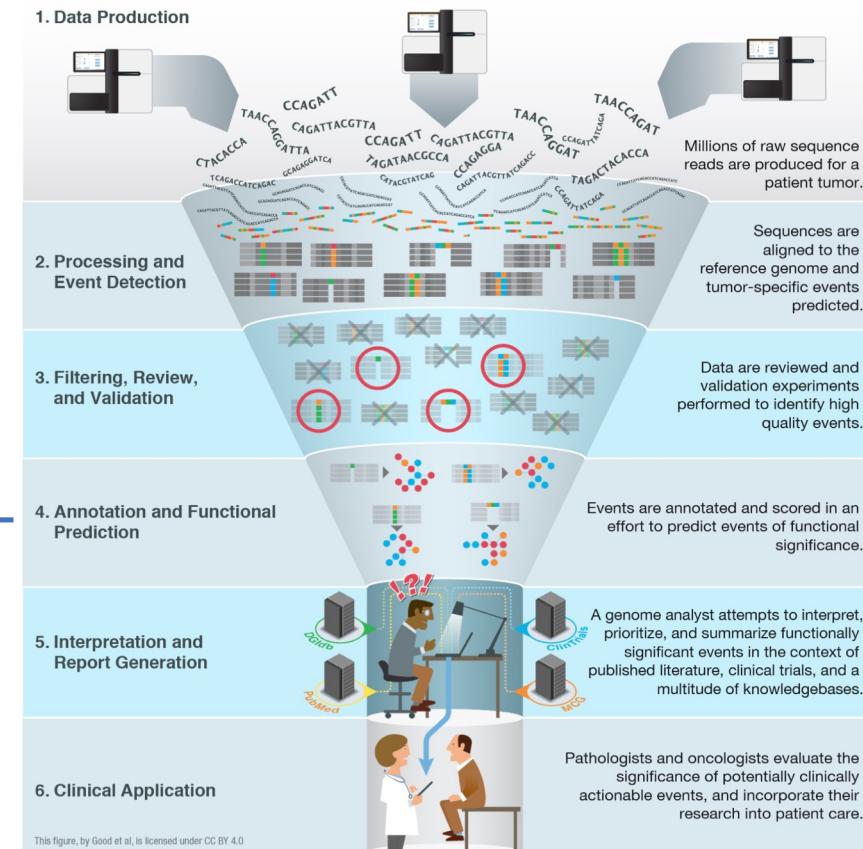


Curation of somatic- and germline variants for clinical (trial) use

Context

Research

- Summarize data types
- Clinical associations
- Validation
- Publication



Clinical

- Minimize FP/FN

Good BM et al. Genome Biology 2014.

Foundation medicine – an example of how to not do it

- In Sweden, broad genomic profiling was not reimbursed in 2020
- Patients go to Docrates (Finland) and bring back FMI reports
- An oncologist at St Göran reached out and asked for assistance to interpret a report from Foundation Medicine
 - Patient wanted Pembrolizumab
- Patient was included into a study at Karolinska and analysed using the ProBio assay

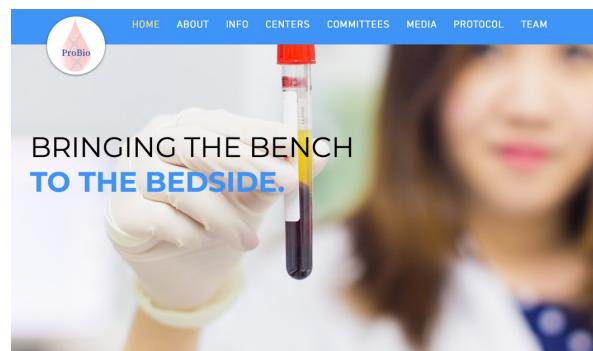
ProBio = a prospective clinical trial in metastatic prostate cancer

STUDY PROTOCOL Open Access

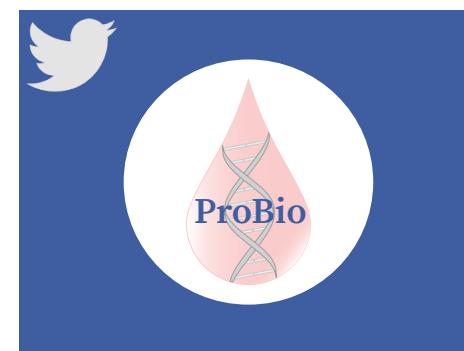
The ProBio trial: molecular biomarkers for advancing personalized treatment decision in patients with metastatic castration-resistant prostate cancer

Alessio Crippa^{1*}, Bram De Laere^{1,2}, Andrea Discacciati¹, Berit Larsson¹, Jason T. Connor^{3,4}, Erin E. Gabriel¹, Camilla Thellenberg⁵, Elin Jänes⁶, Gunilla Enblad⁷, Anders Ullen⁸, Marie Hjälm-Eriksson⁹, Jan Oldenburg¹⁰, Piet Ost¹¹, Johan Lindberg¹, Martin Eklund¹ and Henrik Grönberg¹





► www.probiotrial.org

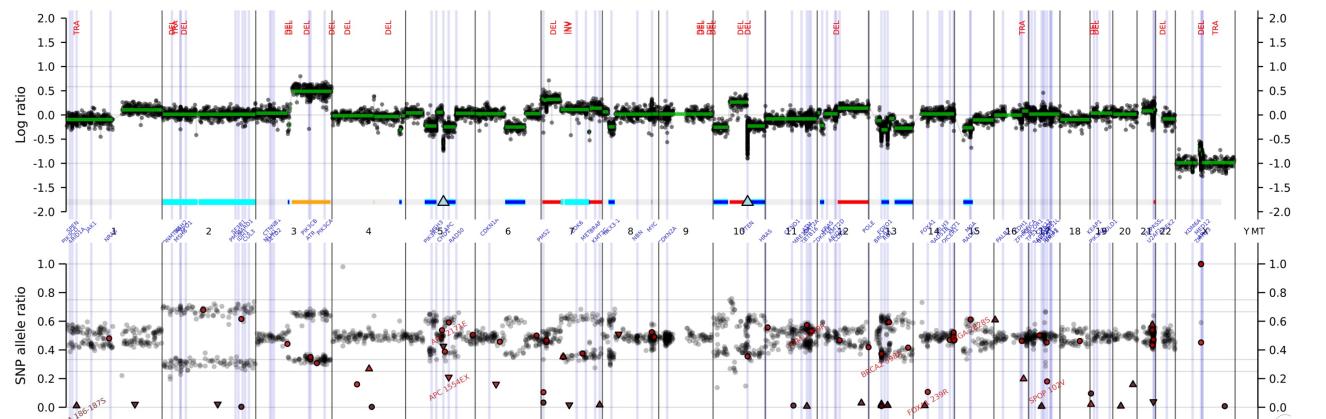


► @ProBioTrial

ProBio = a prospective clinical trial in metastatic prostate cancer

ProBio panel = Custom ctDNA panel for metastatic prostate cancer (1.34 Mb).

- Mutations in 79 genes.
 - Limit of detection: ≥ 0.005
- Structural rearrangements in 7 genes.
 - Limit of detection: ≥ 0.005
- SNP backbone for genome-wide copy-number alterations.
- Homozygous deletions, for selected genes:
 - Limit of detection: 0.08
- Microsatellites to infer MSI.
- Tumor Mutational Burden (TMB) estimation.





FOUNDATIONONE® LIQUID CDx

PATIENT
03-2020-00026951, FI

TUMOR TYPE
Prostate cancer (NOS)
COUNTRY CODE
FI

REPORT DATE
16 Sep 2020
ORDERED TEST #
ORD-0892843-01

ABOUT THE TEST FoundationOne®Liquid CDx is a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

PATIENT

DISEASE Prostate cancer (NOS)

NAME 03-2020-00026951, FI

DATE OF BIRTH [REDACTED]

SEX Male

MEDICAL RECORD # Not given

PHYSICIAN

ORDERING PHYSICIAN [REDACTED]

MEDICAL FACILITY Docrates Syopasairaala

ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN ID 03-2020-00026951 12/12/1954

SPECIMEN TYPE Blood

DATE OF COLLECTION [REDACTED]

SPECIMEN RECEIVED [REDACTED]

Sensitivity for the detection of alterations and genomic signatures is reduced due to sample quality.

Genomic Signatures

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

AR L702H, H875Y

CDK12 K482fs*14

ALK deletion exons 2-12

TP53 C275Y

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

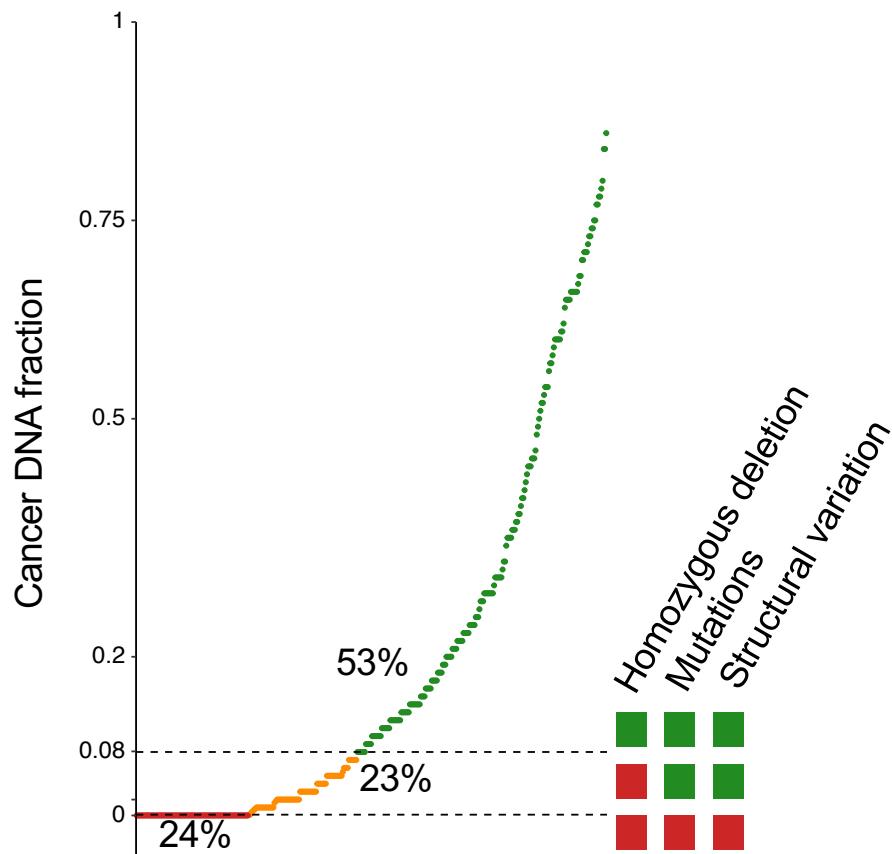
THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

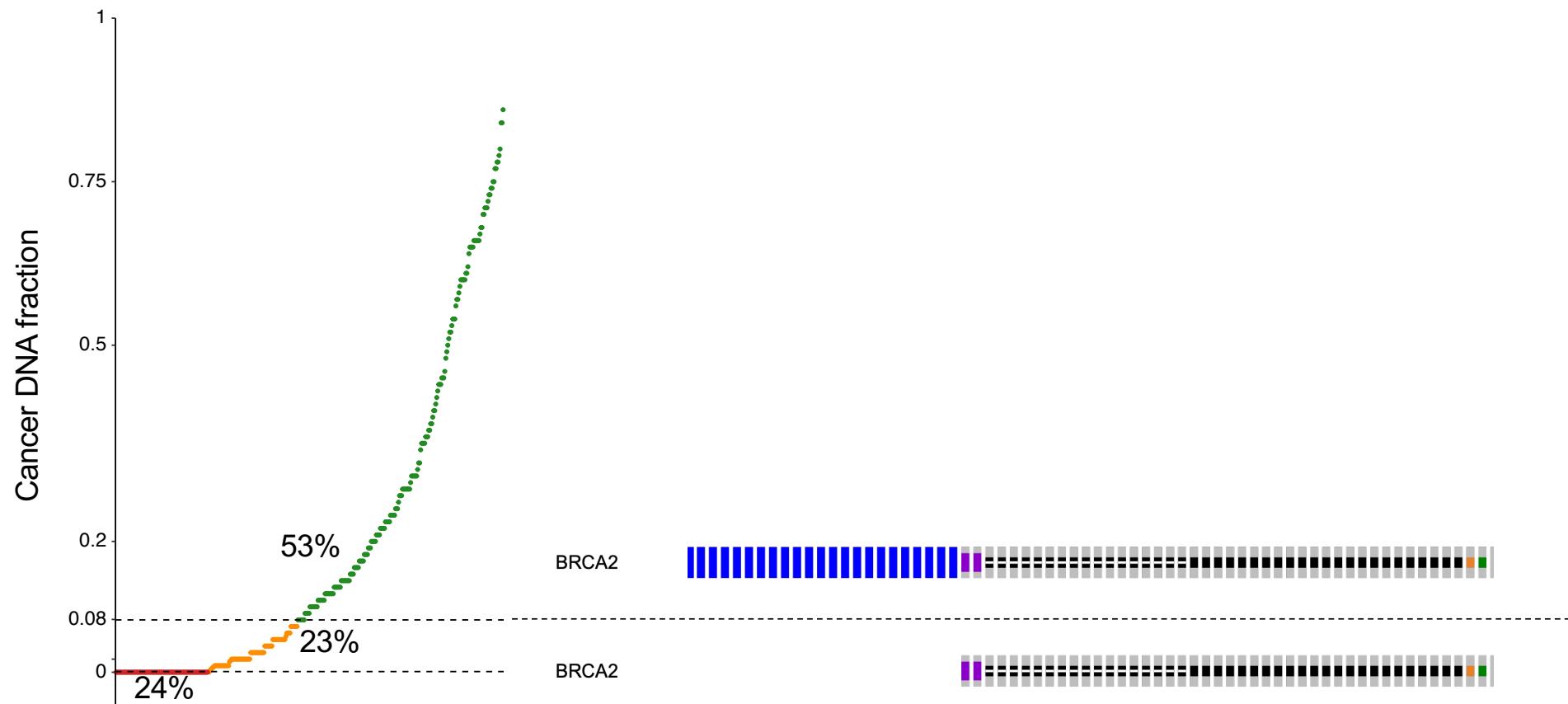
Unable to determine Microsatellite status due to insufficient evidence of genomic instability.

