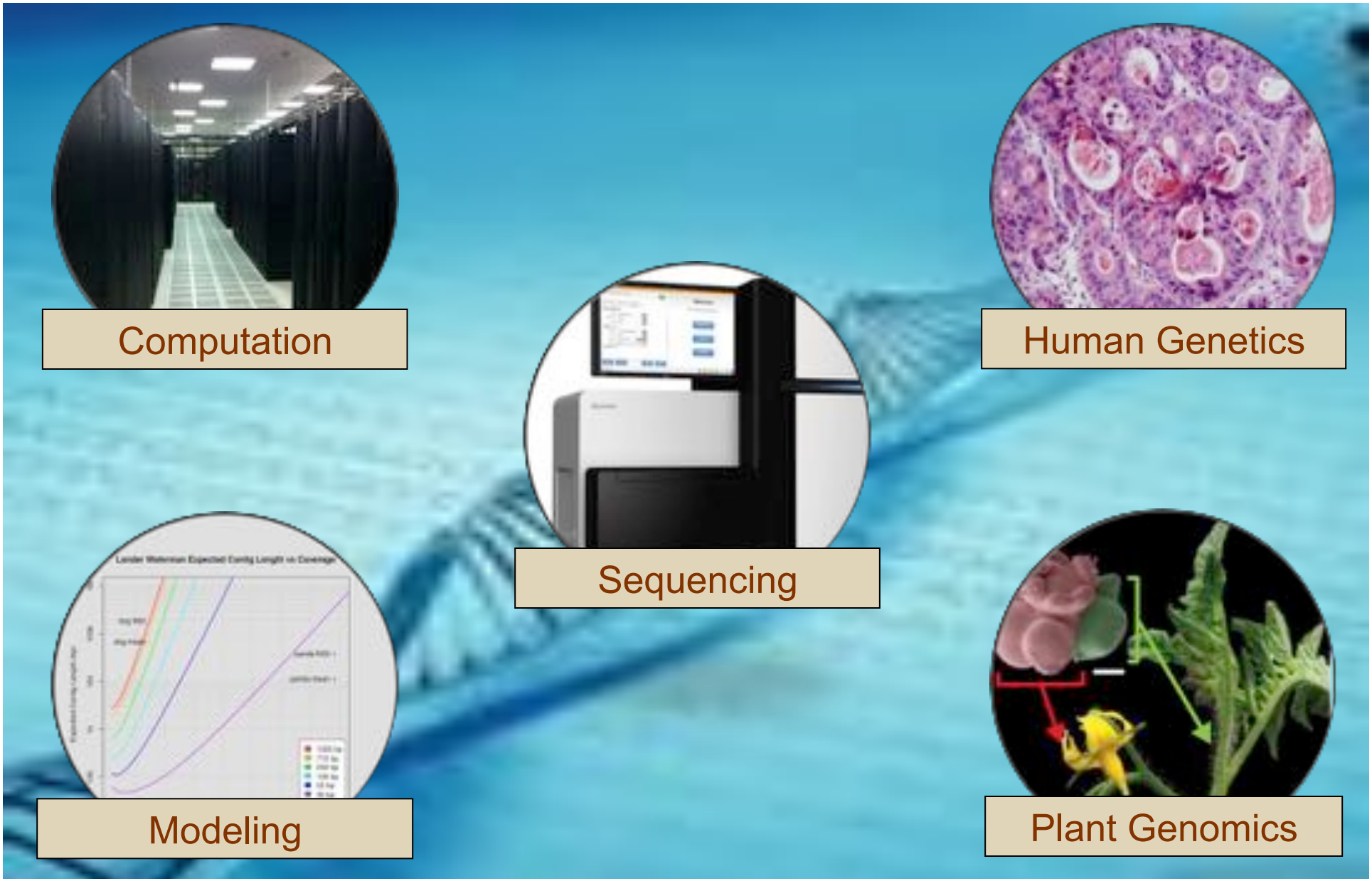
# Next-gen sequence analysis

Michael Schatz

Introduction to Computational Biology
Oct 24, 2013

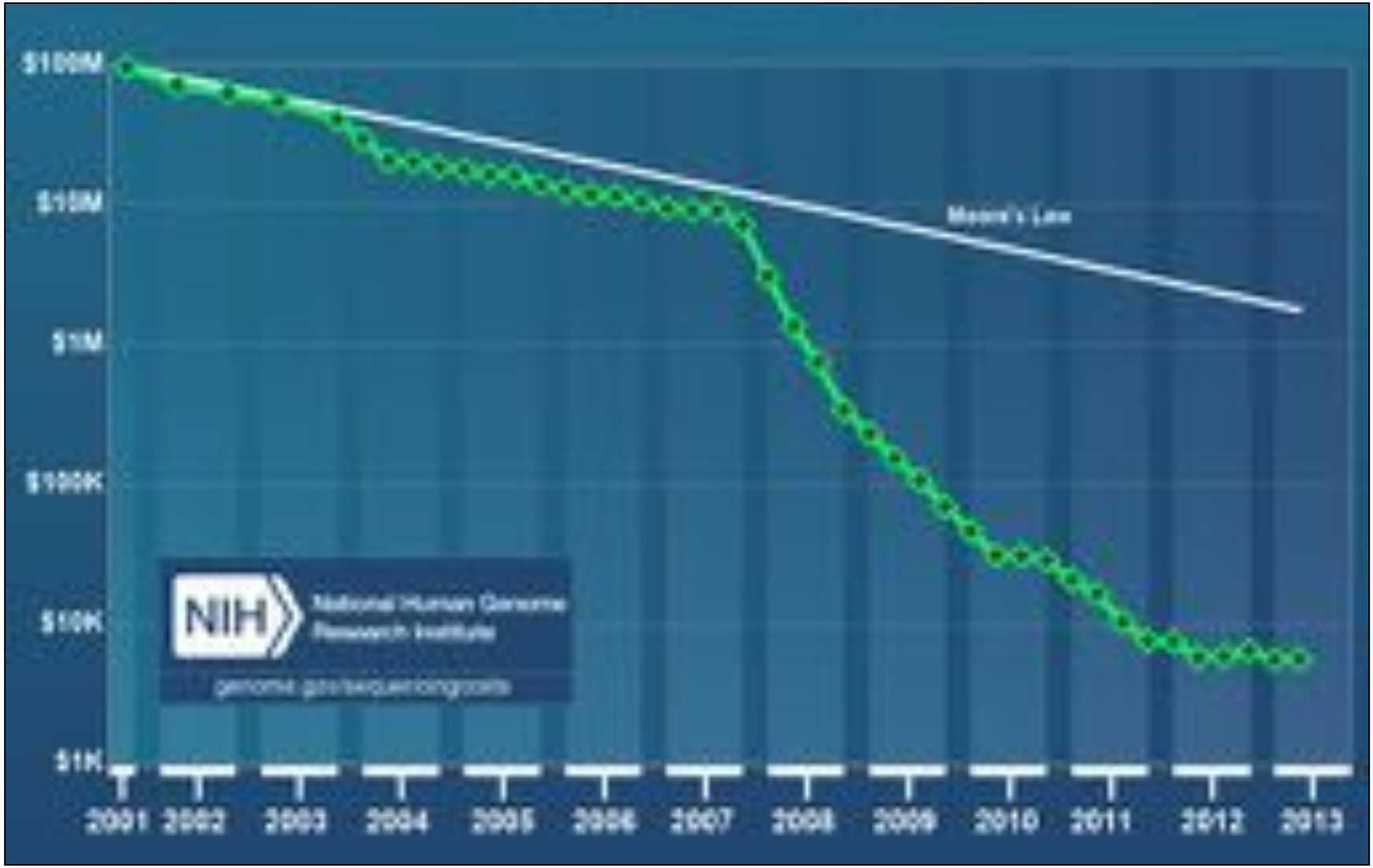
# Schatz Lab Overview



# Outline

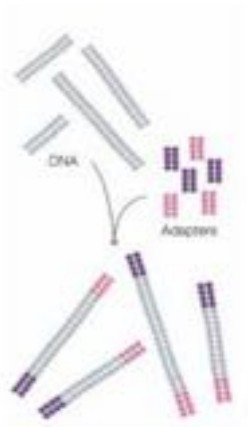
1. Rise of DNA Sequencing
2. Alignment and the BWT
3. Genetics of Autism

