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laboratory medicine.*

PEARLS OF LABORATORY MEDICINE

RNA Sequencing

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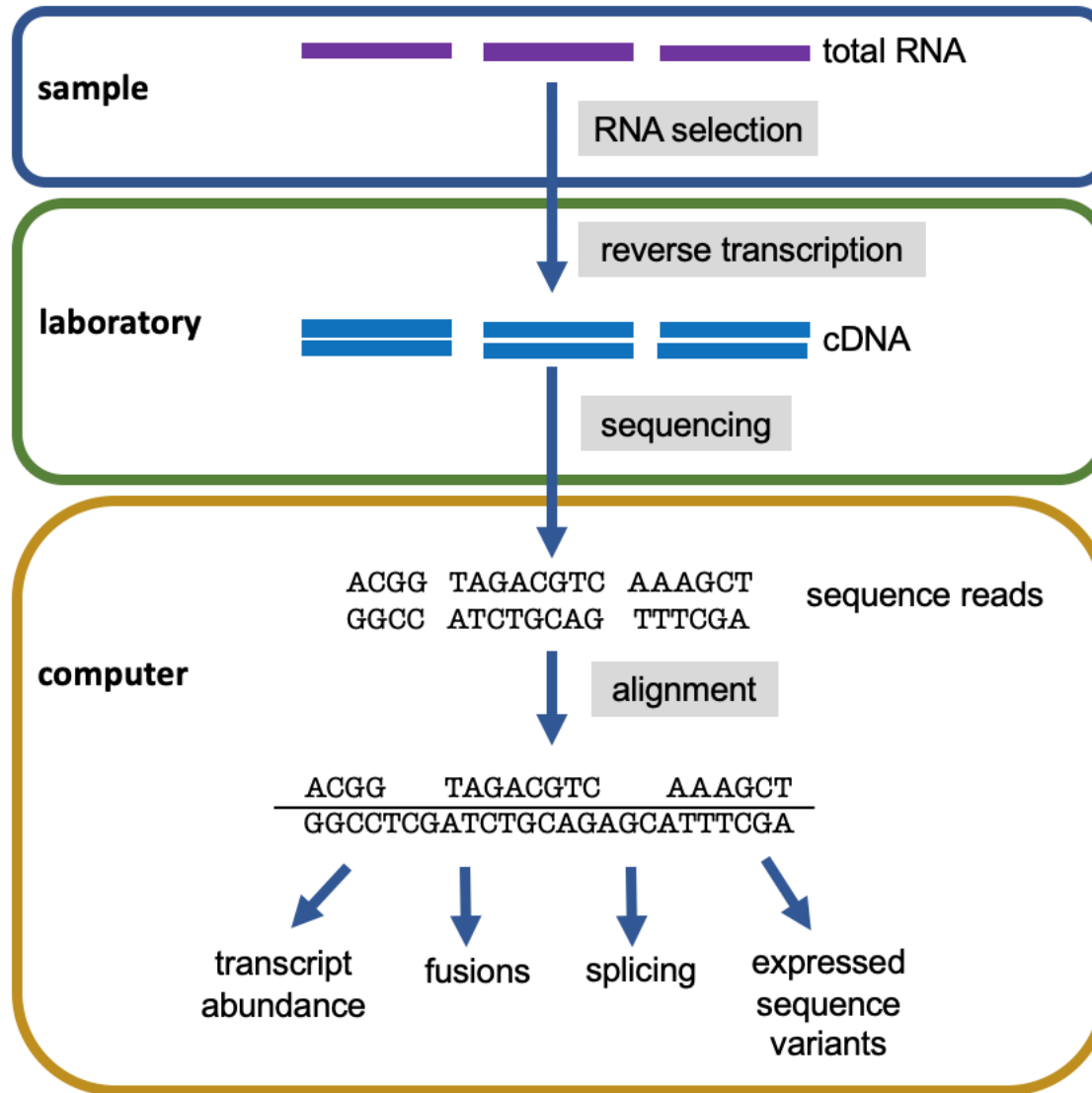
RNA Sequencing

- High throughput sequencing
- Measures transcript sequence and abundance
- Detects multiple RNA populations (coding RNA, lncRNA, miRNA, tRNA)

- Clinical applications
 - Aberrant gene expression
 - Fusions
 - Expressed sequence variants



RNA Sequencing workflow



- Poly-A selection
 - Captures predominantly poly-adenylated (polyA) transcripts
- Ribo-deplete selection
 - Removal of ribosomal RNA (rRNA)
 - Captures both polyA and non-polyA transcripts
- Targeted capture
 - Selection of RNA transcripts by hybridization to complementary probes
- Size Selection
 - Small RNA species (15-30 nucleotides)

➤ Transcript abundance from different RNA selection methods cannot be compared without additional normalization steps.

