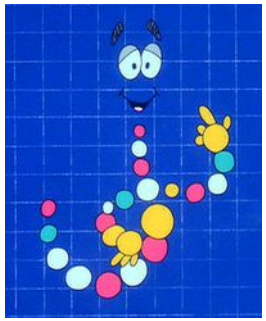
Bioinformatic tales and tools

Tristan M. Carland, PhD
for
SIO Bioinformatic Users Group (SIO-BUG)

CarlandT@gmail.com

Tales and Tools

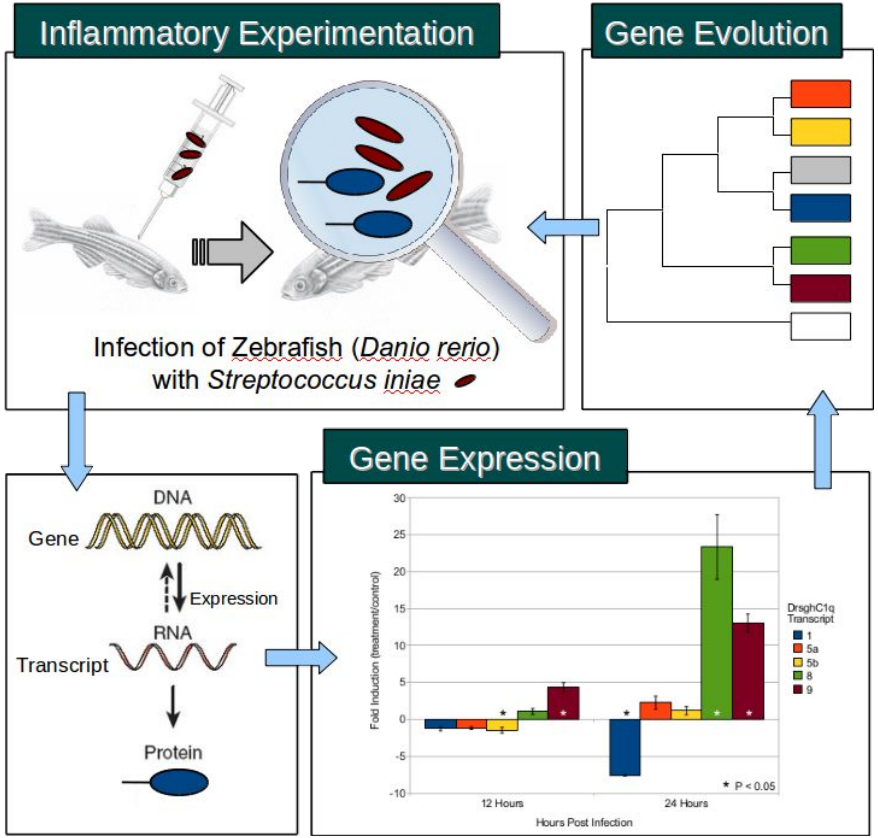
- Career Overview
- Once I was a graduate student
  - Mega, Topali, Chimera, LibreOffice
- The tale(s) of too many projects
  - Unix, BWA, SamTools, GATK, SVS
  - What is a cluster and/or HPC?
- How does cancer work?
- What is it, you do here?
  - Scrum ftw, what is testing?



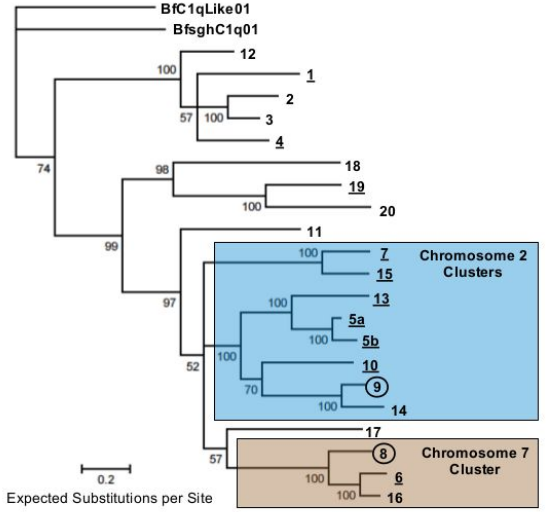
My path to wherever

1. UNCW - Majored in Computer Science
    a. Added a major in Marine Biology (fish are cool)
    b. Went to the Smithsonian (worms are cool)
2. Came to SIO for PhD in Marine Biology
    a. Learned Molecular Biology (in the lab with Lena)
    b. Dabbled in Bioinformatics
3. Postdoc in Genomic Medicine at TSRI
    a. Moved to Human Biology at JCVI
4. Cancer Genomics Investigator with Avera
5. Freelance Consultant in Genomics
6. Software Engineer II (SDET) at Illumina

Once I was a “graduate student”



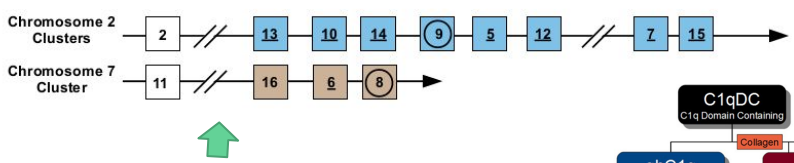
A.



B.

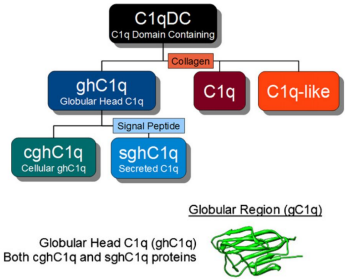
DrsghC1q Gene	Chr.	Exons/ Length	Expression
1	18	3 / 477	Development
2	2	3 / 606	
3	24	3 / 606	
4	23	3 / 600	Development
5a, 5b	2	4 / 624, 642	Development
6	7	3 / 609	Development
7	2	3 / 696	Development
8	7	3 / 678	Infection
9	2	3 / 573	Infection
10	2	3 / 720	
11	7	3 / 636	
12	2	3 / 600	
13	2	4 / 639	Development
14	2	4 / 678	
15	2	6 / 696	Development
16	7	3 / 651	
17	10	5 / 705	Development
18	15	4 / 714	
19	5	3 / 681	Development
20	15	3 / 678	

C.

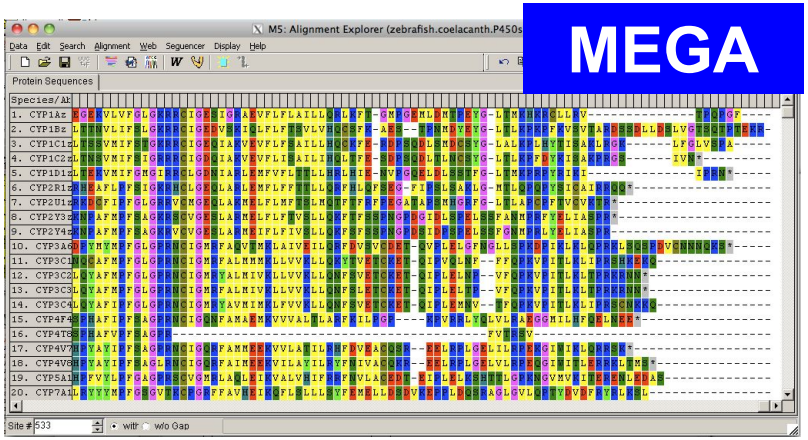


Carland, T. M., Locke, J. B., Nizet, V., & Gerwick, L. (2012). Differential expression and intrachromosomal evolution of the sghC1q genes in zebrafish (Danio rerio). Developmental and Comparative Immunology, 36(1), 31–38.

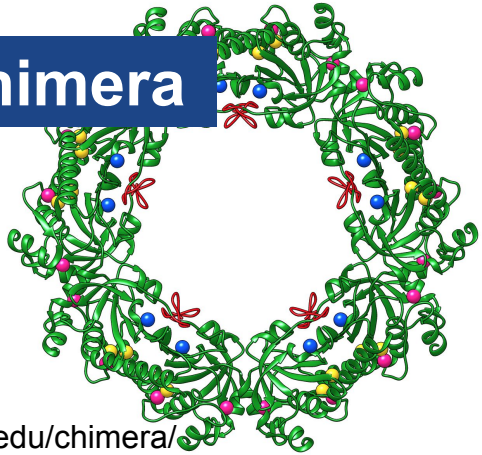
Carland, T. M., & Gerwick, L. (2010). The C1q domain containing proteins: Where do they come from and what do they do? Developmental & Comparative Immunology, 34(8), 785–790.



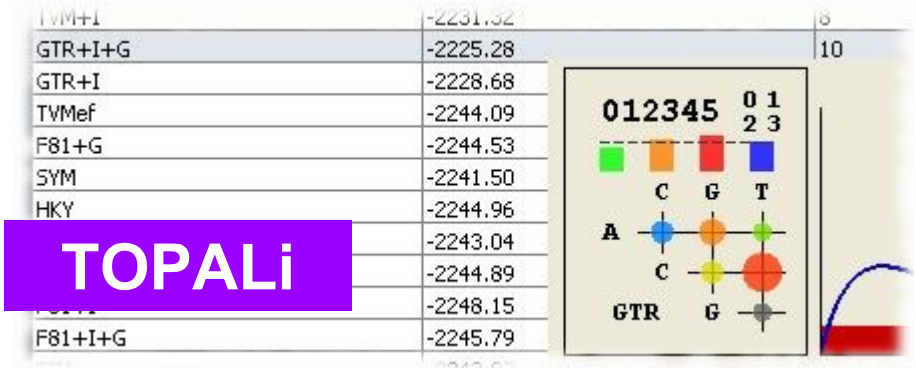
MEGA, Topali, Chimera, InkScape, LibreOffice



