Genome Sequencing:
Applications and Newborn Screening

June 2, 2014

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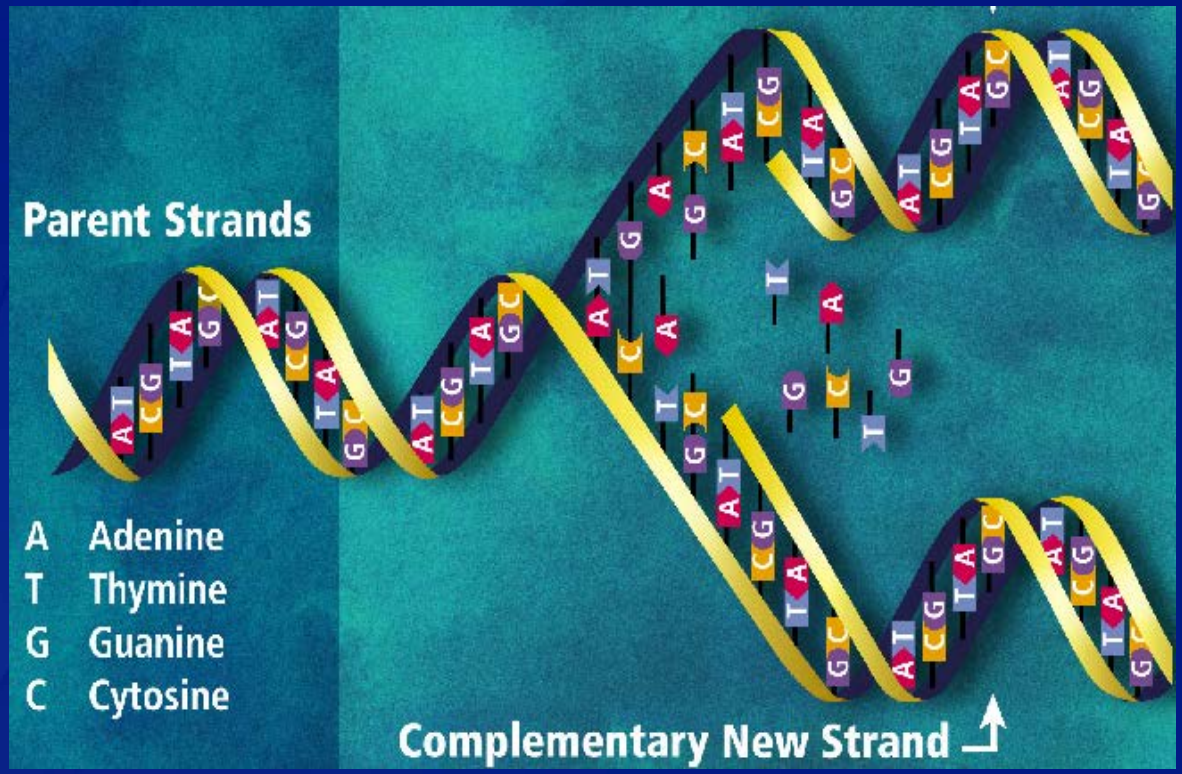
Centers for Disease Control and Prevention

