MAYO CLINIC

Fusion transcript detection in rare genetic disease

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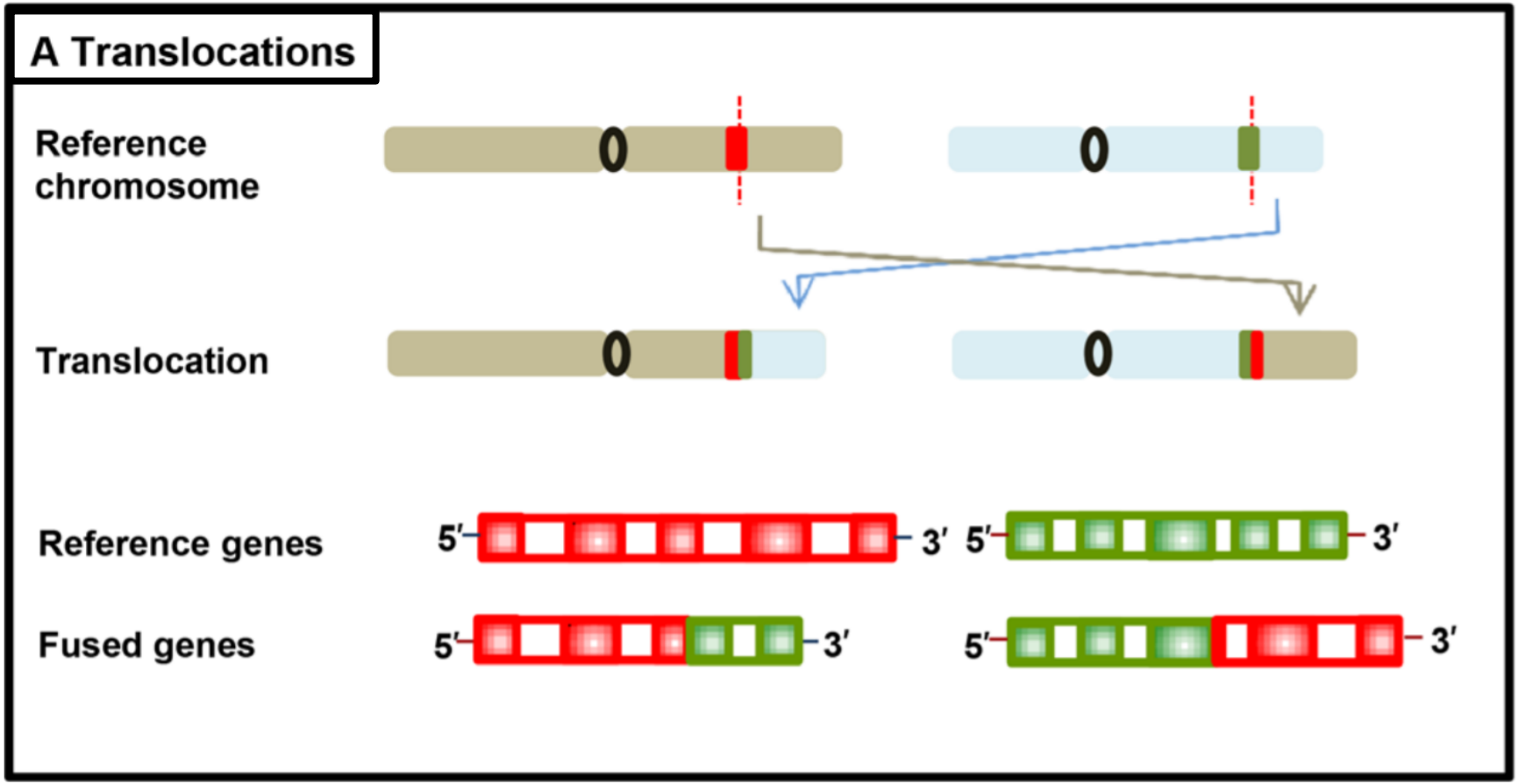
A working definition

- Fusion transcription involves the aberrant conjoining and expression of normally discrete genic material
- Therefore a fusion can be considered *"Aberrantly conjoined and expressed genic material that exists separately under normal conditions"*
- More simply: *pieces of multiple genes are expressed as one*
- Caused by a variety of abnormalities at the DNA level as well as (debatably) at the RNA level

Mechanisms of formation



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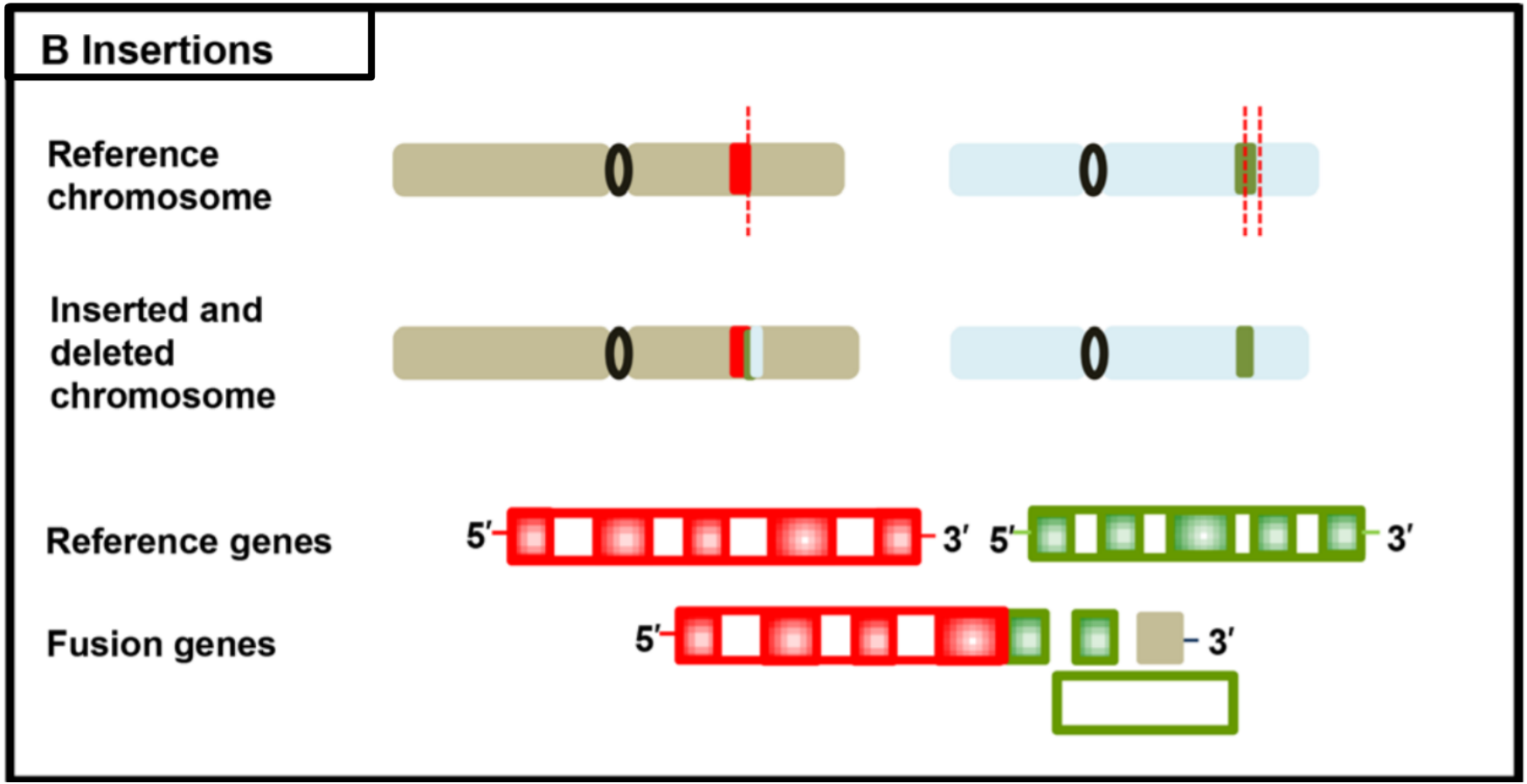

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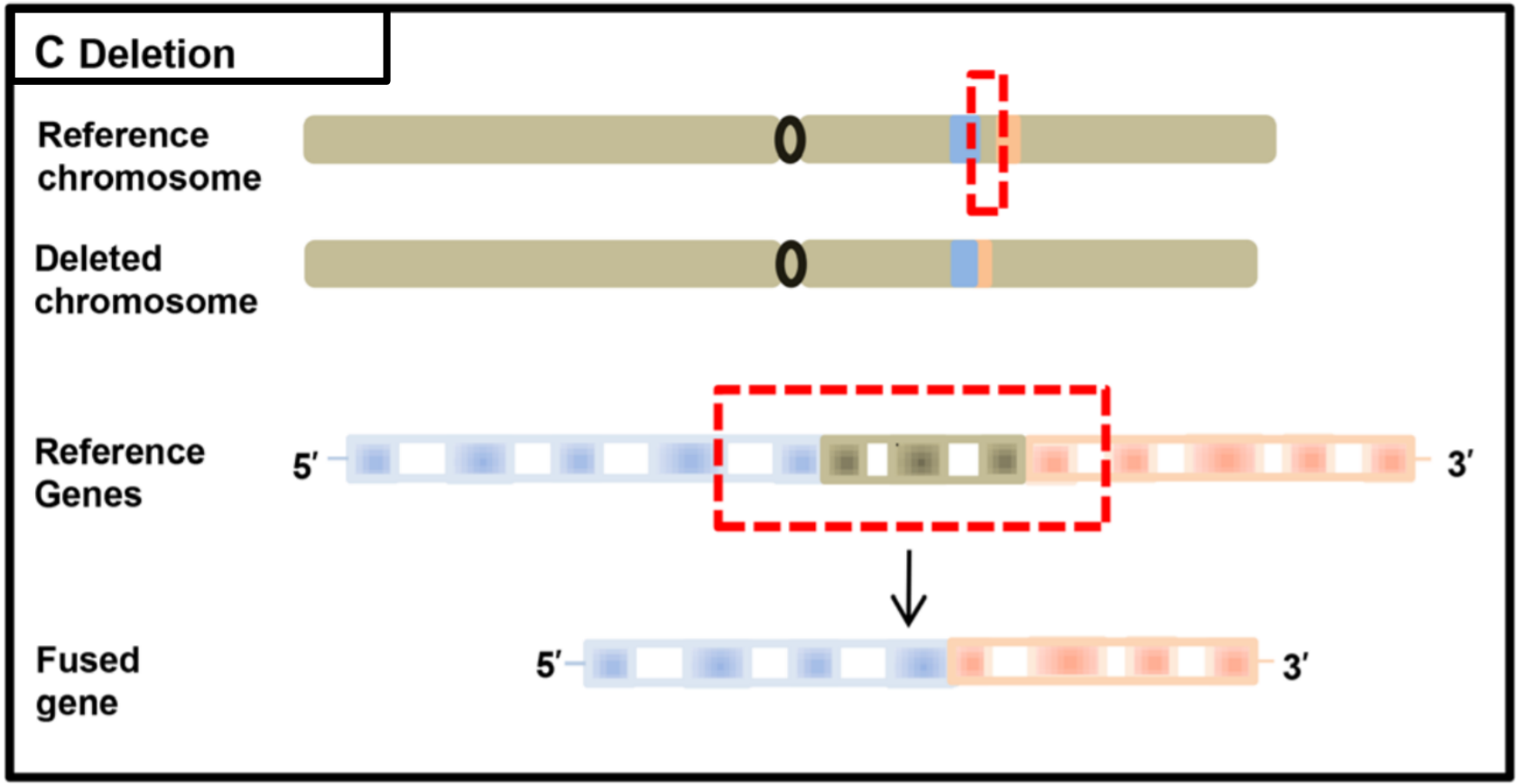

