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PEARLS OF LABORATORY MEDICINE

RNA Sequencing

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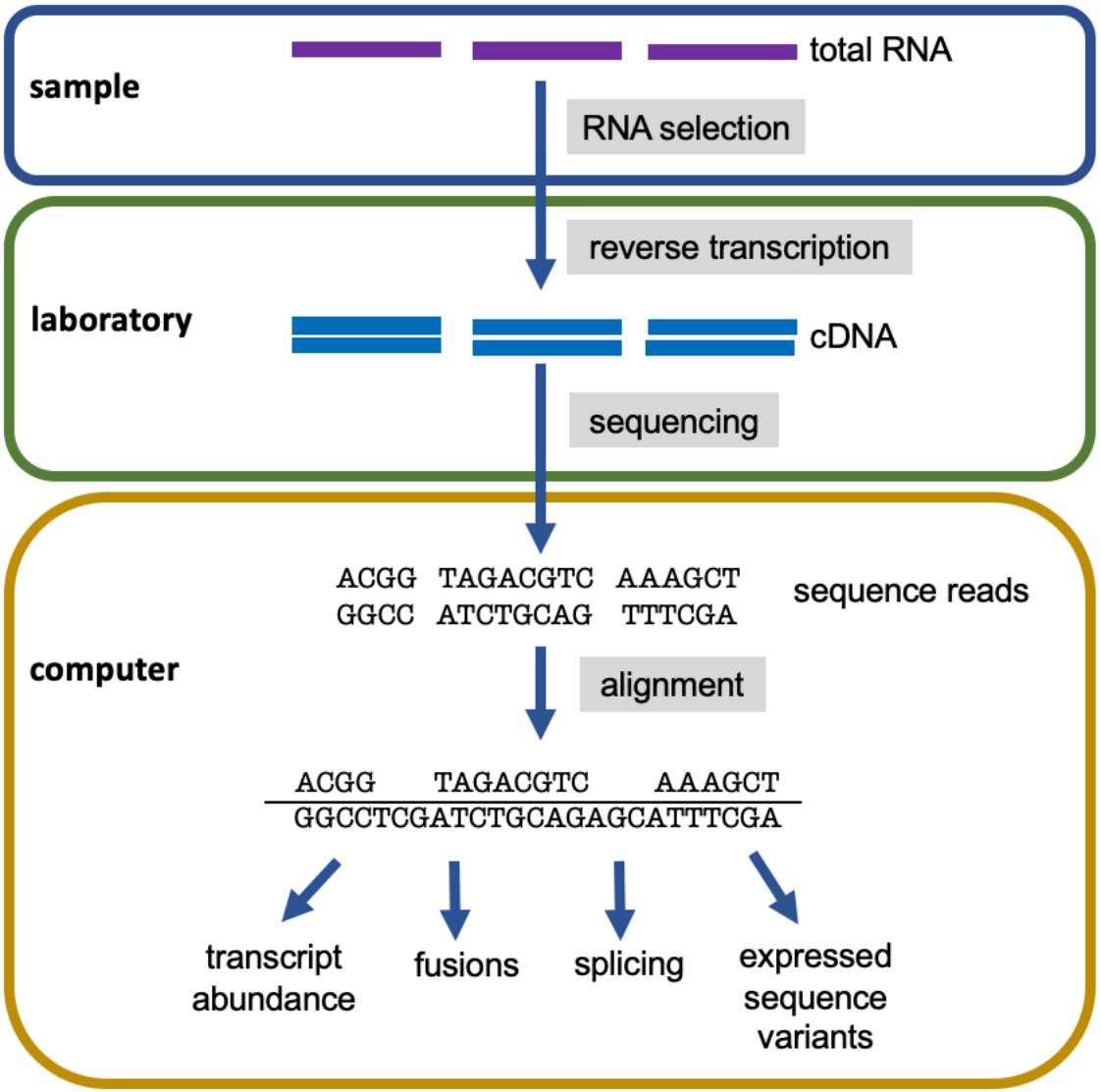
CCTC Clinical Chemistry Trainee Council