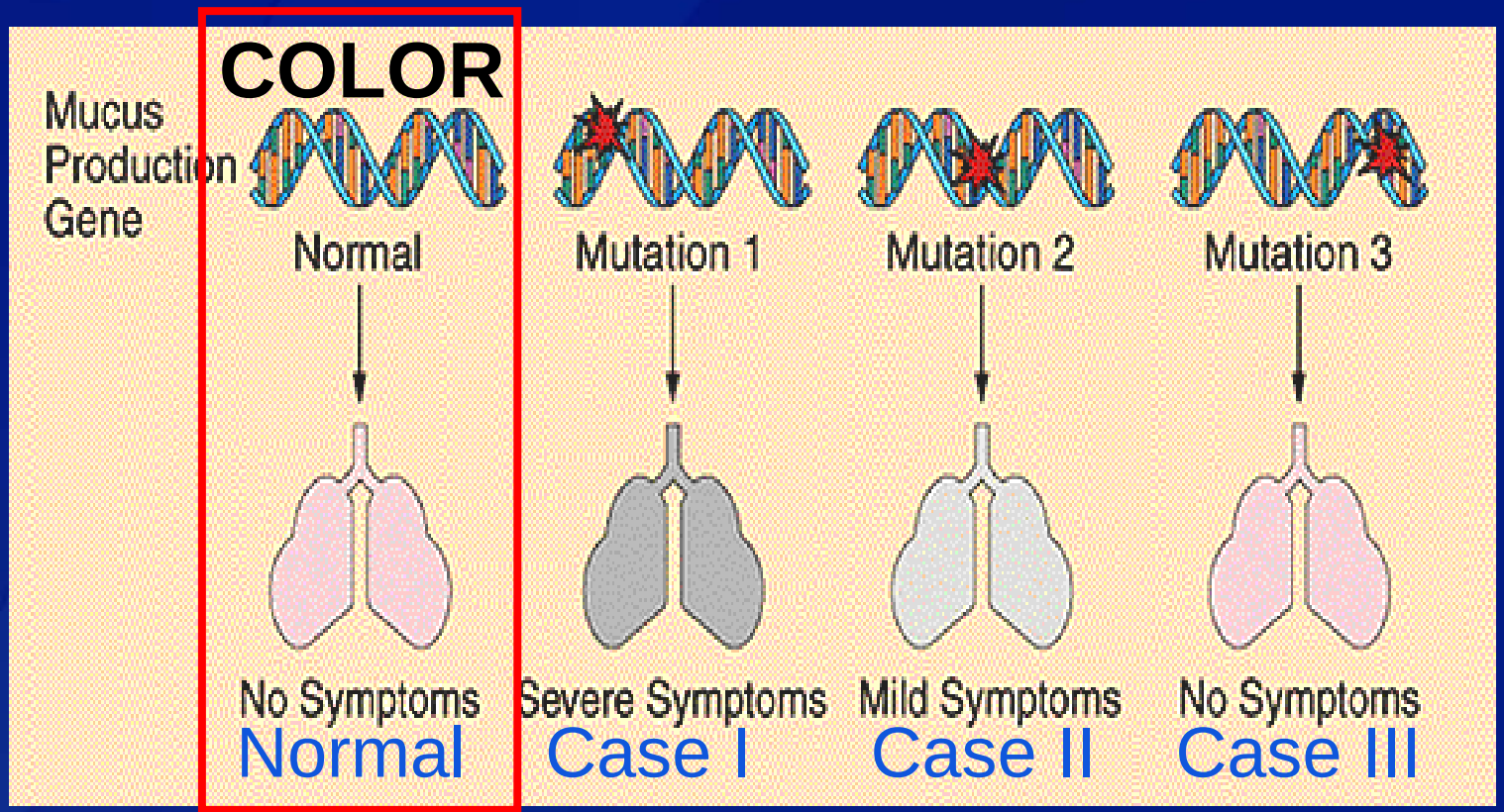
National Center for Environmental Health
Division of Laboratory Sciences