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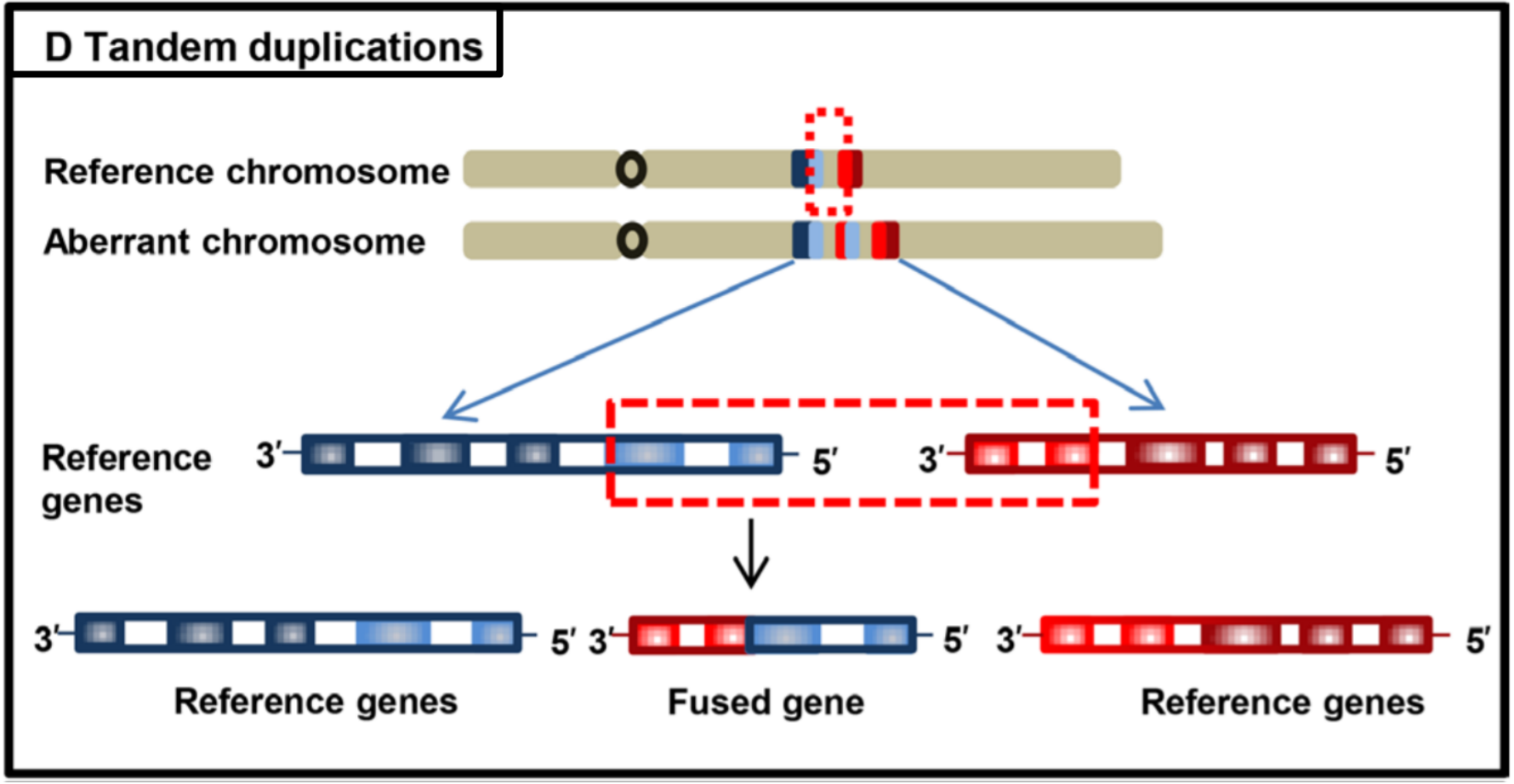

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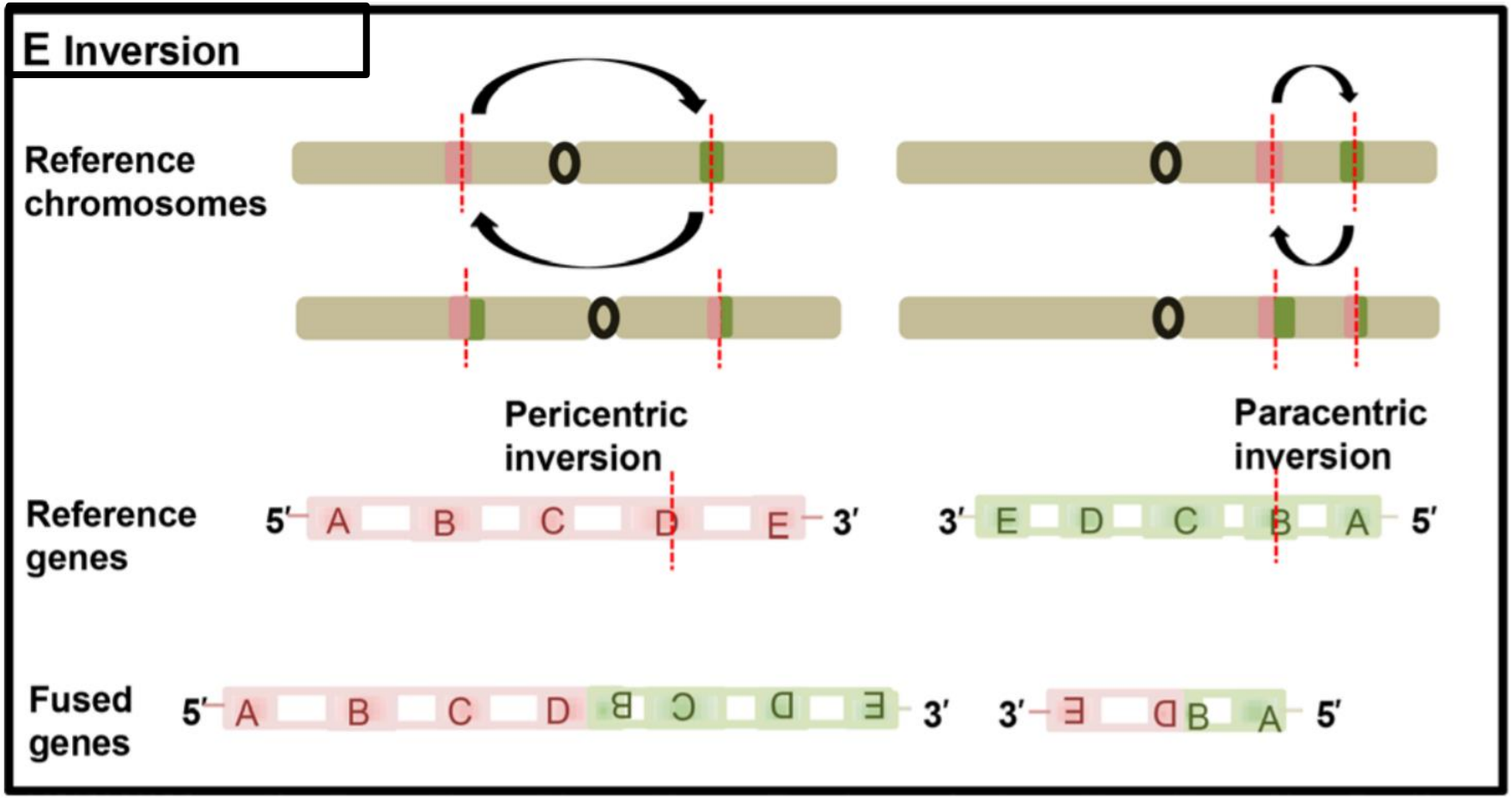

