Wisconsin Newborn Screening Laboratory

Improving IRT/DNA Newborn Screening for Cystic Fibrosis to Reduce Screening False Positives by a New Molecular Strategy

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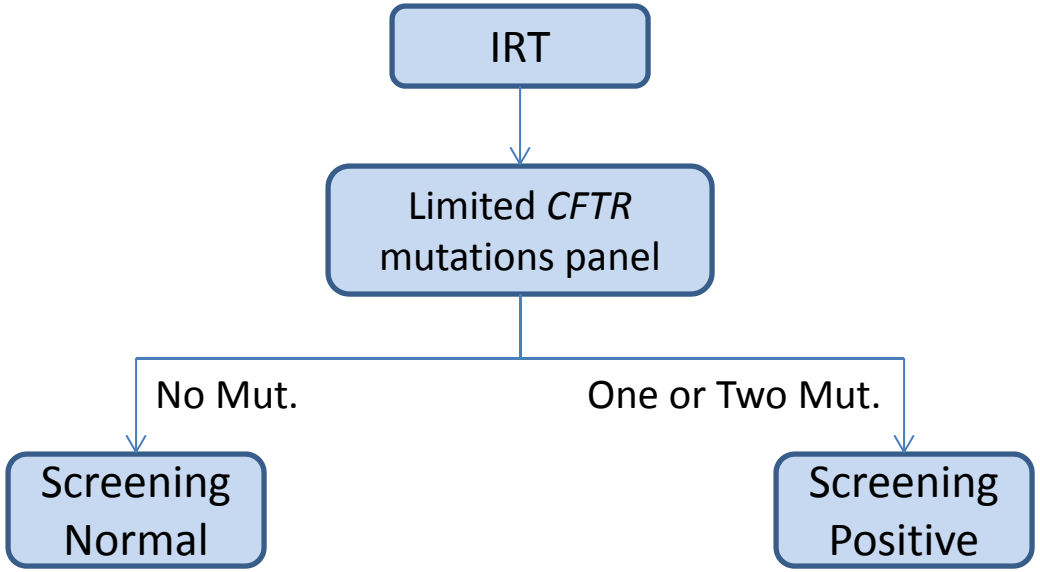
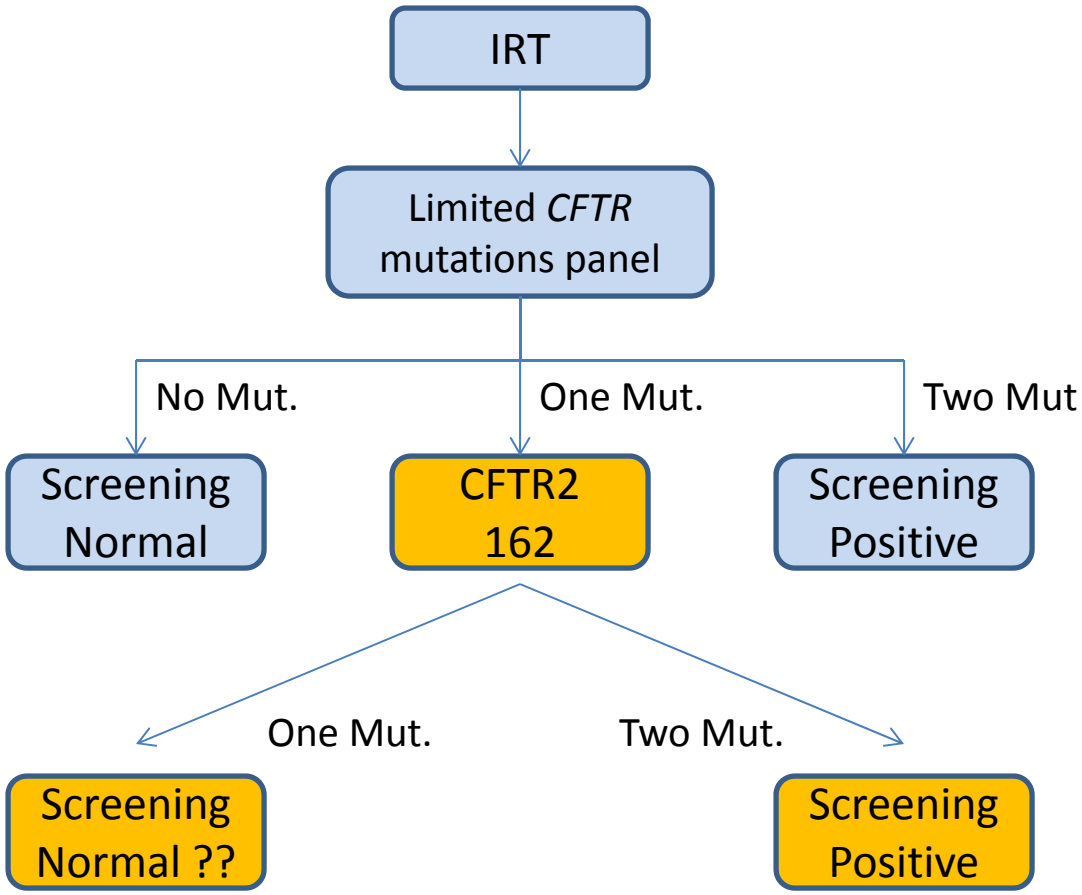
Background

Two Tier IRT/DNA



1. Majority of infants with one mutation identified by NBS are CF carriers.
2. 2,000 mutations of *CFTR* gene are reported in the CF mutation Database, and CFTR2 Project identified the ~160 most common CF disease causing mutations that account for 97% of known CF cases (http://www.cftr2.org/)
3. Advanced next generation sequencing technology makes it possible to simultaneously detect at least 160 mutations, including the common large deletions.

