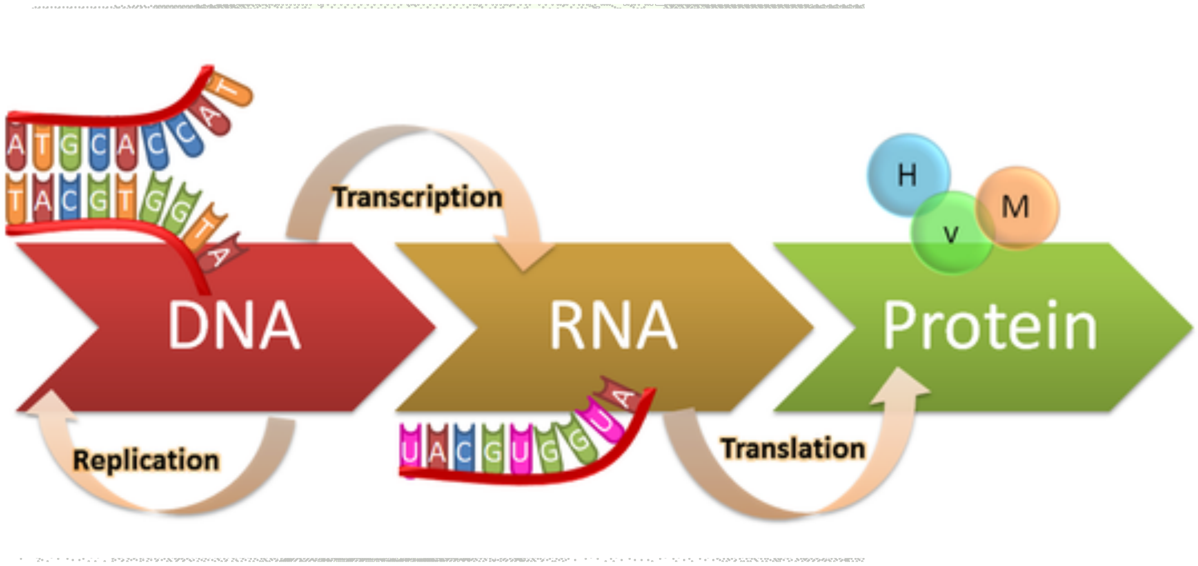
SUMMER INSTITUTE - ONLINE MODALITY CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
June 26	27 9:00-10:00am Virtual Program Orientation for Summer Institute Online Modality	28	29	30	31	July 02
July 03	04 Holiday (Independence Day)	05 8:30-10:00am -Welcome Reception and Buddy Meet & Greet Event	06 Free Day	07 Classes begin! 8:30-11am: BIOL4905 **INTRODUCTION** 8-10:20pm: Afternoon course	08 8:30-11am: BIOL4905 **DNA PREPARATION** 8-10:20pm: Afternoon course	09
10	11 8:30-11am:BIOL4905 **PROTEOMICS I** 8-10:20pm: Afternoon course	12 8:30-11am:BIOL4905 **PROTEOMICS II** 8-10:20pm: Afternoon course	13 8:30-11am:BIOL4905 **PROTEOMICS III** 8-10:20pm: Afternoon course	14 8:30-11am: BIOL4905 **RNA PREPARATION** 8-10:20pm: Afternoon course	15 Virtual Independence Day Activity	16
17	18 8:30-11am:BIOL4905 **qPCR / ROBOTS** 8-10:20pm: Afternoon course	19 8:30-11am:BIOL4905 **DNA Sequence Analysis** 8-10:20pm: Afternoon course	20 **Midterm Break**	21	22 8:30-11am:BIOL4905 **Next Gen. Sequencing** 8-10:20pm: Afternoon course	23
24	25 8:30-11am:BIOL4905 **Microarray I** 8-10:20pm: Afternoon course	26 8:30-11am:BIOL4905 **Nanostring** 8-10:20pm: Afternoon course	27 8:30-11am:BIOL4905 **Automated Microscopy /AFM**	28 8:30-11am:BIOL4905 **Flow Cytometry** 8-10:20pm: Afternoon course	29 FINALS	30
31	August 01 9:00-10:00am: Closing Reception	02	03 Grades available in PAWS			

Legend:

Orange: Courses **Blue: Activities**



nanoString

Direct Expression Profiling
Adapted from

Jesse Gardner's PPT

GSU Biology Core Facility

Supporting Life Sciences at GSU

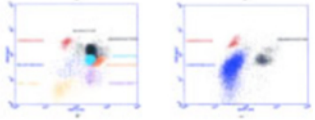
http://biotech.gsu.edu/core_facility/index.html

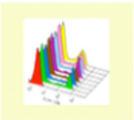
GeorgiaStateUniversity

Flow Cytometry

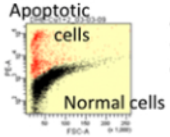
Profiling Cells

Normal Leukaemia patient





See effects of different drugs on Cell cycle



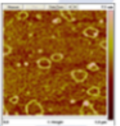
Apoptosis -programmed cell death

Cellular Functions

Atomic Force Microscopy

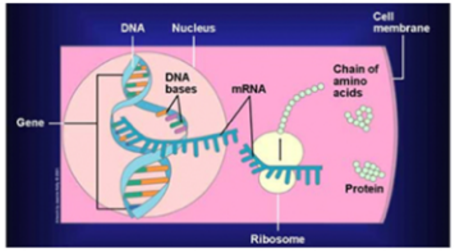
Imaging at the Ångström level

Protein structure analysis



DNA

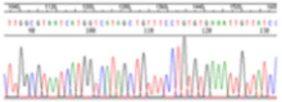
Replication



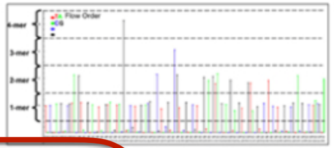
DNA Sequence Analysis:

Profiling DNA

Sanger Sequencing – >800 base pairs/run



High Throughput Genomic Sequencing – 100,000 base pairs/run

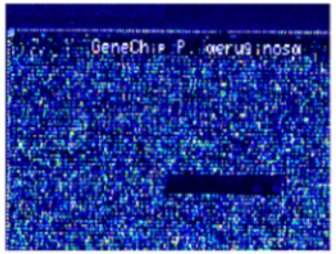


RNA

Expression

Microarray:Analysis

Profiling mRNA



Colour of pin-point dots demonstrates the presence / absence of gene sequences

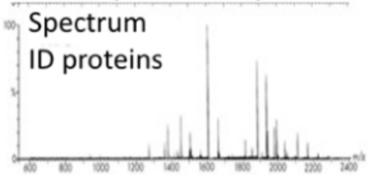
Protein

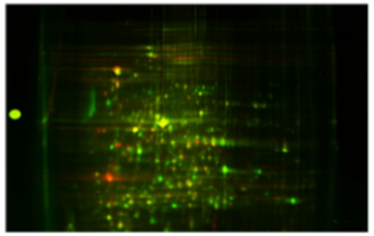
Expression

Mass Spectrometry

Spectrum

ID proteins





Proteomics

Profiling Proteins

2D Protein gel

Protein separation using

Electric charge and molecular weight

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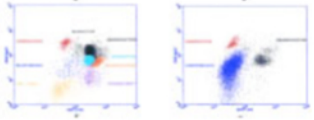

