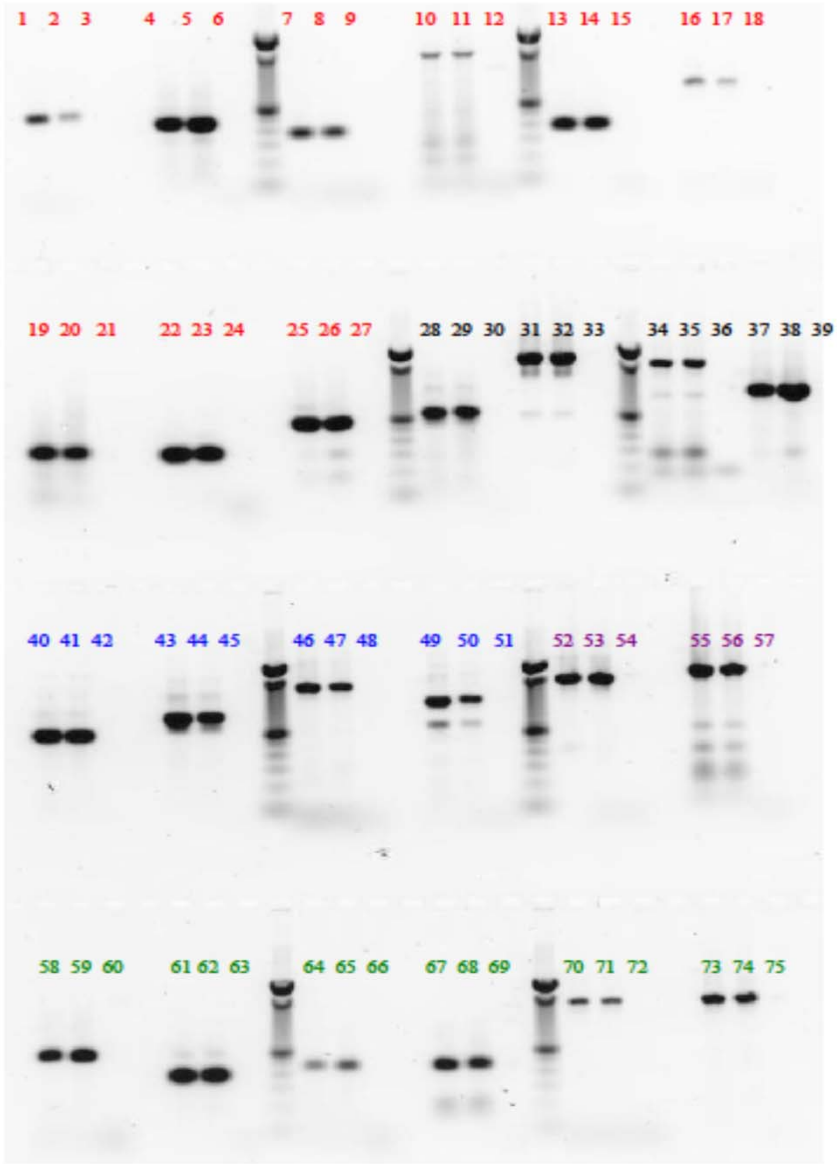
- 5 µL of each amplicon were pooled per sample/individual and sent to our core for "IT Amplicon Sequencing"

- Amplicons were fragmented and barcode adapters ligated (Ion Xpress Plus Fragment Library Kit/Barcode adapters) to generate a library of DNA fragments.

- Pooled barcoded fragments were sequenced on an Ion 314 chip with Ion PGM 200 Sequencing Kit on the Ion Torrent PGM sequencer.

