Whole Exome/Whole Genome Testing
Comparison to Panels

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Disclosures

- I work for a company that offers panel testing and have consulted in the recent past for companies that offer whole exome and whole genome sequencing

Disclaimers

- I will talk about technology and products provided by various commercial entities, but will avoid commercial bias by mentioning brand names
- The field is moving SO RAPIDLY, data and conclusions have a very short half-life

Definitions and Acronyms

- Panels: Sequencing and copy number analysis of a few to a few hundred genes
- WES: Whole Exome Sequencing – the ~2% of the genome that codes for ~ 20,000 proteins
- WGS: Whole Genome Sequencing

The Gettysburg Address

Rlkajgmd wierm mv KsdlijTJnmv ndsFour

scoreqiWlFklsauuuq Fksjjdmncd andruiw wol

vaopbm mSdirseven askjjsdkllQwo yearsgjjgf

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jsdf iobroughtkdsklaoiwq forthjaskkj

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scoreqiWIFklsauuq Fksjjdmncd andruiw wol

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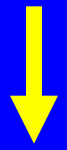
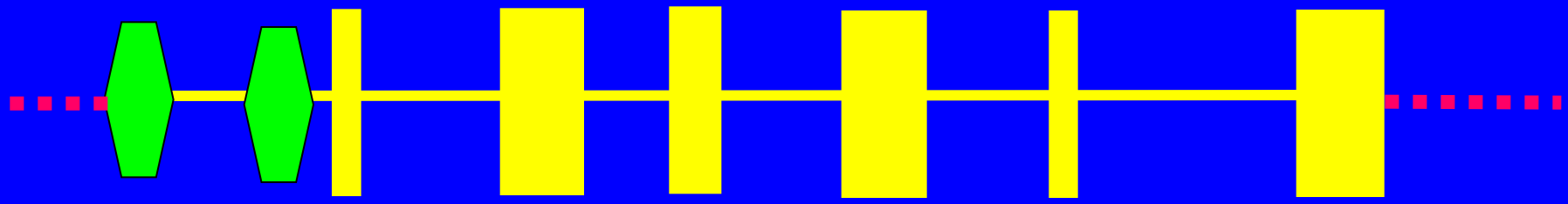


"splicing"

Four score and seven years ago our forefathers brought forth...

Anatomy of a “typical” Gene



Exons (contain the genetic code)

Introns

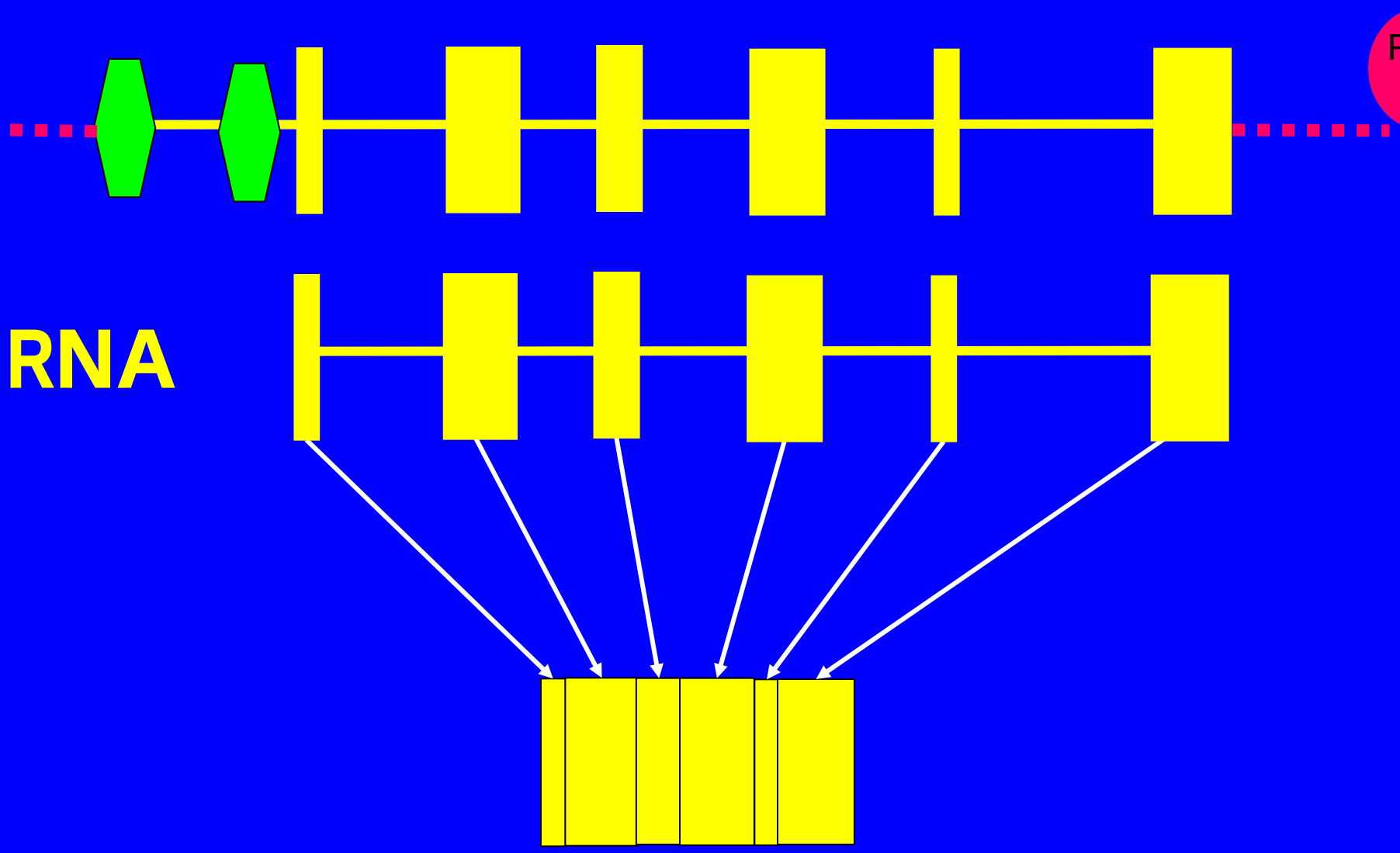
Regulatory regions

~2% of human genome

~20% of human genome

DNA between genes

Gene Expression



Introns excised and exons spliced together to produce the final mRNA for translation into protein