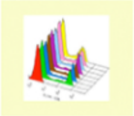

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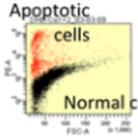

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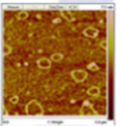

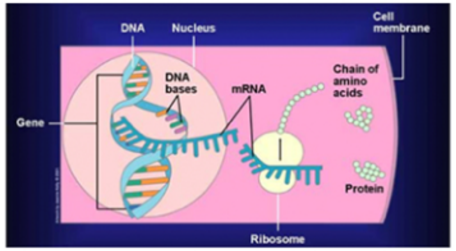

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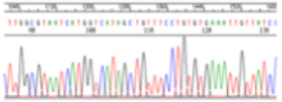

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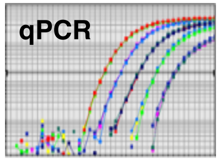

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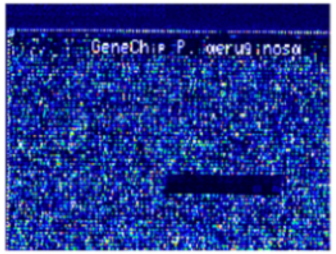

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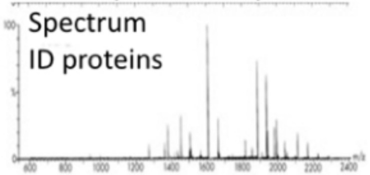
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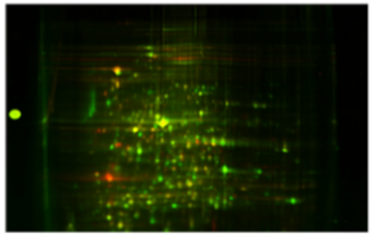

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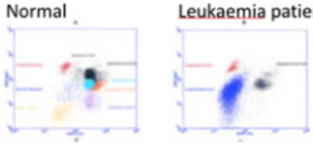

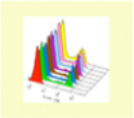

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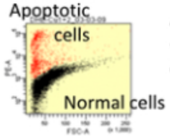

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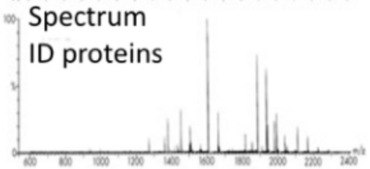

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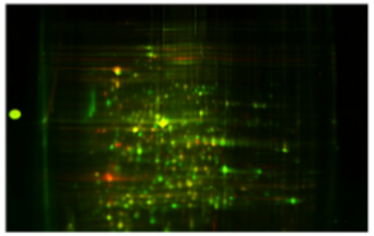

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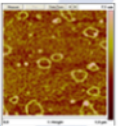

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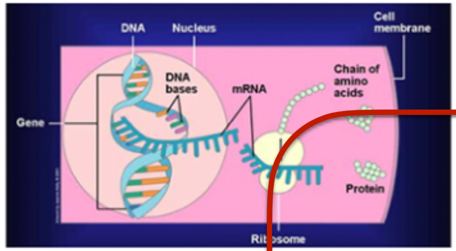

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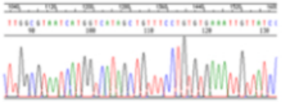

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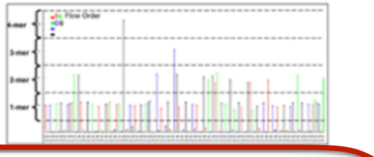

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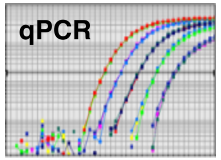

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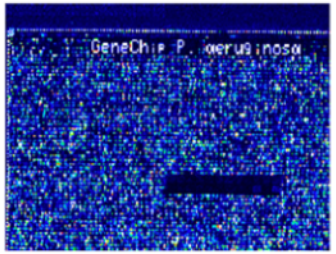

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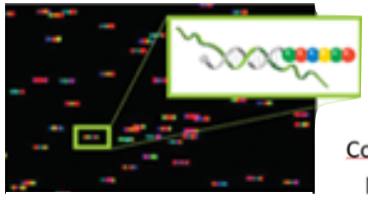


Nanostring

- **Novel chemistry invented in Leroy Hood’s Lab at the Institute for Systems Biology**

Leroy Hood, M.D., Ph.D.

PRESIDENT, INSTITUTE FOR SYSTEMS BIOLOGY, AFFILIATE PROFESSOR, IMMUNOLOGY

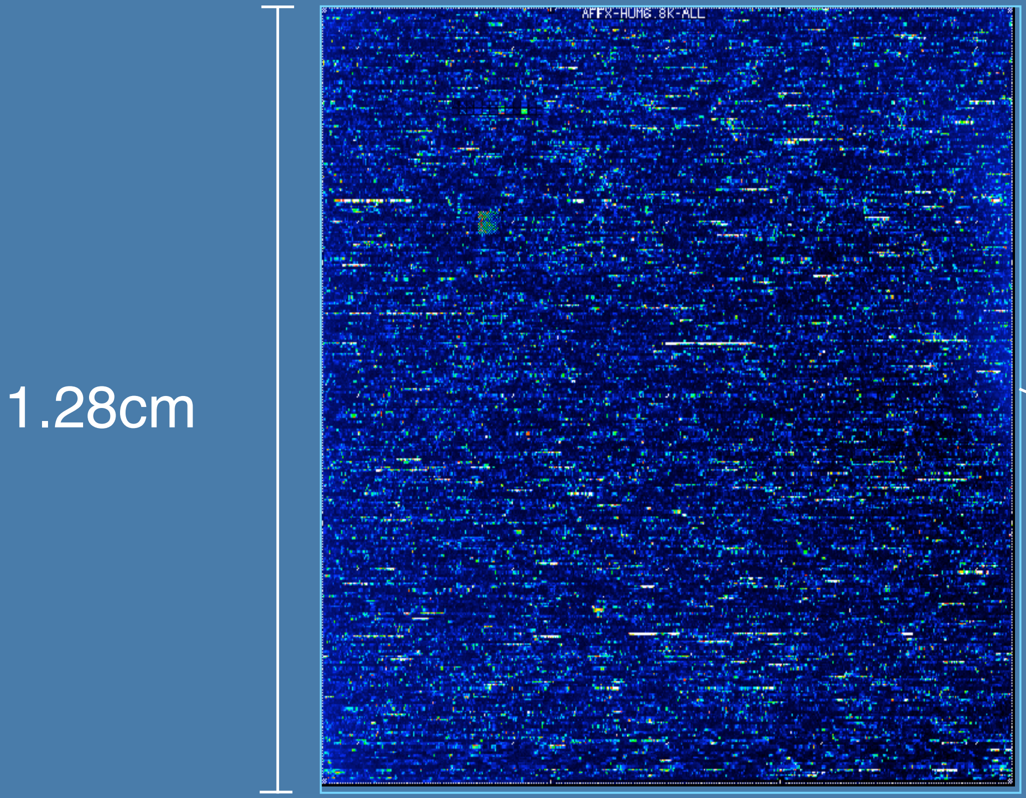
Dr. Hood graduated from the California Institute of Technology (Caltech) with a BS in biology and received his M.D. from the Johns Hopkins Medical School. He returned to Caltech, completing his Ph.D. in 1968. Dr. Hood is President of the Institute for Systems Biology and member of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