# Cost per Genome

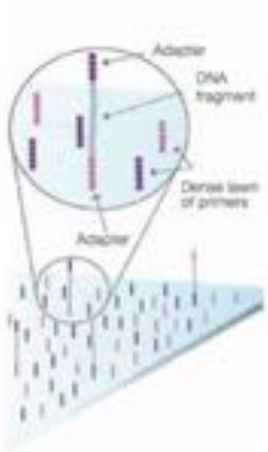



http://www.genome.gov/sequencingcosts/

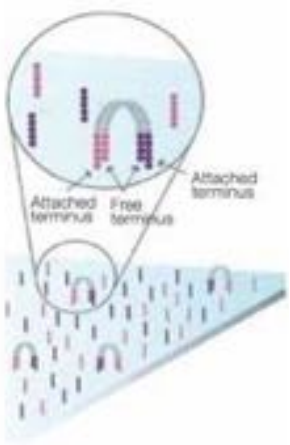
# Illumina Sequencing by Synthesis



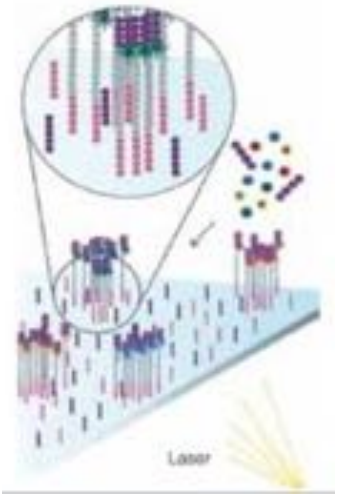

1. Prepare



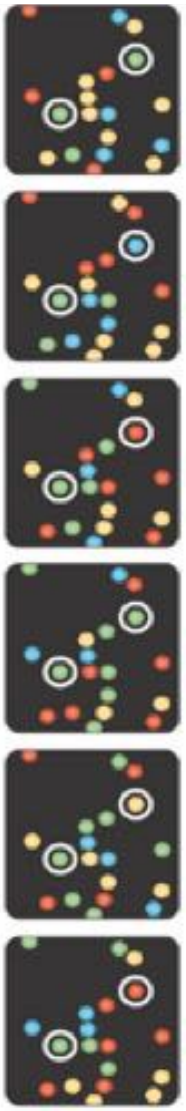

2. Attach




3. Amplify




4. Image



5. Basecall

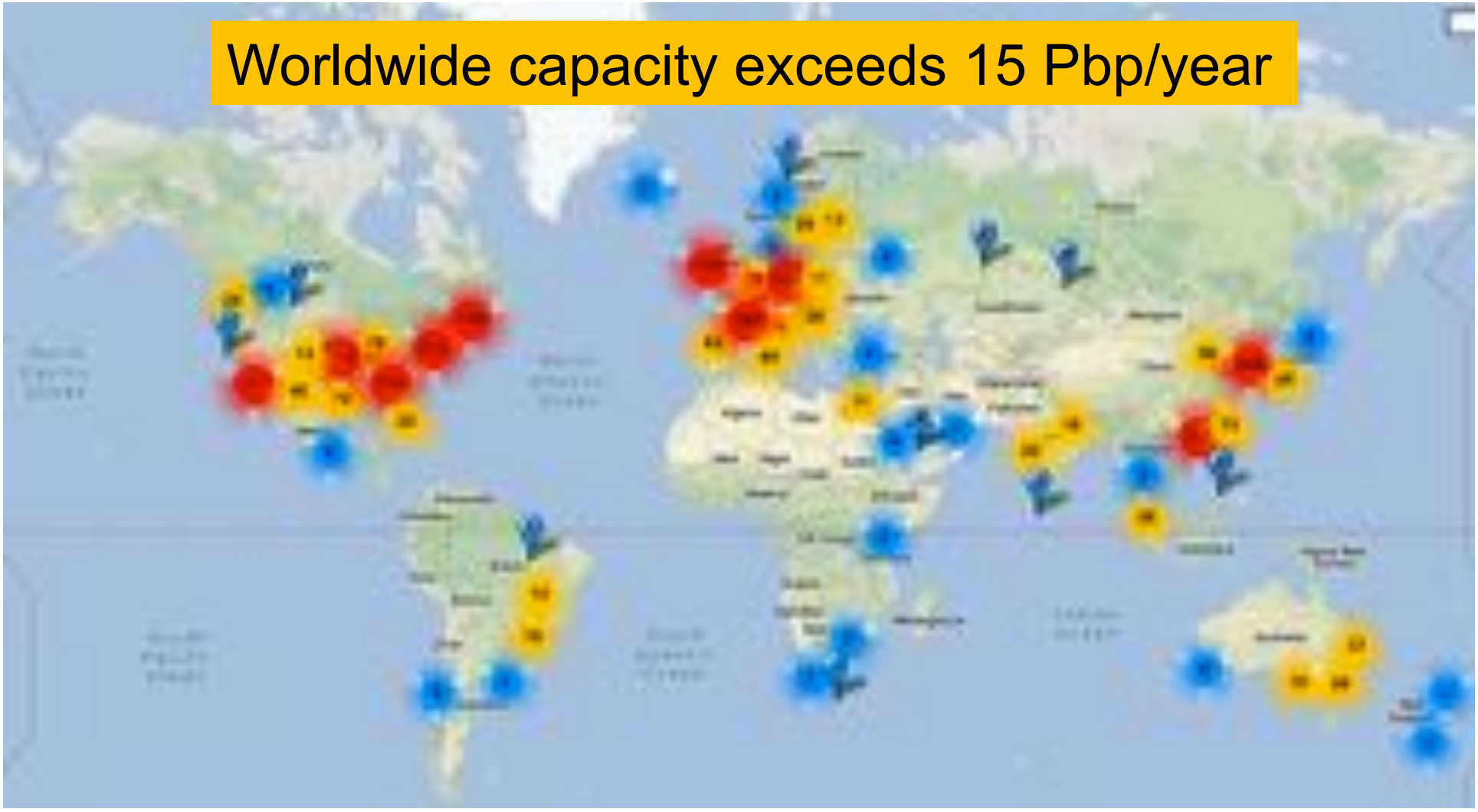
Metzker (2010) Nature Reviews Genetics 11:31-46

http://www.youtube.com/watch?v=l99aKKHcxC4

# Inside the NY Genome Center

Sequencing Capacity: 16 HiSeq 2500 @ 600 Gbp / 11 day = 872 Gbp  / day

# Sequencing Centers




***Next Generation Genomics: World Map of High-throughput Sequencers***
http://omicsmaps.com

# Milestones in Molecular Biology

There is tremendous interest to sequence:

- What is your genome sequence?
- How does your genome compare to my genome?

- Where are the genes and how active are they?
- How does gene activity change during development?
- How does splicing change during development?

- How does methylation change during development?
- How does chromatin change during development?
- How does is your genome folded in the cell?
- Where do proteins bind and regulate genes?

- What virus and microbes are living inside you?
- How has the disease mutated your genome?
- What drugs should we give you?

