How to Multiple Align Huge Number of Pyrosequencing Reads

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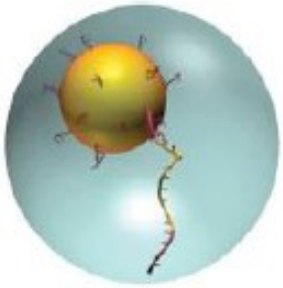
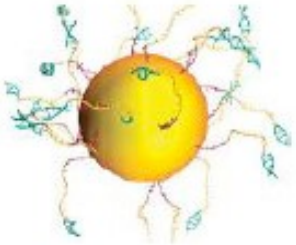
Overview

- Genome Sequencing with Pyrosequencing (454 GS20 Platform)
- Need for Multiple Alignments
- Problem Statement and Assumptions
- Algorithmic approach
- Quality Results
- Ongoing works
- Conclusions and Future work

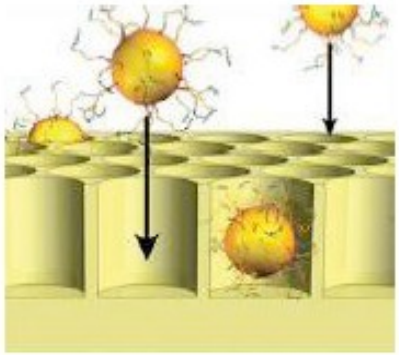
A look at the 454 sequencing technology



DNA fragments are attached to the beads

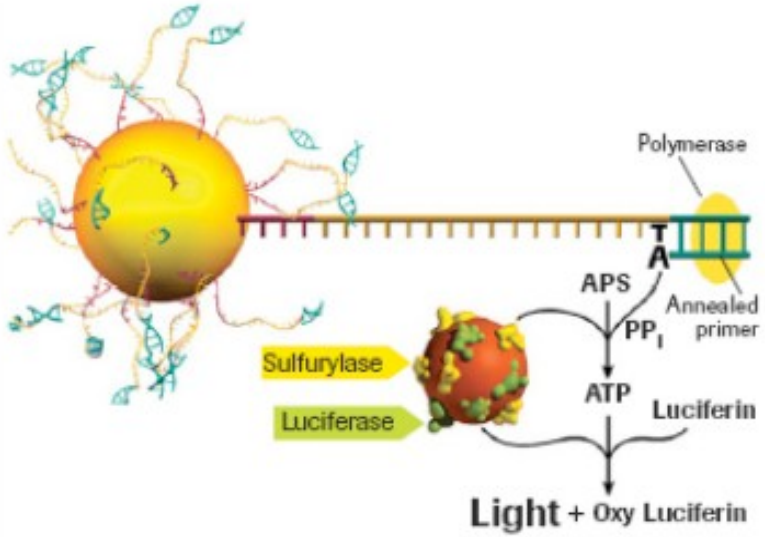


fragments are "amplified"



single beads in ~100.000 wells

DNA is read by detecting light emission associated to base incorporation



Genome Sequencing with pyrosequencing

- Fast and Cheap Sequencing: an important goal
- Pyrosequencing: attractive alternative to current sequencing techniques

