Dottorato di Ricerca in Informatica - Ciclo XXV
Dipartimento di Informatica, Sistemistica e Comunicazione
Facoltà di Scienze Matematiche, Fisiche e Naturali
Università degli Studi di Milano - Bicocca

Algorithms for detecting variations from Next-Generation Sequencing Data

Presentazione Dottorato

30 Settembre 2010

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Tutor: Prof.ssa Lucia Pomello

Outline

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Motivations

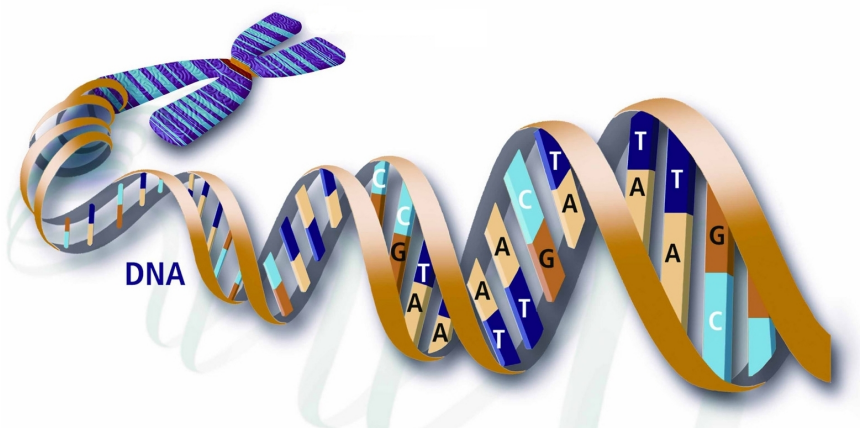
- Revolution in genome sequencing and analysis: from traditional methods to NGS (Next-Generation Sequencing)[1]
- Need to develop novel computational frameworks to analyze NGS data
- Goal: design algorithms to analyze NGS data for detecting sequence variations

[1] Venter J.Craig, Nature (2010), *Multiple personal genomes await*

Genome Sequencing

Determination of the primary structure of a molecule
DNA/RNA $\rightarrow$ sequence of nucleotides

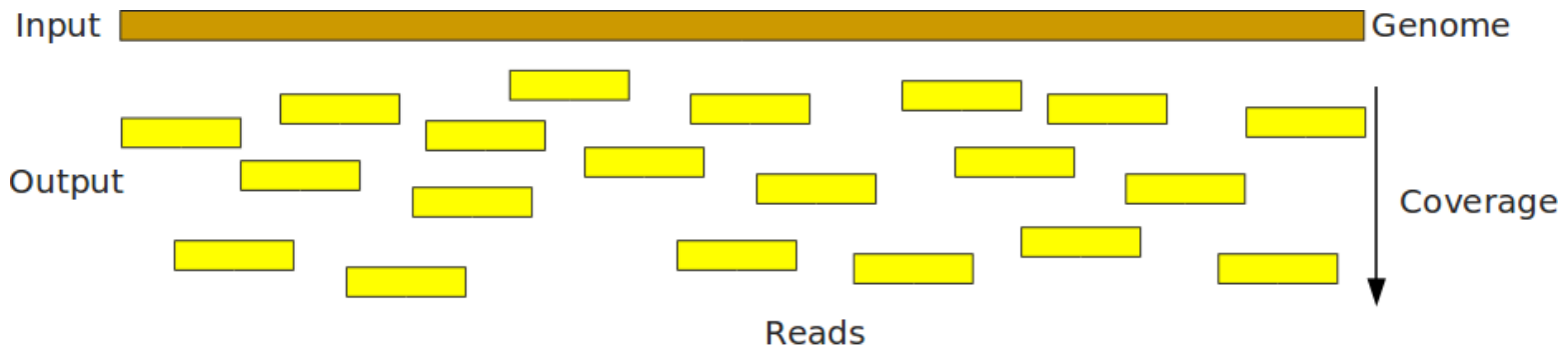
- Traditional Methods (Sanger, 1977)
- Next-Generation Sequencing Methods (2005)



Sanger Vs. Next-Generation Sequencing

- **Sanger (1977)**

- **NGS (2005)**



Sanger Vs. Next-Generation Sequencing

Sanger (1977)

1. Long Reads ($\sim$1000 bp)
2. Low Throughput ($\sim 10^6$ bp/day)
3. Low Coverage ($\sim$ 1x)
4. Expensive (10^3 bp/\$)

NGS (2005)

1. Short Reads (25-300 bp)
2. High Throughput ($\sim 10^9$ bp/day)
3. High Coverage ($>$10x)
4. Low Costs ($> 10^5$ bp/\$)

