Overview of CF and CF Genotyping Platforms

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APHL Molecular Training Course
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National Center for Environmental Health
Division of Laboratory Sciences

Presentation Overview

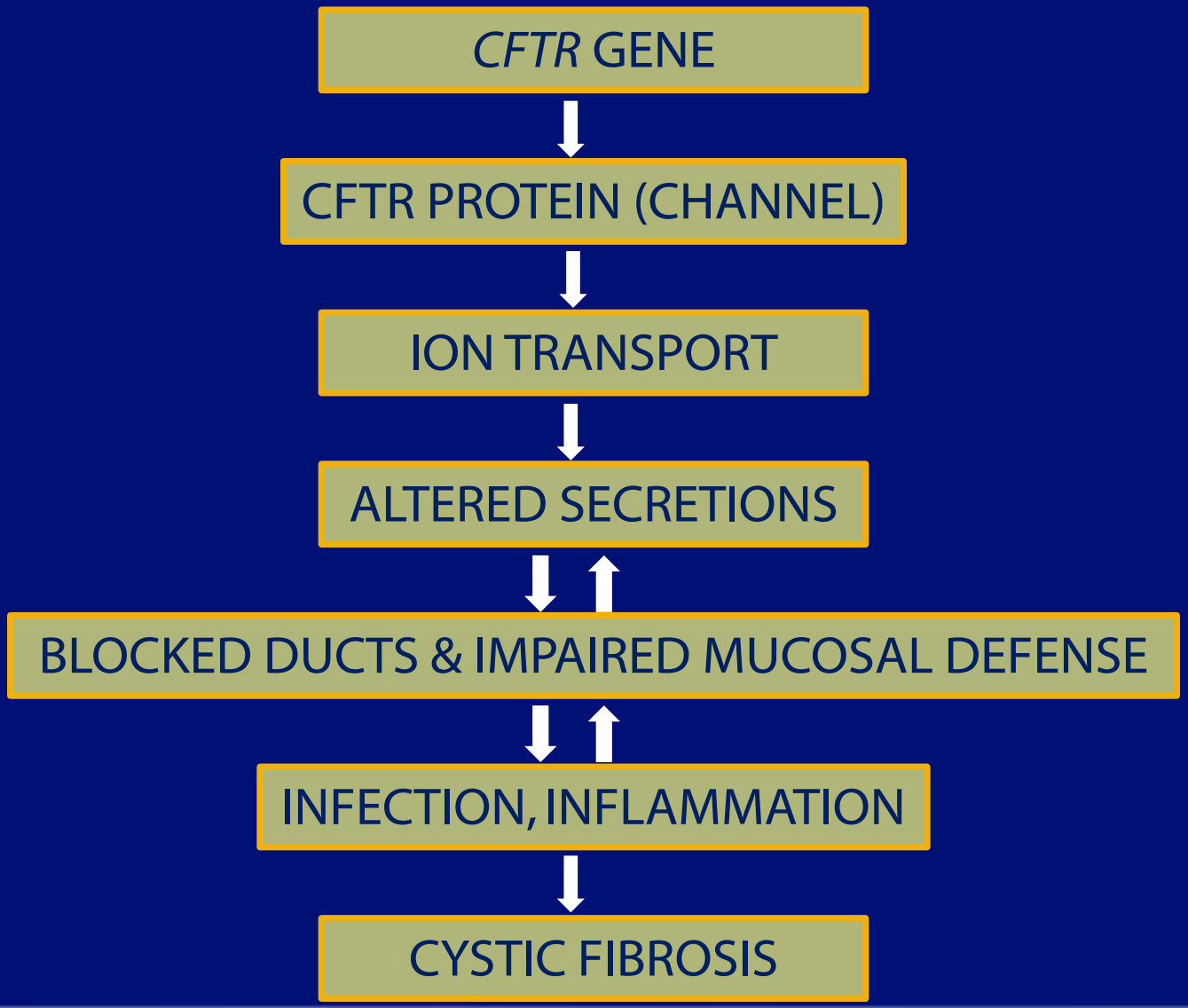
- **Part 1**
  - Brief summary of cystic fibrosis
  - Newborn screening for CF
  - Biochemical assays vs. molecular assays
  - CF screening algorithms in U.S.

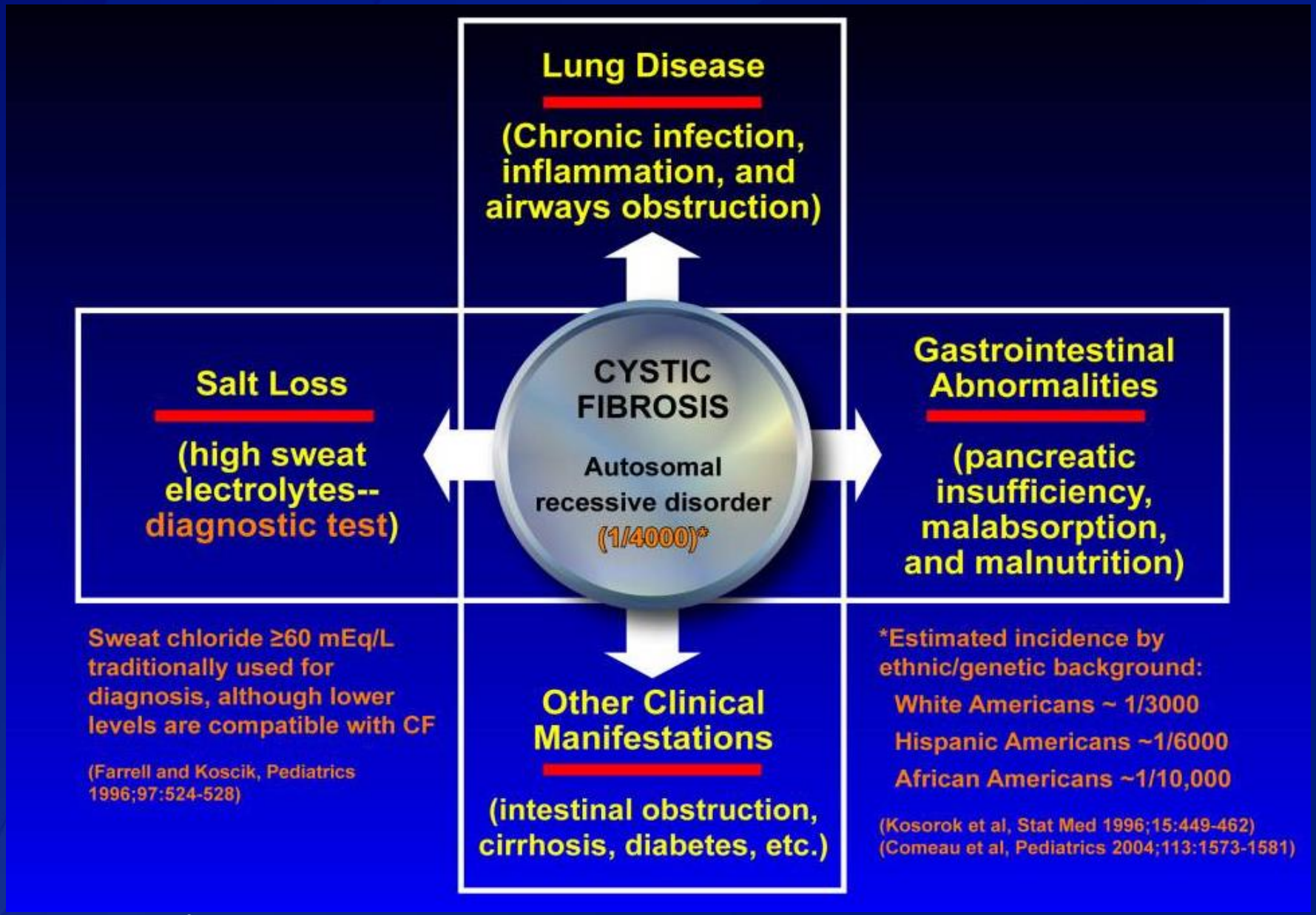
- **Part 2**
  - CFTR gene structure
  - Standard vs legacy mutation nomenclature
  - Description of methods – advantages & limitations

