Analysing *Alu* inserts detected from high-throughput sequencing data

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Before we begin...

Even though I'll only present the minimal amount of biology required to understand my presentation, neglecting some fundamental concepts, I still have to introduce a lot of terms and concepts. So please **feel free to interrupt and ask questions**. It will try my best to avoid leaving you guys feeling like this:

Overview

1. Biology background
   - 1.1 Human genetic diversity
   - 1.2 Molecular biology
   - 1.3 Genomes, the human genome
   - 1.4 High-throughput sequencing and genome assembly
   - 1.5 *Alu* sequences, novel *Alu*s
2. Novel *Alu* insert sequence assembly
   - 2.1 Assembly pipeline
   - 2.2 Applications
     - 2.2.1 Tracing origins of the inserts
     - 2.2.2 Subfamily clustering (not discussed today)
3. Use of novel *Alu*s for measuring genetic distance

Biological background: Human genetic diversity

- *Homo sapiens* originated in Africa
  - Oldest remains found in Ethiopia
- Small group migrated to Arabia, then spread
- Confirmed by much higher genetic diversity between African populations
  - i.e. On average, different populations are much less related to each other
- Roughly inverse relation between distance to Africa and interpopulation genetic diversity

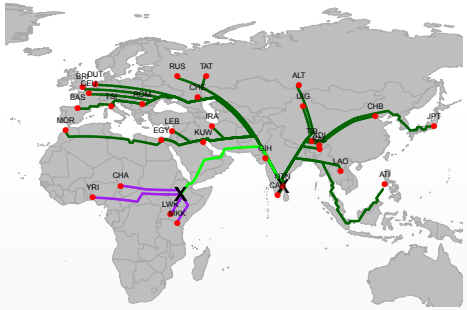
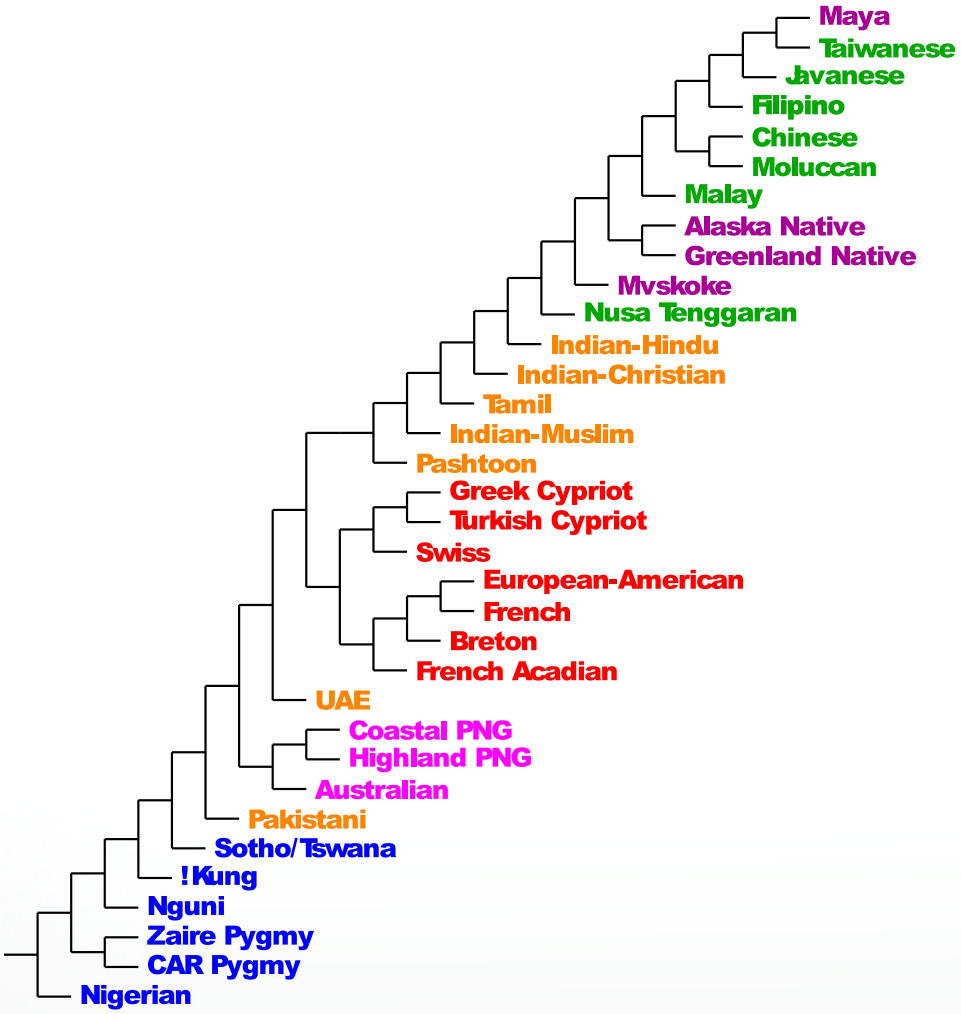


Figure: Melé, Marta, et al. "Recombination gives a new insight in the effective population size and the history of the Old World human populations." Molecular biology and evolution 29.1 (2012): 25-30.