DR. LEE HOOD WRITES 'SECOND OPINION' COLUMNS FOR LOS ANGELES TIMES

Posted on June 20, 2021

ISB Co-founder Dr. Lee Hood is credited with coining the term "systems biology" and has been a longtime advocate of P4 medicine. Now, Hood has been selected by the Los Angeles Times to share his insights in a new weekly op-ed column, called Second Opinion.

GeneChip® Expression Analysis
Hybridization and Staining

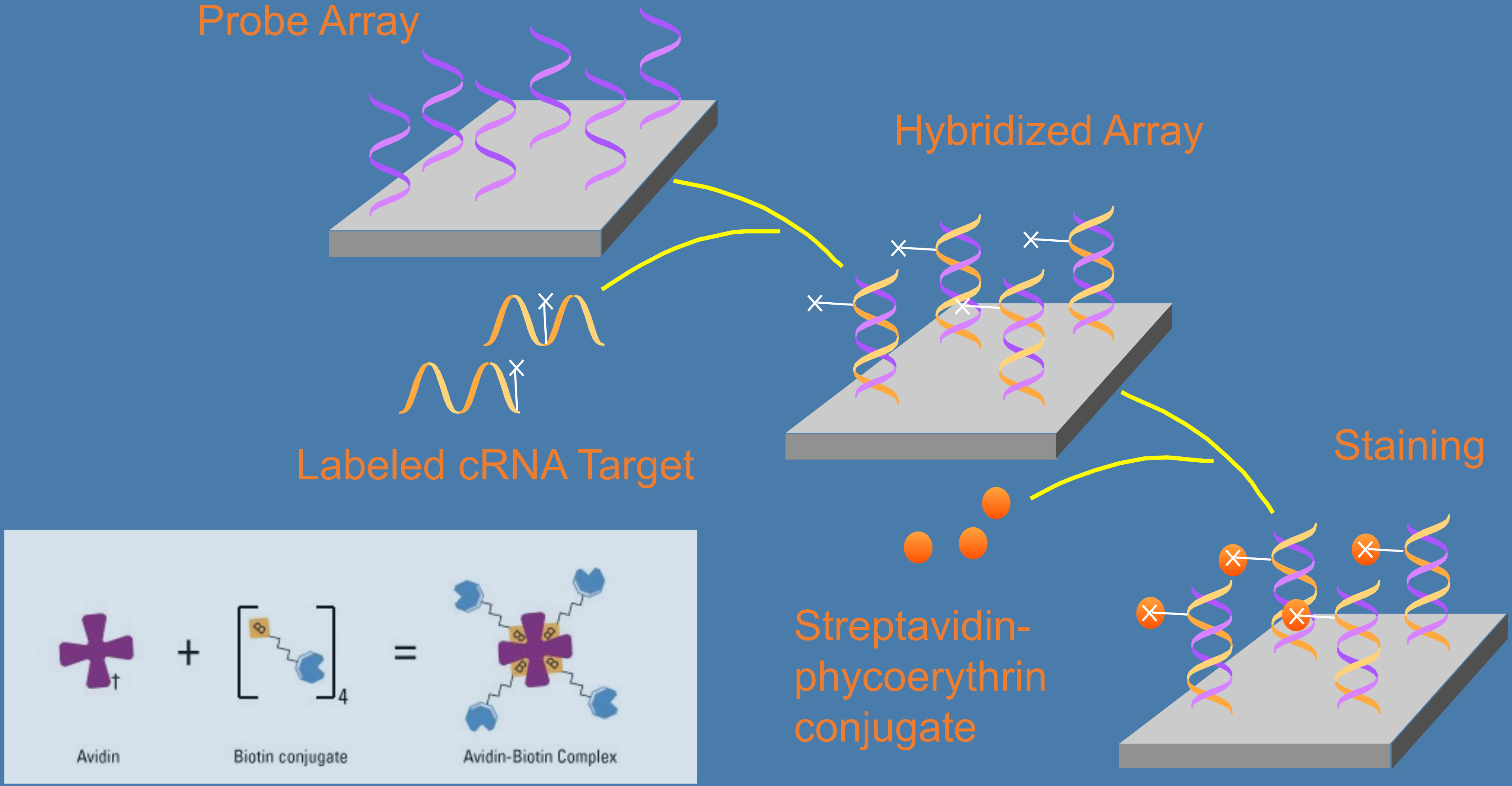


Potentially analyzing > 500,000 different probes complementary to genes of interest

