ACS Cancer Conference 2025

March 12–14 | Phoenix, AZ

Application of NGS Technologies in Oncology

Christie Jett, LCGC

Valley Health - Winchester Medical Center

ACS Cancer Programs

American College of Surgeons

Serve as the liaison to NAPBC for the National Society of Genetic Counselors but all the opinions herein are mine alone and not those of NSGC or my employer

Former member of Cancer Precision Medicine Commons

No financial conflicts of interest to disclose

Objectives

1. Describe evolution of sequencing technologies

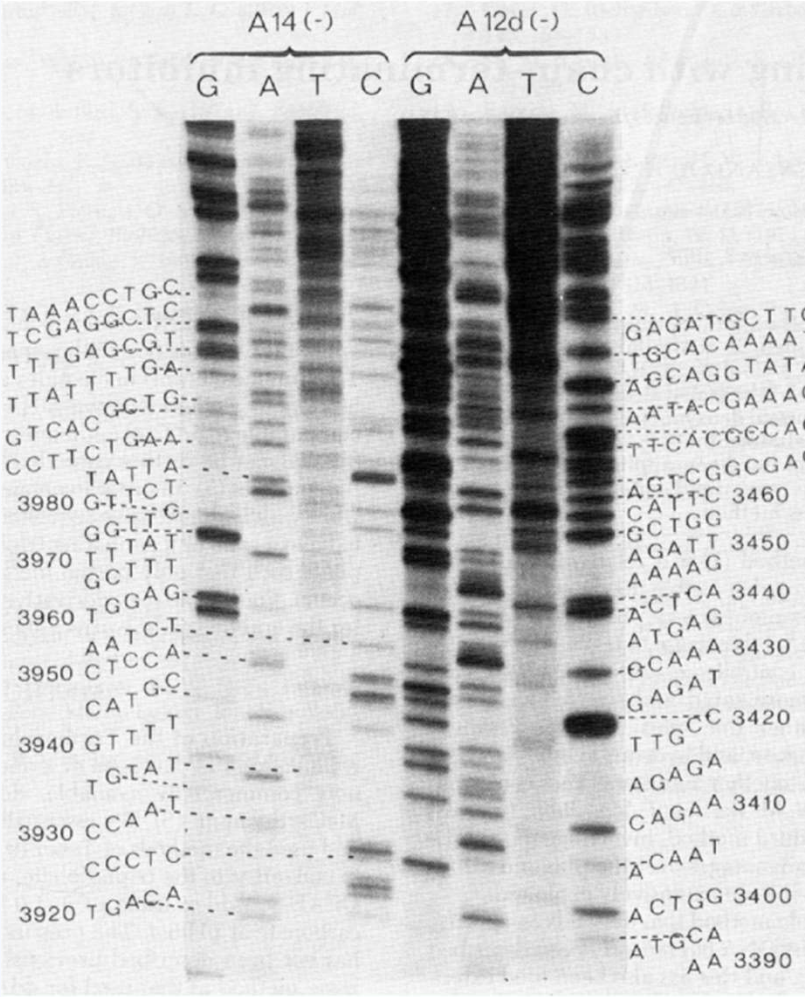
2. Differentiate between germline and tumor applications of sequencing technologies