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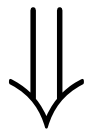
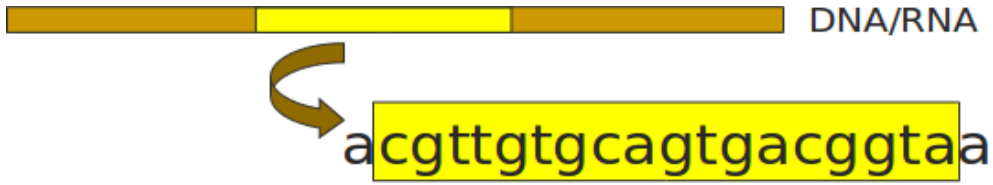


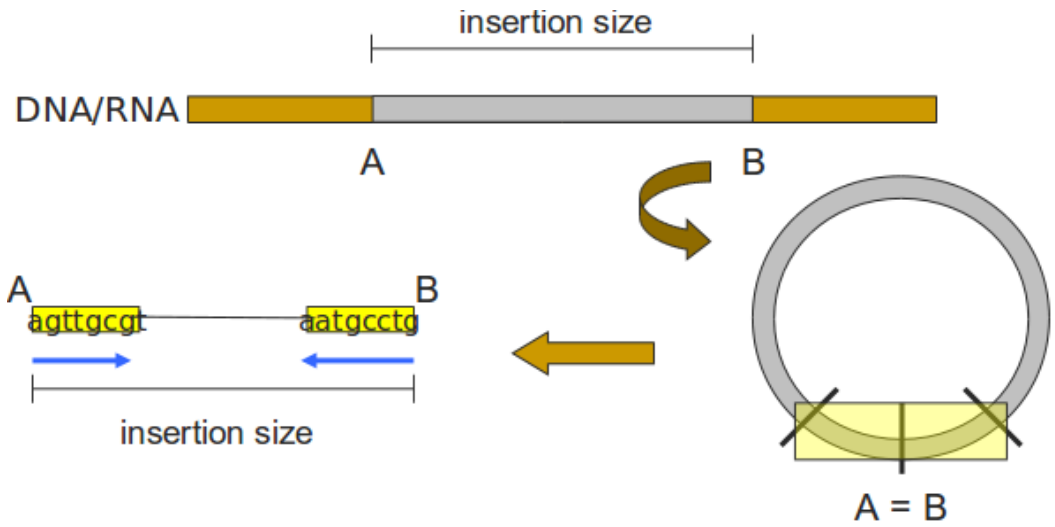
~~Traditional Algorithms, Models and Tools~~

NGS Data

- Short Read

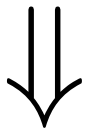


- Paired-Ends



Biological Problems

1. Compare a known genome reference with an unknown genome (but sequenced by NGS)
2. Infer transcripts data using short reads sampled by NGS



Detect Variations

Algorithmic Challenges

- More than 10^9 short sequences $\Rightarrow$ Linear time algorithms
- Need for data compression / succint data structures
- New computational model and data structure for pattern matching
  (es. hashing, Burrows-Wheeler transf., suffix array)[2] [3] [4]

[2] Langmead B, et al., Genome Biology (2009), *Ultrafast and memory-efficient alignment of short DNA sequences to the human genome*

[3] Dalca V.A. and Brudno M., Briefings in Bioinformatics (2010), *Genome variation discovery with high-throughput sequencing data*

[4] Li H. and Homer N., Briefings in Bioinformatics (2010), *A survey of sequence alignment algorithms for next-generation sequencing*

Outline

Computational Problems

1. Identification of differences (Structural Variations) between a known genome (*reference*) and an unknown genome sequenced by NGS (*donor*).

- Biological Motivations:
  - SV are common in human individuals and are related to diversity and disease susceptibility [5] [6]
  - Detecting SV is crucial in medical and biological studies of several diseases

[5] Korbel, et al., Science (2007), *Paired-End Mapping Reveals Extensive Structural Variation in the Human Genome*

[6] Tuzun, et al., Nature Genetics (2005), *Fine-scale structural variation of the human genome*