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



RNA selection methods

- 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)

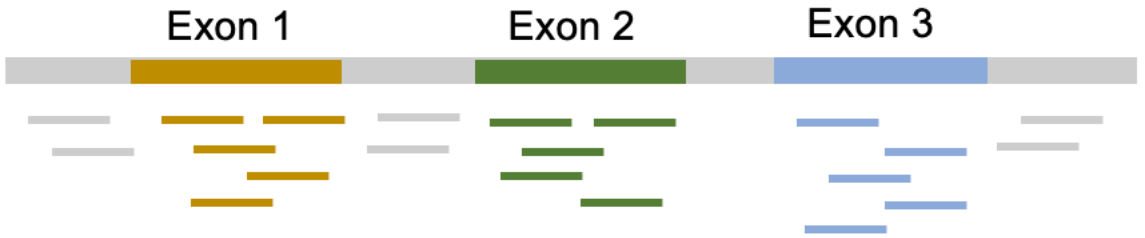
➢ Transcript abundance from different RNA selection methods cannot be compared without additional normalization steps.

Applications

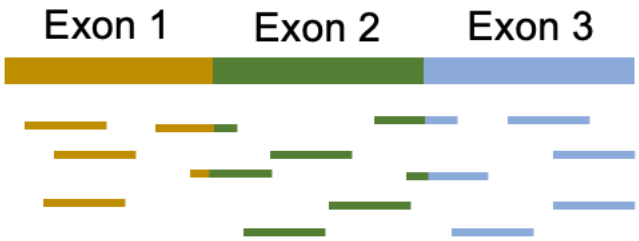
- Gene and exon expression quantification
  - Read counts can be normalized by gene length
- Fusions
  - RNA-seq detects expressed genomic fusions or rearrangements
- Splicing
  - RNA-seq quantifies alternative splicing events and gene isoforms
- Variants
  - RNA-seq data identifies expressed DNA variants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