Biological background: Human genetic diversity



Stoneking, Mark, et al. "Aluinsertion polymorphisms and human evolution: Evidence for a larger population size in Africa." Genome research 7.11 (1997): 1061-1071.

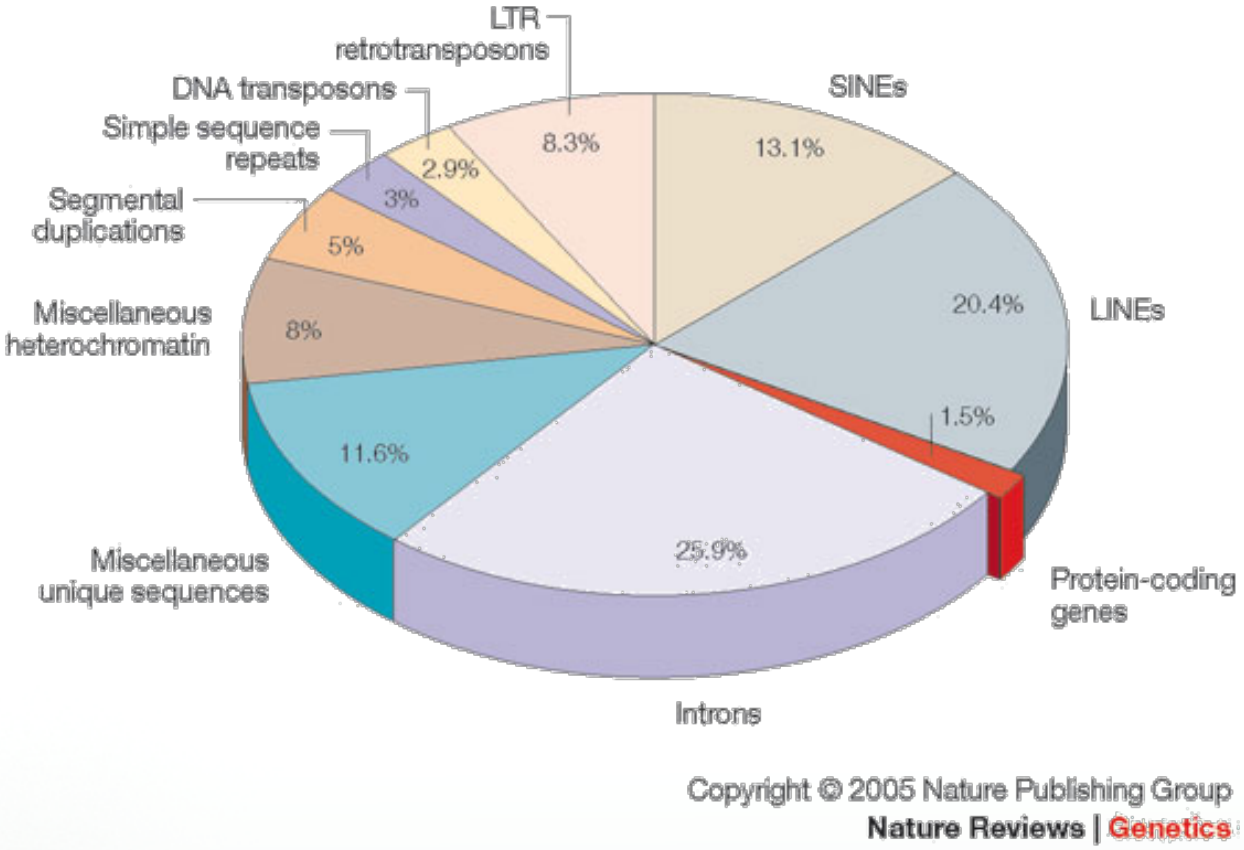
Biological background: Molecular Biology