When DNA is Replicated for Cell Division, Errors can Occur



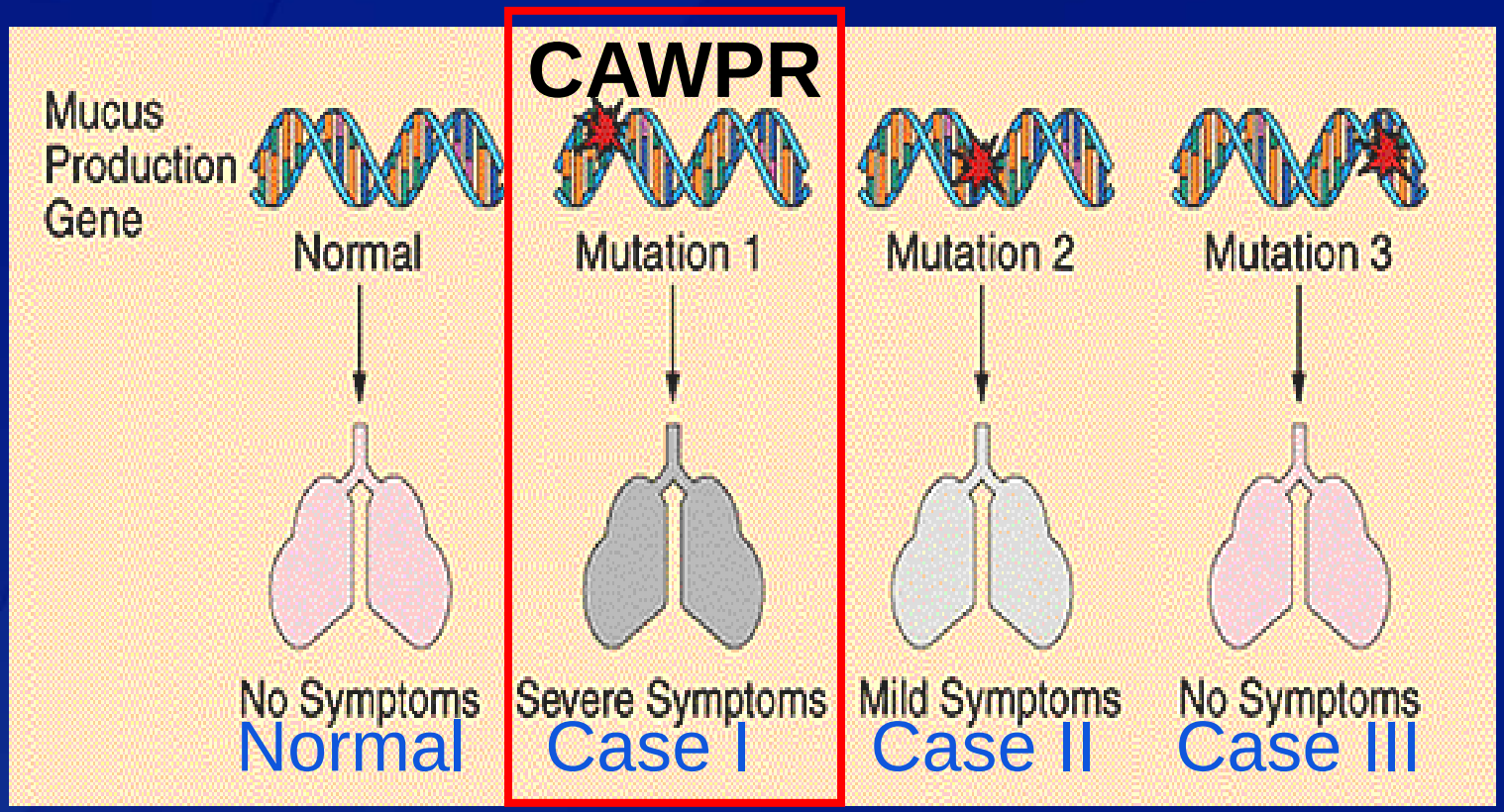
These errors can result in disease

Mutations in the Cystic Fibrosis Gene



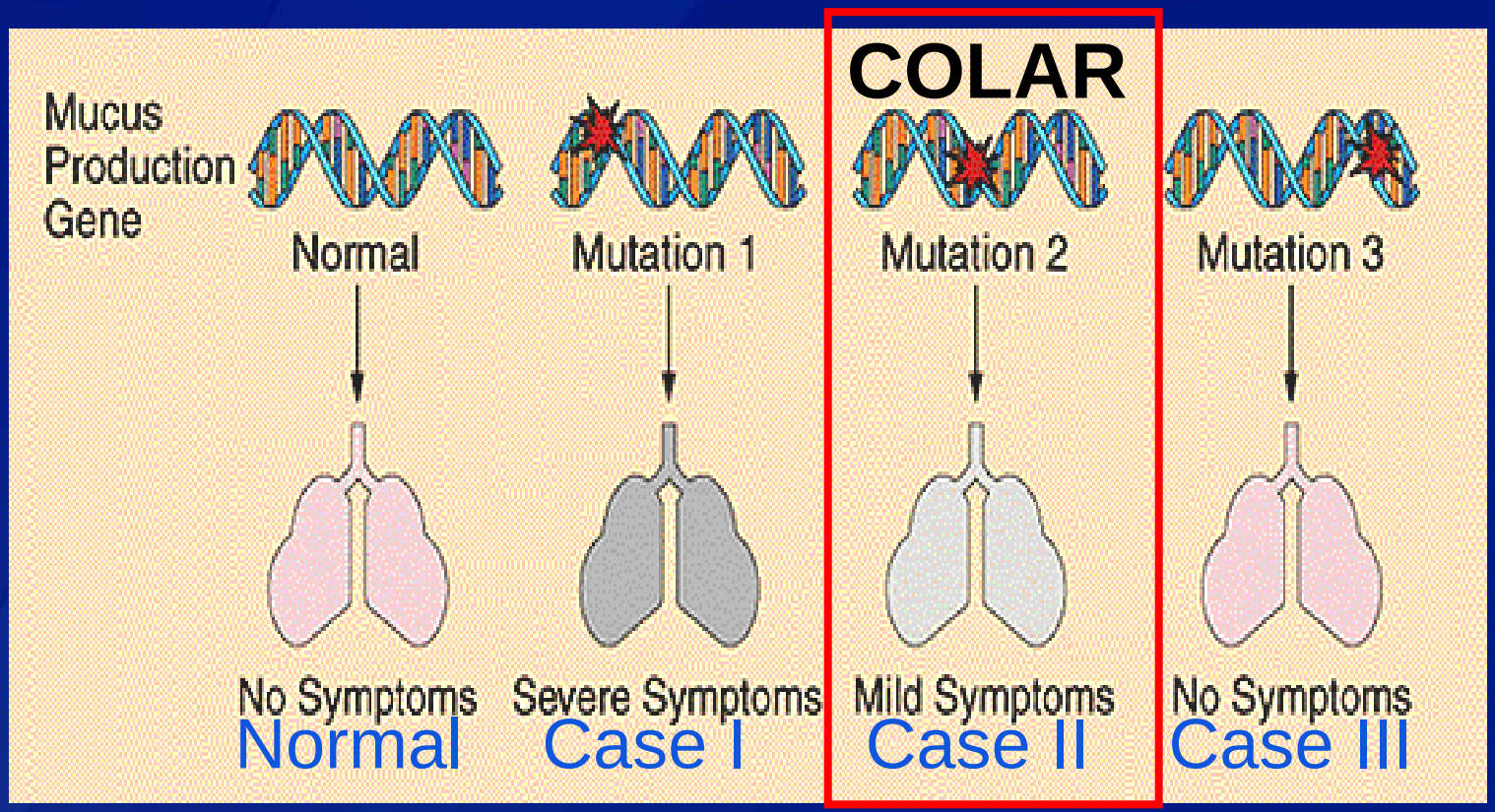
Normal - no changes

Mutations in the Cystic Fibrosis Gene



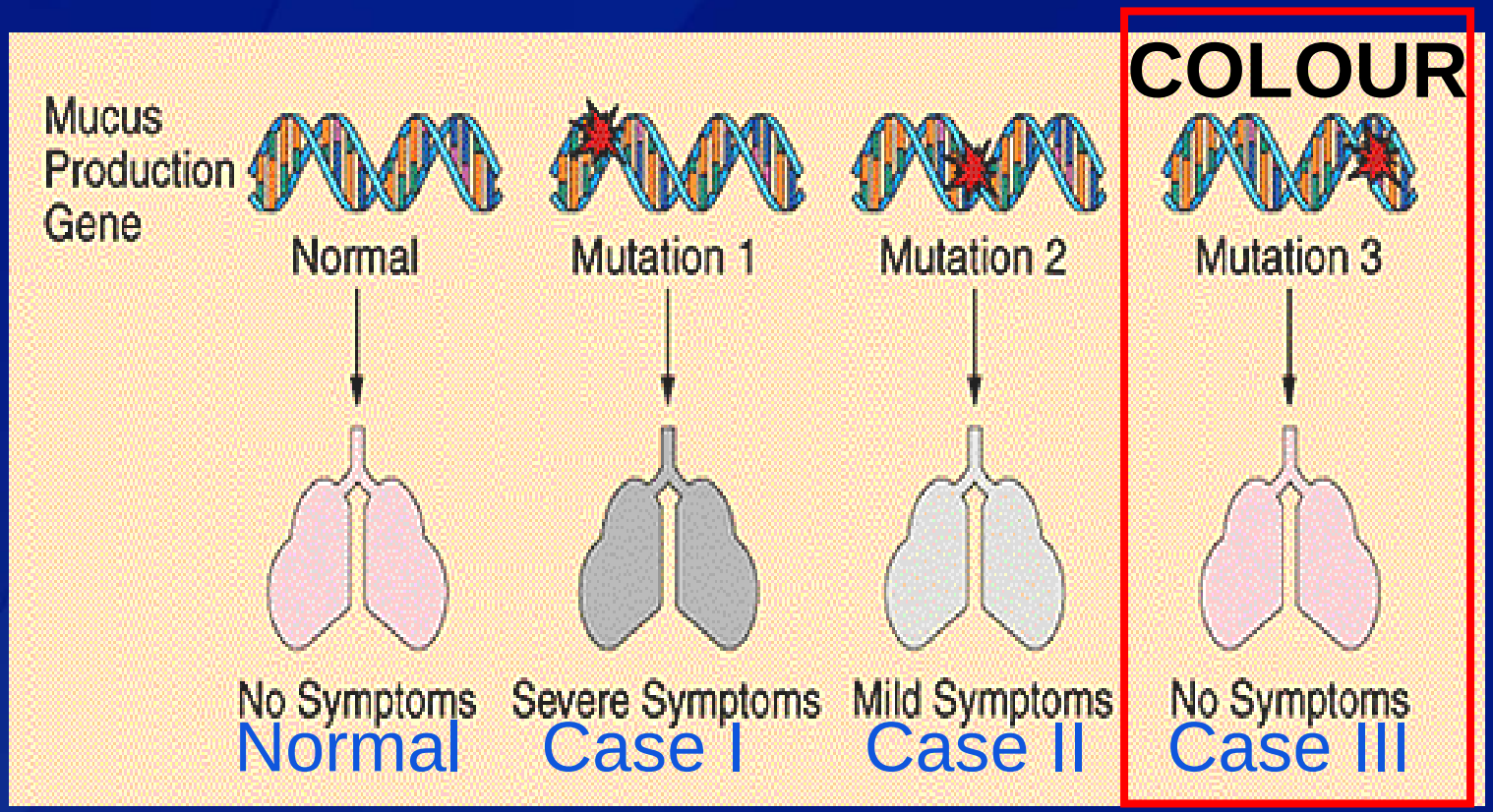
Case I – unrecognizable

Mutations in the Cystic Fibrosis Gene



Case II – typo

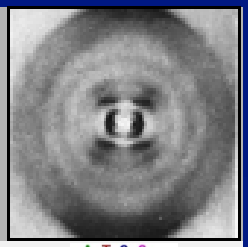
Mutations in the Cystic Fibrosis Gene



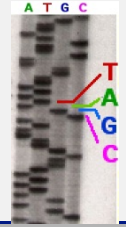
Case III – American vs. British

Molecular Genetics Milestones

- **1952:** Described DNA as a double helix (using x-ray diffraction)