What is Cystic Fibrosis? Disease and Symptoms

- **Chronic disease of the lungs and digestive system**
  - Mutations in the *CFTR* gene (encodes a chloride channel)
  - CFTR channel found in cells producing mucus, sweat, saliva, tears, and digestive enzymes
  - Imbalance of chloride ions into & out of the cell affects mucus consistency
  - Mutations affect production, structure, or stability of the channel
- **Symptoms**
  - Thick, sticky mucus
  - Salty sweat
  - Failure to thrive (pancreatic insufficiency)
  - Many more

From Mutations to Symptoms: Cause & Effect





Slide courtesy of Dr. Phil Farrell

Treatments*

- **Improve Protein Function**
  - Kalydeco – for patients with G551D, G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P or G1349D
  - FDA review of Kalydeco/Lumacaftor combination for people who have 2 copies of F508del
- **Airway Clearance**
  - Manual or mechanical techniques
  - Inhaled medication – mucolytics or hypertonic saline
- **Antibiotics**
  - Oral, intravenous, or inhaled
- **Nutrition**
  - Pancreatic enzymes
  - Monitoring calories

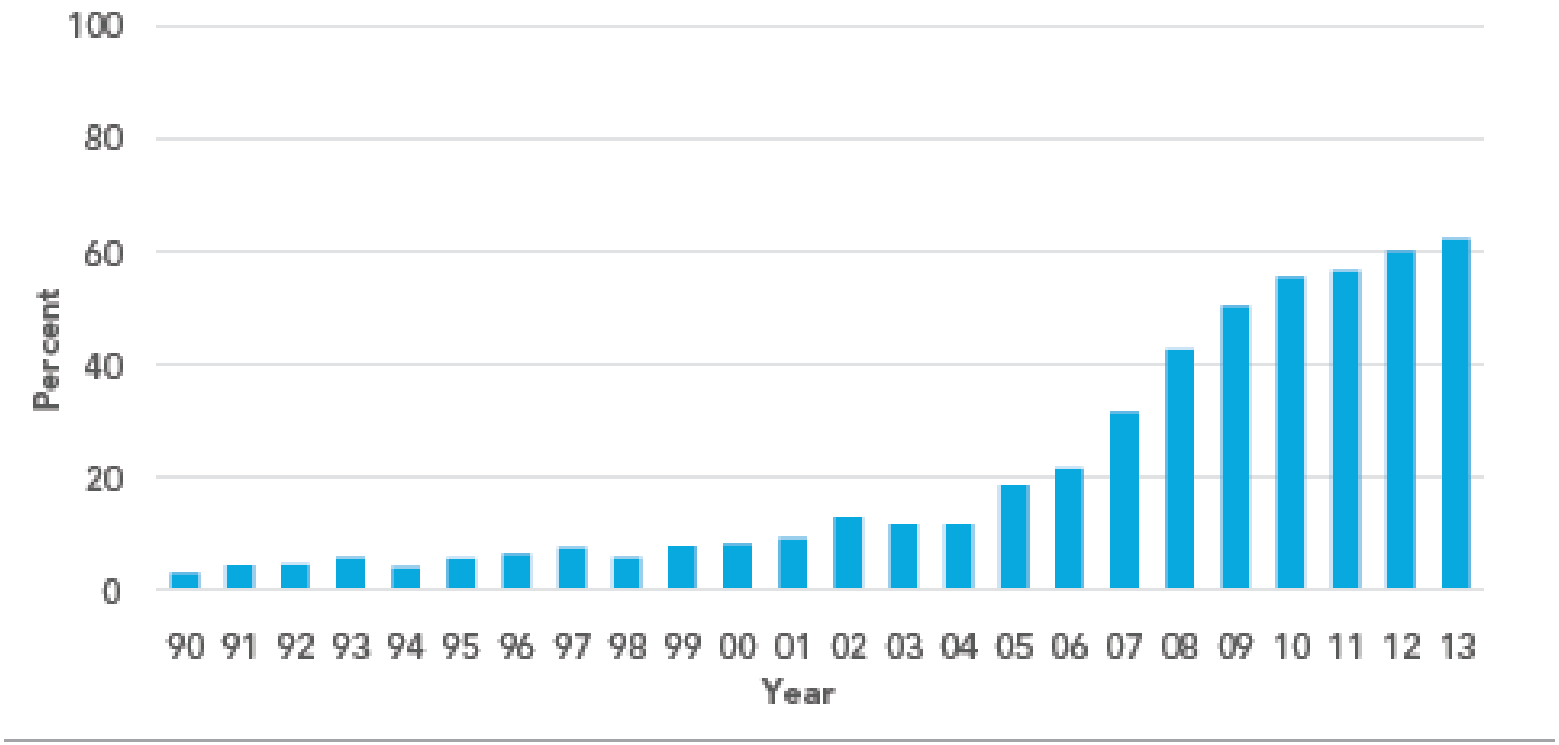
* Information from http://www.cff.org/treatments/Therapies/ accessed February 9, 2015

Why Is CF One of the NBS Disorders?

- **1997 CDC Workshop**
  - Evidence for nutritional benefit; more research needed
- **2003 CDC Workshop**
  - Recommend CF as newborn screening disorder
  - MMWR October 15, 2004 / 53(RR13);1-36
- **2006 Recommended Uniform Screening Panel**
  - CF included as a primary condition

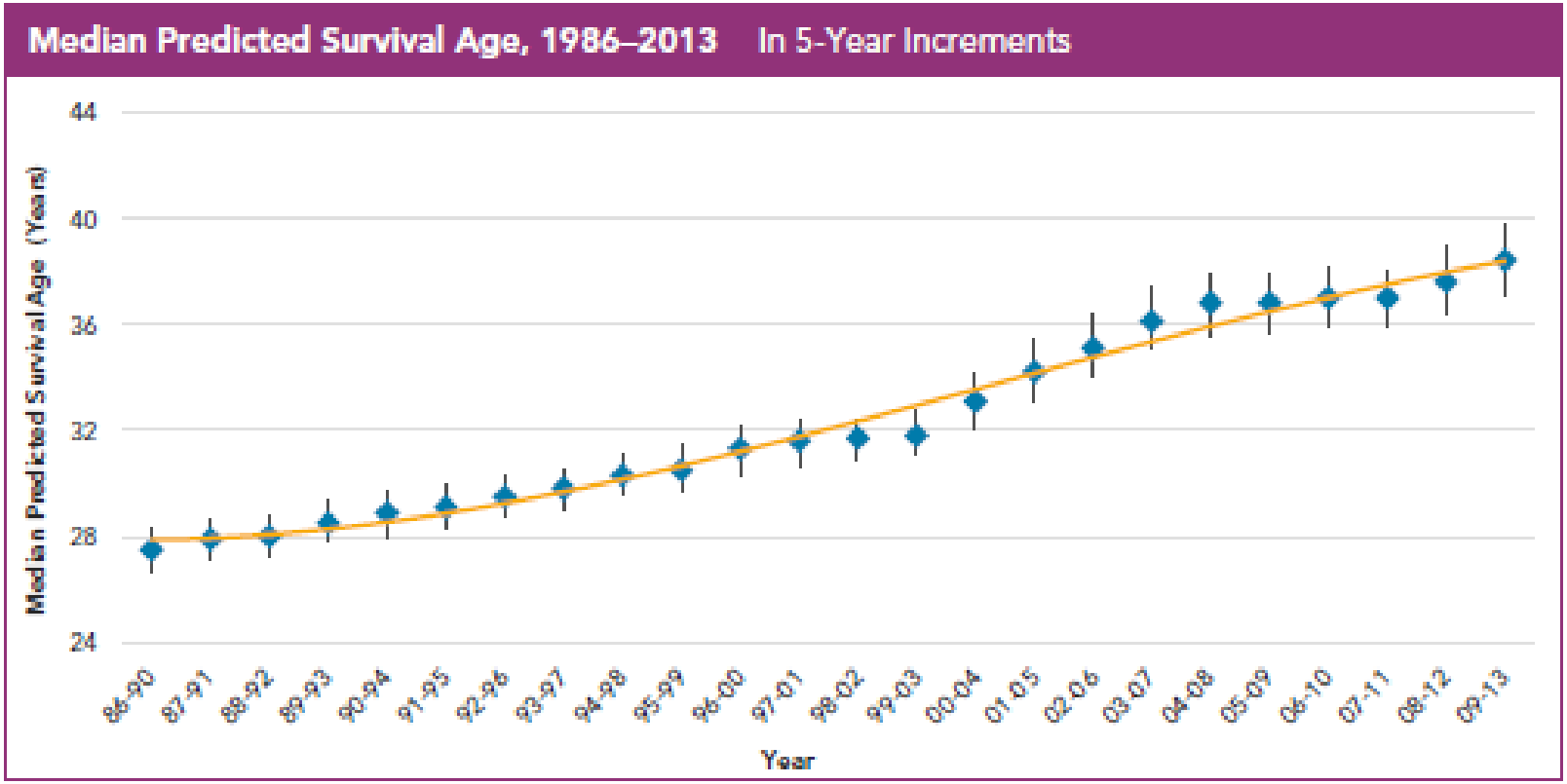
Scientific evidence demonstrated that early diagnosis of CF resulted in better nutritional and health outcomes due to early intervention.