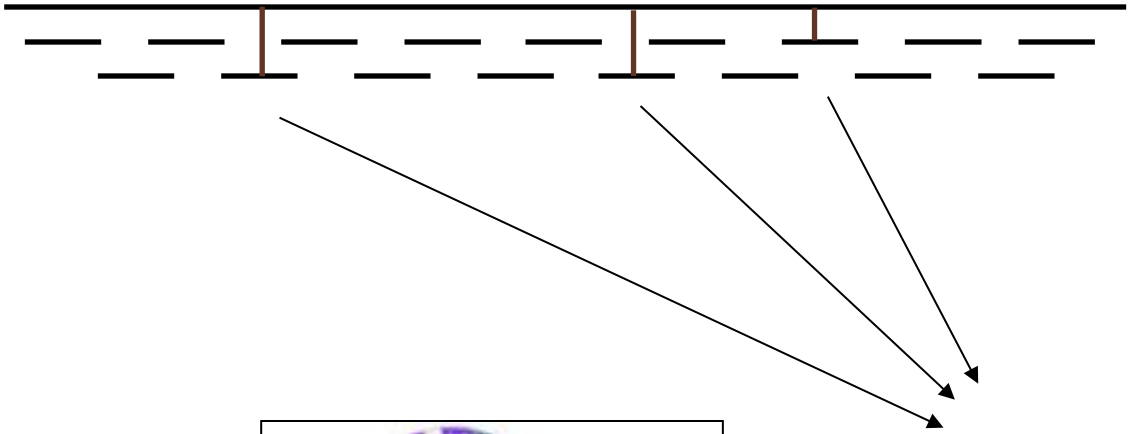
- …

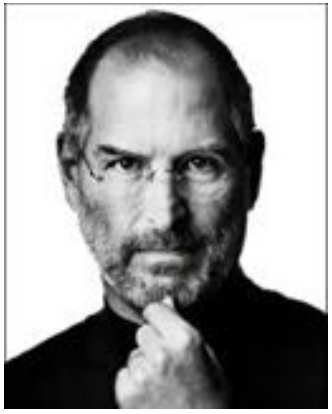
# Outline

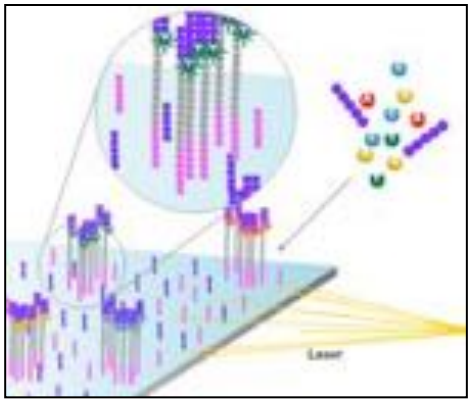
1. Rise of DNA Sequencing
2. Alignment and the BWT
3. Genetics of Autism

# Personal Genomics

How does your genome compare to the reference?







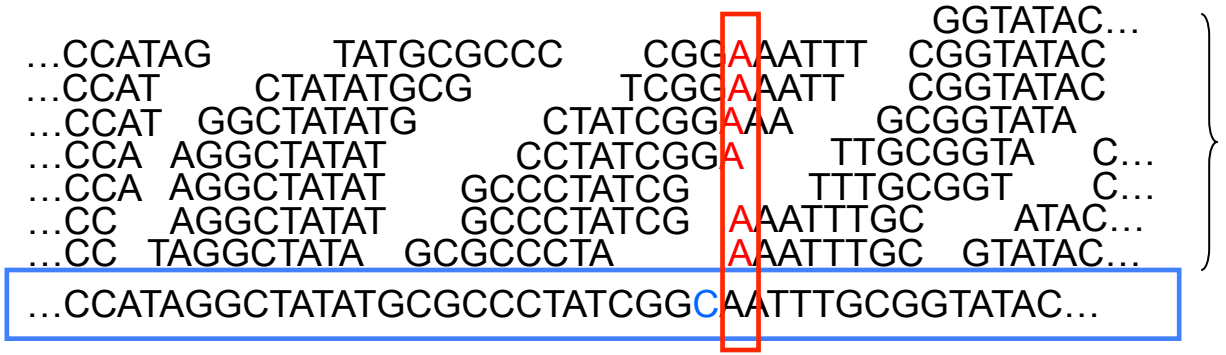
Heart Disease

Cancer

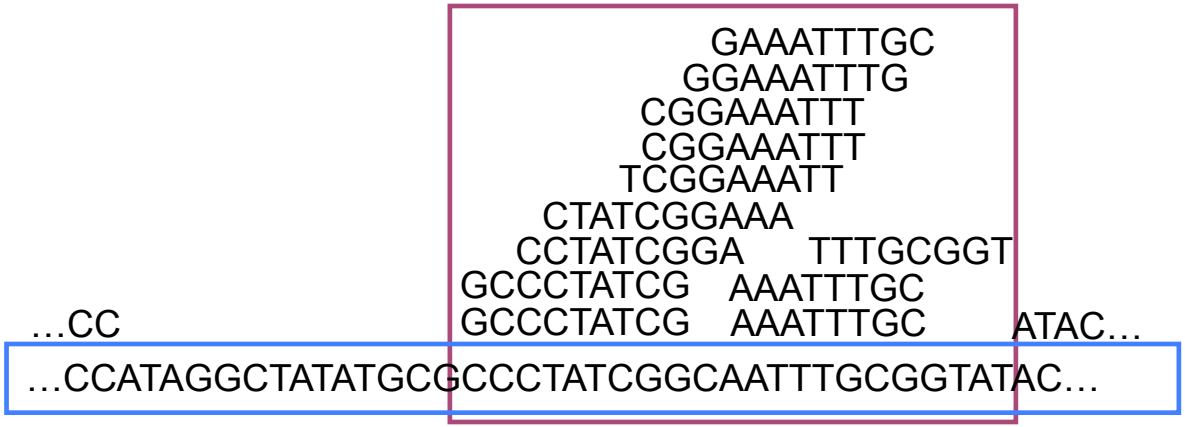
Creates magical technology

# Short Read Applications

- Genotyping: Identify Variations

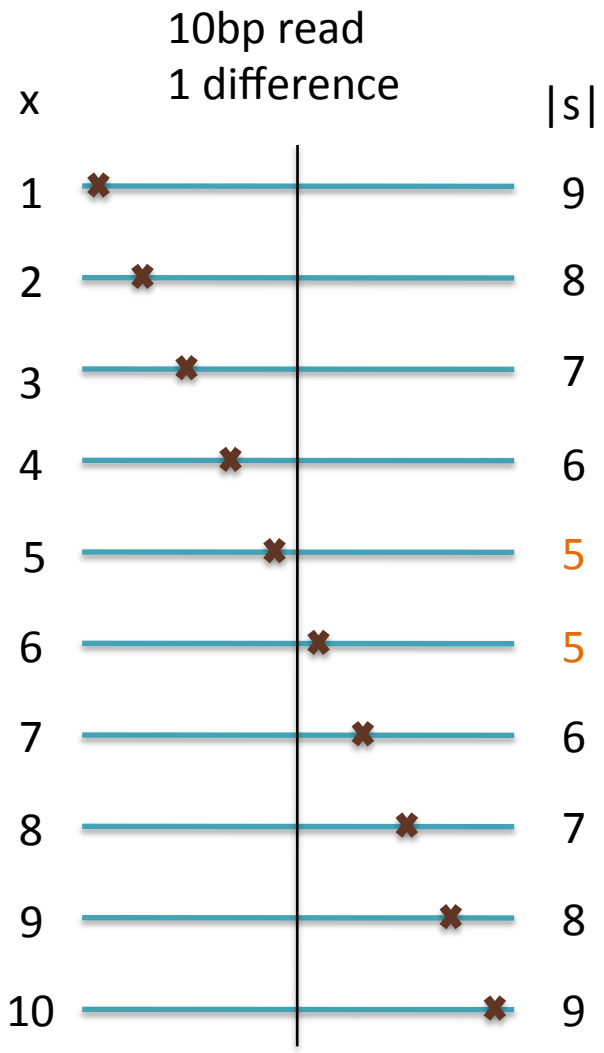



- *-seq: Classify & measure significant peaks



# Seed-and-Extend Alignment

Theorem: An alignment of a sequence of length *m* with at most *k* differences ***must*** contain an exact match at least $s=m/(k+1)$ bp long
(*Baeza-Yates* and Perleberg, 1996)




- Proof: Pigeonhole principle
  - 1 pigeon can't fill 2 holes

- Seed-and-extend search
  - Use an index to rapidly find short exact alignments to seed longer in-exact alignments
    - BLAST, MUMmer, Bowtie, BWA, SOAP, …
  - Specificity of the depends on seed length
    - Guaranteed sensitivity for k differences
    - Also finds some (but not all) lower quality alignments <- heuristic

# Exact Matching Review & Overview

Where is GATTACA in the human genome?