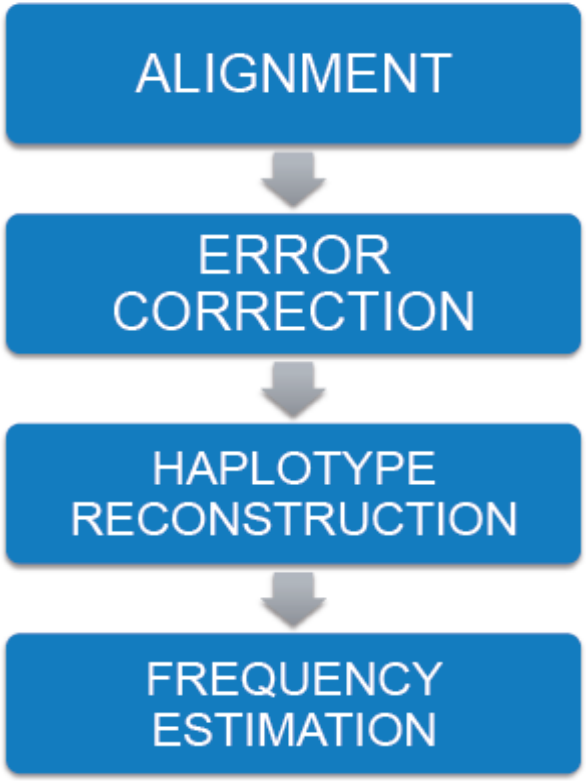
	Sanger	454/Roche
bps per run	~10^5	~10^8
read length	700-1000	~400
cost per run	~1000 $	~15000 $
cost per Mbp	10K $	100 $
accuracy	high	low (in-dels)

Key Issues with pyrosequencing

- Read Length: on average 250-400bp
- Orientation: Original and complement (Ignored for this work)
- Errors
  - Insertions: ~36%
  - Deletions: ~27%
  - Ambiguous Bases: ~21%
  - Substitutions: ~16%

