Genomic and Precision Medicine

Week 2: Applying Genomics to Medicine

Jeanette McCarthy, MD
Robert Nussbaum, MD

UCSF
University of California
San Francisco
advancing health worldwide™

Mendelian Inheritance: Significance

- Mendelian disorders (in the aggregate) are present in ~2-3% of all newborns, although disease may not be manifest for years to decades, if ever!

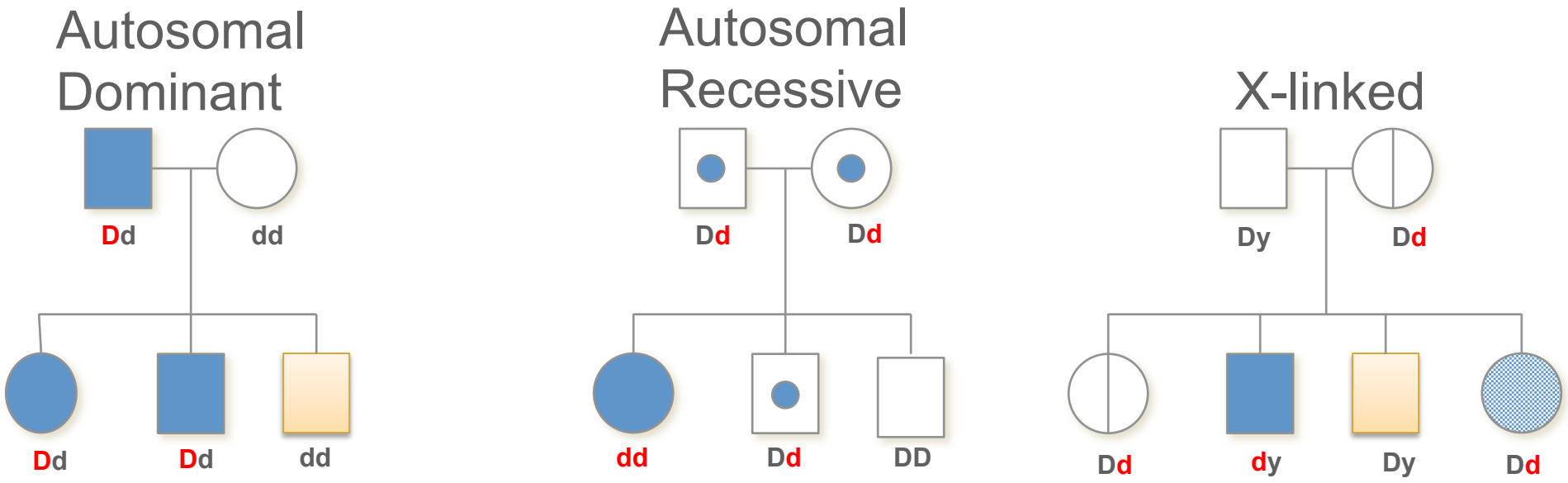
Recognizing a Mendelian Disorder – Red Flags

- Recurs in the Family
- Multiple close relatives are affected
- Tends to be earlier onset than non-Mendelian forms of same disease
- If a Cancer Syndrome, may affect bilateral structures
- Consanguinity

Online Mendelian Inheritance in Man

	Autosomal	X Linked	Y Linked	Mitochondrial	Totals
Genes	13,781	672	48	35	14,536
Disease with genetic basis known	3,758	283	4	28	4,073
Disease with genetic basis unknown	1,569	134	5	0	1,708
Disease suspected of being Mendelian	1,744	115	2	0	1,861
Totals	20,952	1,206	59	65	22,282

Characteristics of a "Typical" Mendelian Disorder



Affected male, female

Unaffected male, female

Affected female, XL, less severe than male

Carrier, unaffected but may manifest disease

Carrier, unaffected and will remain so

Why Do Some Gene Variants Cause Mendelian Patterns of Inheritance?

"Single Gene Defect" (Autosomal)

- Dominant: An alteration in one copy of one gene causes sufficient disruption of normal processes to cause disease. Affects both sexes equally.

- Recessive: Two alterations affecting both copies of a gene cause sufficient disruption of normal processes to cause disease. The alterations may be identical (homozygotes) or may be different (compound heterozygotes). Affects both sexes equally.

Ratios of Affected to Unaffected in a Family Generates Recognizable Mendelian Patterns

Dominant
(Disease Variant = D)

		Affected Dd	
		D	d
Unaffected dd	d	Dd	dd
	d	Dd	dd

Recessive
(Disease Variant = d)

		Carrier Dd	
		D	d
Carrier Dd	D	DD	Dd
	d	Dd	dd

X-linked Inheritance is a Special Case

- Males have only X chromosome, females have two.
- An alteration on the X in a male affects his only copy while it affects only one of the two copies in a female
- One X chromosome, chosen at random, is inactivated, in females
- Most (but not all) of the genes on the inactivated X are silenced
- The proportion of cells with inactive X carrying the normal gene may vary in any given tissue and between carriers
- Some female carriers may be symptomatic, depending on the disorder and X-inactivation pattern

Typical Ratios of Affected to Unaffected within Families Causing an X-linked Mendelian Pattern

X-linked

		Affected Male xy	
		x	y
Noncarrier Female XX	x	Xx	Xy
	x	Xx	Xy

All daughters are carriers
all sons unaffected

X-linked

		Unaffected Male Xy	
		X	y
Carrier Female Xx	X	XX	Xy
	x	Xx	xy

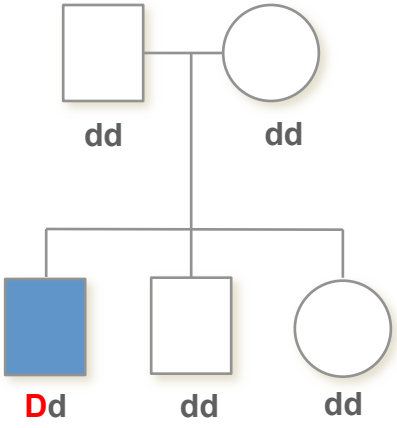
Half of daughters are carriers half of sons affected