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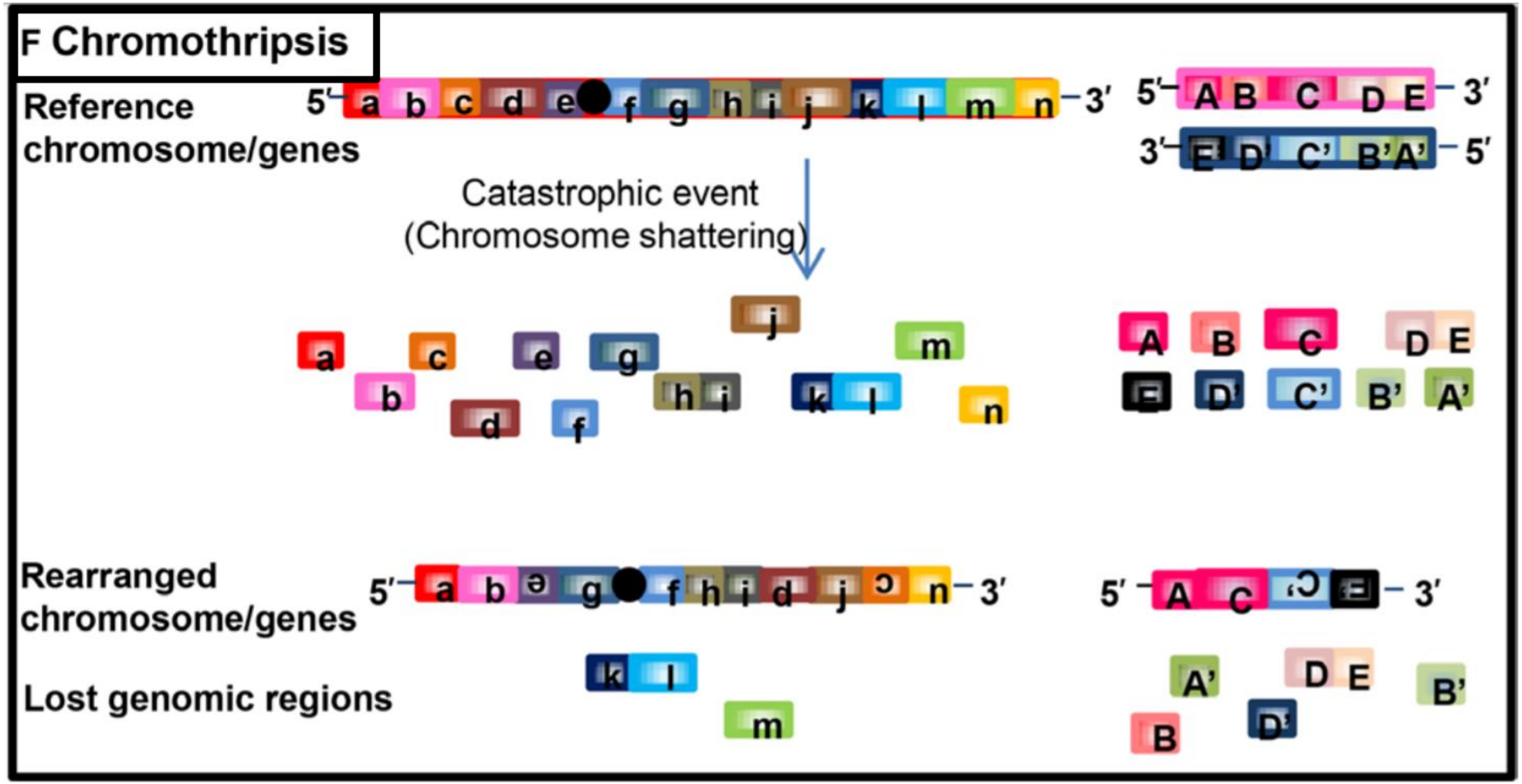
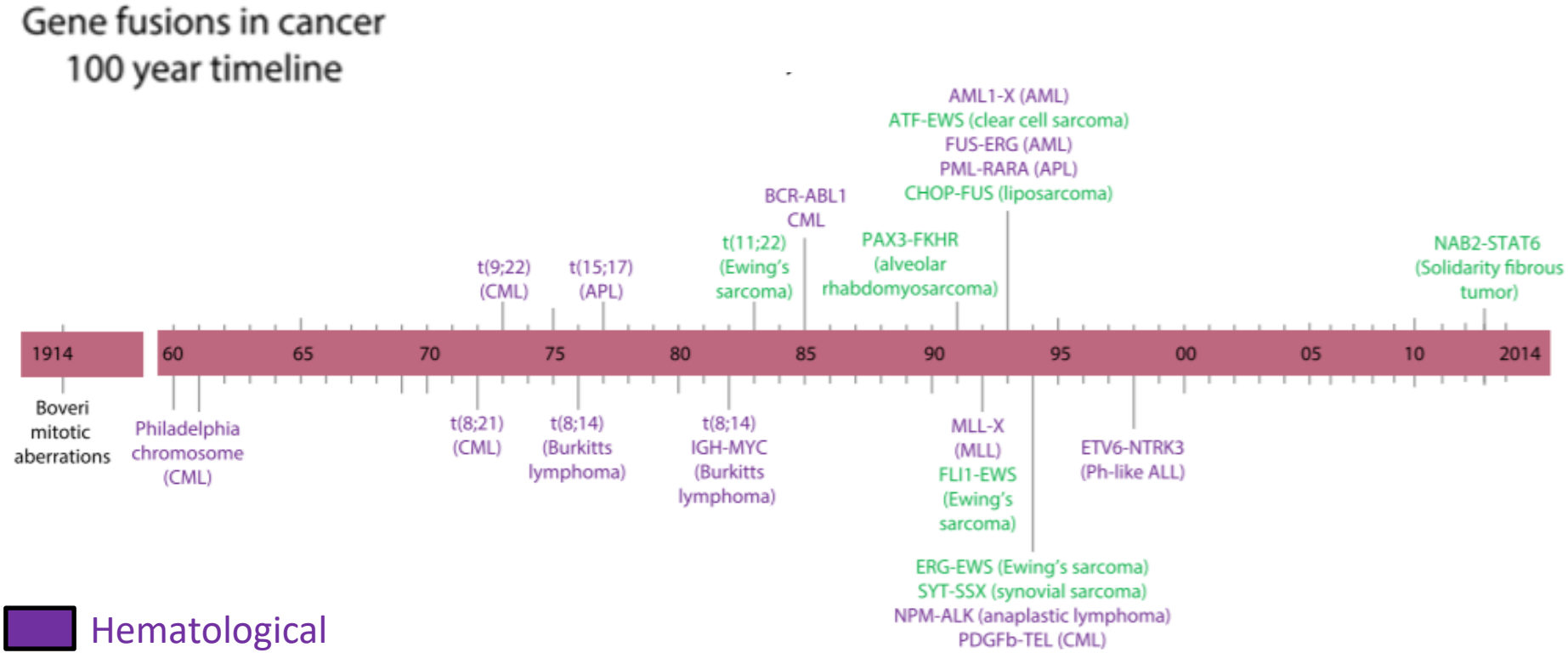


An oncogenic phenomenon?



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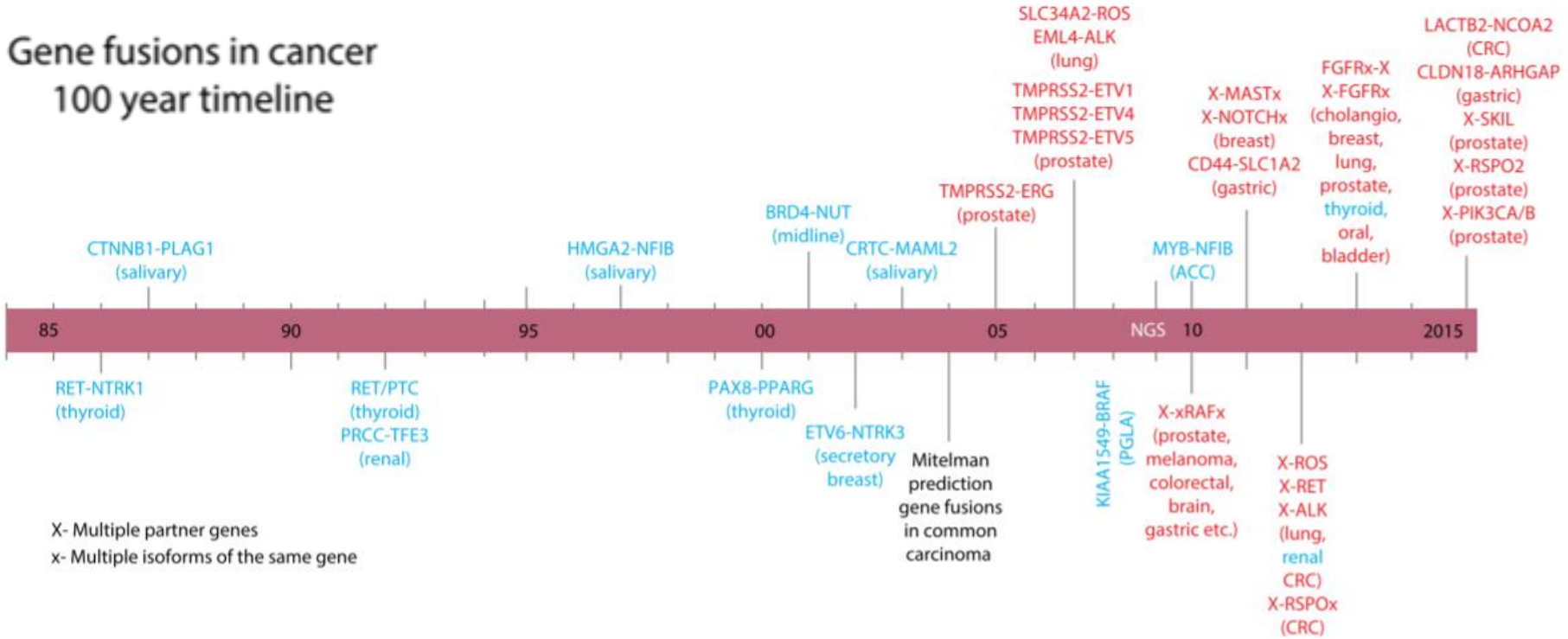


Rare epithelial

Common epithelial

Mechanism of action

- Commonly
  - involve fusion of a downstream kinase
  - or transcription factor
    - with a more highly expressed upstream gene
      - leading to increased expression of the downstream gene or a functional component of it
- Protein formation dependent on in-frame translation

Mechanism of action

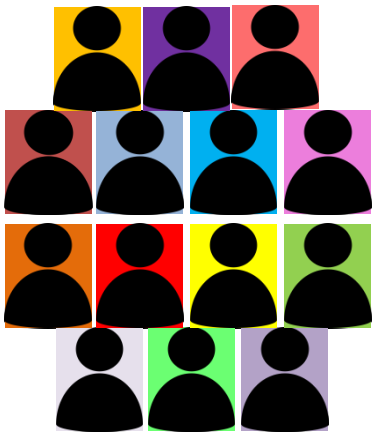
- Most reported gene fusions pertain to gain-of function aberrations imparting neoplastic phenotypes
- Loss of function of tumor suppressors such as TP53 and PTEN have also been identified
- Fusion transcripts are recognized as having diagnostic, prognostic and therapeutic (druggable) relevance in oncology
- Detection of gene fusions is increasingly incorporated into the standard workflow for genomic characterization of tumors in both research and clinical settings

Fusions in inherited disease

- 18-40% unsolved cases are solved by exome sequencing
- RNA-Seq has recently been proposed as a supplementary diagnostic tool
- Cummings *et al.* achieved a 35% diagnostic increase by profiling aberrant splicing and allele specific expression
- Kremer *et al.* added gene expression quantification to the testing repertoire and demonstrated a 10% increase
- Isolated reports exist in the literature of fusion transcripts being detected in cases of brain malformation, intellectual disability, schizophrenia, ASD and more
- Fusion transcription had not been systematically profiled in inherited disease

RNA-Seq in inherited disease

Patient Cohort

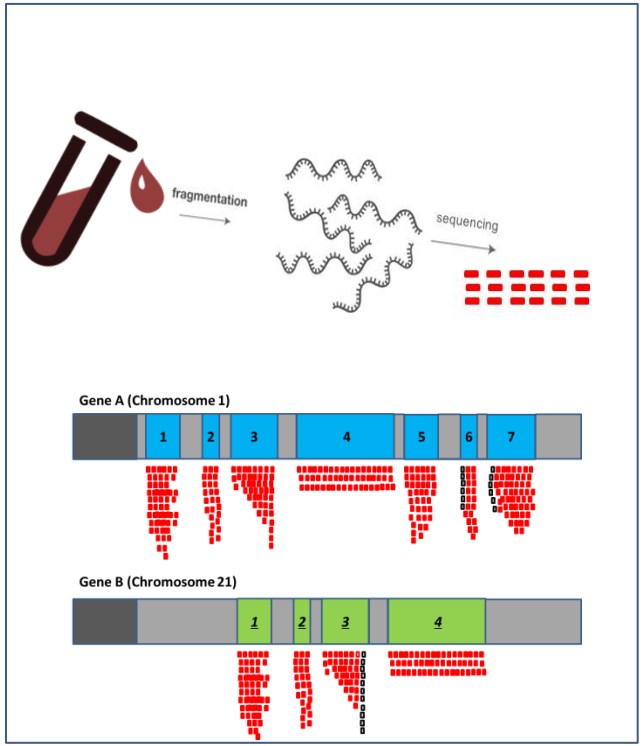


- 47 patients
- Prior exome-sequencing
- 23 M, 24F
- Ages 9 months – 68 years (median 11)
- Diverse phenotypes
  - Neurological
  - Muscular
  - Gastrointestinal
  - Skeletal
  - Connective tissue disorders