- Data Analysis:
    - DNA-Seq workflow of the Partek Genomics Suite
    - CLC Bio workbench software

SCID Ion Torrent Amplicons



KEY

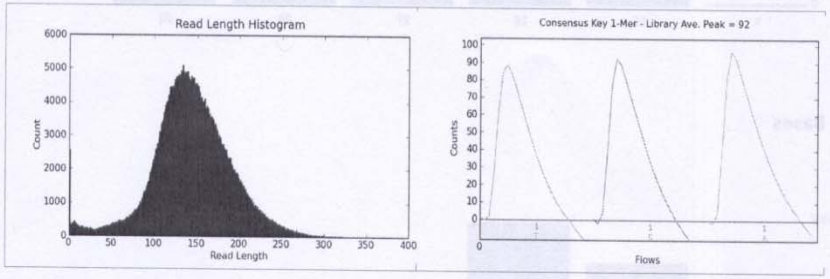
1. ADA_E1a
2. ADA_E1b
3. NTC_E1
4. ADA_E2a
5. ADA_E2b
6. NTC_E2
7. ADA_E3a
8. ADA_E3b
9. NTC_E3
10. ADA_E4-E5a
11. ADA_E4-E5b
12. NTC_E4-E5
13. ADA_E6a
14. ADA_E6b
15. NTC_E6
16. ADA_E7-E9a
17. ADA_E7-E9b
18. NTC_E7-E9
19. ADA_E10a
20. ADA_E10b
21. NTC_E10
22. ADA_E11a
23. ADA_E11B
24. NTC_E11
25. ADA_E12a
26. ADA_E12b
27. NTC_E12
28. RAG1_E1a
29. RAG1_E1b
30. NTC_E1
31. RAG1_E2Aa
32. RAG1_E2Ab
33. NTC_E2A
34. RAG1_E2Ba
35. RAG1_E2Bb
36. NTC_E2B
37. RAG1_E2Ca
38. RAG1_E2Cb
39. NTC_E2C
40. RAG2_E1a
41. RAG2_E1b
42. NTC_E1
43. RAG2_E2a
44. RAG2_E2b
45. NTC_E2
46. RAG2_E3Aa
47. RAG2_E3Ab
48. NTC_E3A
49. RAG2_E3Ba
50. RAG2_E3Bb
51. NTC_E3B
52. IL2_E1-E4a
53. IL2_E1-E4b
54. NTC_E1-E4
55. IL2_E5-E8a
56. IL2_E5-E8b
57. NTC_E5-E8
58. IL7_E1a
59. IL7_E1b
60. NTC_E1
61. IL7_E2a
62. IL7_E2b
63. NTC_E2
64. IL7_E3a
65. IL7_E3b
66. NTC_E3
67. IL7_E4a
68. IL7_E4b
69. NTC_E4
70. IL7_E5-E6a
71. IL7_E5-E6b
72. NTC_E5-E6
73. IL7_E7-E8a
74. IL7_E7-E8b
75. NTC_E7-E8

Report for IT1-92-NBS-SCID1-5-ref092612combined

Library Summary

Based on Predicted Per-Base Quality Scores - Independent of Alignment

Total Number of Bases [Mbp]	68.22
• Number of Q20 Bases [Mbp]	55.87
Total Number of Reads	475,433
Mean Length [bp]	144
Longest Read [bp]	372



Reference Genome Information

Genome Name	*NBS-SCID-AmpliconPanel-092612*
Genome Size	77,713 bases
Genome Version	092612combinedapliconreference
Index Version	tmap-f3

Based on Full Library Alignment to Provided Reference

	AQ20	Perfect
Total Number of Bases [Mbp]	41.82	30.13
Mean Length [bp]	113	87
Longest Alignment [bp]	299	282
Mean Coverage Depth	538.20×	387.70×
Percentage of Library Covered	52%	52%

Read Alignment Distribution

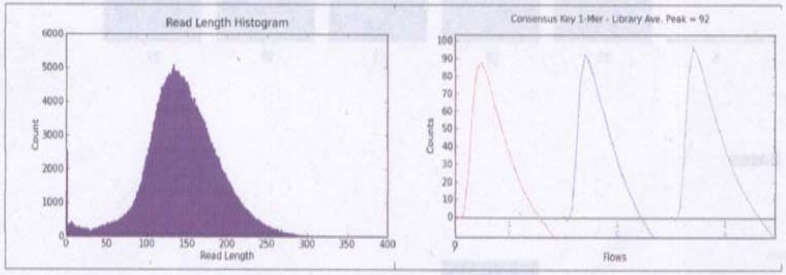
Read Length [bp]	Reads	Unmapped	Excluded	Clipped	Perfect	1 mismatch	≥2 mismatches
50	448,022	18,826	0	0	257,593	114,349	57,254
100	404,953	16,505	0	7,707	134,456	120,144	126,141
150	197,151	7,093	0	12,451	31,808	45,995	99,804
200	47,522	1,425	0	7,641	2,579	5,611	30,266
250	6,555	161	0	2,482	61	212	3,639