Public Health Benefit*



*Cystic Fibrosis Foundation Patient Registry 2013 Annual Data Report, Bethesda, Maryland

Median Predicted Age of Survival*



Median Predicted Age of Survival was 40.7 years in 2013

*Cystic Fibrosis Foundation Patient Registry, 2013 Annual Data Report to the Center Directors, Bethesda, Maryland

How CF Molecular Assays Complicated Your Lives

- New concepts to understand
- New nomenclature & terms
- New methods to learn (DNA extraction, PCR, assays, interpretation)
- Multiple techniques to detect mutations
- Multiple mutation panels
- Unique unidirectional workflow requirements
- Specific environmental Burden of contamination
- Detection of carriers
- Multiple algorithms available to adopt

Comparison of U.S. Mutation Panels

ACMG 23	California	Luminex CFTR IVD 39 v2	Luminex CFTR IVD 60 v2	Hologic CF Inplex 40+4
delF508	delF508	delF508	delF508	delF508
dell507	dell507	dell507	dell507	dell507
G542X	G542X	G542X	G542X	G542X
G551D	G551D	G551D	G551D	G551D
G85E	G85E	G85E	G85E	G85E
N1303K	N1303K	N1303K	N1303K	N1303K
R1162X	R1162X	R1162X	R1162X	R1162X
R334W	R334W	R334W	R334W	R334W
R553X	R553X	R553X	R553X	R553X
W1282X	W1282X	W1282X	W1282X	W1282X
1717-1G>A	1717-1G>A	1717-1G>A	1717-1G>A	1717-1G>A
3120+1G>A	3120+1G>A	3120+1G>A	3120+1G>A	3120+1G>A
3849+10kbC>T	3849+10kbC>T	3849+10kbC>T	3849+10kbC>T	3849+10kbC>T
621+1G>T	621+1G>T	621+1G>T	621+1G>T	621+1G>T
711+1G>T	711+1G>T	711+1G>T	711+1G>T	711+1G>T
A455E		A455E	A455E	A455E
R117H		R117H	R117H	R117H
R347P		R347P	R347P	R347P
R560T		R560T	R560T	R560T
1898+1G>A		1898+1G>A	1898+1G>A	1898+1G>A
2184delA		2184delA	2184delA	2184delA
2789+5G>A		2789+5G>A	2789+5G>A	2789+5G>A
3659delC		3659delC	3659delC	3659delC

CF NEWBORN SCREENING ALGORITHMS*
THE GOOD, THE BAD, AND THE UGLY

IRT/IRT
IRT/DNA
IRT/IRT/DNA
IRT/DNA/EXTENDED GENOMIC ANALYSIS (EGA)

*CLSI. *Newborn Screening for Cystic Fibrosis; Approved Guideline*. CLSI document I/LA 35-A. Wayne, PA: Clinical Laboratory Standards Institute; 2011.

Algorithm 1: IRT/IRT

If IRT level is elevated, a second sample is collected and tested

Advantages

- **Carrier status is not determined**
- **Does not require carrier genetic counseling**
- **Biochemical test easily incorporated into NBS laboratory**

Limitations

- **Best suited to second specimen states**
- **Complicating variables**
  - IRT level variation (increasing age, sick and low birth weight, race/ethnicity)
  - Issues with assay kits have been documented
- **Difficulty setting cut-off limits due to IRT variation**

Algorithm 2: IRT/DNA

If IRT level is elevated, DNA from the blood spot is tested

Advantages

- Second specimen is not required
- Less time to final result (about 1 week)
- Improved detection sensitivity
- Facilitation of follow-up planning
- Facilitation of interpretation of sweat chloride test results
- Reduction of false negatives from high IRT not due to CF

Limitations

- Increased cost for testing and genetic counseling
- More sweat tests for CF carrier infants
- Mutation panel may not reflect population

Algorithm 3: IRT/IRT/DNA

If IRT level is elevated, a second sample is collected and, if it is still elevated, DNA is tested from the second spot

Advantages

- Improved detection sensitivity by lowering IRT cut-offs
- IRT can be done on a subset of second specimens
- Fewer CF carrier infants detected
- Screening can be completed without a second specimen

Limitations

- Best suited to second specimen states
- Need for genetic counseling
- Mutation panel may not reflect population

Algorithm 4: IRT/DNA/EGA

If IRT level is elevated, DNA from the blood spot is tested. If only one mutation is detected, sequencing is performed to determine if a second mutation exists

Advantages

- Only babies with two or more CFTR mutations and/or variants are considered screen positive
- CF carrier infants detected but not referred for sweat chloride testing
- With time, have better understanding of mutations in the population

Limitations

- Higher cost
- Longer time until final screening result

Algorithm Summary

	IRT/IRT	IRT/DNA	IRT/IRT/ DNA	IRT/DNA/ EGA
Carrier status determined	No	Yes	Yes	Yes
New methodoolgy	No	Yes	Yes	Yes
Cost	Neutral	Increased	Increased*	Increased
2nd specimen required	Yes	No	Yes	No
Longer wait until final result	Yes	No	Yes	Yes
Number of sweat chloride tests	Baseline	Increased	Somewhat increased	Decreased

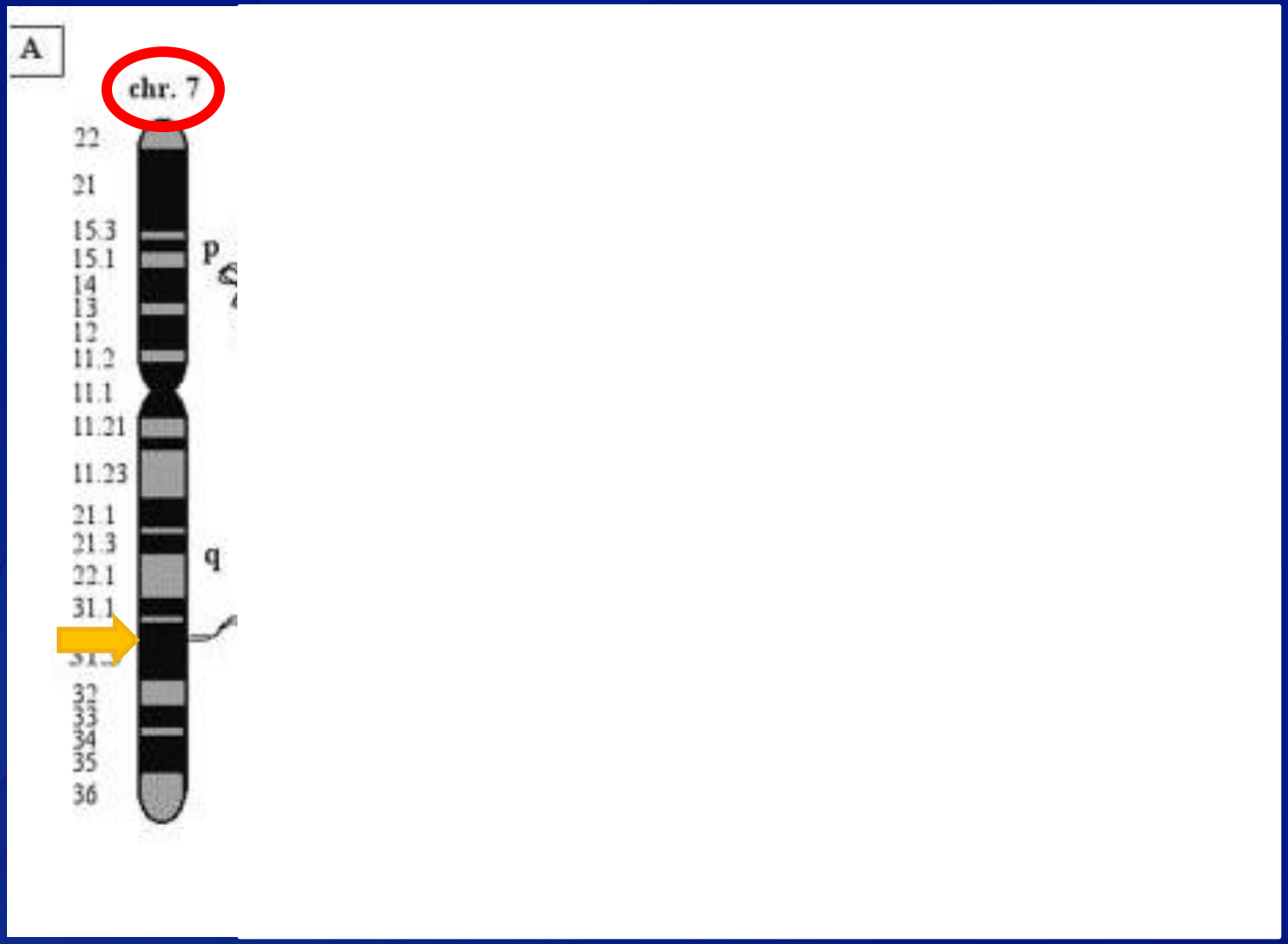
* Theoretically, increase in cost is recouped or decreased if only a subset of 2nd specimens are tested for IRT.