Next Generation Sequencing (NGS) is “Massively Parallel”

- Most platforms attempt to sequence many (many) millions of spatially separated, DNA fragments simultaneously within a flow cell through which reagents can be delivered and removed by cycling
- Methods differ as to read length, accuracy, and throughput

Technologies Are Changing

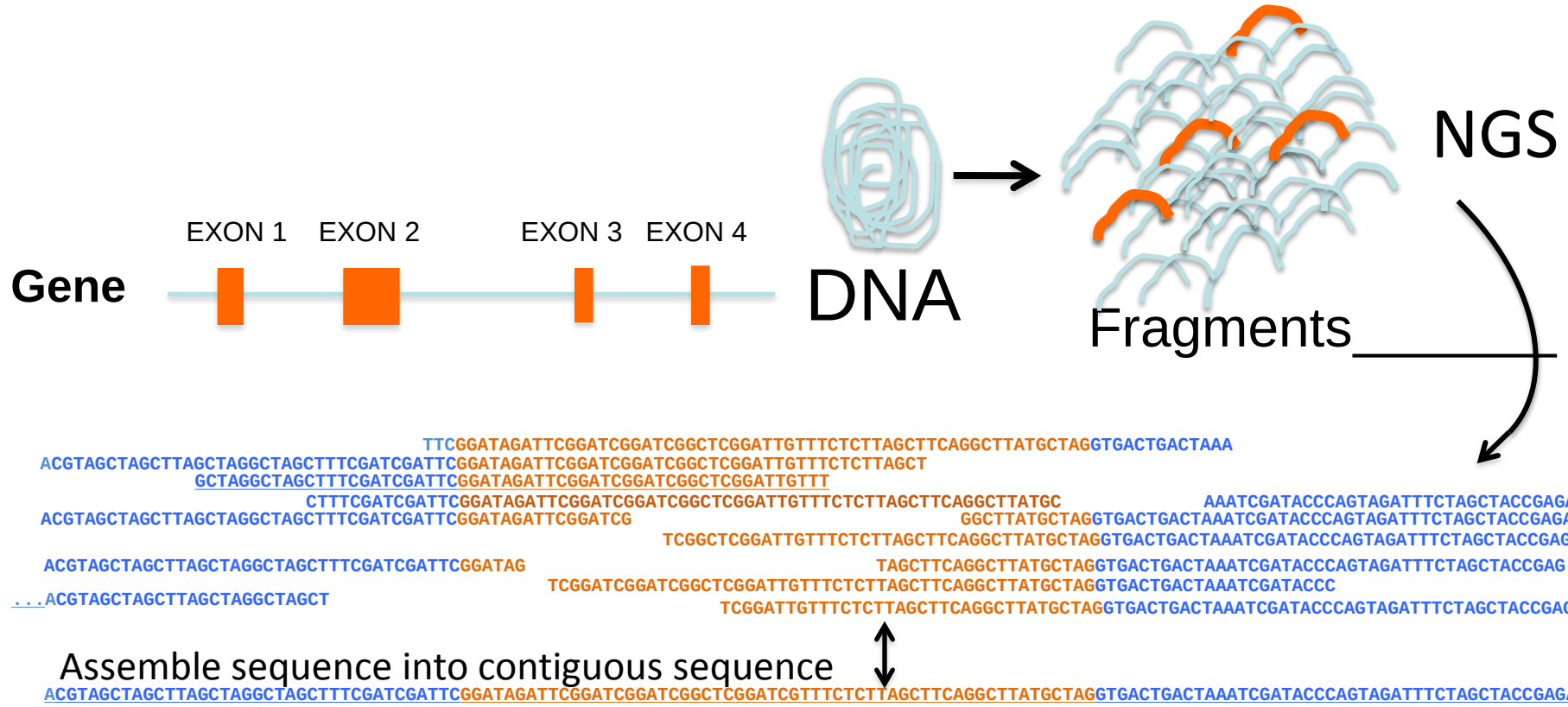
- Panels are in transition from (multiplex) PCR of individual fragments followed by Sanger sequencing to greater use of NGS
- WES/WGS all use NGS

Technologies Are Changing

- Panels by NGS rely on hybrid capture of target exons
- WES usually relies on hybrid capture but a WES can be “extracted” from WGS bioinformatically
- WGS does not rely on hybrid capture