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

Clinical routine testing – ctDNA fraction



Clinical routine testing – ctDNA fraction





FOUNDATIONONE® LIQUID CDx

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03-2020-00026951, FI

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SEX Male

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MEDICAL FACILITY Docrates Syopasairaala

ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN ID 03-2020-00026951 12/12/1954

SPECIMEN TYPE Blood

DATE OF COLLECTION 28 August 2020

SPECIMEN RECEIVED 02 September 2020

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Genomic Signatures

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Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

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GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

due to insufficient evidence of genomic

percentage of circulating-tumor DNA (ctDNA) is based on observed aneuploid instability.

Important, ctDNA fraction high enough to be able to find ALL types of somatic variation



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PHYSICIAN

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MEDICAL FACILITY Docrates Syopasairaala

ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

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Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed

AR L702H, H875Y

CDK12 K482fs*14 →

ALK deletion exons 2-12

TP53 C275Y

Pembro recommendation.
However 1 hit in CDK12 not
enough to recommend Pembro

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

Unable to determine Microsatellite status due to insufficient evidence of genomic instability.

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.



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ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

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SPECIMEN ID 03-2020-00026951 12/12/1954

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ALK deletion exons 2-12

TP53 C275Y

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb →

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Weird to present this TMB, very close to FDA-approved limit to allow PemBro however NO rationale whatsoever for this based on the data presented.

present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.



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Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed please refer to the Appendix

AR L702H, H875Y

CDK12 K482fs*14

ALK deletion exons 2-12

TP53 C275Y

Found with ProBio assay

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

Unable to determine Microsatellite status due to insufficient evidence of genomic instability.

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.



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SEX Male

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ADDITIONAL RECIPIENT None

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PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN ID 03-2020-00026951 12/12/1954

SPECIMEN TYPE Blood

DATE OF COLLECTION 28 August 2020

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Genomic Signatures

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed

AR L702H, H875Y

CDK12 K482fs*14 →

ALK deletion exons 2-12

TP53 C275Y

Found with ProBio assay

AND
CDK12 second hit

AND
CDK12 tandem duplication phenotype

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

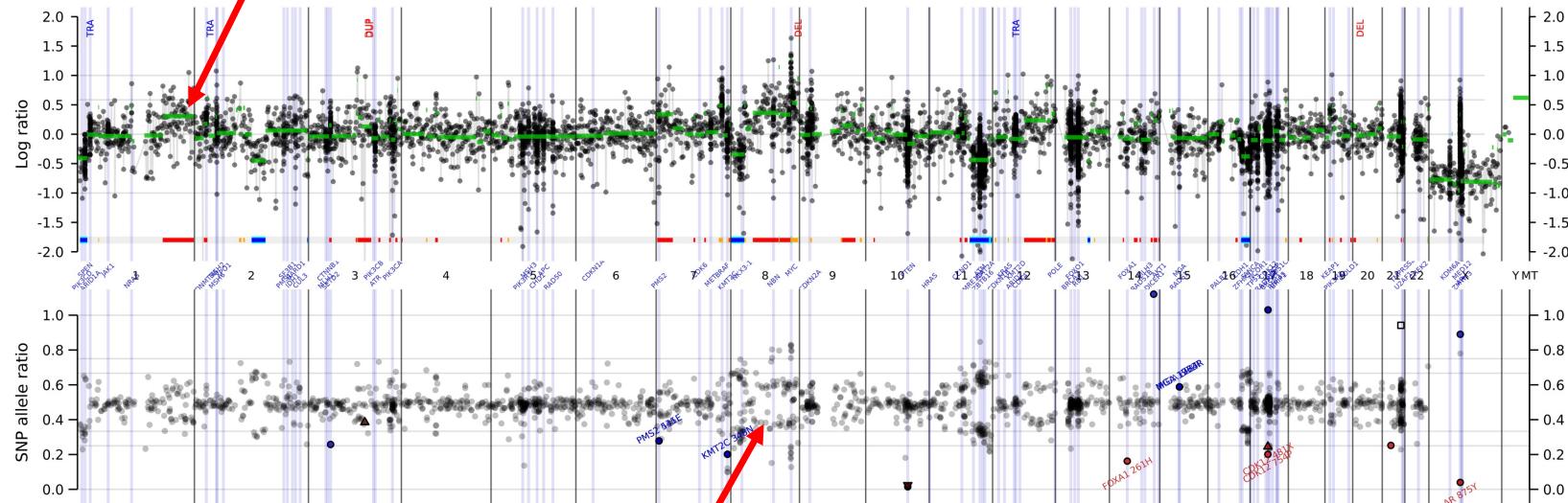
No therapies or clinical trials. See Genomic Signatures section

Unable to determine Microsatellite status due to insufficient evidence of genomic instability.

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

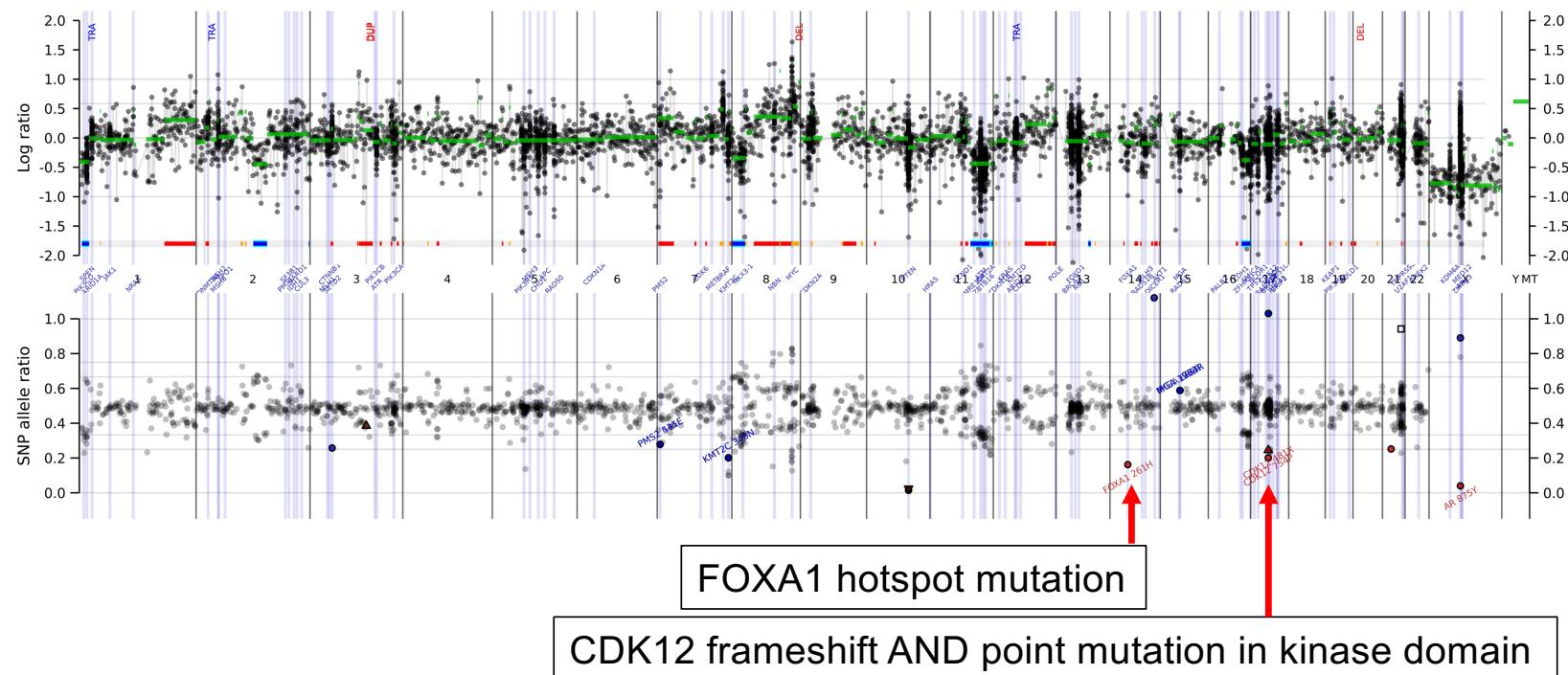
ProBio – liquid biopsy

Genome wide copy number alterations, provided in autoseq curation interface, static and interactive version



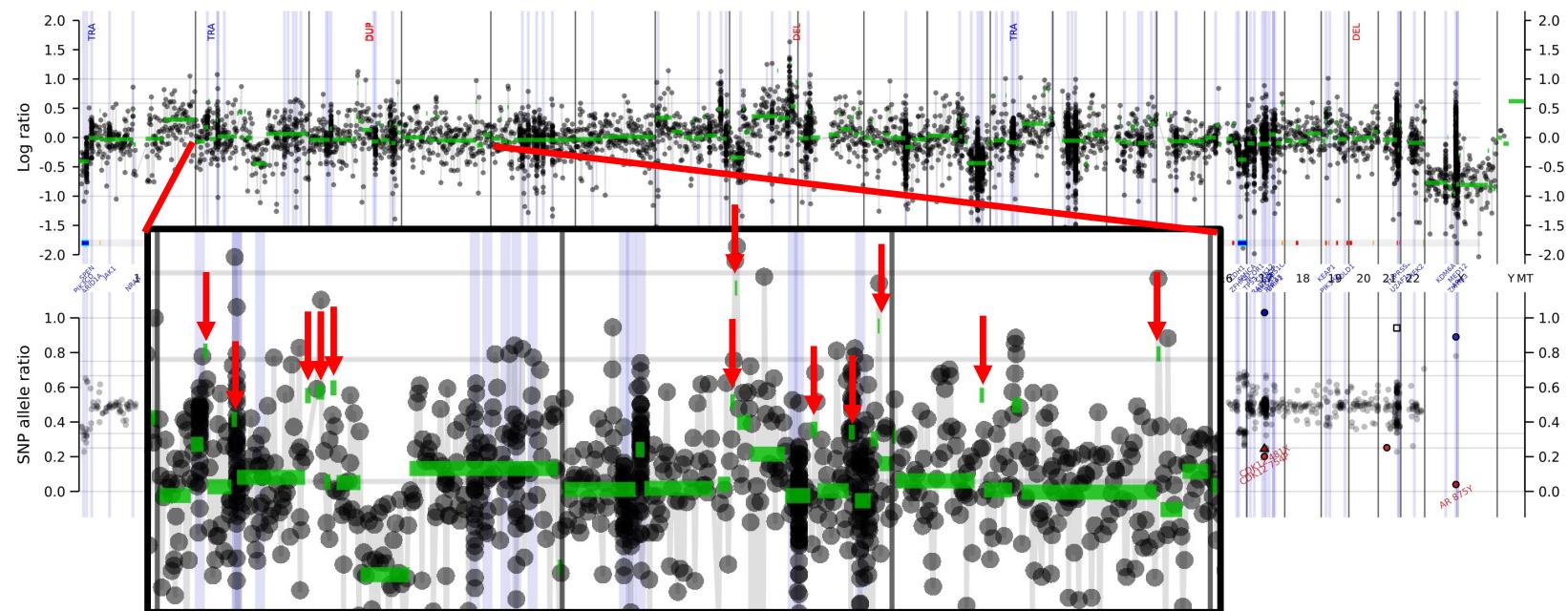
Single nucleotide polymorphism B-allele ratio supporting copy-number data

ProBio – liquid biopsy



ProBio – liquid biopsy

Tandem duplication phenotype = small amplifications genome wide
 Leads to increased amount of fusion proteins = novel antigens on the cell surface.





