COMP90016 Computational Genomics: Single Nucleotide Variants SNPs

Overview

1. What are SNPs?
2. How were they discovered?
3. How are they discovered?
4. A SNP discovery project.

Part 1

WHAT ARE SNPS?

Alleles

- Chromosome pairs
- Two copies of the same chromosome: one from the mother, one from the father
- Similar, but not identical
- In bioinformatics we re-present this as two strings, or one string + a set of differences.

SNPs: some facts and definitions

- SNP == *Single Nucleotide Polymorphism*
- SNPs are differences at single positions between two DNA molecules
  - Molecule 1: AAAAA
  - Molecule 2: AACAA

Important: The double stranded DNA is one molecule!

- In humans:
  - Mother: AAAAAAAAAAAAAGAAA
  - Father: AAAAACAAAAAAAGAAA
  - Population: AAAAAAAAAAAAAAAAA

heterozygous

homozygous

Child genome

- There are Major and minor alleles in a population.

SNPs: more facts

- Due to random mutation and heredity of genomic material SNPs propagate through populations.
- SNPs can be beneficial, detrimental, or inconsequential.

- SNPs occur everywhere in the genome
- Of particular interest in genes and regulating regions
- Change of binding sites or amino acids can be significant to disease

Examples 1 and 2

- How can a SNP be detrimental to a phenotype?
  - Consider an important gene
  - Assume the sequence

    ACAGGAAGC -> TGS

    ACATGAAGC -> T STOP S

Non-synonymous

- How can a SNP be "inconsequential"?
  - As before:

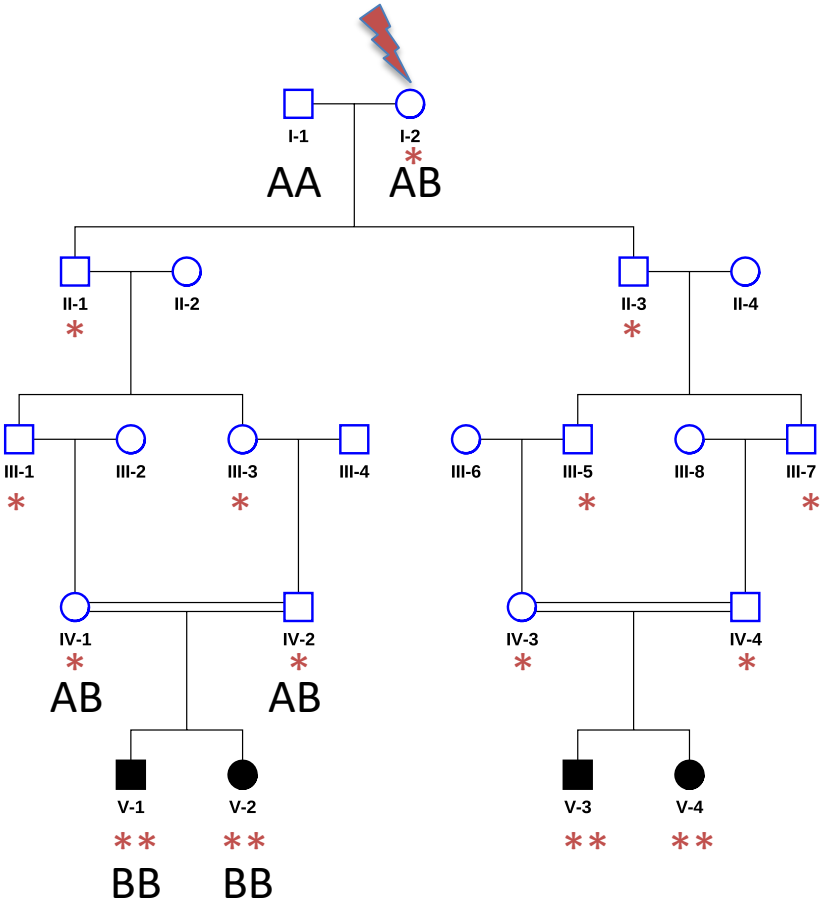
    ACAGGAAGC -> TGS

    ACTGGAAGC -> TGS

Synonymous

Example 3

How do SNPs propagate through a population?



Yet more Definitions…

- There are also SNVs:
  - *Single Nucleotide Variations*
- These are a superset of SNPs.
  - SNPs are only those that are actually found in a population at a certain frequency (typically ≥ 5%).
- In this lecture we are talking about SNPs only (but sometimes the category may be debatable).
- At a specific site we describe DNA by its genotype (AA, AC, AG, …, TT in a diploid genome).
  Confusingly, it is common to describe SNPs as A and B in a population setting (A for the major allele, B for the minor).
- Populations tend to develop linkage: 2 or more neighbouring SNPs being linked by the same haplotype:
  - "If there is a minor allele at position X, there is bound to be a minor allele at positon Y as well"

The Computational Challenge

- There are two computational problems around SNPs:
    1. Discover new variants in a sample. What are the single-base differences between a sample and another/reference? This problem is known as variant calling.
    2. Verify the absence/existence/genotype of known variants within a sample. From prior studies we know about millions of sites in the genome that vary between individuals. Is a sample heterozygous at such a site, or homozygous for minor/major allele? This problem is known as genotyping.

Types of Variants

- There are two kinds of variants that we distinguish:
  1. Germline variants. These are the differences between our alleles and other individuals. These are the variants we are born with .
  2. Somatic variants. These variants are acquired during our lifetime in the DNA of single cells (and may proliferate from there). Such variants can turn our healthy cells into tumour cells.
- Somatic variants are investigated via variant calling. Germline variants may already be known in the population and therefore be validated with genotyping.