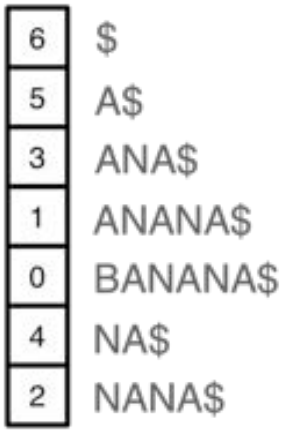
## Brute Force (3 GB)

```
BANANA
BAN
  ANA
    NAN
      ANA
```

Naive

Slow & Easy

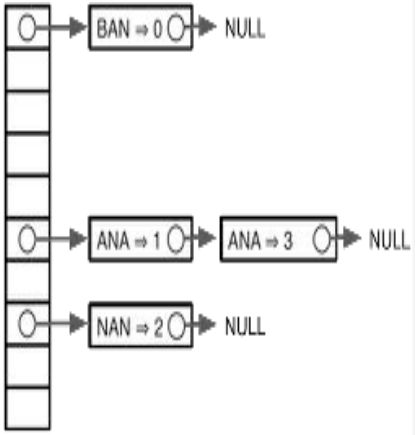
## Suffix Array (>15 GB)




Vmatch, PacBio Aligner

Binary Search

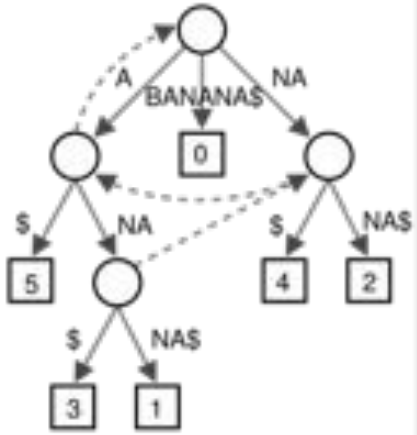
## Hash Table (>15 GB)




BLAST, MAQ, ZOOM, RMAP, CloudBurst

Seed-and-extend

## Suffix Tree (>51 GB)




MUMmer, MUMmerGPU

Tree Searching

*** These are general techniques applicable to any search problem ***

# Algorithmic challenge

How can we combine the speed of a suffix tree (O(|q|) exact match) with the size of a brute force analysis (n bytes)?

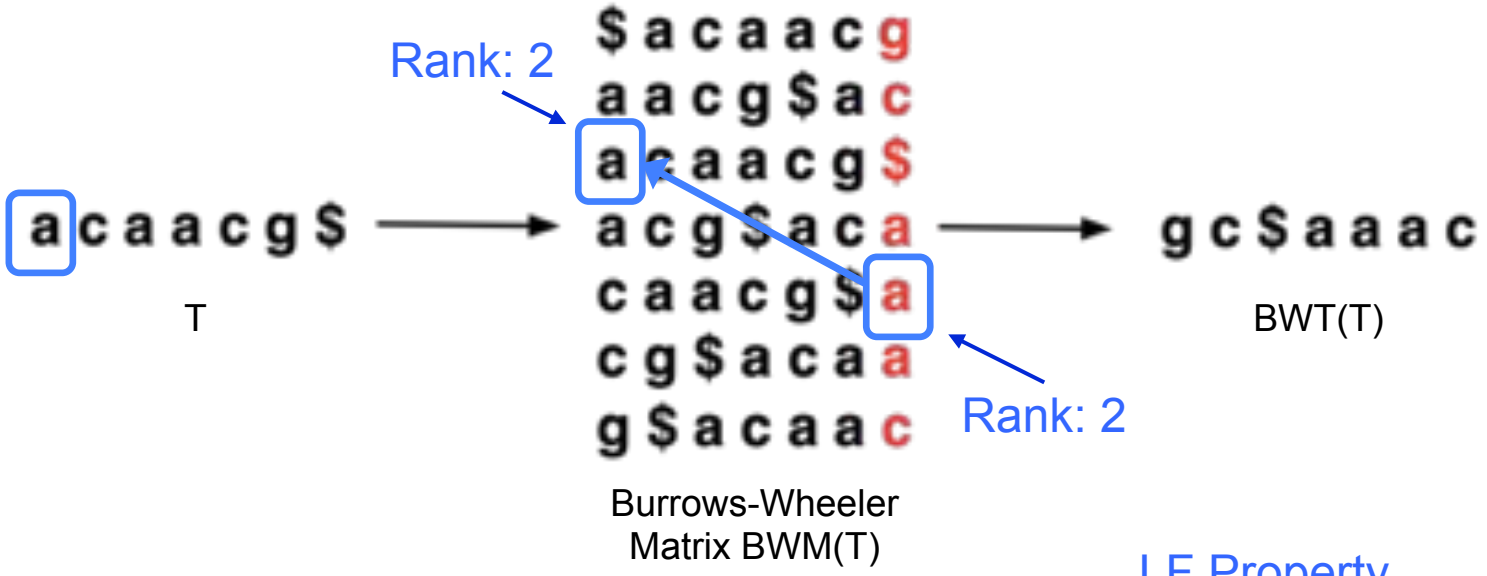
What would such an index look like?

# Fast gapped-read alignment with Bowtie 2

Ben Langmead and Steven Salzberg (2012) Nature Methods. 9, 357–359

# Burrows-Wheeler Transform

- Reversible permutation of the characters in a text




LF Property implicitly encodes Suffix Array

- BWT(T) is the index for T

**A block sorting lossless data compression algorithm.**
Burrows M, Wheeler DJ (1994) *Digital Equipment Corporation.* Technical Report 124

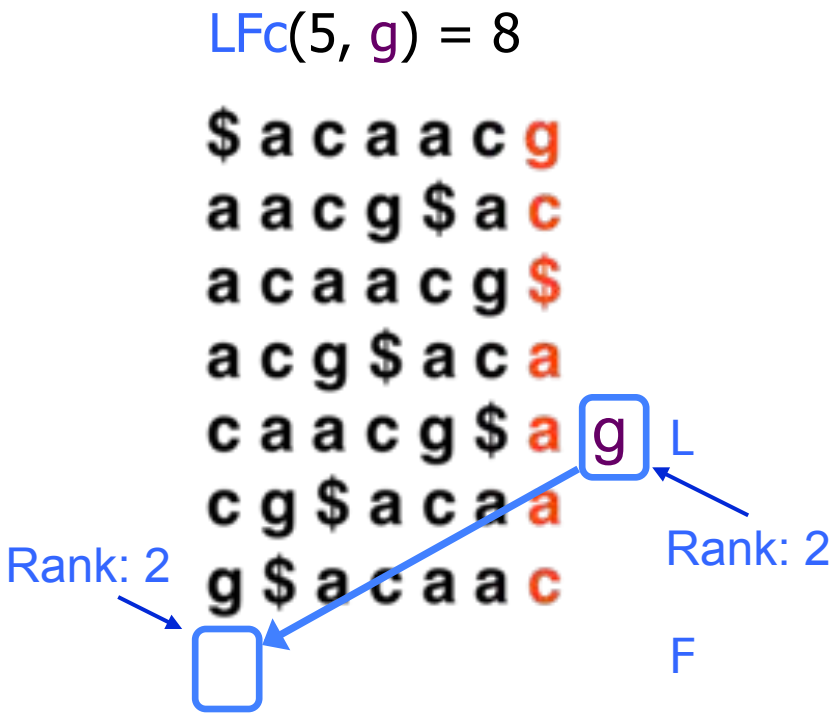
# Burrows-Wheeler Transform

- Recreating T from BWT(T)
  - Start in the first row and apply **LF** repeatedly, accumulating predecessors along the way