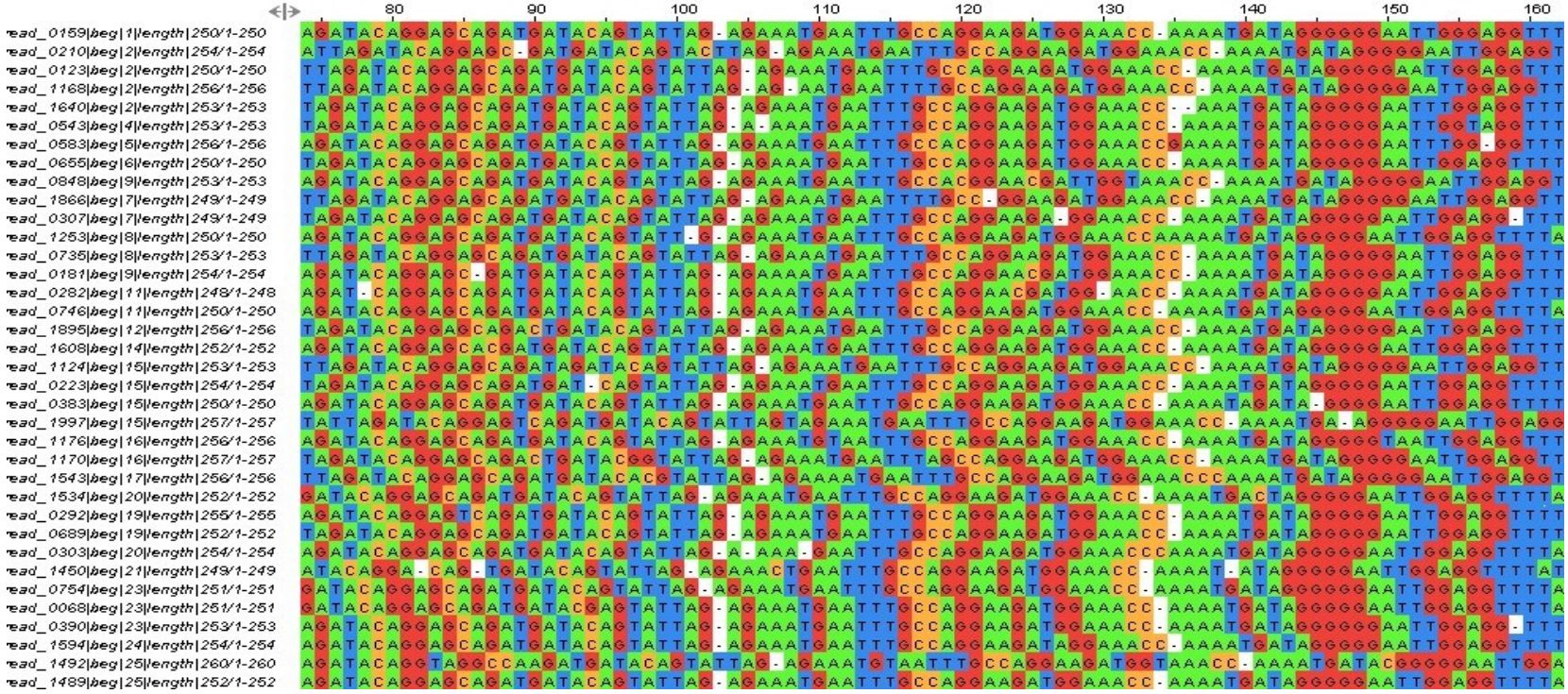
Need for Alignments

- Required as a preprocessing step for number of procedures.
- For example, from reads to haplotypes:



Pairwise alignment

- If reference genome is available, then pairwise alignments can be done
- But don't work :
  - Primarily because of insertions due to pyrosequencing



Issues with Multiple Alignments of short pyrosequencing reads

- Huge number of reads: ~100,000 reads in a single run
- Out of box alignment software are not feasible :
- Because of high complexity. Most are of the order of $O(N^4)$ e.g. Muscle, Clustalw
- Do not give accurate alignments for reads.
  - Primary reason: Do not take into account the position of the reads w.r.t. genome.