THERE IS NO RIGHT WAY OR WRONG WAY FOR CF NEWBORN SCREENING

Cystic Fibrosis Key Points – Part 1

- **CF is caused by mutations in the *CFTR* gene (chromosome 7)**
- **Kalydeco is a drug therapy now available for some CF patients**
- **NBS algorithms used to detect CF**
  - IRT/IRT (no molecular component)
  - IRT/DNA & IRT/IRT/DNA: elevated IRT → *CFTR* mutation(s)
  - IRT/DNA/EGA (elevated IRT → *CFTR* mutations → gene sequencing when only 1 mutation is found)
- **There are several different panels of mutations used by NBS labs that perform a molecular test.**
  - Programs that use a panel include at a minimum the recommended ACMG 23 *CFTR* mutations

CFTR Gene Structure



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CFTR Gene Structure

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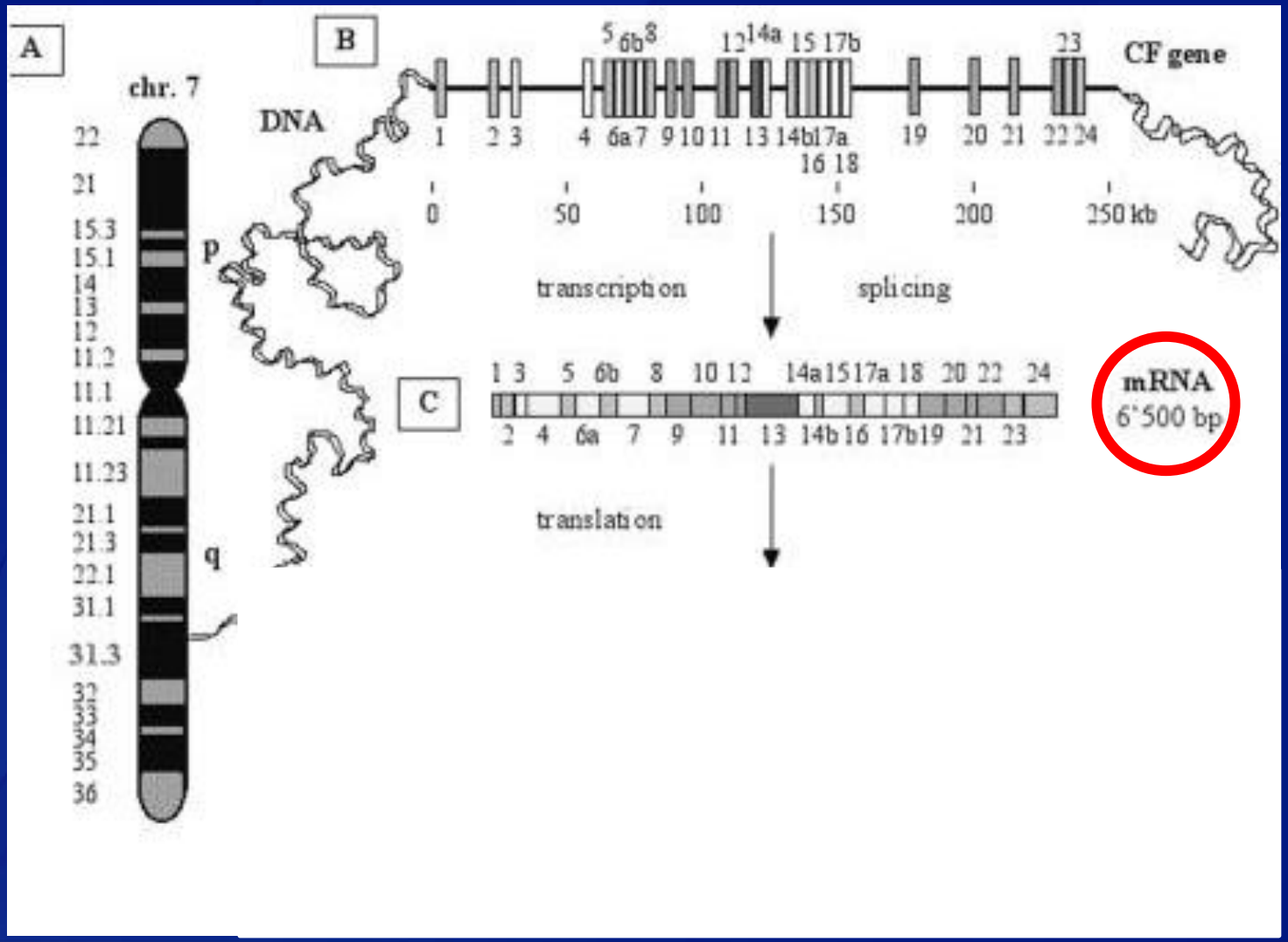
CFTR Gene Structure