3. Consider strengths and limitations of sequencing technologies in cancer care

Sequencing 101

Sanger sequencing

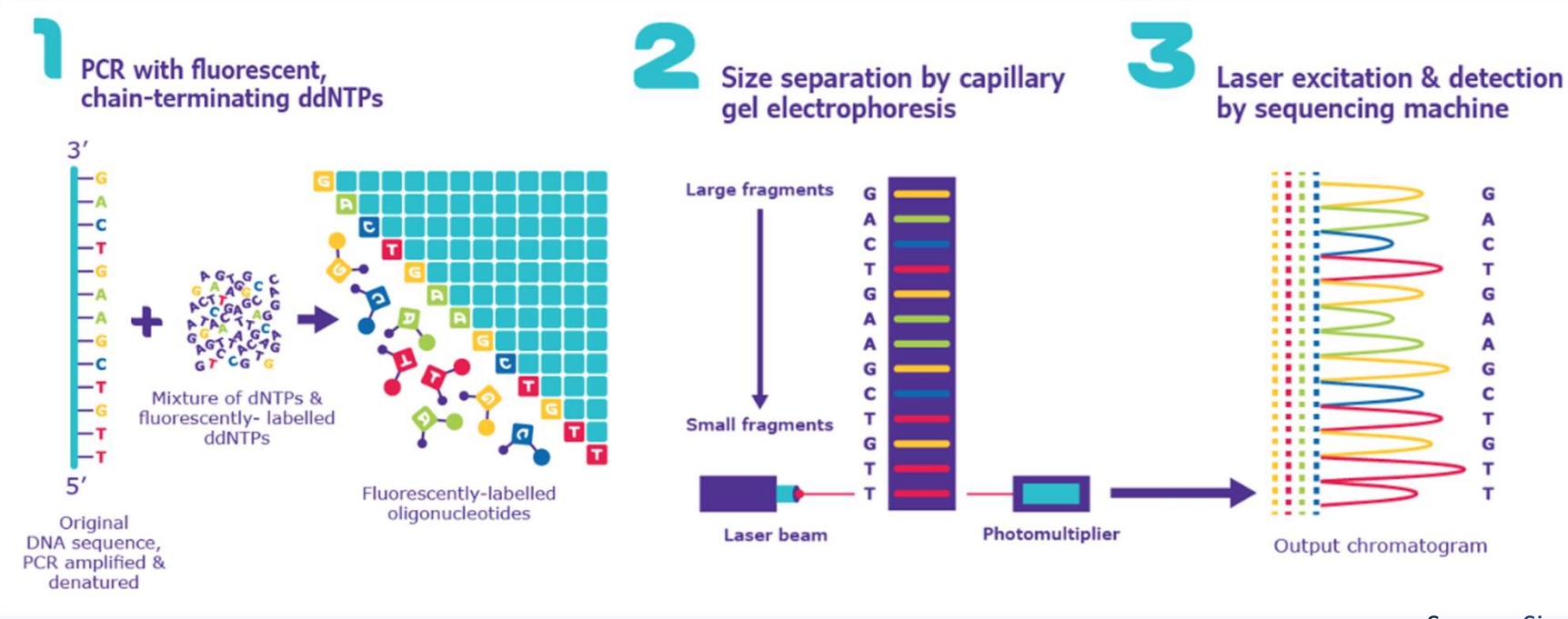
- Developed in the 1970s and still the basis for most next-generation sequencing techniques today
  - Such a revolutionary technique that Frederick Sanger won Nobel prize only a few years after it was introduced
- Too resource-intensive for widespread use
  - Read length limited by ability of electrophoresis to differentiate larger fragments
  - Cost ~ $2.7 billion and took 13 years to sequence the first human genome



Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977 Dec;74(12):5463-7

Sanger Sequencing

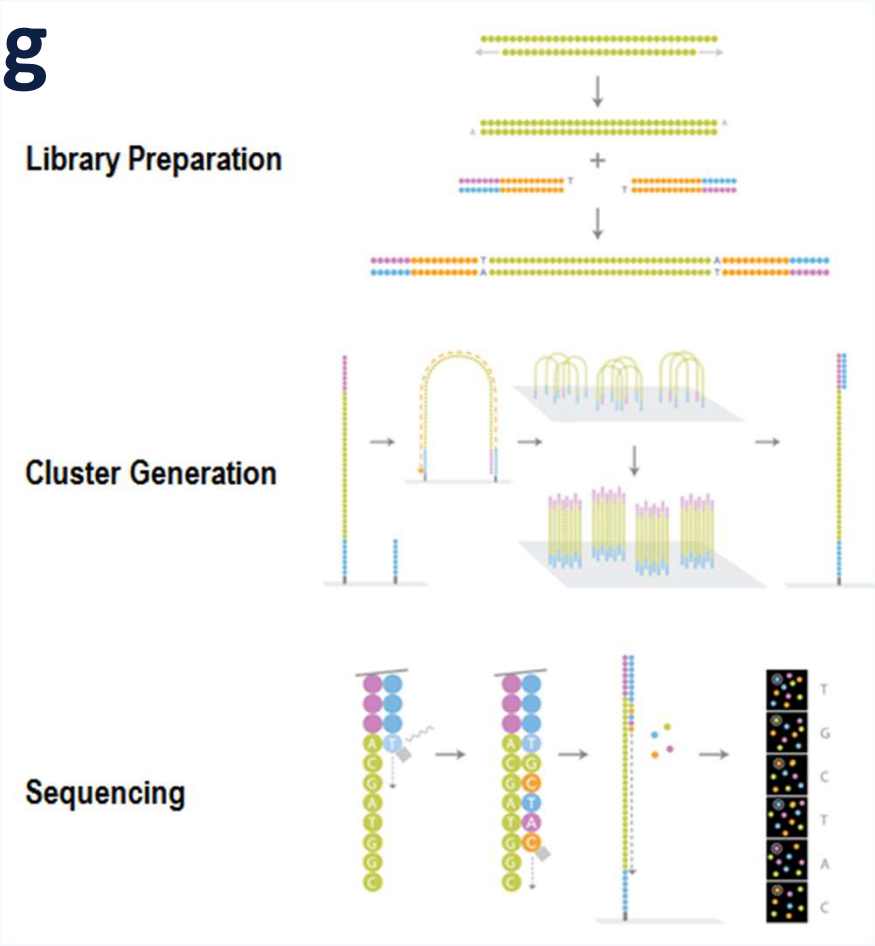
Library preparation Capture/Sequencing Analysis