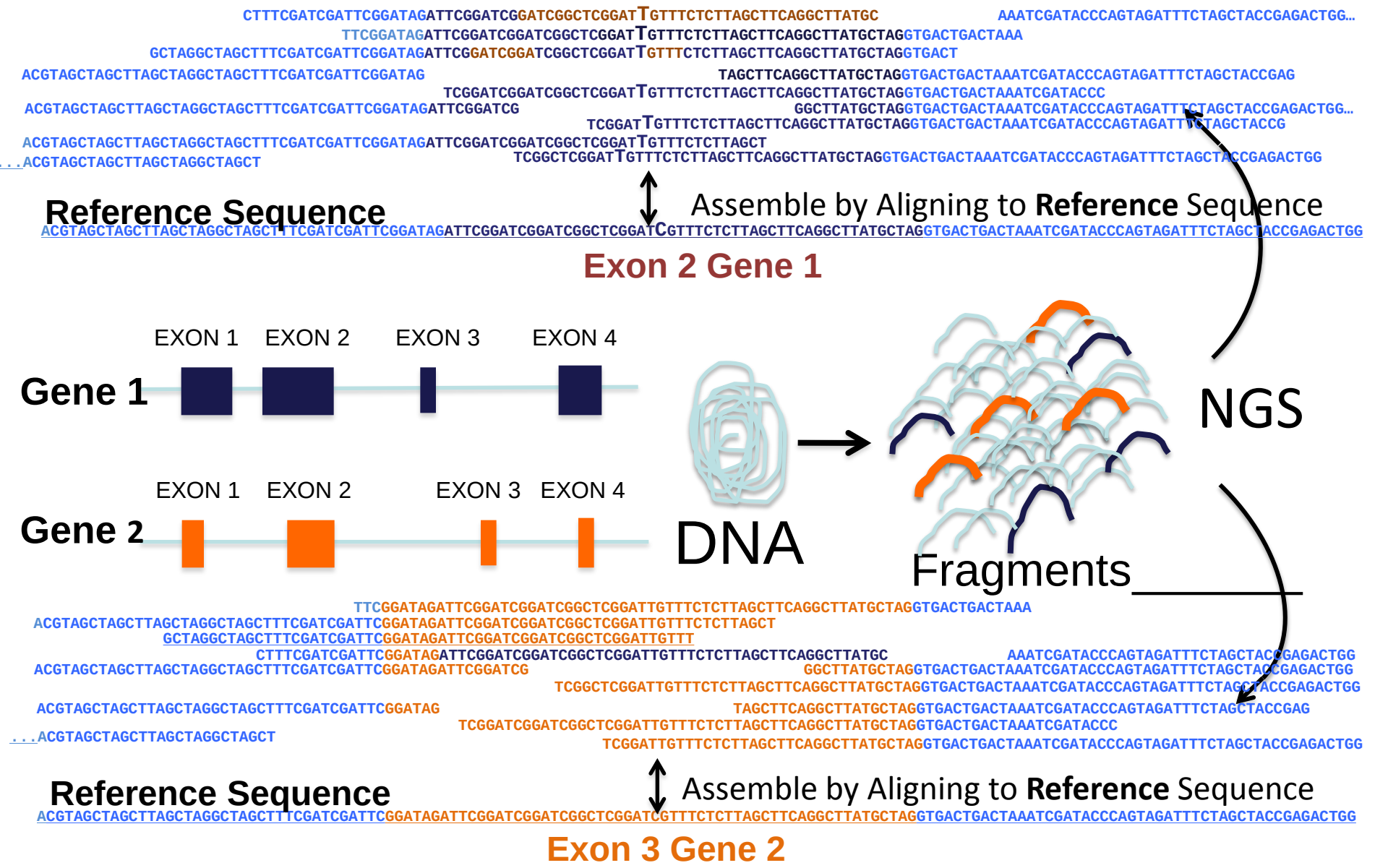
Whole Genome Sequencing

“Holy Grail": De Novo Assembly



Short read lengths and repetitive DNA sequences make this challenging

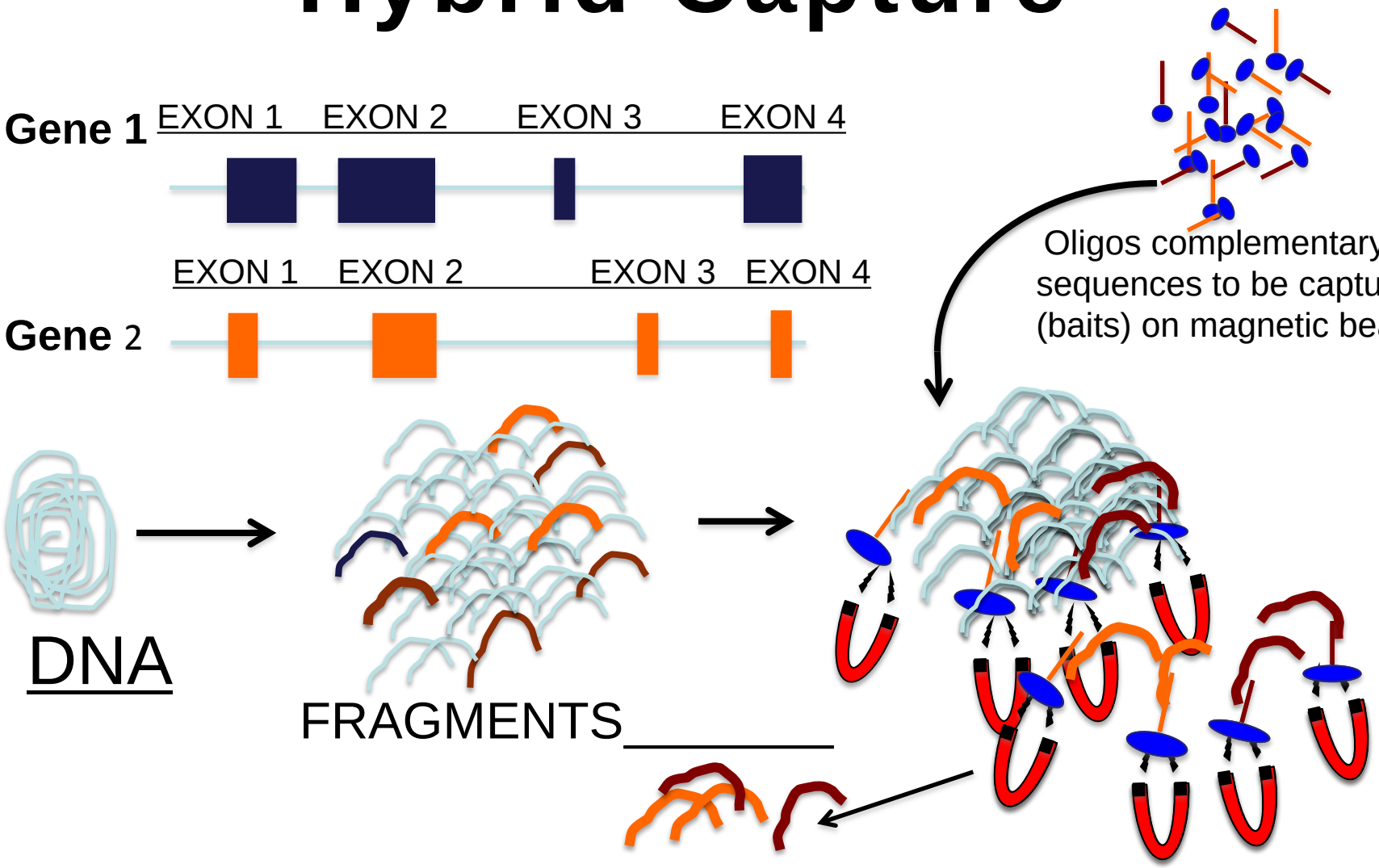
Whole Genome "Re-Sequencing"



Whole Exome Sequencing

- The coding regions plus ~20 bp to either side of the exon in order to see the splicing signals