"Simple" Mendelian Disorders are not so simple

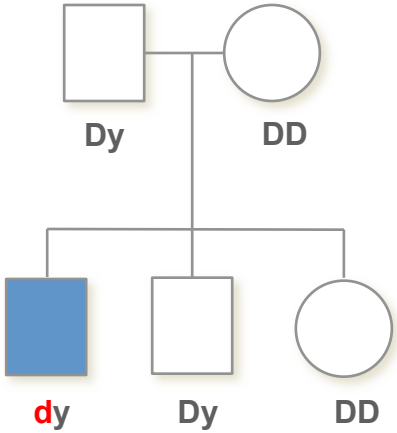
- New Mutation
- Mosaicism – The exception to every cell having the same DNA
- Decreased Penetrance – Disease genotype without phenotype
- Disease in Carriers of Recessive Disorders – Not always "silent"

Situations that Obscure Mendelian Inheritance

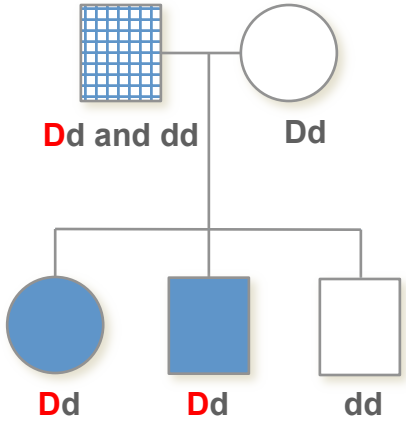
New Mutation AD



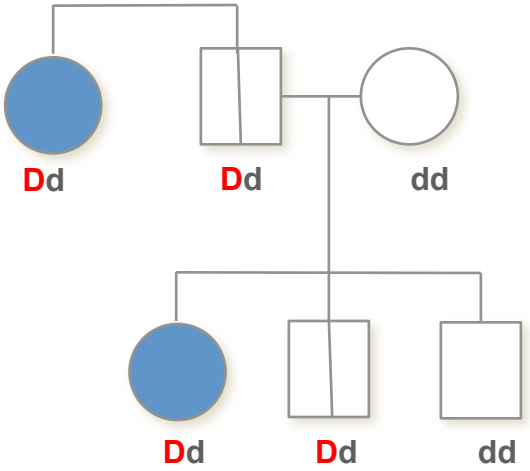
New Mutation XL



Mosaicism Autosomal



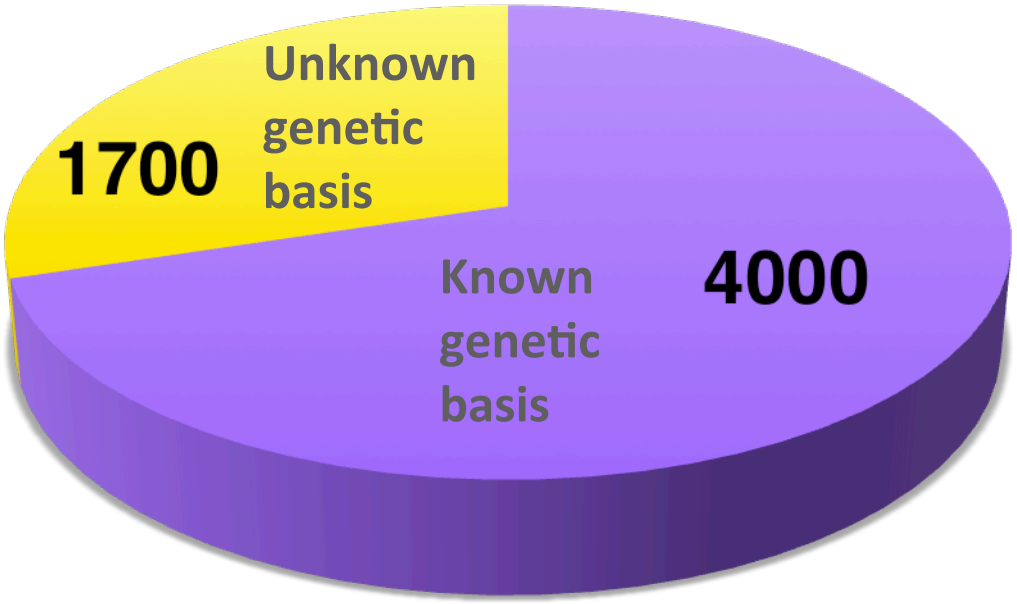
Reduced Penetrance

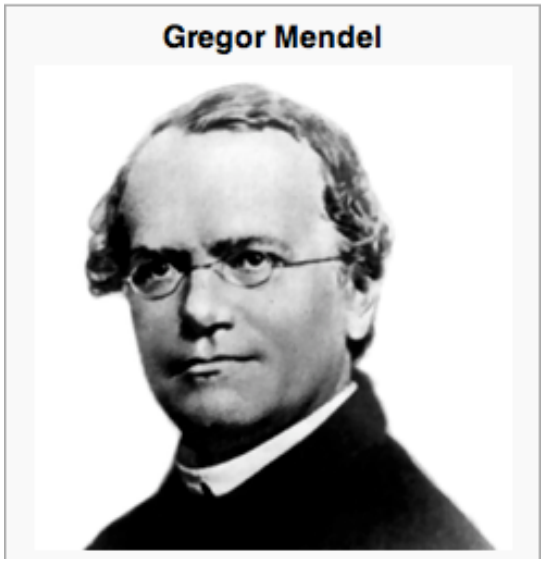


Increased Disease Risk in Carriers of Autosomal Recessive Disorders

- Gaucher Disease: 4-5-fold lifetime risk for Parkinson Disease
- Ataxia Telangectasia: 2-6-fold increased lifetime risk for breast cancer in females
- Cystic fibrosis: Increased risk for chronic sinusitis, bronchiectasis and pancreatitis
- Sickle cell trait: Splenic infarction with altitude, hypoxia or exercise, life-threatening rhabdomyolysis with exercise, renal medullary carcinoma in young adults

How Many Mendelian Disorders Are There?