Source: Sigma Aldrich

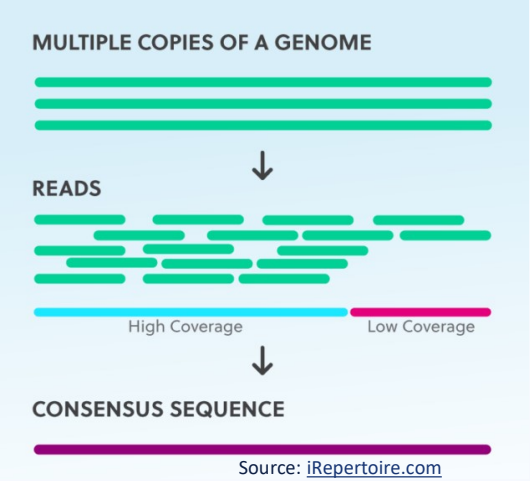
Next-Generation Sequencing

- Sanger sequencing but AT SCALE
  - Massively parallel sequencing
- Library preparation (at scale)
  - Multiplexing = pooling of multiple samples into a single run with the use of unique tags added to each sample
- Capture (at scale)
  - DNA library captured by matrix of oligos affixed to a surface (chip)
- Sequencing (at scale)
  - Pairing of terminal base pair is reversible (rinse and repeat)

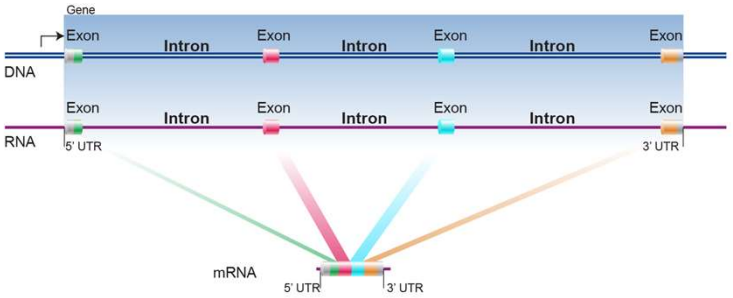


Generating Consensus Sequence

- During analysis reads are aligned to a reference genome to generate a consensus sequence
- Next-gen technologies struggle with deletions, duplications, or splicing variants
  - Clinical tests often combine NGS with other methodologies (MLPA, PCR, Sanger)
  - Paired RNA analysis theoretically allows for detection of splice site variants that fall into deep intronic regions (not typically sequenced)



