Proactive Approaches and Automated Aspects of Variant Classification

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WHEN DECISIONS MATTER

Lab Accuracy

Can the lab find all of the variants (DNA changes)?

Does the lab classify variants (DNA changes) correctly?

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Variant Classification Process

- Process may be manageable for a few novel variants/day, but what about 100 novel variants/day?
  - Hire more Lab Directors/Genetic Counselors
    - Expensive to maintain
    - Limited number of people review each variant – Error prone
  - Outsource to a third party or use off-the-shelf software
    - Expensive to maintain
    - Who is responsible for accuracy and how is it ensured?
  - Develop and validate automation tools
    - Expensive to develop
    - Lab controls accuracy
    - Cost-effective over time

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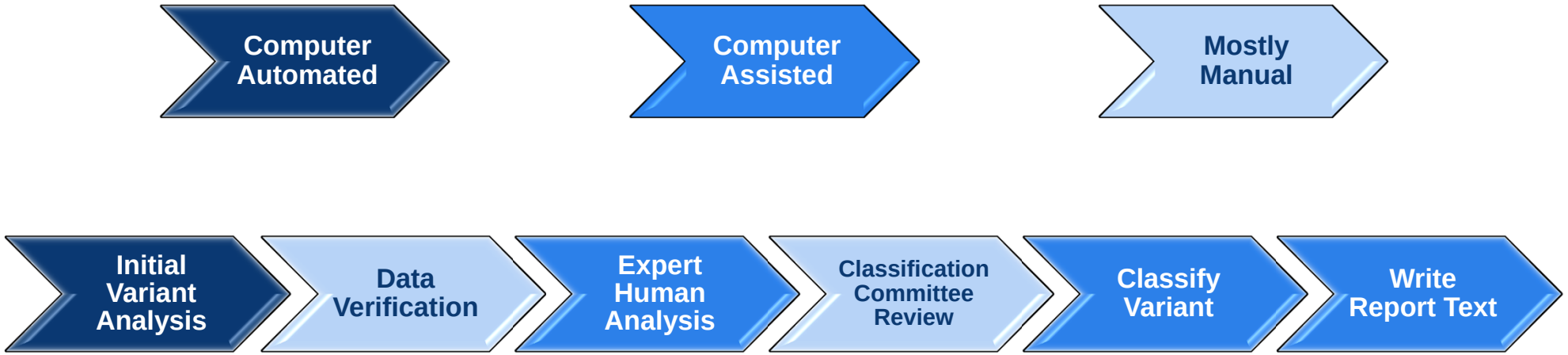
Our Classification Process



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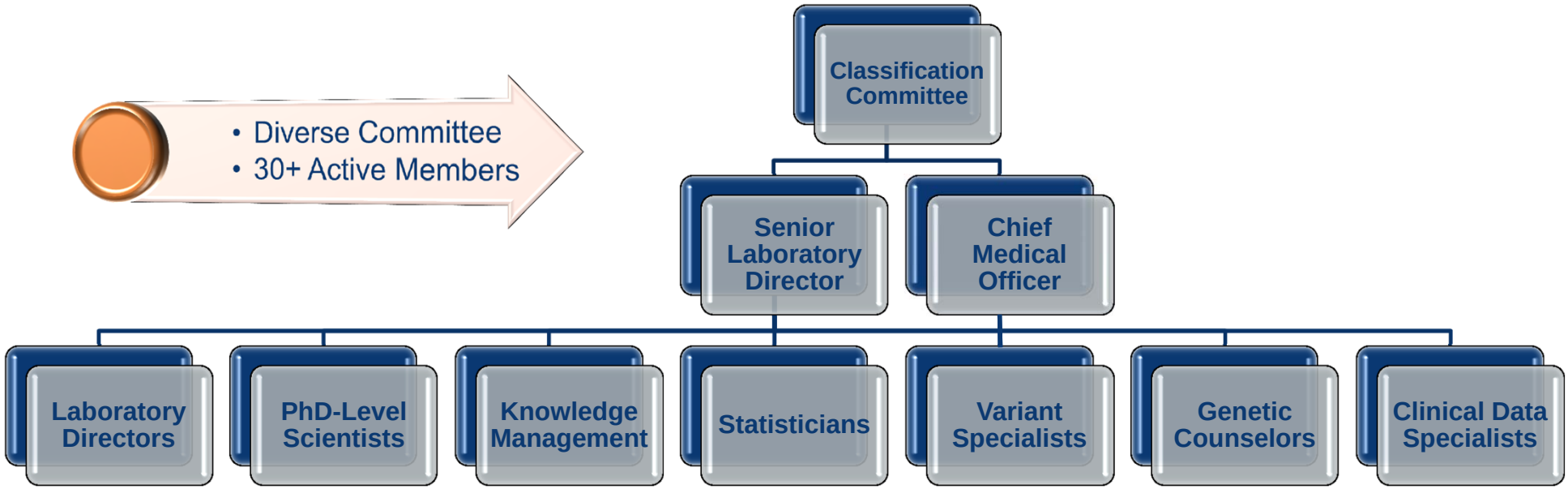
Our Classification Committee

- Diverse Committee
- 30+ Active Members



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VITA (Variant Information Tracking Application)