With this many different disorders caused by different types of alterations in this many genes, how do you know what gene(s) to test, how to test them, and where to find the right test?

Genetic Alterations (Variants) Causing Mendelian Disorders

Type of Variation	Size Range (approx.)	Basis for the Variation	Number of Alleles	Disease Examples
Single Nucleotide (SNV)	1 bp	Substitution of one basepair for another at a particular location in the genome	Usually 2	**Sickle cell disease** Single base change GAG→GTG causes substitution of valine for glutamate at position 6 in the β-globin protein
Insertion/deletion (indel)	1 bp to >100 bp (may be many kb)	*Simple*: Presence or absence of a short segment of DNA between 100-1000 bp in length *Tandem Repeat:* Usually a 2-, 3-, or 4-nucleotide unit repeated in tandem 5-25 times	*Simple*: 2 *Tandem Repeats*: Many, typically 5 or more	**Cystic fibrosis** Deletion of 3 bp deletes a phenylalanine (ΔF508) in the CFTR protein **Huntington disease** There are <36 copies of $(CAG)_n$ normally present is increased to >40, increasing the number of glutamines (encoded by CAG) in the Huntingtin protein
Copy Number (CNV)	10 kb to >1 Mb	Absent or extra copies of a segment of DNA, ranging from 1000-bp to >2 Mb	2 or more	**Charcot-Marie-Tooth 1A Peripheral Neuropathy** Duplication of ~1.5 Mb segment of DNA including the PMP22 gene
Inversions	Few bp to >1 Mb	A DNA segment present in either of two orientations with respect to the surrounding DNA	2	**Hemophilia A** Inversion of ~100 kb segment within the Factor VIII gene

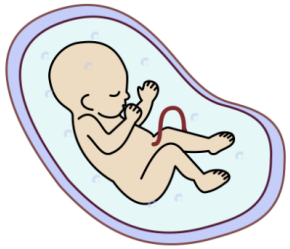
Utility of Genetic Testing

- Make a Diagnosis and Infer Prognosis
- Provide an Explanation and End a "Diagnostic Odyssey"
- Guide Management
- Identify which other Family Members are or are not at Risk for the Disease
- Inform Reproductive Decision Making including Pre-Implantation or Prenatal Diagnosis