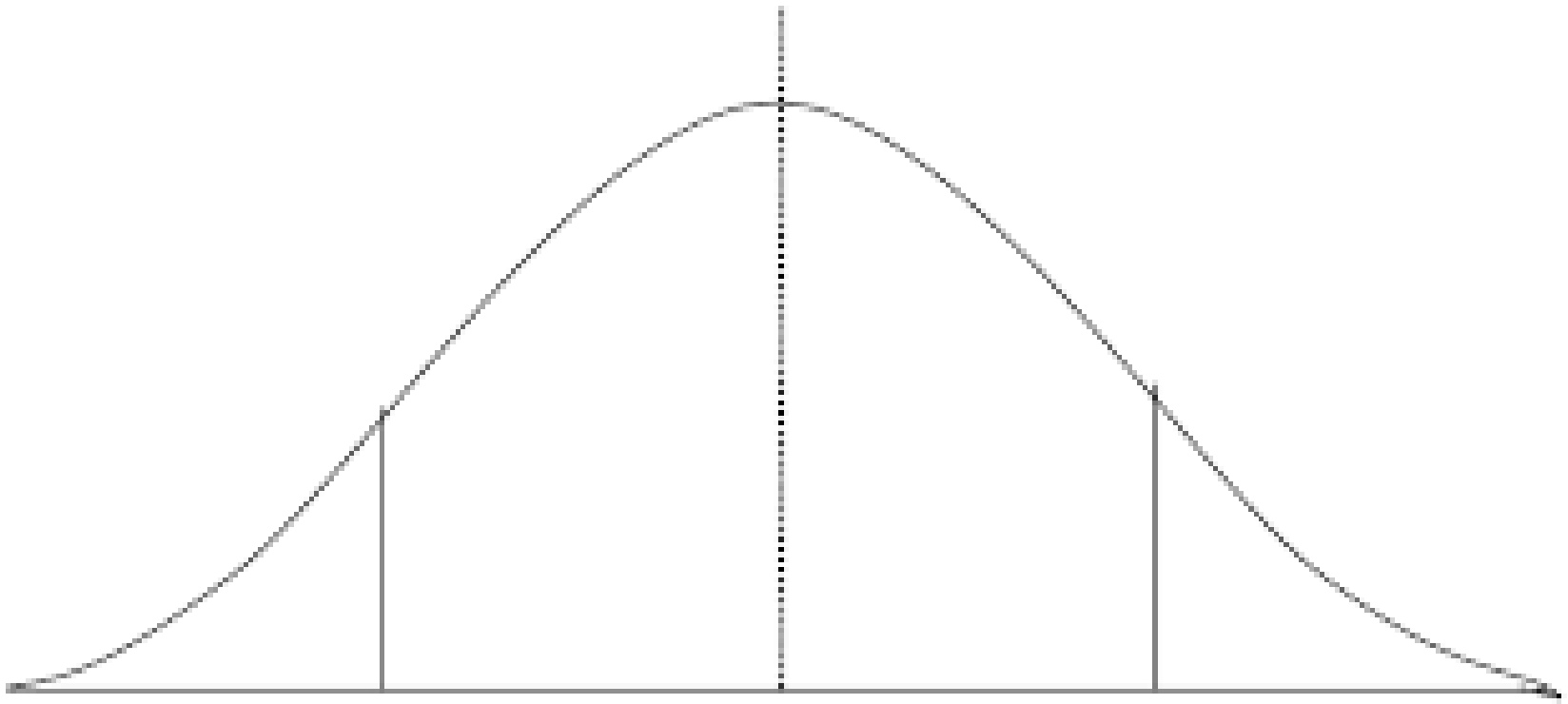
Hybrid Capture



Analytic Validity Issues

- Can you see what’s there?

Coverage – Random Effects

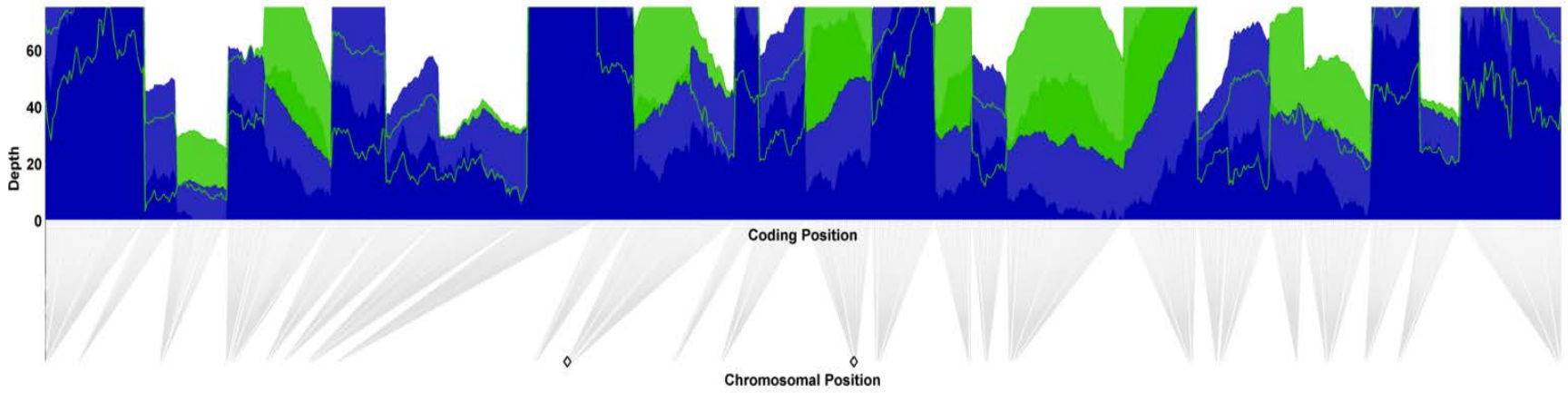


Coverage – Non-Random Effects When Aligning to Reference "Hard-to-Sees"

- Insertions/Deletions of various sizes
- Trinucleotide Repeats
- Repetitive sequences, closely similar pseudogenes, etc.