Oliver *et al.* (2019) https://doi.org/10.1371/journal.pone.0223337

RNA-Seq in inherited disease

RNA-Sequencing

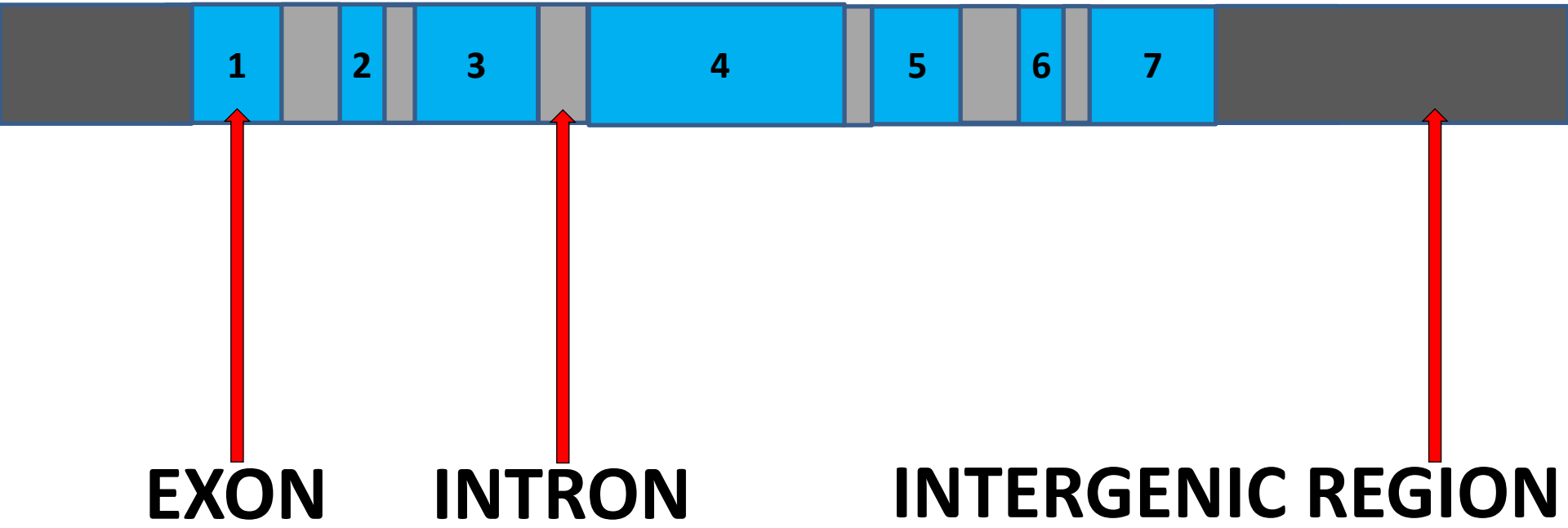


- Patient whole blood
- Illumina HiSeq 2500
- 200 million 100bp PE reads per sample

Fusions in inherited disease

- Fusion detection increased diagnosis of rare disease
  - Two cases confirmed solved
    - SCID
    - Multiple exostoses
  - 4.3% increase in diagnostic yield
  - Experimentally validated existence of fusion events in disease-relevant genes with potential phenotypic relevance in five additional cases

Software solution overview



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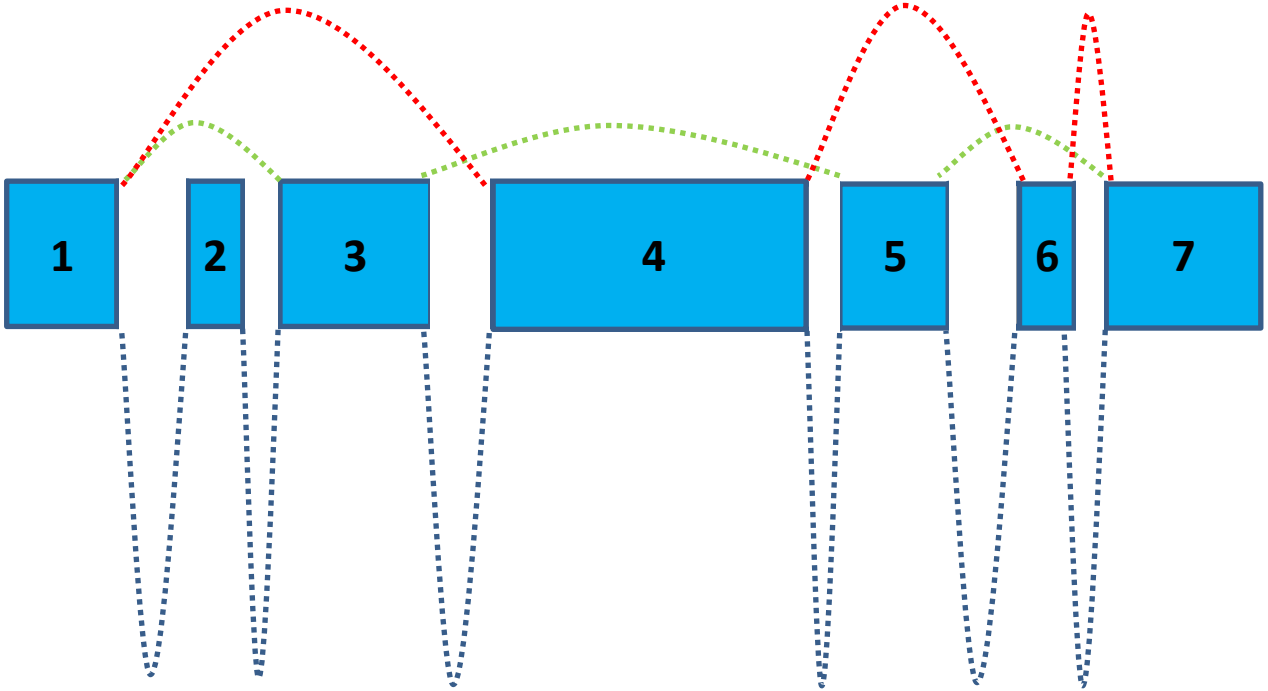

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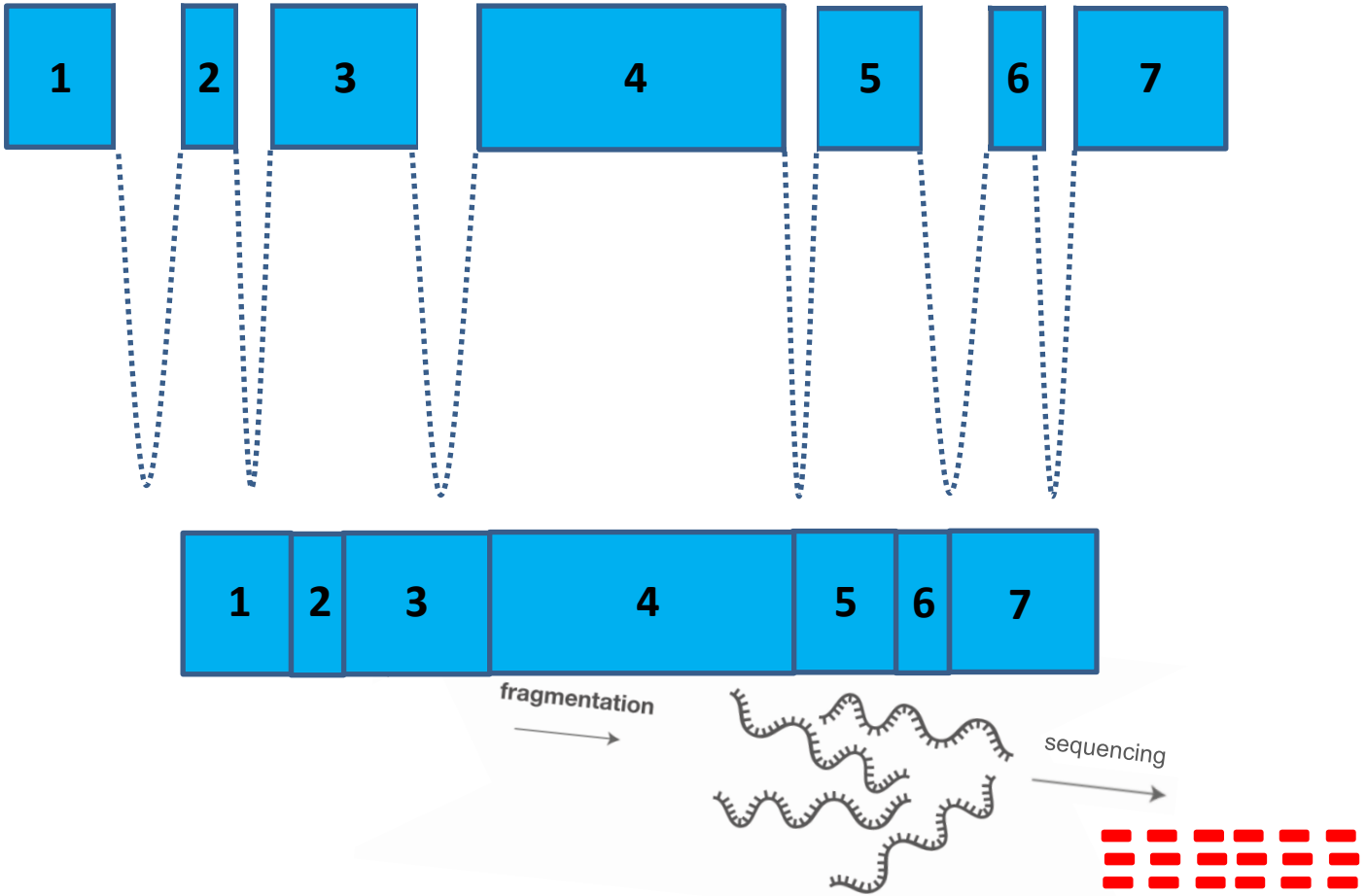