Application: Gene and exon expression quantification

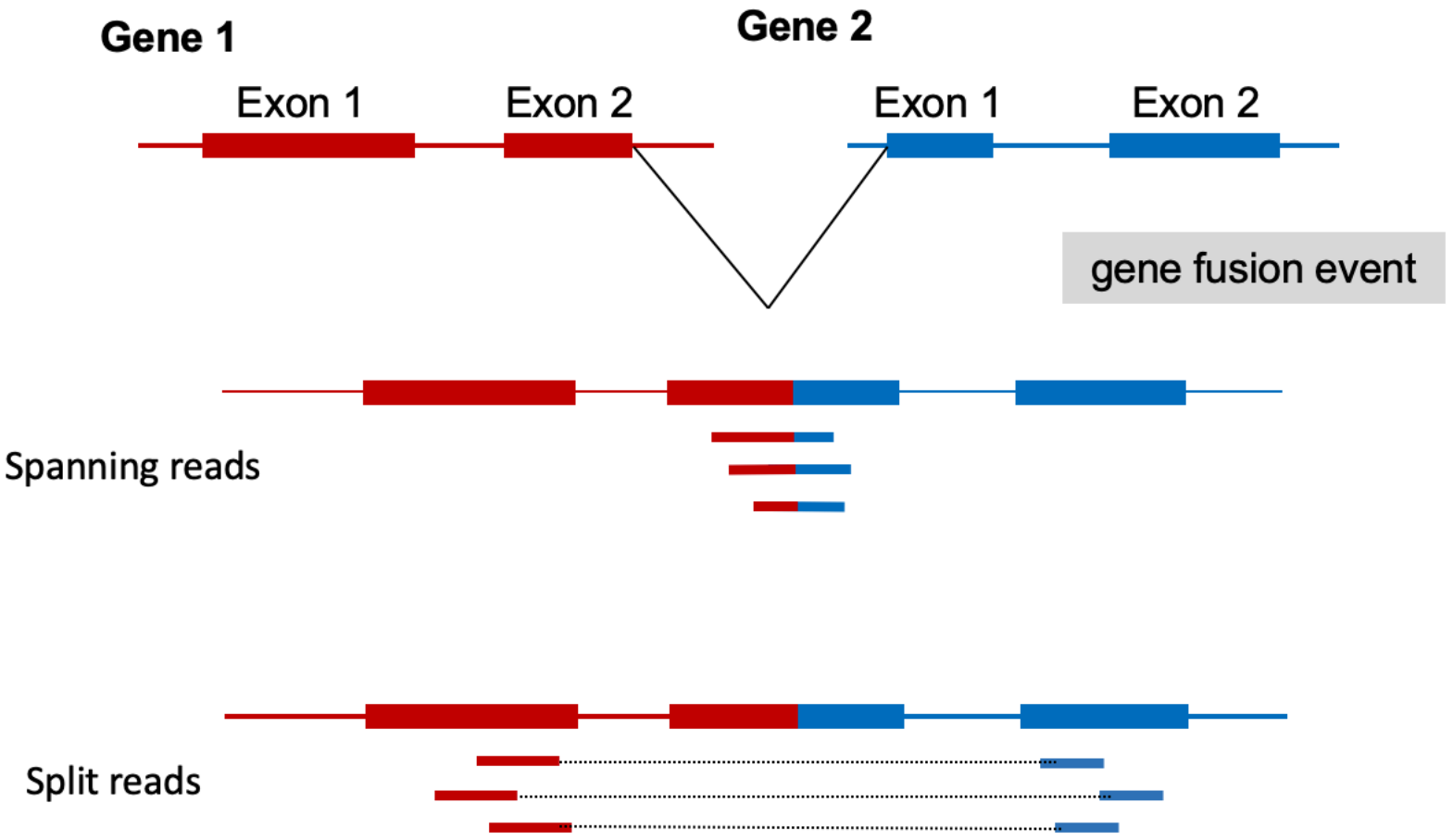
Genome alignment



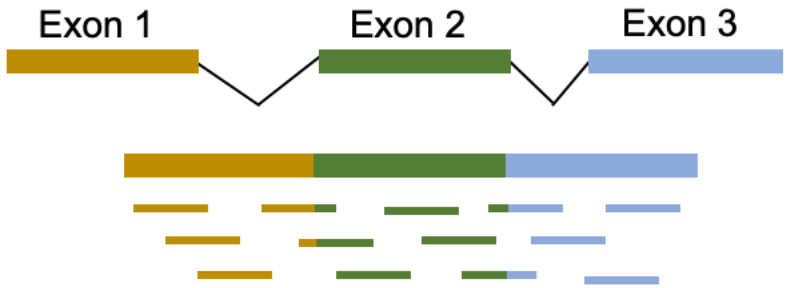
Transcriptome alignment