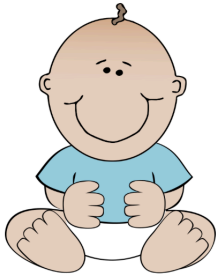
Testing Across the Lifespan

Pre-conception

Prenatal

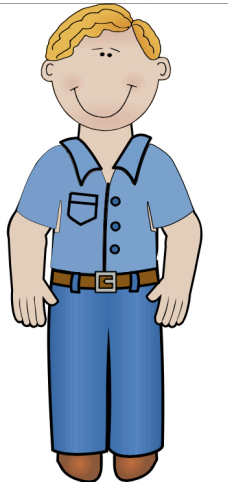


Newborns



Children

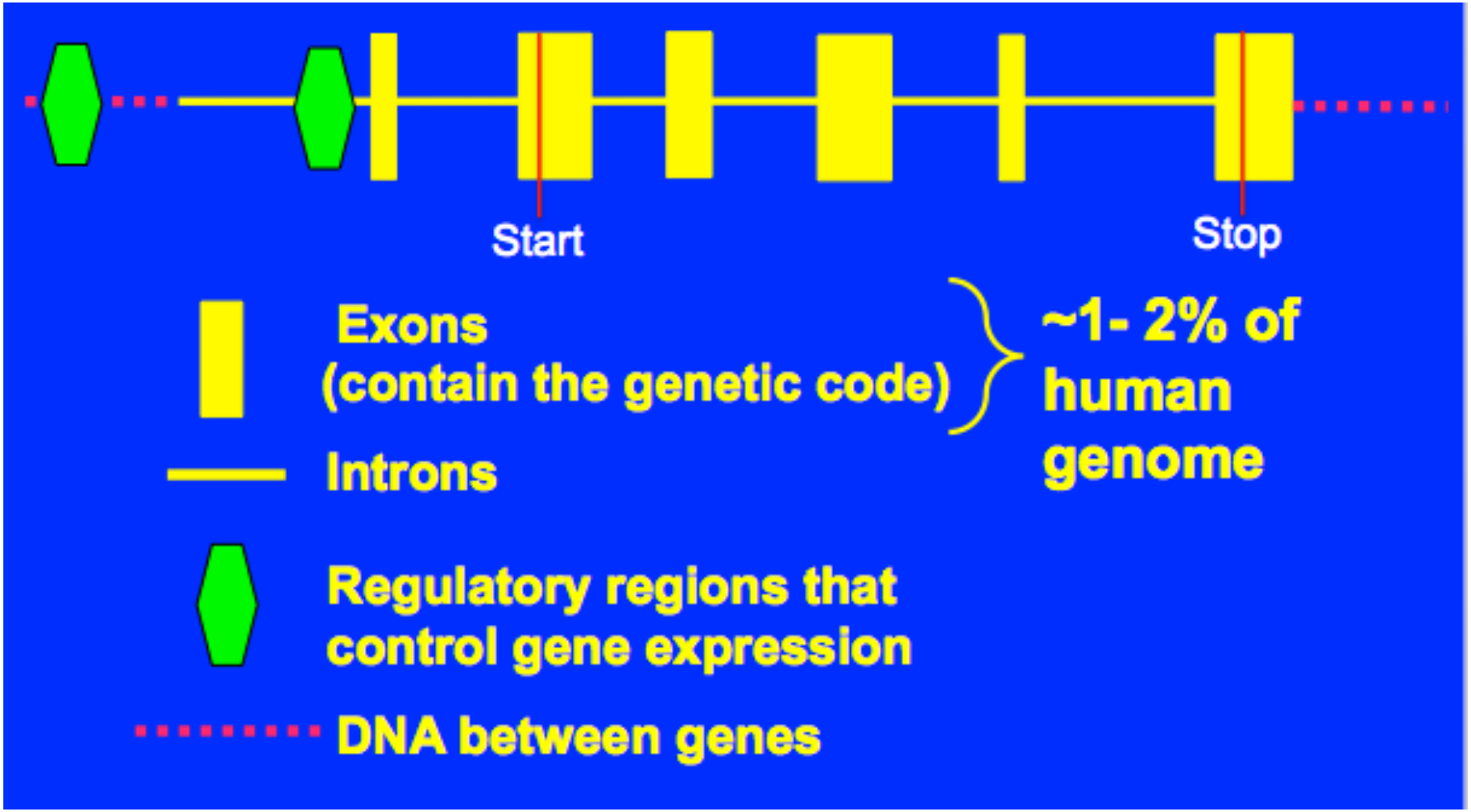
Adults



Matching the Test to the Variant

	Mutations affected 1 - ~100 bp	Mutations affecting >~100 bp
Specific to particular genes	• Single site within a gene • Targeted Gene Sequencing • Sequencing of gene panels • "SNP" Genotyping	• Deletion/Duplication scanning of one or more genes
Whole genome approaches	• Whole Exome Sequencing • Whole Genome Sequencing	• Cytogenomic Array to scan Genome for Copy Number Variants (CNVs)