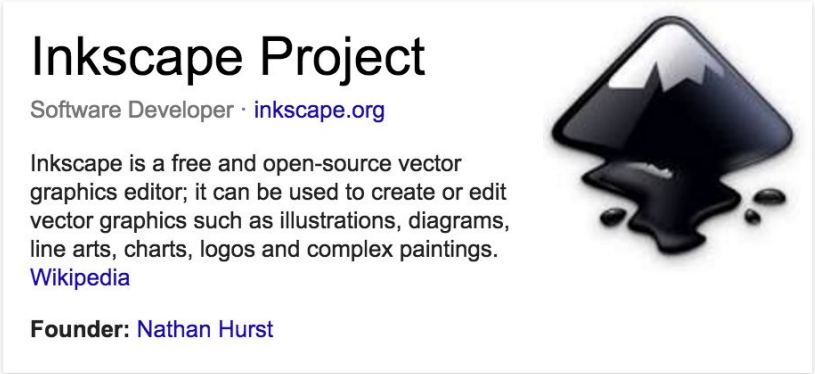
http://www.megasoftware.net/



https://www.cgl.ucsf.edu/chimera/



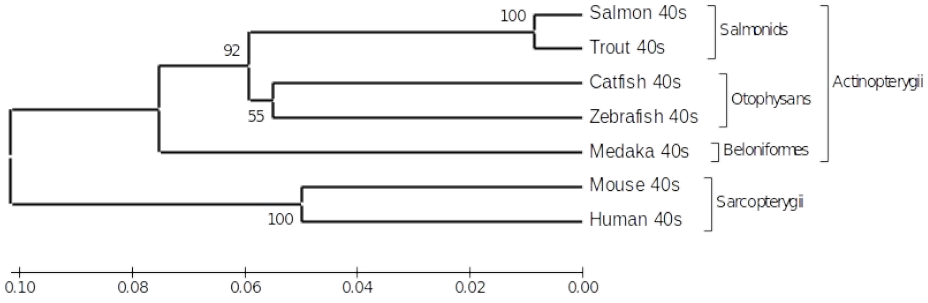
http://www.topali.org

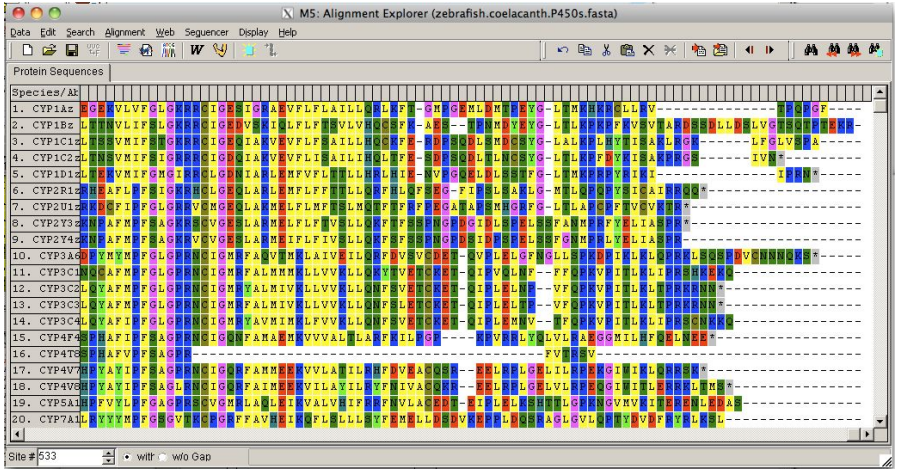


https://inkscape.org/en/

The Three beats of Phylogenetics

- Sequence Choice (NCBI)
  - Organism and/or Gene (family)
- Multiple Sequence Alignment (MEGA)
  - Refine Gap Penalty Parameters
- Phylogenetic Reconstruction (TOPALi)
  - Many ways, many models





~80%

of your time doing bioinformatics will be spent trying to get things to work

just keep swimming

Personalized Genomic Medicine

Scripps Translational Science Institute

The Scripps Research Institute

Scripps

The Creative Destruction of MEDICINE

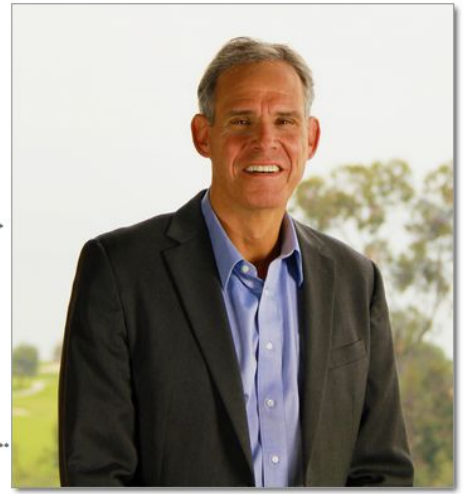
HOW THE DIGITAL REVOLUTION WILL CREATE BETTER HEALTH CARE

Home | Praise | Author | Excerpt | Buy

About Eric Topol

SHARE

Eric Topol, M.D. is professor of genomics and holds the Scripps endowed chair in innovative medicine. He is the director of the Scripps Translational Science Institute in La Jolla, California. Previously, he led the Cleveland Clinic to its #1 ranking in heart care, started a new medical school, and led key discoveries in heart disease. He lives with his family in La Jolla, California.



To learn more about Dr. Topol and his work, please visit his Leigh Bureau Speakers page as well as the Scripps Translational Science Institute, Scripps Health, Scripps Research Institute, and West Wireless Health Institute websites.

Follow Dr. Topol on Twitter, Facebook, and LinkedIn.

Watch his TED talk here.

Dr. Topol on NPR's Science Friday

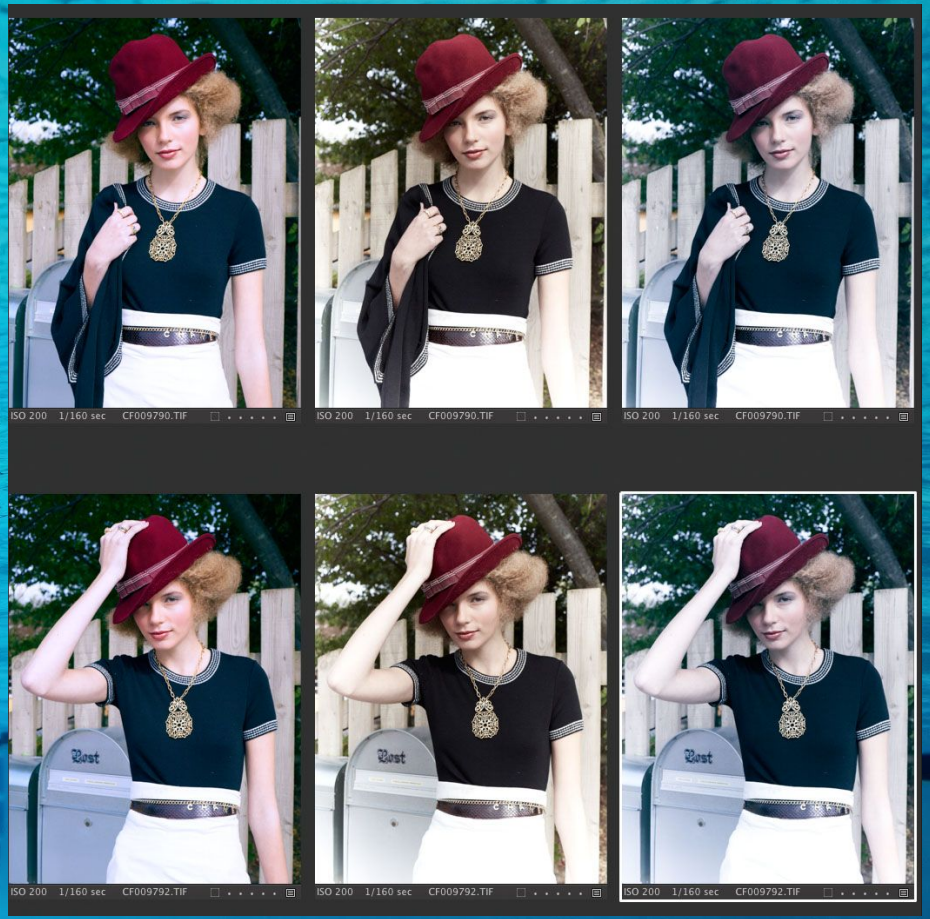
Watch *Medscape's One on One:* Dr. John Reed interviews Dr. Topol about how technology is changing medicine, making diagnosis and treatment faster, better, and more accurate.

So you've had a heart attack

ATCGATCGCGCTGAGCATAGCGATCGCGCCGATCG
ATCGATCGCGCTGAGCATAGCGATCGCGCCGATCG
ATCGATCGCGCTGAGCATAGCGATCGCGCCGATCG
ATCGATCGCGCAGAGCATAGCGATCGCGCCGATCG

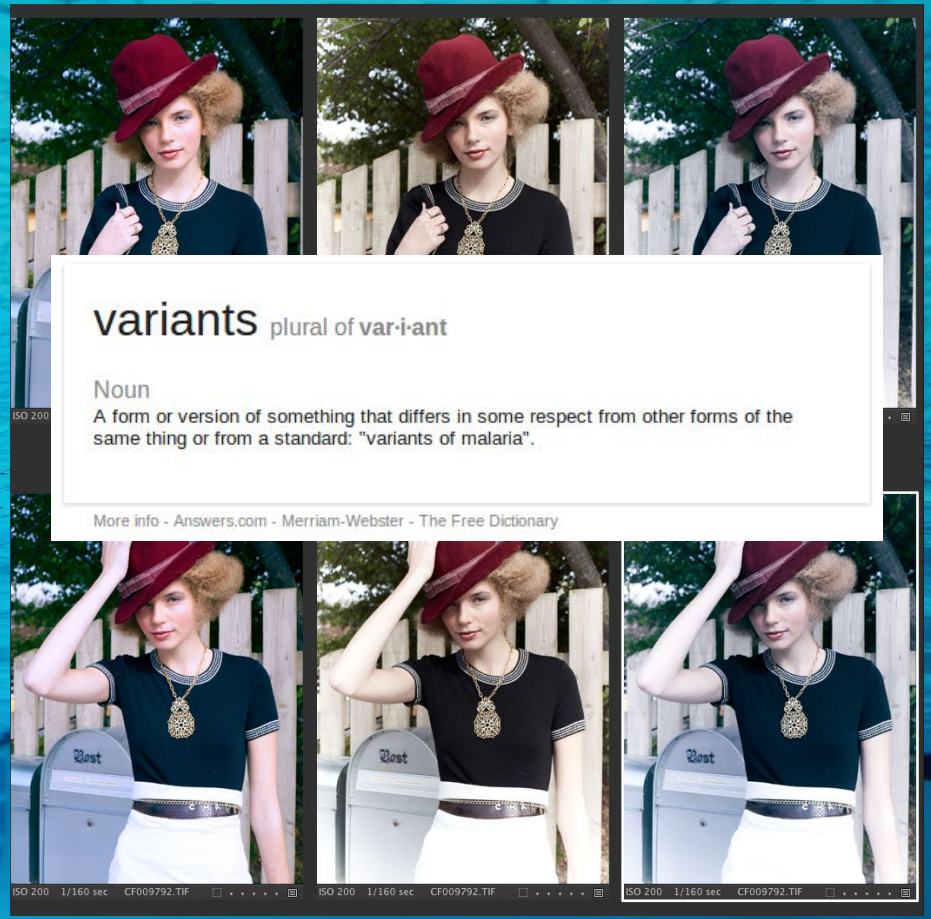
What genomic info should we save? Why?