Report for IT1-92-NBS-SCID1-5-ref122612-nointrons

Library Summary

Based on Predicted Per-Base Quality Scores - Independent of Alignment

Total Number of Bases [Mbp]	68.22
• Number of Q20 Bases [Mbp]	55.87
Total Number of Reads	475,412
Mean Length [bp]	144
Longest Read [bp]	368



Reference Genome Information

Genome Name	*SCID 5 Gene Panel no introns*
Genome Size	21,830 bases
Genome Version	SCID_5_Gene_NoIntrons_v122612
Index Version	tmap-f3

Based on Full Library Alignment to Provided Reference

	AQ20	Perfect
Total Number of Bases [Mbp]	41.64	30.02
Mean Length [bp]	113	87
Longest Alignment [bp]	300	282
Mean Coverage Depth	1,907.60×	1,374.90×
Percentage of Library Covered	100%	100%

Read Alignment Distribution

Read Length [bp]	Reads	Unmapped	Excluded	Clipped	Perfect	1 mismatch	≥2 mismatches
50	448,089	23,651	0	0	256,789	113,739	53,910
100	404,880	20,794	0	6,834	133,903	119,574	123,775
150	197,138	9,101	0	11,867	31,602	45,648	98,920
200	47,595	1,942	0	7,422	2,555	5,529	30,147
250	6,545	219	0	2,442	62	205	3,617