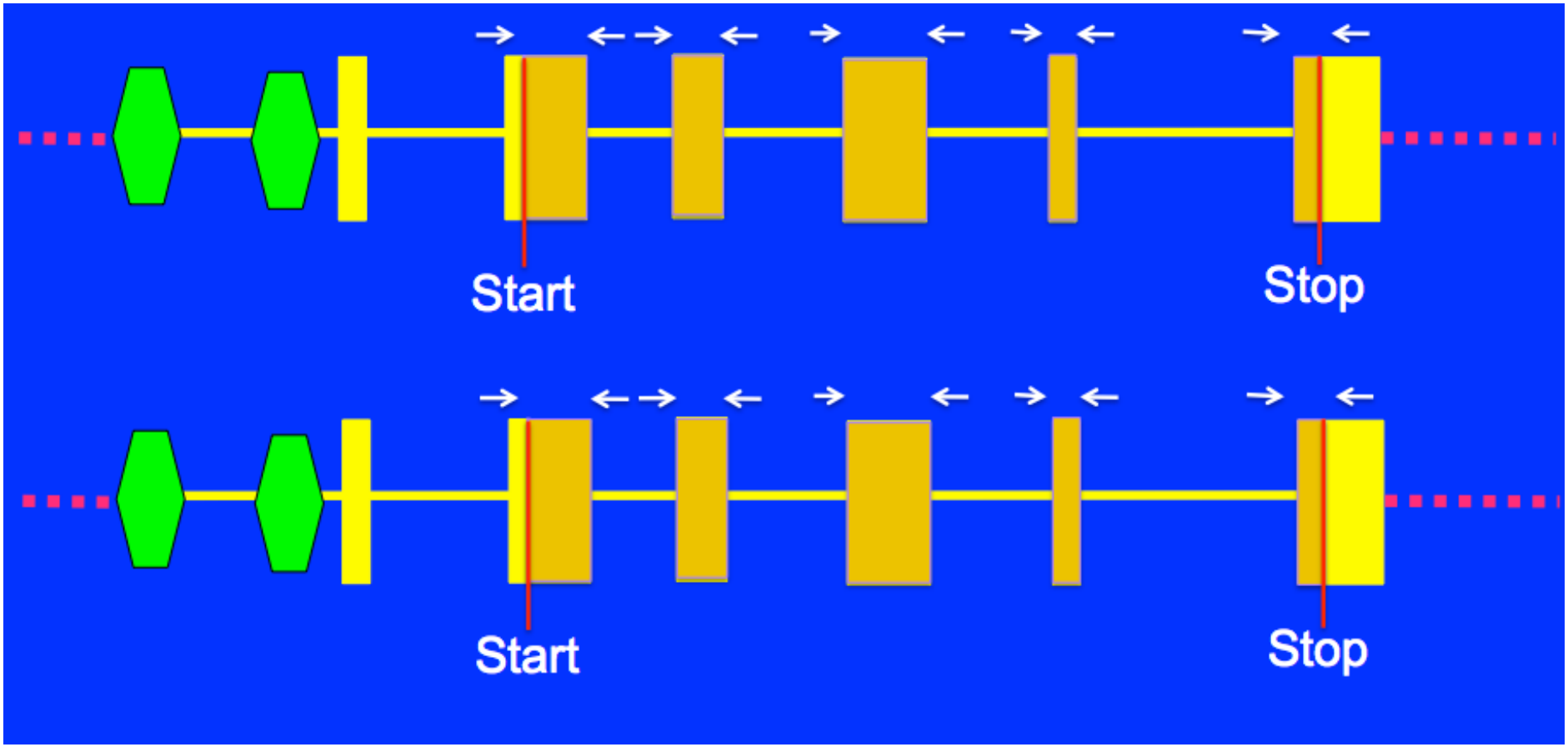
Anatomy of a "Typical" Gene



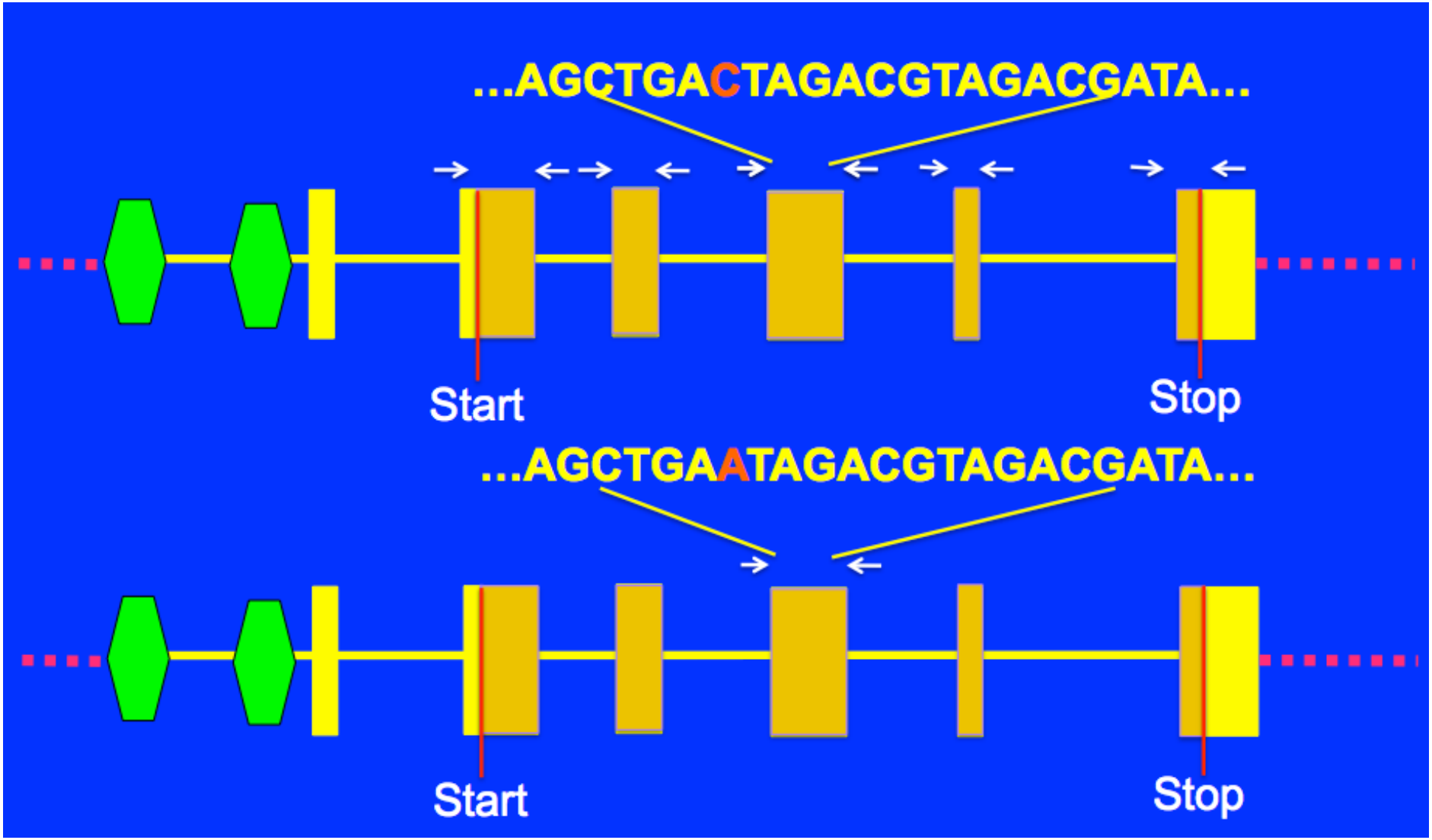
Gene Variants Can:

- Occur in exons to affect the amino acid sequence or to insert premature stop codon
- Affect splicing signals in introns to create novel splice sites or destroy normal splice sites
- Change DNA binding sites for regulatory proteins
- Delete or increase copy number for an entire gene