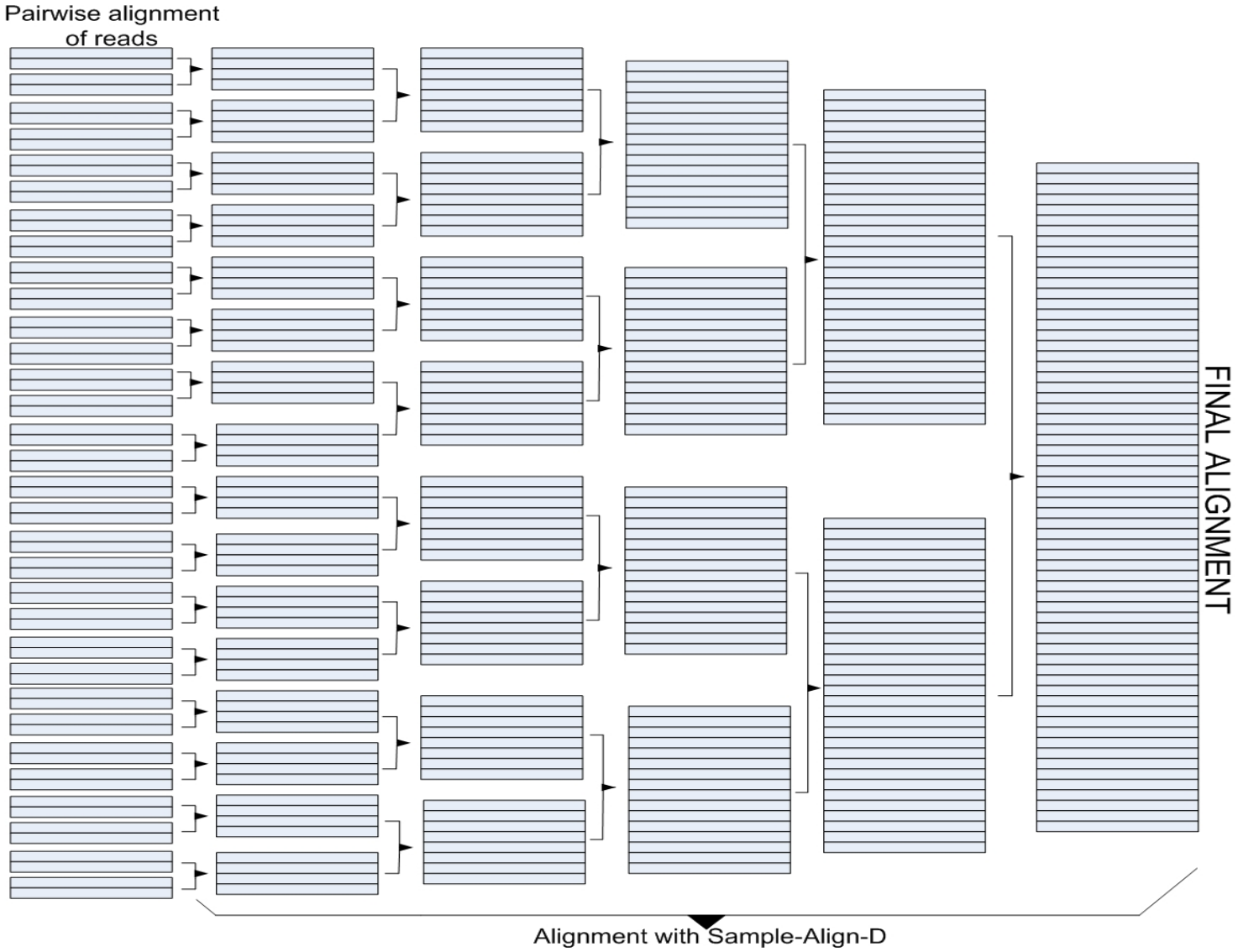
Pyro-Align Algorithm

- Based on the parallel framework of Sample-Align-D algorithm
  - Sample-Align-D gives super-linear speed-ups on multiprocessors; hence gives speed advantages when used on sequential machines
- Assumptions:
  - The original genome or the wildtype is available
  - The reads are in ‘forward’ orientation

Pyro-Align Algorithm sketch

- Align each read to the reference genome, using semi-global alignment.
  - This will place the reads in correct positions
- Do a Hierarchical Progressive alignment:
  - Reorder the reads using the starting positions of the reads to 'generate' a guidance tree.
  - Do pairwise alignments according to the tree.
  - Do profile-profile alignments in a hierarchical fashion

Hierarchical Progressive alignment



Pyro-Align Algorithm Complexity Analysis

- Semi-global alignment = $O(N L_R L_G)$
- Clustering & Reordering = $O(N+NL_G)$
- Pairwisealignments = $O(N L^2_R)$
- Profile-Profile alignments = $O(N \log N L^2_G)$
- Total Asymptotic Complexity= $O(N \log N L^2_G + N L^2_R)$
- Where:
- N= number of reads
- L_R= Average length of reads