Coverage – Non-Random Effects of Hybrid Capture

- **Exons are variably captured by standard exome baits**



Courtesy of Sarah Garcia

Improving Random Coverage costs $$$

- WES sacrifices genome completeness for sake of greater coverage of the most clinically valid portions of the genome at a given cost
- Panels are even more cost-conscious, generating even higher coverage of pertinent genes at much lower cost than WES

Improving Non-Random Coverage costs $$$

- Improve the reference
- Seeing hard-to-do variants requires gene-specific custom solutions that are hard to deploy genome wide
- Modify capture baits and chemistries

Clinical Validity

- Understanding what specific variants mean for health is a **work in progress**
- 20,000 genes, of which ~4,500 have been implicated in disease
- Millions of different variants, some implicated in disease, others not, most unknown
- Most (~85%) variants rare, specific to an ethnic group, a family, or even in individual

Clinical Validity

- Understanding what specific variants in **non-coding portions** of genome is not even in its infancy, it's embryonic
- 98% of the sequence obtained by genome sequencing is in this category
- Many many millions of different variants, some implicated in disease, others not, most unknown

Clinical Utility

- What test is most useful for making a diagnosis or changing management

Panels – Diagnostic Advantages

- Panels target specific indicated genes
- Sequence of exons are all delivered unless otherwise specified
- Del/Dup are all delivered unless otherwise specified

➢ A negative result approaches being a true negative but only for the genes on the panel (Beware of false negatives due to deep intronic mutations

Why Use a Panel?

Use a panel when clinical evaluation suggests a particular diagnosis

Do not order a panel when the diagnosis is unclear or uncertain

WES/WGS - Advantages

- Opportunity to discover new genes involved in known disease phenotypes
- Opportunity to define new disease phenotypes or solve diagnostic dilemmas caused by genes previously not known to cause human disease

WES/WGS – Application to Undiagnosed Disease

- A positive or likely positive result in a characterized gene was identified in 30% of patients (152/500).
- A novel gene finding was identified in 7.5% of patients
- The highest diagnostic rates were observed among patients with ataxia, multiple congenital anomalies, and epilepsy (44, 36, and 35%, respectively).
- Twenty-three percent of positive findings were within genes characterized within the past 2 years.
- The diagnostic rate was significantly higher among with a trio (37%) as compared with a singleton (21%) study.

Farwell et al. (2015) Enhanced utility of family-centered diagnostic exome sequencing with inheritance model–based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med. 2015 Jul;17(7):578-86.

Panels	vs.	Exomes/Genomes
• Complete on a per gene basis • Difficult to stay current given pace of new gene discovery • Best when clinical diagnosis is clear or differential diagnosis is limited and genes involved are known • Rarer and rarer disorders add more and more content that is technically and economically demanding and yet offers testing fewer and fewer patients		• Comprehensive on a genome basis • Difficult to guarantee each gene of interest will be covered each time • Best when clinical diagnosis is obscure and the genes involved are largely unknown (Undiagnosed Diseases) • One test covers most of rare disorders within one assay

Incidental and Secondary Findings

Incidental Findings:

- Results not related to the indication for the test that are discovered in the course of a diagnostic test and may be of medical value

Secondary Findings:

- Results not related to the indication for the test that nonetheless should be deliberately sought after regardless of the indication for the test

Incidental and Secondary Findings Original ACMG Recommendations

American College of Medical Genetics Working Group, 2013

56 genes and 24 conditions that clinical laboratories have an obligation to actively seek out in the course of WES/WGS

- clinicians and laboratory personnel have a fiduciary duty to prevent harm by warning patients and their families about certain Incidental findings
- this duty supersedes patient autonomy
- recommendations include reporting test results for adult-onset conditions to parents of children undergoing WGS/WES, regardless of parent preferences

In essence, ACMG report recommended that certain Incidental Findings be considered Secondary Findings

Incidental Findings – Controversy!!

Burke et al., Genet Med 2013 – Clinical Validity, Utility, and Ethical Concerns

- many of ACMG's 56 genes have an unknown natural history
- ascertainment bias, as mutations were identified in those with disease
- phenotypic spectrum and penetrance not always known
- lack of controlled studies regarding interventions
- prior probability of disease is low – Lowers the PPV
- costs should not be generated if patients do not want results

Clayton et al., Genet Med 2013:

- no case law regarding Incidental Findings discovered from genetic or genomic testing
- "….health providers may face liability if they fail to disclose Incidental Findings that would have offered an opportunity to prevent or alter the course of future disease…"

Incidental and Secondary Findings Revised Recommendations

- 2014: ACMG recommends that patients be given the choice to opt out *before* testing takes place, so that results that they would wish not to receive are not generated.

Receptiveness to Learning Secondary Findings

Shahmirzadi et al., 2014

- 187/200 (93.5%) individuals undergoing diagnostic WES chose to receive one or more categories of Incidental Findings

Sapp et al., 2014

- In children undergoing WES, parents had the most positive attitudes toward learning about variants that predispose to disorders treatable or preventable in childhood.
- They had reservations about learning about predispositions for untreatable adult-onset conditions and carrier status for recessive conditions.

Panels: Incidental Findings

There aren’t any!

Consent - WGS/WES

- Basic genetics (genes, mutations), inheritance patterns
- Penetrance and expressivity
- Types of DNA variants (pathogenic, benign, VUS)
- False negatives
- Genetic Information Nondiscrimination Act of 2008