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MEDICAL FACILITY Docrates Syopasairaala

ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN ID 03-2020-00026951 12/12/1954

SPECIMEN TYPE Blood

DATE OF COLLECTION 28 August 2020

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Sensitivity for the detection of alterations and genomic signatures is reduced due to sample quality.

Genomic Signatures

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

AR L702H, H875Y

CDK12 K482fs*14

ALK deletion exons 2-12 →

Completely irrelevant

TP53 C275Y

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

Unable to determine Microsatellite status due to insufficient evidence of genomic instability.

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.



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COUNTRY CODE
FI

REPORT DATE
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PATIENT

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DATE OF BIRTH [REDACTED]

SEX Male

MEDICAL RECORD # Not given

PHYSICIAN

ORDERING PHYSICIAN [REDACTED]

MEDICAL FACILITY Docrates Syopasairaala

ADDITIONAL RECIPIENT None

MEDICAL FACILITY ID [REDACTED]

PATHOLOGIST Provided, Not

SPECIMEN

SPECIMEN ID 03-2020-00026951 12/12/1954

SPECIMEN TYPE Blood

DATE OF COLLECTION 28 August 2020

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Genomic Signatures

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Gene Alterations

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CDK12 K482fs*14

ALK deletion exons 2-12

TP53 C275Y

False positive clonal hematopoiesis variant

14 Therapies Approved in the EU

20 Clinical Trials

3 Therapies with Lack of Response

GENOMIC SIGNATURES

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

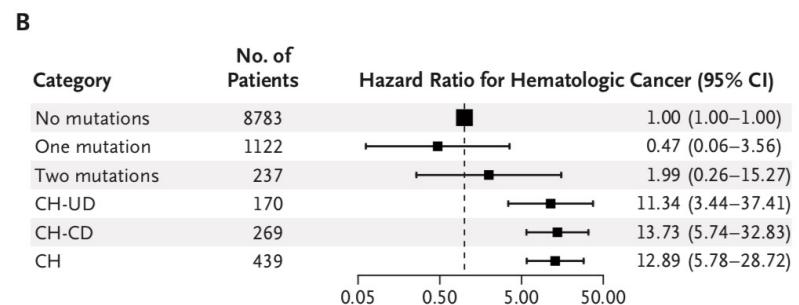
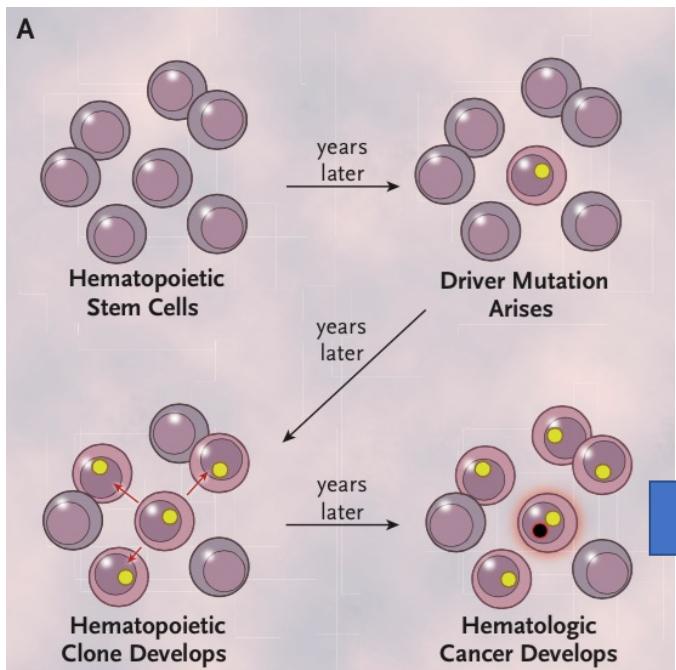
THERAPY AND CLINICAL TRIAL IMPLICATIONS

No therapies or clinical trials. See Genomic Signatures section

Unable to determine Microsatellite status due to insufficient evidence of genomic instability.

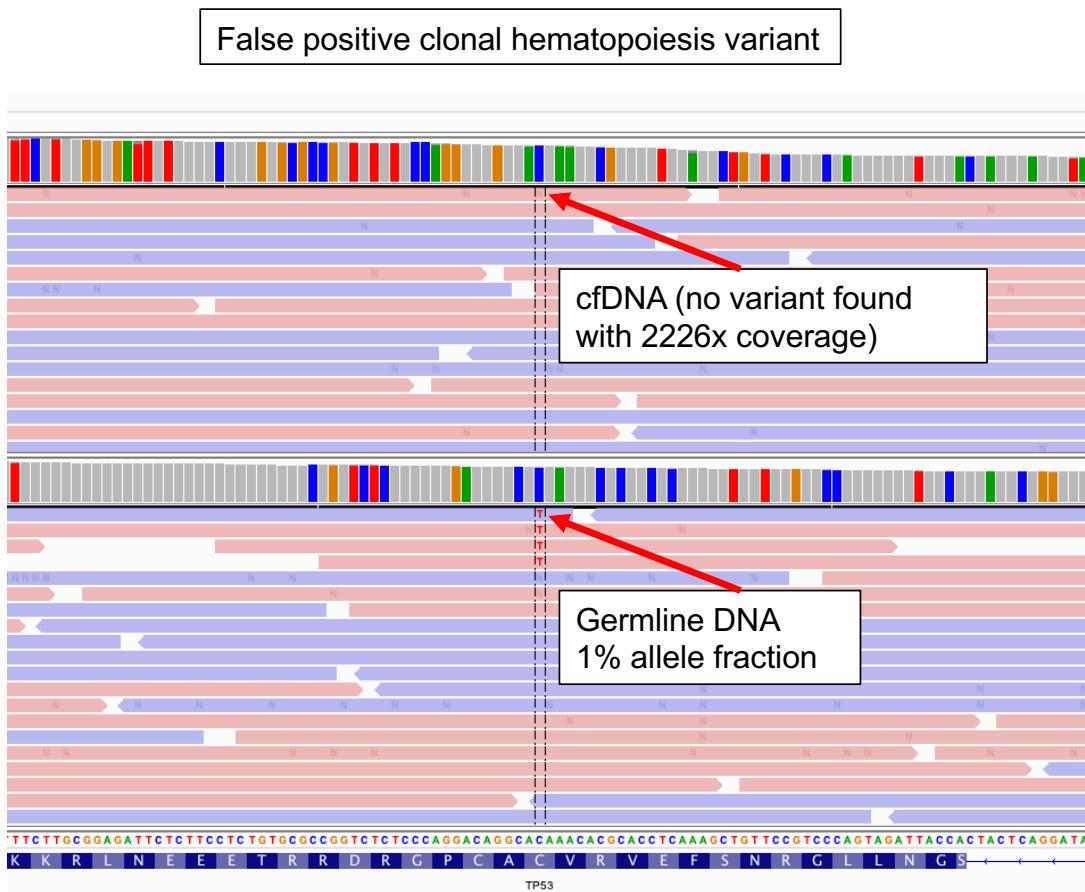
Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

Clonal expansions in white blood cells (CHIP)



Ends up in plasma! 
 Remedy – sequence white blood cell DNA, case closed?

False positive CHIP variant





FOUNDATIONONE® LIQUID CDx

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TUMOR TYPE
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ADDITIONAL RECIPIENT None

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SPECIMEN RECEIVED 02 September 2020

GENOMIC SIGNATURES

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Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

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Genomic Signatures

Blood Tumor Mutational Burden - 9 Muts/Mb

Microsatellite status - Cannot Be Determined

Tumor Fraction - 22%

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

AR L702H, H875Y

CDK12 K482fs*14

ALK deletion exons 2-12

TP53 C275Y

14 Therapies Approved in the EU

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3 Therapies with Lack of Response

Based on the tumor = ctDNA fraction and the TP53 allele frequency (0.24%) the C275Y variant should NOT be presented to a physician as a relevant result on the front page.

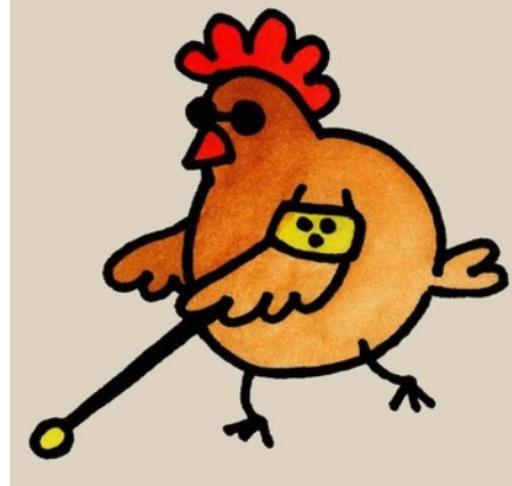
No
Unaltered
Instability

Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

FoundationOne Liquid CDx did not impress

- The Pembro recommendation was correct, for that time, BUT based on the wrong arguments

FoundationOne Liquid CDx



FMI says “there is nothing to do about it” ..

CLINICAL CANCER RESEARCH | PRECISION MEDICINE AND IMAGING

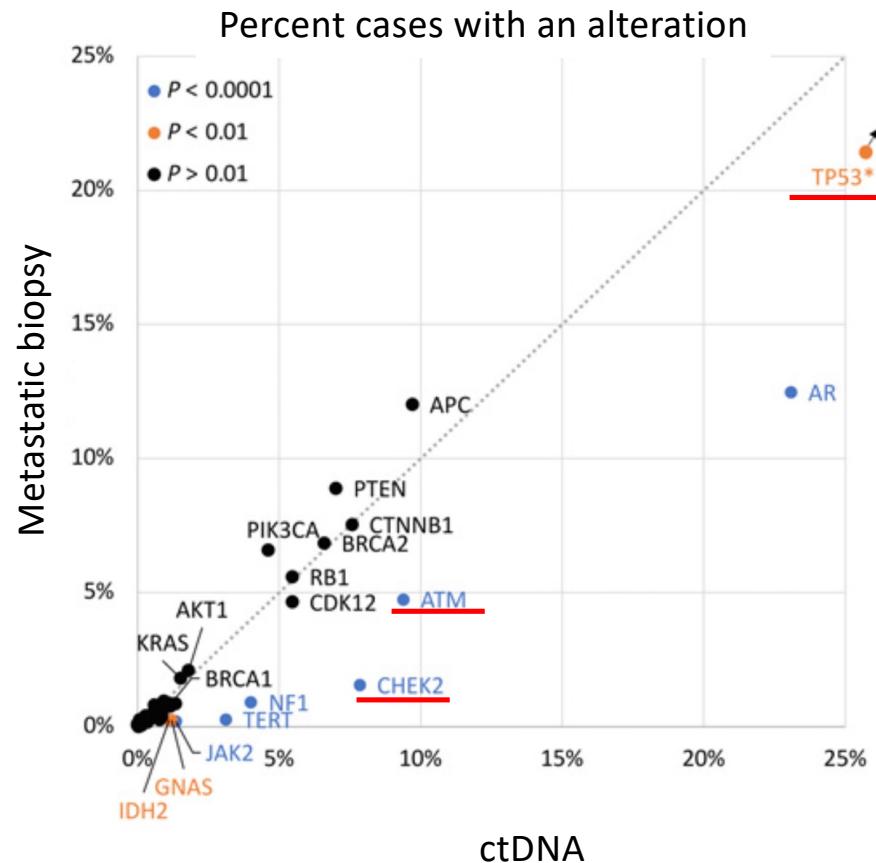
Genomic Analysis of Circulating Tumor DNA in 3,334 Patients with Advanced Prostate Cancer Identifies Targetable BRCA Alterations and AR Resistance

Mechanisms

Hanna Tukachinsky¹, Russell W. Madison¹, Jon H. Chung¹, Ole V. Gjerostrup¹, Eric A. Severson¹, Lucas Dennis¹, Bernard J. Fendler¹, Samantha Morley¹, Lei Zhong¹, Ryon P. Graf¹, Jeffrey S. Ross^{1,2}, Brian M. Alexander¹, Wassim Abida³, Simon Chowdhury⁴, Charles J. Ryan⁵, Karim Fizazi⁶, Tony Golsorkhi⁷, Simon P. Watkins⁷, Andrew Simmons⁷, Andrea Loehr⁷, Jeffrey M. Venstrom¹, and Geoffrey R. Oxnard¹