- Central dogma DNA $\longrightarrow$ RNA $\longrightarrow$ Protein
  - Only in general, several significant exceptions exist
  - DNA stores almost-read-only information. Think of it as a sequence of genes (words) and gene regulators connected by long lengths of junk that may or may not affect these regulators
  - RNA made by transcribing small section of the DNA, can be processed
  - Proteins (and sometimes RNA) perform biological functions
- Genome is collection of chromosomes (DNA molecules) in a cell
  - eg. Humans have two copies of 23 chromosomes (one from each parent)
- When cell divides, DNA replicated so each daughter cell gets a copy
  - Basis of genome sequencing (reading)

Biological background: Genomes

- A chromosome can be represented as a sequence (string) in a five base pair (bp) alphabet $\Sigma = \{A, T, G, C, N\}$
  - Biologically only the first 4 exist, N represents an unknown
  - Represent substrings with coordinates
    - eg. `chr1:12345-12533`
  - This encoding does not represent structure, and other important information
- Consensus sequences for each chromosome from a couple of individuals serves as a reference genome
  - In human reference, variation among contributors not represented
  - An individual can be concisely represented as a list of variants (differences) between him/her and the reference

Biological background: Human genome

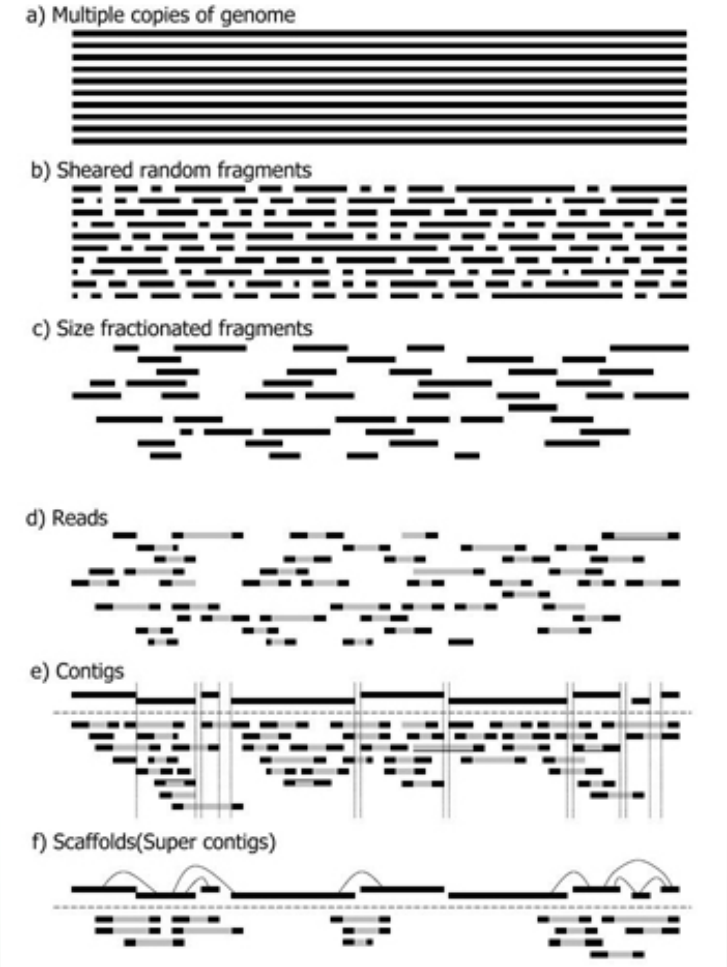


Gregory, T. Ryan. "Synergy between sequence and size in large-scale genomics." Nature Reviews Genetics 6.9 (2005): 699-708.

Biological background: High-throughput sequencing; genome assembly

- A chromosome can't be sequenced in one reaction
- Instead, molecules replicated several times, broken into tiny <100bp pieces, sequenced in parallel to get reads
  - Average number of reads per position called coverage
  - Coverage variable
- Reassemble the original chromosome strings by overlapping the reads
  - If reference sequence available, can align reads to it to help
- Several issues make this non-trivial
  - Don't know which chromosome a given read comes from
  - Repeats make it hard to figure out which reads overlap and how
  - etc.
- Several techniques and algorithms developed

Biological background: High-throughput sequencing; genome assembly



http://www.k.u-tokyo.ac.jp/pros-e/person/shinichi_morishita/shinichi_morishita.htm

QUESTIONS?