Applications

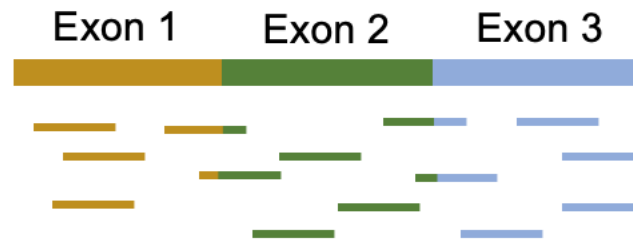
- Gene and exon expression quantification
 - Read counts can be normalized by gene length
- Fusions
 - RNA-seq detects expressed genomic fusions or rearrangements
- Splicing
 - RNA-seq quantifies alternative splicing events and gene isoforms
- Variants
 - RNA-seq data identifies expressed DNA variants or SNPs

Application: Gene and exon expression quantification

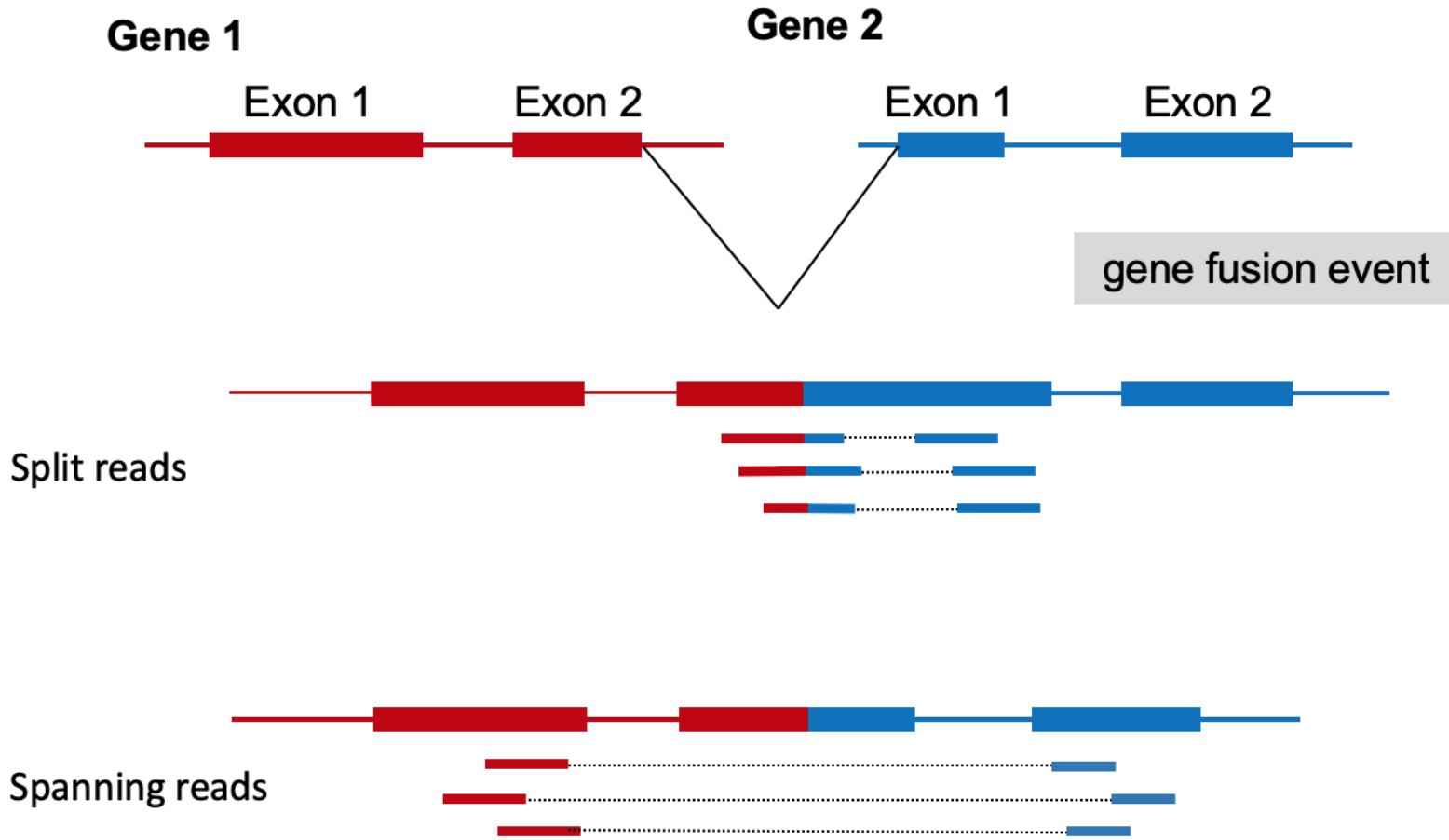
Genome alignment



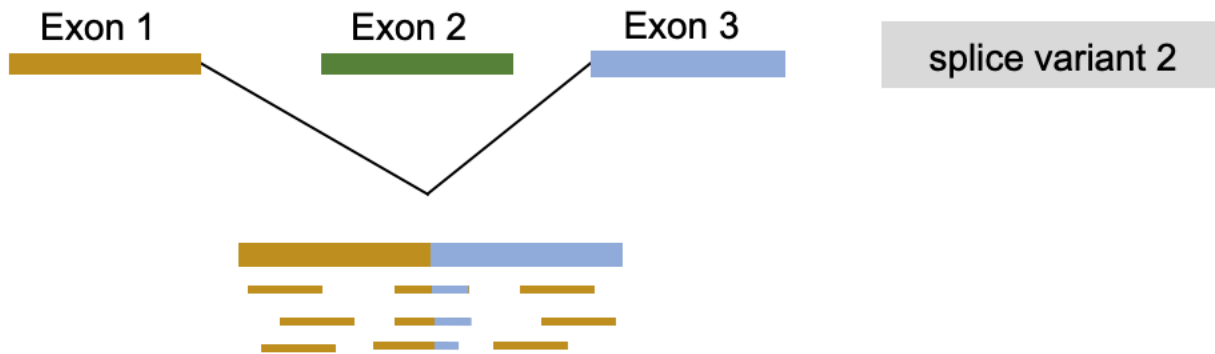
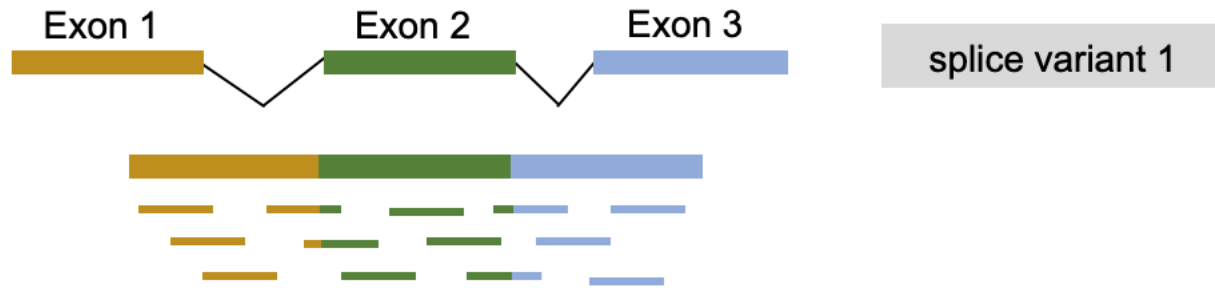
Transcriptome alignment