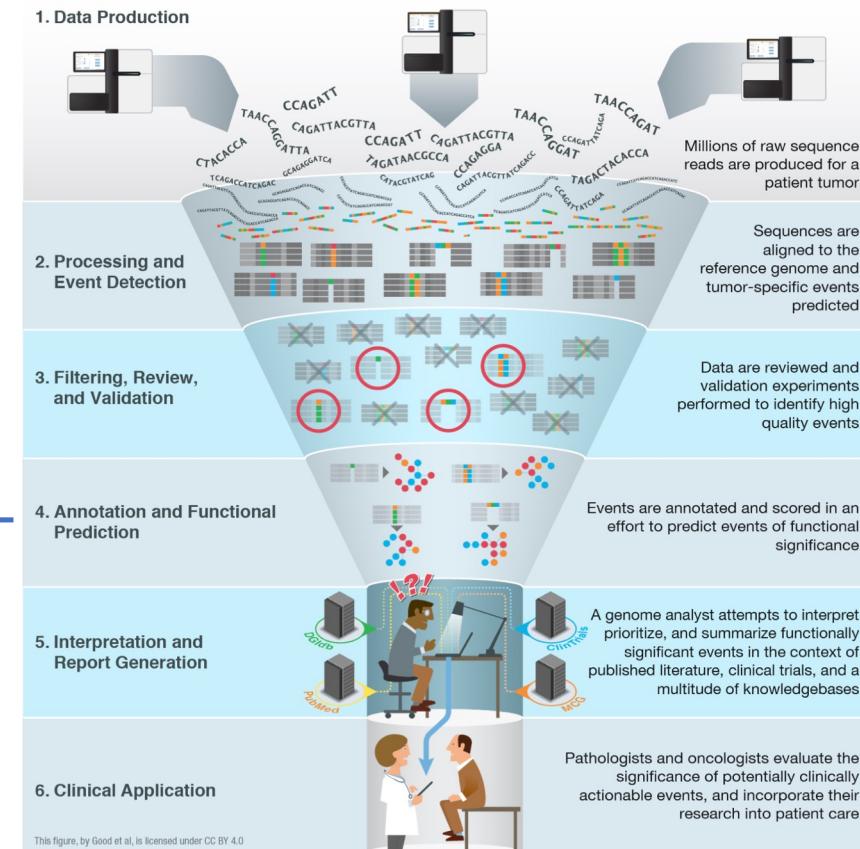
- Triton2/3 + routine testing
- Foundation ACT + FoundationOne Liquid
- No deletions



Context

Research

- Summarize data types
- Clinical associations
- Validation
- Publication



Clinical

- Minimize FP/FN

Good BM et al. Genome Biology 2014.

A proposed procedure

• Purpose

- Manual review is needed to obtain list of high-quality variants
- Proposes a suggestion of standardized manual review

© American College of Medical Genetics and Genomics

ARTICLE | Genetics
inMedicine

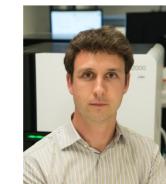
Open

Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples

Erica K. Barnell, BS¹, Peter Ronning, BS¹, Katie M. Campbell, BS¹, Kilannin Krysiak, PhD^{1,2}, Benjamin J. Ainscough, PhD^{1,3}, Lana M. Sheta¹, Shahil P. Pema¹, Alina D. Schmidt, BS¹, Megan Richters, BS¹, Kelsy C. Cotto, BS¹, Arpad M. Danos, PhD¹, Cody Ramirez, BS¹, Zachary L. Skidmore, MEng¹, Nicholas C. Spies, BS¹, Jasreet Hundal, MS¹, Malik S. Sediqzad¹, Jason Kunisaki, BS¹, Felicia Gomez, PhD¹, Lee Trani, BS¹, Matthew Matlock, BS¹, Alex H. Wagner, PhD¹, S. Joshua Swamidass, MD/PhD^{4,5}, Malachi Griffith, PhD^{1,2,3,6} and Obi L. Griffith, PhD^{1,2,3,6}



Malachi Griffith, PhD
Assistant Professor of Medicine
Assistant Professor of Genetics
Assistant Director, MGI

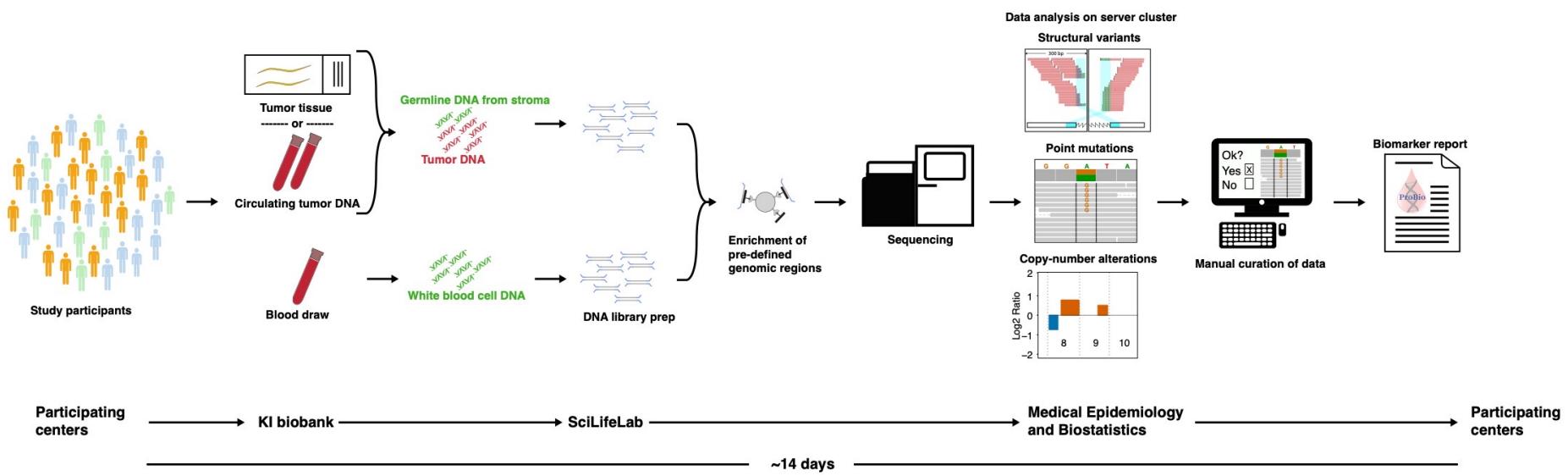


Obi Griffith, PhD
Assistant Professor of Medicine
Assistant Professor of Genetics
Assistant Director, MGI

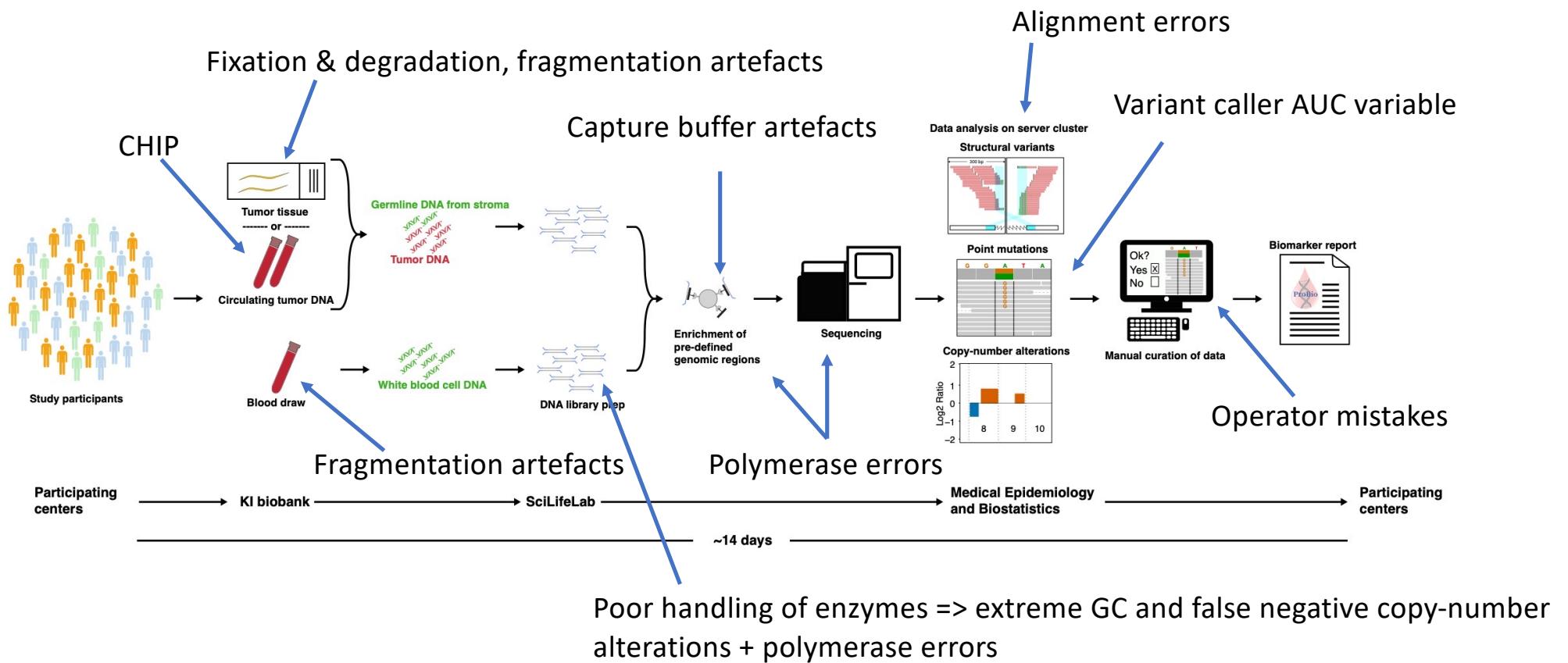
McDonnell Genome Institute, Washington University School of Medicine

Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples, Gen Med, 2018.

What can generate false positive variants?

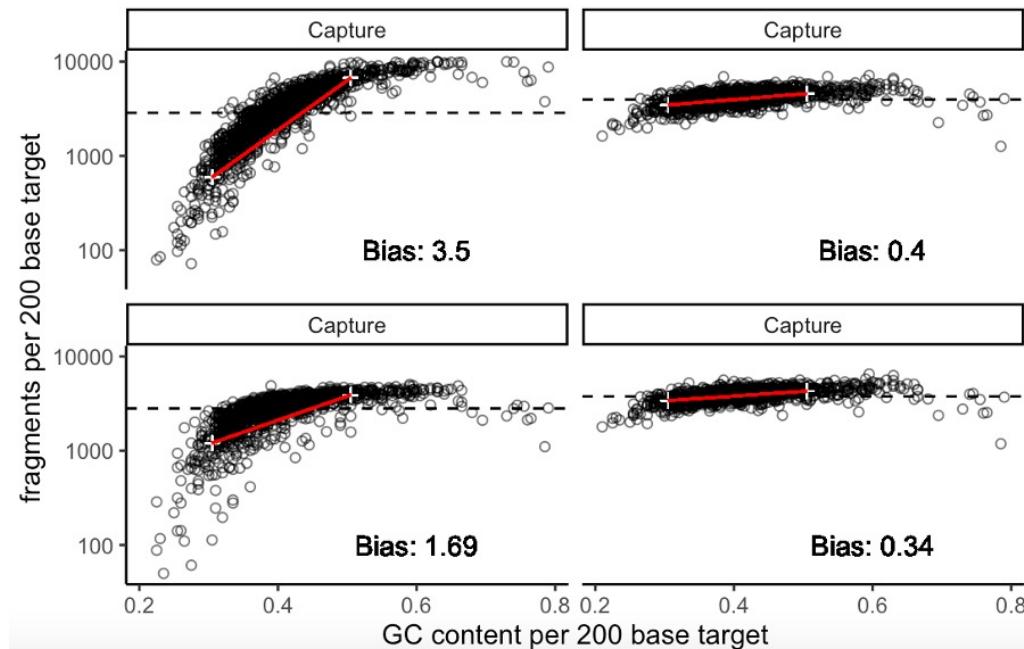


What can generate false positive variants?



What can generate false positive variants?

Thawing library prep enzymes on the bench instead of on ice ...