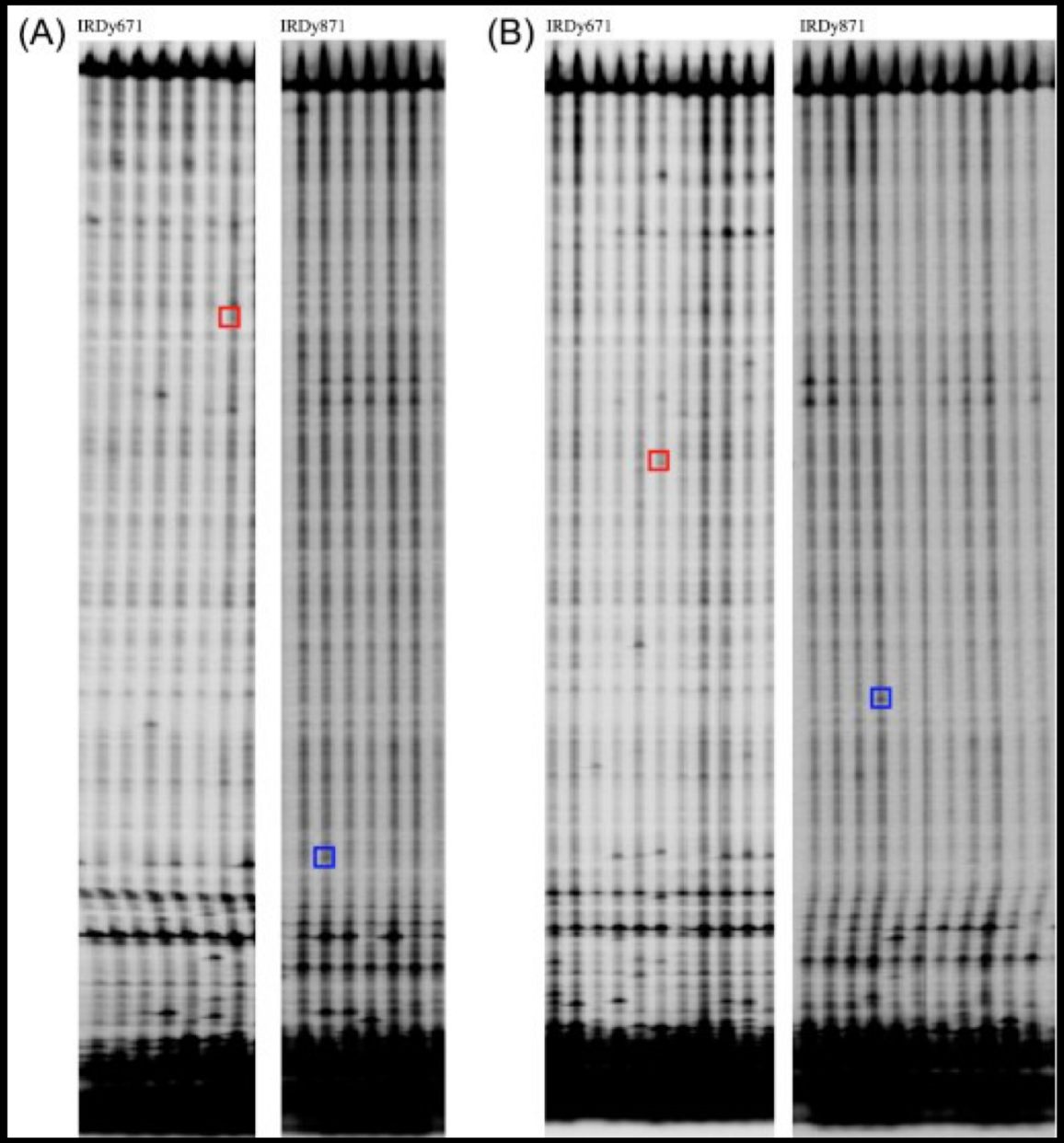
Part 2

HOW TO DISCOVER SNPS – A HISTORY

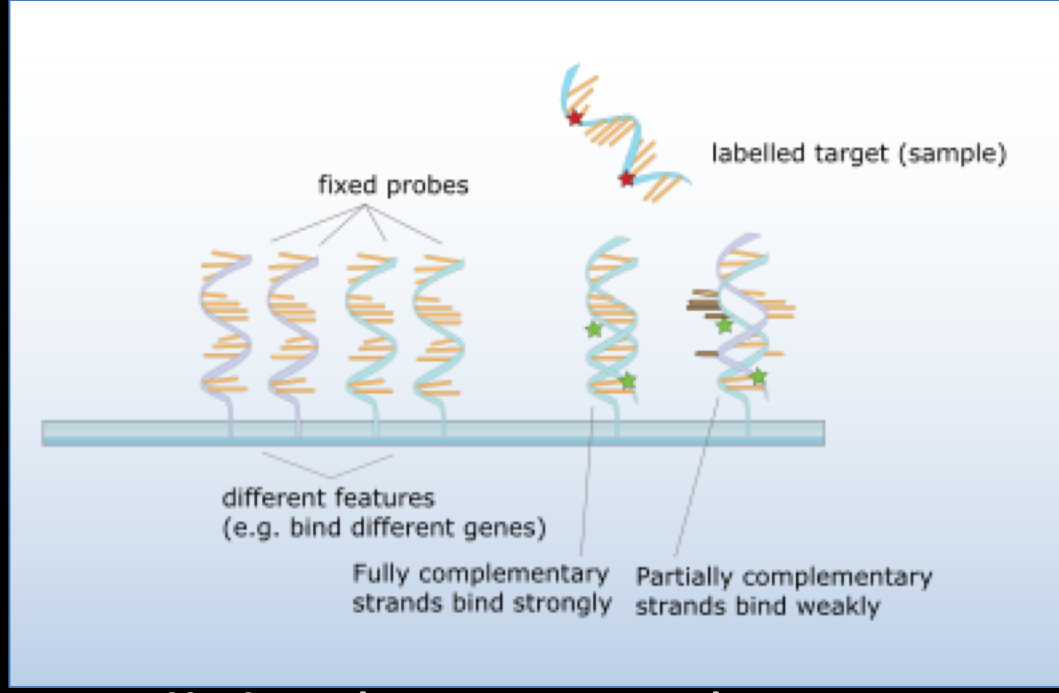
Traditional SNP Discovery (Variant Calling)

- Sanger sequencing of genes -> discovery of slight differences
- A more manual analysis of genomic differences.
- Cataloguing of SNPs led to public data bases and technologies to evaluate SNPs in individuals

SNPs on gel



Traditional SNP Evaluation (Genotyping)

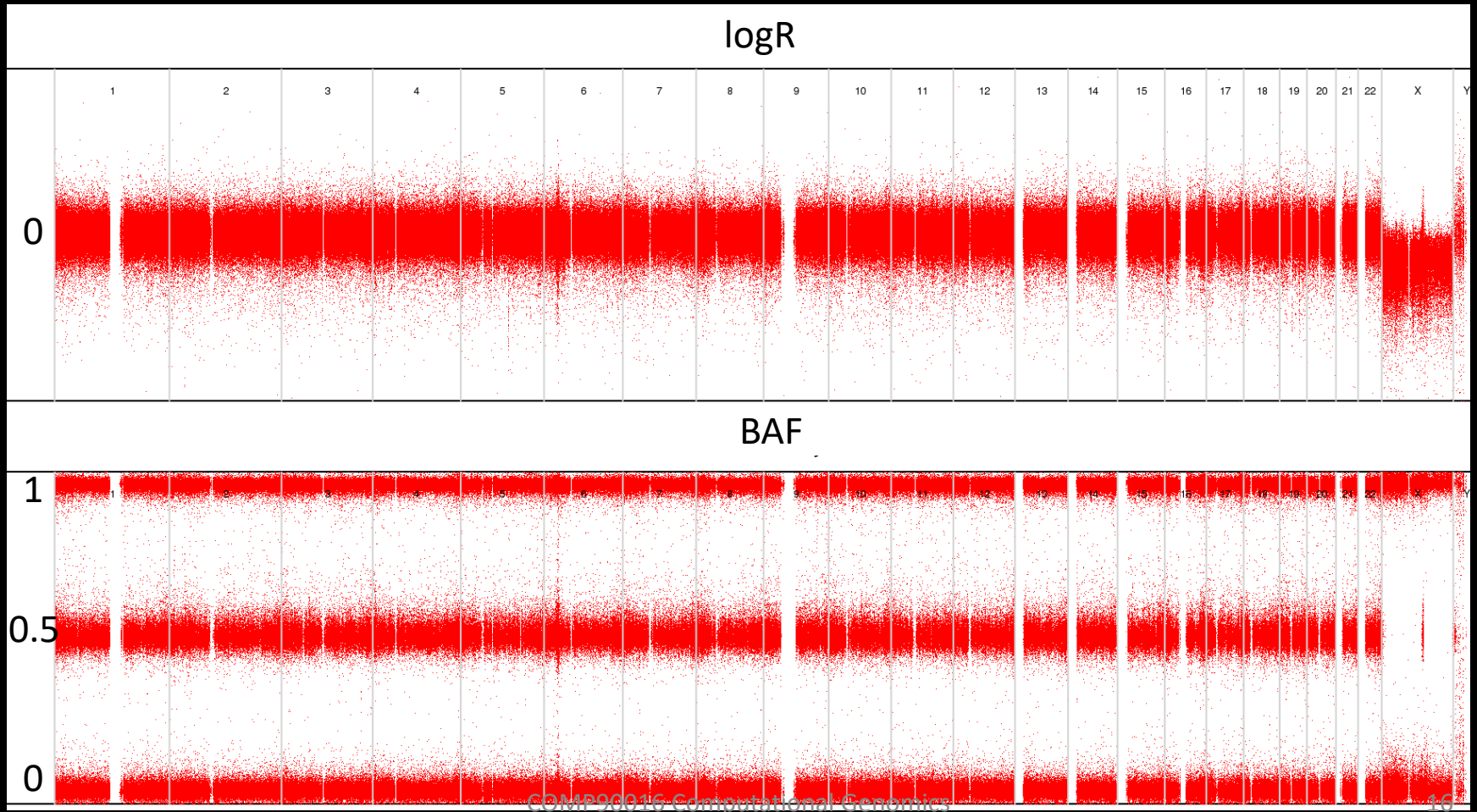


- Micro arrays
- SNP-arrays:

- Probes == different alleles (genotypes)
  - A probe pair has the major and minor allele for a known SNP.
  - By measuring fluorescence the amount of binding to each probe pair can be established.
  - In Bioinformatics, a SNP-array delivers 2n intensity values for n probe pairs.