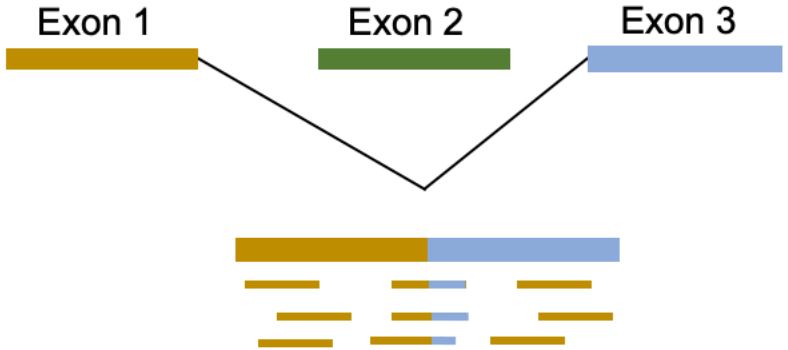
Application: Fusion detection



Application: alternative splicing

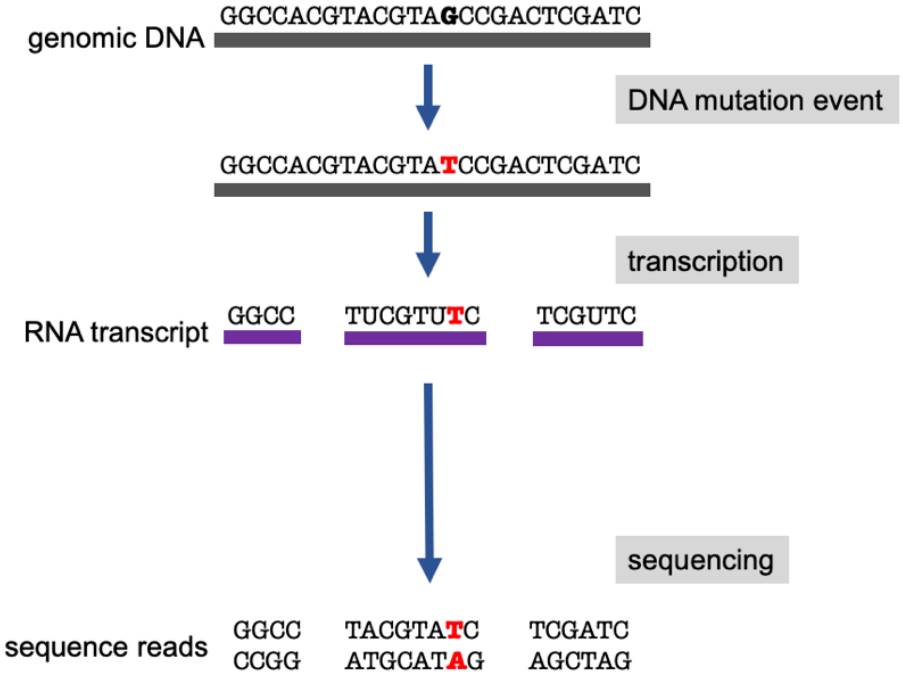


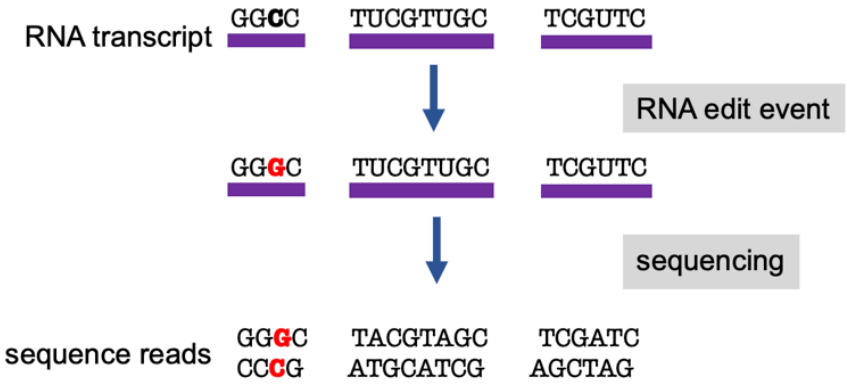
splice variant 1