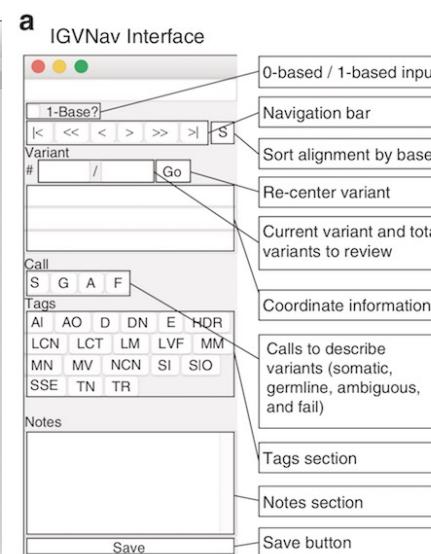
A proposed procedure

- Purpose
 - Manual review is needed to obtain list of high-quality variants
 - Present a suggestion of standardized manual review

- Methods
 - SOP containing
 - 4 different calls
 - Somatic/Germline/Ambiguous/Fail
 - 19 tags + descriptive figures
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 - Accuracy assessed with orthogonal sequencing

Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples, Gen Med, 2018.

IGV-NAV(igator)



b IGVNav Input File

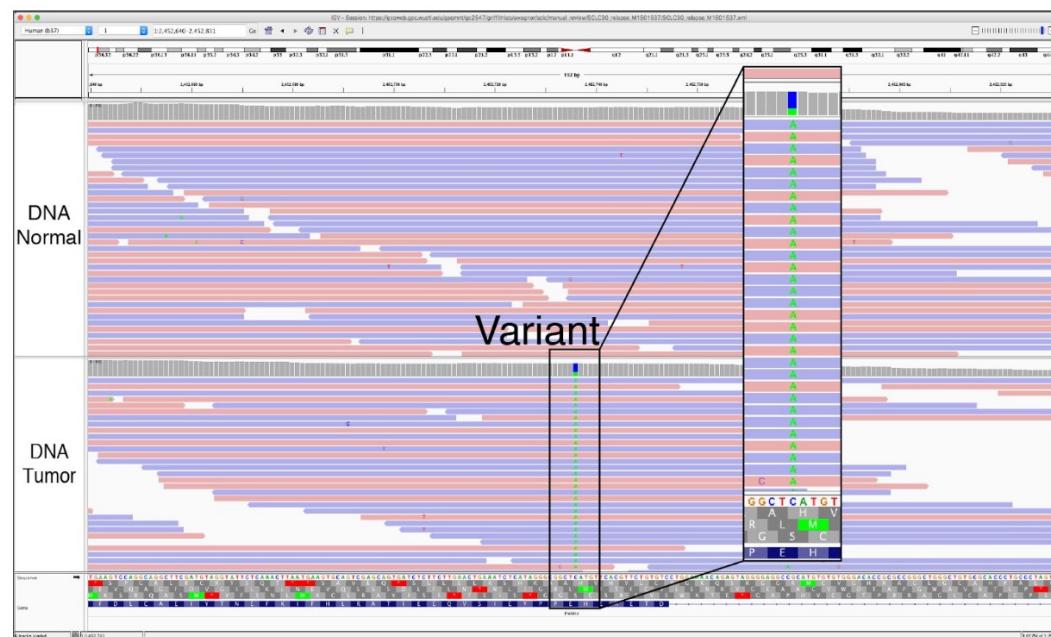
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14	44505849	44505849	A	G			MM
3	67084225	67084225	T	C			
10	26174114	26174114	A	T			SI
10	70753879	70753879	C	A			LVF
10	94227337	94227337	C	T	F		HDR
11	5390168	5390168	T	G			SI
12	100263686	100263686	T	C			
12	122190233	122190233	C	A			
13	109125155	109125155	A	G			
13	23250679	23250679	G	A			SI
13	38691806	38691806	G	A	F		MM

c IGVNav Output File

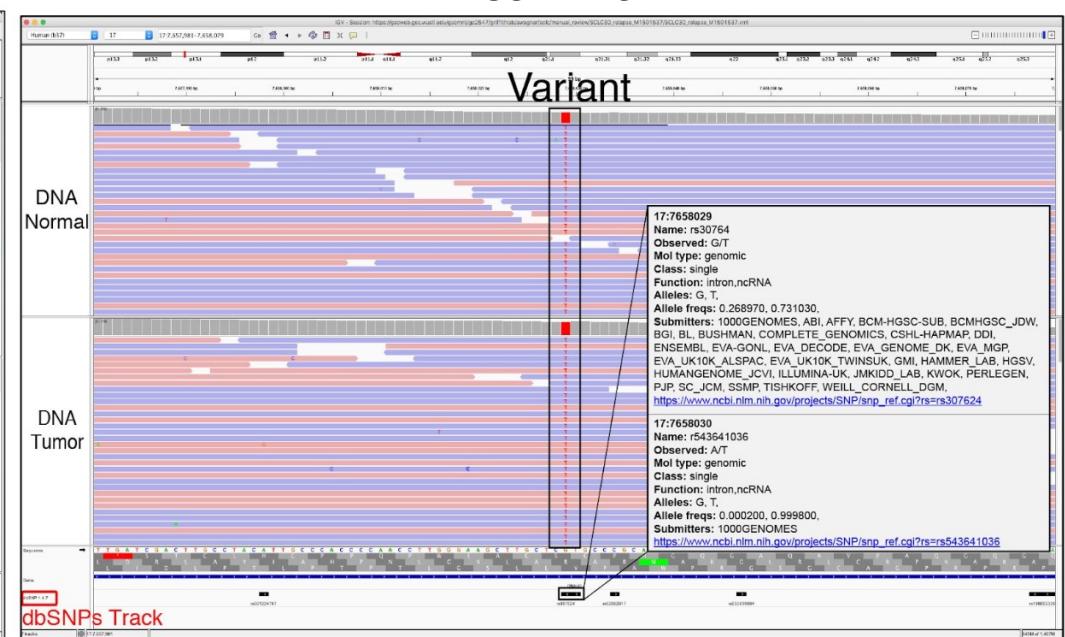
chr	start	stop	ref	var	call	tags	notes
10	26174201	26174201	C	A	G		MM
14	44505849	44505849	A	G	F		
3	67084225	67084225	T	C	A		SI
10	26174114	26174114	A	T	A		SI
10	70753879	70753879	C	A	F		LVF
10	94227337	94227337	C	T	F		HDR
11	5390168	5390168	T	G	F		SI
12	100263686	100263686	T	C	F		SI
12	122190233	122190233	C	A	S		'dinucleotide'
13	109125155	109125155	A	G	A		SI
13	23250679	23250679	G	A	A		SI
13	38691806	38691806	G	A	F		MM