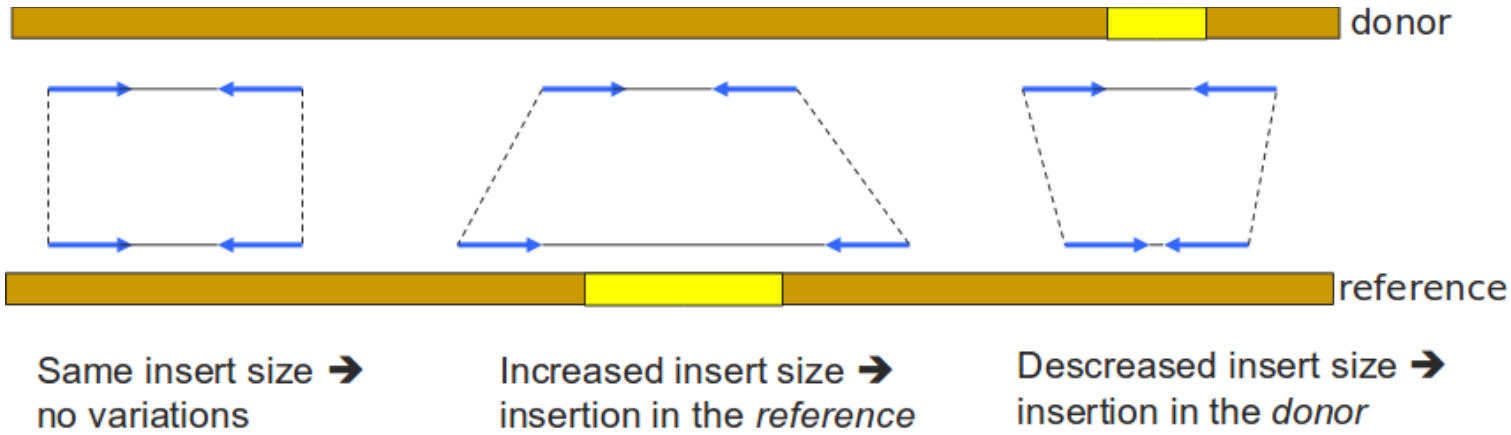
Computational Problems

2. Characterization of variations (i.e alternative splicing events) among different transcripts sequences (sequenced by NGS) of the same gene.

- Biological Motivations:
  - Human genes undergo AS (alternative splicing)
  - AS is the key process in determining transcriptomes diversity

Identification of Structural Variations (SVs)

- Structural Variations (SVs)
  - Insertions
  - Deletions
  - Inversions (>5 Kb)



Identification of Structural Variations (SVs)

- Structural variation discovery using maximum parsimony is NP-hard [7]
- Actual tools consider only one alignment (for each short read) and discard all the others [8]
- Probabilistic frameworks have been designed for the identification of specific SVs

[7] Hormozdiari F., Alkan C., Eichler E., Sahinalp C., Genome research (2009), *Combinatorial algorithms for structural variation detection in high-throughput sequenced genomes*

[8] Medvedev P., Stanciu M., Brudno M., Nature methods (2009) *Computational methods for discovering structural variation with next-generation sequencing*

Identification of Structural Variations (SVs)

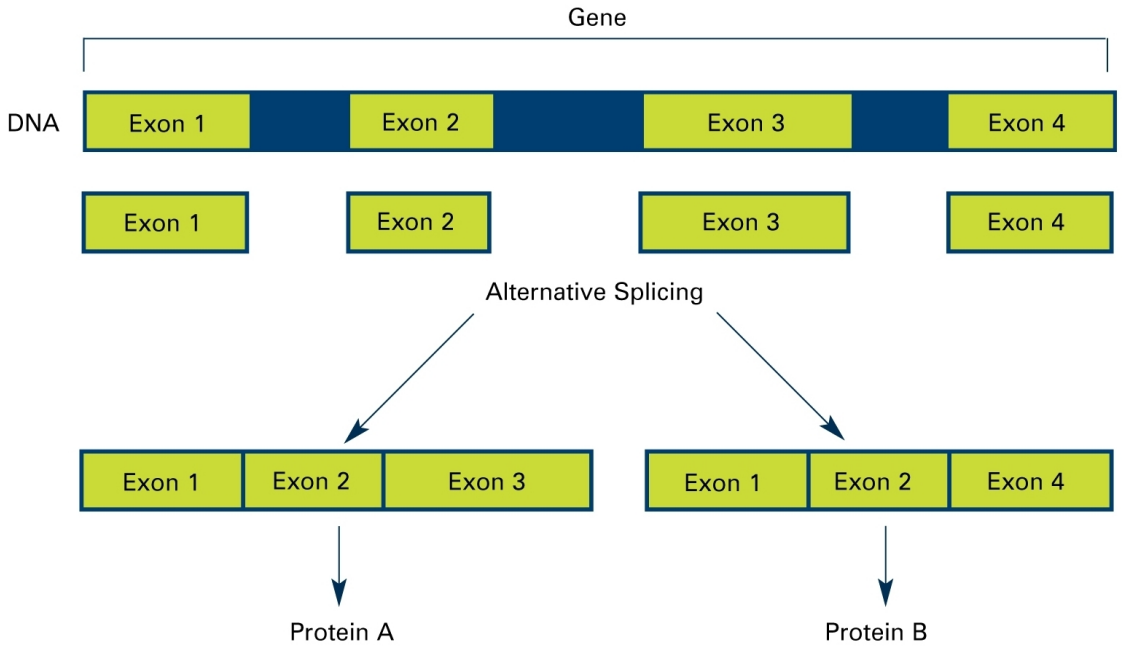
- Problem: predicting structural variations
  - **Input**: a set S of paired-ends (PEs) from a donor genome D and a reference genome R.
  - **Goal**: compute the set of structural variations that explains how D differs from R
- Previous approaches:
  - consider each SV separately
  - adopt probability based formulation
- Our Approach:
  - design a specific tool for PEs
  - develop an integrated approach for all SVs