- **1975:** Devised techniques for DNA sequencing



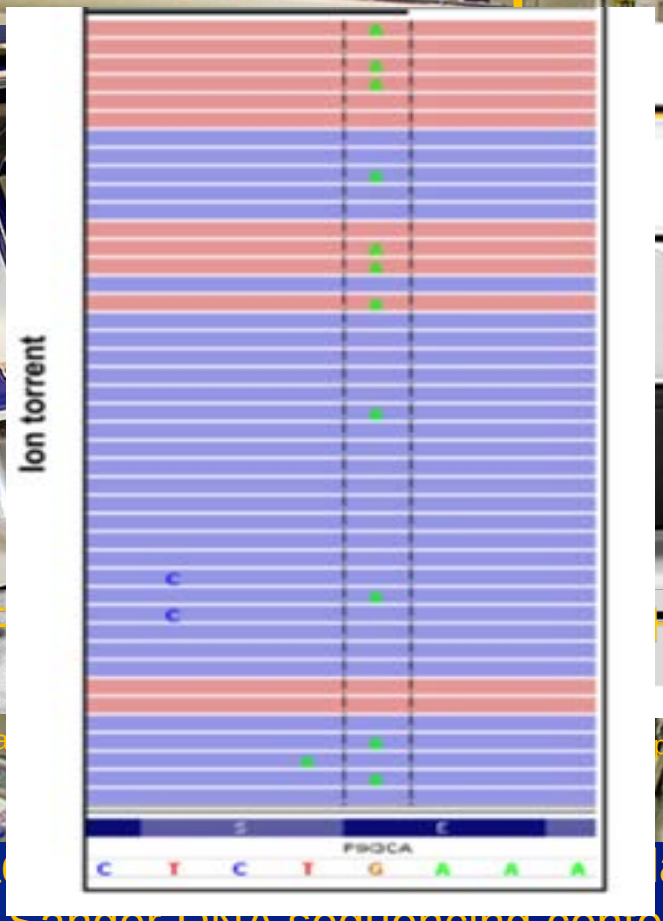
- **1985:** Conducted first polymerase chain reaction (PCR) experiments to amplify specific gene regions

- **2003:** Human Genome Sequencing Project is completed

DNA Sequencing
1975 to 2014 and Beyond

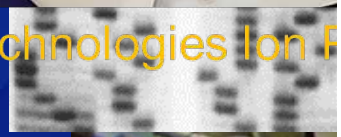
Next Generation Sequencing

Life Technologies Ion P



Singh et al. J Mol Diagn. Vol 15(3), 301-310 (2013)

HiSeq 2500 Sequencer



http://molecularsta

http://www.uvm.edu/~cgep/Education/Sequence.html

The Broa

arge-scale

Sanger DNA sequencing center

Sequencing the Genome

- U.S. Human Genome Project began in 1989 and was completed by 23 collaborating laboratories in 13 years (cost ~$2.7 billion)
- Latest DNA sequencers can sequence a human genome in a few days for less than $5,000
- “2013 is likely to be the year where we see the $1000 genome.” - Daniel Franklin, executive editor of *The Economist*