- Every human genome should be over 99% identical
- Storing the raw data for each sample makes sense
- What should we store to run computations on?
  - We need something to compare easily



What genomic info should we save? Why?

- Every human genome should be over 99% identical
- Storing the raw data for each sample makes sense
- What should we store to run computations on?
  - We need something to compare easily
  - The differences are what define the samples



Variant Call Format

```
##fileformat=VCFv4.1
##FILTER=<ID=LowQual,Description="Low quality">
##FORMAT=<ID=AD,Number=.,Type=Integer,Description="Allelic depths for the ref and alt alleles in the order listed">
##INFO=<ID=MQ0,Number=1,Type=Integer,Description="Total Mapping Quality Zero Reads">
##contig=<ID=1,length=249250621,assembly=b37>
##contig=<ID=2,length=243199373,assembly=b37>
##contig=<ID=GL000206.1,length=41001,assembly=b37>
##contig=<ID=GL000240.1,length=41933,assembly=b37>
##reference=file:///projects/stsi4/HUTs/HT020/scripts/../ref/human_g1k_v37.fasta
#CHROM  POS ID  REF ALT QUAL  FILTER  INFO  FORMAT  HT020en HT020et
1 10177 . A AC  176.41  . AC=2;AF=0.500;AN=4;BaseQRankSum=-1.899;DP=38;FS=8.038;HaplotypeScore=125.8695;MLEAC=2;MLEAF=(
1 10389 . AC  A 288.40  . AC=2;AF=0.500;AN=4;BaseQRankSum=2.179;DP=71;FS=10.976;HaplotypeScore=272.6294;MLEAC=2;MLEAF=(
1 10396 . AC  A 287.40  . AC=2;AF=0.500;AN=4;BaseQRankSum=1.603;DP=68;FS=3.176;HaplotypeScore=269.5334;MLEAC=2;MLEAF=0
1 10439 rs112766696 AC  A 239.40  . AC=2;AF=0.500;AN=4;BaseQRankSum=1.599;DB;DP=50;FS=5.969;HaplotypeScore=236.8964;ML
1 61989 rs77573425  G C 48.17 . AC=2;AF=1.00;AN=2;DB;DP=3;Dels=0.00;FS=0.000;HaplotypeScore=0.0000;MLEAC=2;MLEAF=1.00;
1 569984  . A C 35.20 . AC=1;AF=0.500;AN=2;BaseQRankSum=1.495;DP=10;Dels=0.00;FS=3.680;HaplotypeScore=0.0000;MLEAC=1;ML
1 706330  . A AG  183.59  . AC=3;AF=0.750;AN=4;BaseQRankSum=-0.751;DP=20;FS=0.000;HaplotypeScore=1.8880;MLEAC=3;MLEAF=(
1 714427  rs12028261  G A 743.20  . AC=4;AF=1.00;AN=4;DB;DP=32;Dels=0.00;FS=0.000;HaplotypeScore=0.0000;MLEAC=4;MLEAF=
1 715348  rs3131984 T G 158.02  . AC=4;AF=1.00;AN=4;DB;DP=5;Dels=0.00;FS=0.000;HaplotypeScore=0.0000;MLEAC=4;MLEAF=1.0(
1 723798  rs34882115  CAG C 1003.16 . AC=4;AF=1.00;AN=4;DB;DP=24;FS=0.000;HaplotypeScore=5.4571;MLEAC=4;MLEAF=1.00;MQ=
1 723891  rs2977670 G C 370.27  . AC=4;AF=1.00;AN=4;DB;DP=12;Dels=0.00;FS=0.000;HaplotypeScore=0.0000;MLEAC=4;MLEAF=1.(
```

Variant Call Format

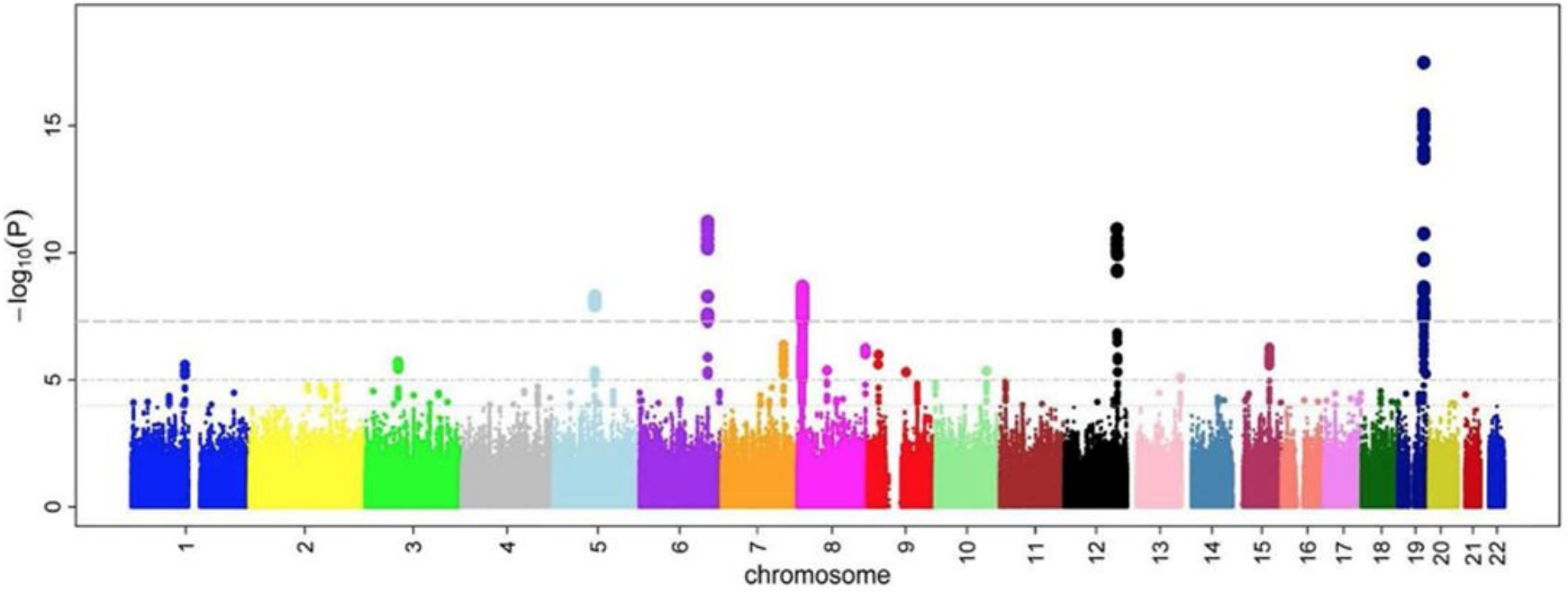
#CHROM	POS	ID	REF	ALT	QUAL	HT020en	HT020et
1	10177	0	A	AC	176.41	0/1:5,4:9:24:67,0,24	0/1:6,5:11:71:147,0,71
1	10389	0	AC	A	288.4	0/1:7,7:14:99:140,0,154	0/1:10,9:22:99:186,0,304
1	10396	0	AC	A	287.4	0/1:6,8:14:99:209,0,124	0/1:11,8:21:99:116,0,270
1	10439	rs112766696	AC	A	239.4	0/1:10,4:14:93:93,0,241	0/1:7,7:17:99:184,0,163
1	61989	rs77573425	G	C	48.17	1/1:1,2:3:6:74,6,0	./.
1	569984	0	A	C	35.2	0/1:6,2:8:62:62,0,137	./.
1	706330	0	A	AG	183.59	0/1:1,3:4:15:80,0,15	1/1:0,5:5:15:142,15,0
1	714427	rs12028261	G	A	743.2	1/1:0,13:13:33:377,33,0	1/1:1,17:19:33:393,33,0
1	715348	rs3131984	T	G	158.02	1/1:0,2:2:6:78,6,0	1/1:0,3:3:9:106,9,0
1	723798	rs34882115	CAG	C	1003.16	1/1:0,13:13:39:547,39,0	1/1:0,11:11:33:492,33,0
1	723891	rs2977670	G	C	370.27	1/1:0,6:6:18:193,18,0	1/1:0,6:6:18:204,18,0
1	725104	rs201386617	C	A	42.42	0/0:1,0:1:3:0,3,33	0/1:1,2:3:24:70,0,24
1	725106	rs200821612	C	G	38.42	0/0:1,0:1:3:0,3,33	0/1:1,2:3:24:66,0,24
1	726202	0	C	T	53.44	0/1:2,2:4:52:55,0,52	0/1:2,1:3:27:27,0,39
1	726481	rs3131980	T	G	205.87	1/1:0,4:4:12:153,12,0	1/1:0,2:2:6:79,6,0
1	729679	rs4951859	C	G	197.26	./.	1/1:0,6:6:18:224,18,0
1	734460	0	TG	T	173.71	1/1:0,3:4:9:109,9,0	1/1:0,3:3:9:100,9,0
1	734491	rs7518433	T	C	47.42	1/1:0,2:2:3:39,3,0	1/1:0,1:1:3:33,3,0
1	734566	rs139489325	G	A	38.41	1/1:0,1:1:3:38,3,0	0/1:3,2:5:28:28,0,104
1	739142	rs2340527	T	A	359.44	0/1:7,9:16:99:214,0,185	0/1:18,8:25:99:174,0,402
1	752566	rs3094315	G	A	844.21	1/1:0,8:8:24:302,24,0	1/1:0,15:15:45:569,45,0
1	752721	rs3131972	A	G	5290.2	1/1:1,67:67:99:2327,181,0	1/1:0,85:85:99:2990,235,0
1	754182	rs3131969	A	G	314.4	1/1:0,5:5:12:155,12,0	1/1:0,6:6:15:186,15,0
1	754192	rs3131968	A	G	123.52	1/1:0,2:2:3:34,3,0	1/1:0,4:4:9:115,9,0
1	754334	rs3131967	T	C	151.02	1/1:0,2:2:6:76,6,0	1/1:0,3:3:9:101,9,0