Image of Hybridized Probe Array

GeneChip® Expression Analysis

Hybridization and Staining


Probe Array

Hybridized Array

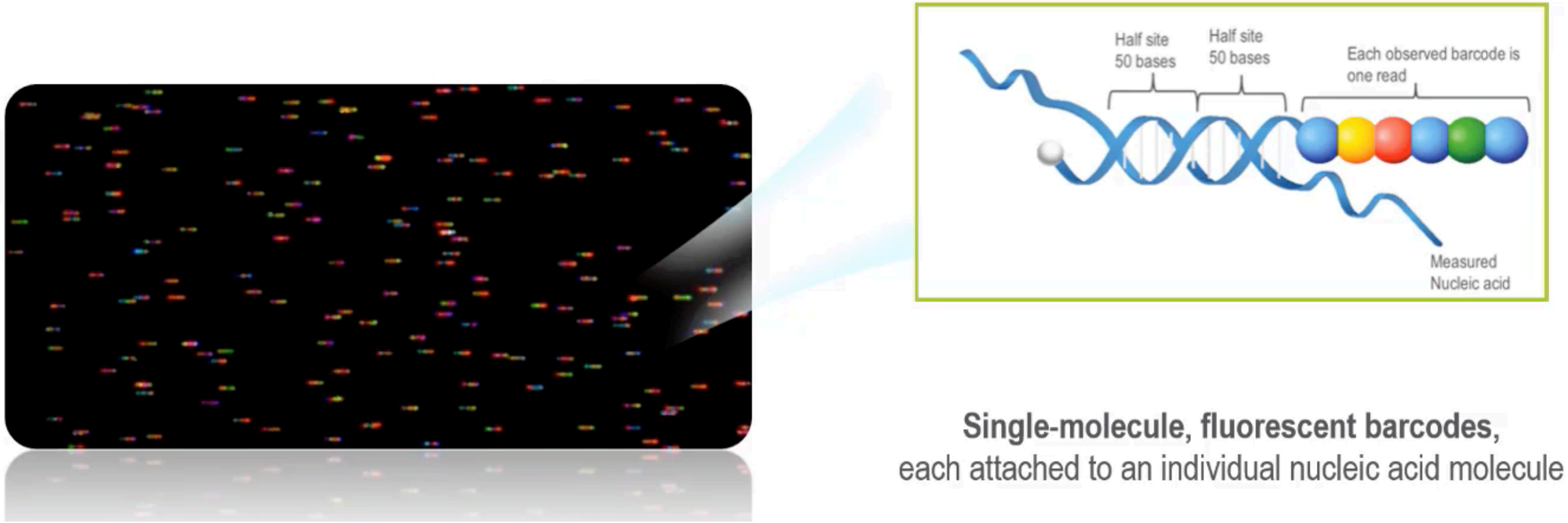
Labeled cRNA Target

Staining

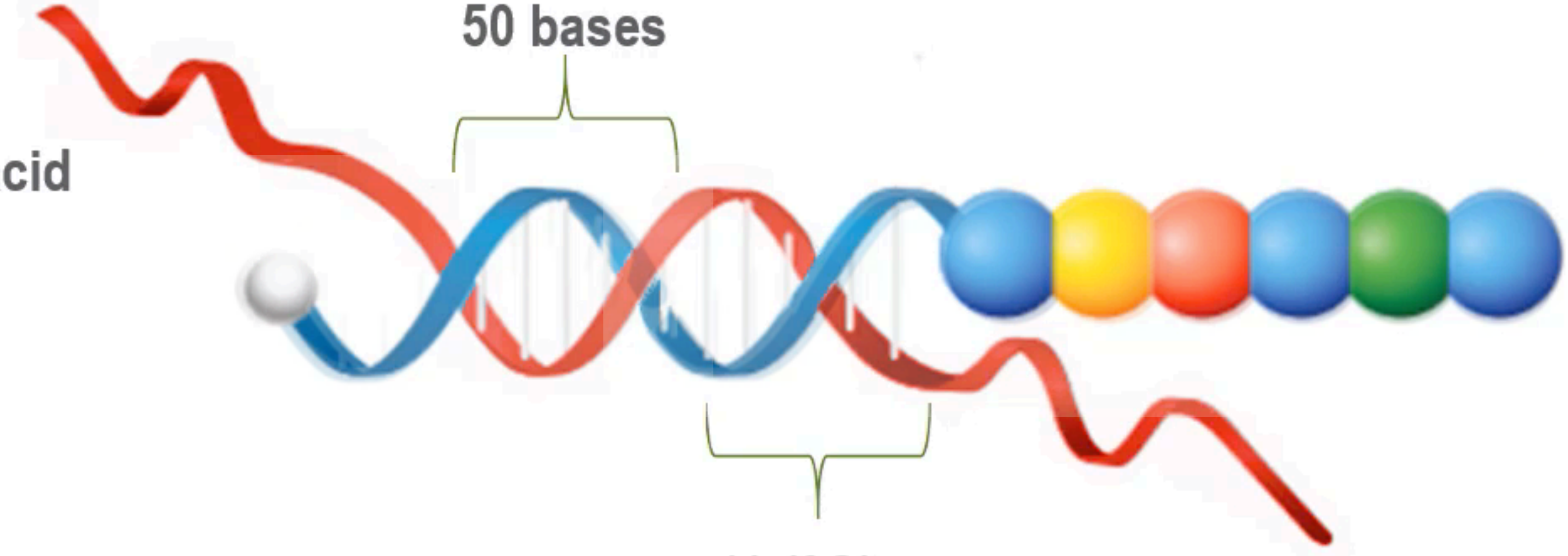
Streptavidin-phycoerythrin conjugate

- Novel chemistry invented in Leroy Hood’s Lab at the Institute for Systems Biology

- **Gene Expression is quantified by directly counting each barcode bound on the slide surface**



Single-molecule, fluorescent barcodes,
each attached to an individual nucleic acid molecule