Proposed IRT/DNA/DNA



Specific Aims

1. Establish a method of simultaneously detecting 162 *CFTR* disease causing mutations ***using dried blood spot routine newborn screening specimens to create IRT/DNA/DNA CF screening opportunity.***

2. Demonstrate that the three-tier IRT/DNA/DNA CF screening protocol would significantly reduce false screening positive results caused by identification of CF heterozygote carrier infants.

3. Demonstrate that it is cost effective to implement the three-tier IRT/DNA/DNA CF screening protocol into routine NBS for CF.

Strategy

1. Capitalize on data from the CFTR2 project.

2. Utilize the Illumina MiSeqDx™ Cystic Fibrosis Solution assay for 162 *CFTR* mutations. (including common deletions)

3. Collaboration with five States (IL, IN, MI,MN, and WI), establishing ***The Great Lakes Consortium for Newborn Screening*** (total more than 500,000 birth per year).

Study Design and Methods

1. Analyze DBS specimens for 162 *CFTR* mutations there is a "high" IRT level and one *CFTR* mutation detected in the routine two tier IRT/DNA screening test (including common large deletions).

2. Verify newly identified mutations by Sanger sequencing at Molecular Quality Improvement Program, CDC NBS & Molecular Biology Branch.

3. DO NOT change routine CF screening/follow-up during the study period, i.e., maintain practices.

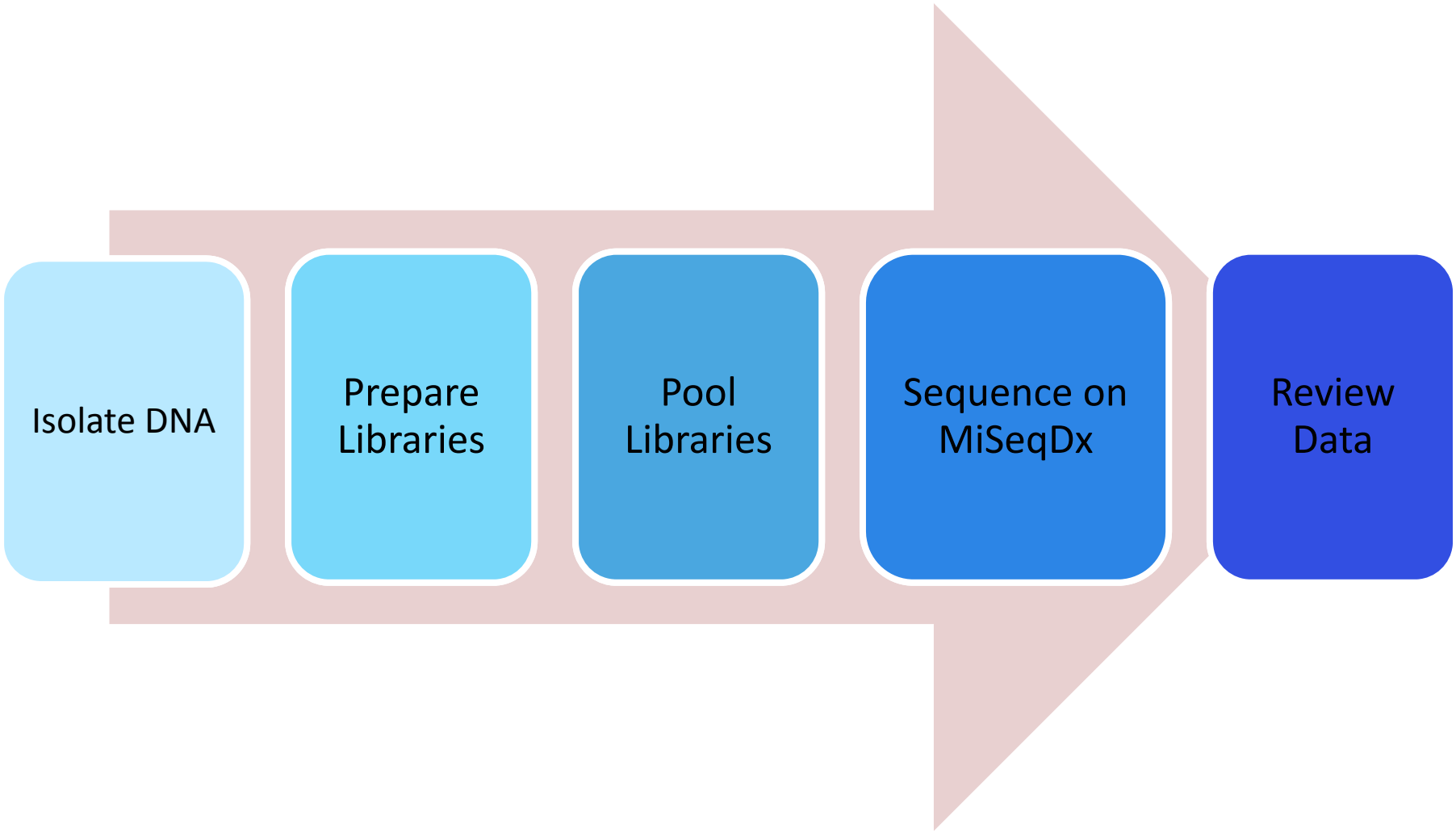
4. Access concordance between the results from sweat test and the number of mutant alleles.