Variant Call Format

#CHROM	POS	ID	REF	ALT	QUAL	HT020en	HT020et
1	10177	0	A	AC	176.41	0/1:5,4:9:24:67,0,24	0/1:6,5:11:71:147,0,71
1	10389	0	AC	A	288.4	0/1:7,7:14:99:140,0,154	0/1:10,9:22:99:186,0,304
1	10396	0	AC	A	287.4	0/1:6,8:14:99:209,0,124	0/1:11,8:21:99:116,0,270
1	10439	rs112766696	AC	A	239.4	0/1:10,4:14:93:93,0,241	0/1:7,7:17:99:184,0,163
1	61989	rs77573425	G	C	48.17	1/1:1,2:3:6:74,6,0	./.
1	569984	0	A	C	35.2	0/1:6,2:8:62:62,0,157	./.
1	714427	rs3131984	G	A	743.2	1/1:0,13:13:33:377,33,0	1/1:0,5:5:15:141,15,0
1	715348	rs3131984	T	G	158.02	1/1:0,2:2:6:78,6,0	1/1:0,3:3:9:106,9,0
1	723798	rs34882115	CAG	C	1003.16	1/1:0,13:13:39:547,39,0	1/1:0,11:11:33:492,33,0
1	725091	rs2977670	G	C	370.2	0/1:1,6:7:18:193,0,18	0/1:0,6:6:18:204,18,0
1	725104	rs201386617	C	A	42.42	0/0:1,0:1:3:0,3,33	0/1:1,2:3:24:70,0,24
1	725106	rs200821612	C	G	38.42	0/0:1,0:1:3:0,3,33	0/1:1,2:3:24:66,0,24
1	726202	0	C	T	53.44	0/1:2,2:4:52:55,0,52	0/1:2,1:3:27:27,0,39
1	726481	rs3131980	T	G	205.87	1/1:0,4:4:12:153,12,0	1/1:0,2:2:6:79,6,0
1	729679	rs4951859	C	G	197.2	0/1:1,6:7:18:193,0,18	0/1:0,6:6:18:204,18,0
1	734460	0	TG	T	173.71	1/1:0,3:4:9:109,9,0	1/1:0,3:3:9:100,9,0
1	734491	rs7518433	T	C	47.42	1/1:0,2:2:3:39,3,0	1/1:0,1:1:3:33,3,0
1	735146	rs139485325	G	A	38.4	0/1:1,1:2:3:38,3,0	0/1:3,2:5:28:28,0,104
1	739142	rs2340527	T	A	359.44	0/1:7,9:16:99:214,0,185	0/1:18,8:25:99:174,0,402
1	743236	rs3094315	G	A	844.2	1/1:0,8:8:24:302,24,0	1/1:0,15:15:45:569,45,0
1	752721	rs3131972	A	G	5290.2	1/1:1,67:67:99:2327,181,0	1/1:0,85:85:99:2990,235,0
1	754182	rs3131969	A	G	314.4	1/1:0,5:5:12:155,12,0	1/1:0,6:6:15:186,15,0
1	754192	rs3131968	A	G	123.52	1/1:0,2:2:3:34,3,0	1/1:0,4:4:9:115,9,0
1	754334	rs3131967	T	C	151.02	1/1:0,2:2:6:76,6,0	1/1:0,3:3:9:101,9,0

Where is it?	What is the reference?	What is an alternative?	Our “normal” sample	Our “tumor” sample	Is it somatic?
1 : 10177	A	AC	0/1	0/1	No
1 : 729679	C	G	./.	1/1	Can’t say
1 : 888234	A	G	0/0	0/1	Yes
1 : 998233	T	A	0/1	0/0	?
1 : 998233	T	A	0/1	1/1	?

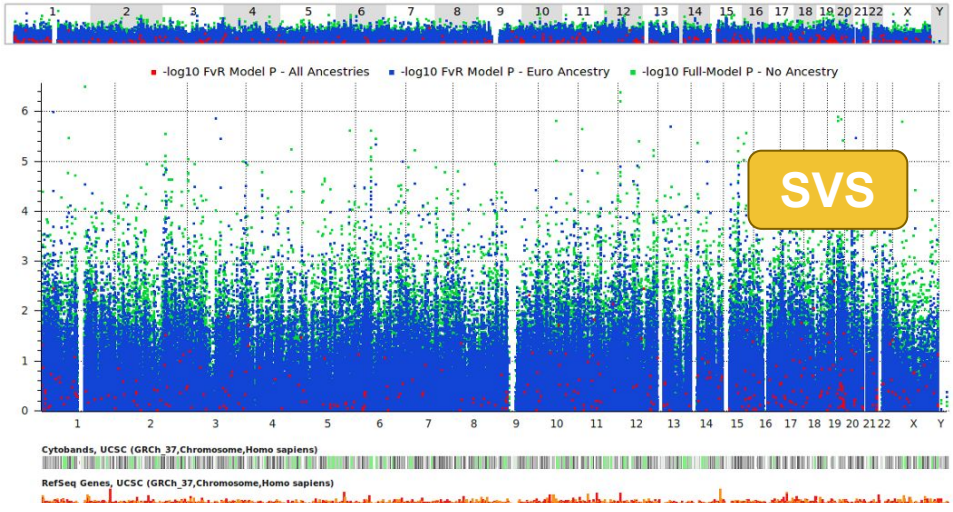
Manhattan Plots - great significance from small variants

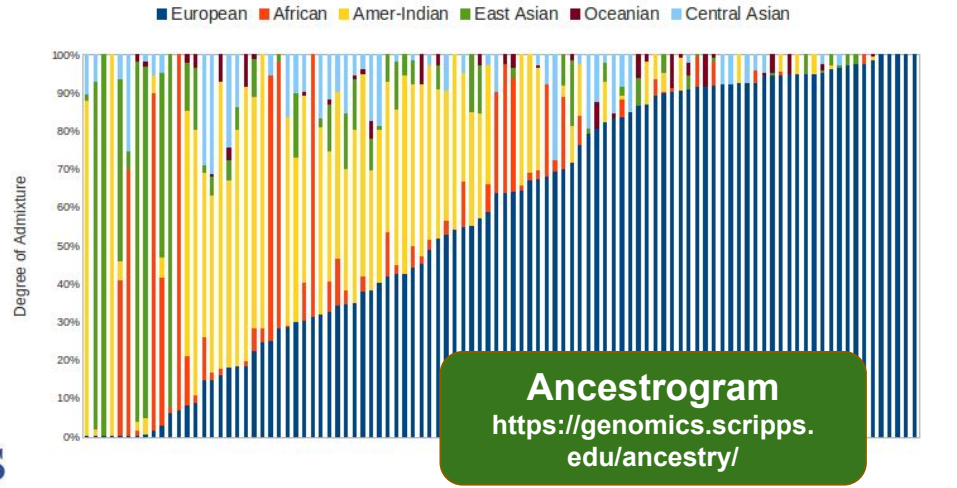


Each dot is a location within the genome, the -log10 P score denotes how well the variation at that position associates with the phenotype of interest.

Projects

- Personalized Cancer Medicine
- Alcoholism in Native Americans
- Group Variant Calling
- Mutated tRNA in Zebrafish
- **Kidney Transplant Exome GWAS**
- Cow Antibody Sequencing
- Eating Disorder Haplotype Study
- Dog Genome Assembly



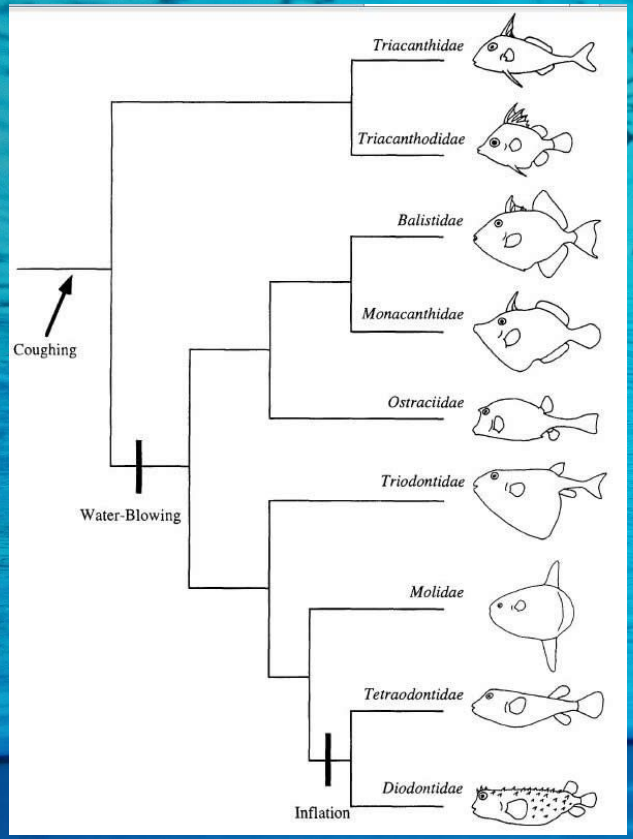


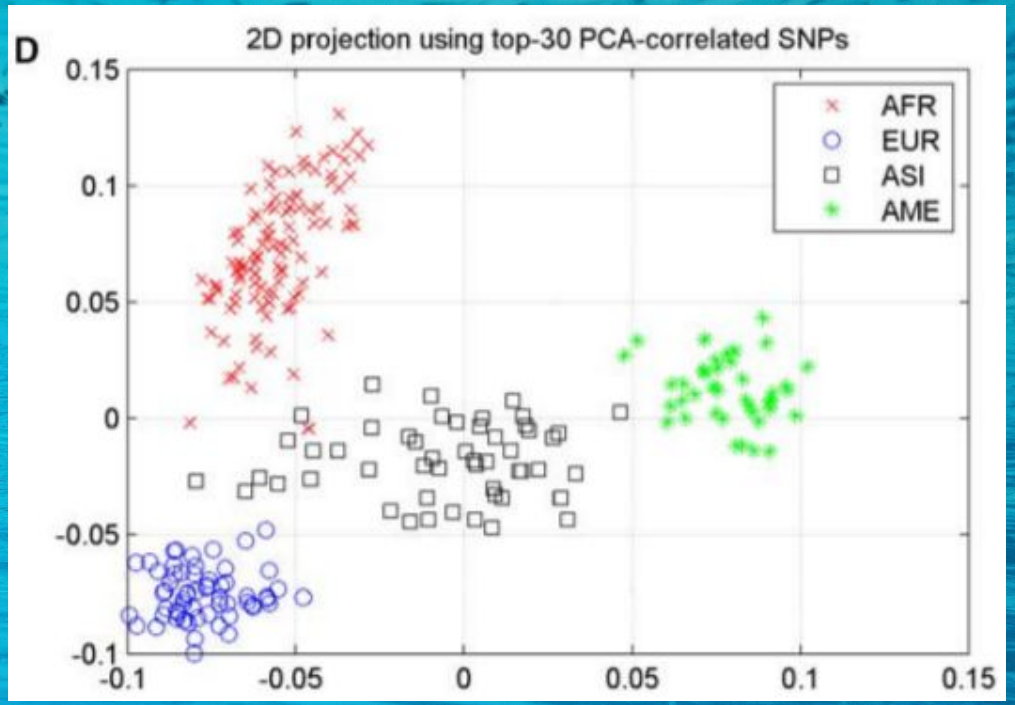
Scripps Translational Science Institute

The Scripps Research Institute

Scripps

Research Idea - Phylogenetic and PCA Clustering





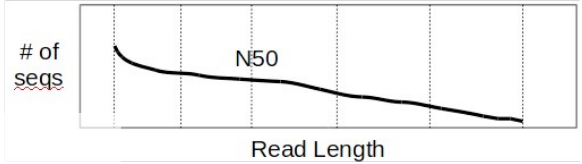
PCA-Correlated SNPs
for Structure Identification
in Worldwide Human Populations