[Decode this BWT string: ACTGA$TTA ]

# BWT Exact Matching

- **LFc**(r, c) does the same thing as **LF**(r) but it ignores r's actual final character and "pretends" it's c:



# BWT Exact Matching

- Start with a range, (**top**, **bot**) encompassing all rows and repeatedly apply **LFc**:

  **top** = **LFc**(**top**, **qc**); **bot** = **LFc**(**bot**, **qc**)

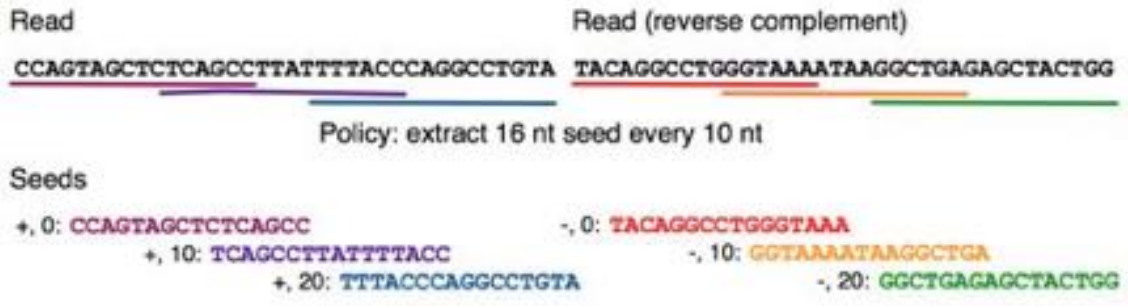
  **qc** = the next character to the left in the query

Ferragina P, Manzini G: Opportunistic data structures with applications. *FOCS. IEEE Computer Society; 2000.*

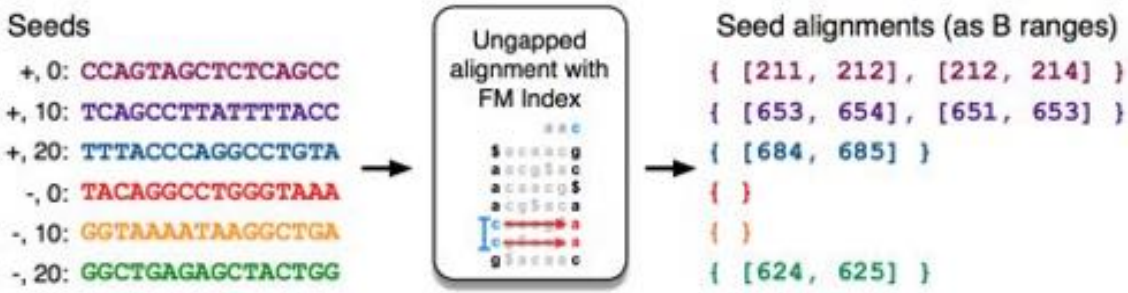
[Search for TTA this BWT string: ACTGA$TTA ]

# Algorithm Overview

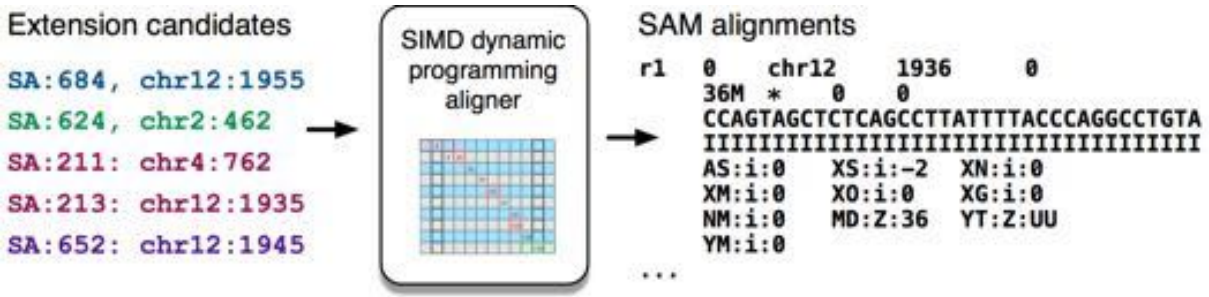
1. Split read into segments



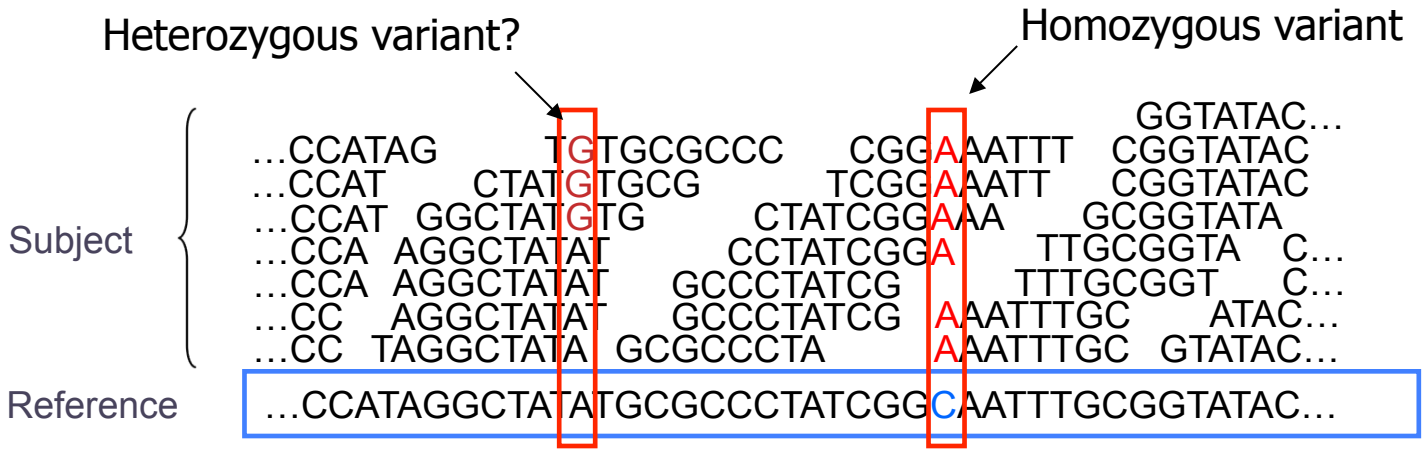

2. Lookup each segment and prioritize




3. Evaluate end-to-end match

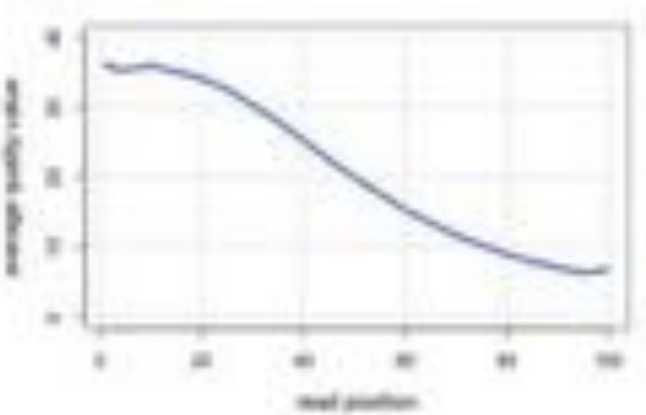


# Genotyping




- Sequencing instruments make mistakes
  - Quality of read decreases over the read length

$$Q_{\text{sanger}} = -10 \log_{10} p$$



- A single read differing from the reference is probably just an error, but it becomes more likely to be real as we see it multiple times
  - Often framed as a Bayesian problem of more likely to be a real variant or chance occurrence of N errors
  - Accuracy improves with deeper coverage