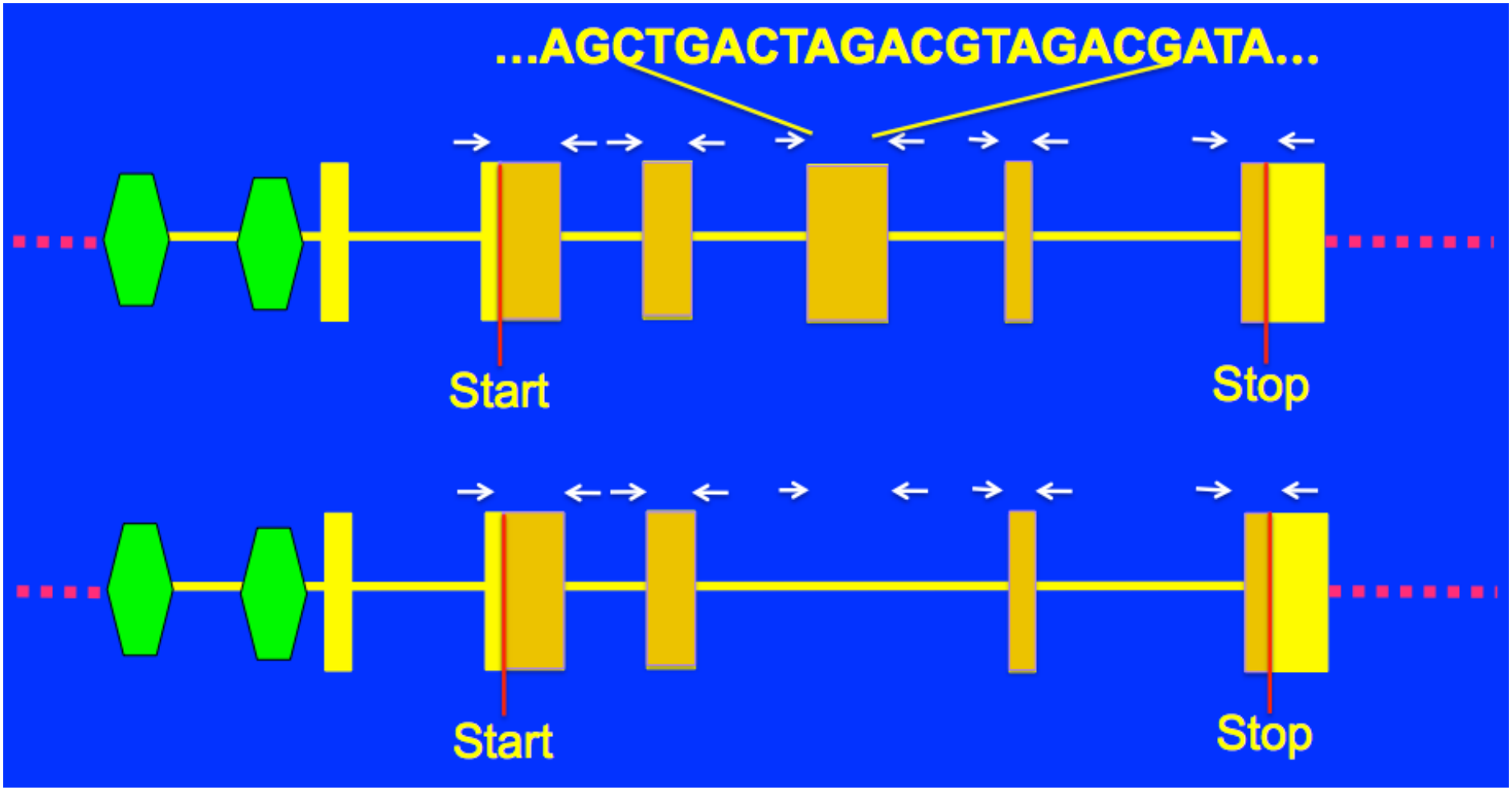
Traditional Sequencing Specific to a Particular Gene