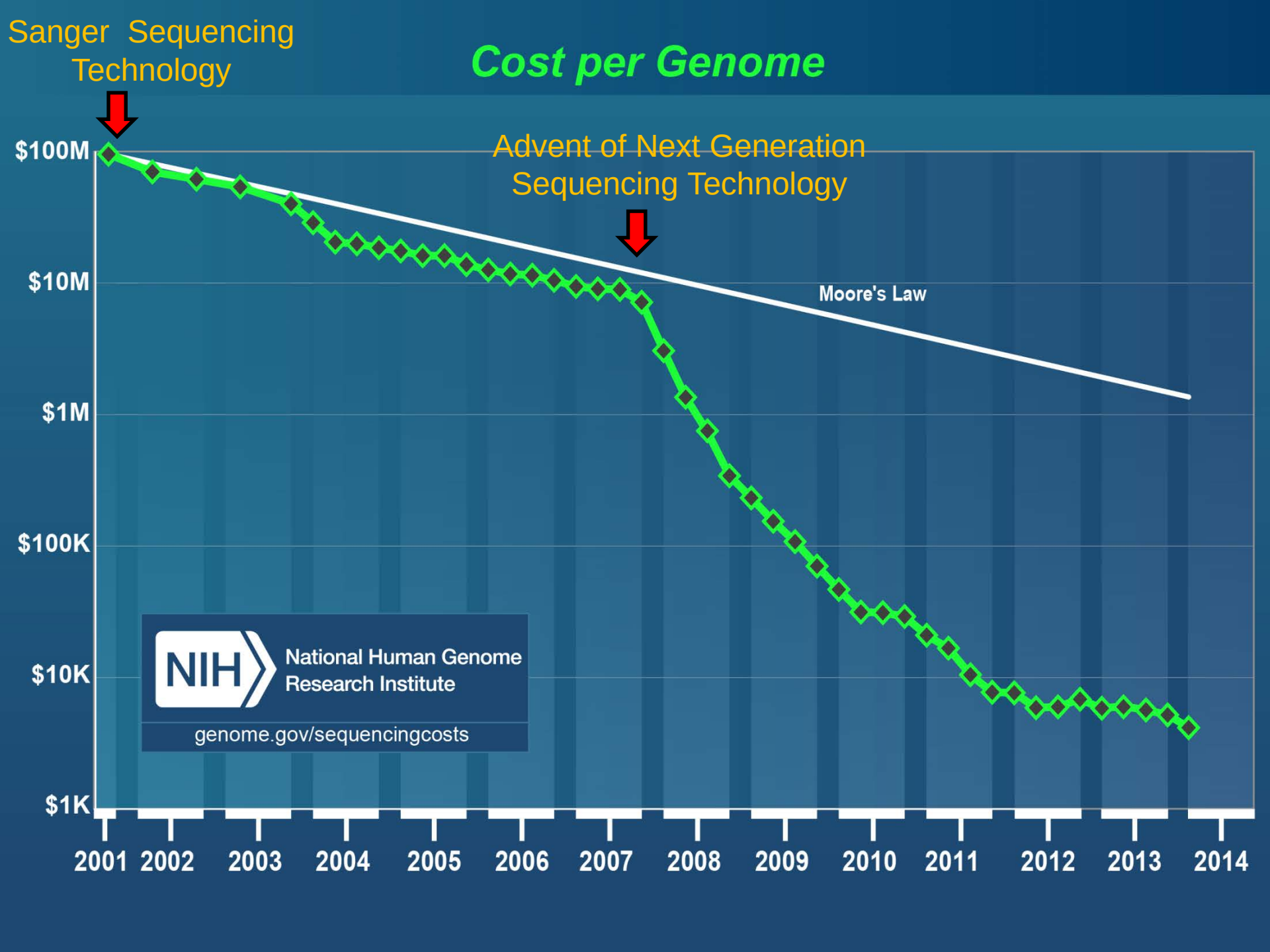
Will Genome Sequencing be like a Healthcare Tsunami?

Or Will We Ride the Wave?

Either way, we are going to get wet…

“…it may soon be easier and cheaper to sequence an entire genome than to test for a number of known mutations.”

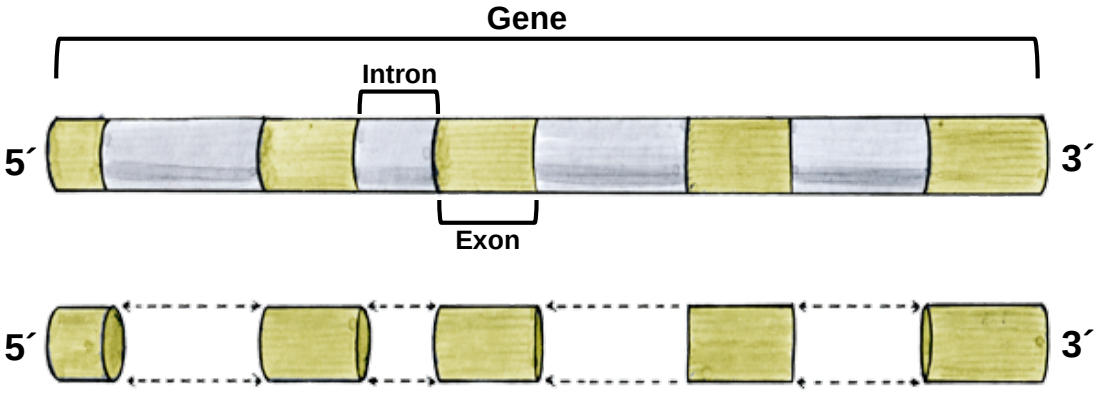
- Foundation for Genomics and Population Health

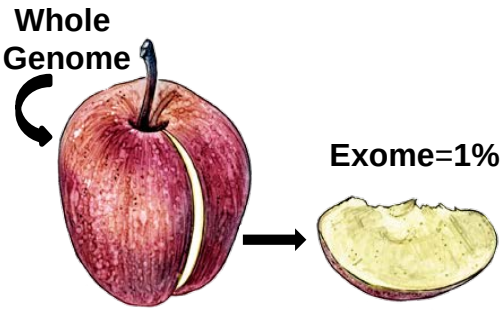

Sanger Sequencing Technology
Cost per Genome
Advent of Next Generation Sequencing Technology
$100M
$10M
$1M
$100K
$10K
$1K
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014