Presents important sequence information					
Allelic/nearby variants	**Functional domains**	**Exon/intron locations**	**Splicing analysis**	**Population data**	**Complex regions**

Automated Computer Analysis

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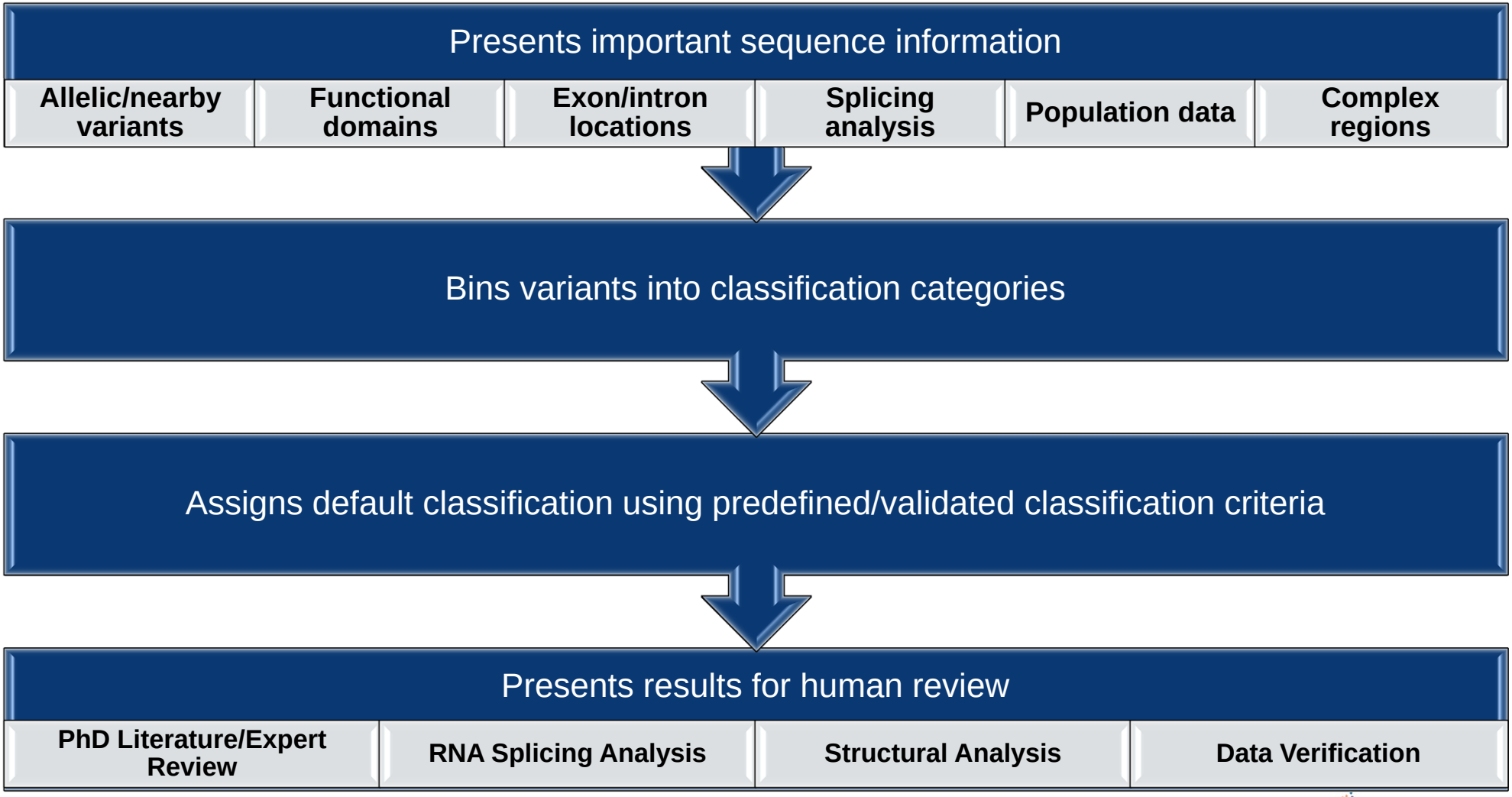
VITA Classification Program



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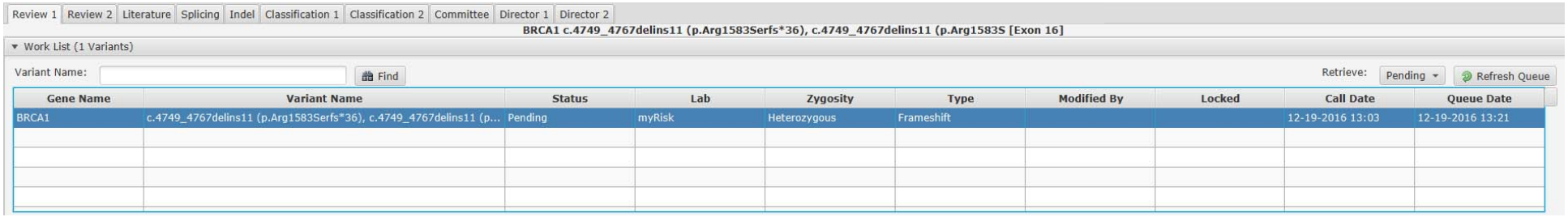
VITA Classification Program