Source: iRepertoire.com



Applying Next-Gen Sequencing in Cancer Care

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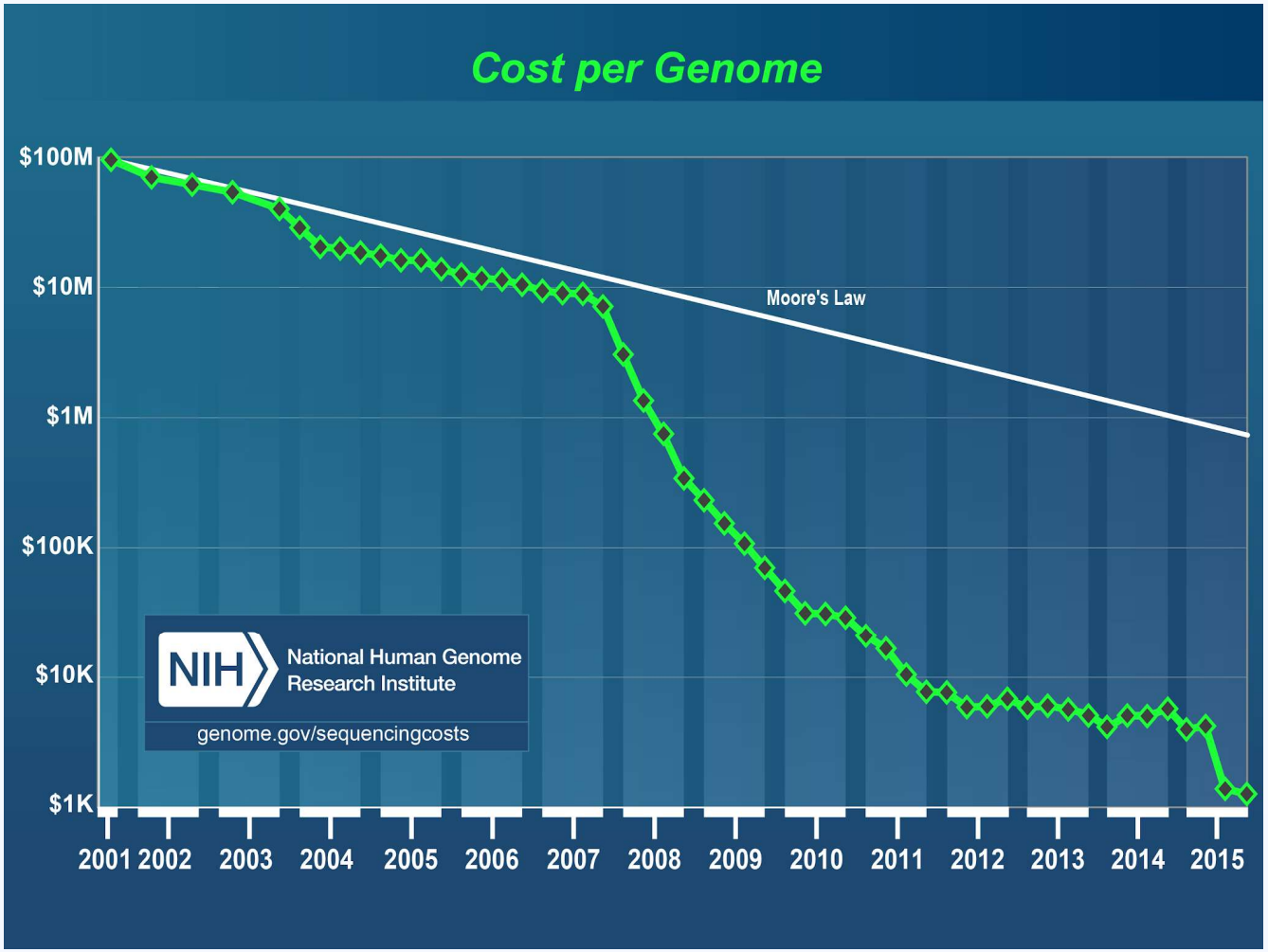
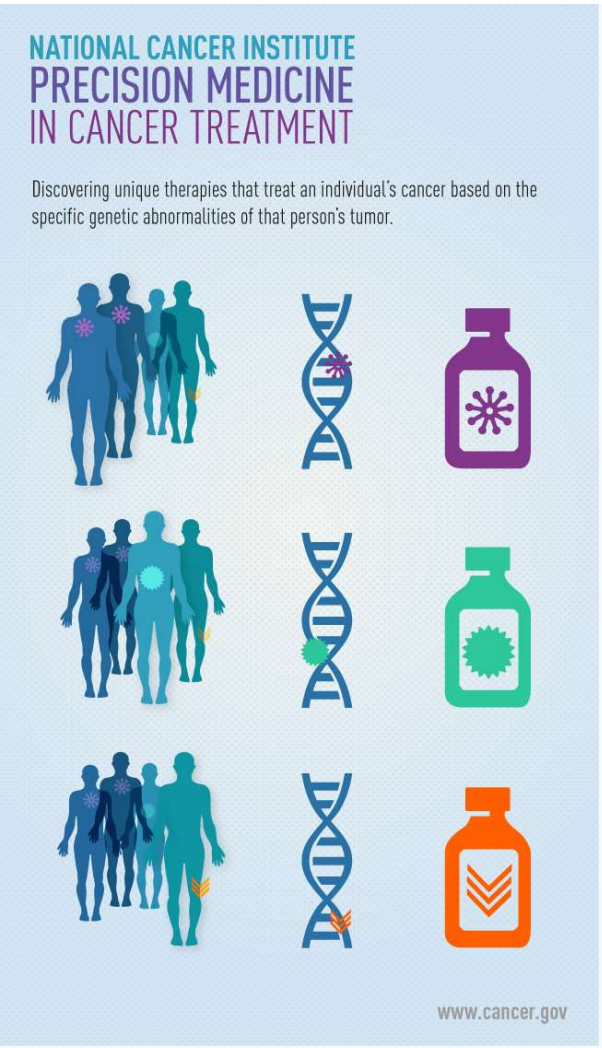