Consent

Rigter et al., 2014

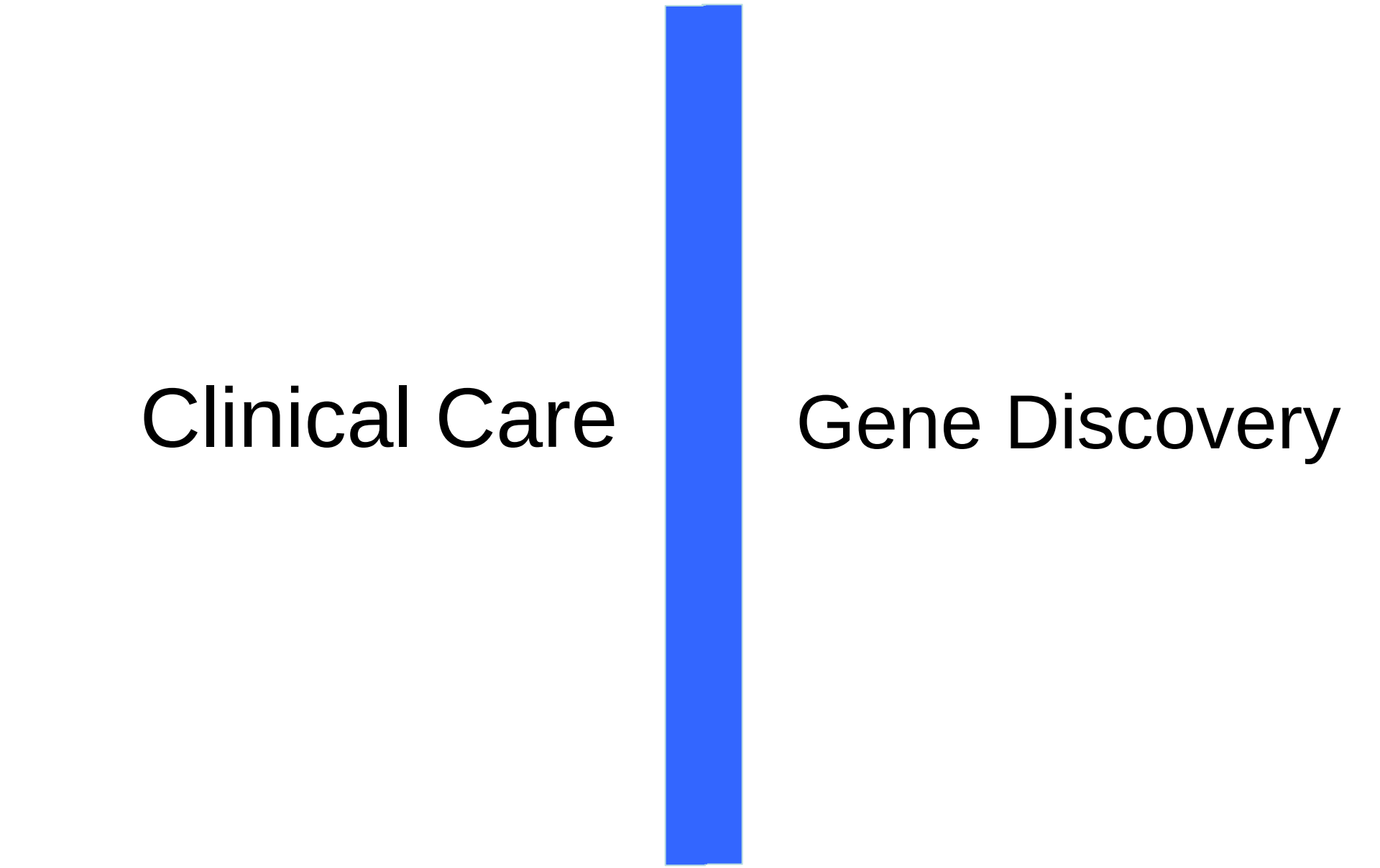
“In any case I think that it's very naïve to think that a patient is more able to choose [which results to receive] when he knows more. There are limits to what patients can comprehend. Decision-making in principle does not get easier, the more elaborately a patient is informed….the quality is important and also a discussion... (ethicist)”

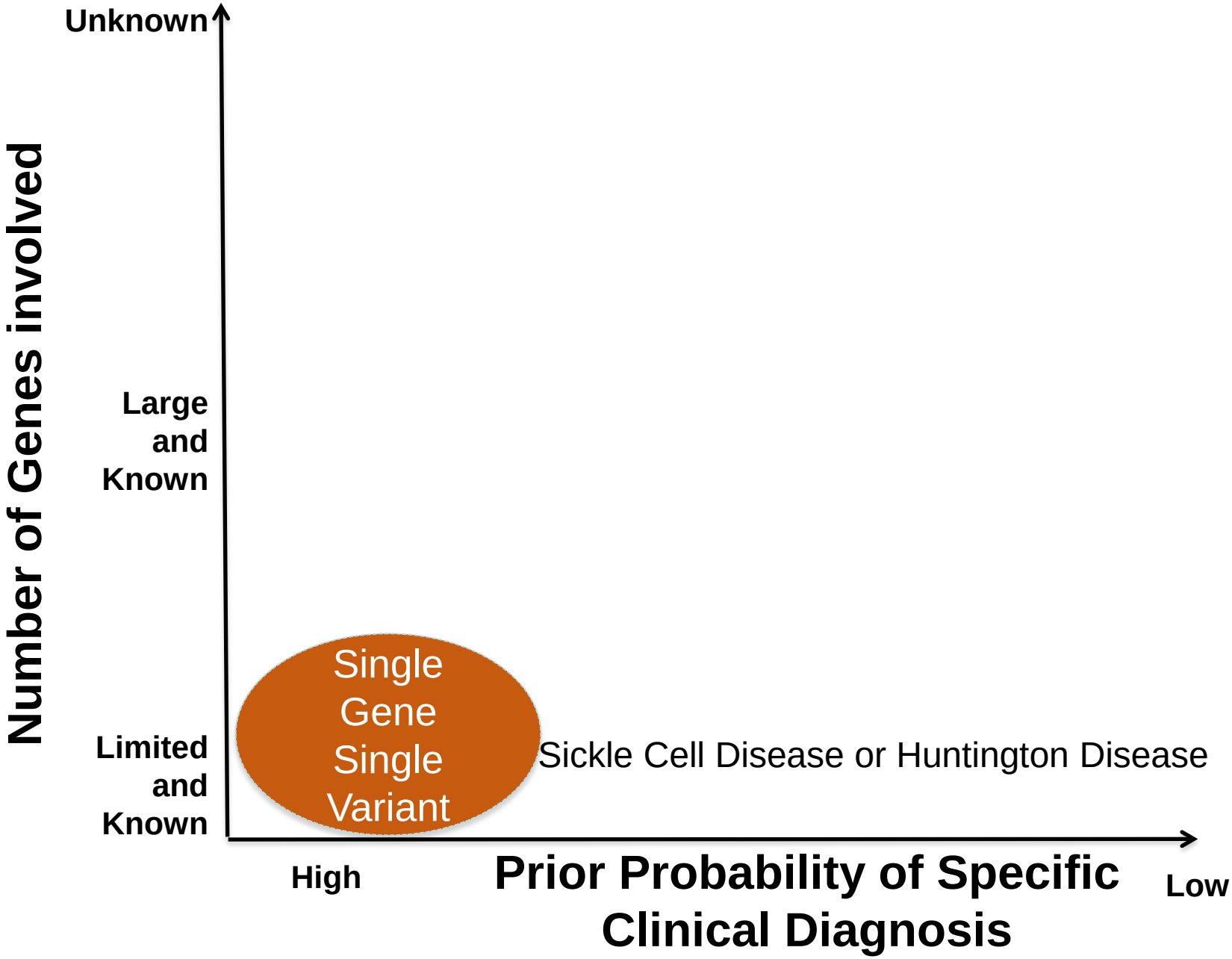
“Can you really give informed consent when you look so widely [at the genome]? Is that manageable for patients?... (patient representative)”