splice variant 2

Application: expressed sequence variants

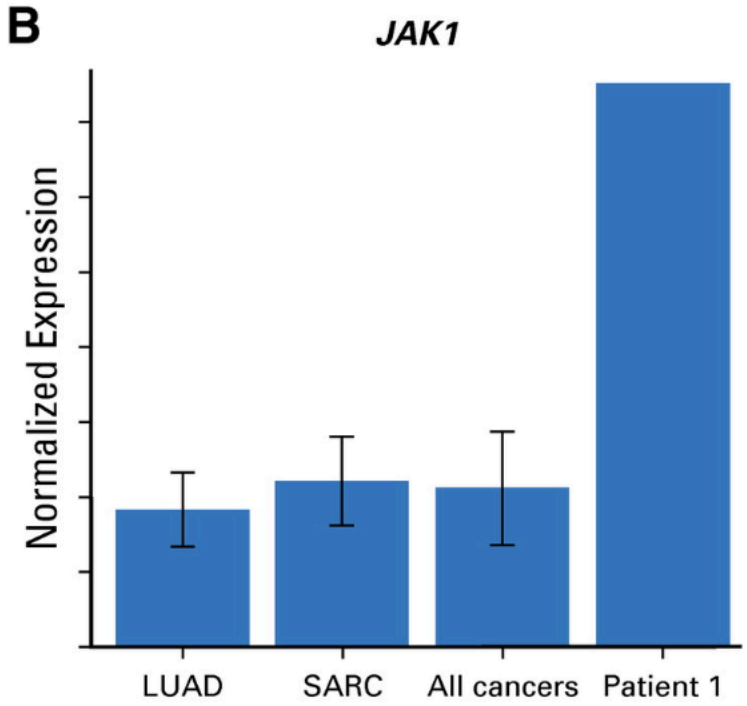


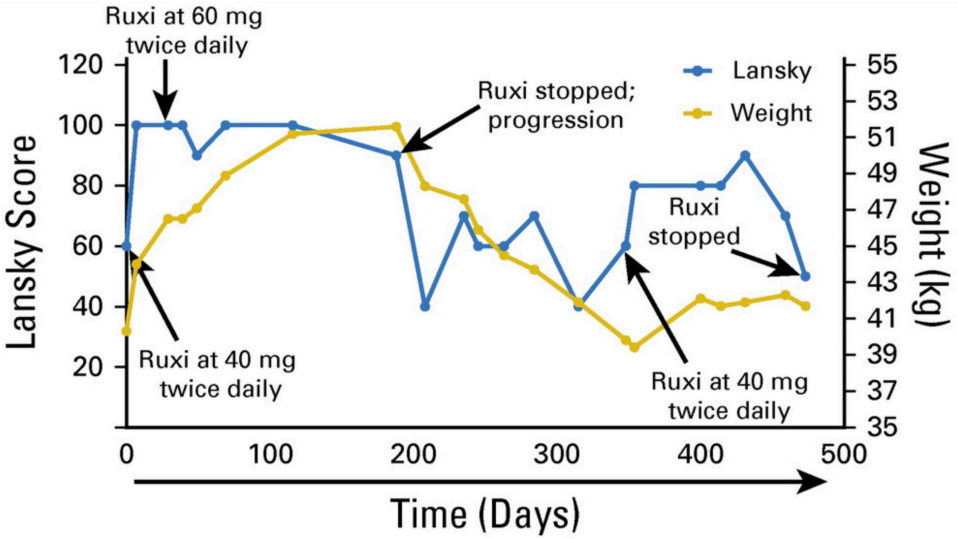


Applications to genomic medicine