Peristera Paschou[1*], Elad Ziv[2,3,4], Esteban G. Burchard[5,6], Shweta Choudhry[7], William Rodriguez-Cintron[8], Michael W. Mahoney[9], Petros Drineas[10]

Projects

- Personalized Cancer Medicine
- Alcoholism in Native Americans
- Group Variant Calling
- Mutated tRNA in Zebrafish
- Kidney Transplant Exome GWAS
- Cow Antibody Sequencing
- Eating Disorder Haplotype Study
- **Dog Genome Assembly!**

Build	Sequences	Total Length	Avg Length	N0	N25	N50	N75	N90	N100
lhm21	3531269	3479947123	985	100	3483	10420	21491	35262	158386
lhm25	5703282	3661489441	641	100	3487	17086	38545	65633	267301
lhm29	7799252	3771706238	483	100	1309	27886	68053	118654	524410
lha29	7758992	3774135425	486	100	1827	30472	74333	130182	678427
lhm31	8795319	3807721776	432	100	500	41348	106387	193997	758707
lha31	8805458	3810569526	432	100	510	43342	112099	203252	975104
lhm33	9858777	3829030194	388	100	108	58868	160049	298292	**1846276**
lha33	9901462	**3833244198**	387	100	108	**60492**	**163721**	**309579**	1314888
lhb33	9899263	3831539101	387	100	108	57915	156252	293855	1312357
lhm37	2313388	2293187894	991	100	1002	2039	3641	5637	23406
lhm41	2098570	2329859104	1110	100	1143	2347	4223	6584	27564
lhm55	1988803	2415984266	**1214**	100	1394	3089	5870	9448	56111
lhm95	**459987**	160020301	347	100	301	341	692	972	8080



- 108bp reads, 500bp inserts
- 108bp reads, 700bp inserts
- 50Bp reads, 500bp inserts
- 36Bp reads, 3kbp inserts

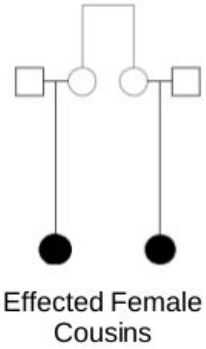
- Total Length 68,904,516,540
  - 68 gbp

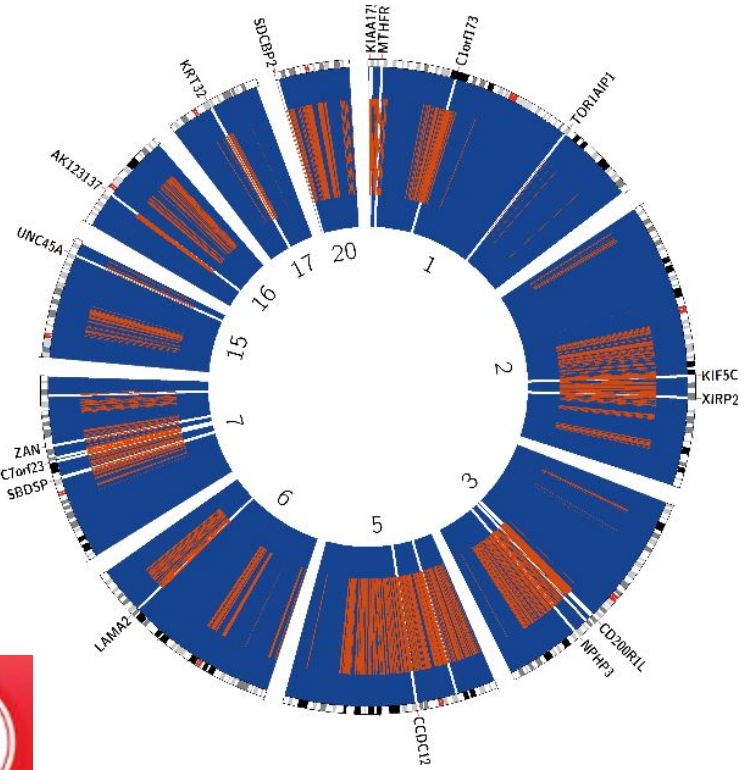
SOAP denovo
http://soap.genomics.org.cn/soapdenovo.html

Scripps Translational Science Institute
The Scripps Research Institute
Scripps

Projects

- Personalized Cancer Medicine
- Alcoholism in Native Americans
- Group Variant Calling
- Mutated tRNA in Zebrafish
- Kidney Transplant Exome GWAS
- Cow Antibody Sequencing
- **Eating Disorder Haplotype Study**
- Dog Genome Assembly





circos.ca

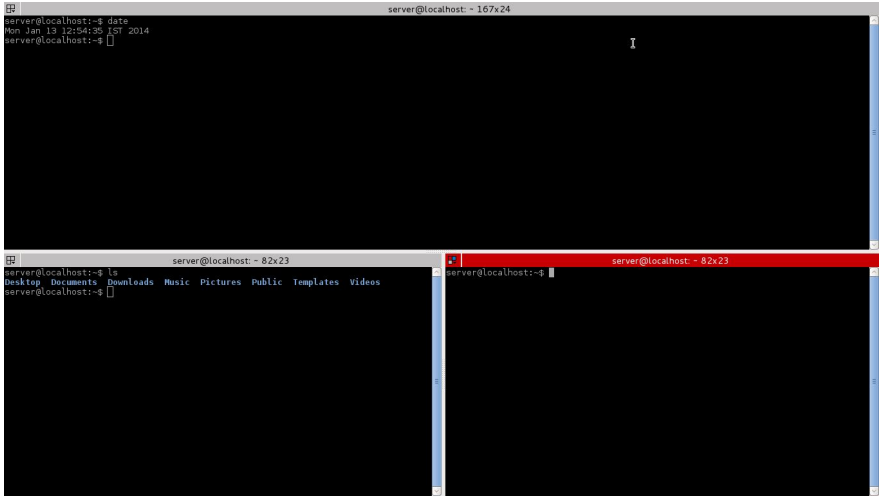
Shih *et al.* 2015 - submitted

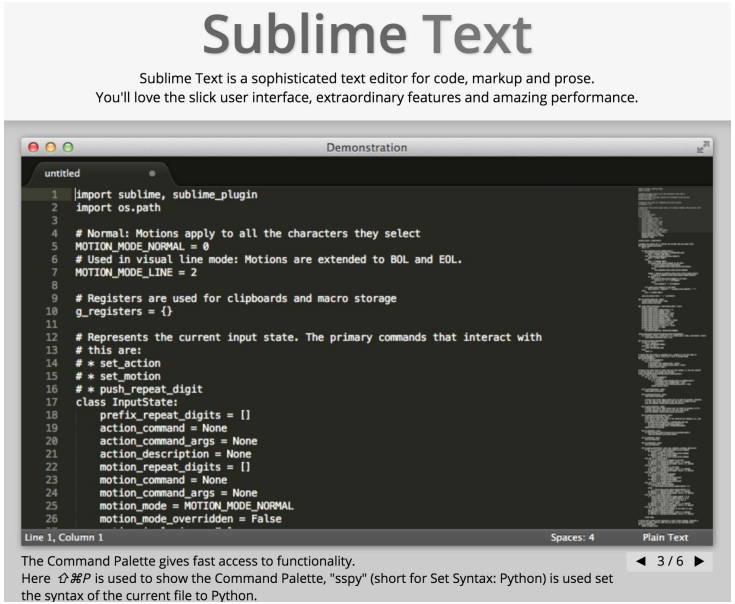
Scripps Translational Science Institute

The Scripps Research Institute

Scripps

Linux, Terminator, Sublime, awk+sed+grep





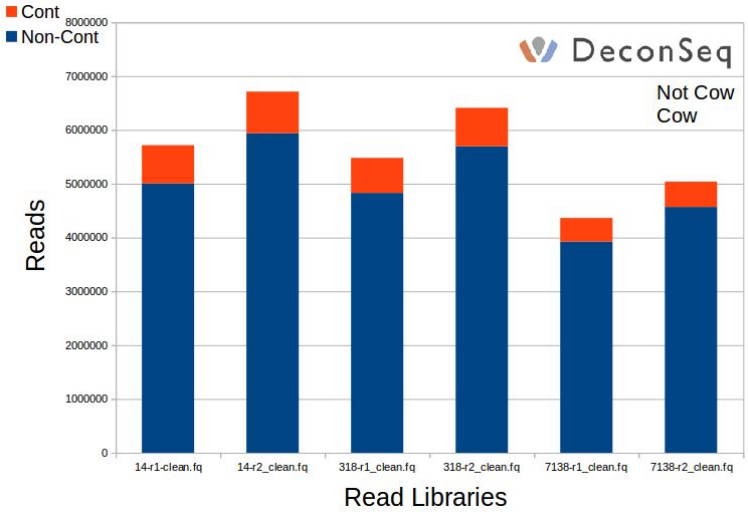
http://www.sublimetext.com/

Grep, AWK, SED, bash/sh/csh - learn them

- Grep - find lines in files
  - grep “thing” <file>
- awk - super versatile but I use it to parse columns and print things
  - awk -F “separator” ‘{print $1” is awesome”}’ <file>
- sed - replace things in files
  - sed -i s/aThing/aNewThing/g <file>
- bash/sh/csh - the shell where you run things
  - You can make scripts in here directly
  - http://tldp.org/HOWTO/Bash-Prog-Intro-HOWTO.html
- Perl would be good to learn (or Python)
  - http://perldoc.perl.org/perlintro.html - I still keep this bookmarked

Projects

- Personalized Cancer Medicine
- Alcoholism in Native Americans
- Group Variant Calling
- Mutated tRNA in Zebrafish
- Kidney Transplant Exome GWAS
- **Cow Antibody Sequencing**
- Eating Disorder Haplotype Study
- Dog Genome Assembly