# Outline

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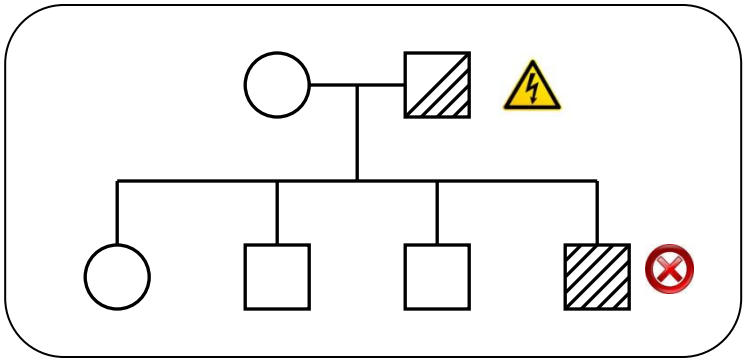
3. Genetics of Autism

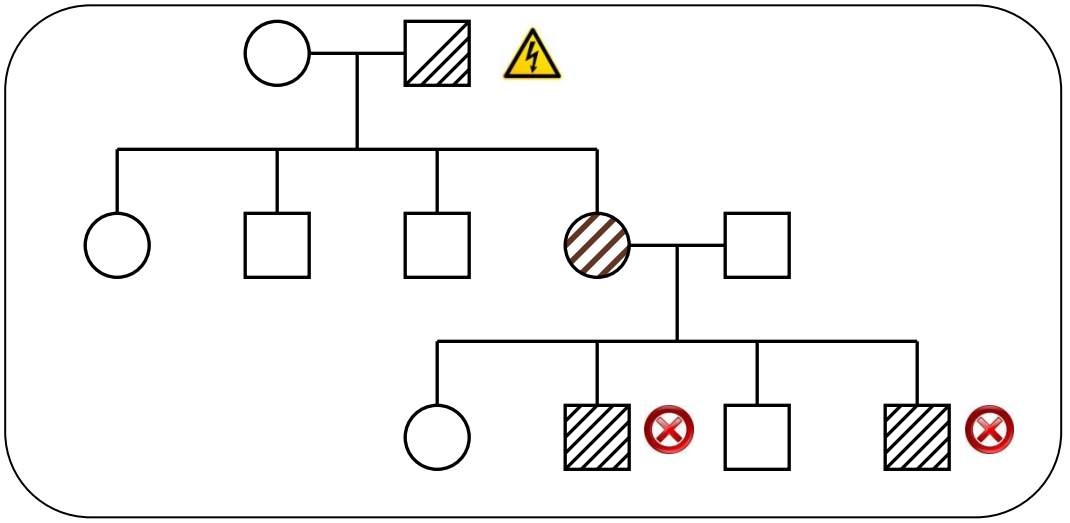
# Unified Model of Autism

Sporadic Autism: 1 in 100



**Prediction**: De novo mutations of high penetrance contributes to autism, especially in low risk families with no history of autism.

Familial Autism: 90% concordance in twins



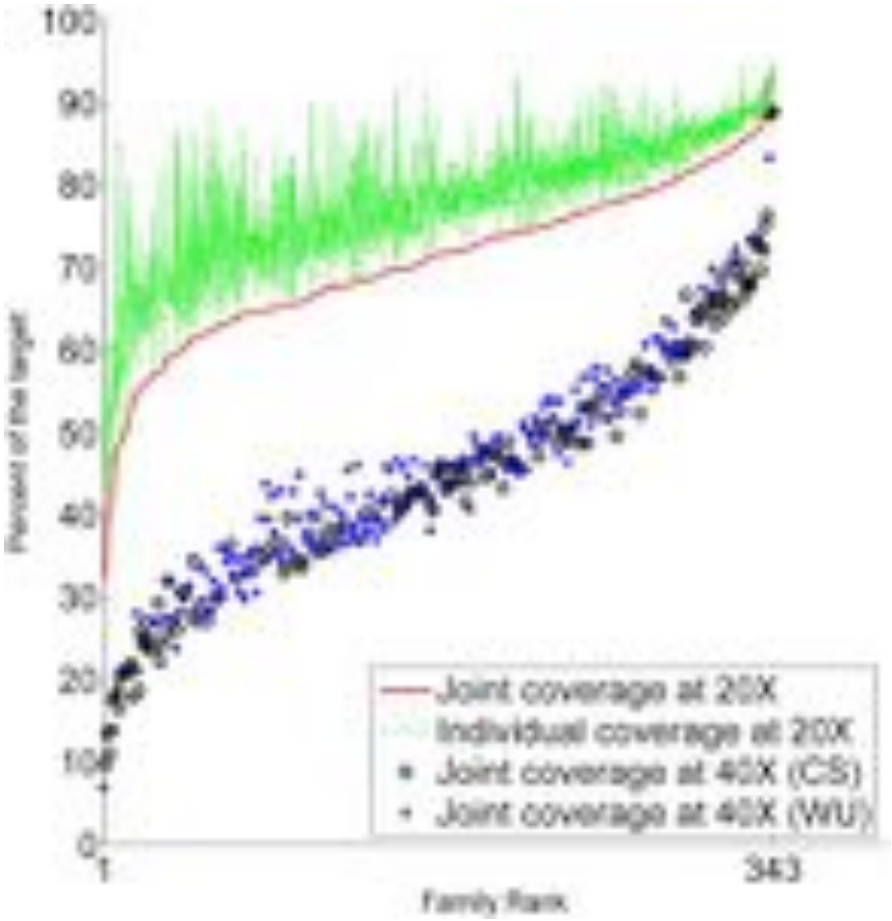
Legend

Sporadic mutation

Fails to procreate

**A unified genetic theory for sporadic and inherited autism**
Zhao *et al.* (2007) *PNAS. 104(31)12831-12836.*

# Exome-Capture and Sequencing




Sequencing of 343 families from the Simons Simplex Collection

- Parents plus one child with autism and one non-autistic sibling
- Enriched for higher-functioning individuals

Families prepared and captured together to minimize batch effects

- Exome-capture performed with NimbleGen SeqCap EZ Exome v2.0 targeting 36 Mb of the genome.
- ~80% of the target at >20x coverage with ~93bp reads

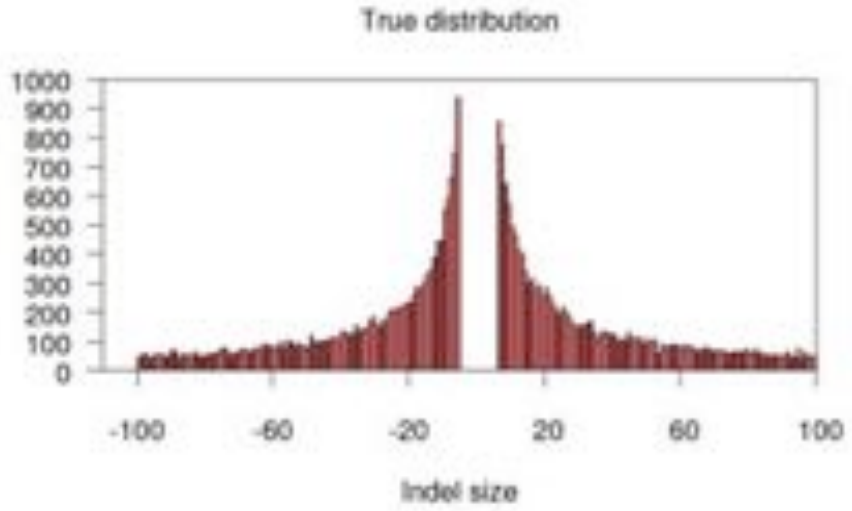
**De novo gene disruptions in children on the autism spectrum**
Iossifov *et al.* (2012) *Neuron*. 74:2 285-299