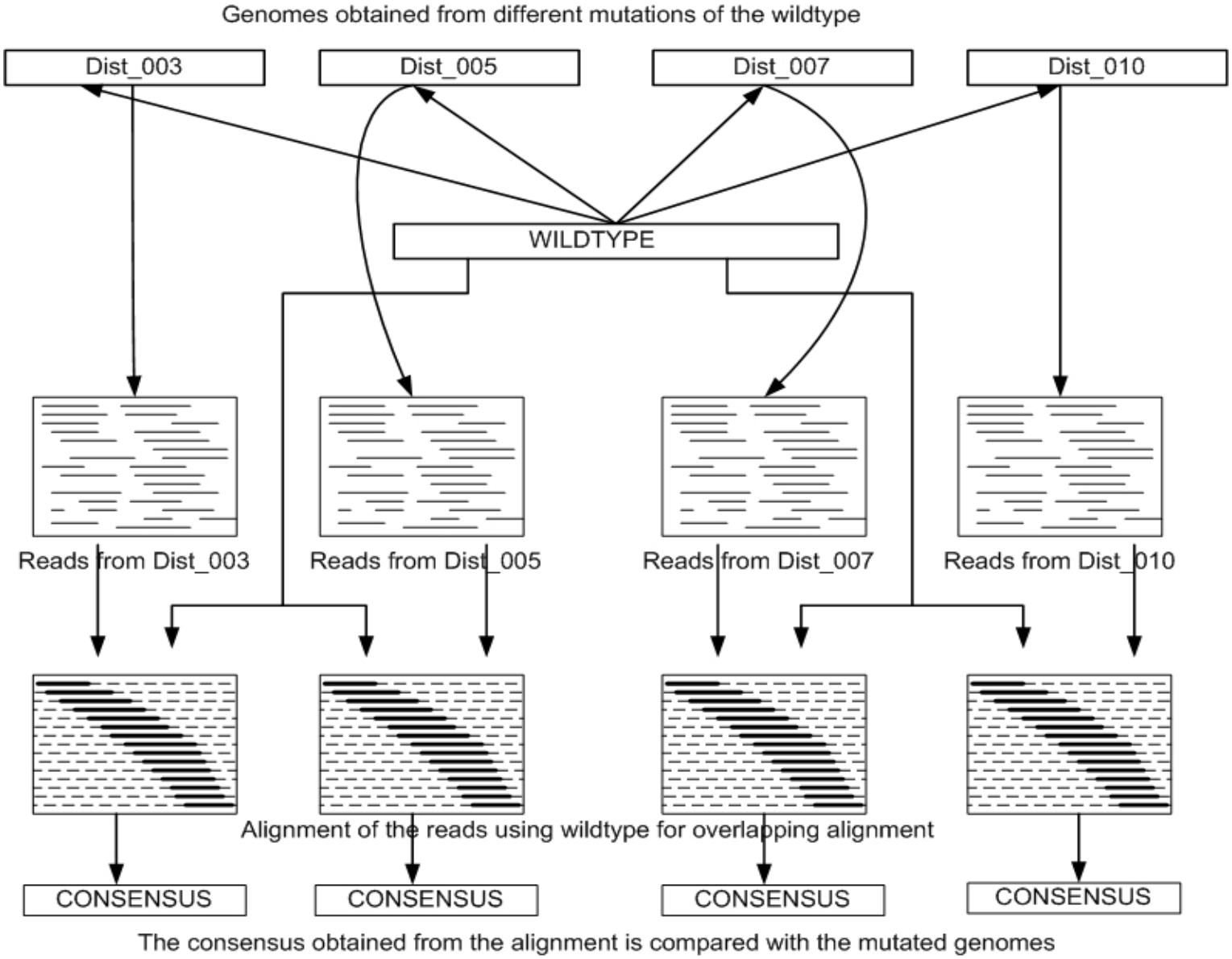
Quality Assessment

- Objective:
  - To access the quality of the alignments w.r.t the original genome
  - Ensure that the system is able to handle reads from multiple haplotypes
- Problems:
  - Ground truth for the alignments is not available(cannot be done with eyeballing due to large number of reads)
  - Standard benchmarks such as Balibase or Prefab cannot be used.