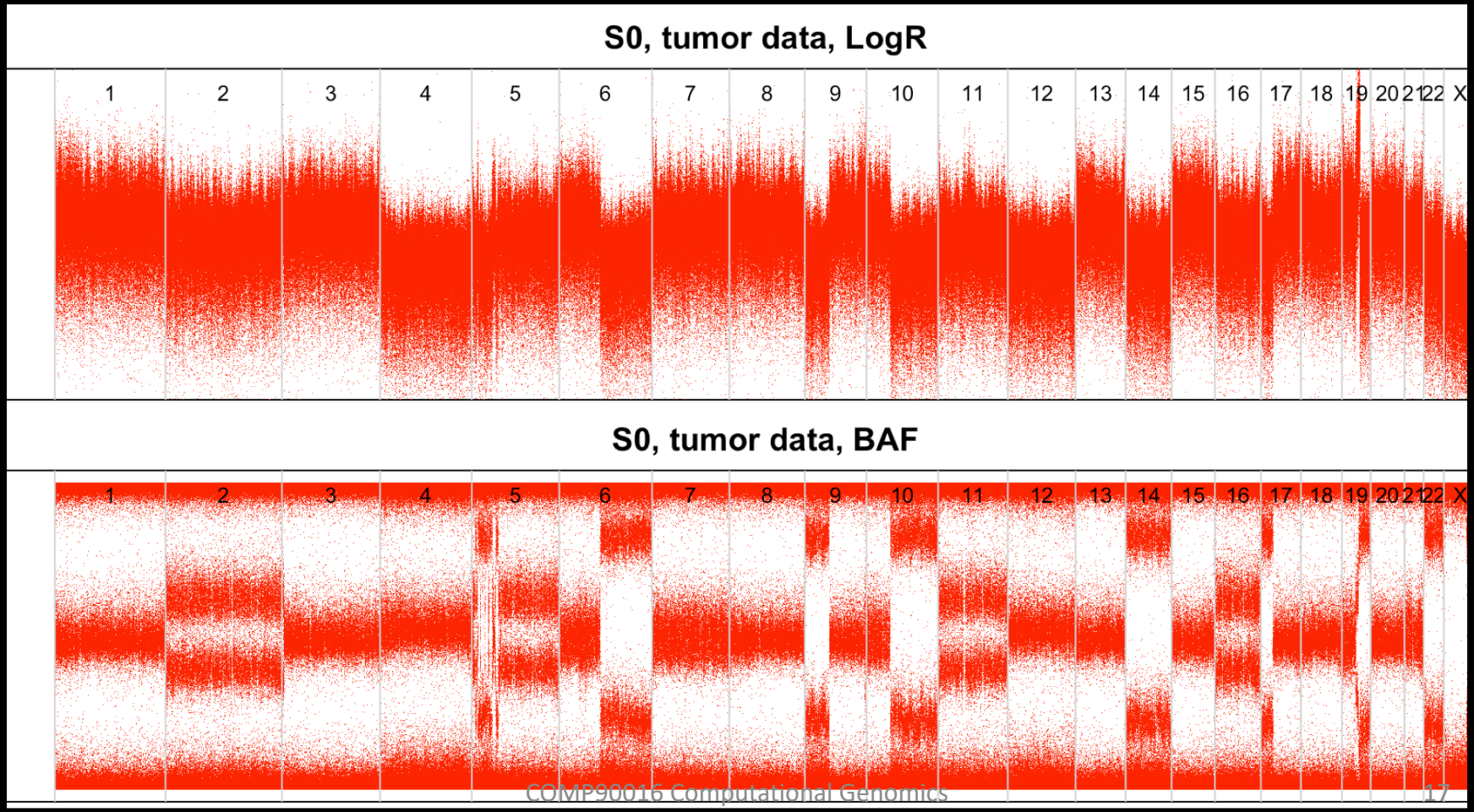
SNP Array Data

Let the array intensities be represented by I_{kj} with k an integer in {1..n} and j in {1,2}. Define logR:=log2((I_{k1}+I_{k2})/mean I), and BAF:=I_{k1}/(I_{k1}+I_{k2})



SNP Array Data in Cancer

Let the array intensities be represented by I_{kj} with k an integer in {1..n} and j in {1,2}. Define logR:=log2((I_{k1}+I_{k2})/mean I), and BAF:=I_{k1}/(I_{k1}+I_{k2})



Knowledge about SNPs

- dbSNP (https://www.ncbi.nlm.nih.gov/SNP/)
- "As of build 131 (available February 2010), dbSNP had amassed over 184 million submissions representing more than 64 million distinct variants for 55 organisms, including *Homo sapiens*, *Mus musculus*, *Oryza sativa*, and many other species" *
- "As of 23 July 2013, dbSNP listed 62,676,337 SNPs in humans." *

* Wikipedia

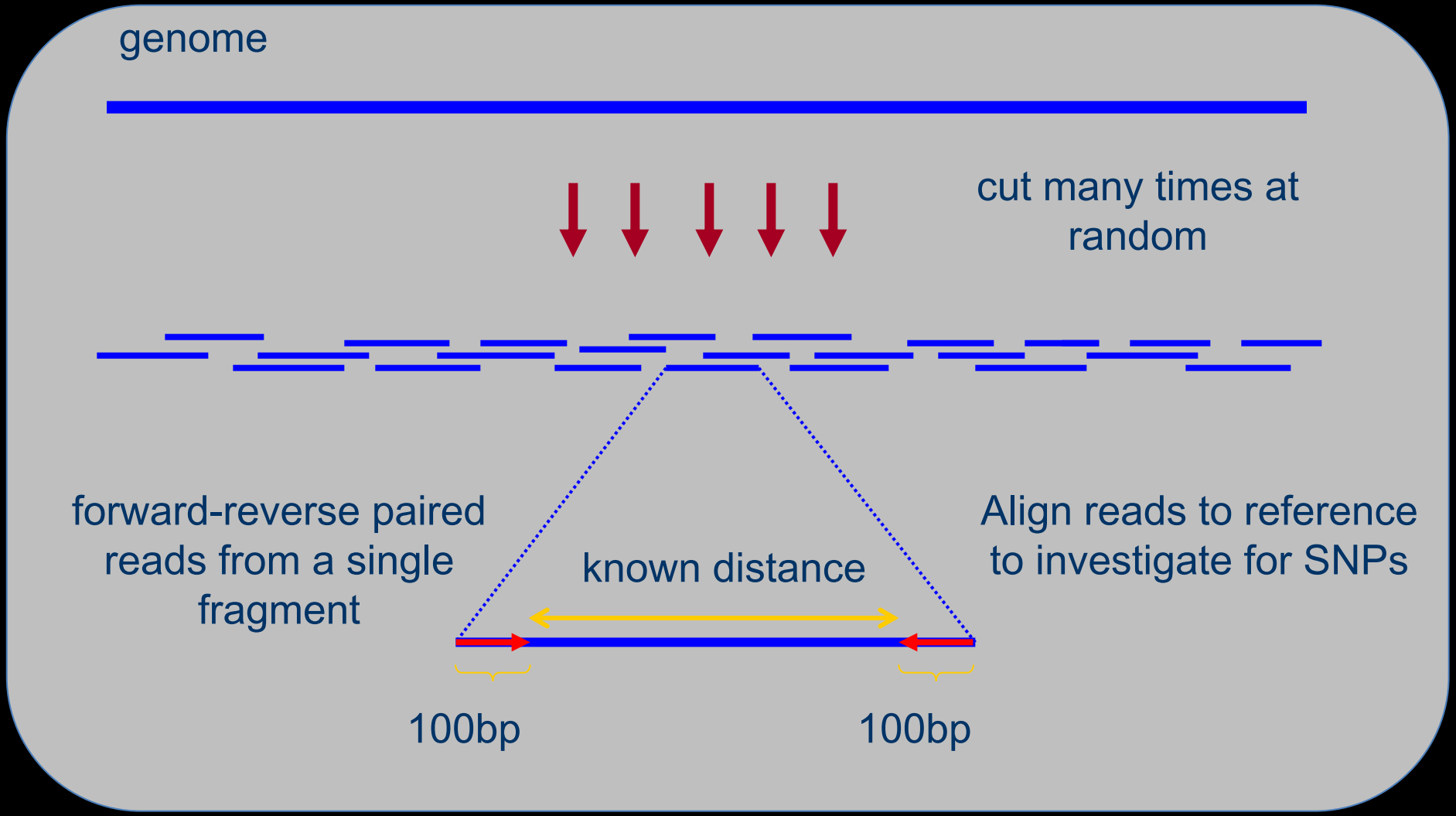
Summary

- SNP: chr-a, position b, genotype c, allele frequency d
- CGAGTACGGGCTGCAGGCATACACT[A/G]AAGTGAAAACTGTGAGTGTGGGACC
  Chromosome: 12:112241766
  Gene:ALDH2
  Functional Consequence: missense
  Clinical significance: drug-response
  Validated: by 1000G,by cluster,by frequency,by hapmap
  Global MAF: A=0.0574/124
- Discovery dates back to the 1950s, but new insights have accelerated over the decades.