- Well Controlled Program Ensures Quality
  - Queue system ensures all analyses are performed before classification committee review and final classification
  - Computer enforces classification verification by multiple individuals
  - Computer alerts users of unexpected classifications
  - Computer provides auditable trail of all data and review notes

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Identify and Evaluate Literature

- Critical questions:
  - When and how often should we evaluate the literature?
  - What tools should we use?
    - PubMed
    - Google
    - Third party software
    - Laboratory-developed tools

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Literature Review

- Literature lists are generated daily by an automated algorithm that includes:
  - Searches by multiple gene names
  - Searches alternative nomenclatures (i.e., HGVS vs. BIC)
- The Algorithm:
  - Removes redundant citations
  - Provides URLs to publications
  - Highlights search terms found in each reference
  - Sorts by most relevant citation
- Process and algorithm tested and validated to ensure identification of relevant literature

Esterling L *et al.* Comparison of a Literature Search Algorithm and Curated Publication Database with the Literature Content of Locus Specific Databased. Presented at ACMGG Annual Meeting, March 2016.

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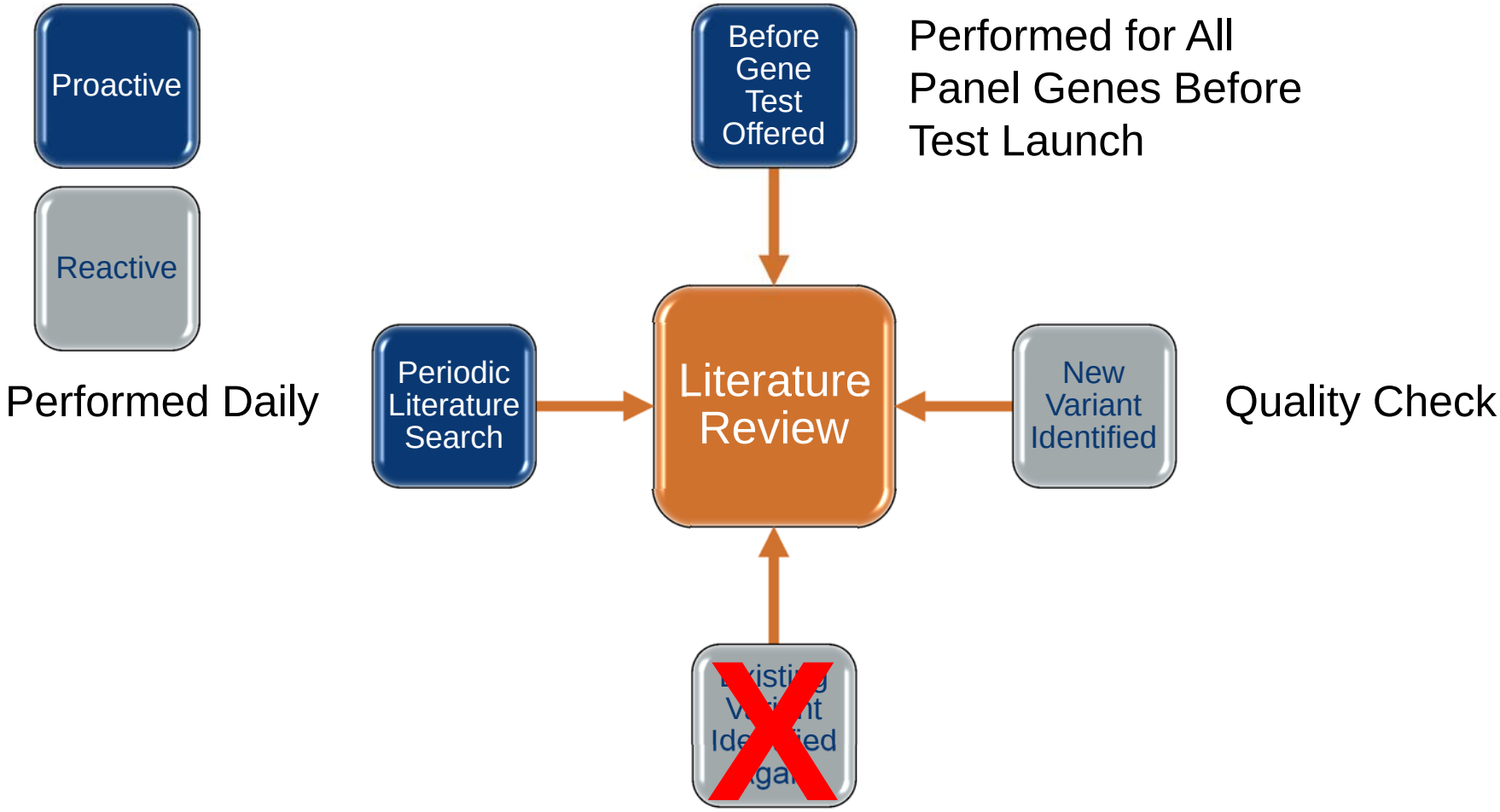
Proactive vs. Reactive Literature Review