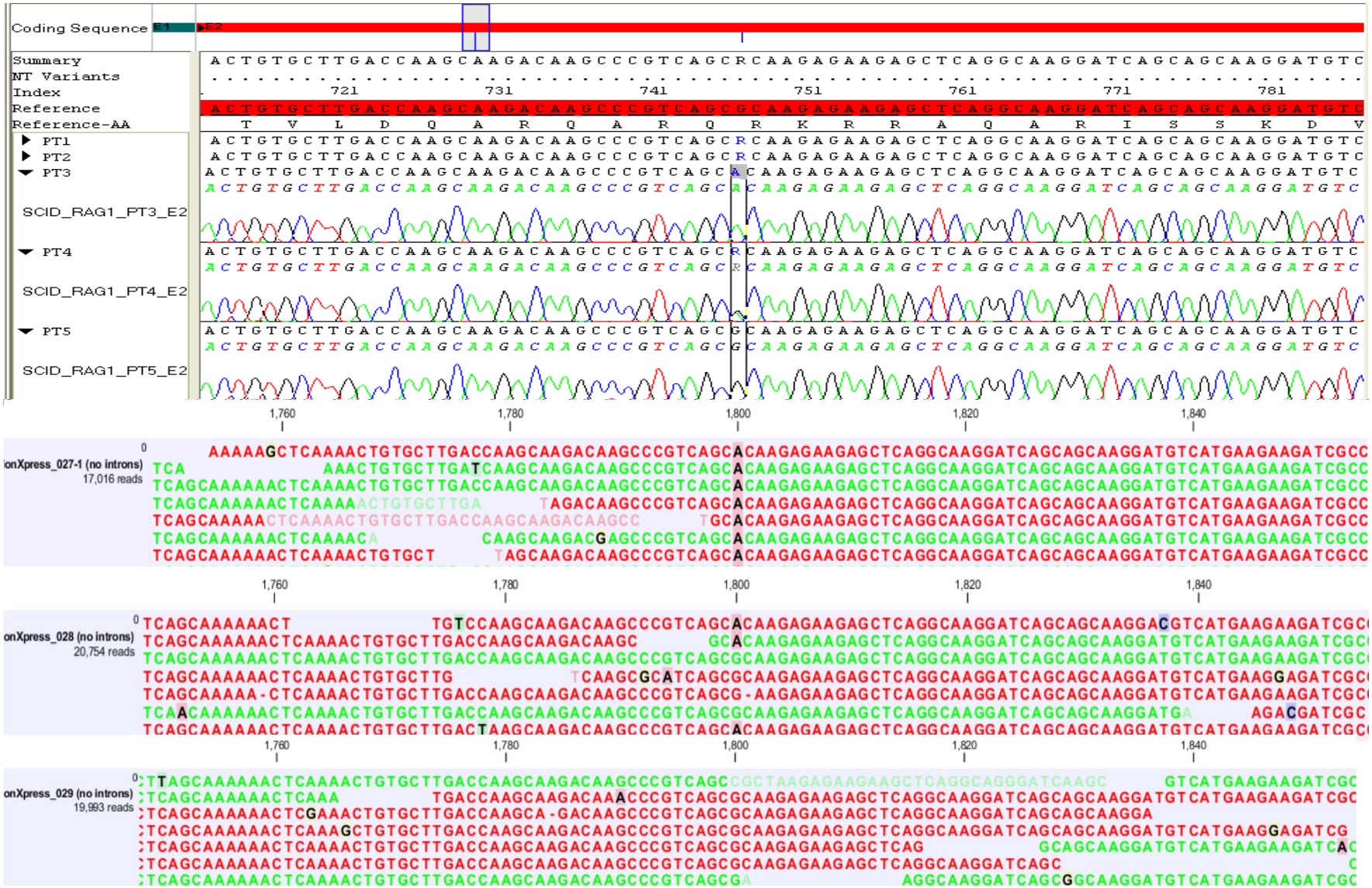
Data Analysis: Torrent Suite 2.2

Based on Full Library Alignment to Provided Reference **(Complete genomic sequences**)		
	AQ20*	Perfect**
Total Number of Bases [Mbp]	41.82	30.13
Mean Length [bp]	113	87
Longest Alignment [bp]	299	282
Mean Coverage Depth	538.20x	387.7x
Percentage of Library Covered	52%	52%
* Greatest length at which error rate is 1% or less. ** Longest perfectly aligned segment		

Based on Full Library Alignment to Provided Reference **(Amplicons only sequences)**		
	AQ20*	Perfect**
Total Number of Bases [Mbp]	41.64	30.02
Mean Length [bp]	113	87
Longest Alignment [bp]	300	282
Mean Coverage Depth	1,907.60x	1,374.90x
Percentage of Library Covered	100%	100%
* Greatest length at which error rate is 1% or less. ** Longest perfectly aligned segment Saavedra-Matiz et al. Clin Chem 59:7 (2013)		