MiSeqDx Cystic Fibrosis System

- 162 DNA mutations including (IUO version*)
  - 127 single nucleotide mutations
  - 32 insertion / deletion mutations
  - 2 large deletions
  - PolyTG / PolyT region

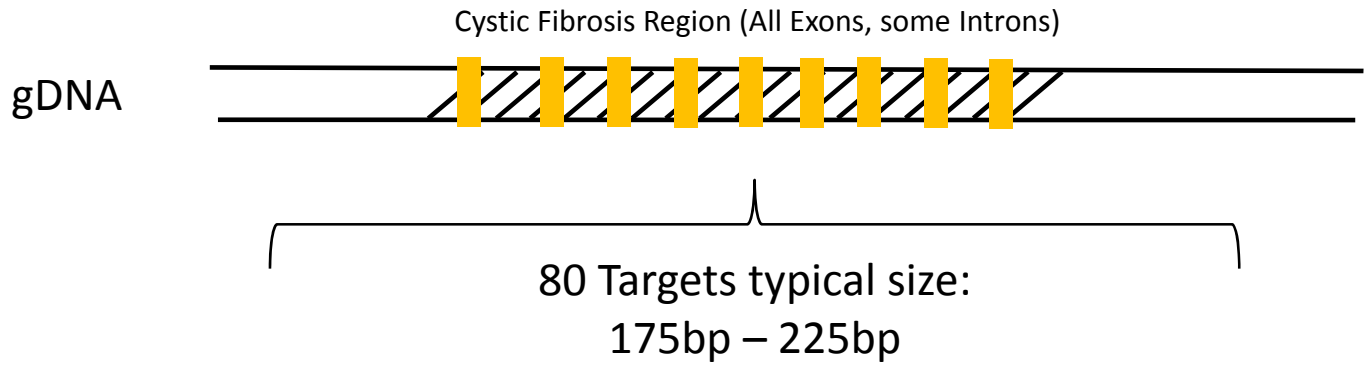
*Product is currently under FDA review.