Using IGV to evaluate variants

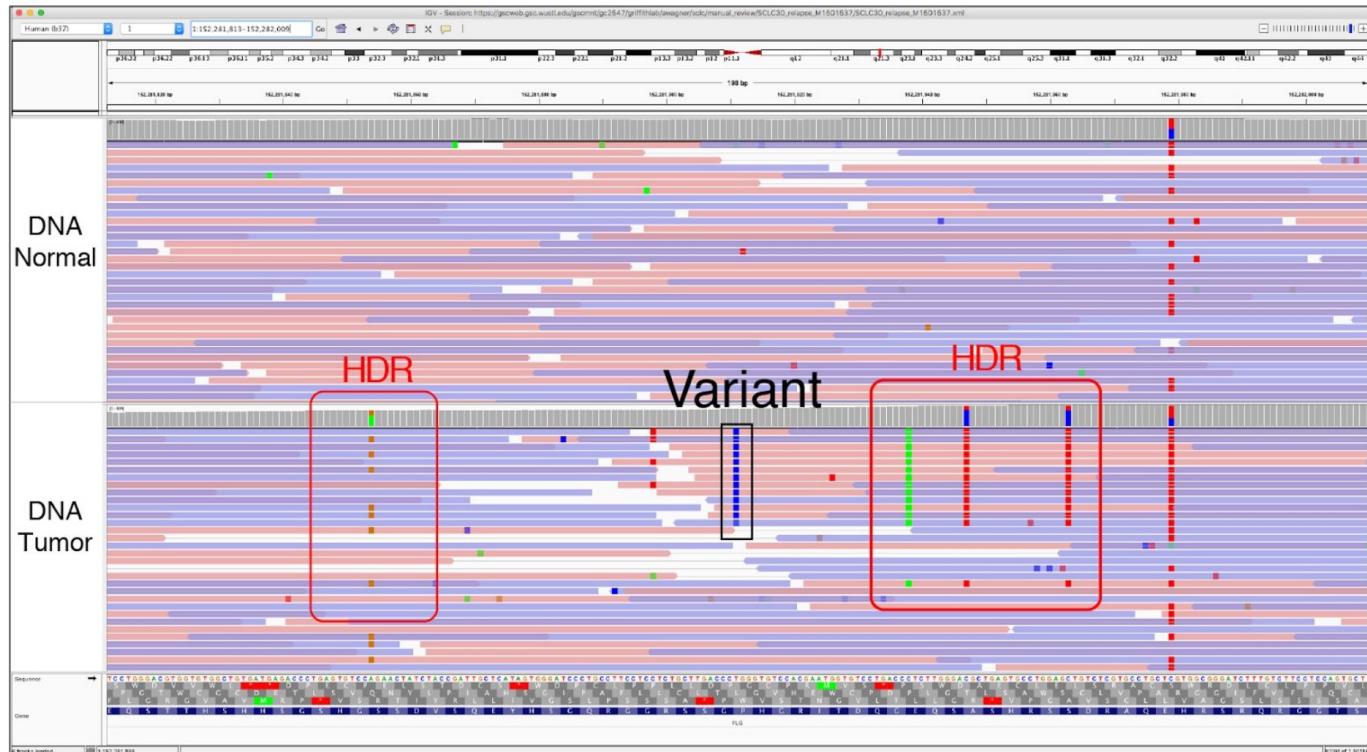
Somatic



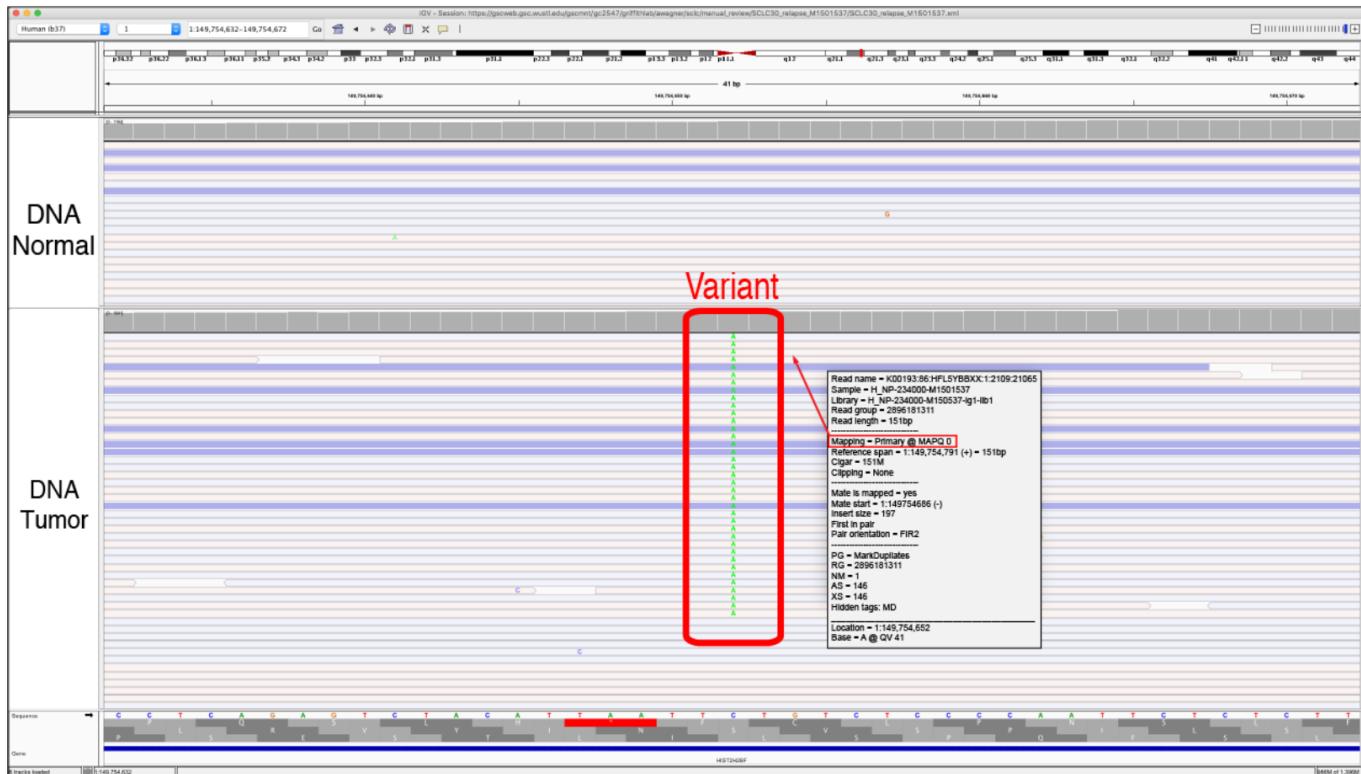
Germline



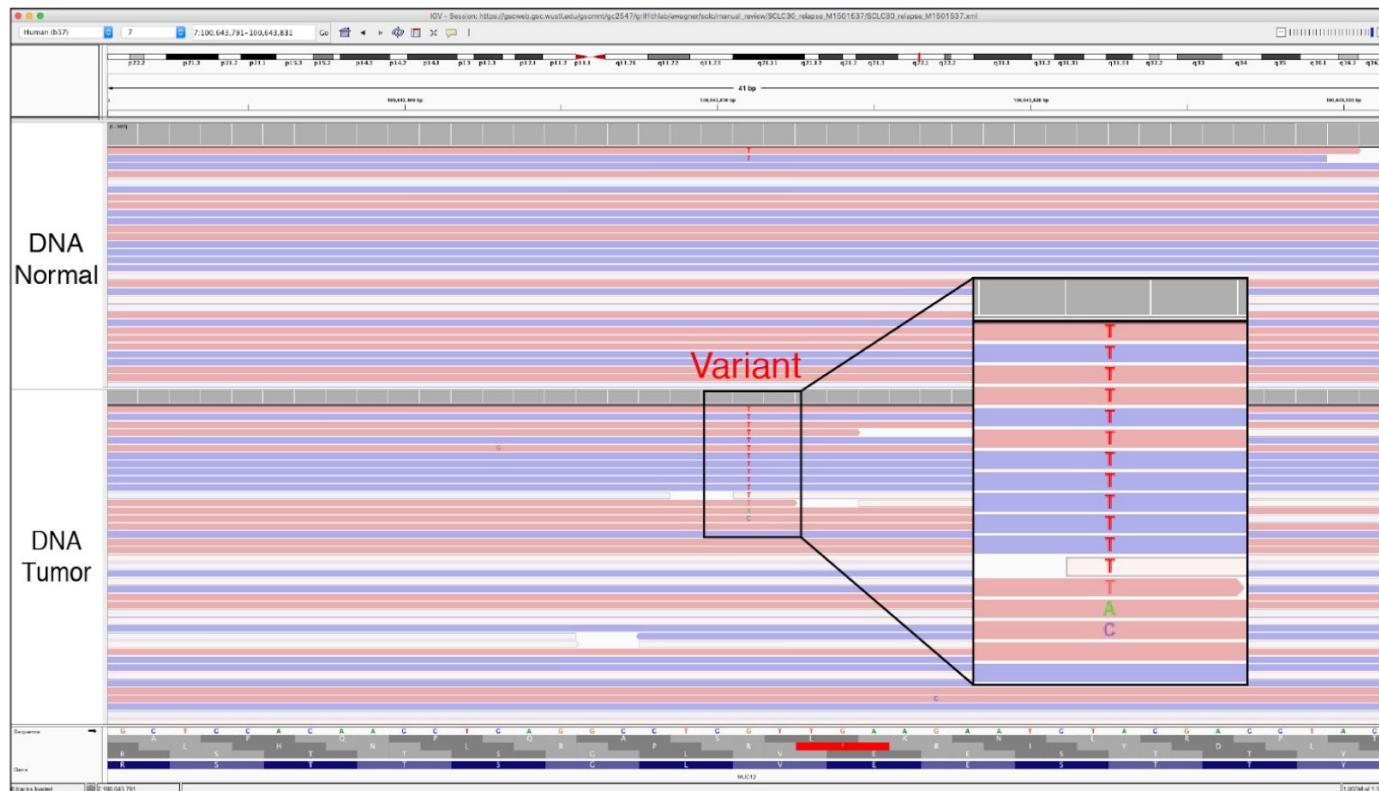
Using IGV to evaluate variants



Using IGV to evaluate variants

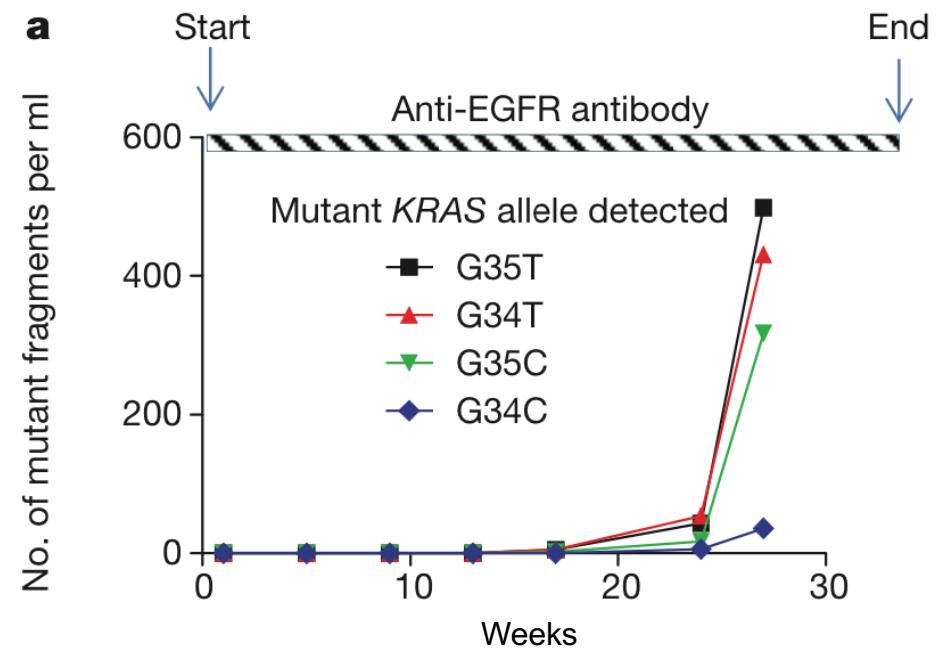


Using IGV to evaluate variants



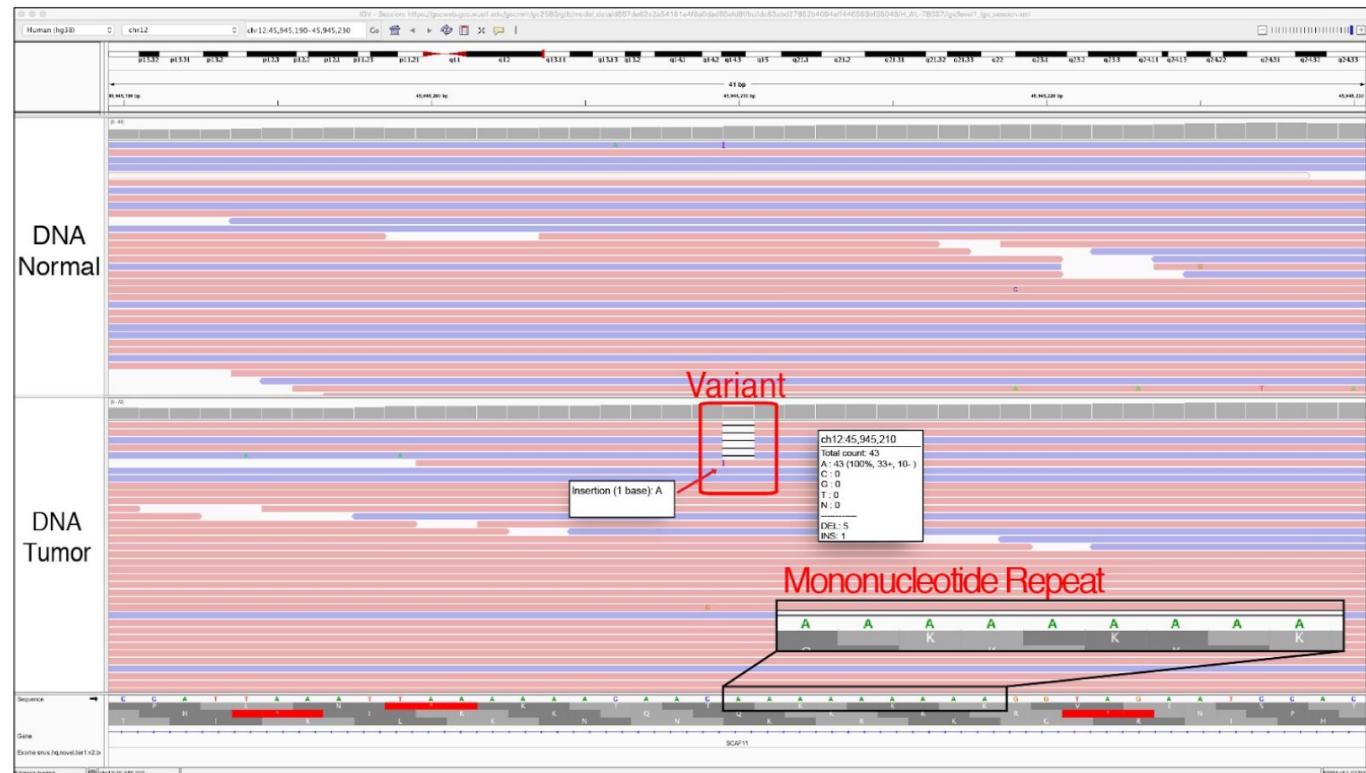
Why targeted mono-therapy fail in metastatic disease

- Metastatic patients basically always contain resistant clones to targeted monotherapy
 - Inevitable due to random errors during cell-division
 - Combinations the way forward hitting on orthogonal functions.
 - Side effects ...

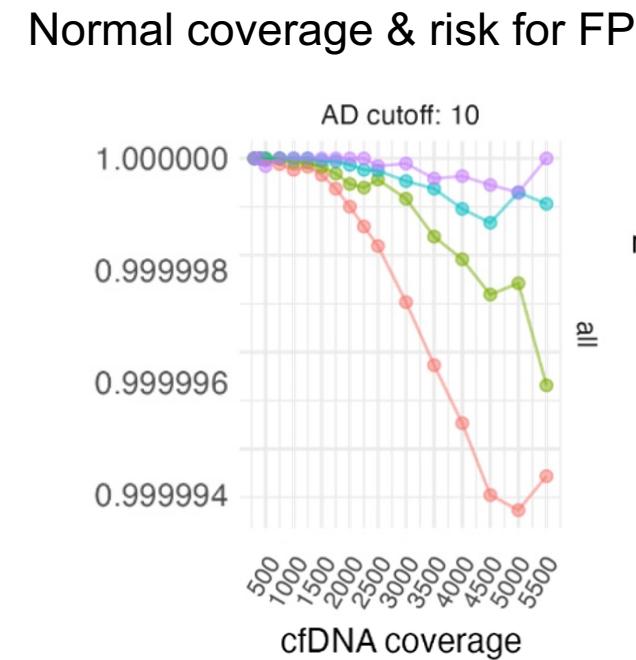
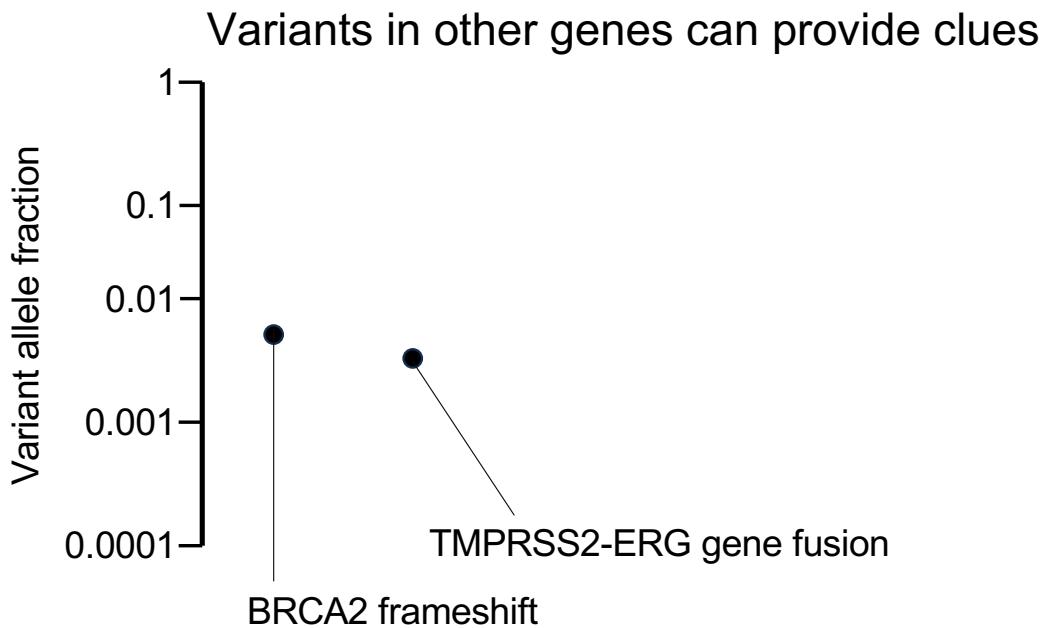