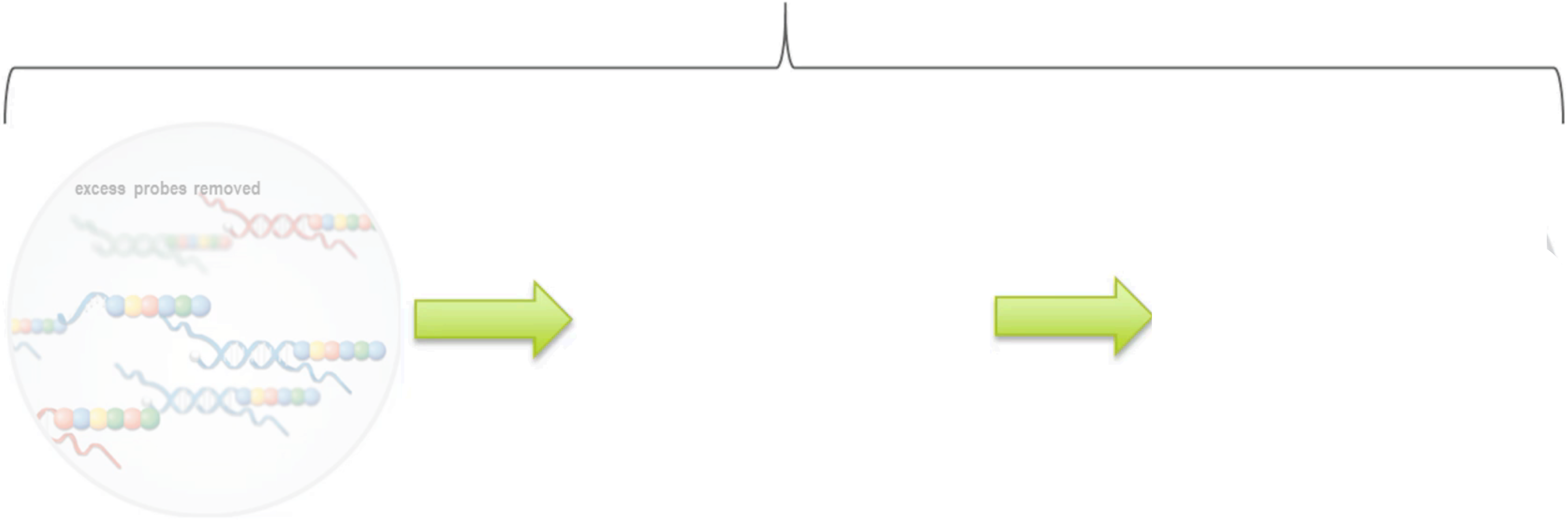


Nucleic acid

Half Site
50 bases

Half Site
50 bases

Automated instrumentation



Purification

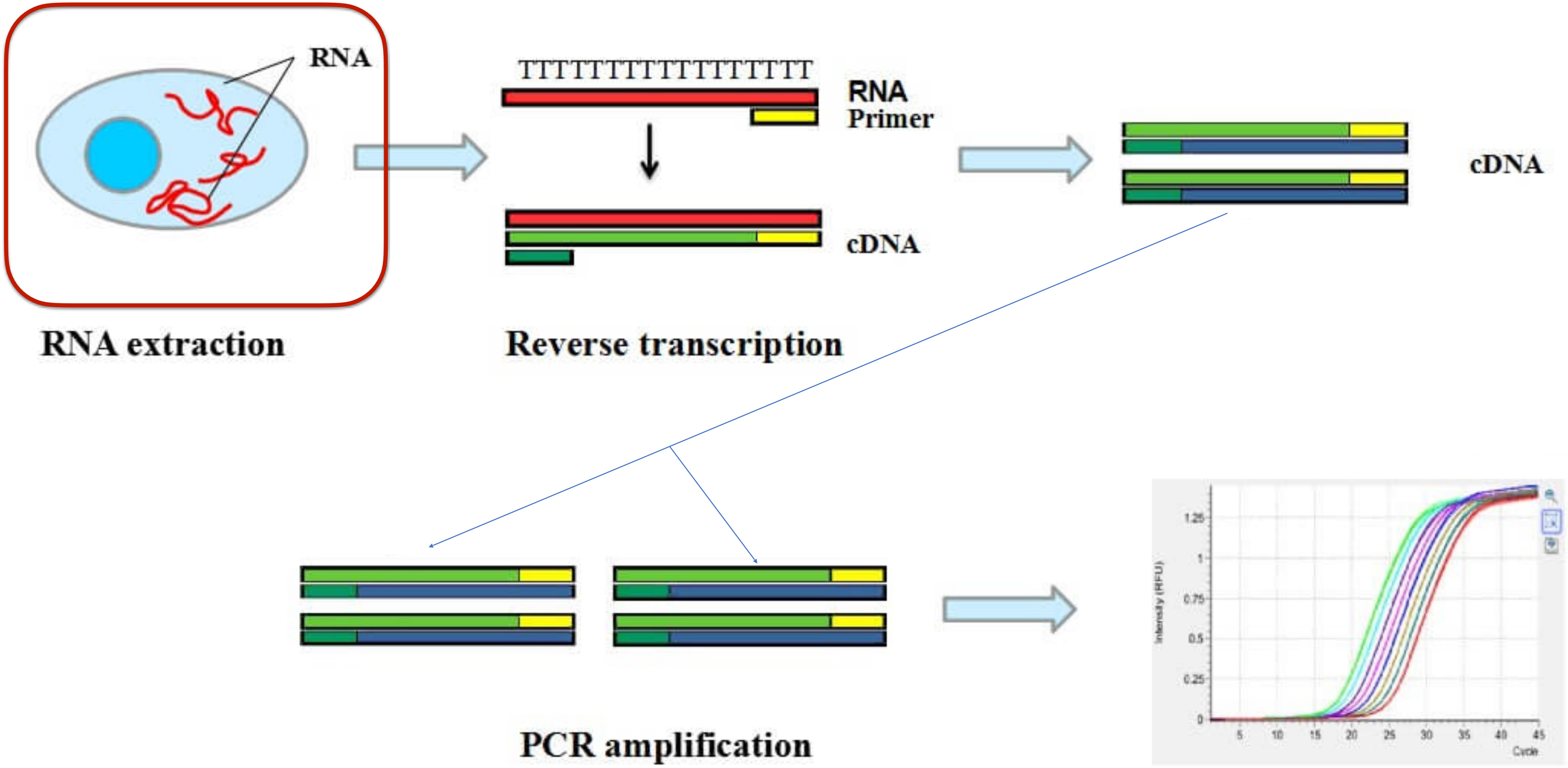
Alternative Methods


https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr.html

- RT-qPCR (Polymerase)
  - cDNA
  - qPCR
  - Pitfalls

- nanoString (no Polymerase)