Cystic Fibrosis Workflow Overview

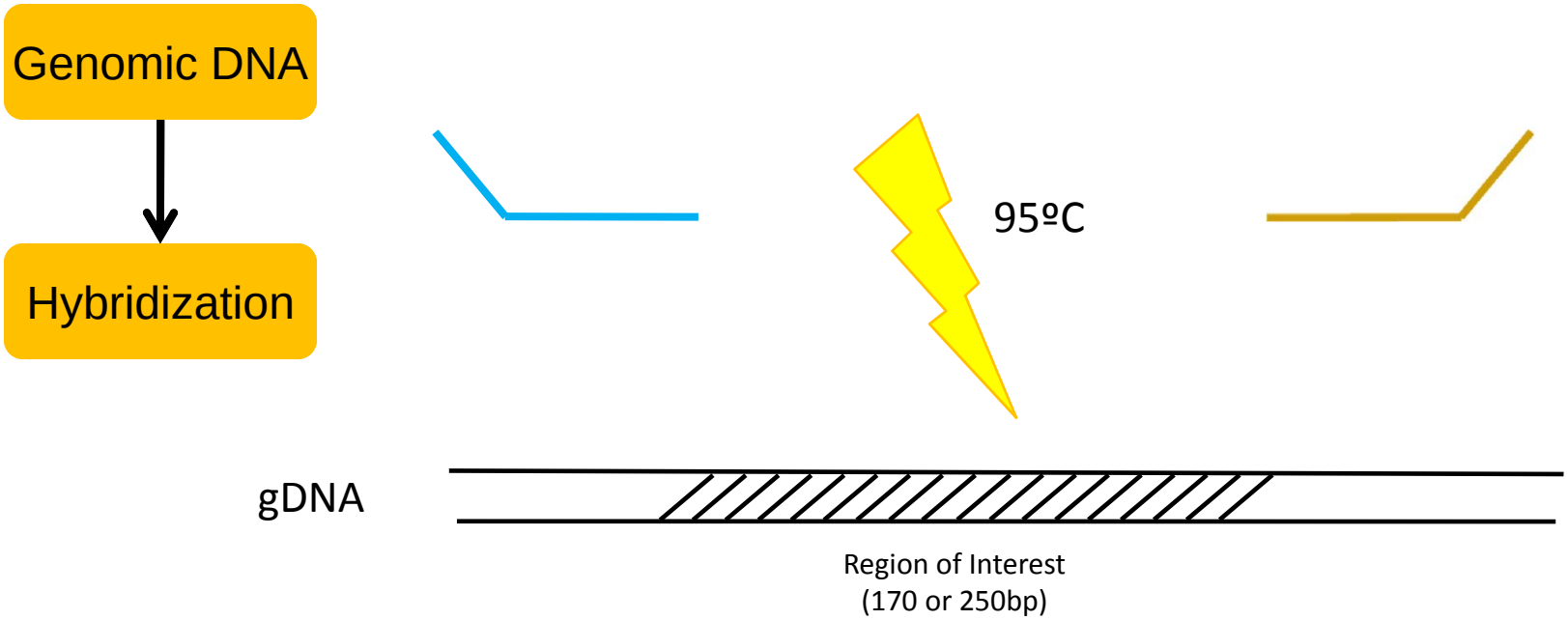


Cystic Fibrosis Assay Overview

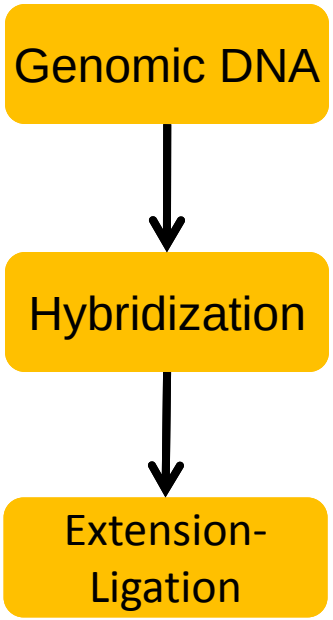
Genomic DNA

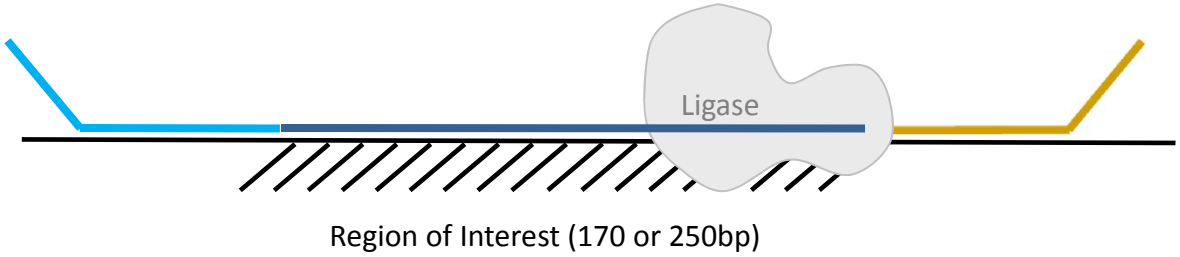


Cystic Fibrosis Assay Overview



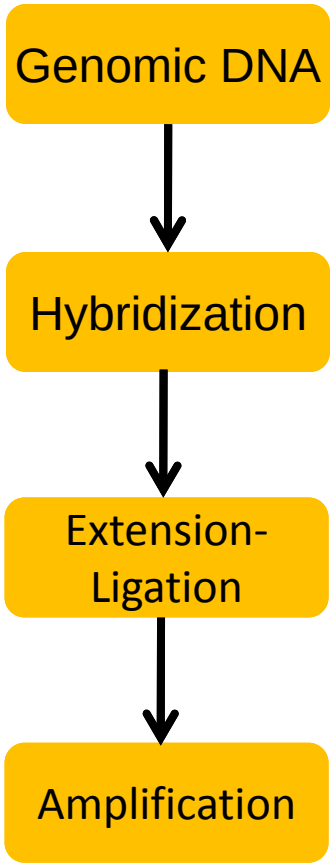
Cystic Fibrosis Assay Overview

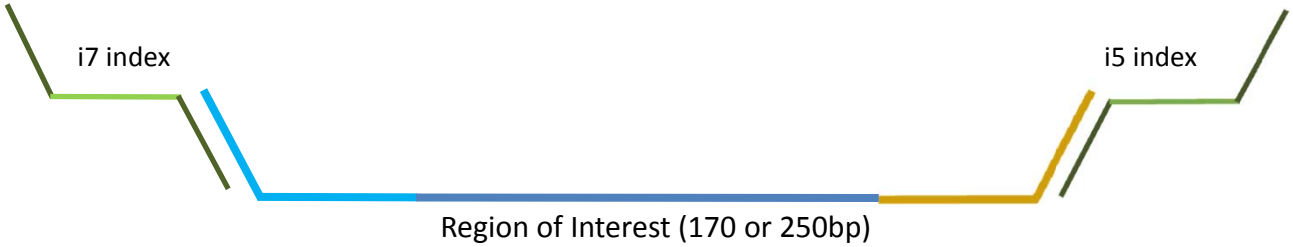




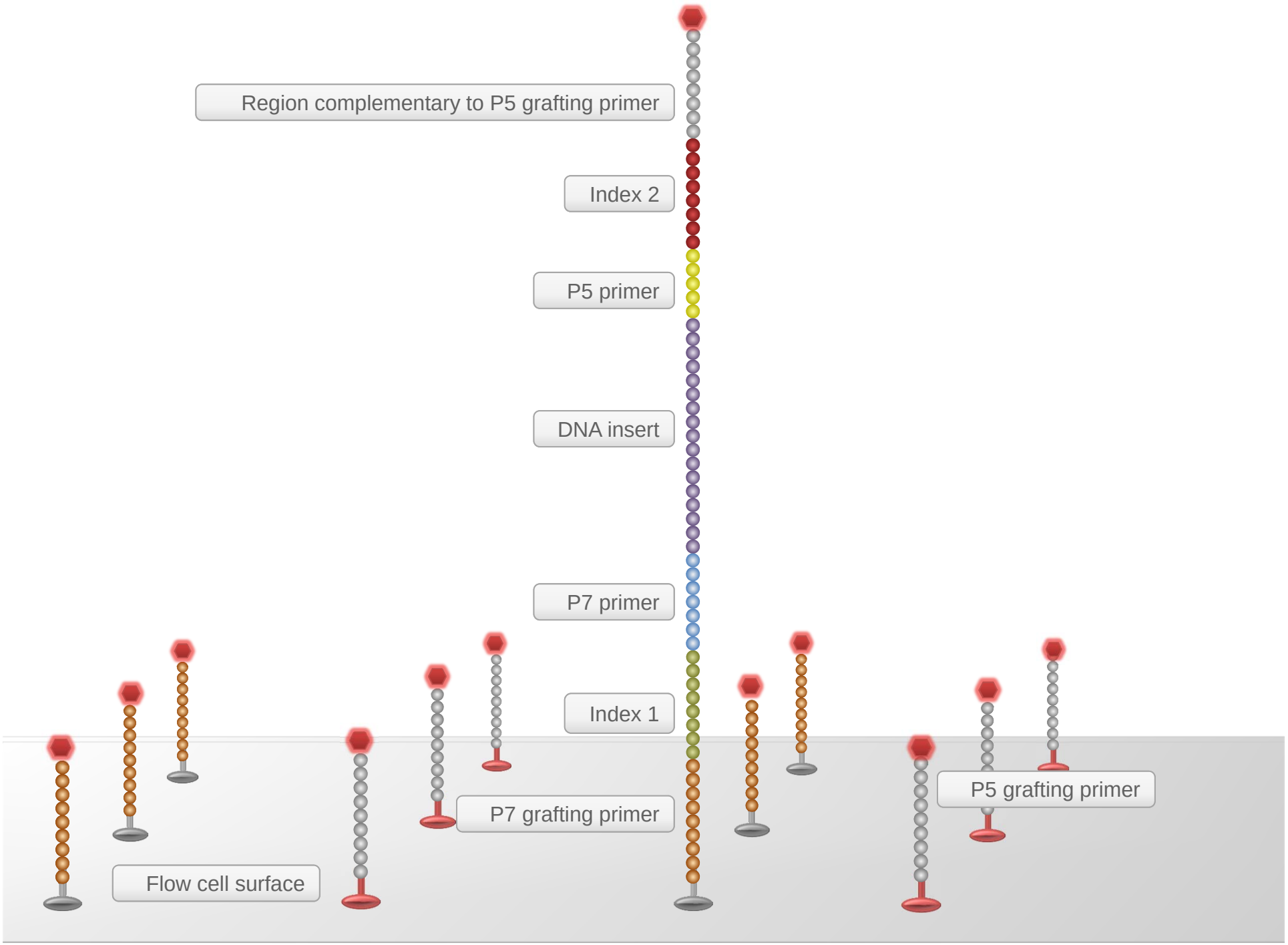
Cystic Fibrosis Assay Overview

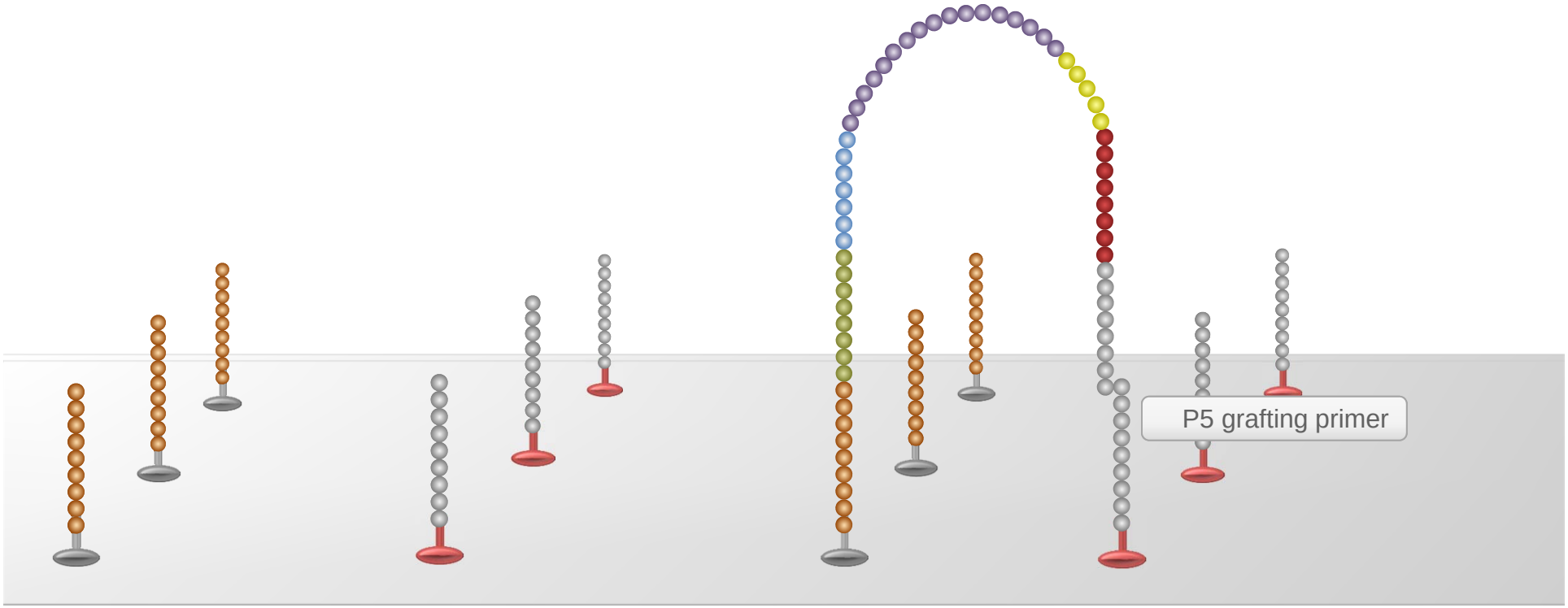
How the assay works