Quality Assessment

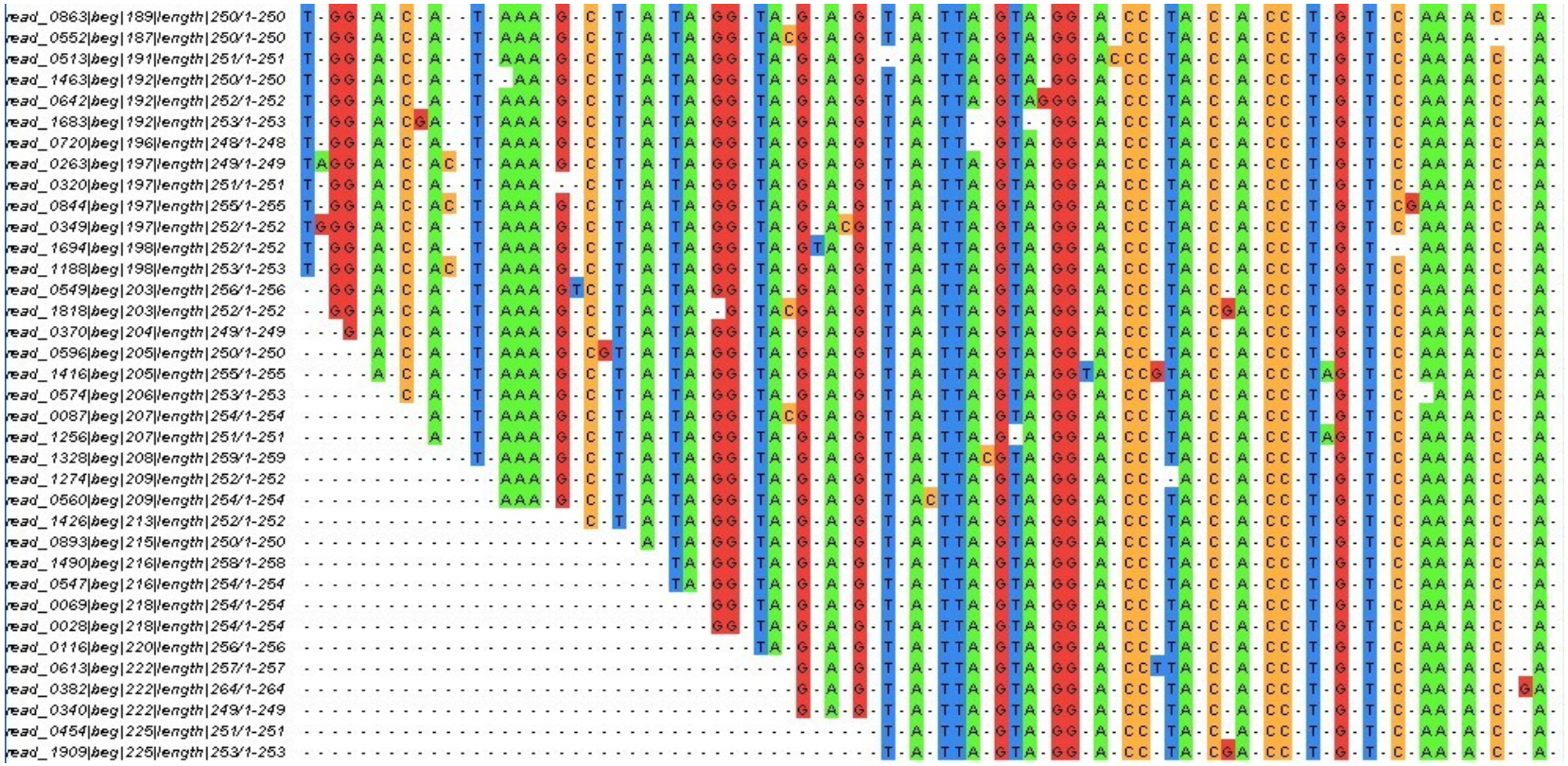
- We choose HIV pol gene with length of 1970bp as wildtype for the experiments
- Four sets of genomes from the wildtype are produced at 3%, 5%, 7% and 10% mutations.
- These mutated genomes are used to produce the reads.
- The reads are aligned and the consensus from the reads is compared with the reference genome.

Quality Assessment



Results

- Successful alignment of reads from mutated genome reads as well as the mixture.



Results

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Results

Ongoing Works—Sketch of parallelization of pyro-Align

- Proposed Approach:

➢ In parallel on multiple processors, align each read to the reference genome, using semi-global alignment.

➢ Do a Parallel Hierarchical Progressive alignment using sample-align-D

➢ We expect super-linear speedups for parallel pyro-align giving enormous advantage in terms of timing and memory

Conclusions and Future work

- A low complexity algorithm for aligning huge number of pyrosequencing reads is presented.
- Successfully aligned the reads from mutated and mixture of mutated genomes.
- Presented the quality assessment and compared with pairwise alignments
- We are working on parallelization of the algorithm

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Questions ??