Part 3

CURRENT STATE OF SNP CALLING

Whole Genome Shotgun Sequencing



SNPs and Sequencing

Samtools Mpileup

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… Samtools Mpileup

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Computational Strategy and Challenges

- One strategy: parse the Mpileup output and decide if there is a SNP at any given position
- Challenges with this approach:
  - Sequencing Errors
  - Mappability
- Computational opportunities:
  - Expectations on frequencies
- Necessary coping strategies:
  - Cleaning the data
  - Modeling assumptions

Dealing With Sequencing Errors: Utilization of Quality Scores

- Sequencing platforms include a quality string along with the read string, which reflects the confidence in each base.
- They make use of Phred scores*:
  - $Q = -10 \log_{10}(P)$
  - These scores are encoded in Ascii by offsetting the score by an integer (in Illumina 33 or 64):
    - A Base quality of 40 is encoded as Ascii 104: “h”

* Ewing B, Hillier L, Wendl MC, Green P. (1998): Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 8(3):175–185. PMID 9521921

Phred Scores and SNP calling

Phred Quality Score	Probability of incorrect base call	Base call accuracy
10	1 in 10	90%
20	1 in 100	99%
30	1 in 1000	99.9%
40	1 in 10,000	99.99%
50	1 in 100,000	99.999%
60	1 in 1,000,000	99.9999%

"Typically, analyses would first involve a filtering step in which only high-confidence bases would be kept. The most common cutoff used would be a Phred-type quality score of Q20 (QPhred = 20). "*

*Genotype and SNP calling from next-generation sequencing data (Nat Rev Gen, PMC3593722)

Calling Genotypes

"… call a heterozygous genotype if the proportion of the non-reference allele is between 20% and 80%; otherwise, a homozygous genotype would be called. This is a fairly standard procedure and works well when the sequencing depth is high (>20×), so that the probability of a heterozygous individual falling outside the 20–80% range is small."*

*Genotype and SNP calling from next-generation sequencing data (Nat Rev Gen, PMC3593722, 2011)

Probabilistic Genotyping (Modelling Assumptions)

The cutoff solution to calling genotypes is problematic, because it does not reflect uncertainties in the calls. Neither does it allow for incorporation of prior knowledge.

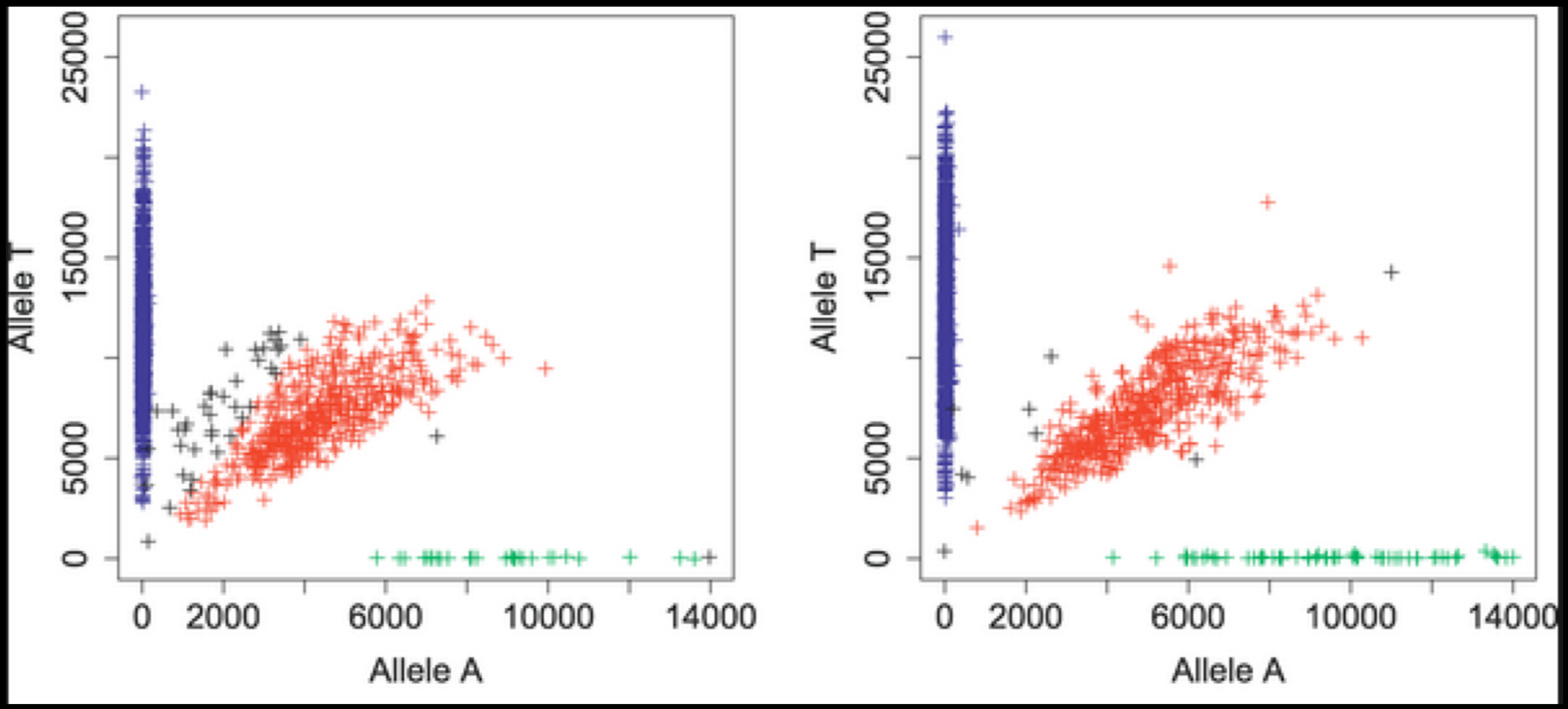
$$P(E_i|D) = \frac{P(D|E_i)P(E_i)}{P(D)} = \frac{P(D|E_i)P(E_i)}{\sum_j P(D|E_j)P(E_j)}$$

Use Bayes' theorem to give each genotype a posterior probability.
This helps to investigate the data more holistically.
Prior knowledge finds its way into genotyping:

- known population alleles give higher prior to some alleles.
- some allele changes are more likely than others, this too can be modeled.
- framework can facilitate a range of scenarios: haploid genomes, diploid, …

Further reading: *SNP calling using genotype model selection on high-throughput sequencing data,* Bioinformatics (2012) 28 (5): 643-650.
doi: 10.1093/bioinformatics/bts001

Genotyping of SNPs



A Method to Address Differential Bias in Genotyping in Large-Scale Association Studies
Plos Genetics 2007: http://dx.doi.org/10.1371/journal.pgen.0030074