Identification of Structural Variations (SVs)

- Algorithmic Solution
  - PEs are aligned to the reference genome R (> 1 locations and > 1 orientations) and clustered into
    - Concordant mapped PEs $\Rightarrow$ Donor $=$ Reference
    - Discordant mapped PEs $\Rightarrow$ Structural Variations
  - Discordant PEs are analyzed to detect
    - Insertion / Deletion, Inversion, Other complex cases
- Issues
  - Large SVs are hard to detect
  - Detecting combinations of different SVs

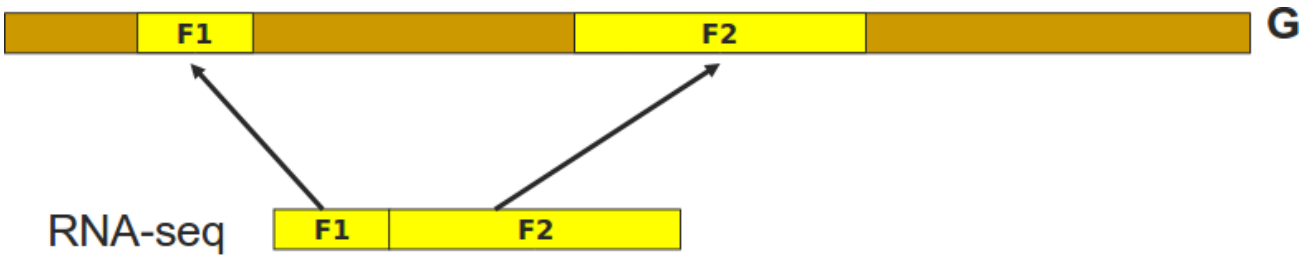
Characterization of Alternative Splicing Events

- Alternative Splicing



Characterization of Alternative Splicing Events

- No techniques based on short reads comparison
- No characterization of differences of transcripts
- Developed algorithms map the NGS data into the given reference genome to infer splice junctions[9] [10]



[9] Bryant, et. al., Bioinformatics (2010) *Supersplatspliced RNA-seq alignment*

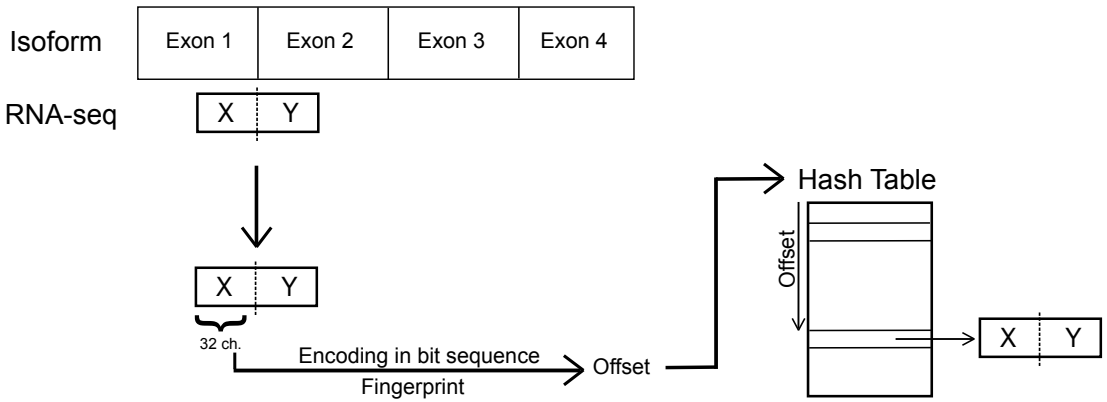
[10] Trapnell, et. al., Bioinformatics (2009) *TopHat: discovering splice junctions with RNA-Seq*

Characterization of Alternative Splicing Events

- Problem: inference of alternative splicing (AS) events
  - **Input**: a set of short reads from transcripts of a gene
  - **Goal**: graph representation of AS events (genome scale)
- Previous approaches:
  - detect splice junctions
  - validate transcripts
- Our Approach:
  - detect differences (which are few) and discard similarities (too many)
  - no alignment to the reference genome

Characterization of Alternative Splicing Events

- Algorithmic Solution
  - We index short reads with a hash table in order to:
    1. assembly chains of short reads to compose Exons
    2. identify junction points of Exons



- Issues
  - Not unique identification of splicing junction
  - Some AS events are hard to characterize
  - Needs for a topological sort of Exons

Outline

Conclusions