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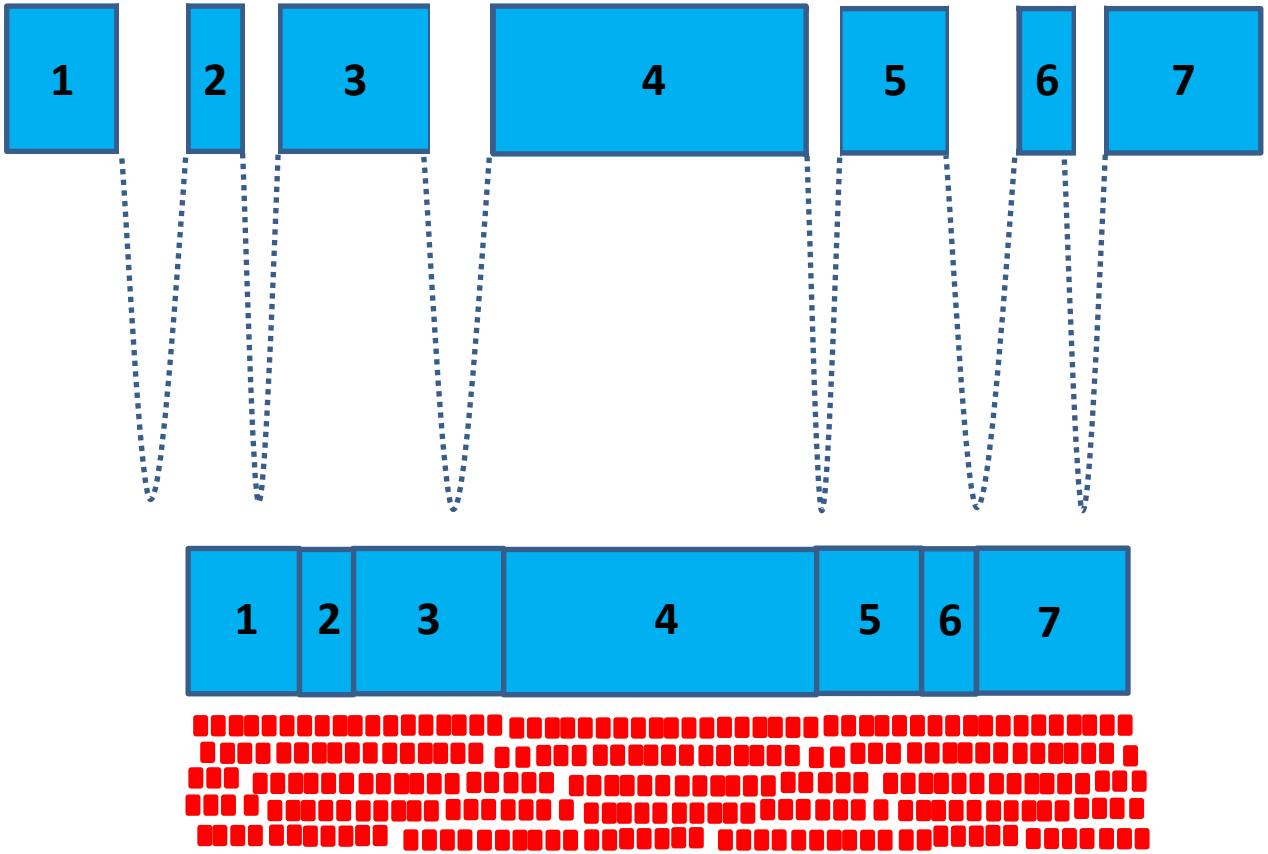

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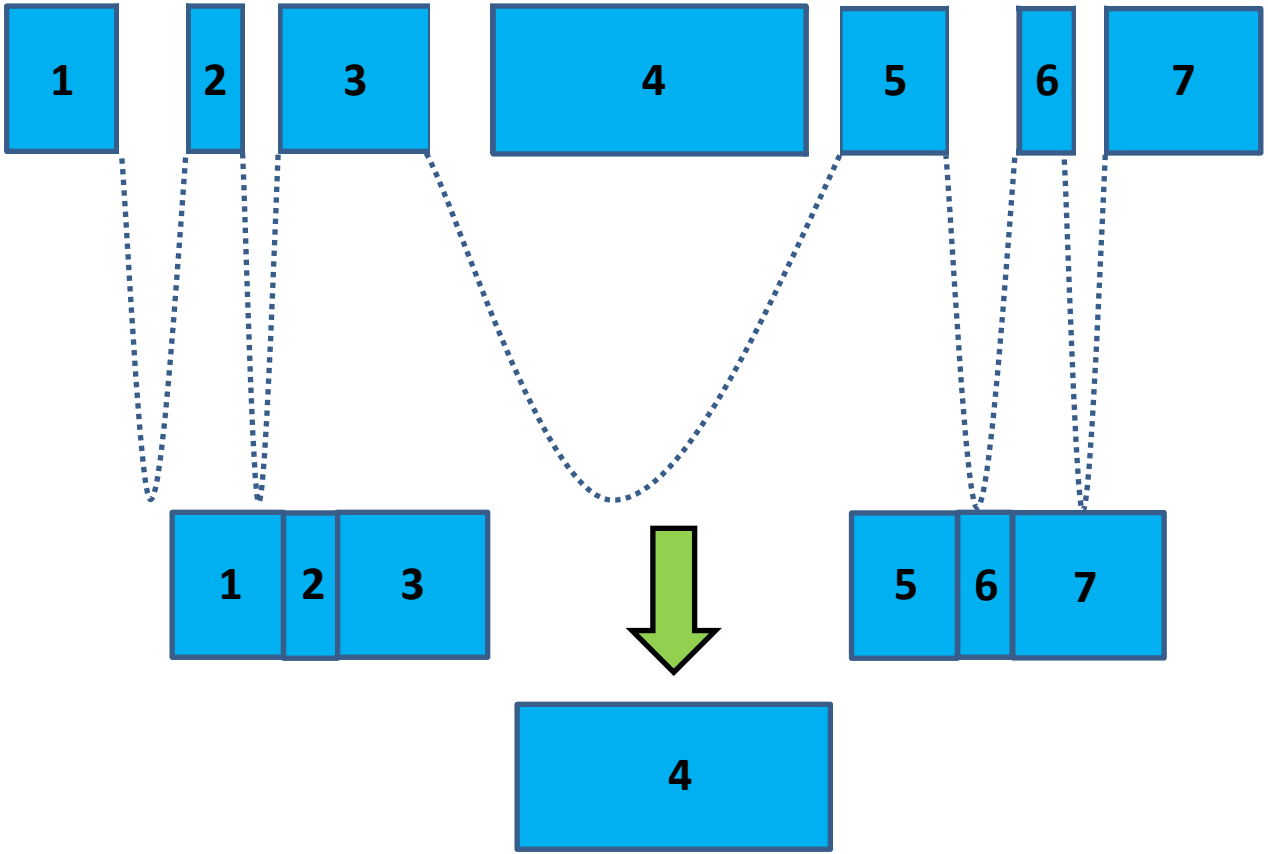

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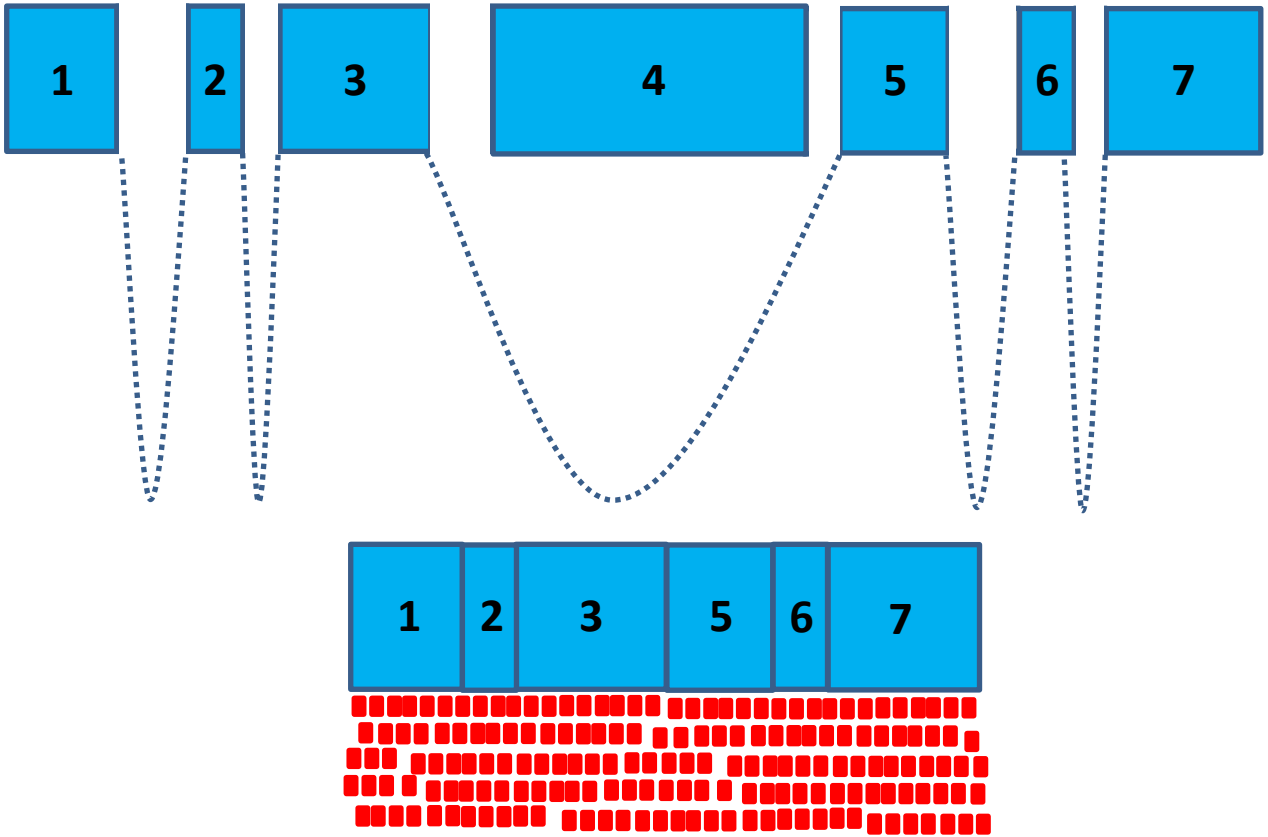

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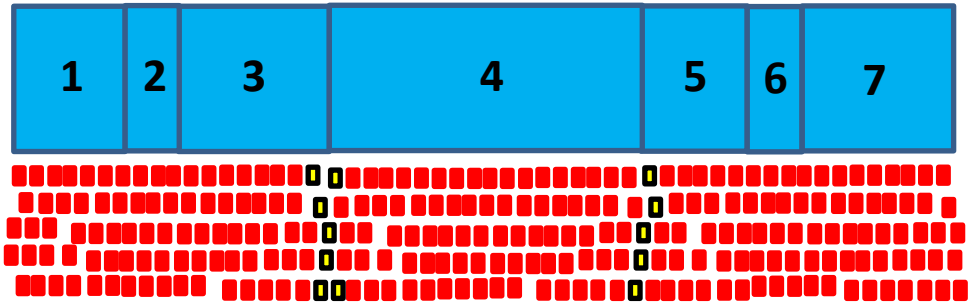