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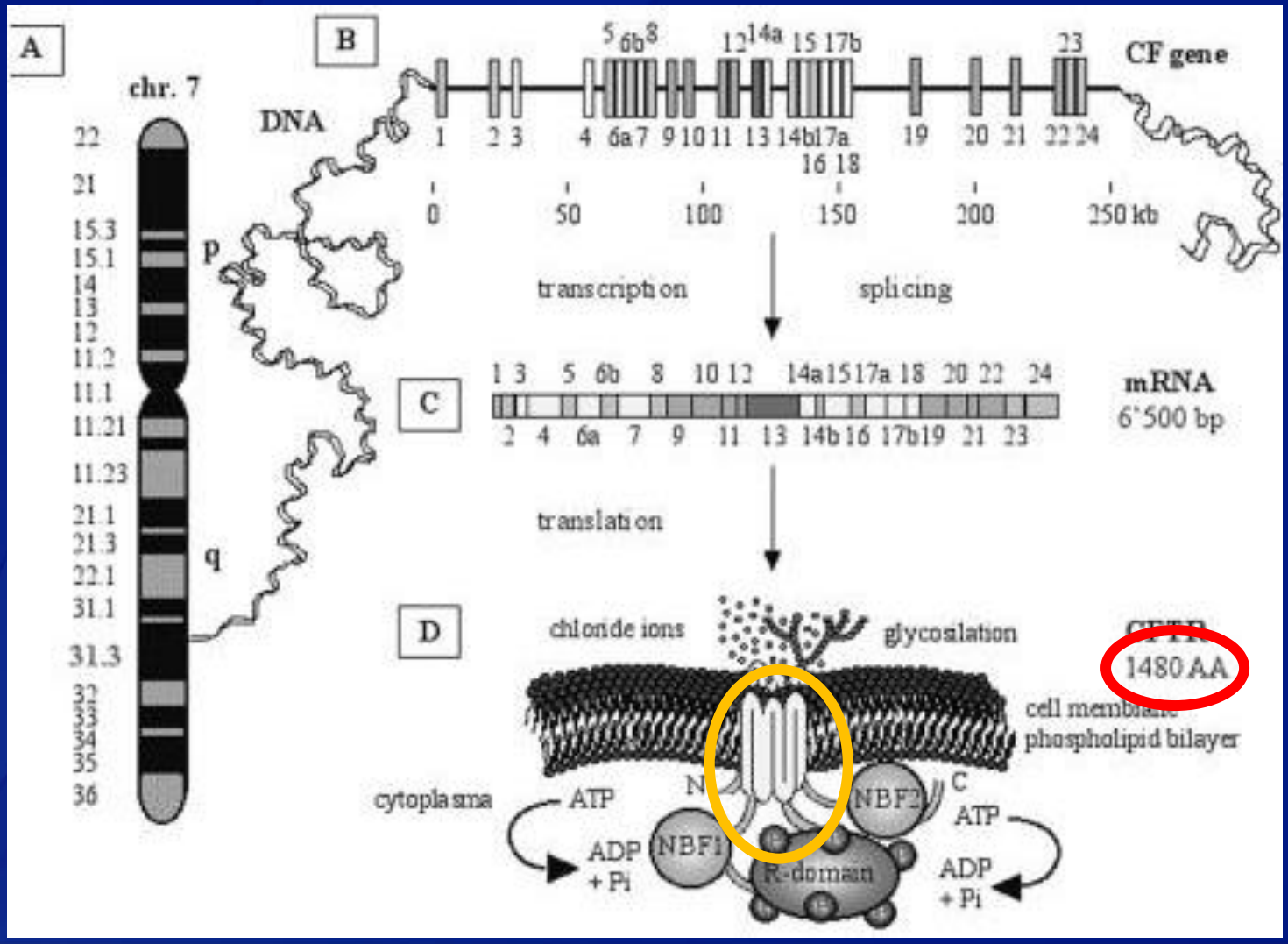

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HGVS vs. Legacy Nomenclature

- **Human Genome Variation Society guidelines** facilitate uniform and standard nomenclature of DNA and protein sequence variants
- **HGVS** nomenclature recommends
  - Sequence variations should be described at the DNA level
  - DNA name: "g" for genomic or "c" for cDNA followed by nucleotide number(s) affected by the change
    - may be an insertion, deletion or substitution
  - Protein name: "p" followed by the affected amino acid, the aa number and the substitution
- **Legacy** nomenclature
  - DNA names used for intron mutations, deletions, and insertions
  - Protein names used for both substitution and nonsense mutations

CFTR HGVS Nomenclature

CFTR

xTAG CF Kit Name/ Legacy Name	HGVS DNA Name	HGVS Protein Name	Legacy Exon or Intron	Exon or Intron
dF508	c.1521_1523delCTT	p.Phe508del	Exon 10	Exon 11
dI507	c.1519_1521delATC	p.Ile507del	Exon 10	Exon 11
G542X	c.1624G>T	p.Gly542X	Exon 11	Exon 12
G85E	c.254G>A	p.Gly85Glu	Exon 3	Exon 3
2869insG	c.2737_2738insG	p.Tyr913X	Exon 15	Exon 17
3120G>A	c.2988G>A	no protein name	Exon 16	Exon 18
3199del6	c.3067_3072delATAGTG	p.Ile1023_Val1024del	Exon 17a	Exon 19
3791delC	c.3659delC	p.Thr1220LysfsX8	Exon 19	Exon 22

Exon Changes

https://www.luminexcorp.com/prod/groups/public/documents/lmnxcorp/cf-nomenclature-whitepaper.pdf

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dI507	c.1519_1521delATC	p.Ile507del	Exon 10	Exon 11
G542X	c.1624G>T	p.Gly542X	Exon 11	Exon 12
G85E	c.254G>A	p.Gly85Glu	Exon 3	Exon 3
2869insG	c.2737_273[illegible]ins[illegible]	p.Tyr913X	Exon 15	Exon 17
3120G>A	c.2988G>A	no protein name	Exon 16	Exon 18
3199del6	c.3067_3072delATAGTG	p.Ile1023_Val1024del	Exon 17a	Exon 19
3791delC	c.3659delC	p.Thr1220LysfsX8	Exon 19	Exon 22

Insertions

Ex:

AGGTACCTG ATCGCTGAA

AGGTACCTGGATCGCTGAA

CFTR HGVS Nomenclature

CFTR

xTAG CF Kit Name/ Legacy Name	HGVS DNA Name	HGVS Protein Name	Legacy Exon or Intron	Exon or Intron
dF508	c.1521_152[del]TT	p.Phe50[del]	Exon 10	Exon 11
dI507	c.1519_152[del]TC	p.Ile50[del]	Exon 10	Exon 11
G542X	c.1624G>T	p.Gly542X	Exon 11	Exon 12
G85E	c.254G>A	p.Gly85Glu	Exon 3	Exon 3
2869insG	c.2737_2738insG	p.Tyr913X	Exon 15	Exon 17
3120G>A	c.2988G>A	no protein name	Exon 16	Exon 18
3199del6	c.3067_307[del]TAGTG	p.Ile1023_Val102[del]	Exon 17a	Exon 19
3791delC	c.365[del]	p.Thr1220LysfsX8	Exon 19	Exon 22

Deletions

Ex:

AGGTACCTCTTGCTGAA

AGGTACCT GCTGAA

CFTR HGVS Nomenclature

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xTAG CF Kit Name/ Legacy Name	HGVS DNA Name	HGVS Protein Name	Legacy Exon or Intron	Exon or Intron
dF508	c.1521_1523delCTT	p.Phe508del	Exon 10	Exon 11
dI507	c.1519_1521delATC	p.Ile507del	Exon 10	Exon 11
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G85E	c.254G>A	p.Gly85Glu	Exon 3	Exon 3
2869insG	c.2737_2738insG	p.Tyr913X	Exon 15	Exon 17
3120G>A	c.2988G>A	no protein name	Exon 16	Exon 18
3199del6	c.3067_3072delATAGTG	p.Ile1023_Val1024del	Exon 17a	Exon 19
3791delC	c.3659delC	p.Thr1220[illegible]fsX8	Exon 19	Exon 22

Frameshift Deletion

Ex:

Thr Glu Gly Gly Asn Ala Ile Leu Glu

ACA GAA GGT GGA AAT GCC ATA TTA GAG

Lys Lys Val Glu Met Pro Tyr STOP

A-AG AAG GTG GAA ATG CCA TAT TAG AG

CFTR HGVS Nomenclature

CFTR

xTAG CF Kit Name/ Legacy Name	HGVS DNA Name	HGVS Protein Name	Legacy Exon or Intron	Exon or Intron
dF508	c.1521_1523delCTT	p.Phe508del	Exon 10	Exon 11
dI507	c.1519_1521delATC	p.Ile507del	Exon 10	Exon 11
G542X	c.162[illegible]G>T	p.Gly542X	Exon 11	Exon 12
G85E	c.25[illegible]G>A	p.Gly85Glu	Exon 3	Exon 3
2869insG	c.2737_2738insG	p.Tyr913X	Exon 15	Exon 17
3120G>A	c.298[illegible]G>A	no protein name	Exon 16	Exon 18
3199del6	c.3067_3072delATAGTG	p.Ile1023_Val1024del	Exon 17a	Exon 19
3791delC	c.3659delC	p.Thr1220LysfsX8	Exon 19	Exon 22

Substitutions

Ex:

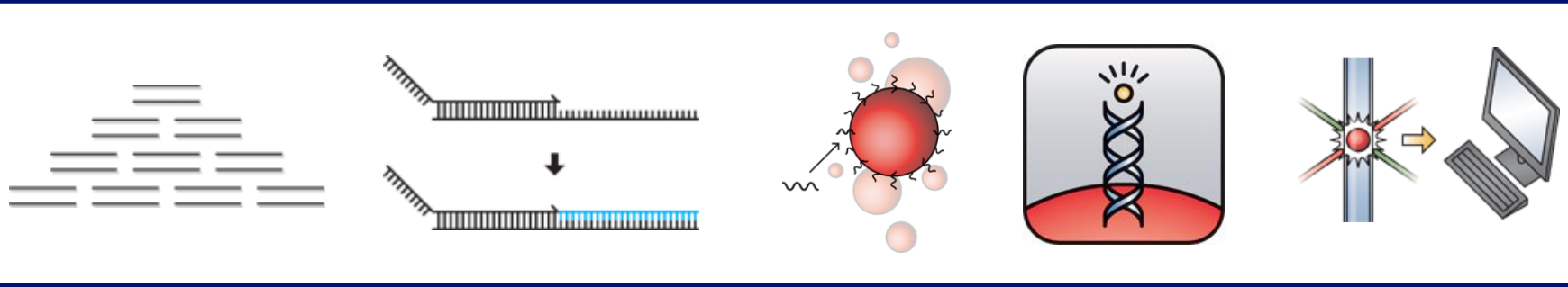
AGGTACCTGATCGCTGAA

AGGTACCTAATCGCTGAA

Assays to Detect Mutations in *CFTR*

- **Single mutation detection (F508del)**
  - Gel based assays to discriminate size differences
  - Fluorescent detection Taqman assay
- **Multiplex mutation detection**
  - xTAG CF Assay – Luminex Corporation
    - xTAG 39
    - xTAG 60
  - InPlex CF Assay – Hologic (Invader technology)
    - InPlex – 23 mutations
    - InPlex – 40 mutations
  - DNA sequencing
    - Unlimited within amplicons

xTAG Cystic Fibrosis Assay Technology
Luminex Corp



Multiplex PCR Rxn Amplicon Treatment

Allele-specific Primer Extension

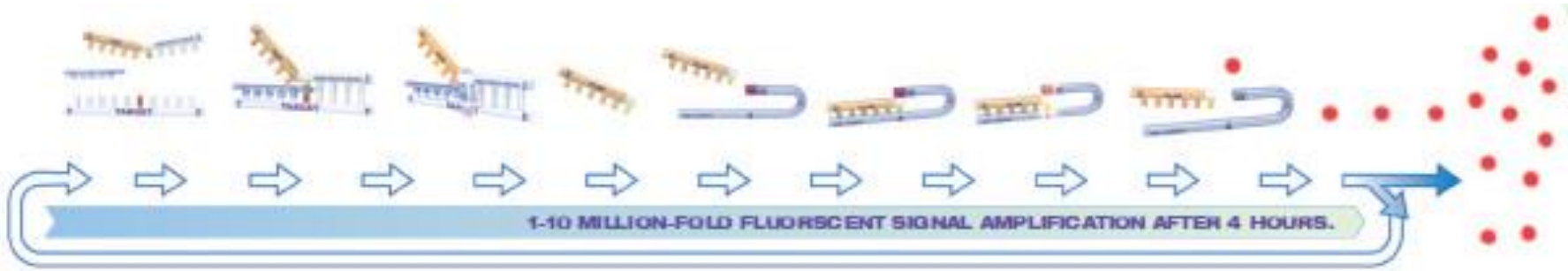
Bead Hybridization

Reporter Addition

Data Acquisition

https://www.luminexcorp.com/Products/Assays/ClinicalDiagnostics/xTAGCysticFibrosis/

InPlex CF Molecular Assay Technology
Hologic



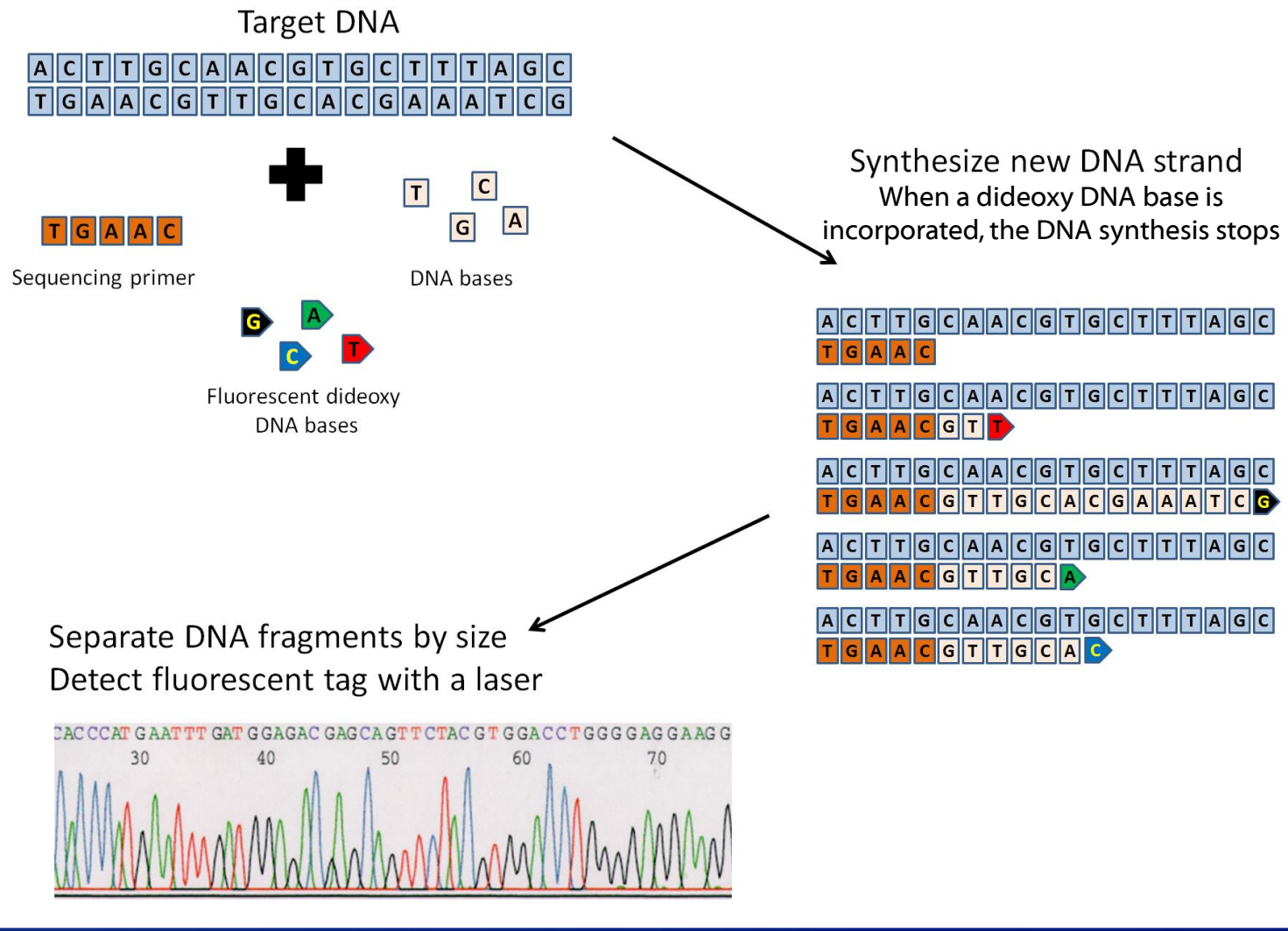
Probe and Invader Oligo binds to specific DNA sequences creating a flap which is then cleaved when the desired sequence is present.

Flaps combine with fluorescence resonance energy transfer (FRET) probe generating a fluorescent signal.