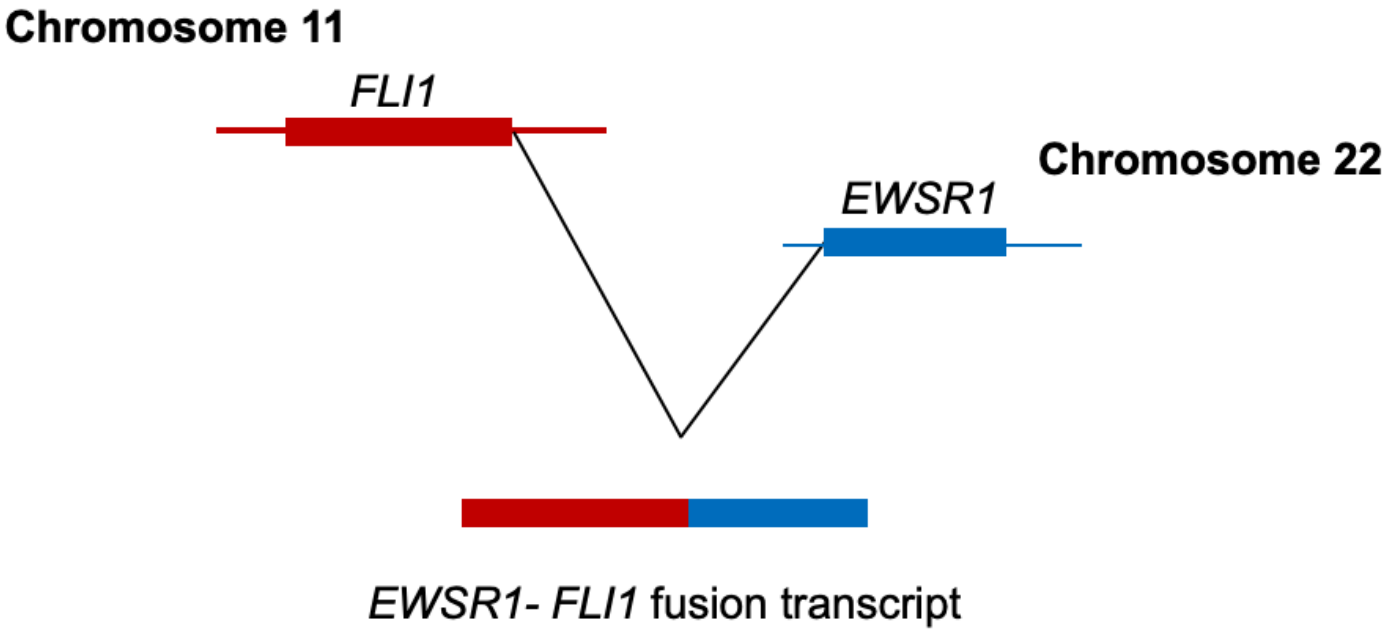
- Comparative gene expression analysis
  - Example: tumor vs. normal tissue
- Diagnostic fusions
- Alternative or novel splicing in disease
- Disease-associated variants