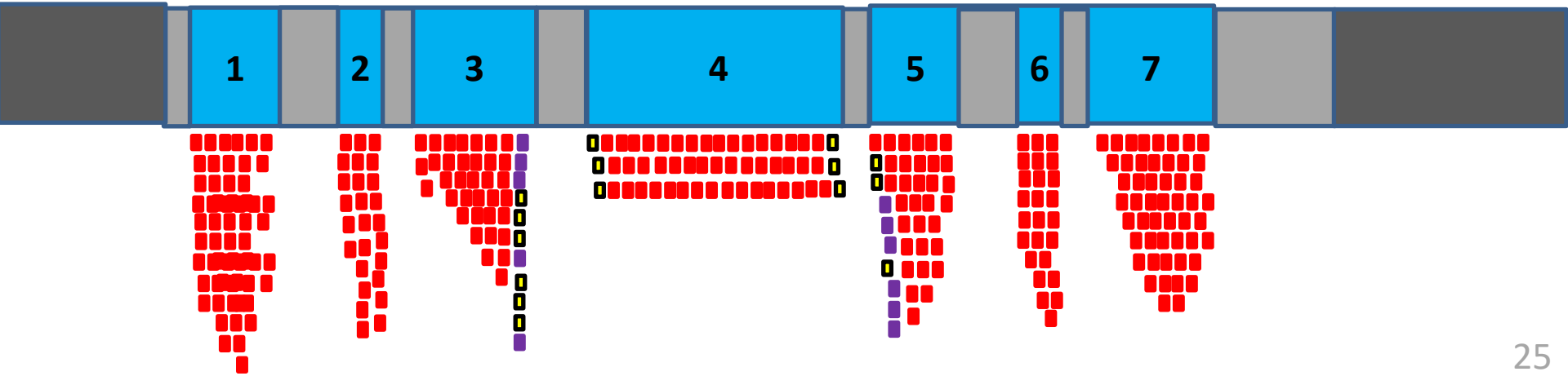

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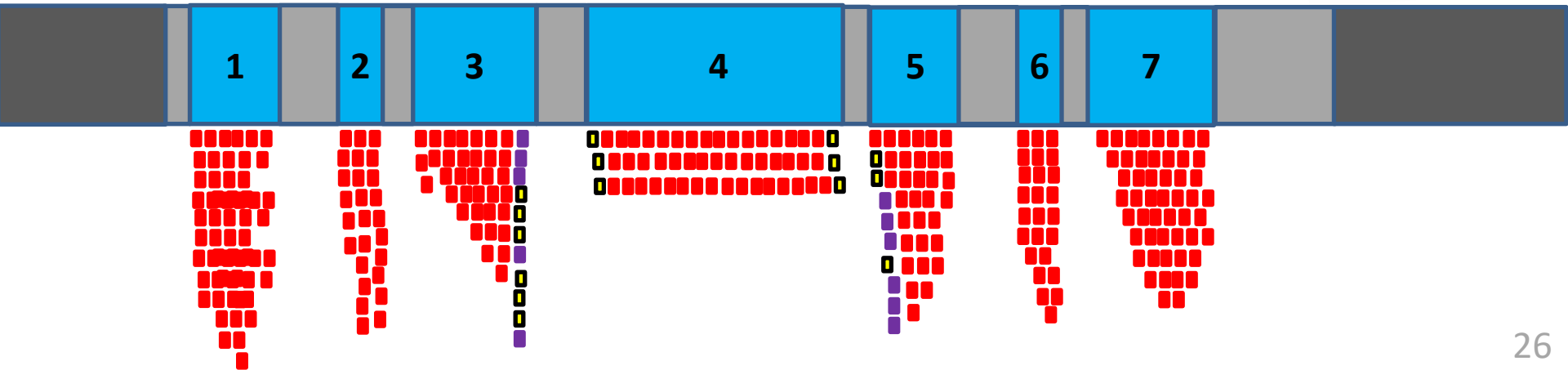

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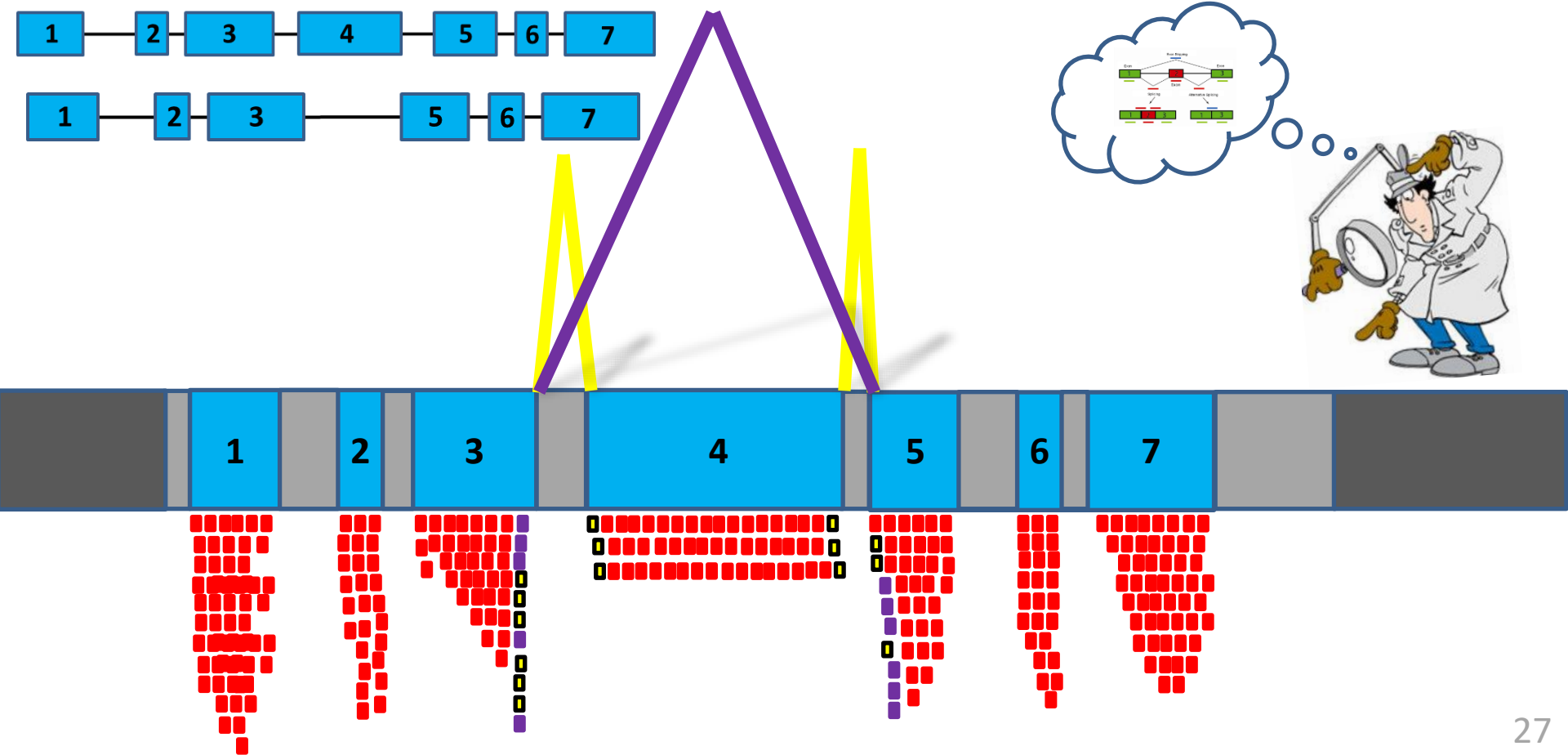
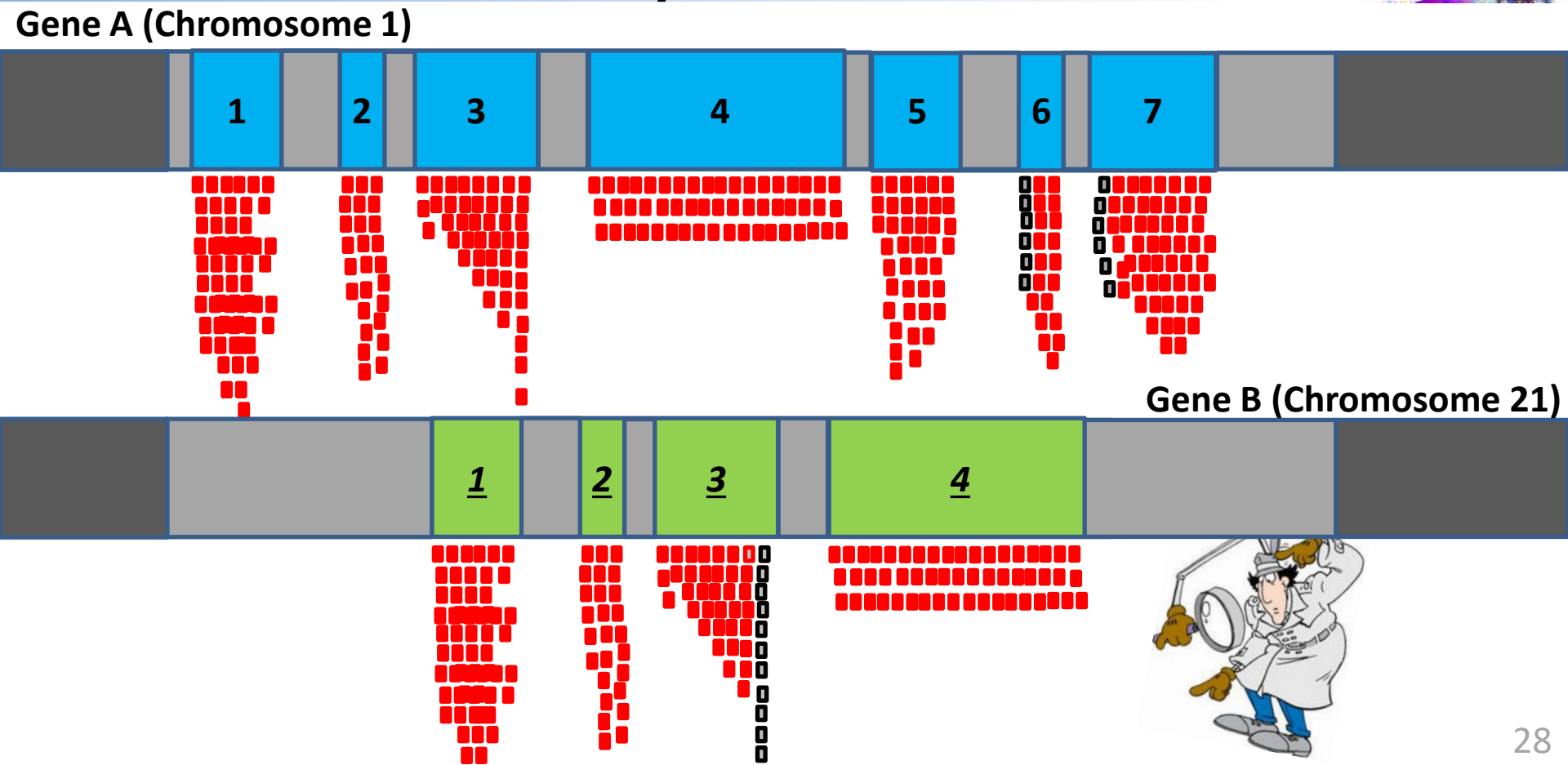