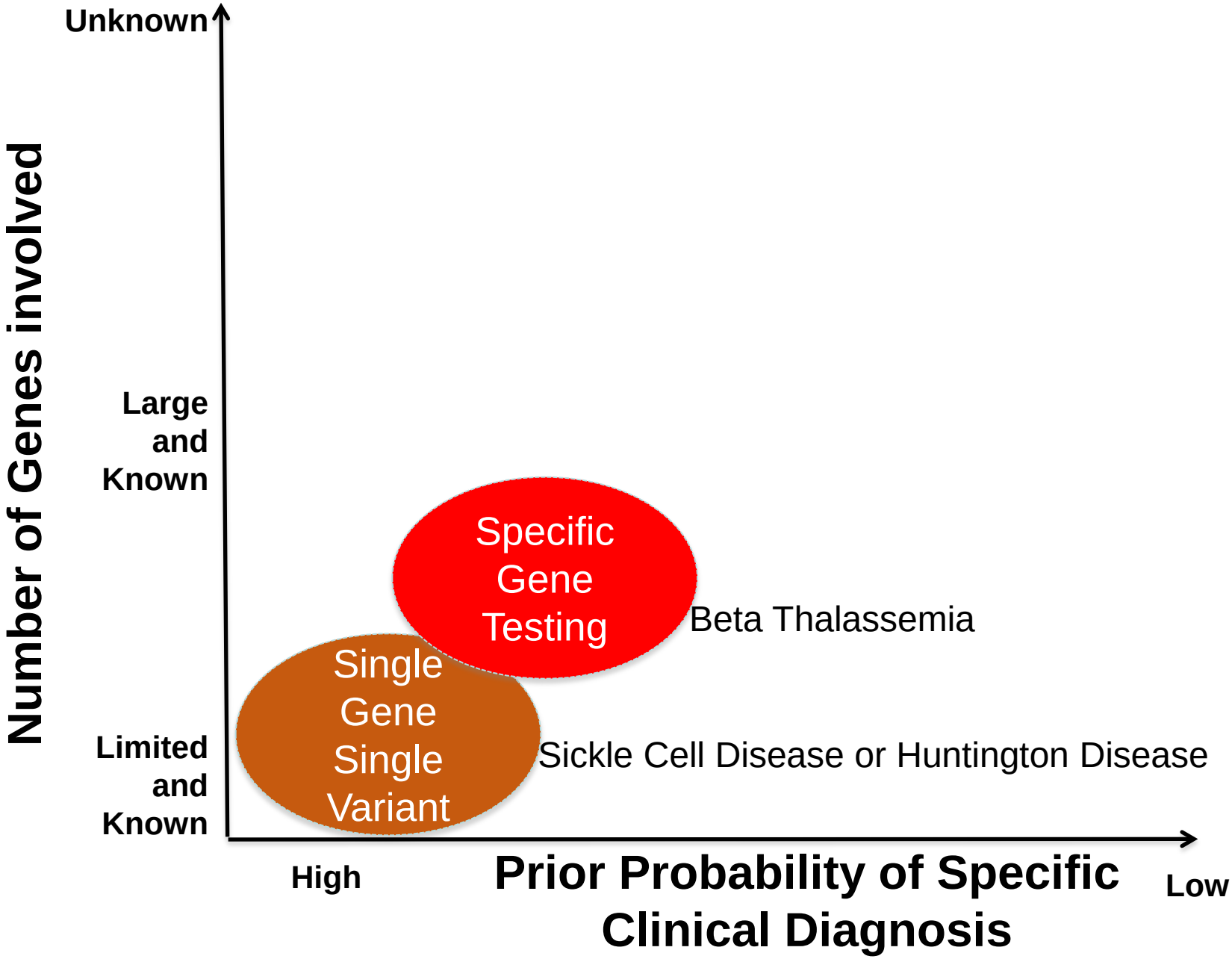
Tabor et al., 2012

“The part of me that was saying ‘Hurry up, let’s get on with it’ was in conflict with the part of me that says, ‘Well, this is good, they’re doing it properly.’ ”