	Gene	Loc.	c.DNA	Protein	REF. Allele	Allele* variants	Counts	Coverage	Freq	For / Rev Balance	rs #	Clinical Interp.
S2	ADA	E4	c.239A>G	p.Lys80Arg	A	G/A	268/218	486	55.1/44.9	0.42/0.46	11555566	Unknown
S2	ADA	E6	c.534A>G	p.Val178Val	A	A/G	349/297	649	53.8/45.8	0.50/0.40	244076	Unknown
S1	IL7	I1	c.82+16G>C		G	C/G	418/389	808	51.7/48.1	0.47/0.49	1353252	Unknown
S3	IL7	I1	c.82+16G>C		G	C/G	253/227	481	52.6/47.2	0.47/0.42	1353252	Unknown
S4	IL7	I1	c.82+16G>C		G	C	775	776	99.9	0.49	1353252	Unknown
S5	IL7	I1	c.82+16G>C		G	C/G	396/379	777	51.0/48.8	0.49/0.49	1353252	Unknown
S1	IL7	E2	c.197T>C	p.Ile66Thr	T	T/C	959/692	1656	57.9/41.8	0.47/0.43	1494558	Unknown
S3	IL7	E2	c.197T>C	p.Ile66Thr	T	T/C	590/408	999	59.1/40.8	0.43/0.45	1494558	Unknown
S4	IL7	E2	c.197T>C	p.Ile66Thr	T	C	1103	1114	99	0.44	1494558	Unknown
S5	IL7	E2	c.197T>C	p.Ile66Thr	T	T/C	793/582	1377	57.6/42.3	0.46/0.49	1494558	Unknown
S1	IL7	E4	c.412G>A	p.Val138Ile	G	A/G	838/816	1655	50.6/49.3	0.50/0.48	1494555	Pathogenic
S3	IL7	E4	c.412G>A	p.Val138Ile	G	G/A	536/478	1014	52.9/47.1	0.50/0.49	1494555	Pathogenic
S4	IL7	E4	c.412G>A	p.Val138Ile	G	A	1298	1301	99.8	0.49	1494555	Pathogenic
S5	IL7	E4	c.412G>A	p.Val138Ile	G	A/G	785/770	1555	50.5/49.5	0.50/0.49	1494555	Pathogenic
S1	IL7	E4	c.495C>T	p.His165His	C	C/T	891/840	1732	51.4/48.5	0.48/0.47	2228141	Silent
S4	IL7	E6	c.731C>T	p.Thr244Ile	C	T/C	89/81	170	52.4/47.6	0.48/0.40	6897932	Unknown
S5	IL7	E6	c.731C>T	p.Thr244Ile	C	C/T	49/48	97	50.5/49.5	0.45/0.44	6897932	Unknown
S3	IL7	E8	c.1066A>G	p.Ile356Val	A	G/A	178/172	350	50.9/49.1	0.45/0.48	3194051	Unknown
S4	IL7	E8	c.1066A>G	p.Ile356Val	A	G/A	221/203	424	52.1/47.9	0.43/0.50	3194051	Unknown
S5	RAG1	E1	c.-65A>G	5'-UTR	A	G/A	480/442	922	52.1/47.9	0.49/0.45	872053	Unknown
S1	RAG1	E2	c.746 G>A	p.Arg249His	G	G/A	215/164	379	56.7/43.3	0.49/0.48	3740955	Unknown
S2	RAG1	E2	c.746 G>A	p.Arg249His	G	G/A	309/168	477	64.8/35.2	0.41/0.50	3740955	Unknown
S3	RAG1	E2	c.746 G>A	p.Arg249His	G	A	226	226	100	0.38	3740955	Unknown
S4	RAG1	E2	c.746 G>A	p.Arg249His	G	G/A	150/144	294	51.0/49.0	0.49/0.47	3740955	Unknown
S5	RAG1	E2	c.2880A>G	p.Ala960Ala	A	G/A	520/463	983	52.9/47.1	0.50/0.47	1980131	Silent
S5	RAG2	E1	c.-526T>C	Promoter	T	C	773	781	99	0.48	73455545	Unknown

***Sanger Concordance: 100%** **Data Analysis: CLC Bio Workbench Software**



***RAG1* E2: c.746 G>A = p.Arg249His**

Gene	Name	Chr.	Ref Seq ID
ADA	Adenosine Deaminase	20q13.12	NG_007385.1
IL2RG	Inteleukin 2 Receptor Gamma	Xq13.1	NG_009088.1
IL7RA	Inteleukin 7 Receptor Alpha	5p13	NG_009567.1
RAG1	Recombination Activaring Gene 1	11p13	NG_007528.1
RAG2	Recombination Activaring Gene 2	11p13	NG_007573.1
JAK3	Janus Kinase 3	19p13.1	NG_007273.1
CD3D	CD3 Antigen Delta Subunit	11q23	NG_009891.1
CD3E	CD3 Antigen Epsilon Subunit	11q23	NG_007373.1
CD3Z	CD3 Antigen Zeta Subunit	1q22-q23	NG_007384.1
CD45	Prot-Tyrosine Phosphatase Rec-Type C	1q31-q32	NG_007730.1
DCLRE1C	DNA Cross-Link Repair Protein 1C	10p13	NG_007276.1
STA5B	Signal Transducer/Activator Transciption 5B	17q11.2	NG_007271.1
DNA PKC	DNA Dependent Protein Kinase Catalytic Subunit	8q11	NG_023435.1
AK2	Adnylate Kinase 2	1p34	NG_016269.1
DNA LIGASE IV	DNA Ligase 4	13q33-q-34	NG_007396.1
CERNUNNOS	Non-Homologous End Joining Factor 1 (NHEJ1)	2q35	NG_007880.1
CORONIN 1A	Coronin Like Protein A	16p11.2	NG_023415.1
CHH/RMRP	Cartilage Hair Hypoplasia	9p21-p12	NG_017041.1