Fusion transcripts



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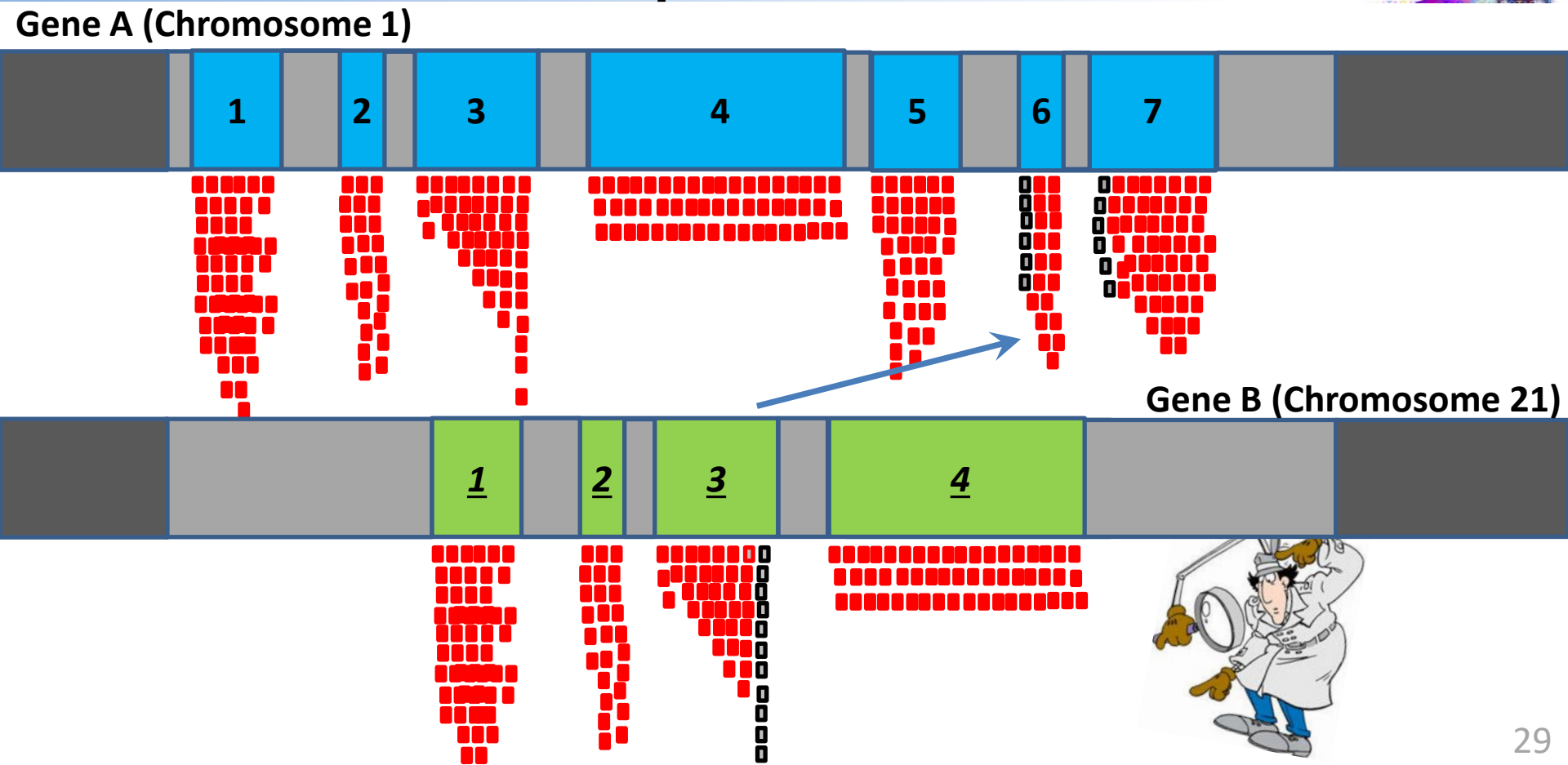

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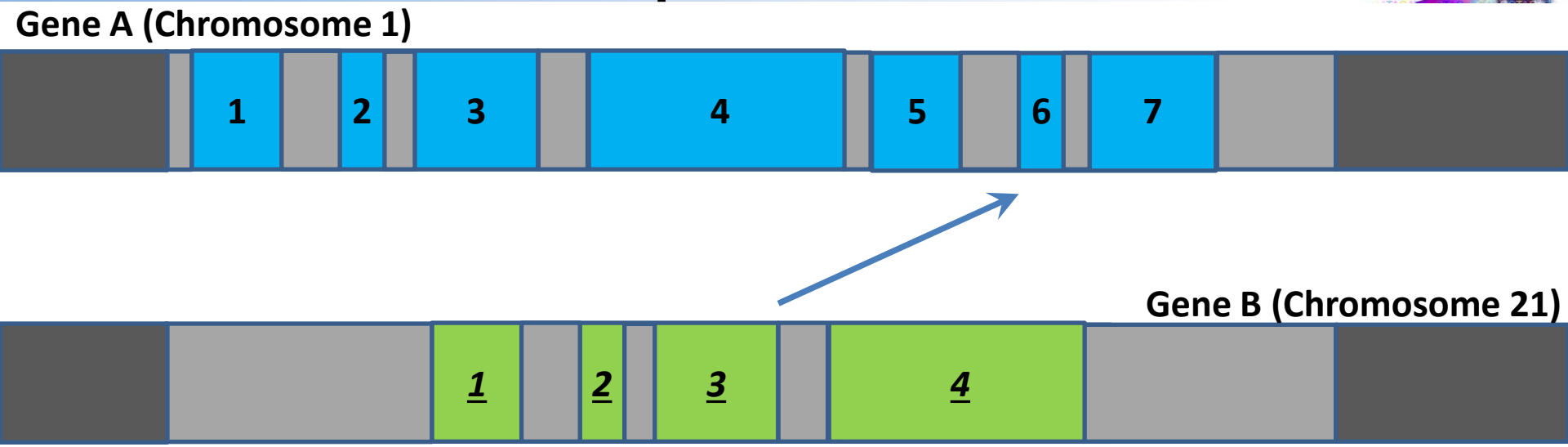

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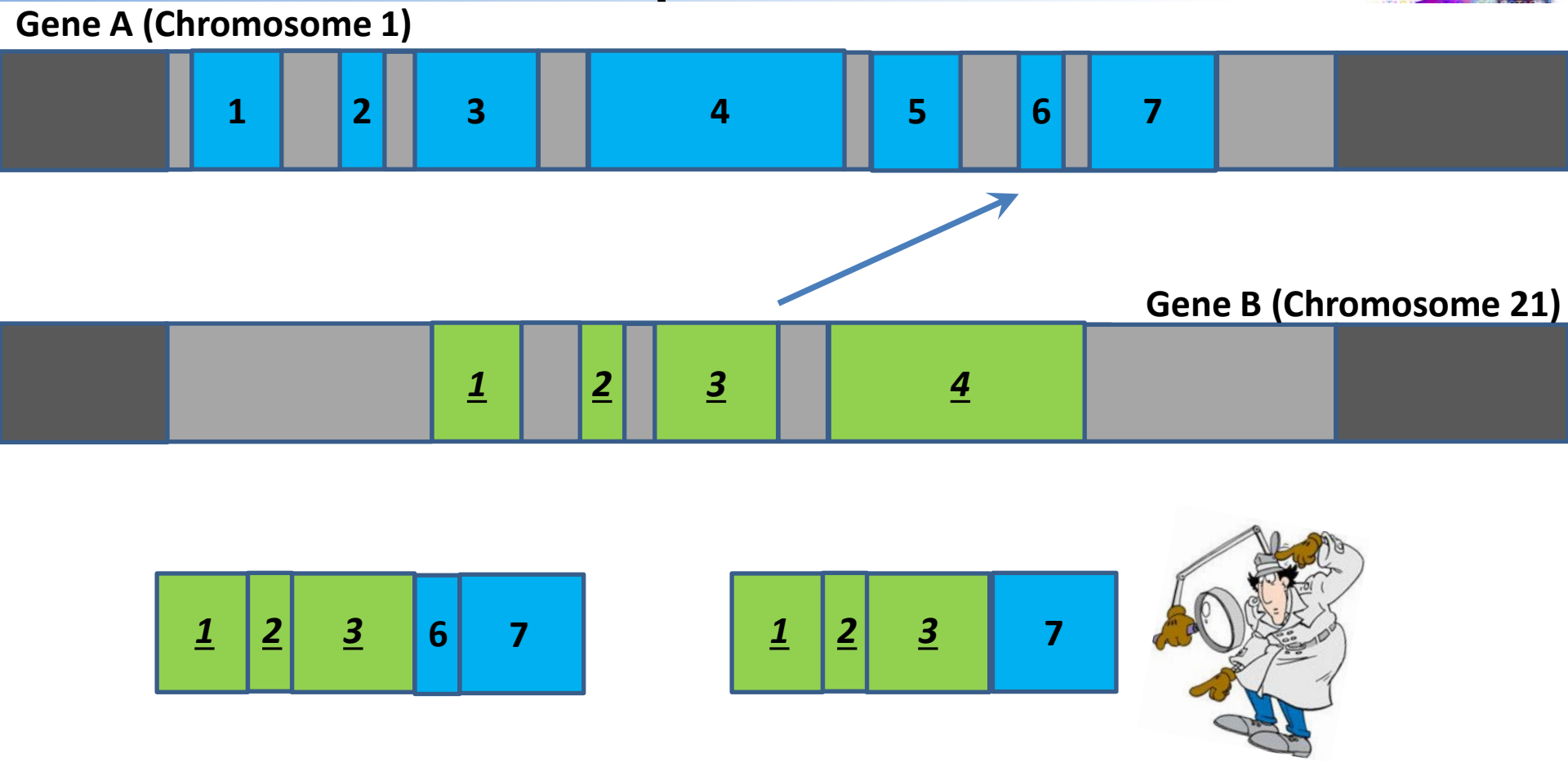


Fusion calling challenges

- Complicated by the many false positive candidates resulting from:
  - alignment artifacts such as multi-mapping of reads owing to homologous (pseudogenes) and/or repetitive sequences
  - sequencing artifacts due to errors in library generation (particularly ligation and PCR artifacts) and sequencing

- Incorporating these considerations, and additional bioinformatics filters, various bioinformatics pipelines have been developed to help prioritize fusion candidates from next-generation sequencing (NGS) data

- "Read-through" transcription of neighboring genes occurs frequently in normal cells

- Common non-pathogenic fusion events between distal genes are known to exist due to distinct polymorphic haplotypes

Fusion calling challenges

- Numerous software solutions exist for fusion detection
  - e.g. STAR-Fusion, Tophat-Fusion, PRADA, Fusioncatcher

- Technical comparisons demonstrate limited overlap and no caller is fully inclusive
  - Partially because FPs are abundant & outputs require filtering
  - Filters are trained using *in-silico*, tumor or cell-line data & performance falters on alternative data types