The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers, Nature, 2012
 Evolutionary dynamics of cancer in response to targeted combination therapy, ELife, 2013

Using IGV to evaluate variants



Potential remedy



A proposed procedure

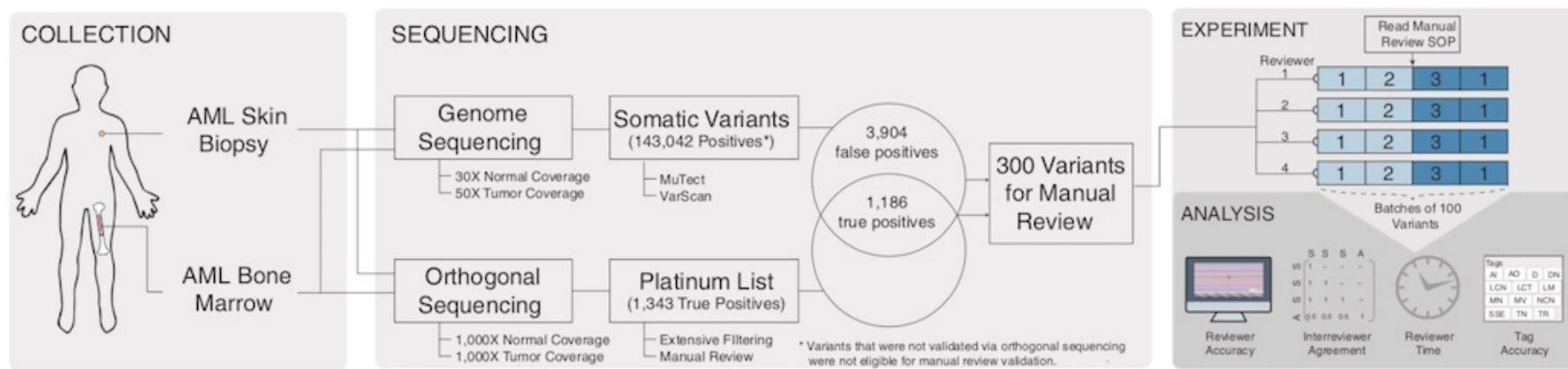
- Purpose
 - Manual review is needed to obtain list of high-quality variants
 - Present a suggestion of standardized manual review

- Methods
 - SOP containing
 - 4 different calls
 - Somatic/Germline/Ambiguous/Fail
 - 19 tags + descriptive figures
 - Data features, common artifacts, providing support for call
 - 4 reviewers classified the variants
 - prior to SOP
 - after reading SOP
 - Accuracy assessed with orthogonal sequencing

Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples, Gen Med, 2018.

Results

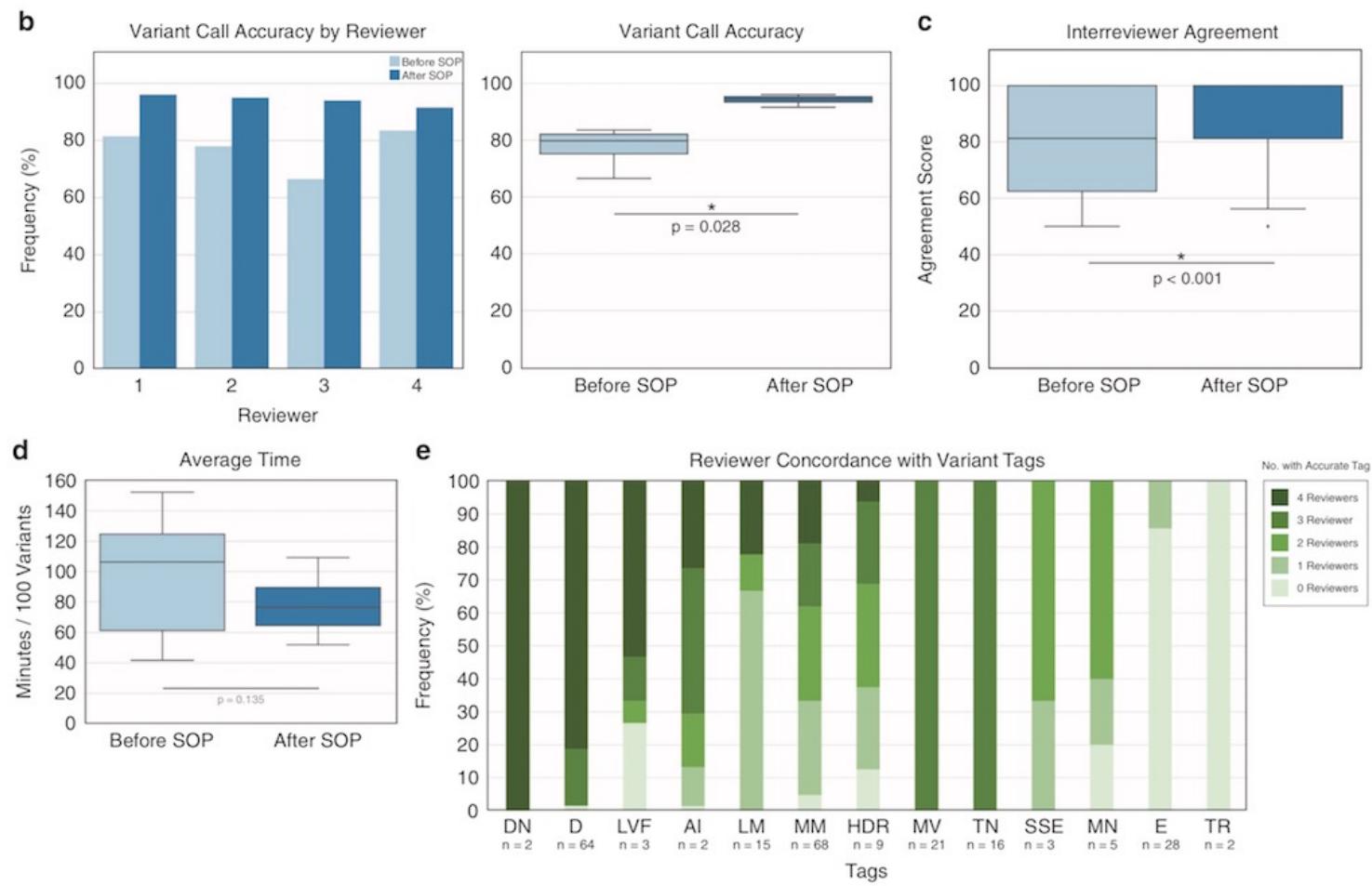
a



- Blinded novice reviewers manually reviewed 200 variants in two batches
- Important to use multiple tags to motivate the “Call”
- To fail a variant, the reviewer must confidently determine that the variant was called because of a sequencing or analysis artifact.

$$\text{Accuracy} = \frac{\text{correct classifications}}{\text{all classifications}}$$

Results



Why manual when it can be automated ..

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ARTICLE | **Genetics in Medicine**

Open

Standard operating procedure for somatic variant refinement of sequencing data with paired tumor and normal samples

Erica K. Barnell,^{BS¹}, Peter Ronning,^{BS¹}, Katie M. Campbell,^{BS¹}, Kilannin Krysiak,^{PhD^{1,2}}, Benjamin J. Ainscough,^{PhD^{1,3}}, Lana M. Sheta,¹, Shahil P. Pema,¹, Alina D. Schmidt,^{BS¹}, Megan Richters,^{BS¹}, Kelsy C. Cotto,^{BS¹}, Arpad M. Danos,^{PhD¹}, Cody Ramirez,^{BS¹}, Zachary L. Skidmore,^{MEng¹}, Nicholas C. Spies,^{BS¹}, Jasreet Hundal,^{MS¹}, Malik S. Sediqzad,¹, Jason Kunisaki,^{BS¹}, Felicia Gomez,^{PhD¹}, Lee Trani,^{BS¹}, Matthew Matlock,^{BS¹}, Alex H. Wagner,^{PhD¹}, S. Joshua Swamidas,^{MD/PhD^{4,5}}, Malachi Griffith,^{PhD^{1,2,3,6}} and Obi L. Griffith,^{PhD^{1,2,3,6}}

Generate a lot of data

Machine learning →

nature genetics

TECHNICAL REPORT

<https://doi.org/10.1038/s41588-018-0257-y>

A deep learning approach to automate refinement of somatic variant calling from cancer sequencing data

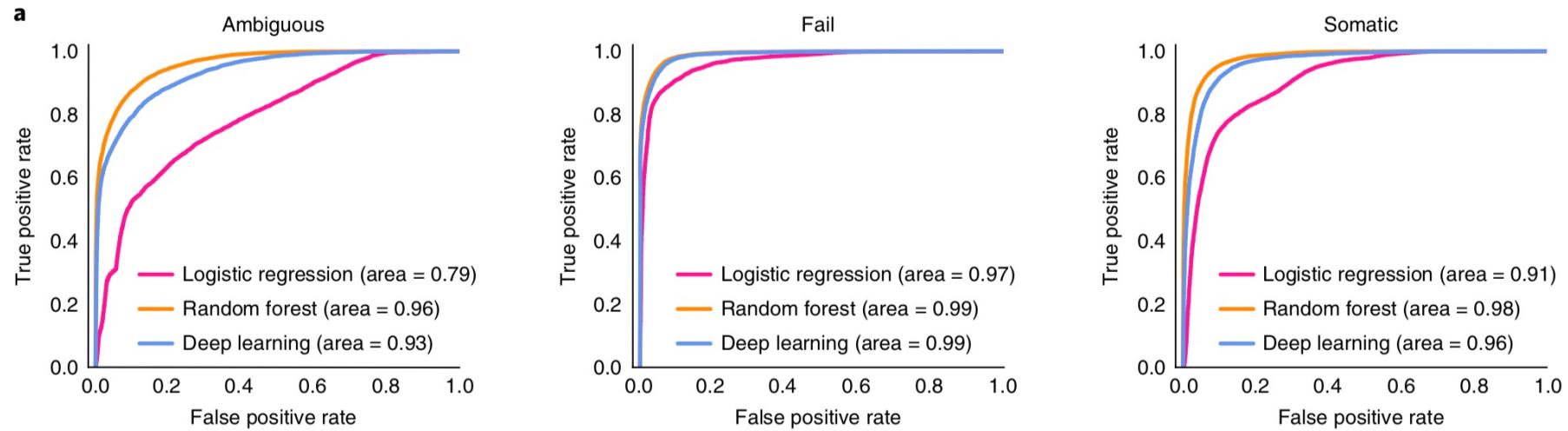
Benjamin J. Ainscough^{①,2,12}, Erica K. Barnell^{②,1,12}, Peter Ronning¹, Katie M. Campbell^{③,1}, Alex H. Wagner^{④,1}, Todd A. Fehniger^{⑤,2,3}, Gavin P. Dunn^④, Ravindra Uppaluri^⑤, Ramaswamy Govindan^{②,3}, Thomas E. Rohan^⑥, Malachi Griffith^{⑦,1,2,3,7}, Elaine R. Mardis^{⑧,9}, S. Joshua Swamidas^{⑩,11*} and Obi L. Griffith^{⑨,1,2,3,7*}

Improve speed!