Fluorescent Detection

http://www.inplexcf.com/laboratory/inplextechnology.html

CFTR DNA Sanger Sequencing



Case Study of a Newborn with Elevated IRT

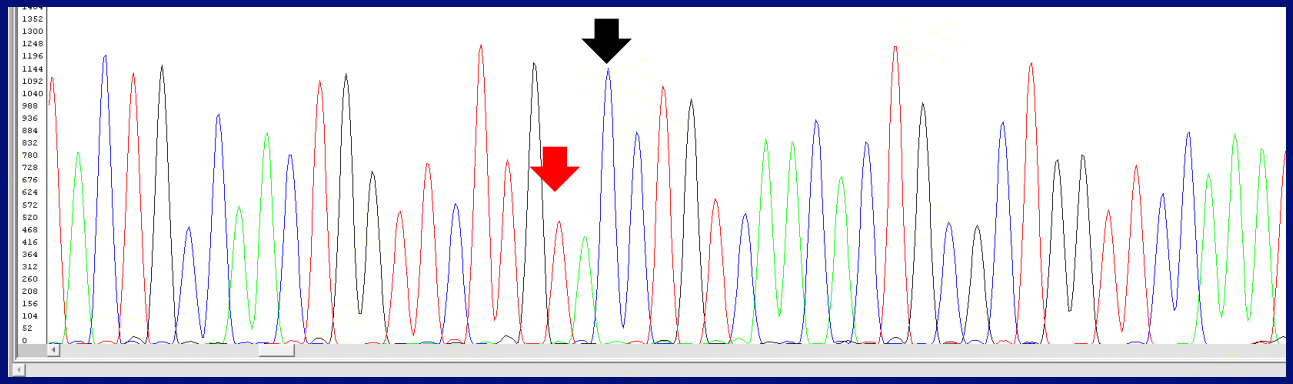
- **DBS was detected with elevated IRT above the 4% Cutoff → Reflex to 2nd Tier Mutation Testing**
- **Initial Assay: Luminex xTAG 39**
  - Two probes representing mutations Y1092X C>G and Y1092X C>A failed both the initial and repeat run
  - Repeat specimen was requested with the same results

Variation	Call	Raw Signals (MFI)		Background (MFI)		Net Signals (MFI)		Allelic Ratios		AR Thresholds			Notes and explanations
		Wt Allele	Mut Allele	Wt Allele	Mut Allele	Wt Allele	Mut Allele	Wt Allele	Mut Allele	WT Call	Wt Present	Mut Present	
2789+5G>A	WT	6438.0	62.0	68.5	85.0	6369.5	0.0	1.00	0.00	0.85	0.25	0.25	
3120+1G>A	WT	7454.5	178.0	53.0	44.0	7401.5	134.0	0.98	0.02	0.85	0.25	0.25	
Y1092X-C>G	No Call	220.0	84.0	43.0	77.0	177.0	7.0			0.75	0.25	0.30	Variation failed: signal(s) inadequate
└ Y1092X-C>A	No Call		112.0		60.5		51.5					0.30	Variation failed: signal(s) inadequate

- **Secondary Assay: Inplex CF - 40**
  - No mutations detected – both Y1092X probes gave a normal result

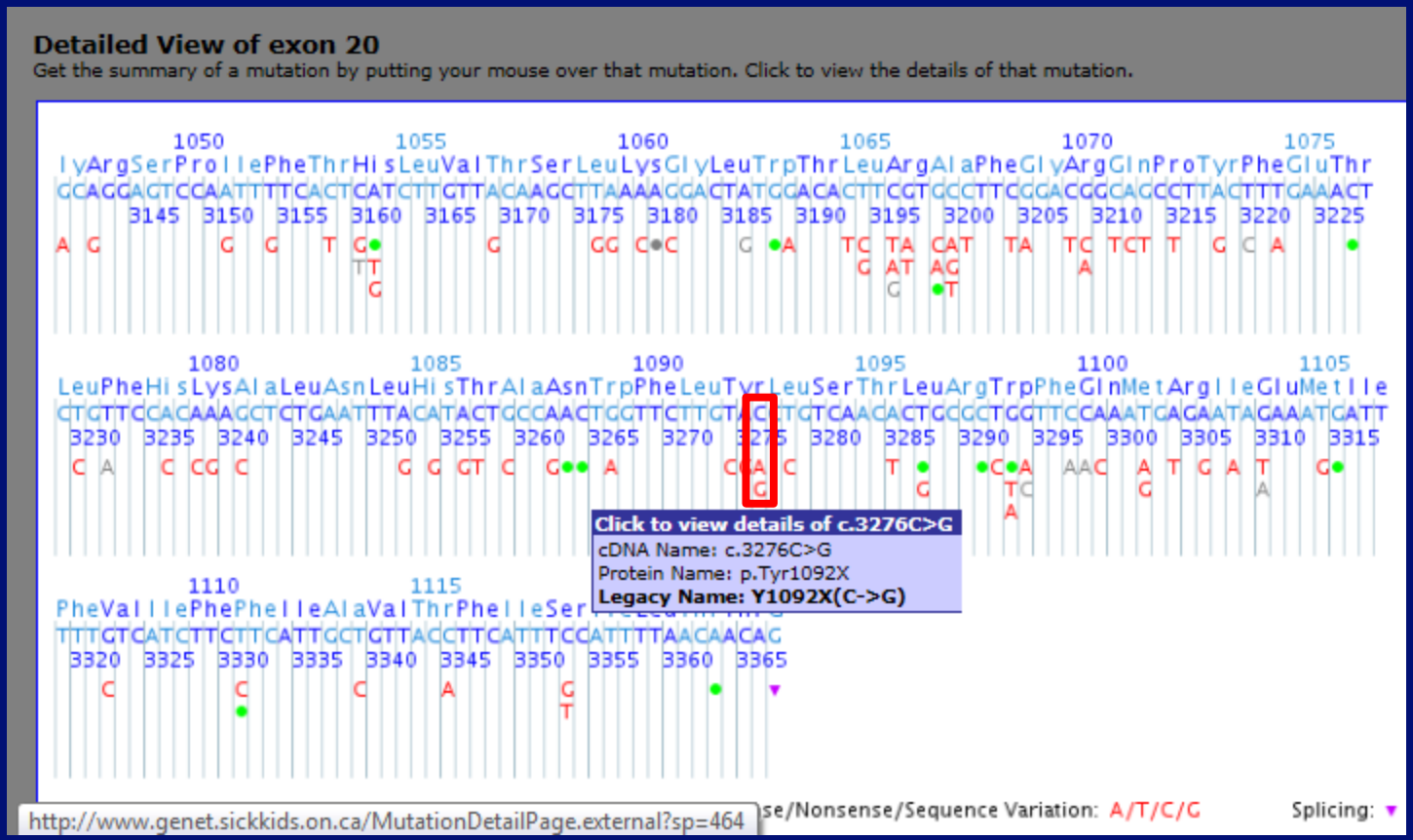
Case Study of Newborn with Elevated IRT

- **DBS was detected with elevated IRT above the 5% Cutoff → Reflex to 2nd Tier Mutation Testing**
- **Assay: Luminex xTAG 39**
  - Two probes representing mutations Y1092X C>G and Y1092X C>A failed both the initial and repeat run
  - Repeat specimen was requested with the same results
- **Sample sent for DNA sequencing of Exon 20**
  - Baby was "homozygous" for Y1092H T>C