  - Bar-codes
  - Hybridization
  - Analysis

RT-qPCR



RNA extraction

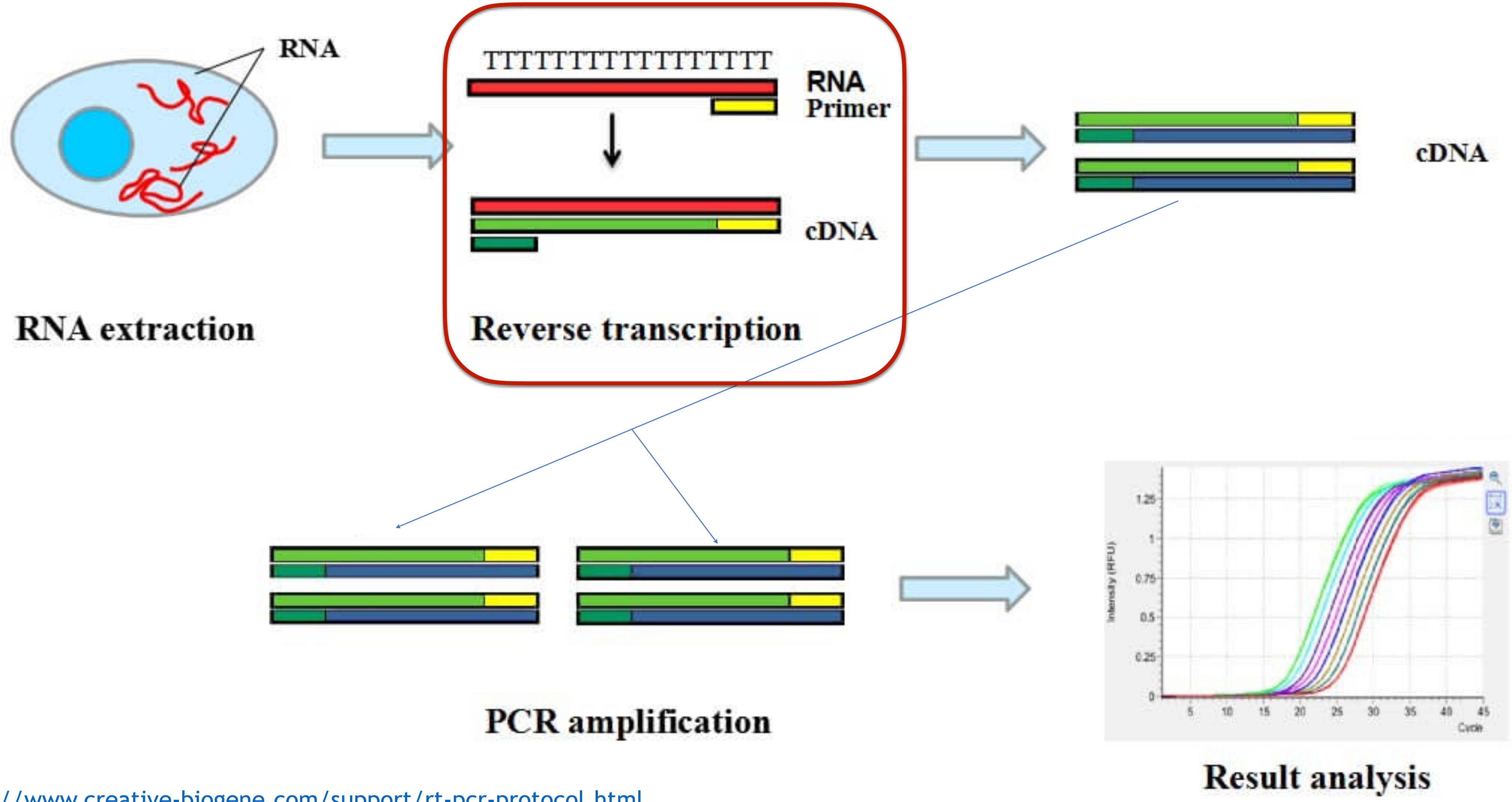
Reverse transcription

PCR amplification

Result analysis

https://www.creative-biogene.com/support/rt-pcr-protocol.html

RT-qPCR



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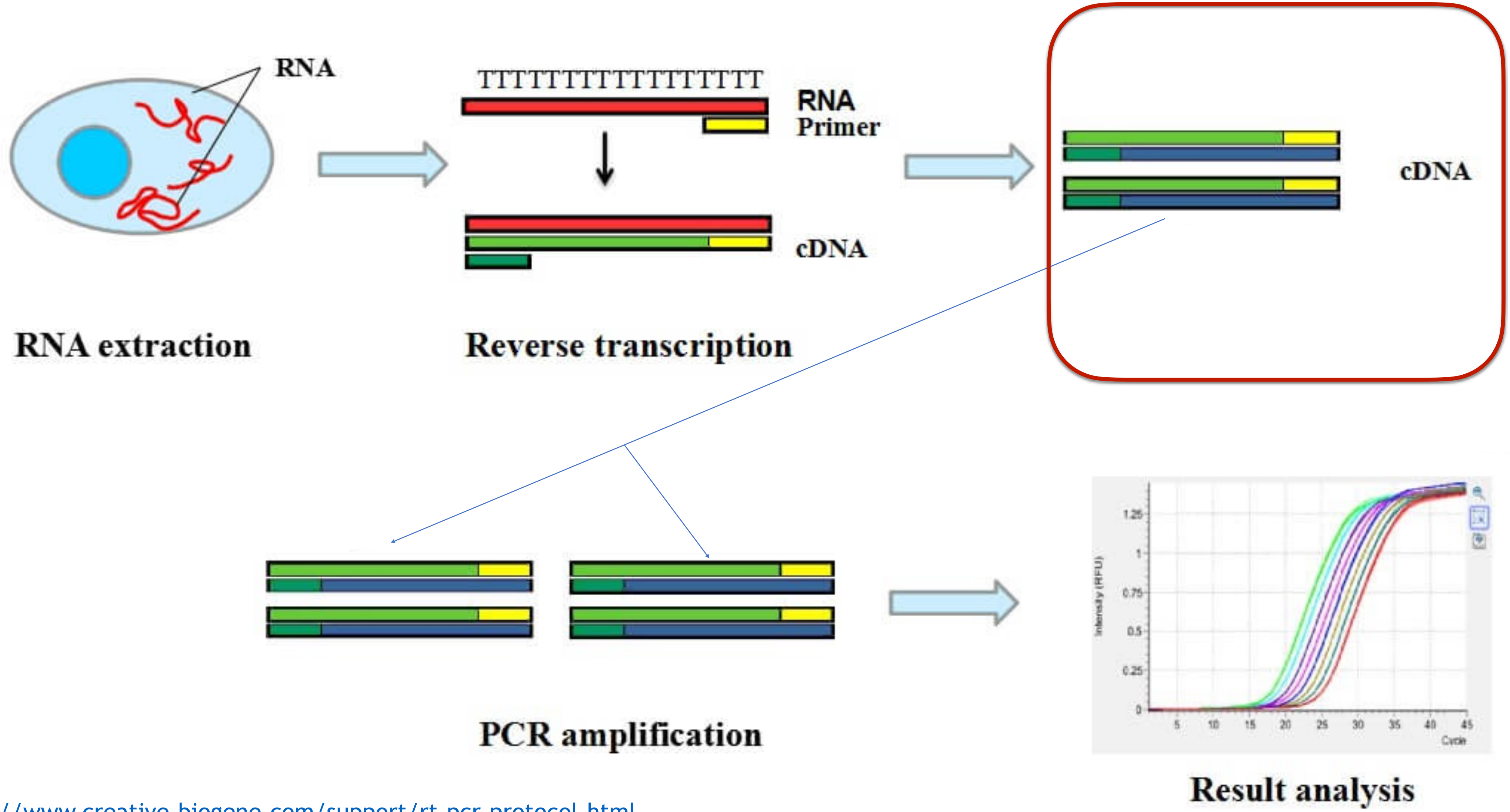