SNP calling – an example algorithm: Somatic Sniper

- Somatic Sniper is designed to identify variants in a tumour/normal setting – that means variants that are present in one sample (tumour) but not the other (normal) to investigate causative changes.
- SS applies a mathematical model to establish true difference in the two samples genotypes.
- SS offers parameterised quality filtering of data:
  - Minimum mapping quality
  - Minimum base quality
- Example output:
  - #CHROM POS ID REF ALT QUAL FILTER INFO FORMAT NORMAL TUMOR
  - scf_1107000998904 225 . G A . . . GT:IGT:DP:DP4:BCOUNT:GQ:JGQ:VAQ:BQ:MQ:AMQ:SS:SSC 0/0:0/0:4:1,1,0,2:2,0,2,0:1:.:0:33:40:36:0:. 1/1:1/1:7:0,3,2,2:4,0,3,0:65:.:65:37:42:44:2:30
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  - scf_1107000998904 1765 . T C . . . GT:IGT:DP:DP4:BCOUNT:GQ:JGQ:VAQ:BQ:MQ:AMQ:SS:SSC 1/1:1/1:18:2,2,14,0:0,14,0,4:117:.:117:36:43:44:1:. 0/0:0/0:13:8,2,3,0:0,3,0,10:118:.:226:33:42:41:2:84

Somatic Sniper details

a) Probability estimation for somatic variants:

$$S = -10\log_{10}\left(\frac{\sum_{i=0}^{9} P(T|H_i)P(N|G_i)P(H_i|G_i)P(G_i)}{\sum_{j=0}^{9}\sum_{k=0}^{9} P(N|G_k)P(T|H_j)P(H_j|G_k)P(G_k)}\right)$$

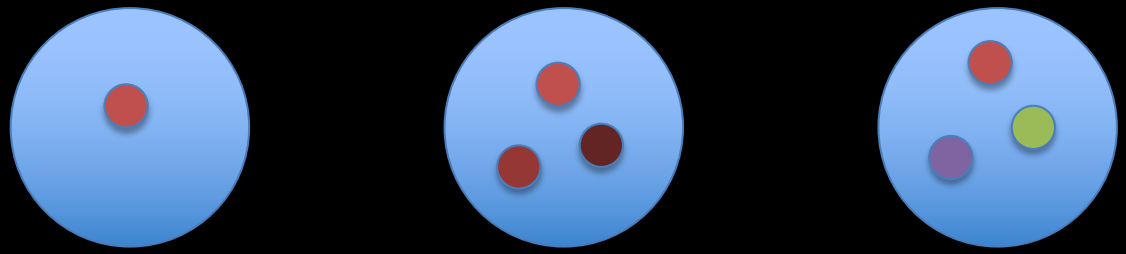
b) Quality checks to exclude false positives:
   1. Site is >10 bp from a predicted indel of quality >=50.
   2. Maximum mapping quality at the site is >=40.
   3. Fewer than three SNV calls in 10bp window around the site.
   4. Site is covered by at least three reads.
   5. Consensus quality >=20.
   6. SNP quality >=20.
   7. No entry in dbSNP.
   8. No LOH calls

For further details check "**SomaticSniper: identification of somatic point mutations in whole genome sequencing data**" by Larson et al.

Modeling assumptions

- Samples can come from haploid, diploid, or polyploid organisms:

- Samples can come from single cells, clonal populations of cells, or heterogeneous populations



Ploidy Implications

- What frequencies do we expect for variants in a…
  - Haploid genome (E. Coli)?
  - Diploid genome?
  - Triploid genome (seedless watermelon (o_O)?
  - Tetraploid genome?

Clonality Implications

- For a diploid organism, what do we expect for variant frequencies in a…
  - Even mixture of two samples?
  - Uneven mixture of two samples?
- Clonality as well as ploidy can vary throughout the genome!
- This is a topic to come in the structural variant lectures.

Application in Bioinformatics: Additional Challenges

- Problems with big data:
  - Potentially millions of variants in output
  - Too many to eyeball for meaningful results
- Filtering of output
- Annotation of output
- Superimposing experimental design to discover results of interest.

Part 4

A BIOINFORMATICS EXAMPLE

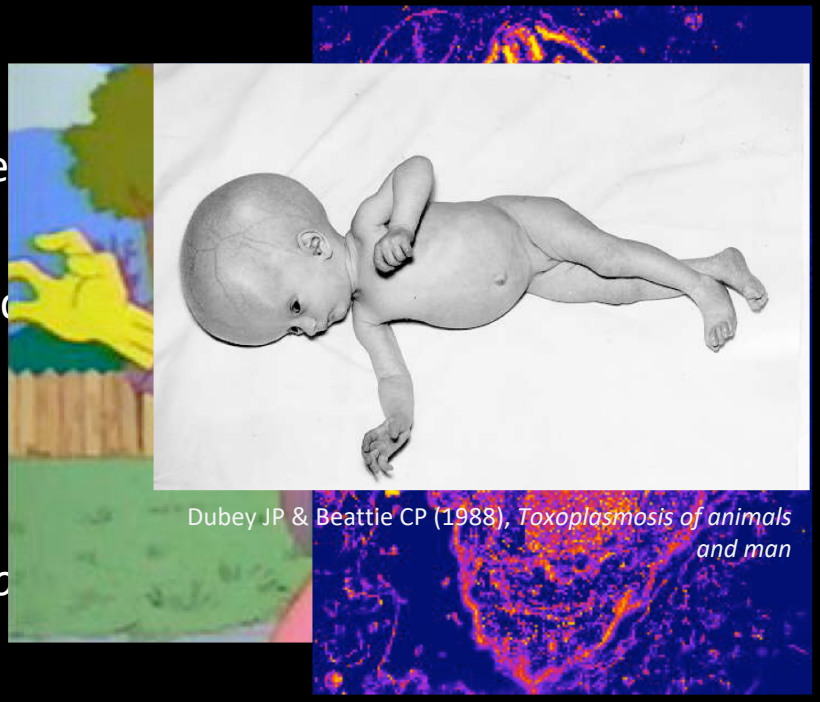
A Project: Toxoplasma Gondii

A project at WEHI with Dr. Chris Tonkin and PhD candidate James McCoy.

Research into signaling pathways of a parasite.