Identify and Evaluate Literature

- Knowledge Management – PhD Scientists
  - Review all literature
  - Create a written summary
  - Alert Laboratory Directors and subject experts if significant literature is identified
- Subject Matter Experts – PhD Scientists
  - mRNA splicing analysis – mRNA splicing experts
  - Structural analysis – Structural Biologists
  - Functional analyses –Biochemists
  - Segregation analysis –Geneticists
  - Statistical analysis –Statisticians
  - Population data – Statisticians and Population Geneticists

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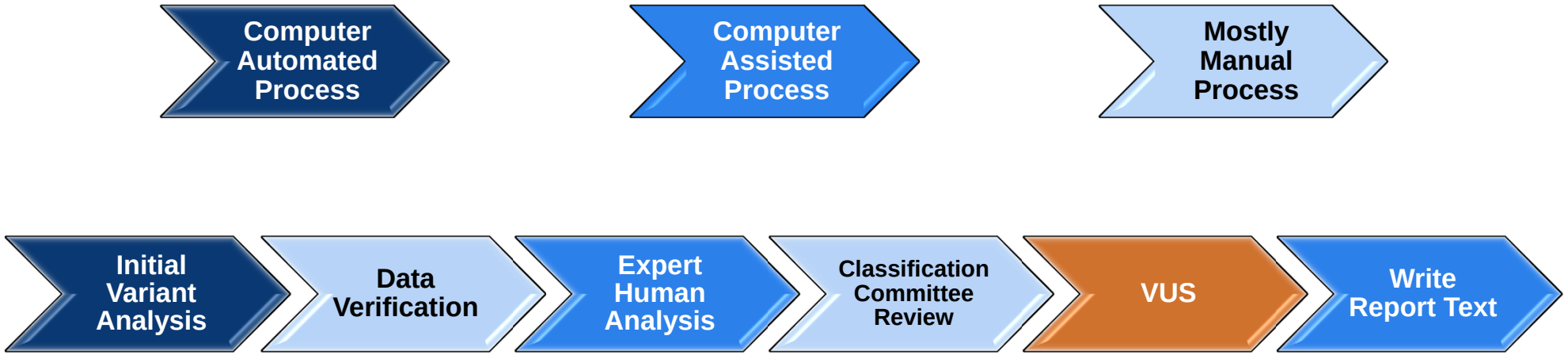
Variant Reclassification



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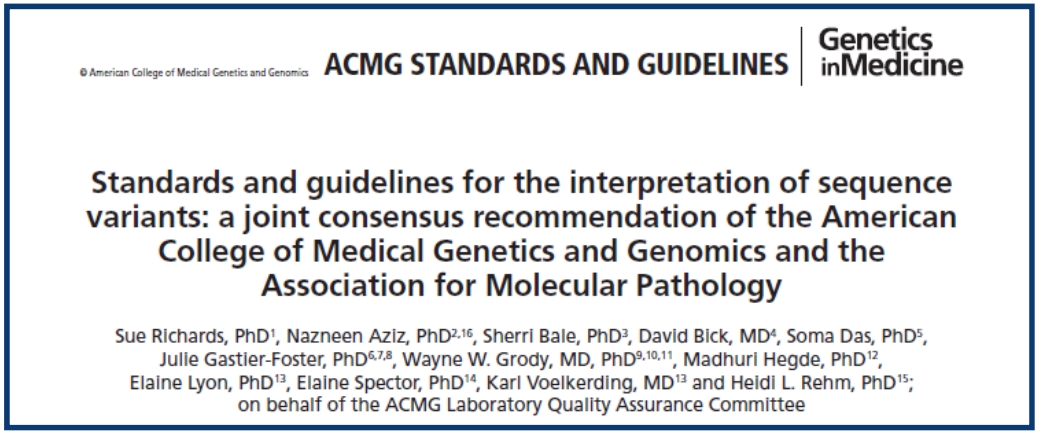
Variant Reclassification

- Variant reclassification has been historically “reactive”



- ACMG classification guidelines are reactive
  - Don’t generate novel data
  - Recommend how to analyze data you already have
- “Proactive” reclassification is critical – Generate our own data
  - Most VUS will never be reclassified if we wait for data to come to us

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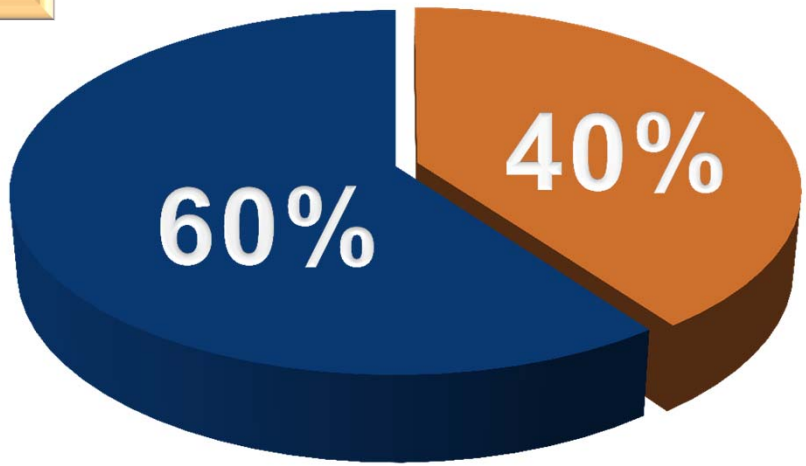
Reclassification Techniques