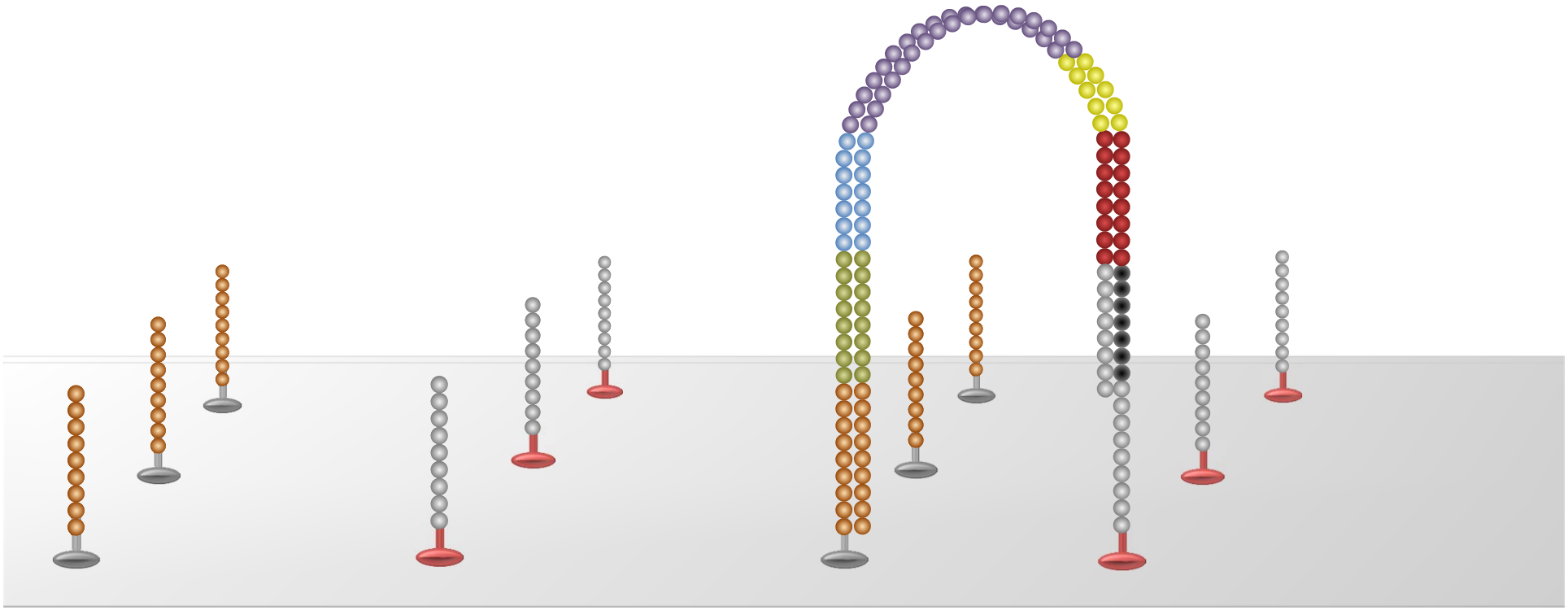


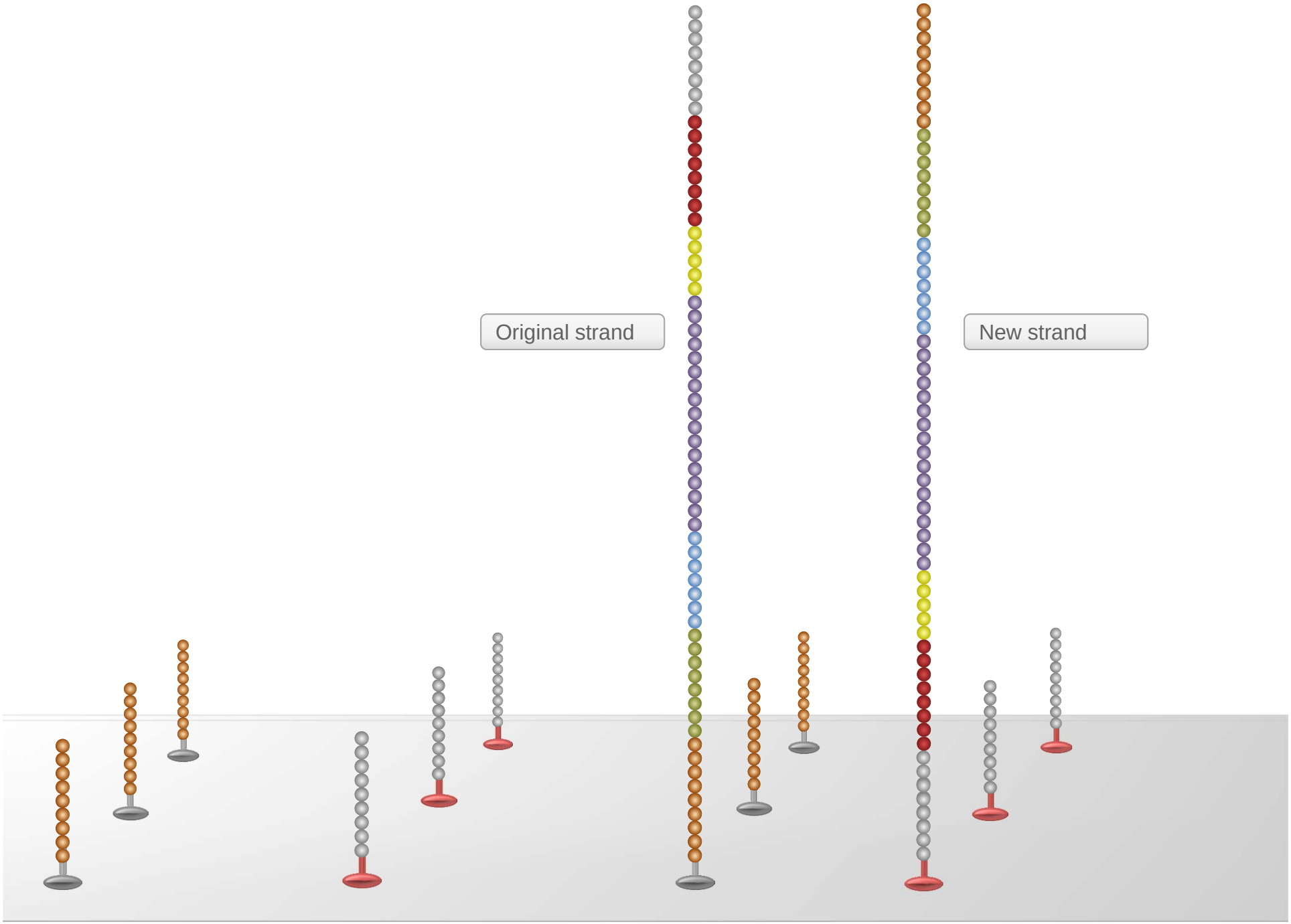


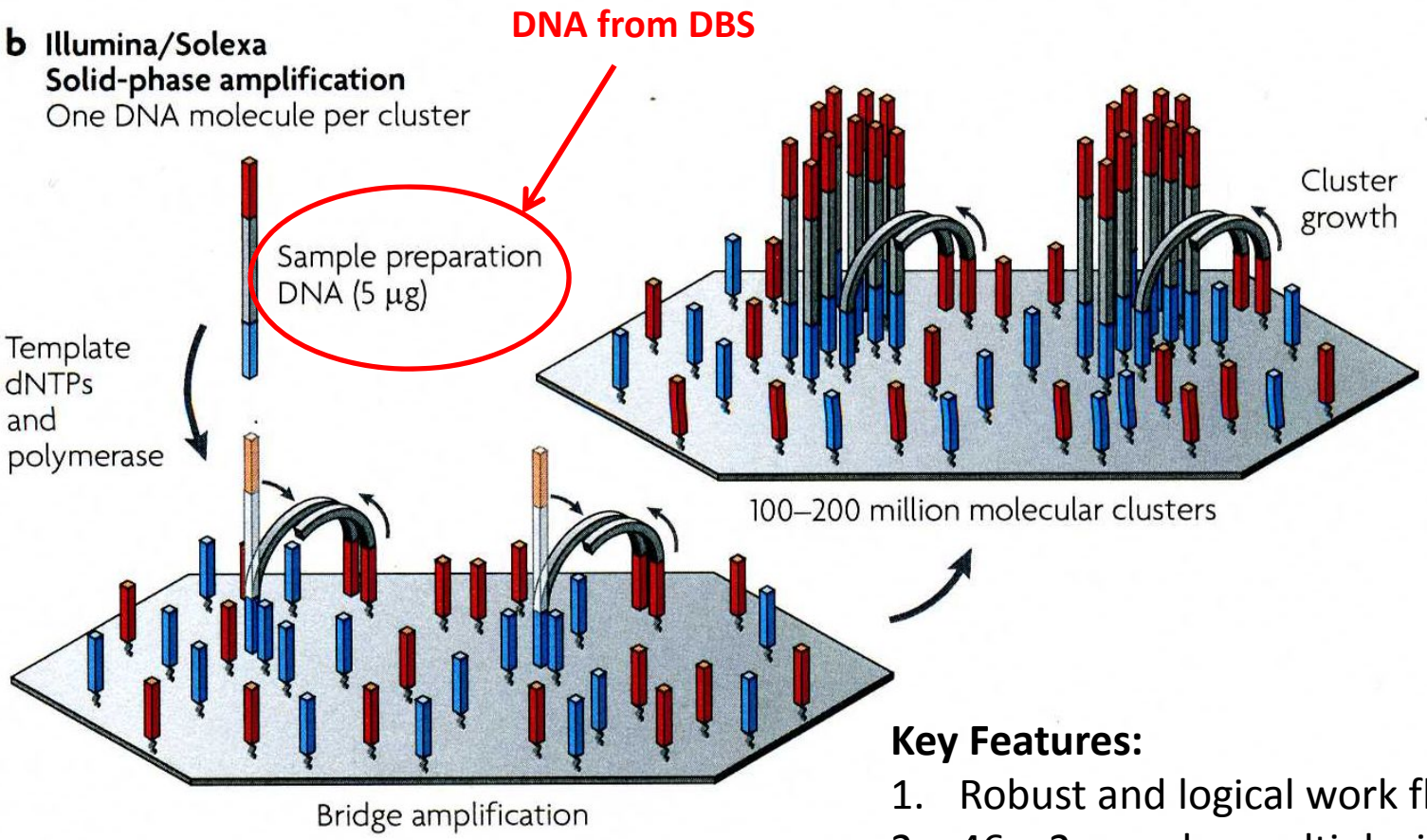
1. Add primers for dual-indexing
2. PCR Amplification
3. PCR Clean up


Region complementary to P5 grafting primer
Index 2
P5 primer
DNA insert
P7 primer
Index 1
P7 grafting primer
P5 grafting primer
Flow cell surface


P5 grafting primer




Original strand
New strand



Key Features:

1. Robust and logical work flow
2. 46 + 2 samples multiplexing platform