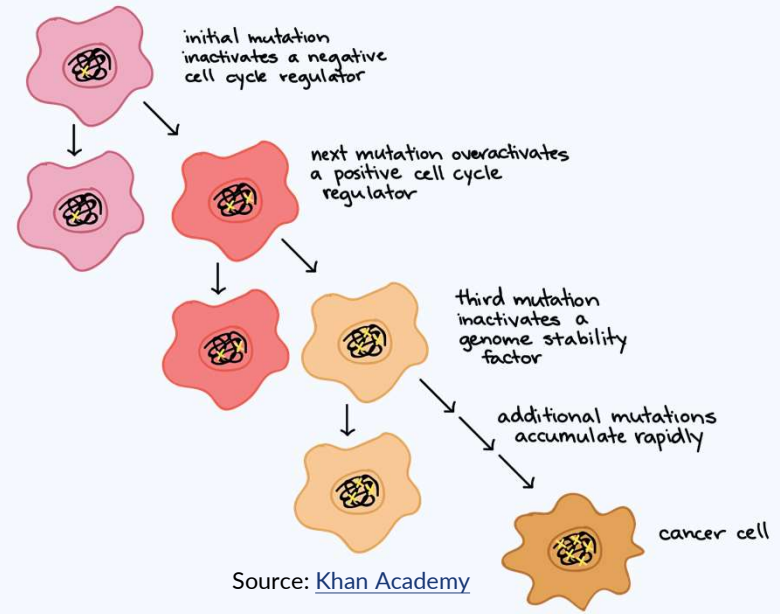


Promise of Precision Medicine

- Advent of cheaper and faster sequencing technologies has brought us closer to the promise of precision medicine
  - "Biomarkers" refers to DNA, proteins, molecules and other byproducts from the tumor
- Can sequence normal DNA, tumor DNA, or circulating cell-free DNA
  - Utilizing same technology and terminology has led to significant confusion

Inherited/Germline

- Present in every cell of the body (including tumor cells)
- Can be inherited and passed onto offspring
- Identification of baseline risks allows for personalized cancer surveillance and mitigation *prior* to onset of cancer



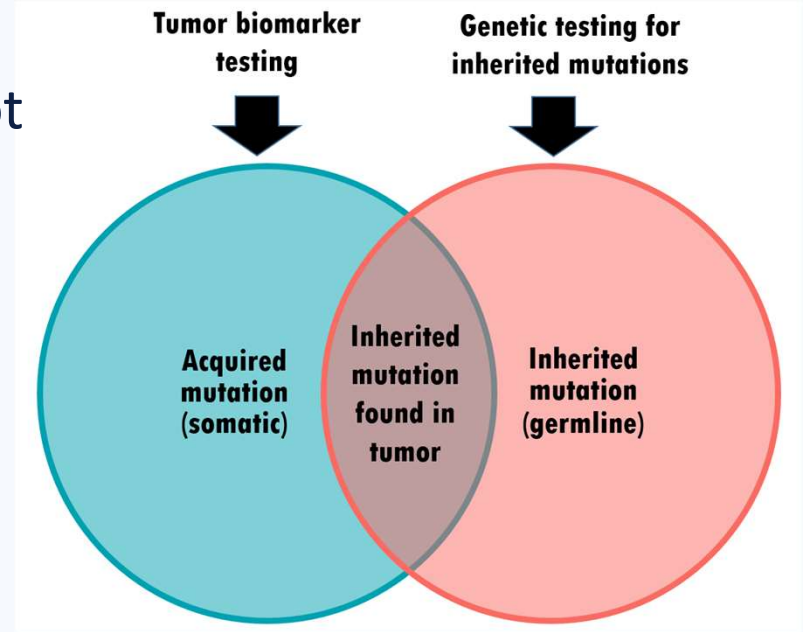
Source: Khan Academy

Acquired/Somatic

- Present in only a subset of cells (tumor)
- Cannot be inherited or passed onto offspring
- Identification of oncogenic driver mutations allows for targeted therapies, avoidance of ineffective therapies *after* cancer develops