Performed by Myriad

- Pheno® analysis
- MCO® analysis
- *in*Site™ RNA Laboratory

- Proactive Methods
- Reactive Methods



Performed by Myriad / Possibly by Other Labs

- Literature evaluation
- Population frequency
- In-house segregation analysis
- In-house protein structural analysis
- In-house *in trans* co-occurrence

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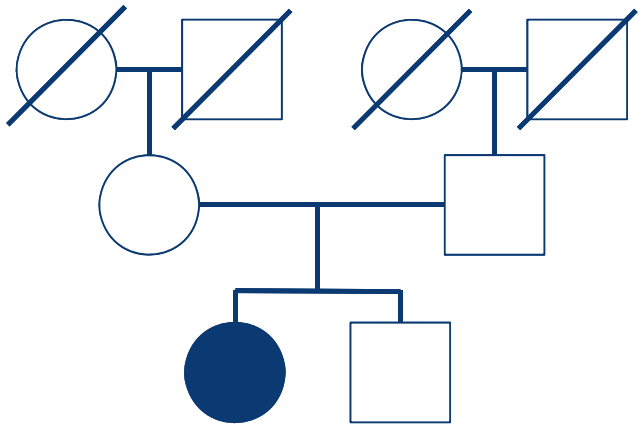
In-House Segregation Analysis

- Classical approach to reclassification
- Limited utility for cancer genetics
  - High phenocopy rate
  - Reduced penetrance for many genes
  - Smaller American family sizes
- Our approach
  - Proactively target variants
    - “Close” to being reclassified
    - Other lines of evidence available
  - Proactively reach out to families and offer free family analysis
  - Store data in custom pedigree program
  - Perform statistical analysis customized to small pedigrees



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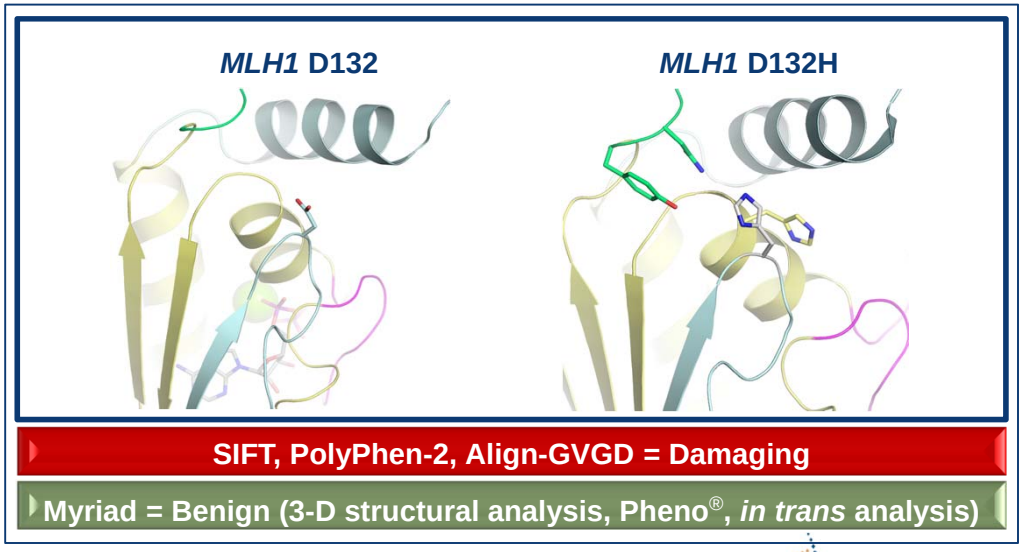
In-House Protein Structural Analysis

- Diagnostic labs generally can't generate crystal structures
- Crystal structures are publicly available – Protein Data Bank (PDB), Protein Data Bank, Europe (PDBe), etc.
- Knowledgeable Structural Biologist required
  - Validate current crystal structures before use
  - Develop additional structures
    - *MLH1* N-terminus – Wu H, Zeng H, Lam R, Tempel W, Kerr ID, Min J (2015). Acta Crystallogr F Struct Biol Commun 71, p981-5.
  - Map variants



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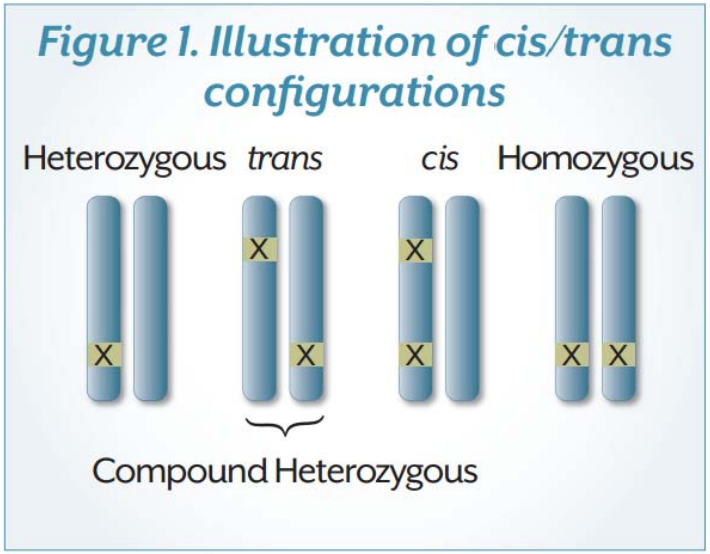
In-House *In Trans* Co-occurrence Analysis