Targeted Mutation Detection

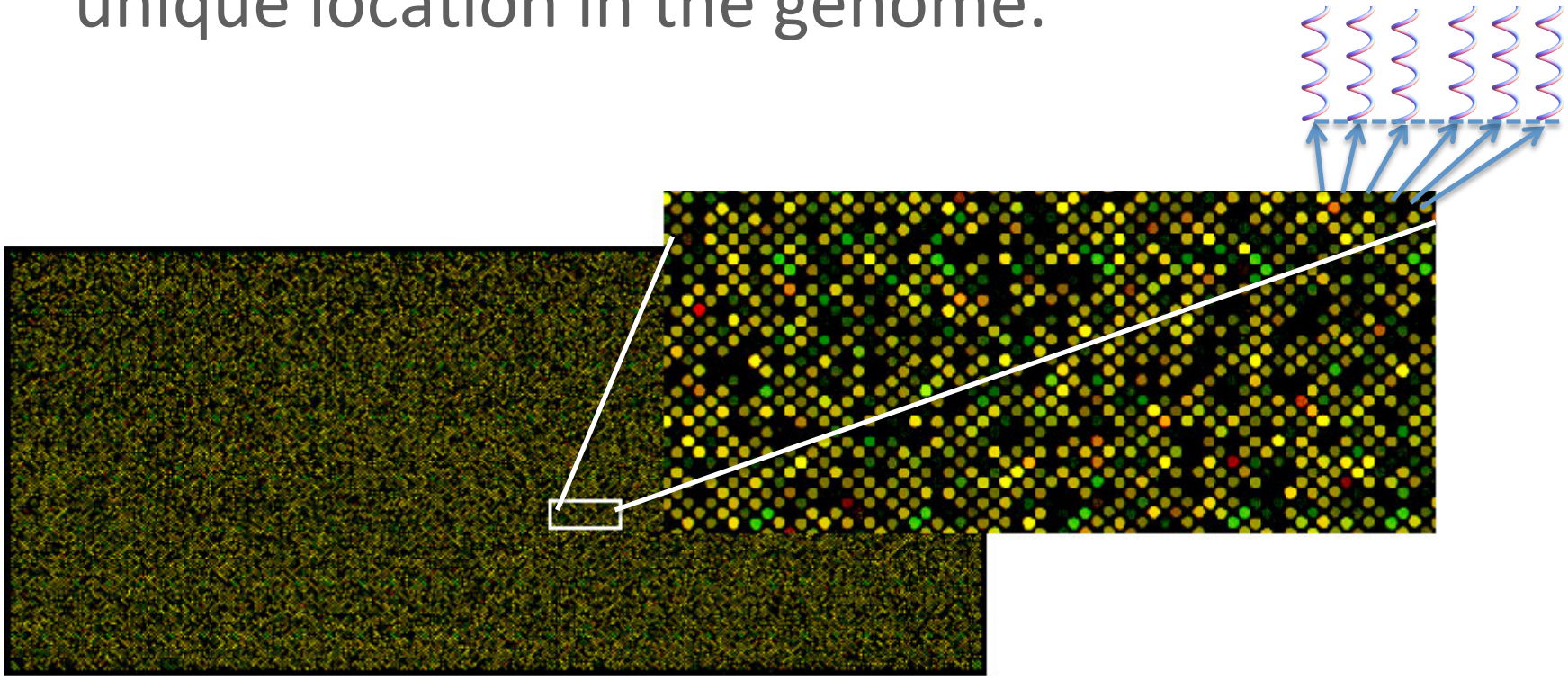


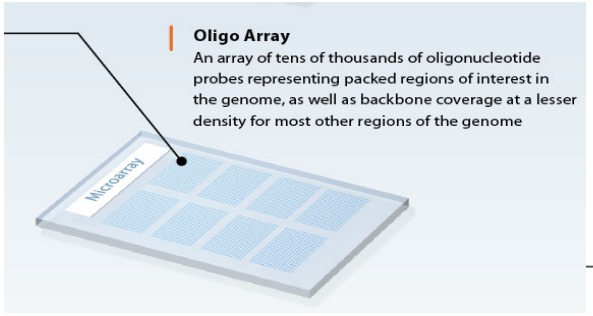
Traditional Sequencing Can Miss a Deletion/Duplication

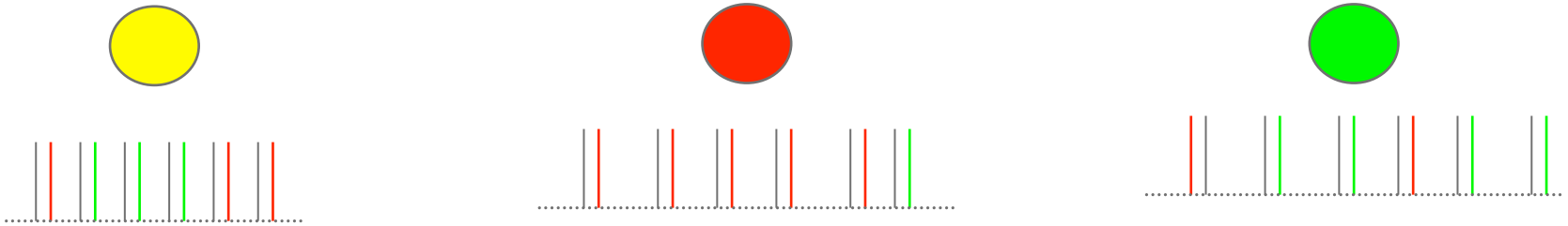


Cytogenomic Array

- Each spot contains copies of a short strand of single-stranded DNA that is specific to a unique location in the genome.







NORMAL
Control= Patient
No imbalance
Log_2 Ratio = 0

LOSS OR DELETION
Control Patient
Imbalance detected
Log_2 Ratio = ~ - 1.0

GAIN OR DUPLICATION
Patient Control
Imbalance detected
Log_2 Ratio = ~+ 0.5

AMA Ethical Guidelines for Genetic Testing in Children

- For childhood onset condition that IS preventable or treatable, testing should be offered or, in some cases, be required.
- For childhood onset condition that is NOT preventable or treatable, parents generally should have choice whether to have their children tested.
- For adult onset condition that is NOT preventable or treatable, genetic testing of children should not be undertaken.
- Genetic testing for carrier status should be deferred.
- Testing of children for the benefit of a family member should not be performed unless the testing is necessary to prevent substantial harm to the family member.

http://www.ama-assn.org/ama/pub/physician-resources/medical-ethics/code-medical-ethics/opinion2138.page

Next Generation Sequencing Changes the Testing Paradigm

STAY TUNED to Future Sessions

Finding Laboratories that Perform Genetic Testing

In the United States, Canada, and Europe (partial):

GeneTests	http://www.genetests.org/tests/
Genetic Test Registry:	https://www.ncbi.nlm.nih.gov/gtr/
Eurogentests:	http://eurogentest.org

These registries list >2800 different sequencing and deletion/ duplication tests ranging from a targeted single gene test to gene panels that examine many genes simultaneously.

Quiz: Question

TRUE or FALSE:

Targeted gene sequencing will detect all the alterations responsible for Mendelian disease

Quiz: Answer

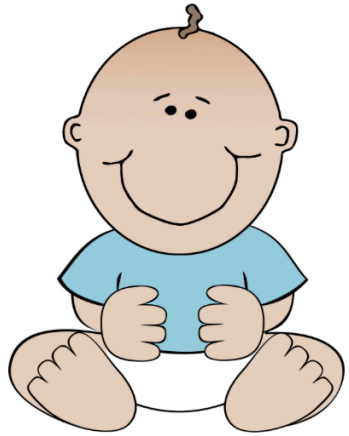
FALSE

Targeted sequencing may miss deletions or duplications or inversions, depending on where the amplification primers are positioned with respect to the location of the disease-causing alteration.

Newborn Screening

Why Do We Need Newborn Screening?

- Many disorders are not obvious or readily diagnosable in a newborn, leading to delay in instituting effective interventions that can prevent or ameliorate irreparable harm



Criteria for Newborn Screening

1. An important health problem whose natural history is understood.
2. Facilities for diagnosis and treatment are available
3. There should be a suitable and acceptable test and treatment.
4. A latent or early symptomatic stage exists during which intervention improves outcomes.
5. The cost of case-finding (including diagnosis and treatment) is economically balanced in relation to possible expenditure on medical care as a whole.

Newborn Screening is a Program, not a Test

Successful newborn screening requires:

- Acquiring the samples and performing the test,
- Having a system for notifying families whose infants test positive,
- Providing follow-up definitive testing and, if confirmed,
- Instituting appropriate management and services.