# Variation Detection Complexity

## SNPs + Short Indels

High precision and sensitivity

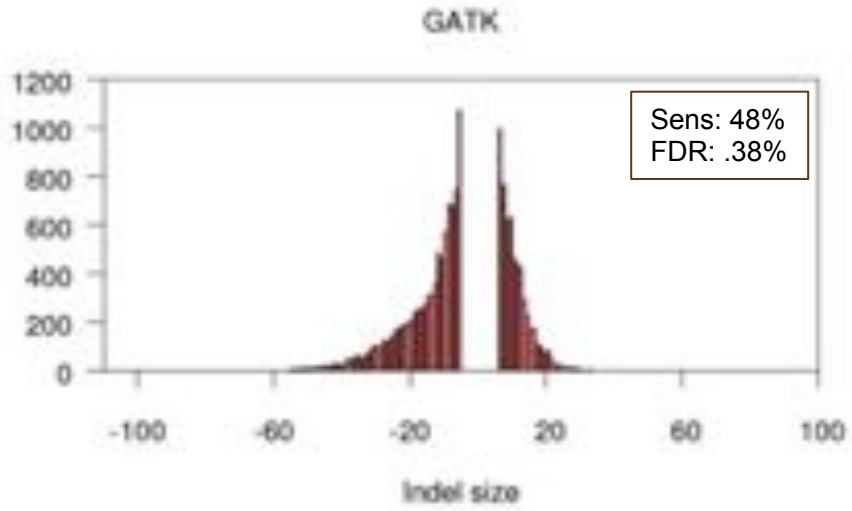
```
..TTTAGAATAG-CGAGTGC...
      ||||||| ||||
      AGAATAGGCGAG
```




## "Long" Indels (>5bp)

Reduced precision and sensitivity

```
..TTTAG--------AGTGC...
  |||||         |||||
  TTTAGAATAGGC  |||||
        ATAGGCGAGTGC
```




Analysis confounded by sequencing errors, localized repeats, allele biases, and mismapped reads

# Scalpel: Haplotype Microassembly

DNA sequence **micro-assembly** pipeline for accurate detection and validation of *de novo* mutations (SNPs, indels) within exome-capture data.

Features

1. Combine mapping and assembly
2. Exhaustive search of haplotypes
3. De novo mutations

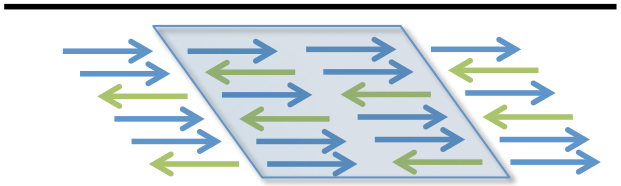
NRXN1 *de novo* SNP
(auSSC12501 chr2:50724605)

**SCALPEL: Micro-assembly approach to accurately detect de novo and transmitted indel mutations within exome-Capture data**

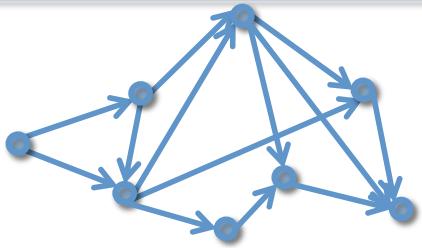
Narzisi, G, O'Rawe, J, Iossifov, I, Lee, Y, Wang, Z, Wu, Y, Lyon, G, Wigler, M, Schatz, MC (2013) *In preparation*

# Scalpel Pipeline

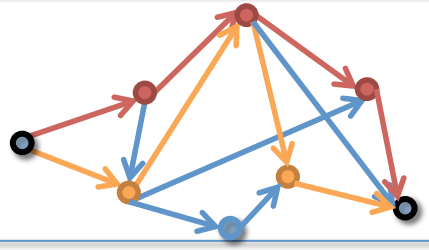
Extract reads mapping within the exon including (1) well-mapped reads, (2) soft-clipped reads, and (3) anchored pairs



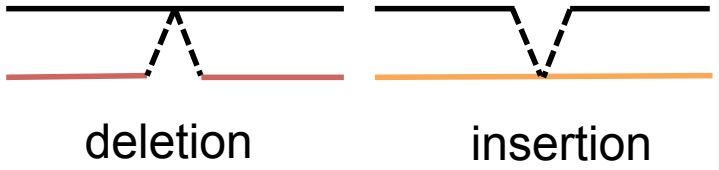
Decompose reads into overlapping *k*-mers and construct de Bruijn graph from the reads



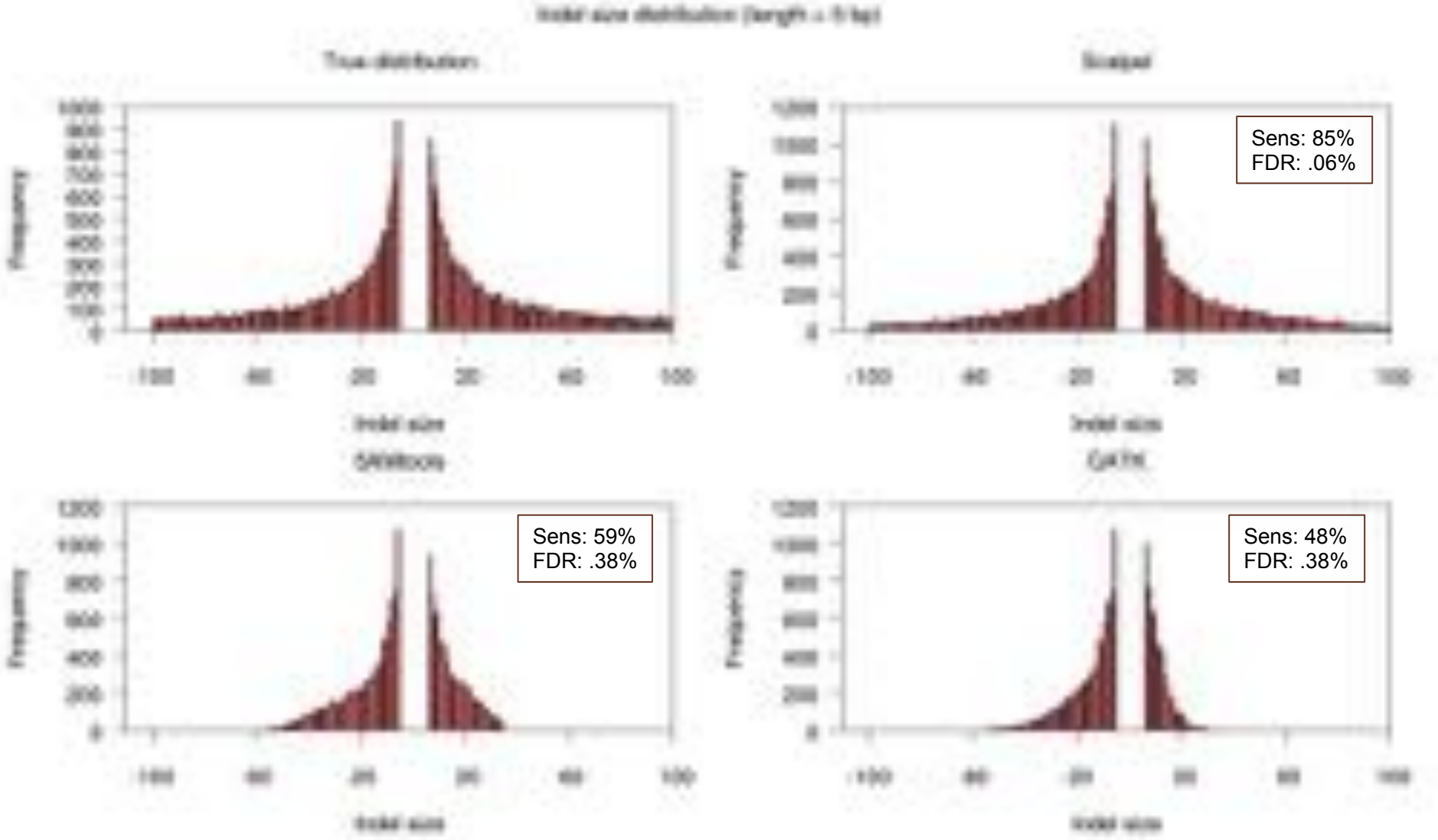
Find end-to-end haplotype paths spanning the region