- For many genes, pathogenic variant homozygosity or compound heterozygosity is either lethal or results in a severe phenotype
- Can be used to downgrade variant classification
- We proactively determine phase
  - Offer free family analysis
  - Construct and validate haplotypes
    - Computer determines haplotypes
    - Family testing not required
  - Computer immediately informs classification committee of co-occurrences



Fernandes PH *et al.* Validation of utilizing *in trans* co-occurrence or homozygosity to downgrade the classification of genetic variants in the *BRCA1, BRCA2* and Lynch syndrome genes. Presented at ACMGG Annual Meeting, March 2015.

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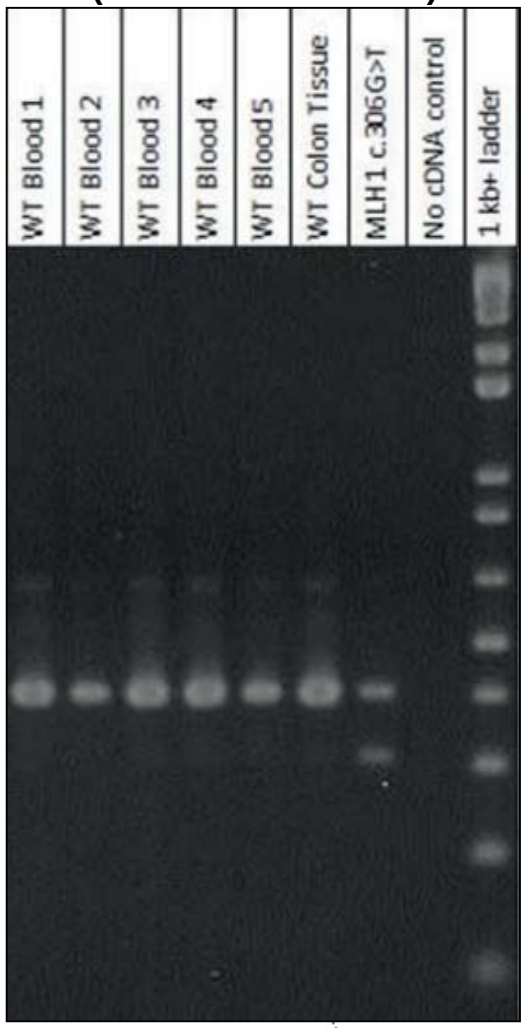
In-House RNA Splicing Analysis

- *In silico* RNA splicing algorithms
  - Flag potential variant-associated splicing defects
  - Often inaccurate
  - Cannot determine full vs. partial splicing defects
  - Additional data required
- Our approach
  - Identify variants with a high likelihood of disrupting RNA splicing
  - Offer free RNA testing to patients
  - Use results to upgrade variants

***MLH1* c.306G>T**
(Last Base of Exon 3)



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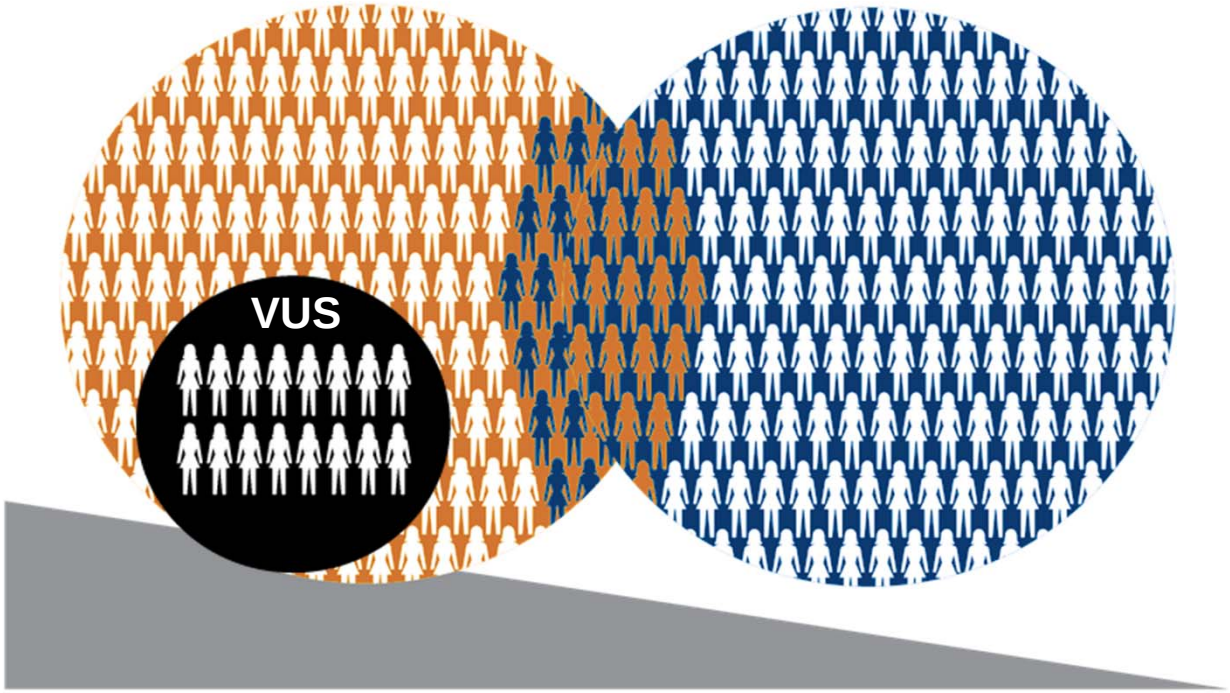
Pheno® Analysis