Newborn Screening – Example

Phenylketonuria (PKU)

- Deficiency of the enzyme phenylanalanine hydroxylase prevents conversion of phenylalanine (an essential amino acid) to tyrosine
- If left untreated, blood and brain levels of phenylalanine soar and lead to intellectual and developmental disabilities (IDDs)
- Prior to screening, PKU was one of the leading causes of IDD in the U.S.
- Treating with phenylalanine-restricted diet from birth eliminates IDD
- Today, PKU has been virtually eliminated as a cause of IDD in this country

Newborn Screening is (mostly) "Phenotype Based" and not "Genotype Based"

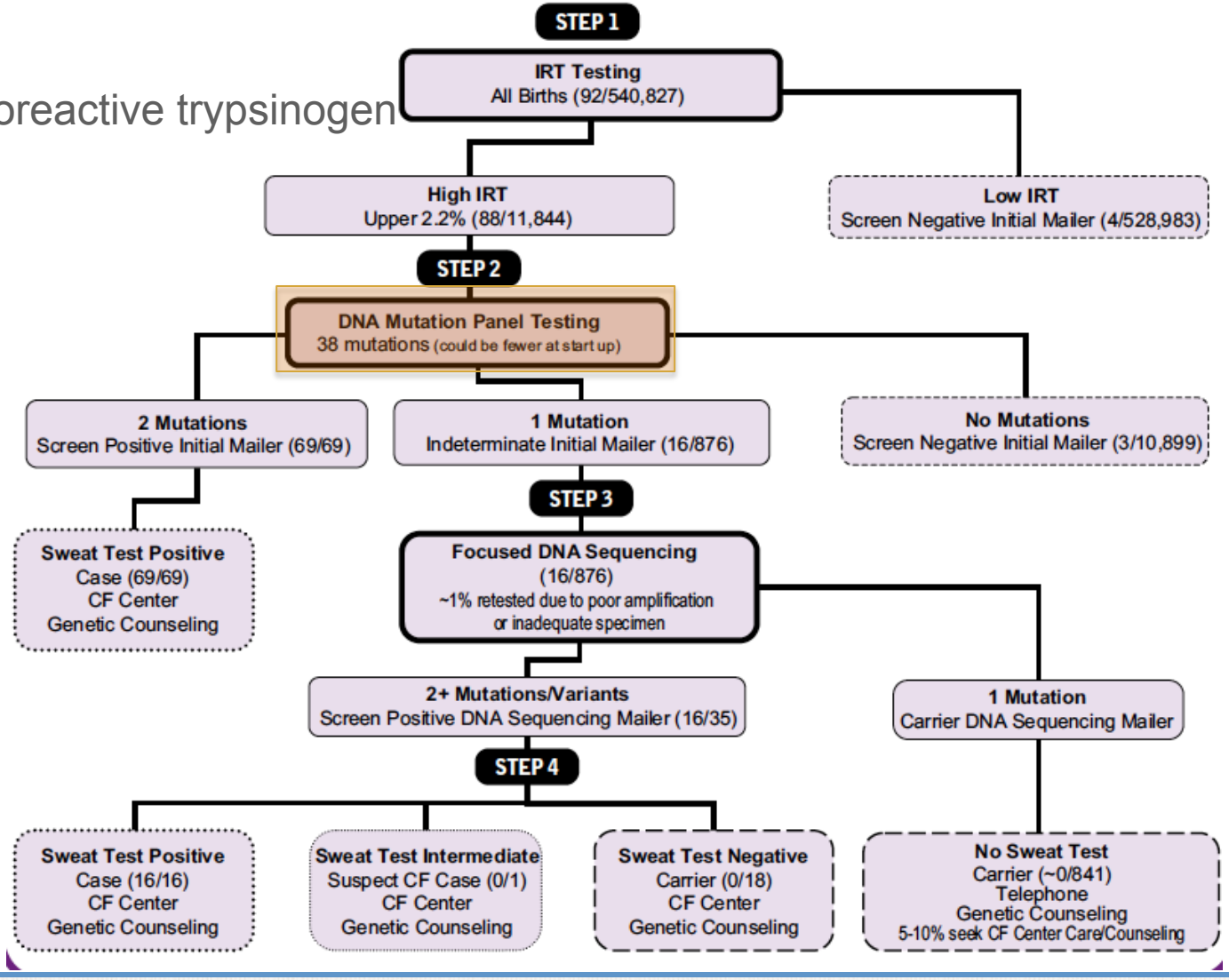
- Deafness
- Cystic Fibrosis
- Hypothyroidism
- Galactosemia
- Hemoglobinopathies
- Fatty Acid Disorders
- Organic Acid Disorders
- Amino Acid Disorders
  (>40 disorders by Tandem Mass spectroscopy)
- Congenital Adrenal Hyperplasia
- Biotinidase deficiency
- Severe Combined Immunodeficiency (T-cell lymphopenia)

Newborn Screening – Scope

- Varies by state
- Uniform panel of 31 core disorders and 26 secondary disorders currently recommended by the US Secretary's Advisory Committee on Heritable Disorders in Newborns and Children
- 2003: all but 4 states screening for only 6 conditions
- 2013: all states screening for more than 30

Example: California Cystic Fibrosis Newborn Screening Includes Gene Testing

IRT-Immunoreactive trypsinogen



Newborn Screening – A Success Story

- Virtually all of the 4,000,000 babies born in the United States receive some degree of screening but which tests are done differs between states.
- 12,000 babies in the United States screen positive and are confirmed to have one of the disorders for which screening is done
- Every $1 spent on screening results in savings of $10-20 by reducing medical costs, averting expenses for special services, and increased productivity
- Screening is available in many other countries, but not all, with a varying menu of conditions for which screening is provided

Future of Newborn Screening – Many Contentious Issues

- Should we be adding more disorders and do we need to better define, refine, or expand criteria for utility?
- Should we doing screening to find parents at risk for disease in future children early enough to inform their reproductive decision making?
- Introduction of DNA sequencing – adjunct to or replacement for some phenotypic screening?