DeconSeq

edwards.sdsu.edu/deconseq/

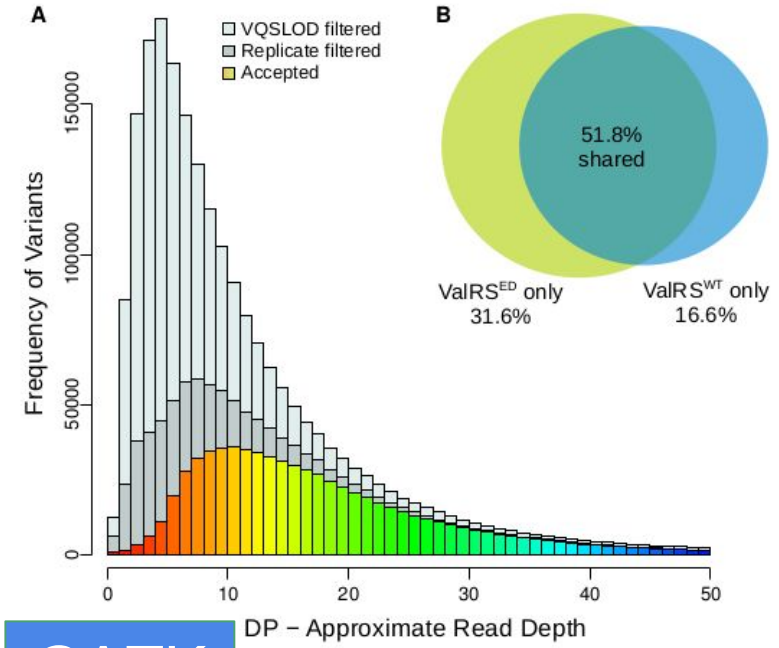
Scripps Translational Science Institute

The Scripps Research Institute

Scripps

Projects

- Personalized Cancer Medicine
- Alcoholism in Native Americans
- Group Variant Calling
- **Mutated tRNA in Zebrafish**
- Kidney Transplant Exome GWAS
- Cow Antibody Sequencing
- Eating Disorder Haplotype Study
- Dog Genome Assembly



GATK

RStudio

InkScape

Song *et al.* 2016 - in prep, again
Carland *et al*. 2016 - maaaaaybe

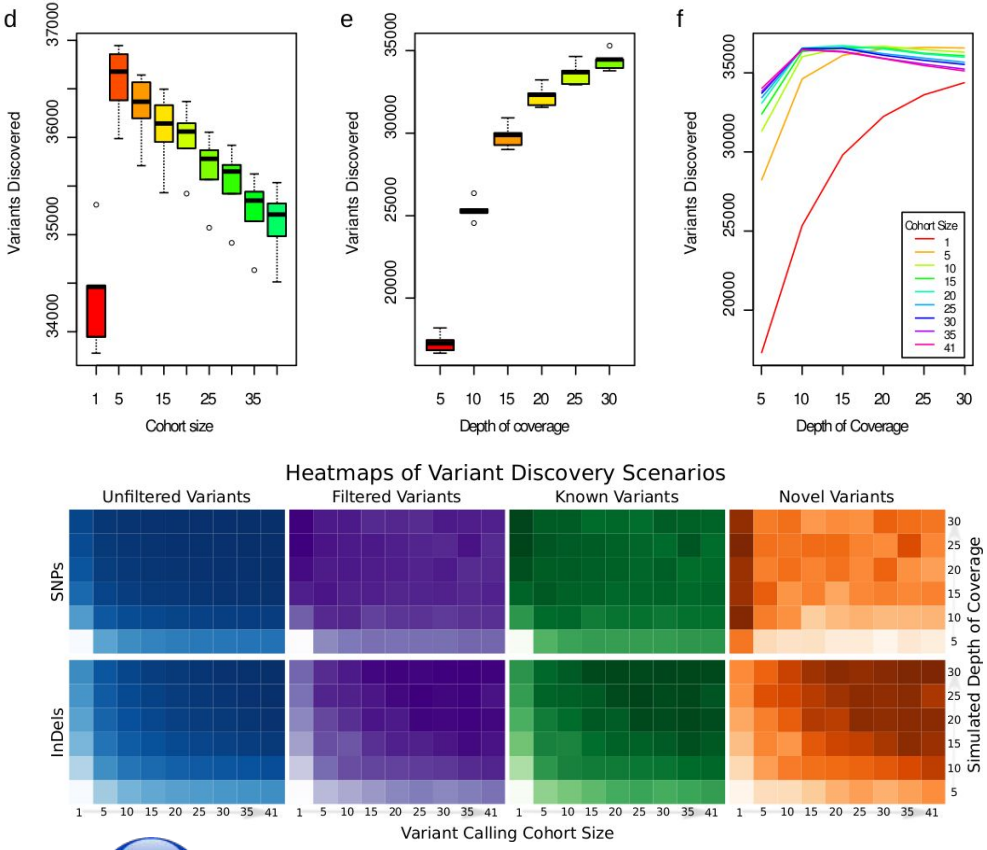
Scripps Translational Science Institute

The Scripps Research Institute

Scripps

Projects

- Personalized Cancer Medicine
- Alcoholism in Native Americans
- **Group Variant Calling**
- Mutated tRNA in Zebrafish
- Kidney Transplant Exome GWAS
- Cow Antibody Sequencing
- Eating Disorder Haplotype Study
- Dog Genome Assembly