Biological background: *Alus*

ggccgggcgcggtggctcacgcctgtaatcccagcactttgggaggccgaggcgggcgg

- ∼45% of human genome composed of repetitive elements
  - Retrotransposons (LINEs, SINEs), other short repeats, etc.
- *Alus* are most common SINE, >1 million copies
- Replicate by reverse transcribing RNA to DNA then inserting into genome, 1 replication every ∼10 births
- ∼99% inactive in humans, *Alu*Y subfamily active
- Since lack of *Alu* rarely means that insertion didn't happen, good for calculating genetic distance
- Have been linked to certain disorders
- Traditionally detected through wet lab methods
- *Alu* inserts that are not present in the reference human genome called novel inserts

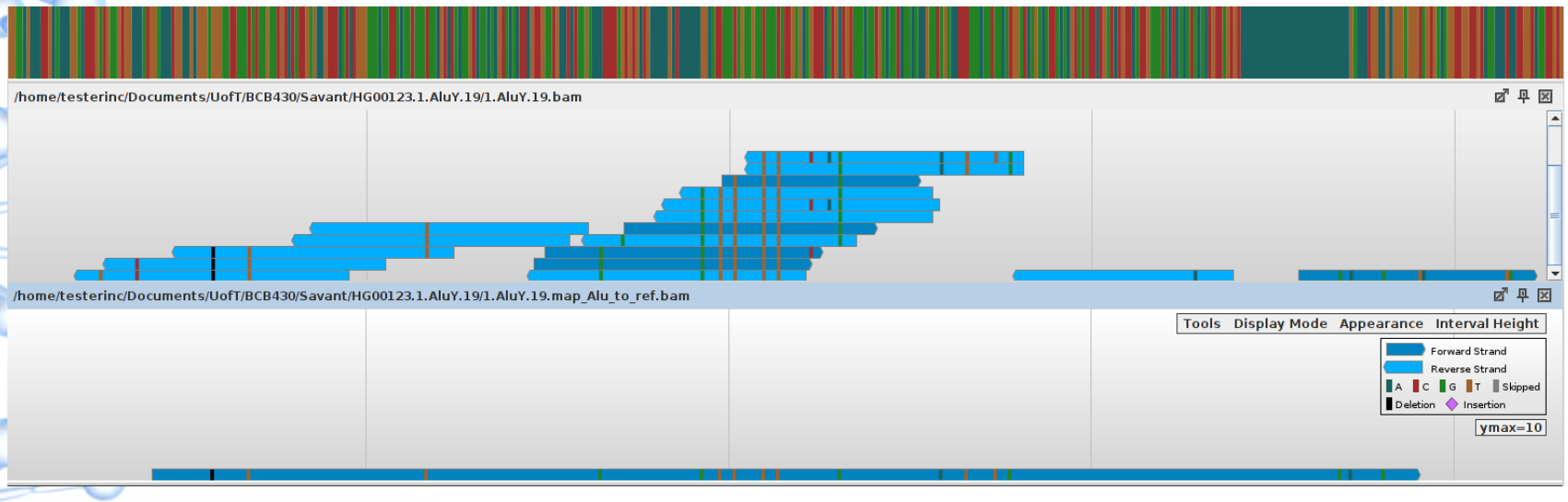
Biological background: Detecting novel *Alu*s

- Recently, programs developed for detecting novel *Alu* inserts from HTS data
  - Last year, Matei and I developed `alu-detect`
- All of these programs suffer from high false negative rates
  - Happens to be that coverage around *Alu*s is naturally low
  - Also dependent on result filtering
- Previous programs (including `alu-detect`) have only outputted lists of coordinates and subfamily identities of detected insertion events, not the actual sequences of the inserts

Assembling *Alu*s: Pipeline

- To assemble the sequence of a novel *Alu* insert, we need the reads
- `alu-detect` extended to report which reads used as evidence for novel inserts
- Pipeline of standard computational biology tools used to assemble the sequence
  - Aligned the reads to the consensus sequence of its *Alu* subfamily
  - Used read quality and alignment quality scores to determine significant differences between the reference and the reads
  - Merged the changes into the reference
- Since coverage was too low (not enough reads to make differences significant), data from several individuals was combined

Assembling *Alus*: Pipeline



Assembling *Alu*s: Origins of the inserts

- Aligned the constructed inserts back to the reference
- Since *Alu* sequences are very similar and have high copy number, several hits for each query
- Selected hit with highest alignment score
- Results need to be investigated further
  - High proportion of inserts originate from single source
  - Most common *Alu* subfamily in data is the previously reported most active
  - However, second most common subfamily in data not the second most active subfamily
  - Possible cause(s)