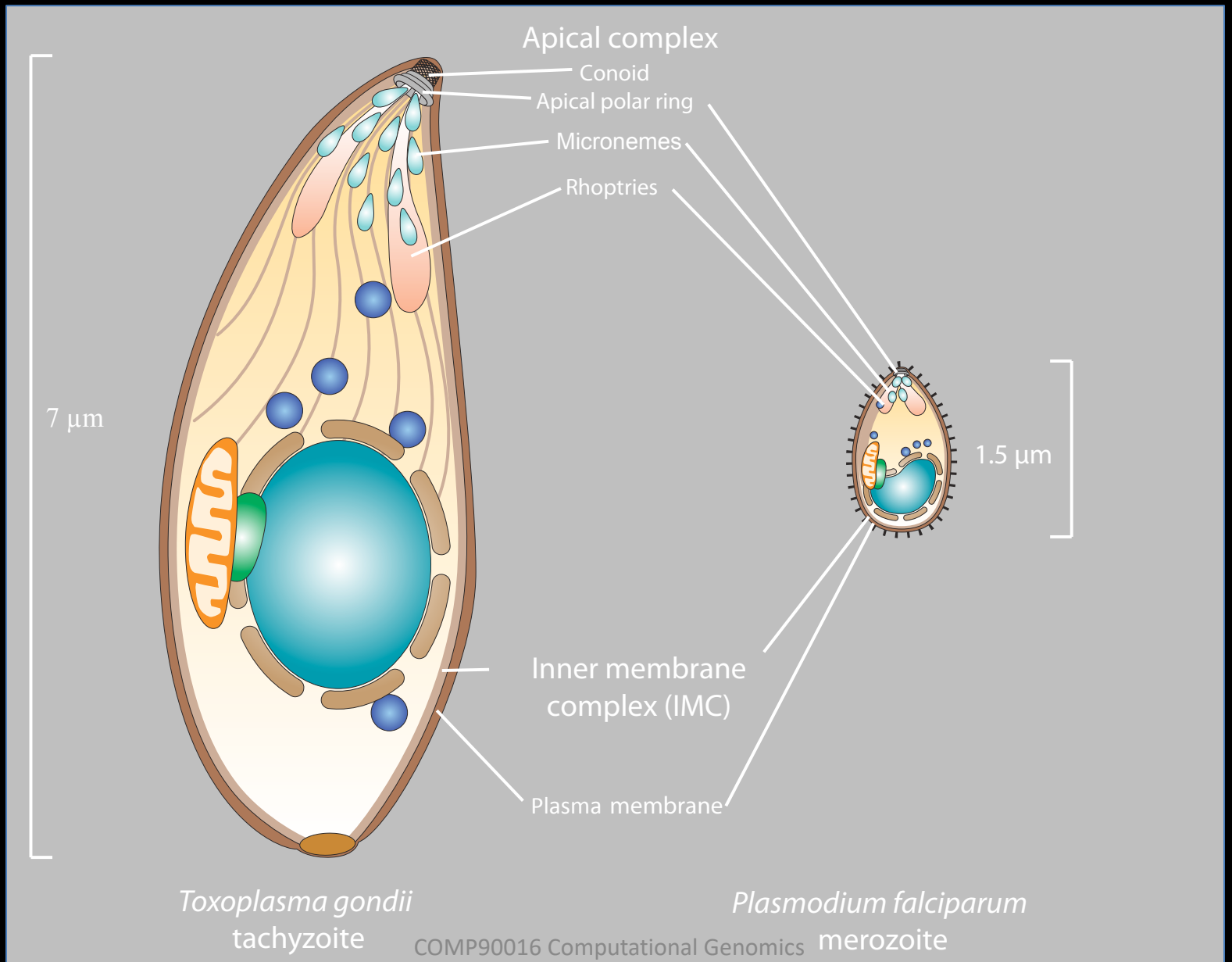
Toxoplasma gondii in disease

- "Crazy cat lady" parasite
  - Sexual life cycle in cats
  - Humans accidental secondary hosts
- 40 – 80% of individuals in a population infecte
- Common cause of disease in vulnerable indivi
  - Birth defects/stillbirth
  - Neurological disease in AIDS patients
- Member of phylum Apicomplexa (with *Plasmo*
  - Valuable model organism



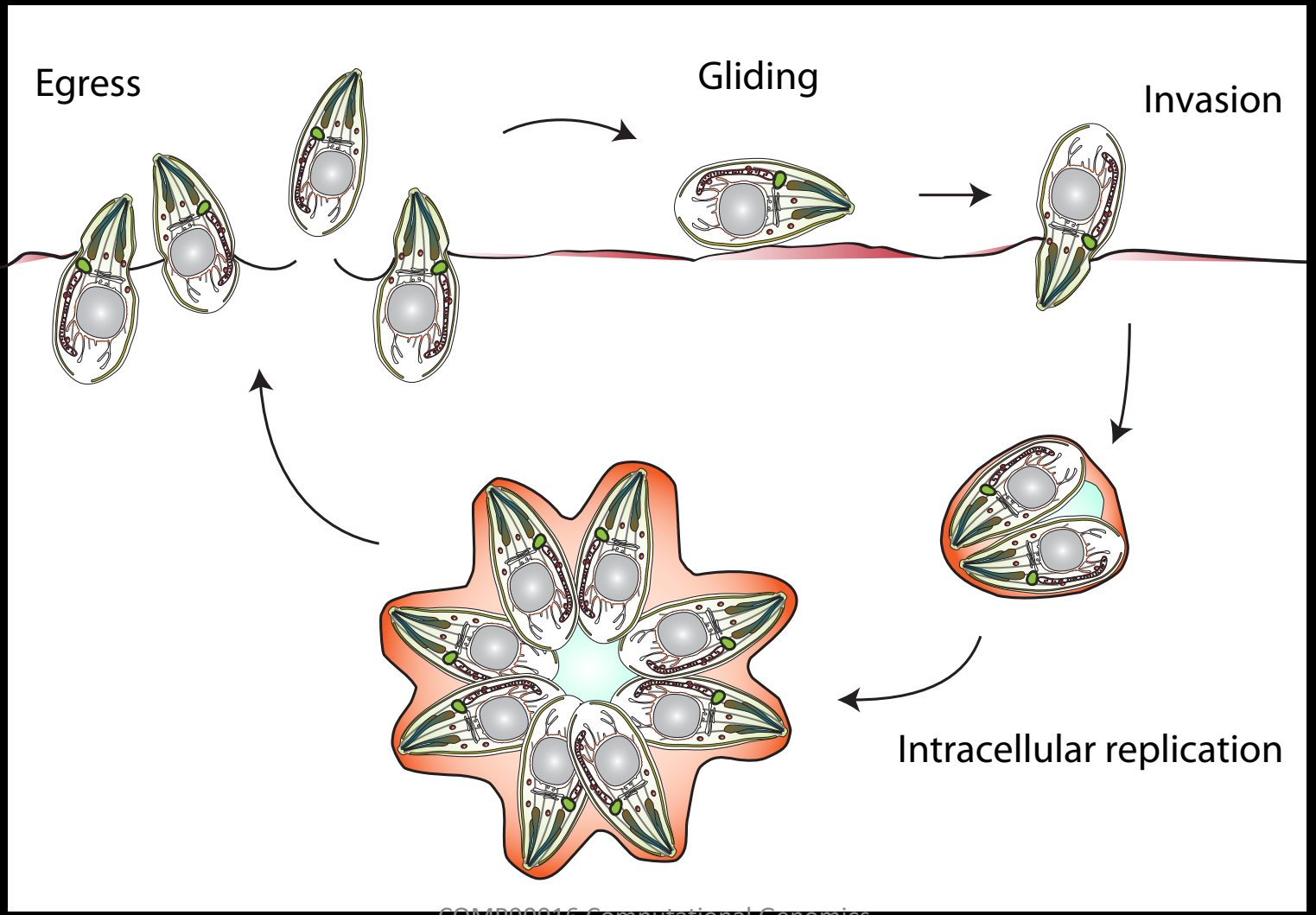
Dubey JP & Beattie CP (1988), *Toxoplasmosis of animals and man*