Scripps Translational Science Institute

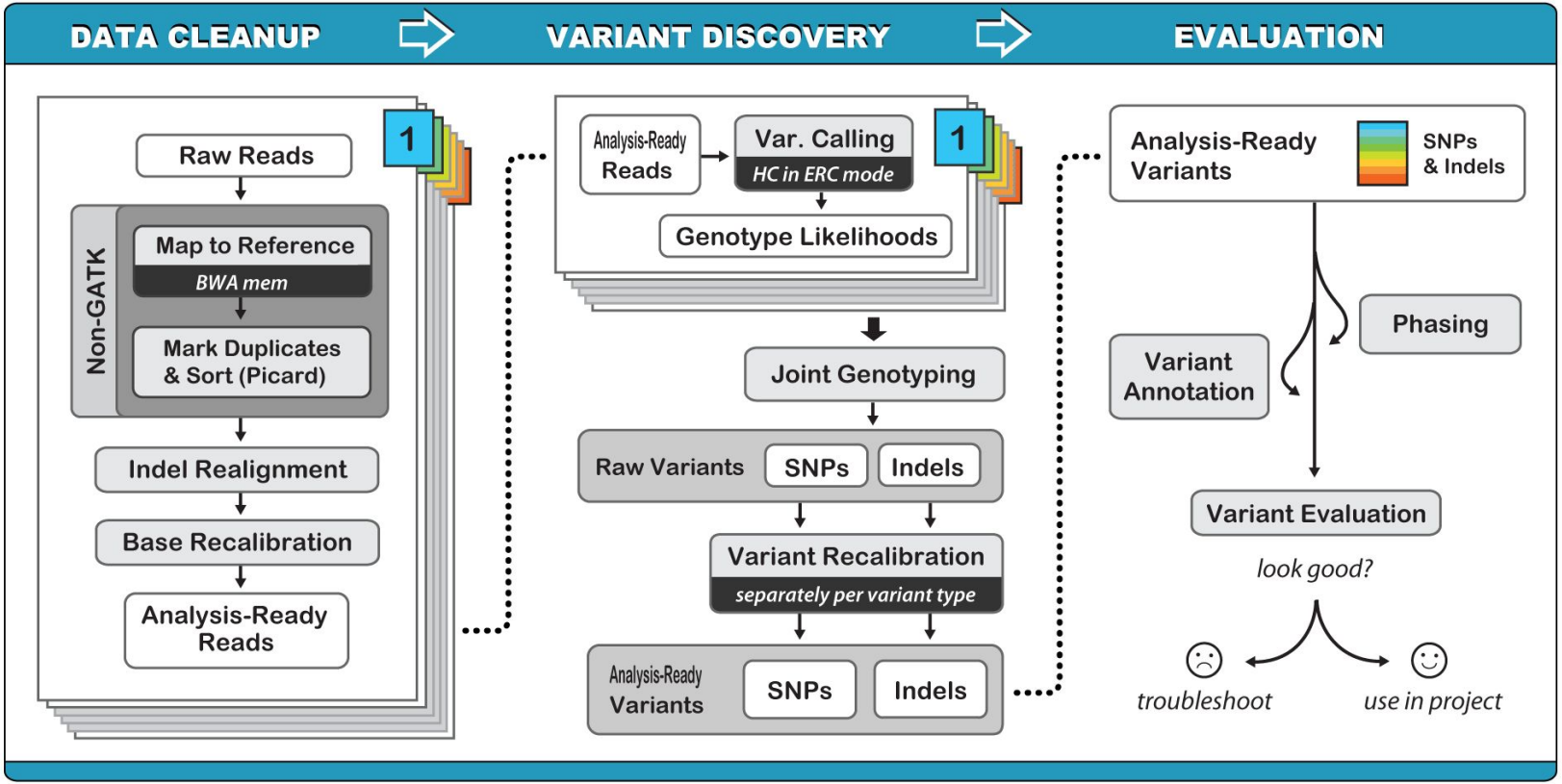
The Scripps Research Institute

Scripps

RStudio

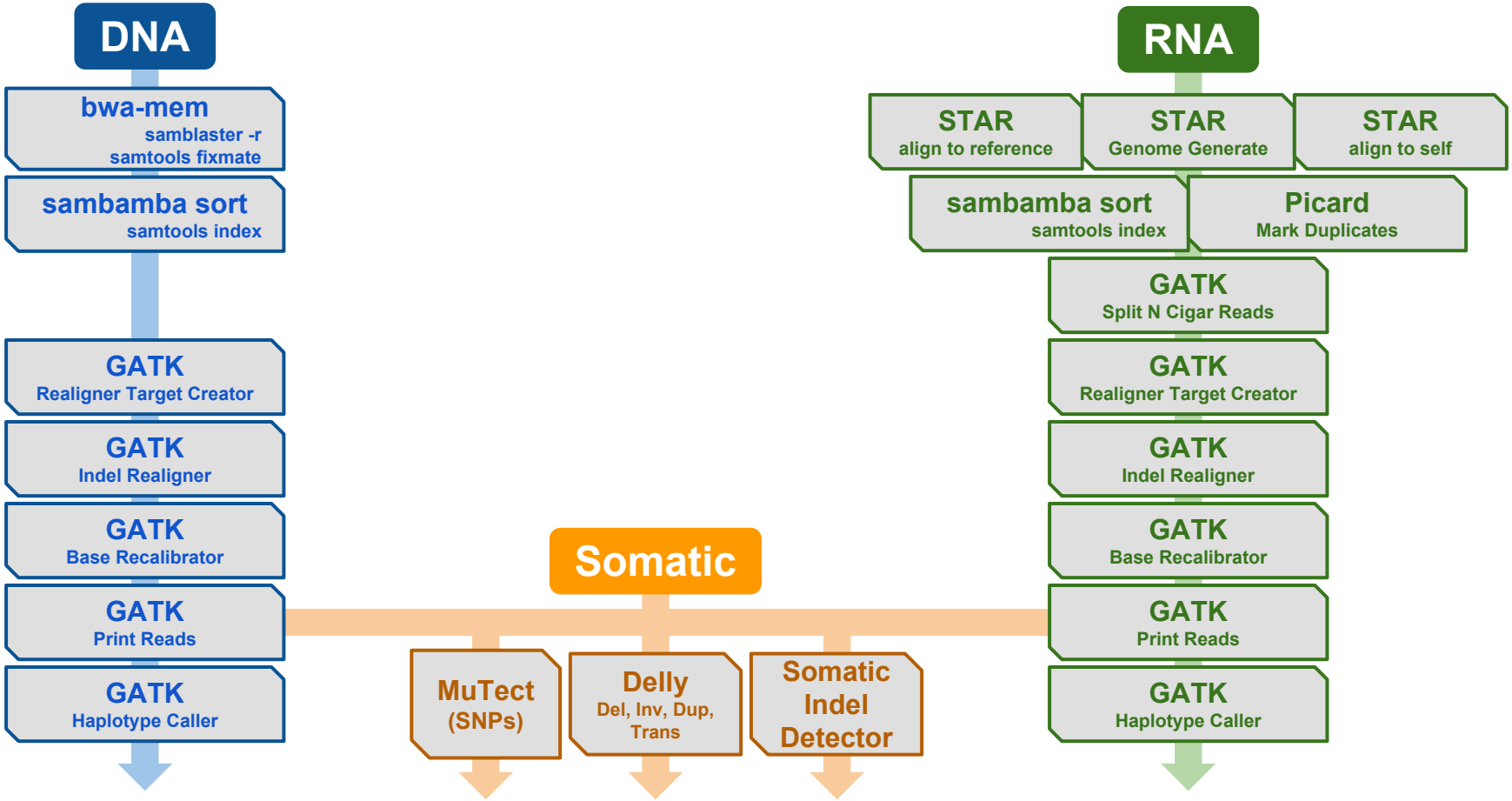
Carland *et al.* 2015 - in prep..

Genome Analysis ToolKit - Best Practices



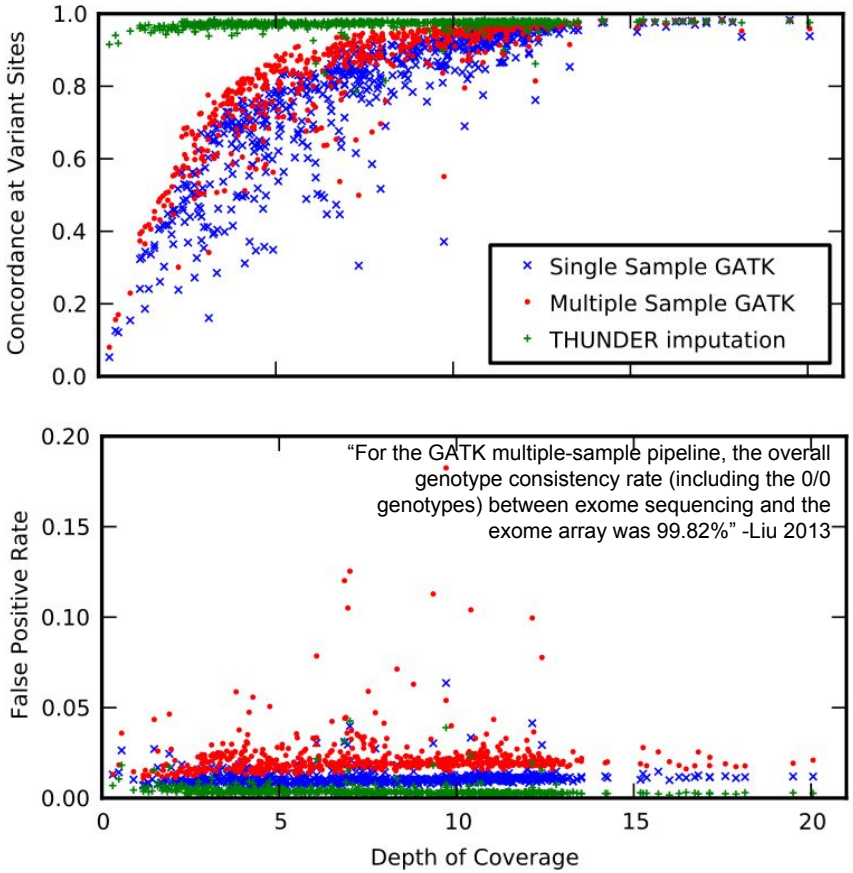
https://www.broadinstitute.org/gatk/guide/best-practices

My latest GATK Best Practices pipeline



Projects

- Personalized Cancer Medicine
- **Alcoholism in Native Americans**
- Group Variant Calling
- Mutated tRNA in Zebrafish
- Kidney Transplant Exome GWAS
- Cow Antibody Sequencing
- Eating Disorder Haplotype Study
- Dog Genome Assembly



Scripps Translational Science Institute

The Scripps Research Institute

Scripps

Bizon *et al. BMC Genomics* 2014, **15**:85
http://www.biomedcentral.com/1471-2164/15/85

High Performance Computing

(see blackboard for details)

Projects

- **Personalized Cancer Medicine**
- Alcoholism in Native Americans
- Group Variant Calling
- Mutated tRNA in Zebrafish
- Kidney Transplant Exome GWAS
- Cow Antibody Sequencing
- Eating Disorder Haplotype Study
- Dog Genome Assembly

Scripps Translational Science Institute

The Scripps Research Institute

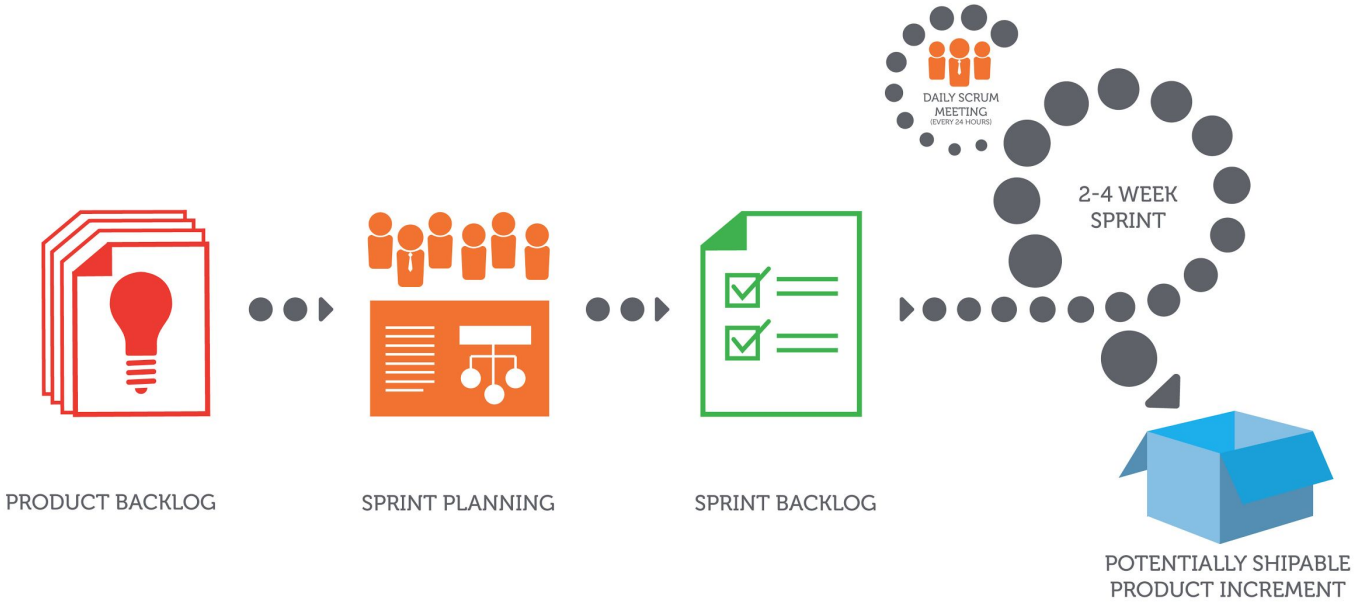
Scripps

Cancer, how to beat it

(see blackboard for details)

What is it, you do here?

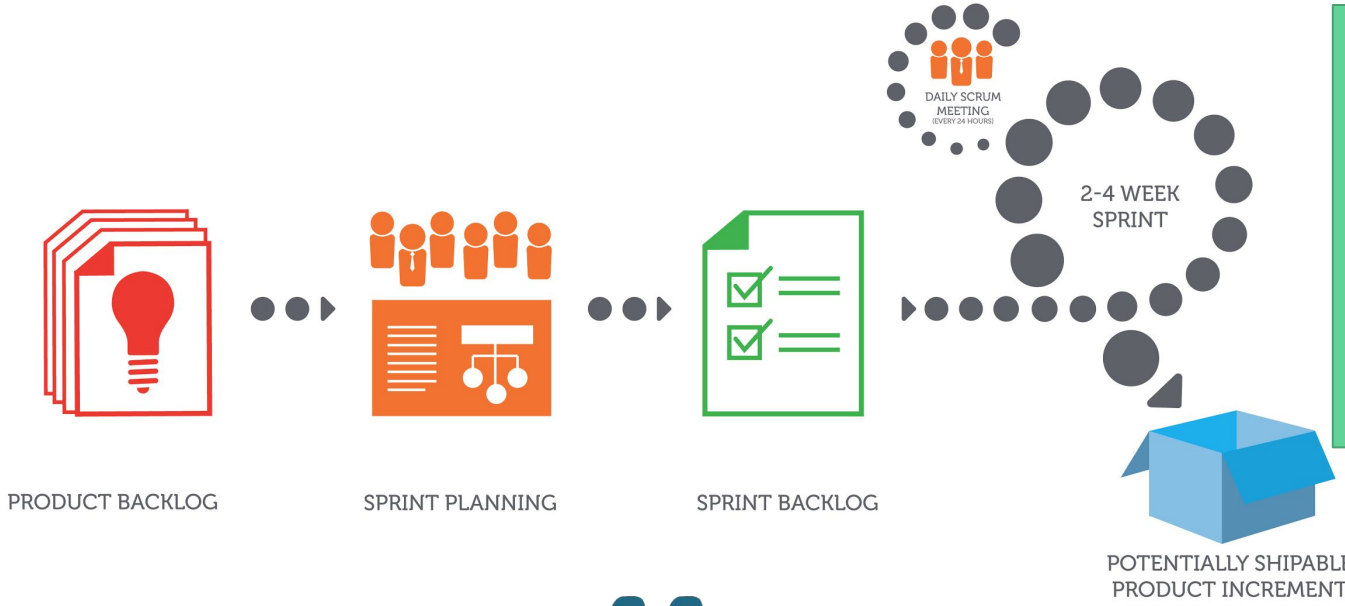
Software Engineer II, Software Development Engineer in Test



How scrum saves the world

What is it, you do here?