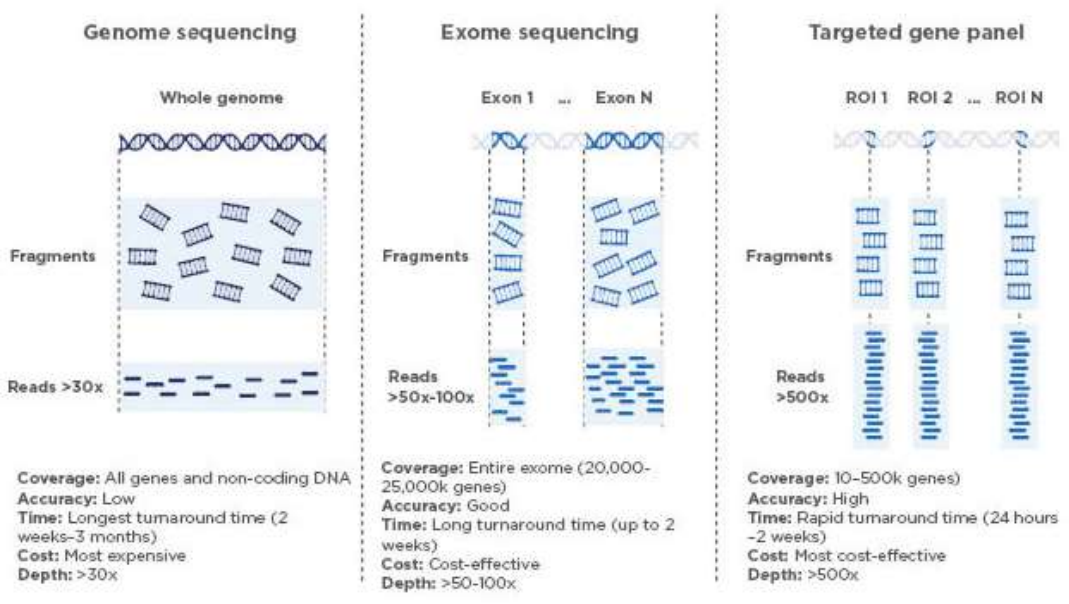
Identification of Germline Mutations on Tumor Testing

- While germline mutations *may* be identified, they can easily be missed or not reported
- Variant Allele Frequency (VAF)
  - Expected 40-60% VAF for germline variants
  - Allelic dropout, post-treatment reversion, sample heterogeneity can lead to lower than expected VAFs
- Intentional filtration
  - Some labs may intentionally filter out germline variants that do not have therapeutic impact



Balancing Read Depth and Coverage

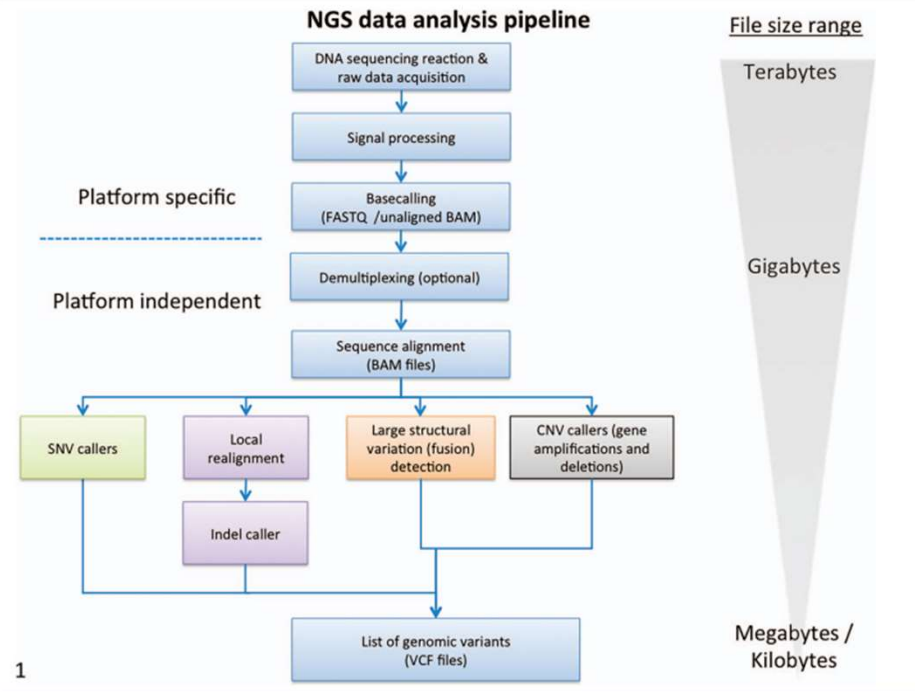
- Read depth = number of times specific region is sequenced
  - Higher read depth gives higher confidence that "call" is correct (true signal instead of noise)
  - Lower read depth allows for broader coverage across genome
- The broader the panel, the higher the likelihood that a variant will be missed
  - Laboratories may "turn up" read depth in regions of interest
  - Tumor panels are designed to maximize detection of actionable variants, not germline variants