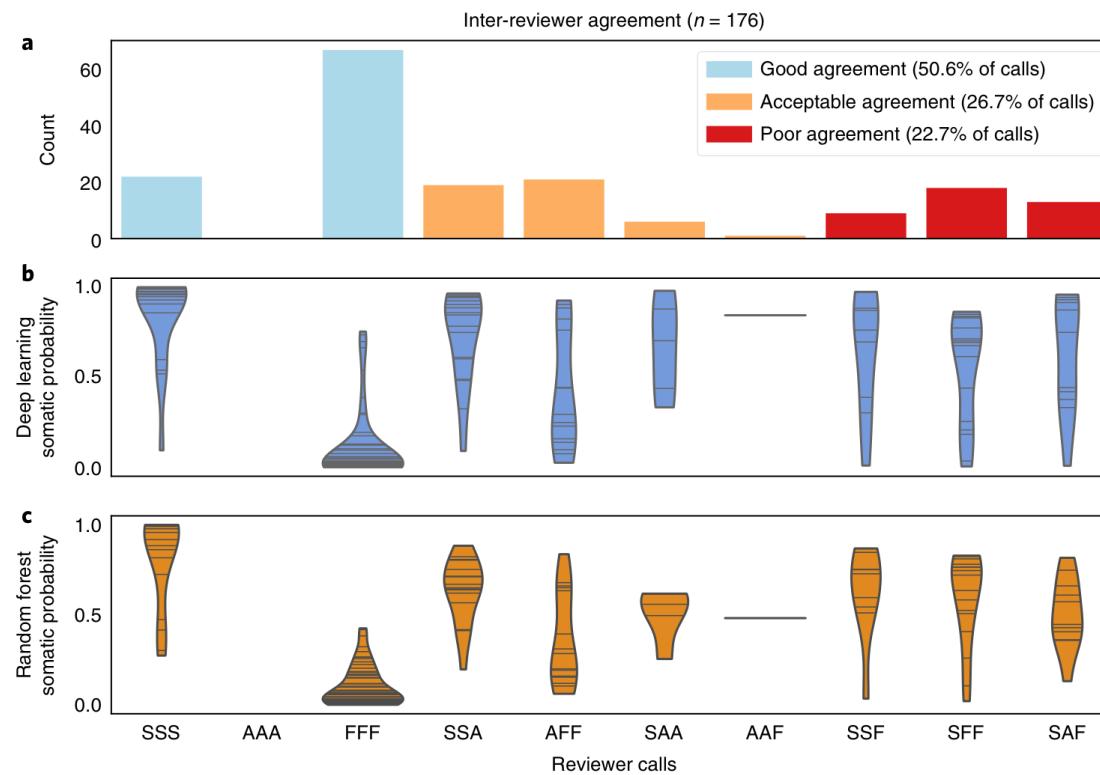
Why manual when it can be automated ..

- Potential of using machine learning to replace manual assessment
 - Training data set:
 - 41,000 variants from 21 studies
 - 440 cases derived from nine cancer subtypes.
 - Paired tumor and normal samples
 - Estimated manual effort: 585 hours
 - 71 features was assigned to train the model
 - For example: cancer type, sample type, tumor read depth, normal read depth, tumor VAF, normal VAF, base quality, mapping quality

Good enough?

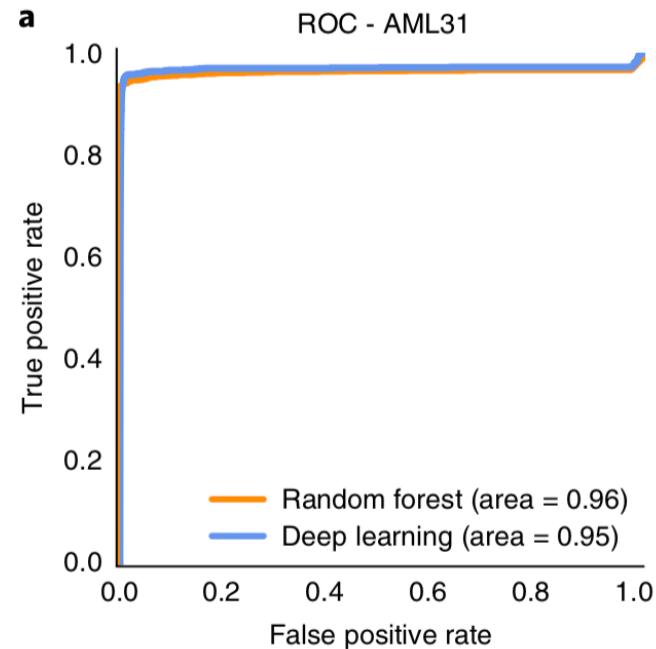


When reviewers agree - performance is good

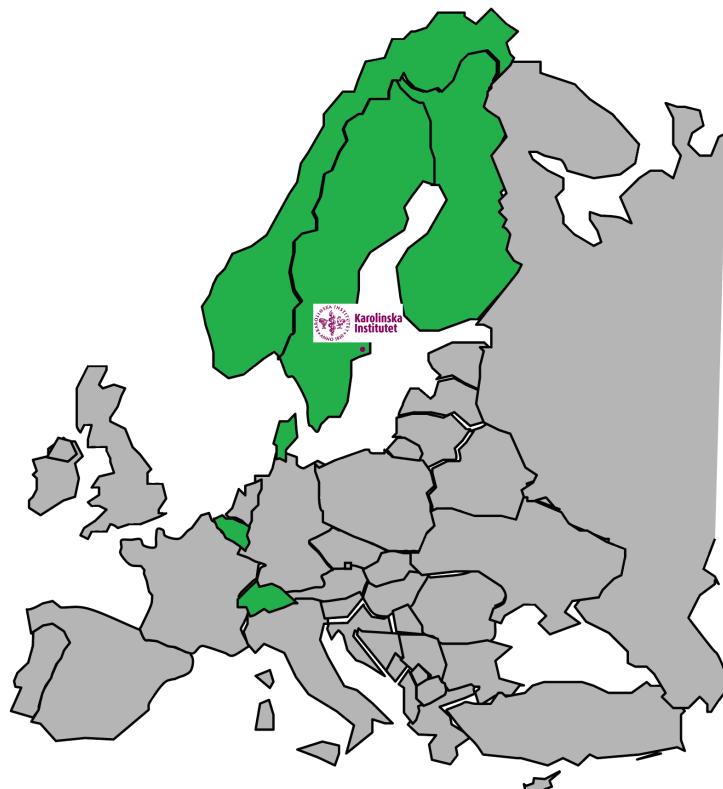


Validation

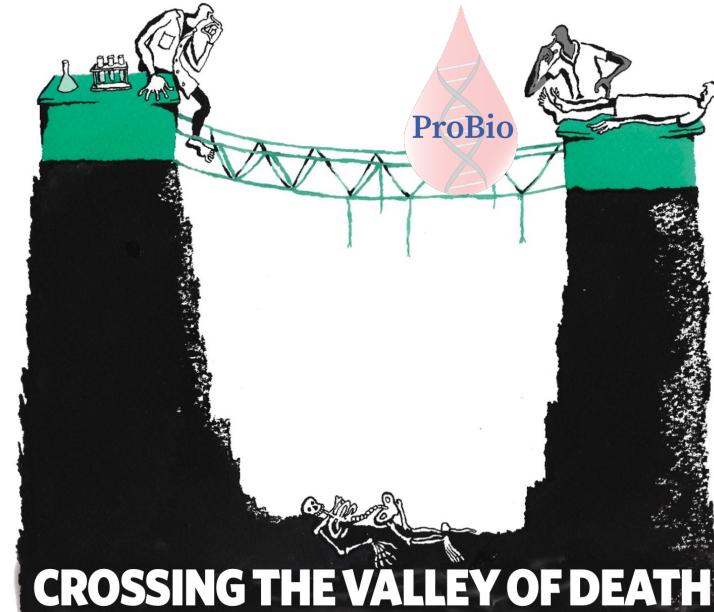
- Independent validation of an AML case.
 - 192,241 putative somatic variants
 - All variants validated using targeted sequencing
 - Better truth set = better ROC curve



How to solve the challenge problem for studies and routine?

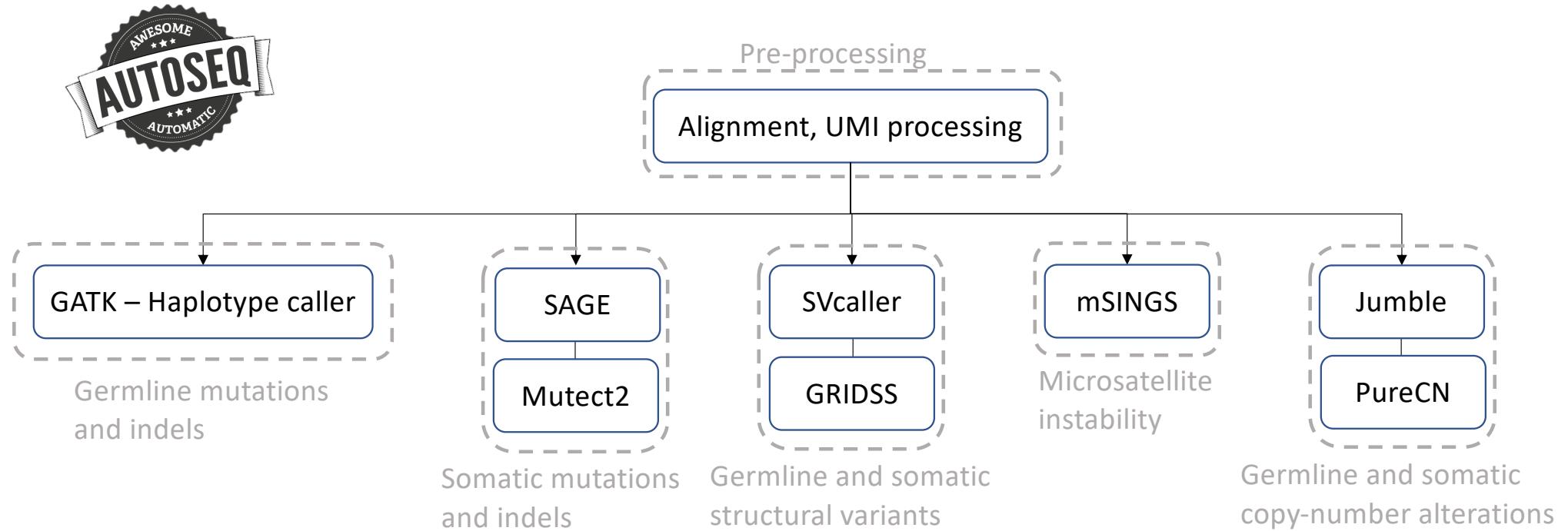


Research



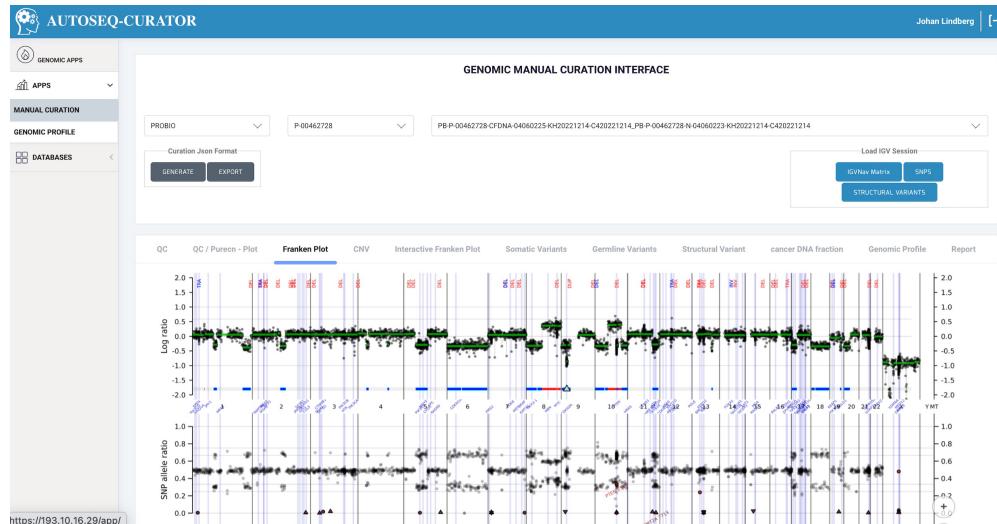
Routine diagnostics

AutoSeq Pipeline



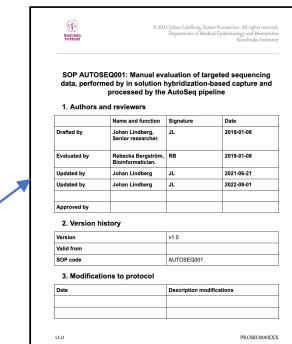
Curation (examination) of the results

Curation interface

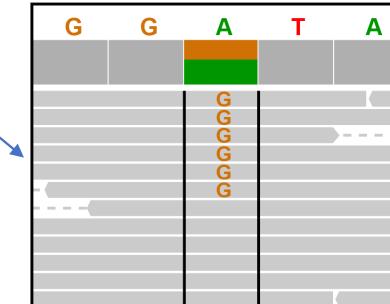


Everything available by a click of a button.

SOP in google cloud



Variant assessment



Curation took 1 – 4 hours before the development of the curation interface.
Now it takes 15 – 30 min.

Joint curation - PB597