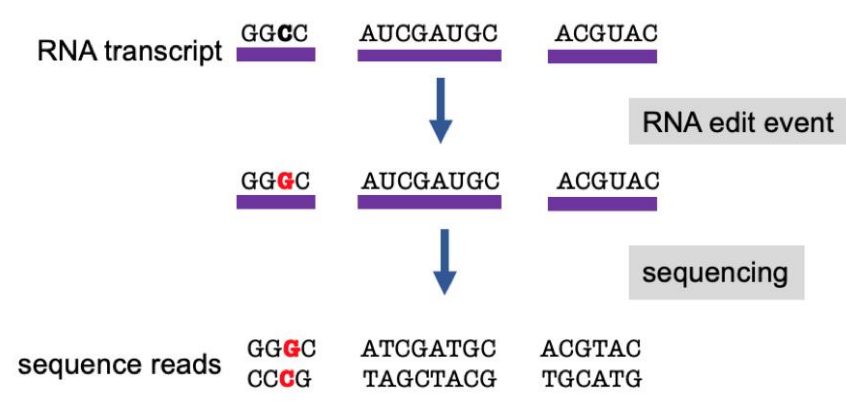
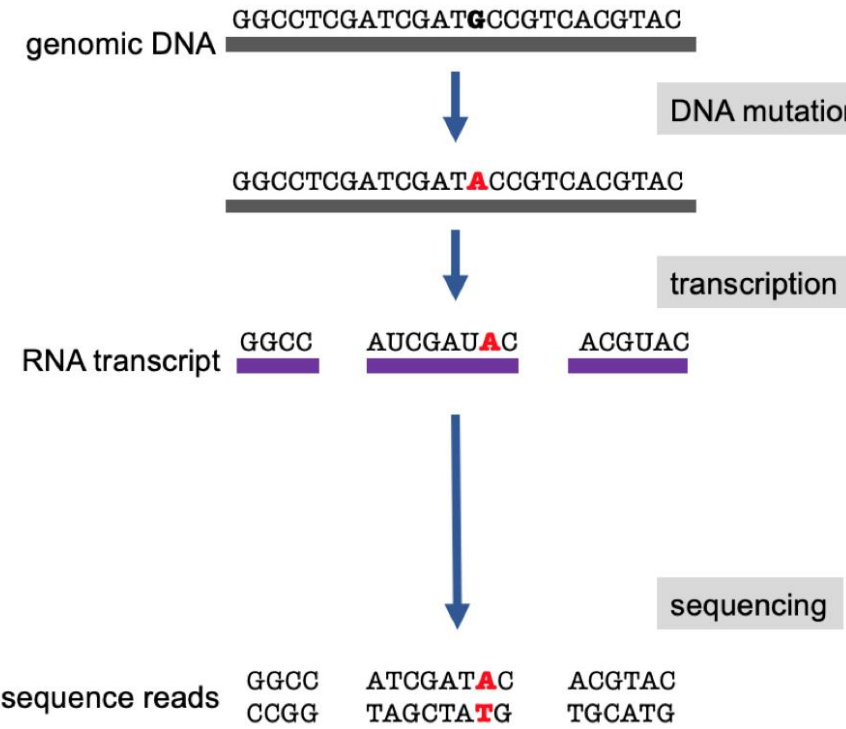
Application: Fusion detection



Application: alternative splicing



Application: expressed sequence variants

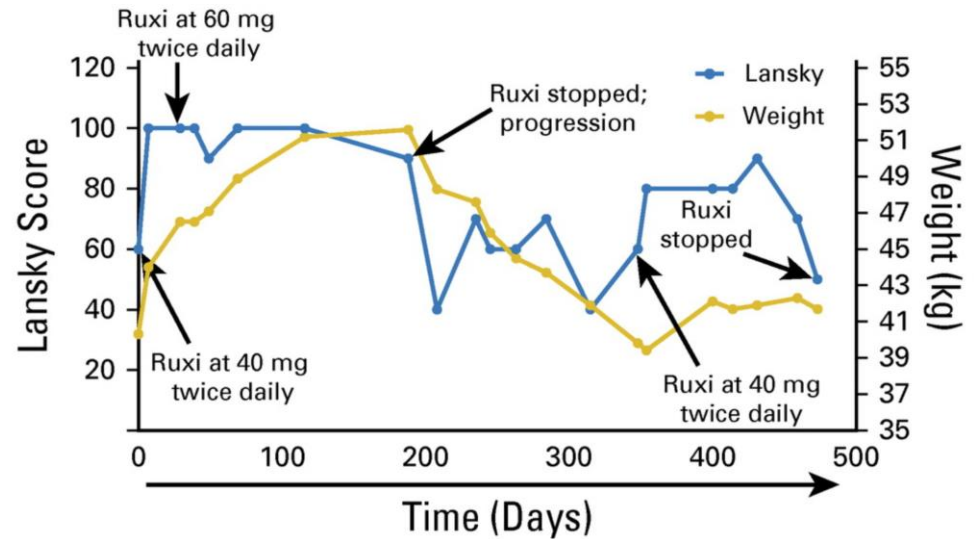
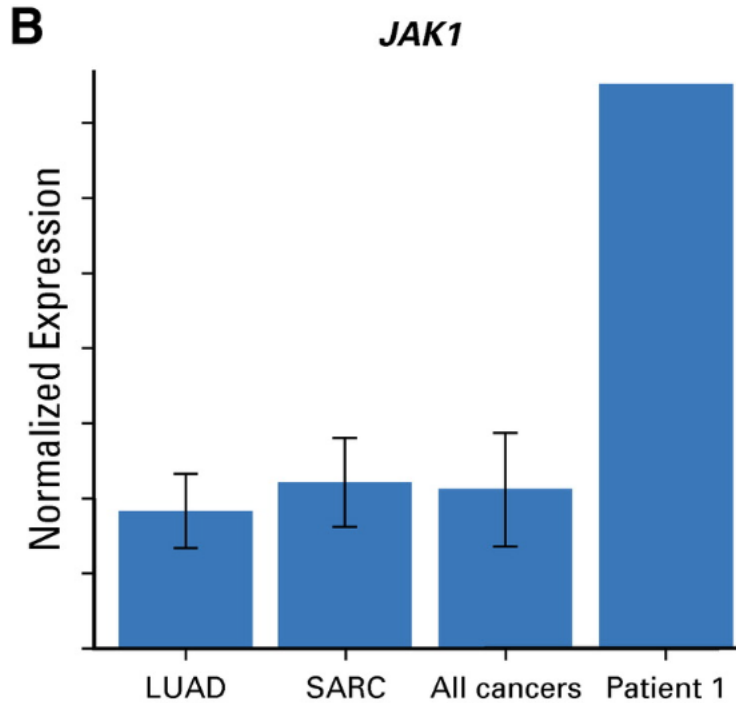