Source: Duraes et al. Demystifying the Discussion of Sequencing Panel Size in Oncology Genetic Testing. European Medical Journal 7.2. 2022;7[2]:68-77

New problem? Data management!

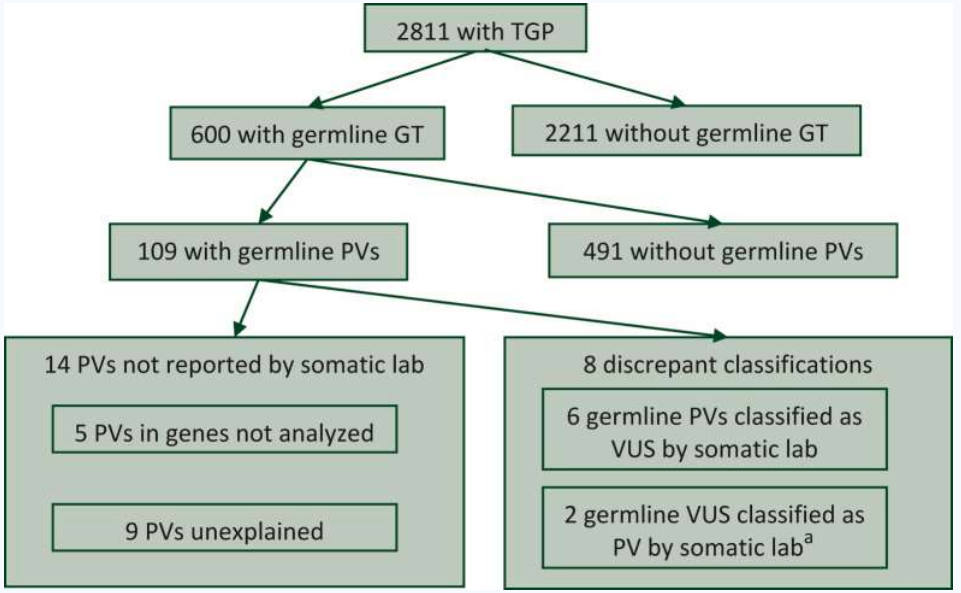
- Single next-gen run will generate massive amounts of data (terabytes!), which leads to high noise to signal ratio
  - Post-test analysis and clean-up is now critical and most expensive part of testing
- Sequencing platforms are largely comparable… but the post-test analysis may not be
  - This makes comparing results between labs extremely difficult as many labs develop primers, software, algorithms in-house



Source: Roy S et al. Next-Generation Sequencing Informatics: Challenges and Strategies for Implementation in a Clinical Environment. Arch Pathol Lab Med. 2016 Sep;140(9):958-75