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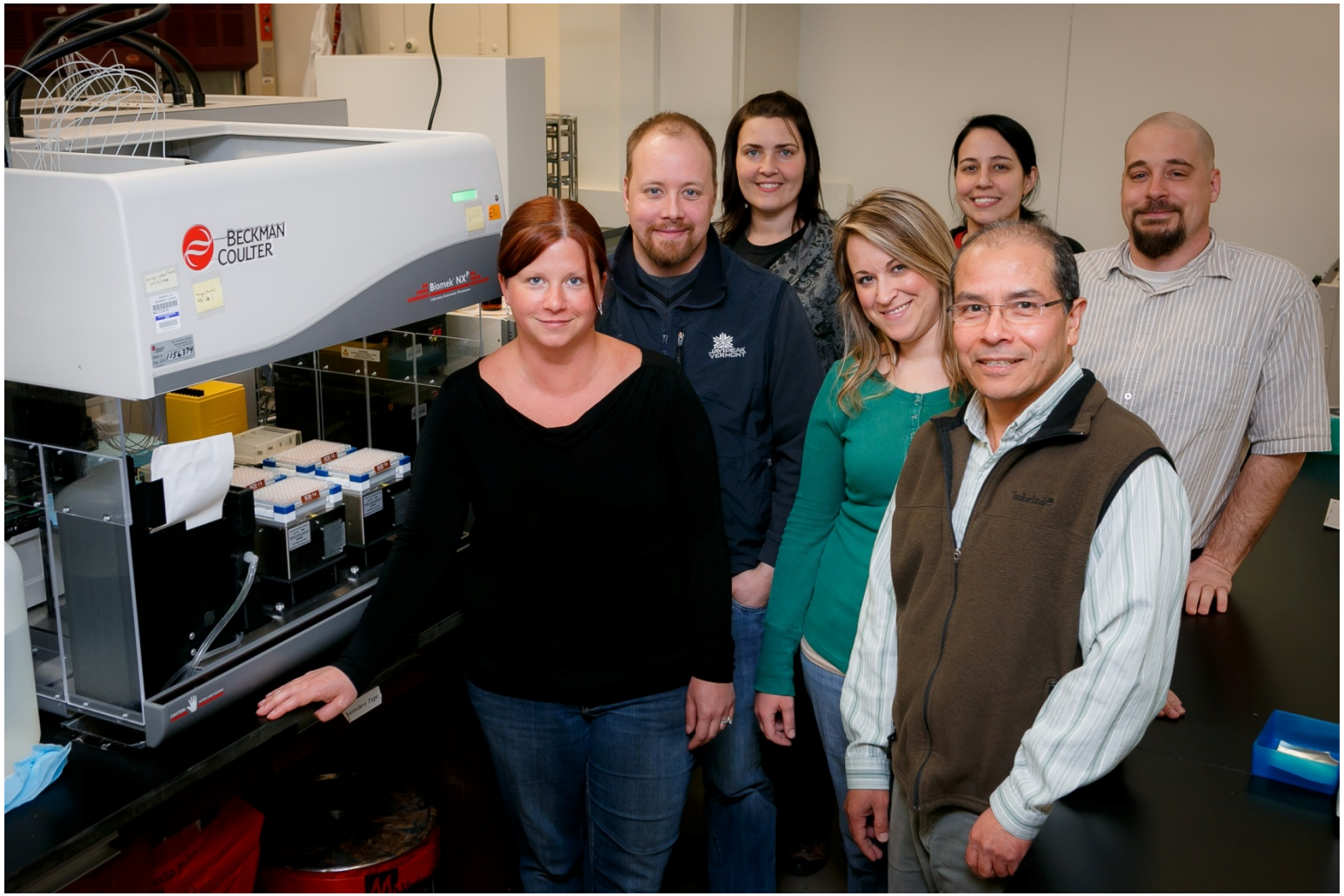
Variant Caller Reports	Mapped Reads	Reads On-Target	Bases On-Target	Read Depth	1x Coverage	20x Coverage	100x Coverage	Variants Detected
IonXpress_017	95,160	97.88%	96.30%	107.71	54.412%	47.438%	31.352%	59
IonXpress_022	116,197	98.21%	96.63%	140.15	54.909%	49.845%	39.002%	51
IonXpress_032	98,000	97.74%	96.22%	122.51	55.139%	50.831%	41.782%	51
IonXpress_040	126,069	96.72%	95.52%	148.35	98.015%	90.565%	55.072%	100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