Sequencing the Human Genome

- **Genome is comprised of 3 billion bases**
- **Exome is approximately 1% of the genome**
  - Includes DNA segments that contain genes
  - Genes hold the recipe for all the body's proteins

Genome vs. Exome





Genome Sequencing for Clinical Diagnosis

The NEW ENGLAND JOURNAL of MEDICINE

Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders

Yaping Yang, Ph.D., Donna M. Muzny, M.Sc., Jeffrey G. Reid, Ph.D., Matthew N. Bainbridge, Ph.D., Alecia Willis, Ph.D., Patricia A. Ward, M.S., Alicia Braxton, M.S., Joke Beuten, Ph.D., Fan Xia, Ph.D., Zhiyv Niu, Ph.D., Matthew Hardison, Ph.D., Richard Person, Ph.D., Mir Reza Bekheirnia, M.D., Magalie S. Leduc, Ph.D., Amelia Kirby, M.D., Peter Pham, M.Sc., Jennifer Scull, Ph.D., Min Wang, Ph.D., Yan Ding, M.D., Sharon E. Plon, M.D., Ph.D., James R. Lupski, M.D., Ph.D., Arthur L. Beaudet, M.D., Richard A. Gibbs, Ph.D., and Christine M. Eng, M.D.

Molecular Diagnosis using Genome Sequencing

- Exome sequencing to achieve a clinical diagnosis
- Recent NEJM study made a molecular diagnosis in 62 of 250 cases (25%)
- Marked improvement over testing single genes or gene panels currently used

http://www.jsonline.com

Limitations of Sequencing
No Method is Perfect

- Deletions removing large segments of DNA, rearrangements or copy number variations will not be detected
  - Examples of newborn diseases resulting from large deletions: Cystic fibrosis and Congenital Adrenal Hyperplasia
- Person to person DNA variation can give inaccurate results due to mis-priming
- Traditional sequencing has an error rate of 1/10,000 to 1/100,000 - Next Gen sequencing is considerably higher

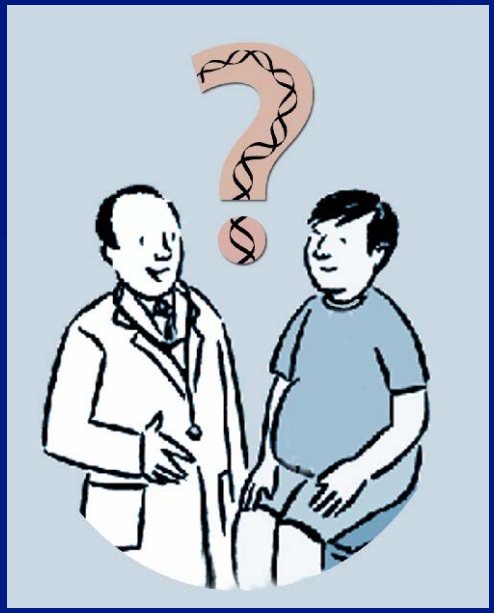
Challenges of Genome/Exome Sequencing

- **Major Challenge: Determining whether any given variant is pathogenic**
- **ACMG defined 5 categories to classify variants:**
  - Known pathogenic
  - Likely to be pathogenic
  - Unknown significance
  - Likely to be benign
  - Benign

- **Knowledge accruing daily, however the medical impact of most variants is unknown**

Genome Sequencing Results

- **What data should be returned to patients and physicians?**
- **Factors to consider**
  - Patient autonomy
  - Patient privacy
  - Physician liability
  - Clinical laboratory guidelines for reporting



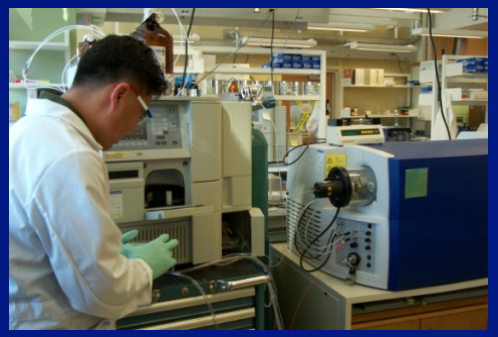
Characteristics of Newborn Disorders Include

- Significant disease
- Prevention possible
- Not evident until harm is done
- Mass testing methods available
- Benefits justify costs