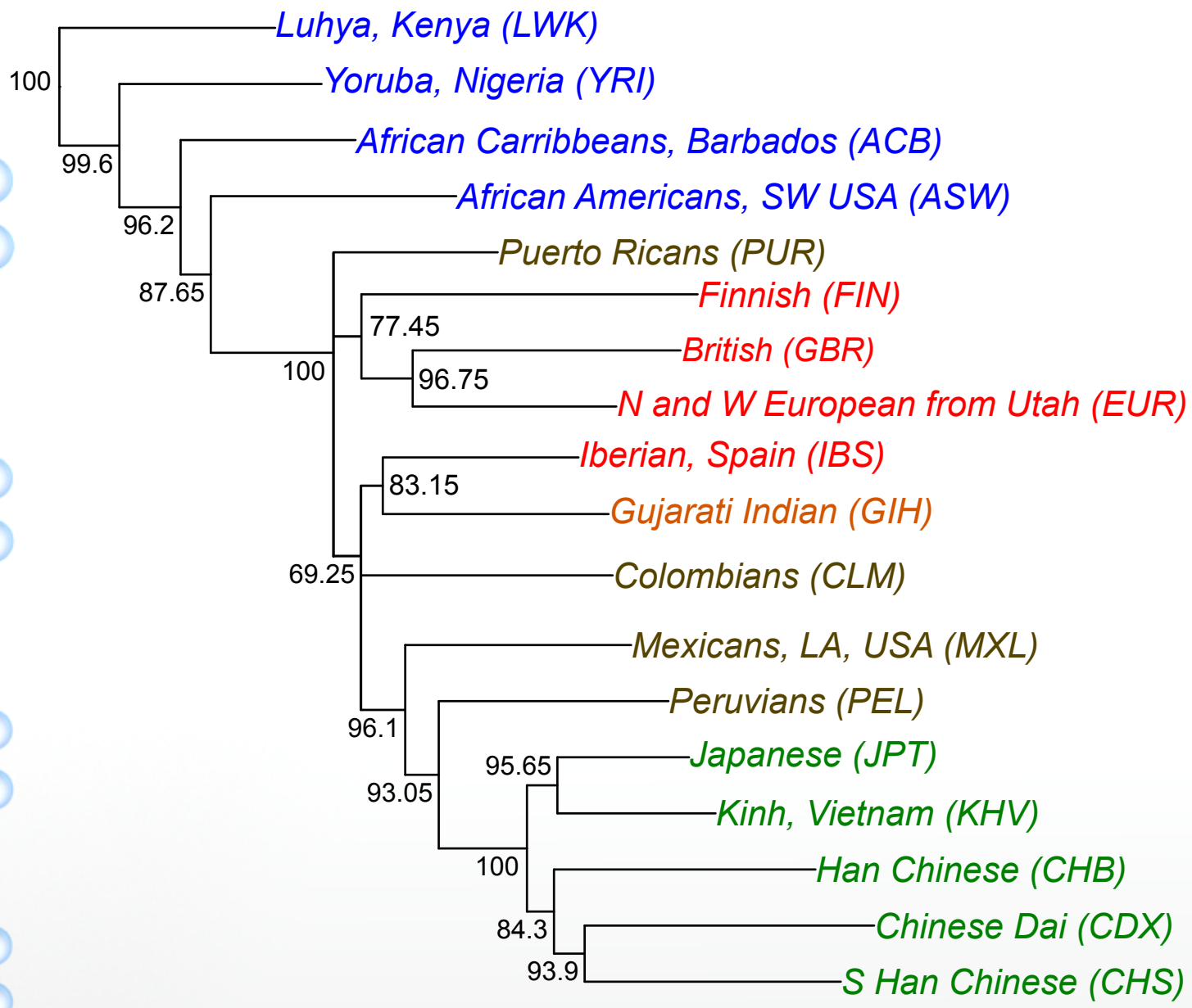
$$\left.\begin{array}{c}\text{Flawed method?}\\ \text{Garbage in, garbage out?}\end{array}\right\}\text{most likely}$$

Actual result?

Alu frequencies as genetic distance

- Detected novel *Alu* inserts in 166 individuals distributed across 19 ethnic groups from the 1000 Genomes project
- For each insert, calculated frequency of that insert in each group
  - Each insert represented as a 19-dimensional vector of probabilities
  - Equivalently, each population represented by a 50-dimensional vector of values in $[0, 1]$.
- Picked the top 50 inserts with the highest frequency variances
  - PCA also attempted, but results were much worse
- Clustered using neighbour-joining with the Cavalli-Szforza distance and bootstrapped with $B = 1000$ samples.
- Future work
  - See if these vectors are correlated to vectors of frequencies for other kinds of genetic variants

Population clustering by genetic distance



Thank you for your time

and now...

COOKIES!