Align assembled sequences to reference to detect mutations

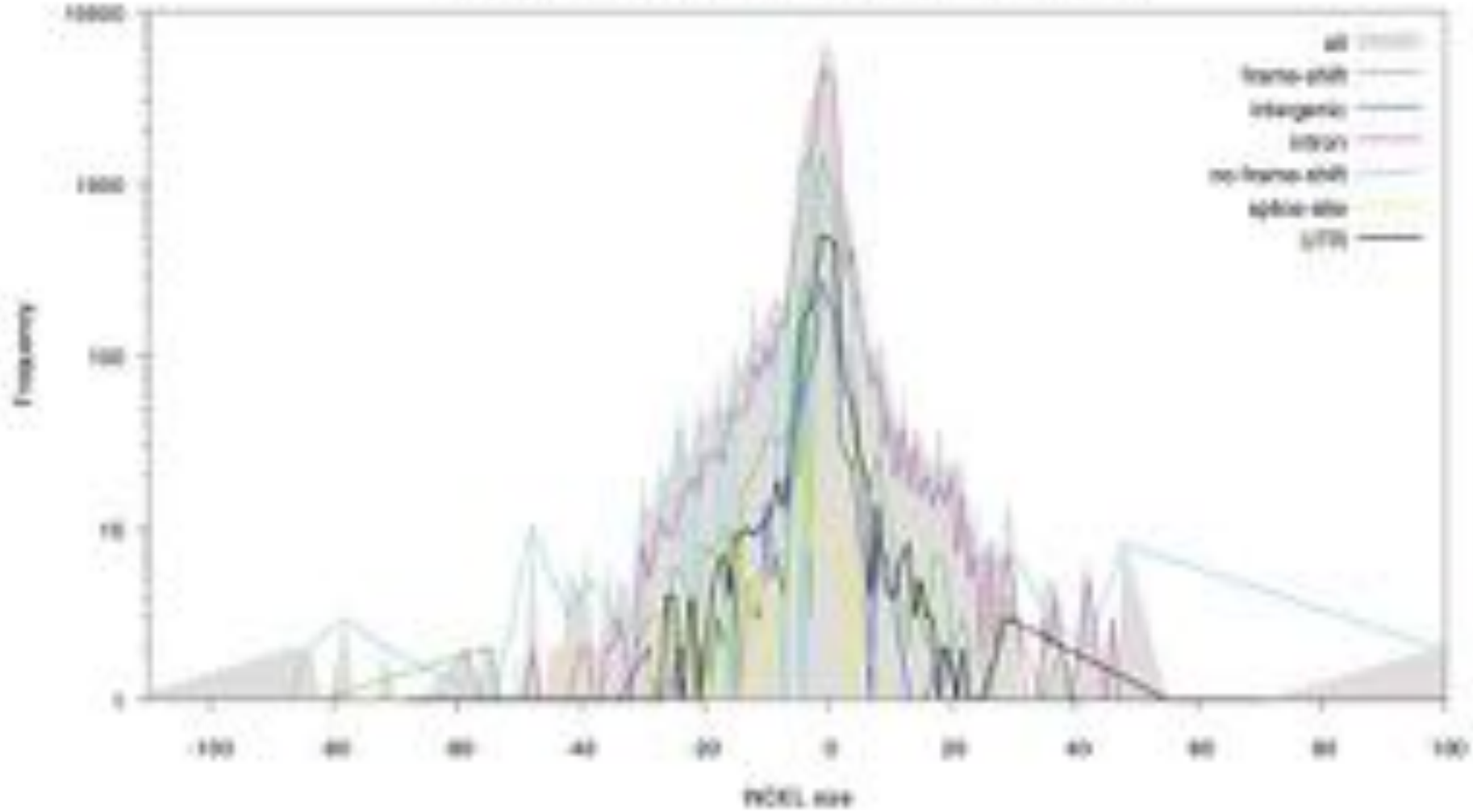


# Simulation Analysis




Simulated 10,000 indels in a exome from a known log-normal distribution

# Revised Analysis of the SSC

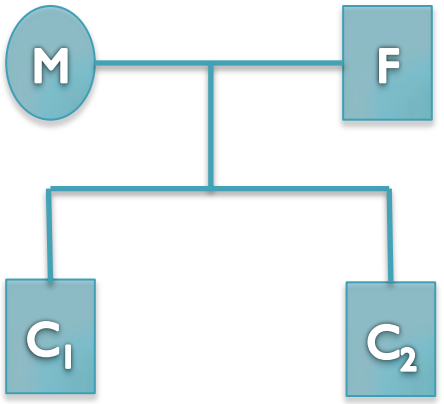


Constructed database of >1M transmitted and de novo indels
Many new gene candidates identified, population analysis underway

# De novo mutation discovery and validation

**Concept**: Identify mutations not present in parents.

**Challenge**: Sequencing errors in the child or low coverage in parents lead to false positive de novos




```
Ref:     ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...

Father:  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Mother:  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Sib:     ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Aut(1):  ...TCAGAACAGCTGGATGAGATCTTAGCCAACTACCAGGAGATTGTCTTTGCCCGGA...
Aut(2):  ...TCAGAACAGCTGGATGAGATCTTACC------CCGGAGATTGTCTTTGCCCGGA...
```

6bp heterozygous deletion at chr13:25280526 ATP12A

# De novo Genetics of Autism

- In 343 family quads so far, we see significant enrichment in de novo ***likely gene killers*** in the autistic kids
  - Overall rate basically 1:1 (432:396)
  - 2:1 enrichment in nonsense mutations
  - 2:1 enrichment in frameshift indels
  - 4:1 enrichment in splice-site mutations
  - Most de novo originate in the paternal line in an age-dependent manner (56:18 of the mutations that we could determine)
- Observe strong overlap with the 842 genes known to be associated with fragile X protein FMPR
  - Related to neuron development and synaptic plasticity

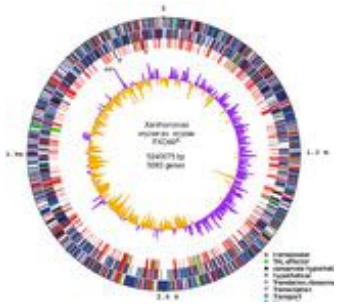
**De novo gene disruptions in children on the autism spectrum**
Iossifov *et al.* (2012) *Neuron*. 74:2 285-299

# Summary

I'm interested in answering biological questions by developing and applying novel algorithms and computational systems

- Interesting biological systems: human diseases, foods, biofuels
- Interesting biotechnology: new sequencing technologies
- Interesting computational systems: parallel & cloud technology
- Interesting algorithms: assembly, alignment, interpretation

Also extremely excited to teach the next generation of scientists in the WSBS, URP, and high school programs



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*See you at*

*Genome Informatics*

*Oct 30 – Nov 2*

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