Software Engineer II, Software Development Engineer in Test



80%
of the value
comes from
20%
of the work
do that part first

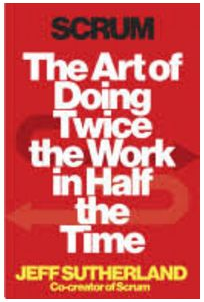
Trello

todoist

Learn Scrum immediately

- Make a backlog
  - Organize it into stories
- What can you do in two weeks
  - Plan the two weeks
  - Debrief the two weeks
  - How can we make the next two weeks better?
- **One** task at a time
- Meet daily for 15 minutes
  - Same time, same place
- Work <40 hours per week

Scrum: The Art of Doing Twice the Work in Half the Time [Book]



Shop now

Sponsored

$14.99 · Google Play

Free shipping, no tax

View all sellers and prices

Product details

Author: Jeff Sutherland

Publisher: Crown Publishing Group

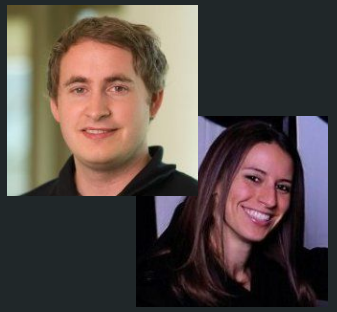
Pages: 256

Format: ebook

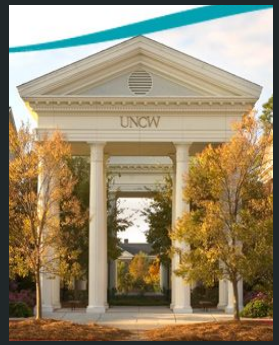
Publication Date: 2014

ISBN: 0385346468

View more details



Acknowledgements



illumina®

PersImmune

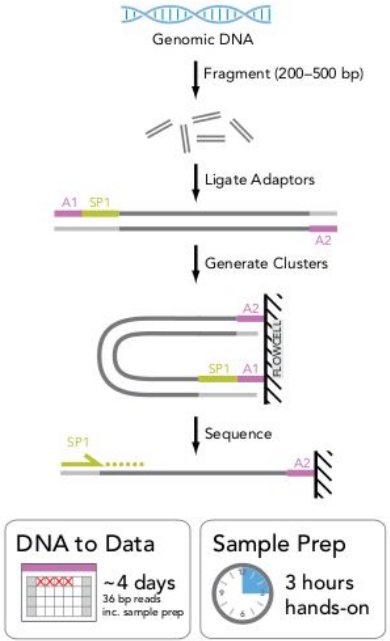
Immunotherapy, Personalized

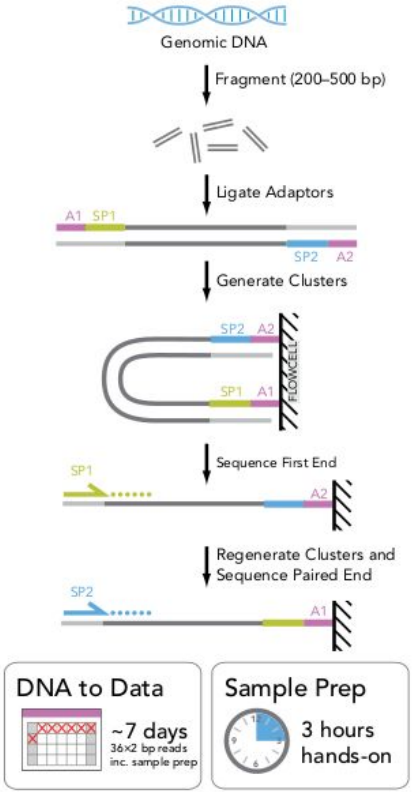
Avera

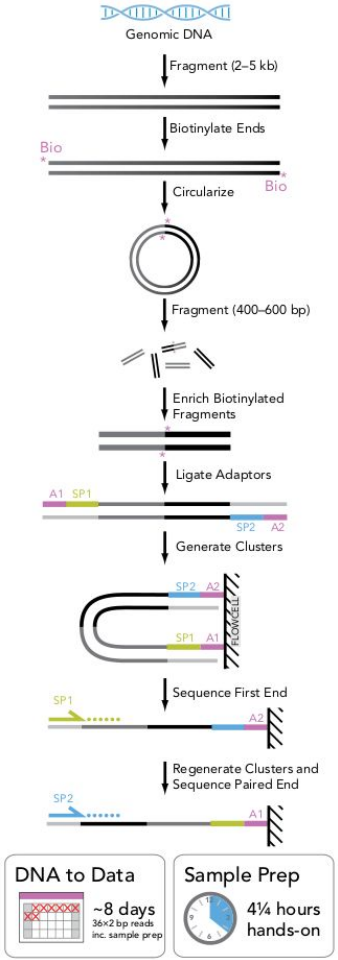
The Scripps Research Institute

More illumina® Sequencing

- Single End
- Paired End
- Mate Pair

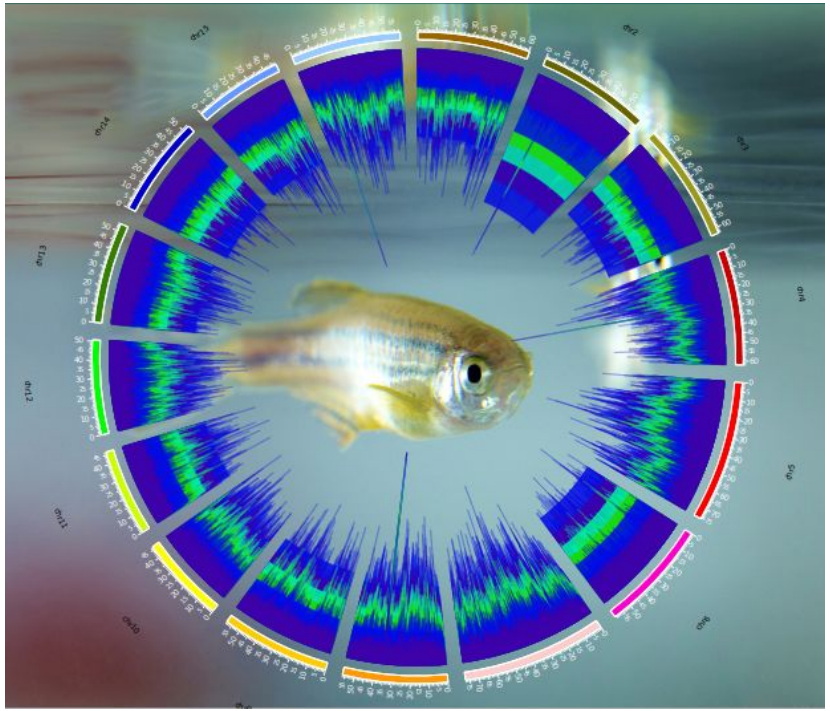


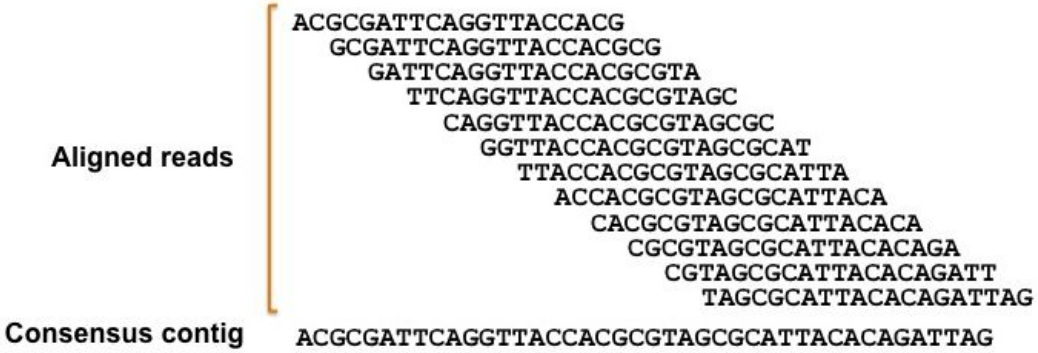




What is depth of coverage?

- Coverage - how much of the genome is covered
- Depth - how deep is a given base pair covered
- Depth of Coverage - state both..





Tools to get moving - platform independent

- Illumina BaseSpace - candy store of tools, either it works or you can’t do it
  - Link - feels like the app store for bioinformatics, beware older versions of tools
- Galaxy - many places including Globus
  - Link - nice for building pipelines, very appropriate for short term analyses
- Ugene - Mentioned last time, great all around resource
  - Link - platform independent, excellent youtube series (link)
  - Now contains full suite for NGS analysis (not that I’ve tried it)
- MEGA - It was your homework for a reason
  - Link - great for phylogenetics or to just poke at sequences and alignments
- Secure Shell Chrome Extension
  - Link - Get to the command line from your browser, not great for file access, get an FTP

Tools and what they do

- Phylogenetics
  - MEGA, TOPALi, Genious et al, Ugene, Websites Galore
- Alignment tools
  - MEGA (really, go get it), Ugene, Clustal website, other websites
  - BWA, BowTie, STAR, Isaac,
- Quality Control
  - Qualimap, FastQC,
- Assembly
  - SOAP, Velvet, Abyss, Arachne
- Scripting is a language and a tool
  - R (+ggplot2), Perl, Python, Circos (is Perl or R), BedTools, etc
- Pipeline Handlers
  - Build your own in Bash/Python/Perl
  - Ptolemy or GridNexus
  - OmicsPipe (caugh)
  - BCBio
  - Galaxy or Globus
- For purchase....
  - GoldenHelix + VarSeq ($$ but great)

To *really* dig in..

- Upscale Text Editor
  - NotePad++ (link) excellent windows tool
  - Sublime (link) beloved Mac and Linux tool
- Ubuntu (link) or Linux Mint (link) or Bio-Linux (link) or Ubuntu Gnome (link)
  - VirtualBox (link) will let you run linux inside of Windows, or really anything inside of anything
  - Parallels (link) will let you run linux (and windows) inside of MacOs
- SSH Secure Shell Client
  - Link - Might want this if you're in Windows, it's the command line and a file transfer in one
- Learn Unix and Python
  - Unix - underlying software of Linux and Mac, very powerful and dynamic (link)
  - Python - popular scripting language, requires clean code to work (link)
  - R - Similar to MatLab but Free and more widely used, get R-Studio (link)