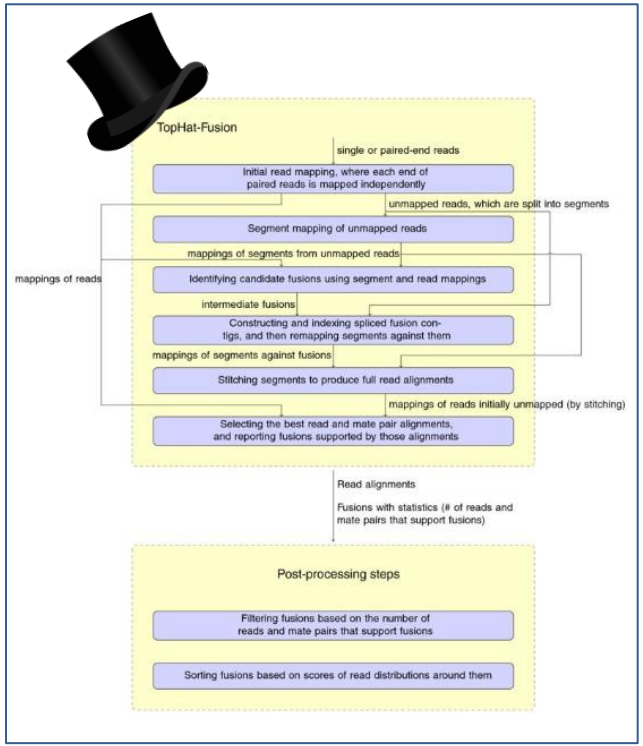
- It is recommended to select a caller on the basis of the data being profiled however none are trained on inherited disease

Fusion calling challenges

- Any attempt to detect fusions in inherited disease thus requires:
  - Inherent sensitivity
  - A means of deprioritizing biologically and phenotypically unimportant fusion candidates

Filtering / Prioritization

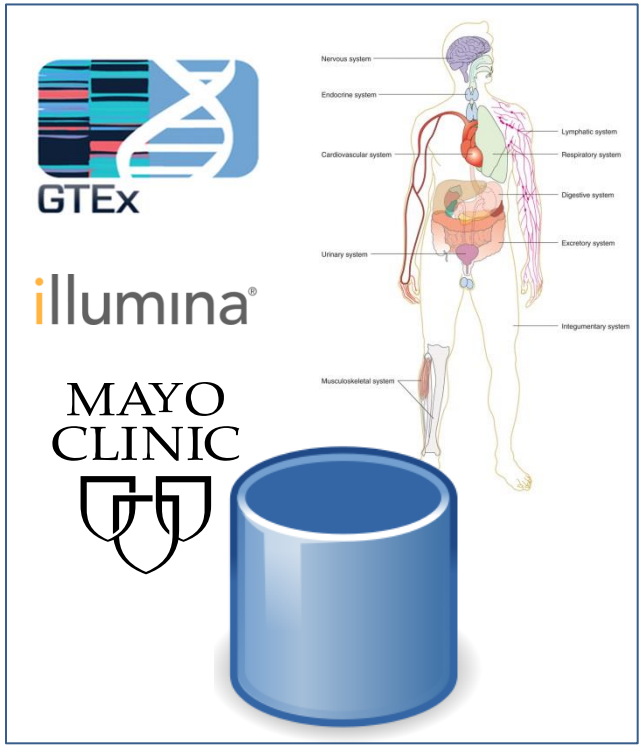
Read support (basic)



- TopHat Fusion (Kim & Salzberg 2011)
  - Equally applicable to other callers
- Omitted all TopHat filtering steps (cancer cell-line derived)
- Employed a very minimal depth filter (2 reads)

Filtering / Prioritization

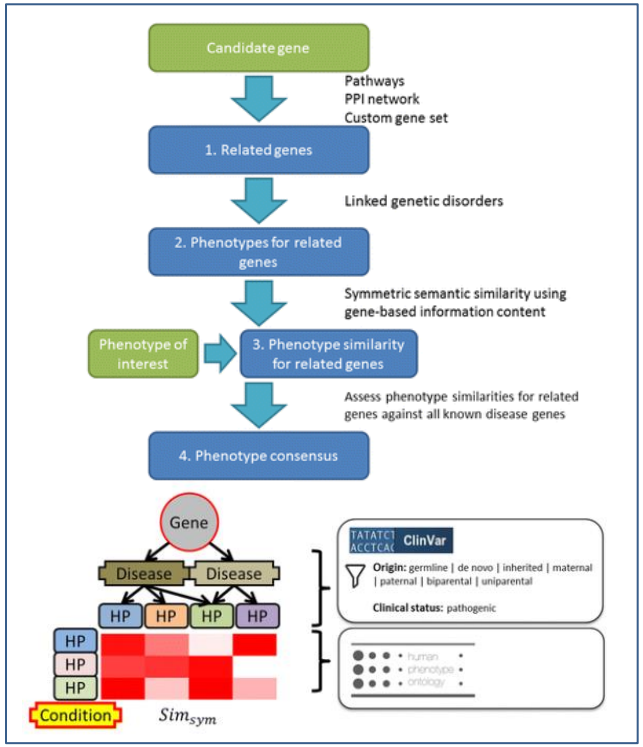
Normal DB comparison



- Compared fusion candidates to a database of candidates from normal tissues
- Fusion calling on samples from GTEx, Illumina Human Bodymap, Mayo Clinic
- Approx. 800 samples, 30 tissues
- Any fusion candidates occurring in DB or more than one cohort sample were categorized as normal/recurrent

Filtering / Prioritization

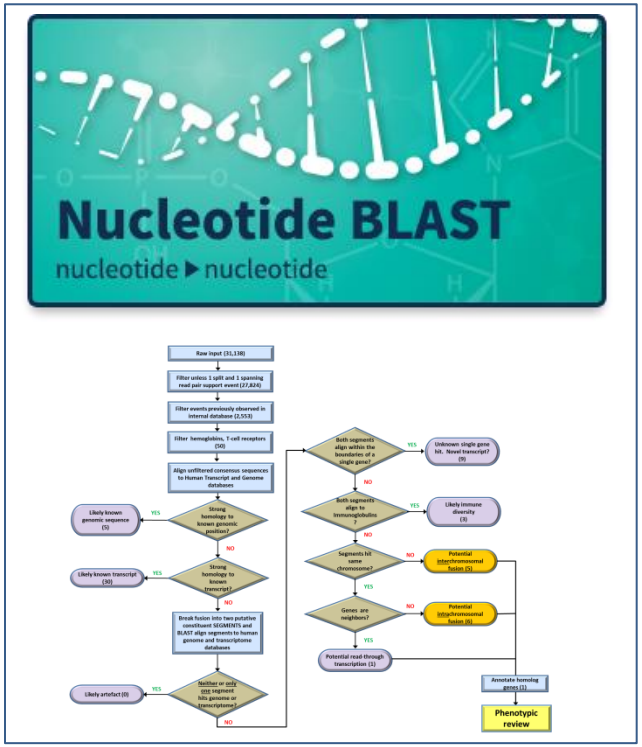
Phenotypic Prioritization



- Dual approach
  - Manual (Literature, OMIM, Genecards)
  - *In-silico*
    - *PCAN: phenotype consensus analysis to support disease-gene association (Godard & Page, 2016)*
- Generated phenotypically prioritized events for follow-up validation

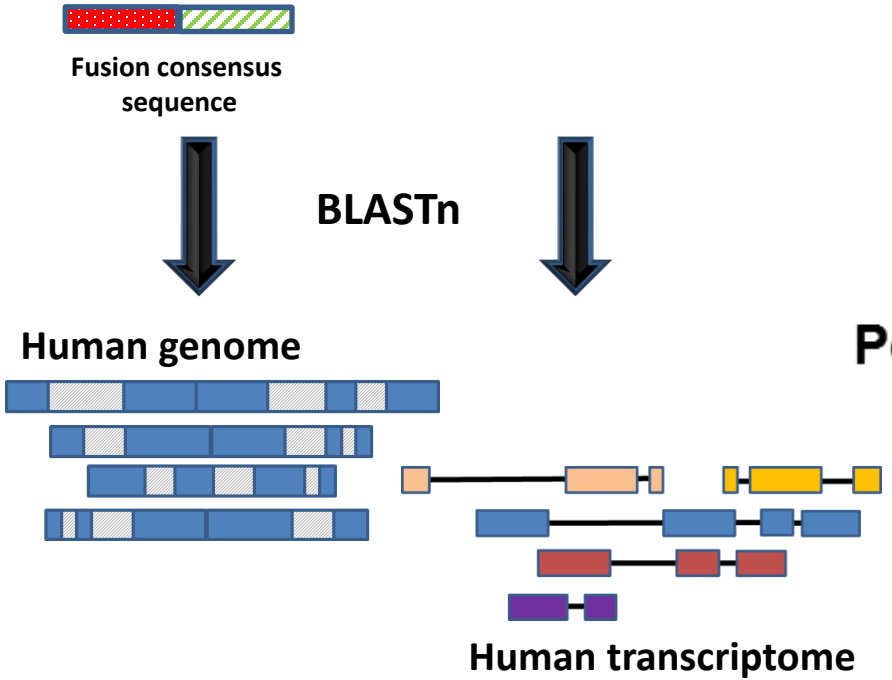
Filtering / Prioritization

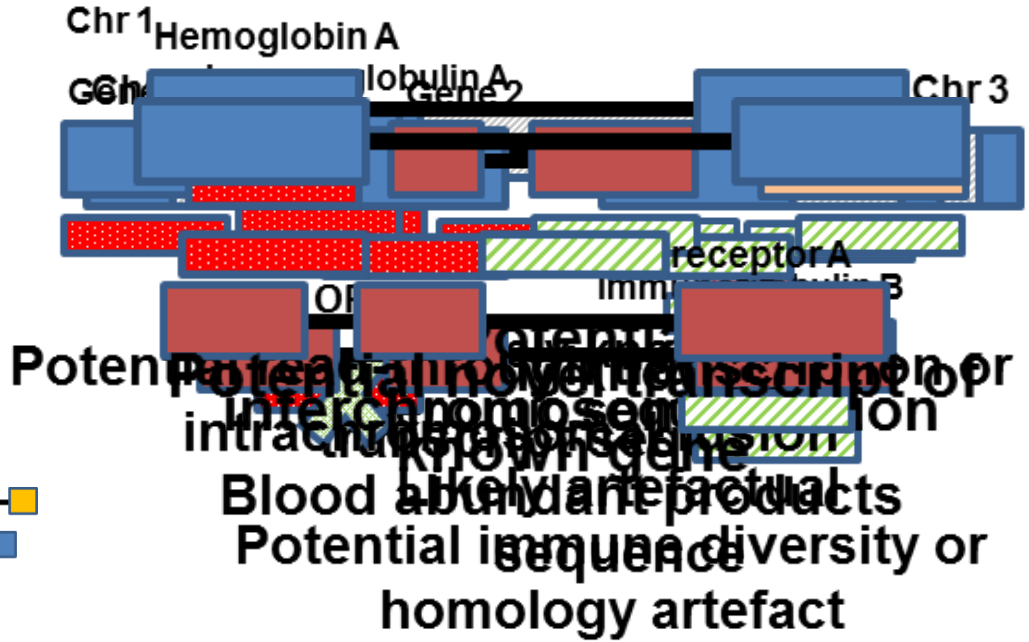
BLAST categorization



- Fusion consensus sequences generated by TopHat Fusion used as input
  - Algorithm dependent
- Devised custom categorization pipeline based on BLASTn
- Categorization logic based on best alignments

Candidate Categorization





Now let's try it…

Questions