3. Dual indexing identification
4. Immediate result w/o informatics

Michael L. Metzker, *Nature Review Genetics*, 2010

MiSeqDx™ Cystic Fibrosis System Validation

- 68 **DBS** specimens with known mutations studied (generally detected through NBS)
  - 48 unique CF-causing mutations
  - 45 Wisconsin patients with two known mutations
  - 23 specimens from CDC NBS & Molecular Biology Branch
- DBS specimens were de-identified
- Results showed 100% concordance with each sample allele call rate at 100%
- Assay validated on two different DNA isolation methods

Potential Advantages of the proposed IRT/DNA/DNA method

- Sweat tests contribute 11.1% of the cost for diagnosis of CF through NBS, and this could be reduced to ~1% if the 3-tier method is successful.
- Avoid unsuccessful follow-up (missed/failed sweat tests).
- Another advantage would be eliminating the parental costs/travel and some anxieties, especially when QNS sweat tests occur.

Current Study Status

- **Wisconsin Site**
  - Obtained IRB approval
  - Completed assay validation using **DBS**
  - On-going MiSeqDx™ Cystic Fibrosis Solution assay on specimens with one mutation
  - Evaluate the assay performance on DNA eluted from DBS w/o washing step.

- **Collaboration Sites**
  - Three states obtained IRB approval.
  - One state scheduled specimens shipping.
  - One state IRB pending

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It all starts with a healthy baby!

Quality care helps keep them healthy!