Slide Courtesy of Mike Glass
Washington State Dept of Health

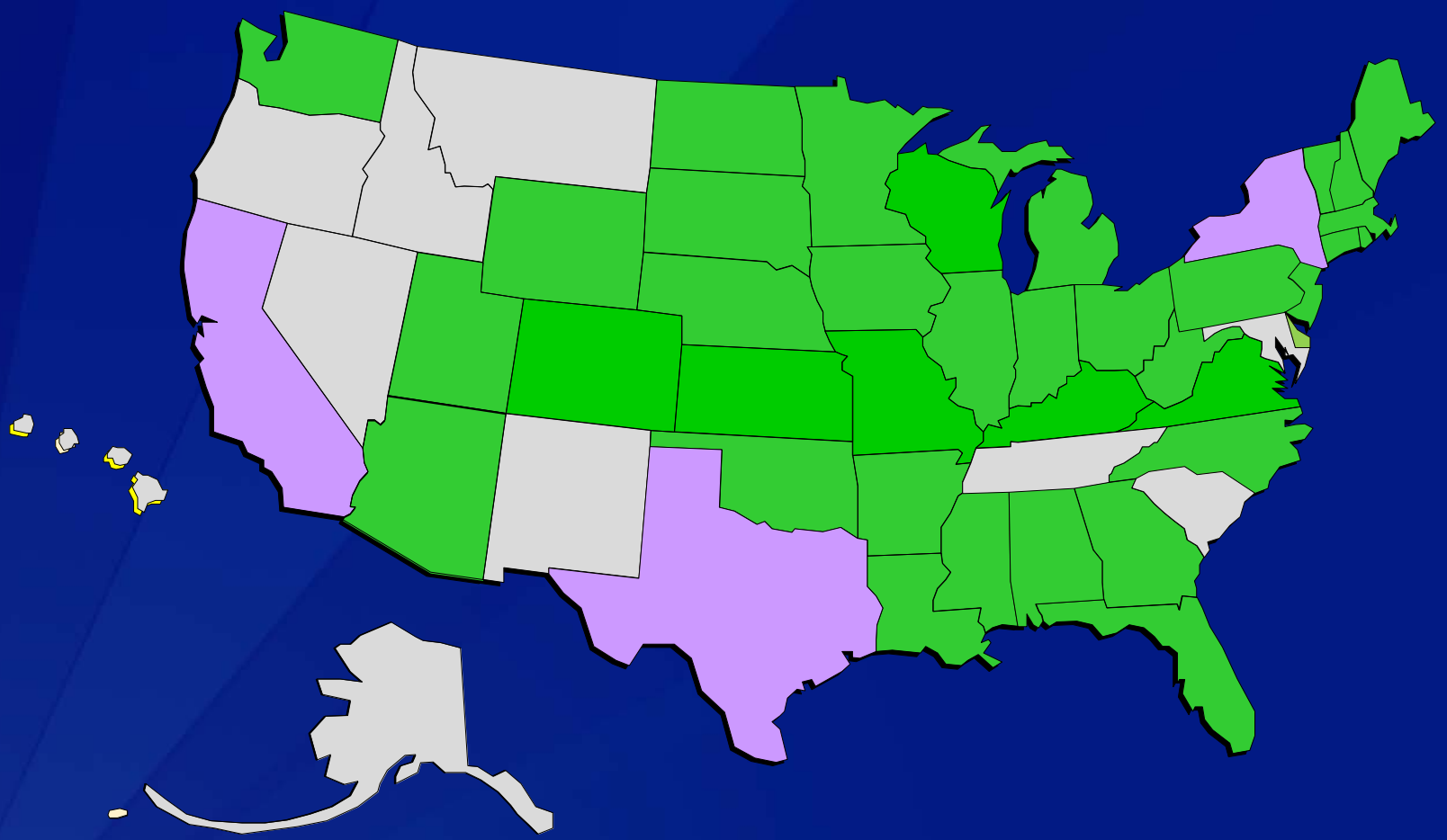
Molecular Testing in Newborn Screening Laboratories

- **Second tier molecular tests**
  - Increase sensitivity or specificity of primary assay
    - Cystic fibrosis
  - Clarify an ambiguous result
    - Hemoglobinopathies
  - Supplemental “Just in Time” assay
    - Galactosemia



- **Primary molecular test**
  - When no other assay is available – Severe Combined Immunodeficiency (SCID)

NBS Molecular Testing Status: 2014



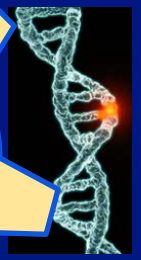
41 states offer a molecular test
3 states use targeted DNA sequencing

Future of Sequencing in Newborn Screening…

- Targeted gene sequencing to identify mutations associated with diseases that have treatable or preventable outcomes
- Mutation specific drugs
  - Kalydeco – treats Cystic Fibrosis patients with specific muations
    - G551D, G178R, S549N, S549R, G551S, G1244E, S1251N, S1255P & G1349D
- Exome and Genome sequencing will be explored over the next few years by NICHD grant recipients

Translating Research to Medicine and Public Health

Research



Medicine

Public Health

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
E-mail: cdcinfo@cdc.gov Web: www.cdc.gov