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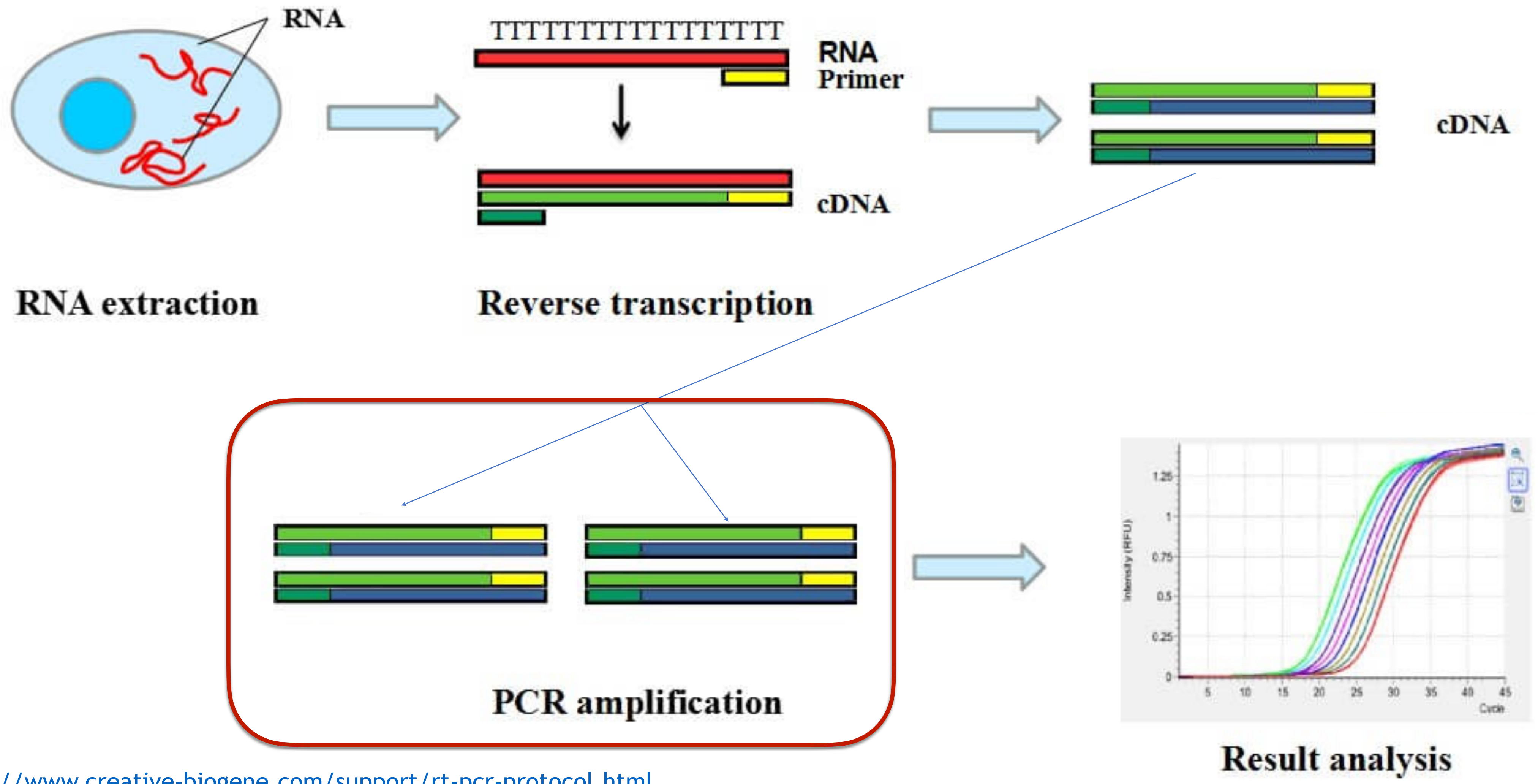

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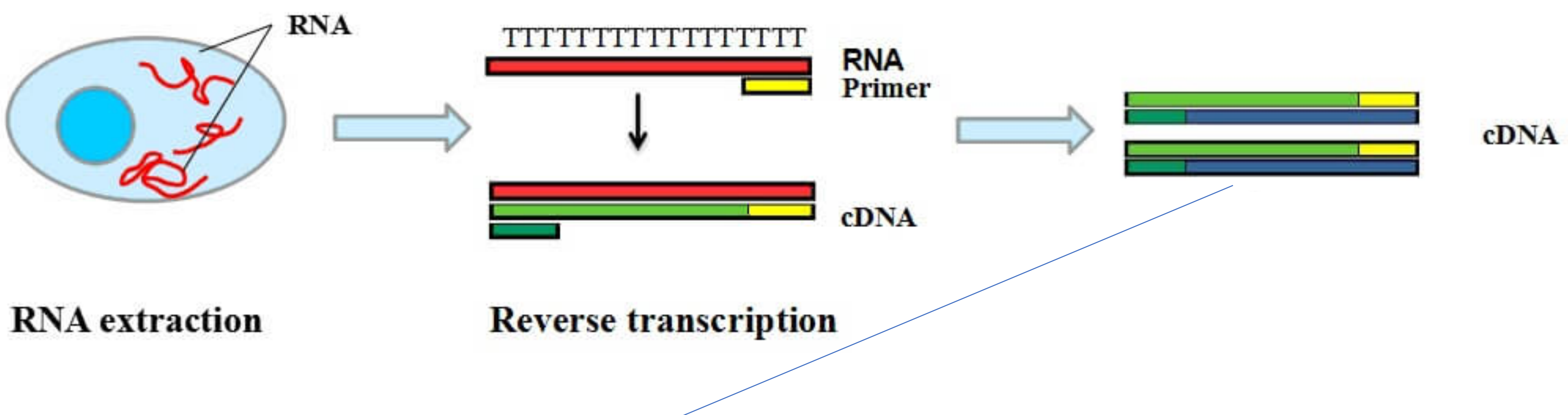

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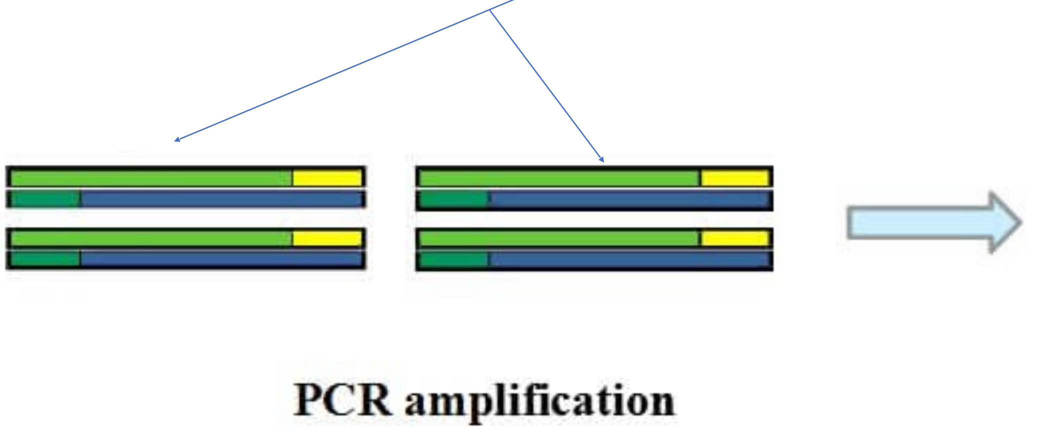
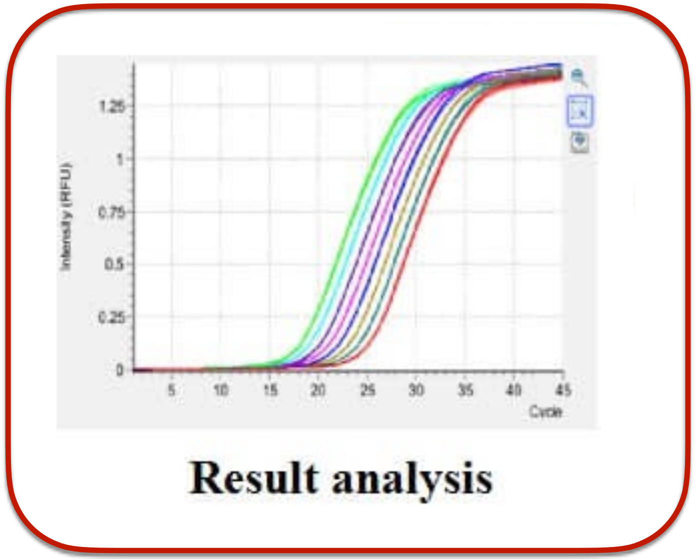