Known Mutations in CFTR Exon 20

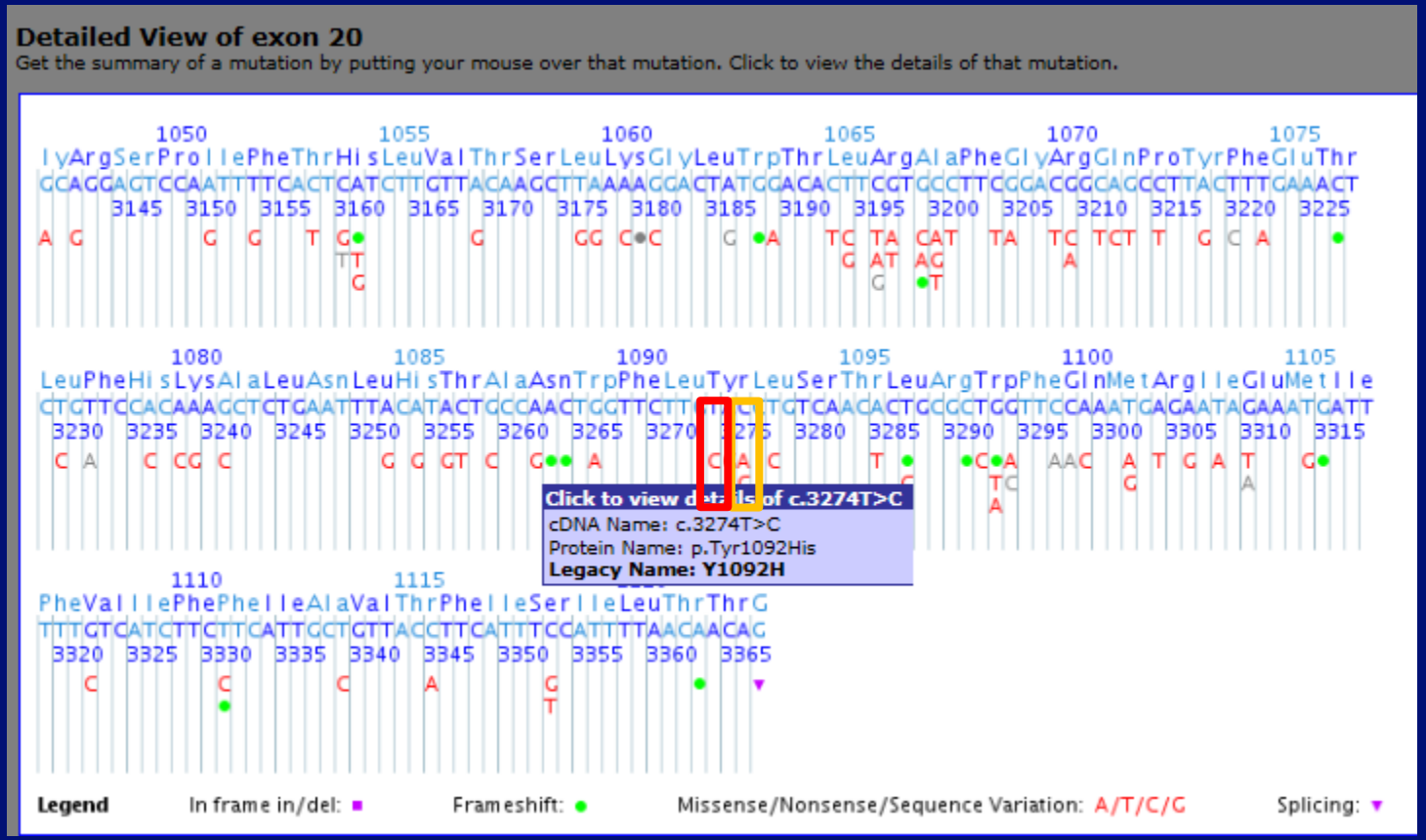
CFTR



CFTR Mutation Database: http://www.genet.sickkids.on.ca/

The Good, The Bad and The Ugly…

CFTR



Y1092H's proximity to Y1092X resulted in a failure of the Luminex Y1092X probes to hybridize.

Is this Case Study Done??

- **Was the baby homozygous or hemizygous for Y1092H T>C?**
  - hemizygous is when there is only 1 member of a chromosome segment rather than the usual 2
- **Could there be a large deletion of Exon 20????**
- **How could this be determined????**
  - **Approach 1:** Sequence Exon 20 in both parents to see if they both have Y1092H T>C
  - **Approach 2:** Perform a molecular deletion assay such as MRC Holland's MLPA which can detect 1 versus 2 copies of Exon 20

Case Study Take Home Messages

- **Assay failures can offer important information**
- **No assay can catch everything**
- **Assays used in newborn screening labs do not detect most large deletions**
- **Know your state's policies**
  - What is your program responsible for and what is diagnostics responsible for in your state?
  - How do you communicate your findings in the most meaningful way to diagnostic partners?

Cystic Fibrosis Key Points Part 2

- **HGVS nomenclature describes the nature of the mutation which is different from the legacy nomenclature previously used for *CFTR* mutations.**
  - Eg. F508del (legacy) vs. c.1521_1523delCTT (HGVS)
- **There are two commonly used technologies used in the U.S. to detect a panel of *CFTR* mutations**
  - InPlex CF Assay from Hologic – probe hybridization and invader technology
  - xTAG CF Assay from Luminex – primer extension

Thank you!

Newborn Screening

***Saving* Lives.**

***Promoting* Healthier Babies.**

***Protecting* our Future.**

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333

Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348

Visit: www.cdc.gov | Contact CDC at: 1-800-CDC-INFO or www.cdc.gov/info

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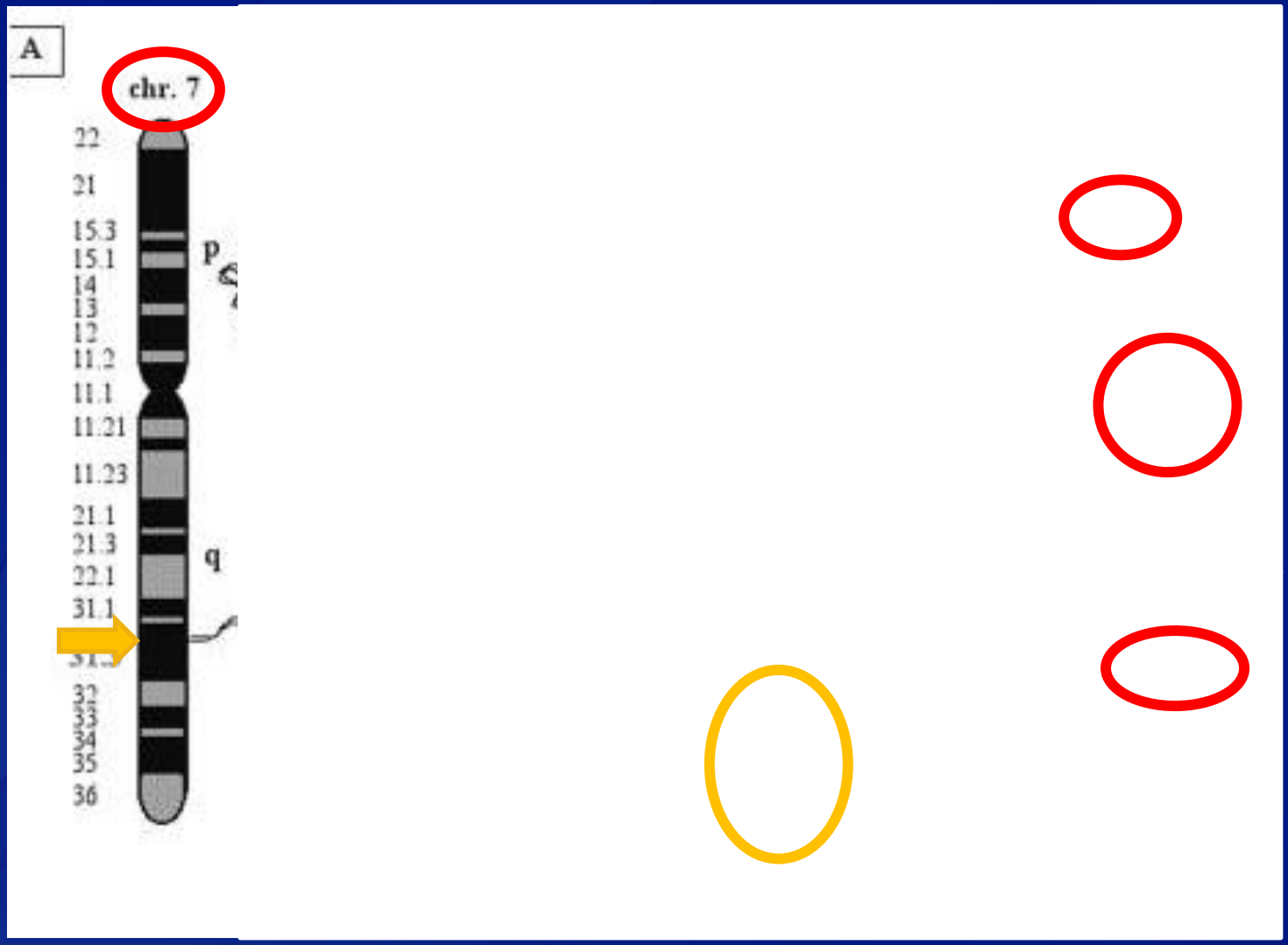
- Exon changes
- Deletions
- Insertions
- Substitutions
- Frameshifts

CFTR Gene Structure



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