Severity of Personal and Family History of Cancer

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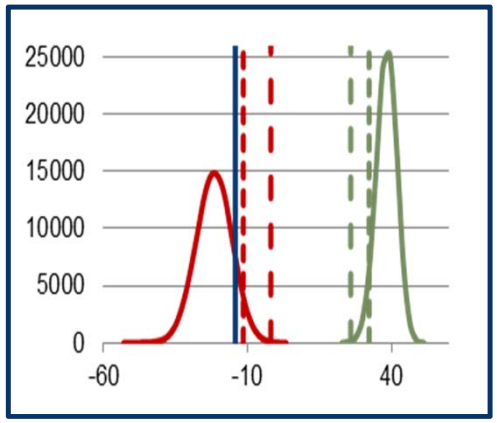
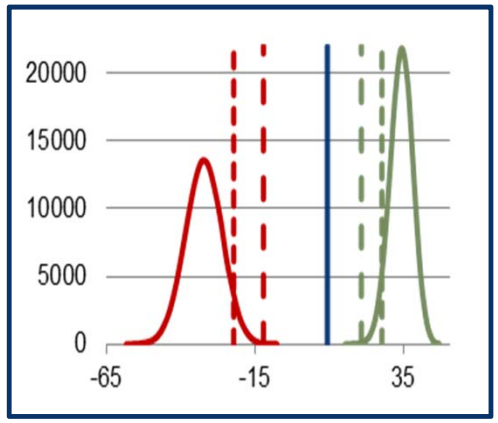
Pheno® Analysis



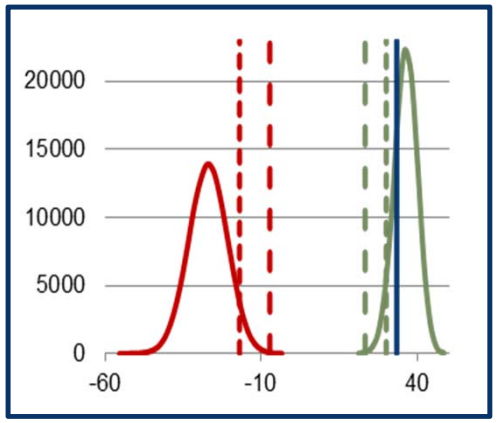
BRCA1 p.Cys61Gly

Pathogenic



BRCA1 p.Arg1699Gln

Lower Penetrance



BRCA1 p.Lys355Lys

Benign

- Pruss D *et al.* Development and validation of a new algorithm for the reclassification of genetic variants identified in the *BRCA1* and *BRCA2* genes. Breast Cancer Res Treat. 2014;147(1):119-32.
- Morris B *et al.* Classification of genetic variants in genes associated with Lynch syndrome using a clinical history weighting algorithm. BMC Genet. 2016 Jul 1;17(1):99.

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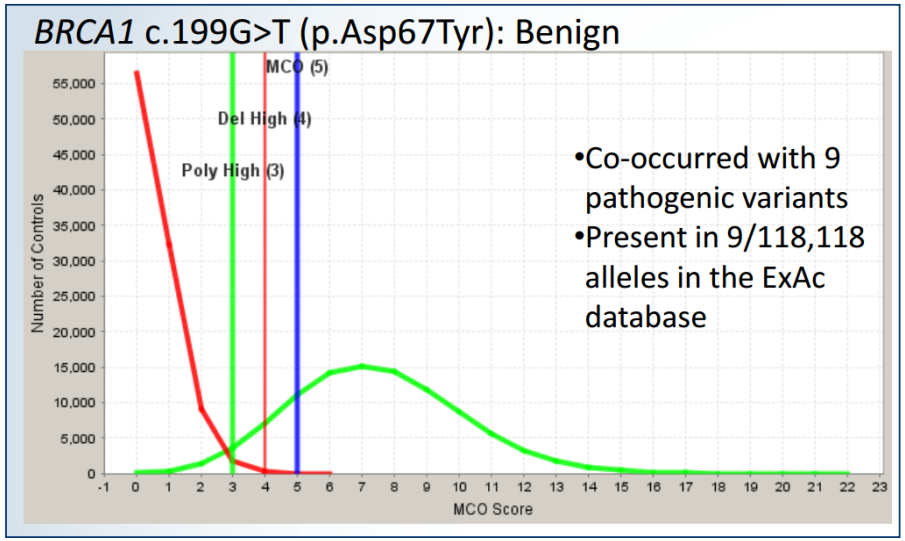
Mutation Co-occurrence (MCO)® Analysis