Conservation of apicomplexan invasion components



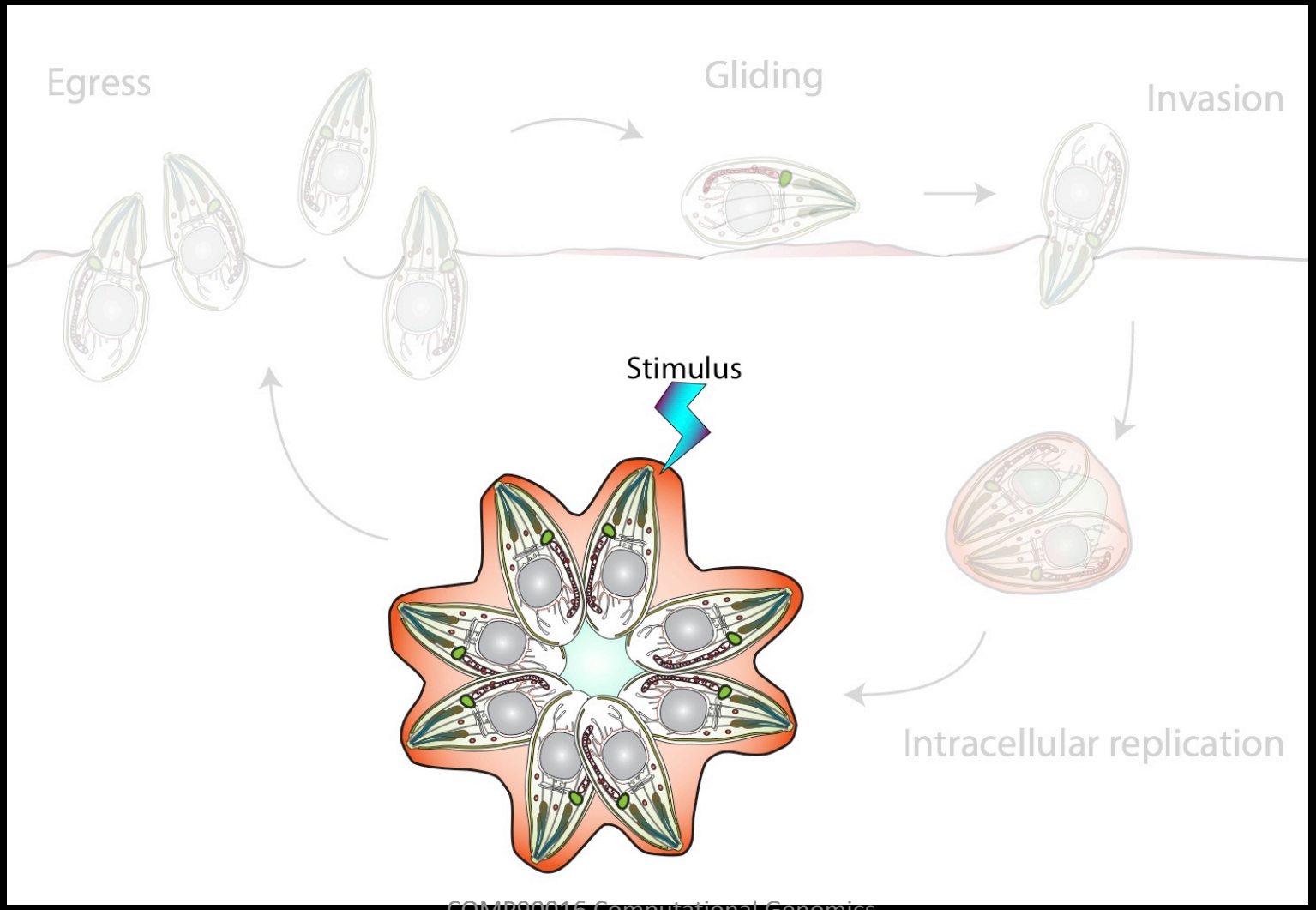
Gliding motility in Apicomplexa

- Substrate-dependent motility and invasion regulated by calcium signalling



Gliding motility in Apicomplexa

- Substrate-dependent motility and invasion regulated by calcium signalling



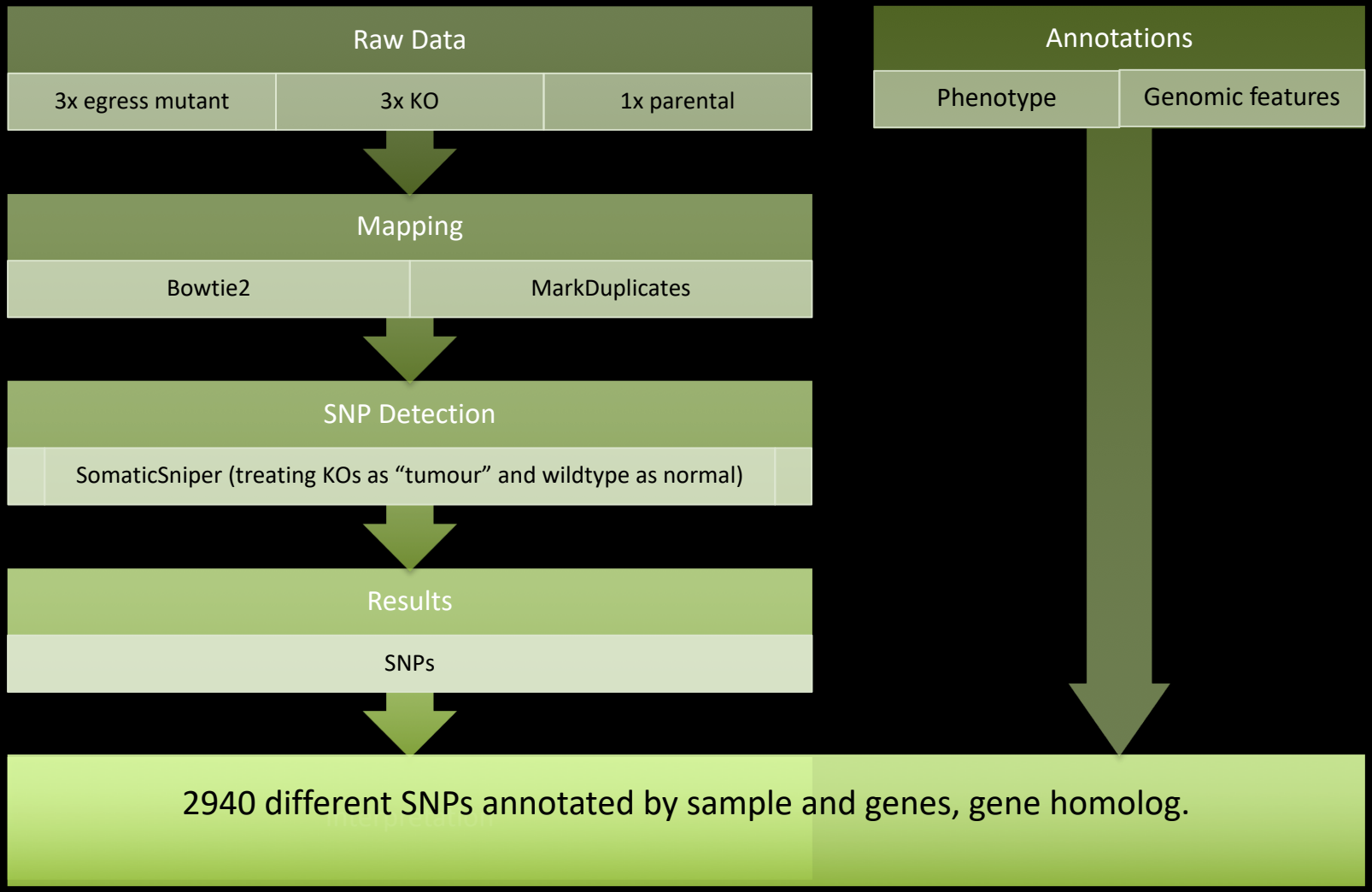
Project Overview

- Summary:
  - Knockout toxoplasma cannot egress from host cell
  - Mutagenize and isolate clones that can egress
  - Sequence mutant, KO & wildtype
  - SNP calling
  - Investigate gene candidates responsible for phenotype

Analysis overview

- Reference:
  - Toxoplasma Gondii GT1: ~61Mbp
- Data:
  - 3x KO mutant (no egress), 3x KO mutant (restored egress), 1x wildtype
  - Illumina HiSeq runs to 30x each
- Analysis:
  - Read alignment
  - SNP calling
  - Annotations
  - Evaluation

NGS analysis pipeline



Evaluating the results

- Of the 2940 SNPs…
  - 172 lie within genes
  - 83 of those result in non-synonymous changes
  - Finally, only 3 fulfill our experimental expectation: they are present in all the egress restored samples, but none of the other KOs.
- One of the affected genes is known to be related to surpressing calcium signaling in other related species.
- The result gene candidate was then validated and investigated further in the lab.
- *A Forward-genetic Screen Identifies a Negative Regulator of Rapid Ca2+-dependent Cell Egress in the Intracellular Parasite Toxoplasma gondii.*
  J Biol Chem. 2017 Mar 3. pii: jbc.M117.775114. doi: 10.1074/jbc.M117.775114.