Human Genetics




Unknown
Number of Genes involved
Large and Known
Limited and Known
Single Gene Single Variant
Sickle Cell Disease or Huntington Disease
High
Prior Probability of Specific Clinical Diagnosis
Low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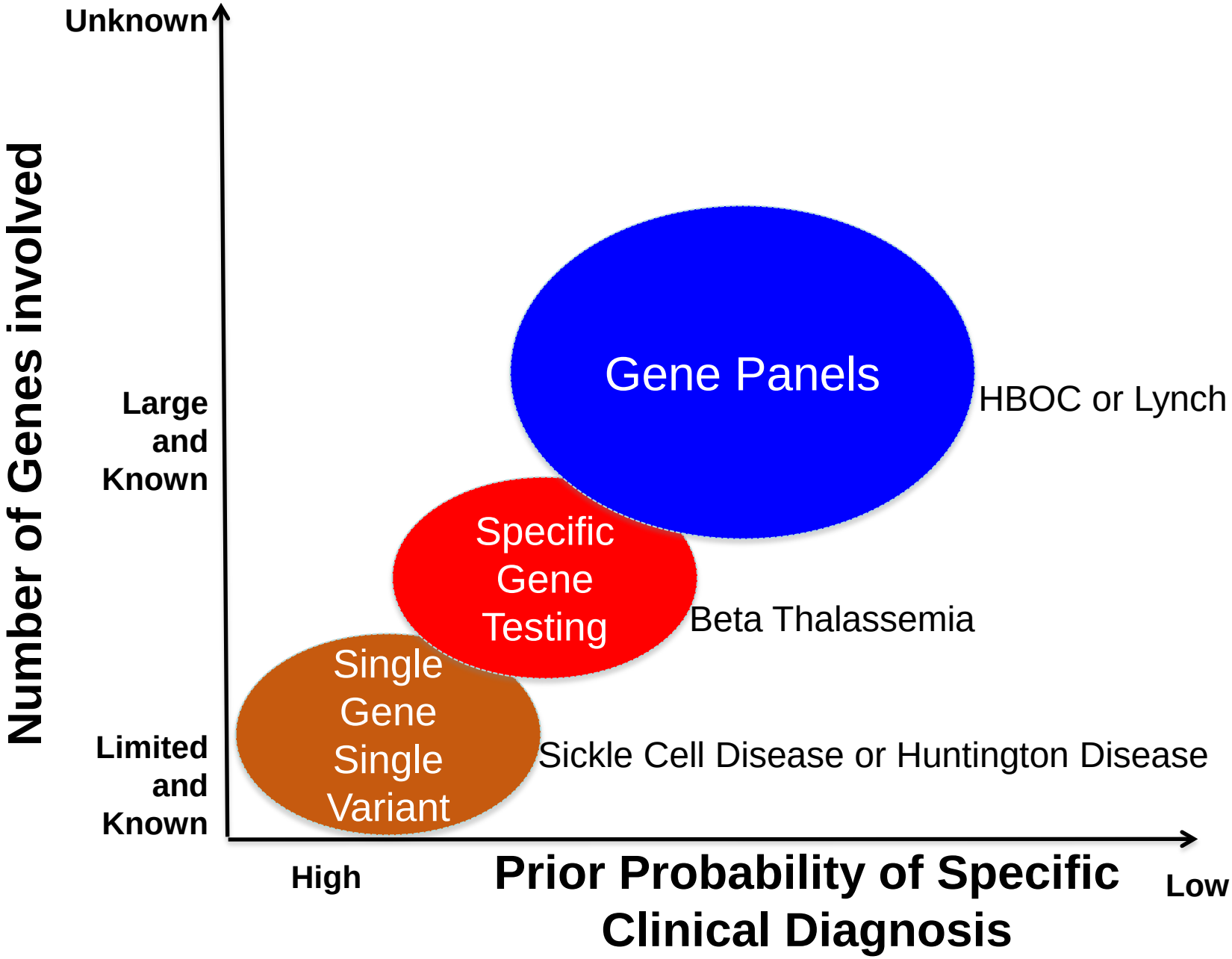
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Limited and Known
Gene Panels
HBOC or Lynch
Specific Gene Testing
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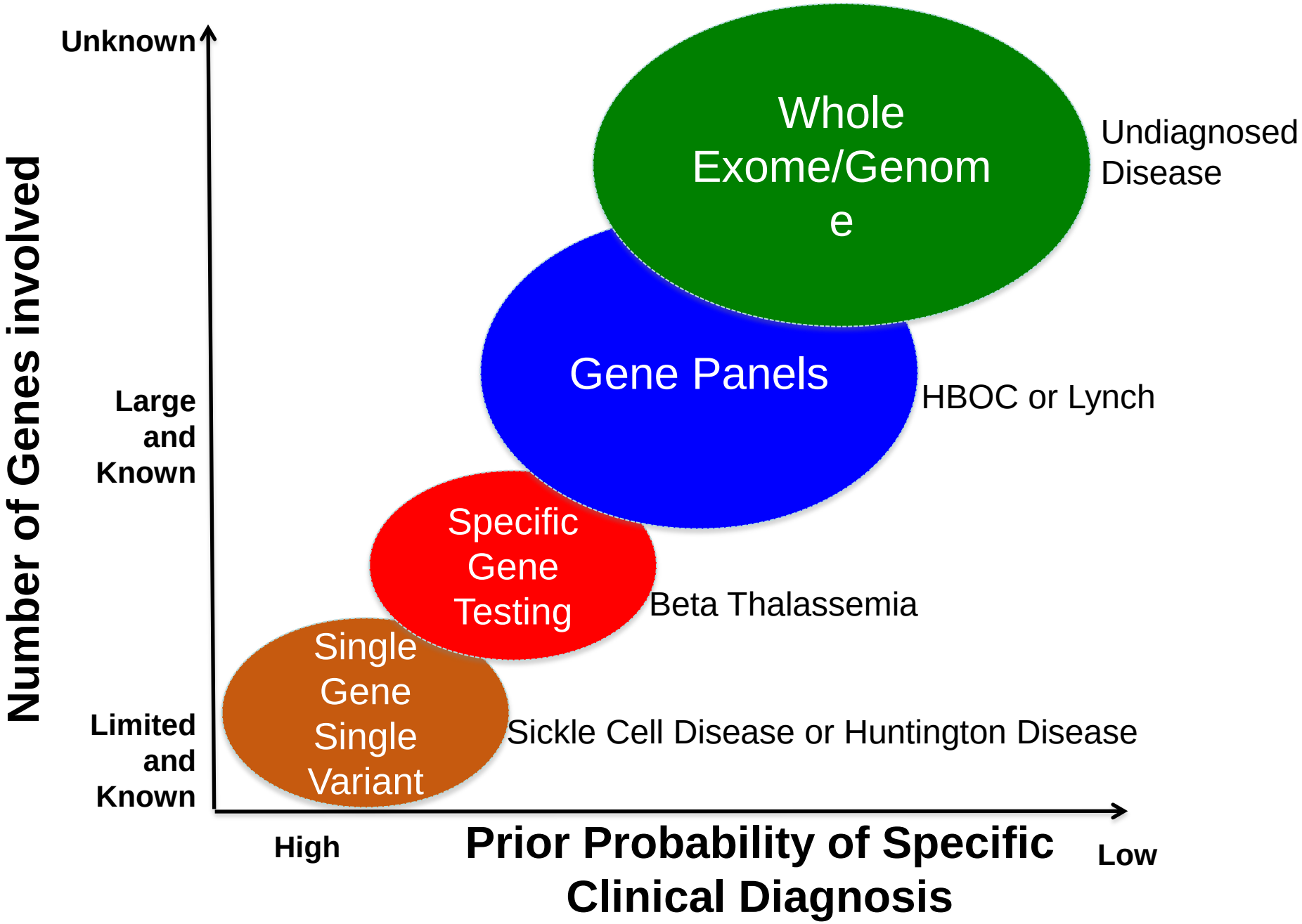
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Undiagnosed Diseases