Applications to genomic medicine

- Comparative gene expression analysis
 - Example: tumor vs. normal tissue
- Diagnostic fusions
- Alternative or novel splicing in disease
- Disease-associated variants



Genomic medicine: comparative gene expression analysis



Reprinted with permission. © 2018 American Society of Clinical Oncology. Newton, Y, et al. Comparative RNA-sequencing analysis benefits a pediatric patient with relapsed cancer. JCO PO 2, 2018: 1-16.



Genomic medicine: diagnostic fusions

Chromosome 11



FLI1

Chromosome 22



EWSR1



EWSR1-FLI1 fusion transcript

Emerging applications in germline diseases

- Detection of extreme gene expression
 - Non-coding dysregulation can cause overexpression
 - Detectable using outlier analysis
- Identification of mono-allelic expression (MAE)
 - Important when only 1 allele harbors a mutation
- Aberrant splicing defects
 - Specific splice isoforms linked to disease

Kremer LS, Bader DM, Mertes C, et al. Genetic diagnosis of Mendelian disorders via RNA sequencing. *Nat Commun* 2017;15824.

Germline disease: aberrant gene expression

- Gene expression above physiological levels can cause rare disorders
 - Epigenetic dysregulation
 - Variants in promotor or enhancer regions
- Marie-Charcot-Tooth disease
 - Caused by PMP22 duplication, leading to overexpression detectable by RNA-seq
- Identification of overexpression using larger datasets
 - Z-score cutoff
 - Outlier in a normal distribution ($1.5 \text{ IQR} + Q3$)

van Paassen BW, van der Kooi AJ, van Spaendonck-Zwarts KY, et al. PMP22 related neuropathies: Charcot-Marie-Tooth disease type 1A and hereditary neuropathy with liability to pressure palsies. Orphanet journal of rare diseases. 2014 Dec;9(1):38.