SNP Calling from Sequencing Data Summary

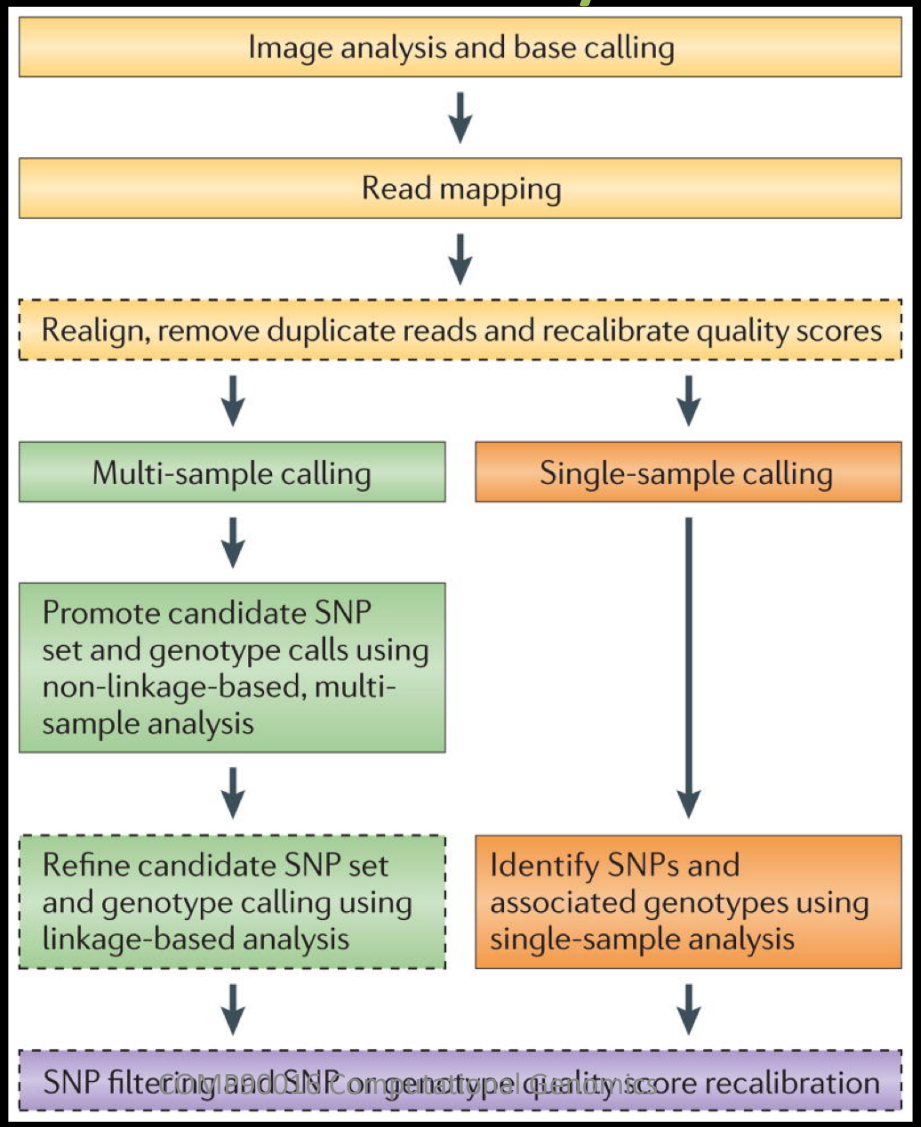
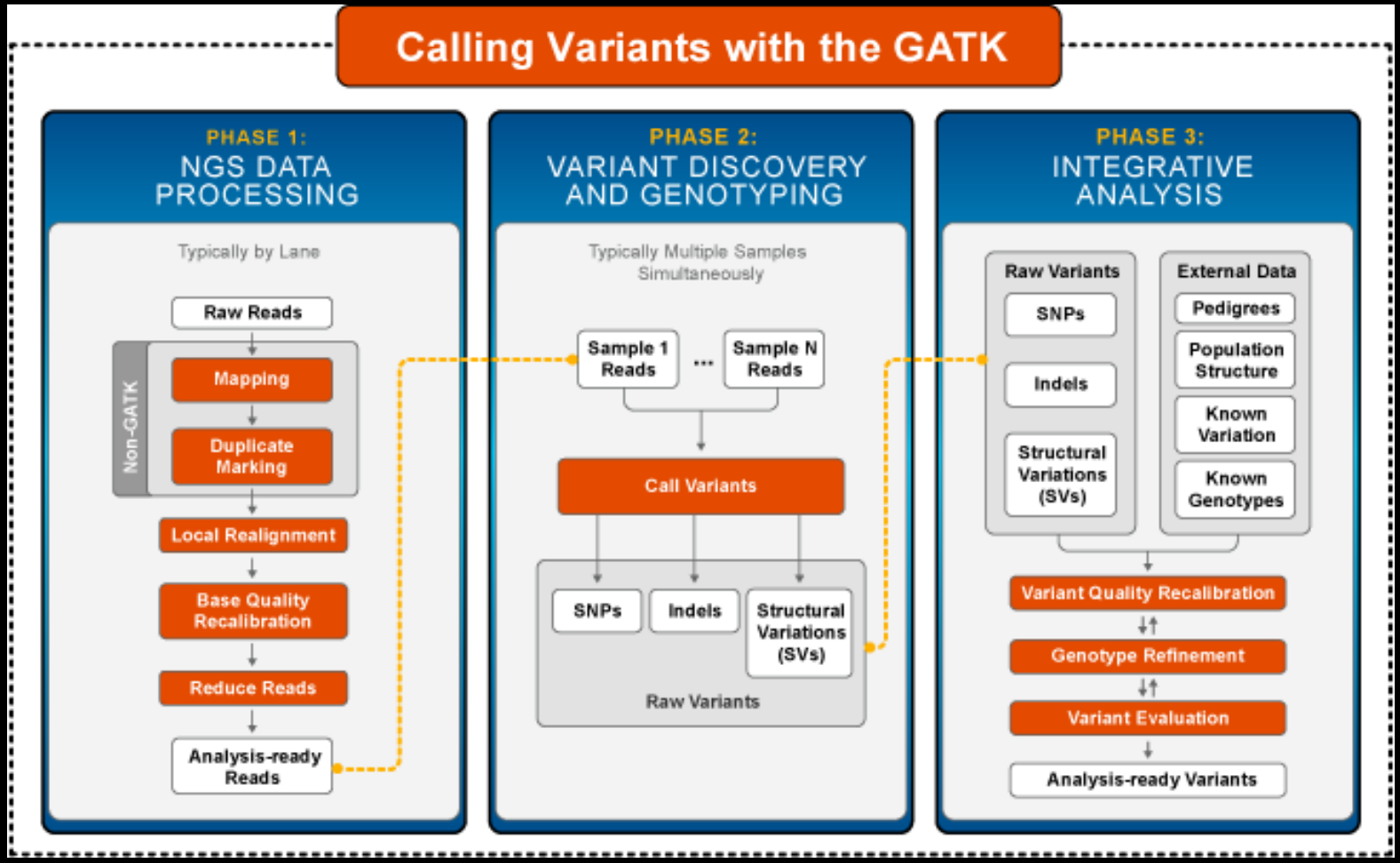


Image from *Genotype and SNP calling from next-generation sequencing data* (Nat Rev Gen, PMC3593722)

Further reading
https://en.wikipedia.org/wiki/SNV_calling_from_NGS_data

Variant Calling and Genotyping as Done by the Broad Institute



Final Words on the state of SNP Calling

- SNP calling is challenging in general and depending on setting due to:
  - Sequencing errors
  - Mappability
  - PCR duplicates
  - Heterogeneous population of cells
  - Levels of clonality within population
- Therefore, a common practice is to use several SNP callers on the data and investigate variants that are called by more than one tool.

Variant Call Format (VCF)

```
##fileformat=VCFv4.0
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=1000GenomesPilot-NCBI36
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples
   With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM  POS     ID  REF ALT QUAL FILTER INFO FORMAT NA00001
20      14370   .   G   A 29 PASS NS=3;DP=14;DB GT:GQ:DP 0|0:48:1
20      17330   .   T   A 3 q10 NS=3;DP=11;AF=0.017 GT:GQ:DP 0|0:49:3
20      1110696 .   A   G,T 67 PASS NS=2;DP=10;AA=T GT:GQ:DP 1|2:21:6
20      1230237 .   T   . 47 PASS NS=3;DP=13;AA=T GT:GQ:DP 0|0:54:7
…
```

Conclusion

- SNPs are one of the most abundant and easiest to detect variations between individuals of an organism.
  - The variations are catalogued and distinguished as major and minor alleles.
- Hopefully, you have gained some understanding of why they are interesting in general, and what technologies exist to analyse them.
  - Detection and confirmation methods evolved with technological advances.
- Project: beneficial (to the organism) SNPs are identified to further understanding of signaling in the parasite.
  - Single nucleotide changes can have a significant impact on phenotypes.