- It is highly unlikely, but not impossible, to carry 2 pathogenic mutations
  - In same gene *(in cis* or *in trans*), *or*
  - In 2 different genes in the same pathway
    - Example: *BRCA1* and *BRCA2*
- MCO analysis measures the statistical significance of a variant co-occurring multiple times with one or more pathogenic mutations



Coffee B *et al.* Utilization of Mutation Co-occurrence (MCO) Analysis as Evidence for Benign and Likely Benign Variant Classification. Presented at ACMGG Annual Meeting, March 2015.

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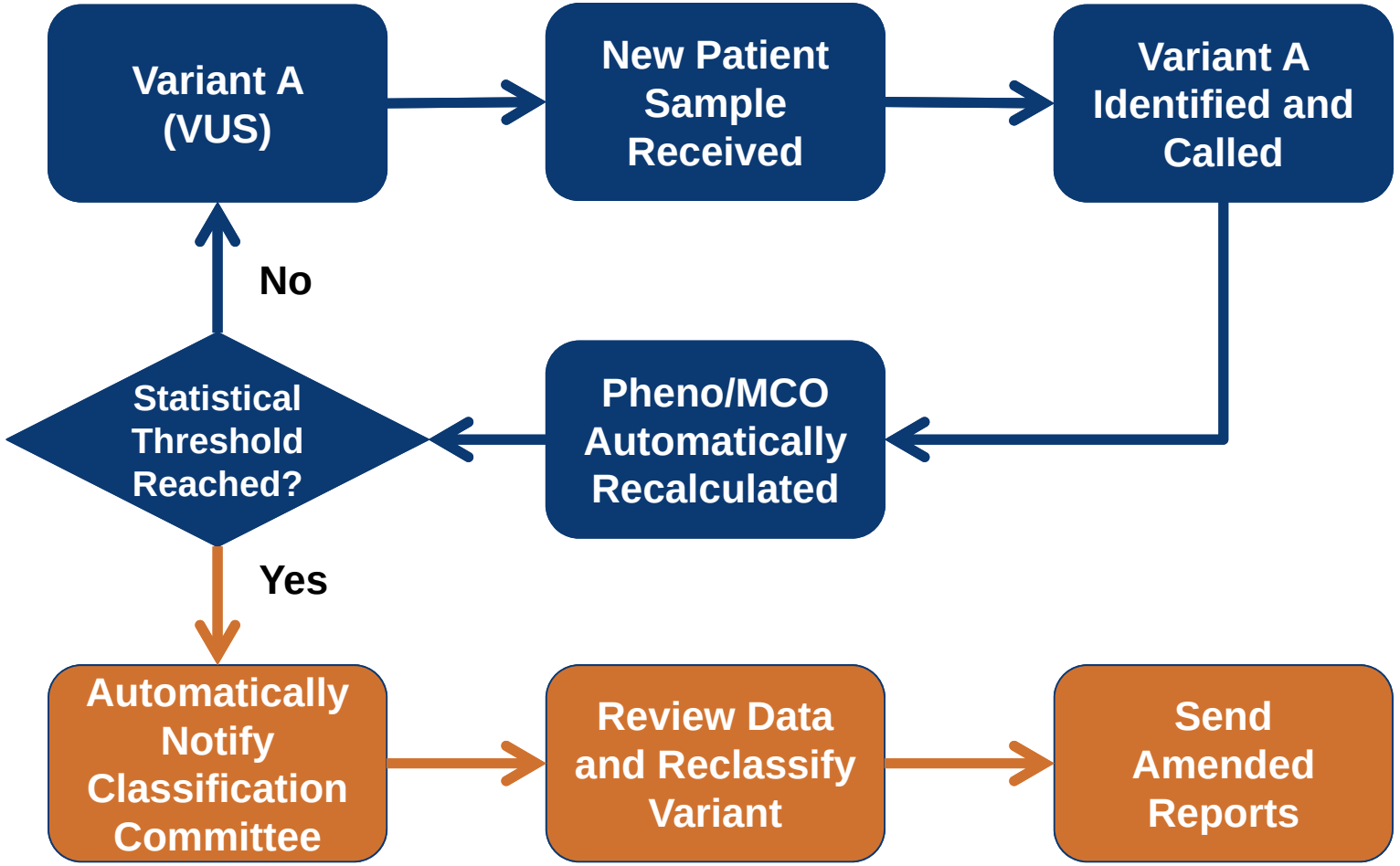
Pheno® and MCO® Automation – 24/7



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Summary

- Larger gene panels require a robust approach to variant classification and reclassification
- ACMG classification guidelines
  - Address how to evaluate data already obtained
  - Do not address how to generate novel variant data
- Laboratories should proactively develop novel classification technologies and offer these to patients/families undergoing testing as part of standard-of-care
- Novel and proactive technologies will advance the science of variant classification, resulting in more definitive test results and improved patient outcomes

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