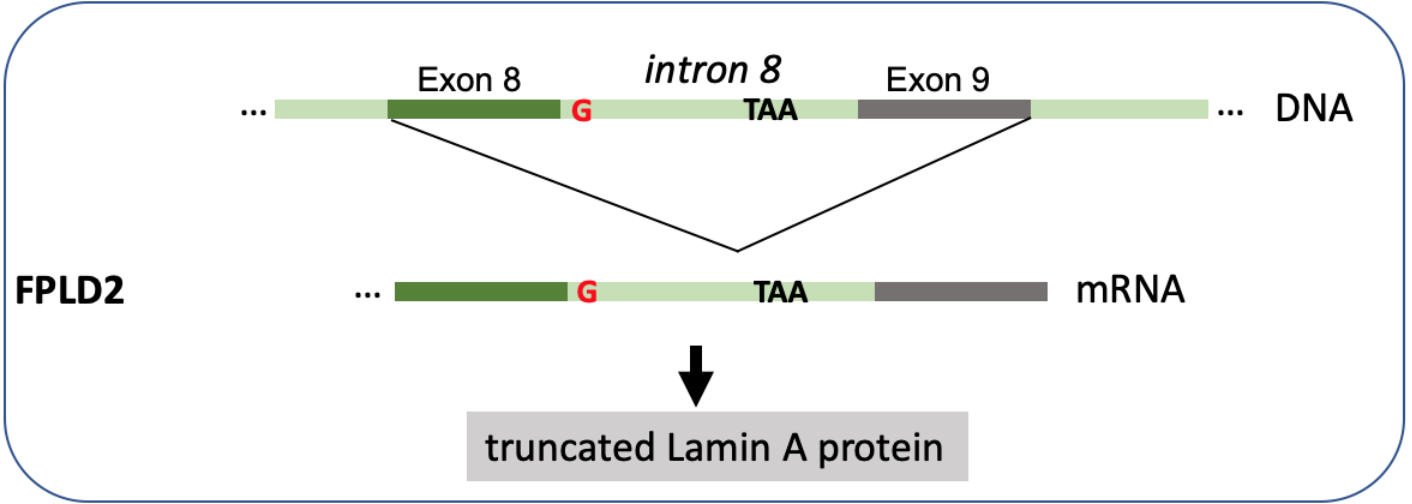
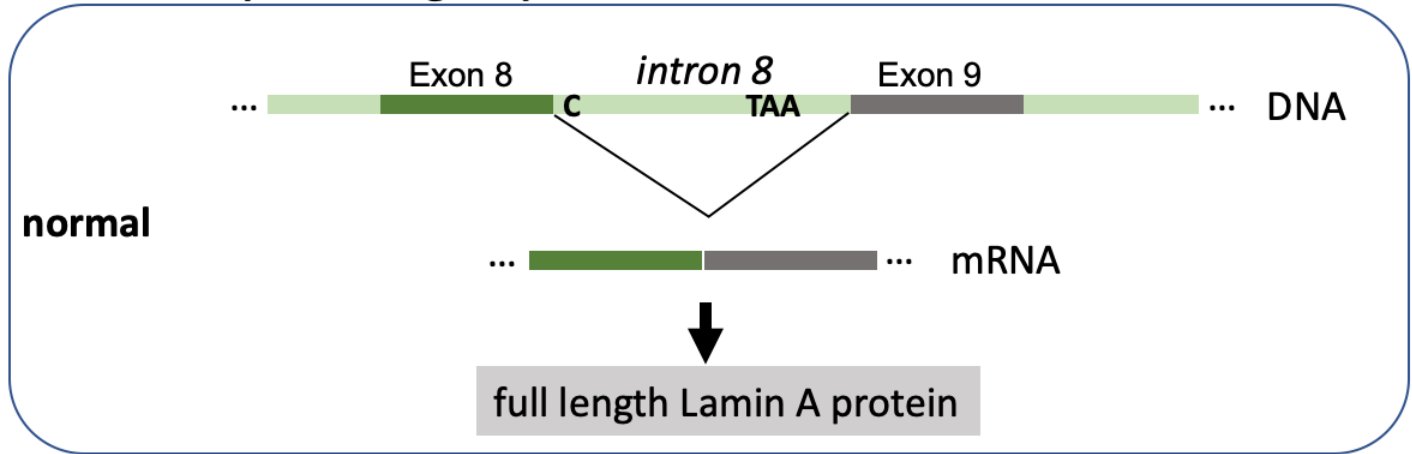
Germline disease: mono-allelic expression (MAE)

- Thrombocytopenia with absent radii (TAR)
 - Reduction in platelet number
 - Absence of radius bone in forearm
- Caused by compound heterozygous variants in *RBM8A* gene
 - One allele is deleted
 - The other allele has reduced expression due to a non-coding variant

Albers CA, Paul DS, Schulze H, et al. Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome. *Nat Genet* 2012;44(4):435.

Aberrant splicing in familial partial lipodystrophy type 2 (FPLD2)

LMNA (Lamin A gene)



Conclusion

- RNA sequencing is a promising technology in the clinic
- Applications across genomic medicine and germline diseases
- Developing field with upcoming applications



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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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- **Expert Testimony:** No disclosures
- **Patents:** No disclosures



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