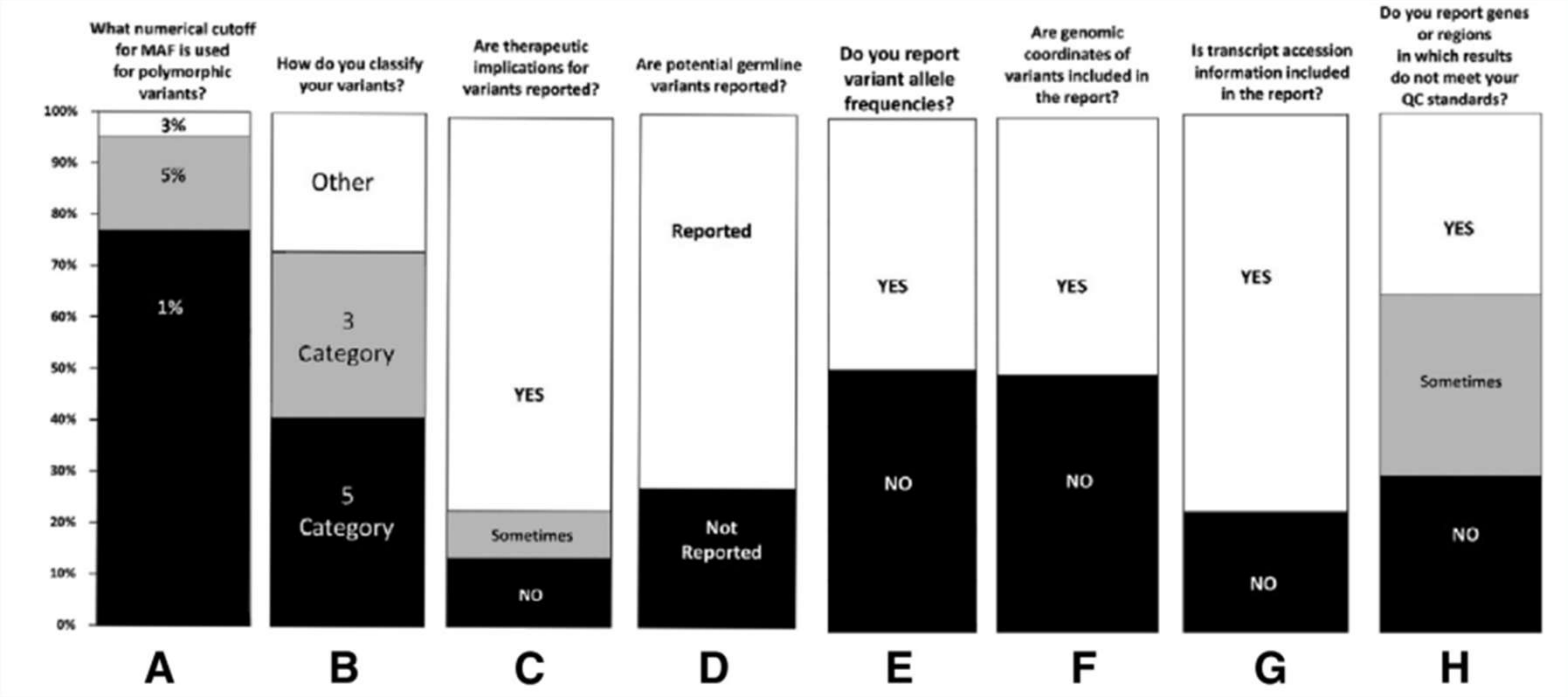
Discrepancies in Variant Interpretation

- Per NCCN guidelines, suspected germline mutations should be confirmed by separate analysis in a CLIA-certified lab
  - Confirmation alone is not enough because variant classification may differ (7%)
- Due to large number of aberrations, germline and somatic nomenclature may also differ
  - Recommend full gene sequencing rather than site-specific analysis for confirmation



Source: Pauley K, et al. Discrepancies between tumor genomic profiling and germline genetic testing. ESMO Open. 2022 Aug;7(4):100526

Discrepancies in Reporting Practices



Source: Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. Li, Marilyn M. et al. The Journal of Molecular Diagnostics, Volume 19, Issue 1, 4 - 23

Takeaways

- Next-gen sequencing is revolutionary but built on the principles of Sanger sequencing
  - You don't have to throw out what you already know!
- Sequencing is cheap. Analysis is not.
  - Genetic tests are like MRIs – just because you *can* doesn't mean you should
  - More is not always better
- Genetic counseling mantra: The *right* test in the *right* patient at the *right* time
  - Collaboration amongst specialties is increasingly important in cancer care

Thank you!

Christie Jett, MS, LCGC
540-446-2800 | cjett@geneticsupportfoundation.org

Resources

Cancer Precision Medicine Commons

Forman A, Sotelo J. Tumor-Based Genetic Testing and Familial Cancer Risk. Cold Spring Harb Perspect Med. 2020 Aug 3;10(8)

Lincoln SE, et al. Yield and Utility of Germline Testing Following Tumor Sequencing in Patients With Cancer. JAMA Netw Open. 2020 Oct 1;3(10):e2019452

Pauley K, et al. Discrepancies between tumor genomic profiling and germline genetic testing. ESMO Open. 2022 Aug;7(4):100526

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. Li, Marilyn M. et al. The Journal of Molecular Diagnostics, Volume 19, Issue 1, 4 - 23