Genomic medicine: comparative gene expression analysis





Genomic medicine: diagnostic fusions



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

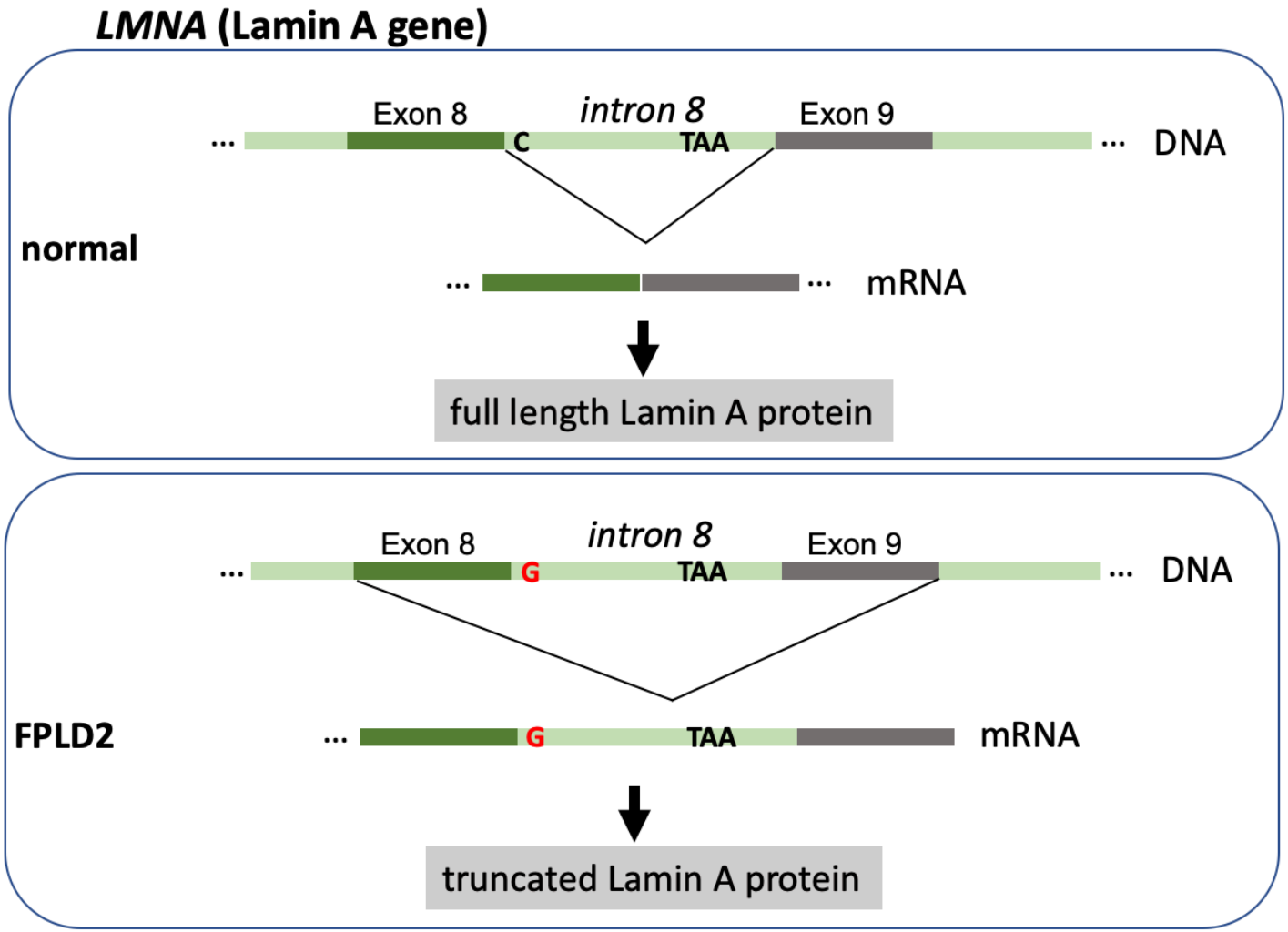
- Gene expression above physiological levels can cause rare disorders
  - Epigenetic dysregulation
  - Variants in promotor or enhancer regions
- Identification of expression outliers using larger datasets
  - Z-score cutoff
  - Outlier in a normal distribution (1.5 IQR + Q3)

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)



Conclusion

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

References

1. Newton Y, Rassekh SR, Deyell RJ, et al. Comparative RNA-sequencing analysis benefits a pediatric patient with relapsed cancer. JCO PO 2018;2:1-16.
2. Romeo, Salvatore, and Angelo P. Dei Tos. Soft tissue tumors associated with EWSR1 translocation. Virchows Arch 456.2 2010;219-234.
3. Kremer LS, Bader DM, Mertes C, et al. Genetic diagnosis of Mendelian disorders via RNA sequencing. Nat Commun 2017;15824.
4. 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.
5. Morel CF, Thomas MA, Cao H, et al. A LMNA splicing mutation in two sisters with severe Dunnigan-type familial partial lipodystrophy type 2. J Clin Endocrinol Metab 2006;*91*(7):2689-2695.

Disclosures/Potential Conflicts of Interest

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

- **Employment or Leadership**:
- **Consultant or Advisory Role:**
- **Stock Ownership:**
- **Honoraria:**
- **Research Funding:**
- **Expert Testimony:**
- **Patents:**

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