PCR amplification



Result analysis

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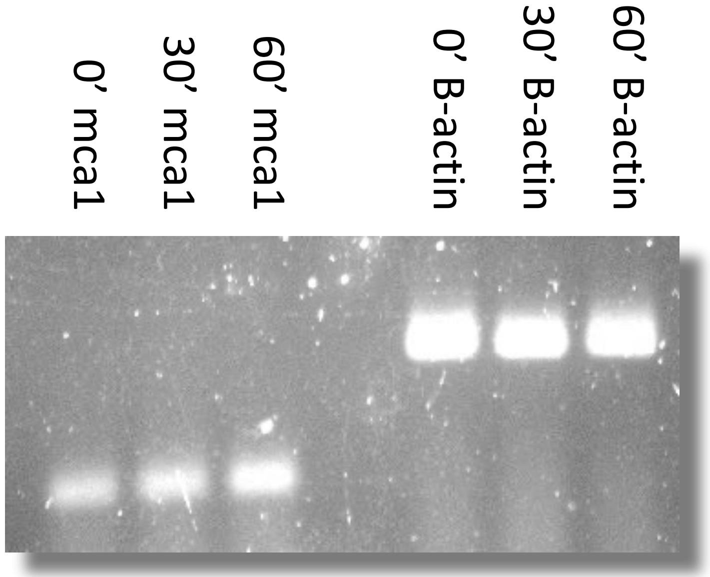
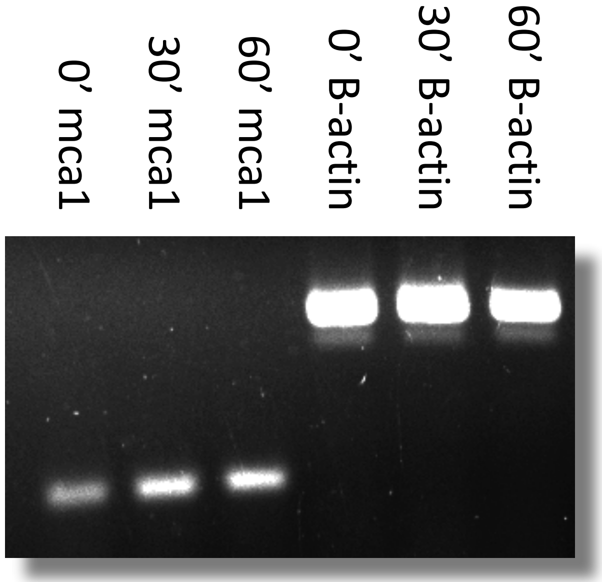
Metacaspase-1 (mca1) was induced by yeast acute exposure to the heavy metals chromium and cadmium

Chromium Exposure



Cadmium Exposure



Relative Quantification	0 min	30 min	60 min
Chromium exposure	1.00	1.34	1.51
Cadmium exposure	1.00	1.66	1.56

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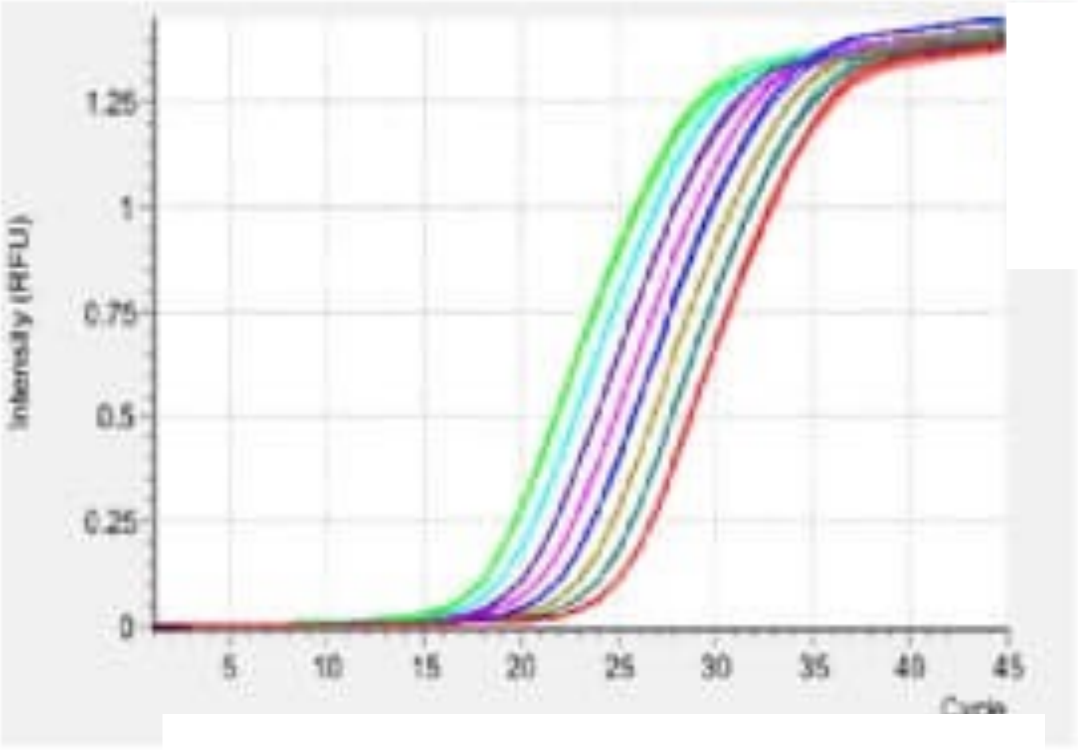


qPCR does provide for multiplex analysis

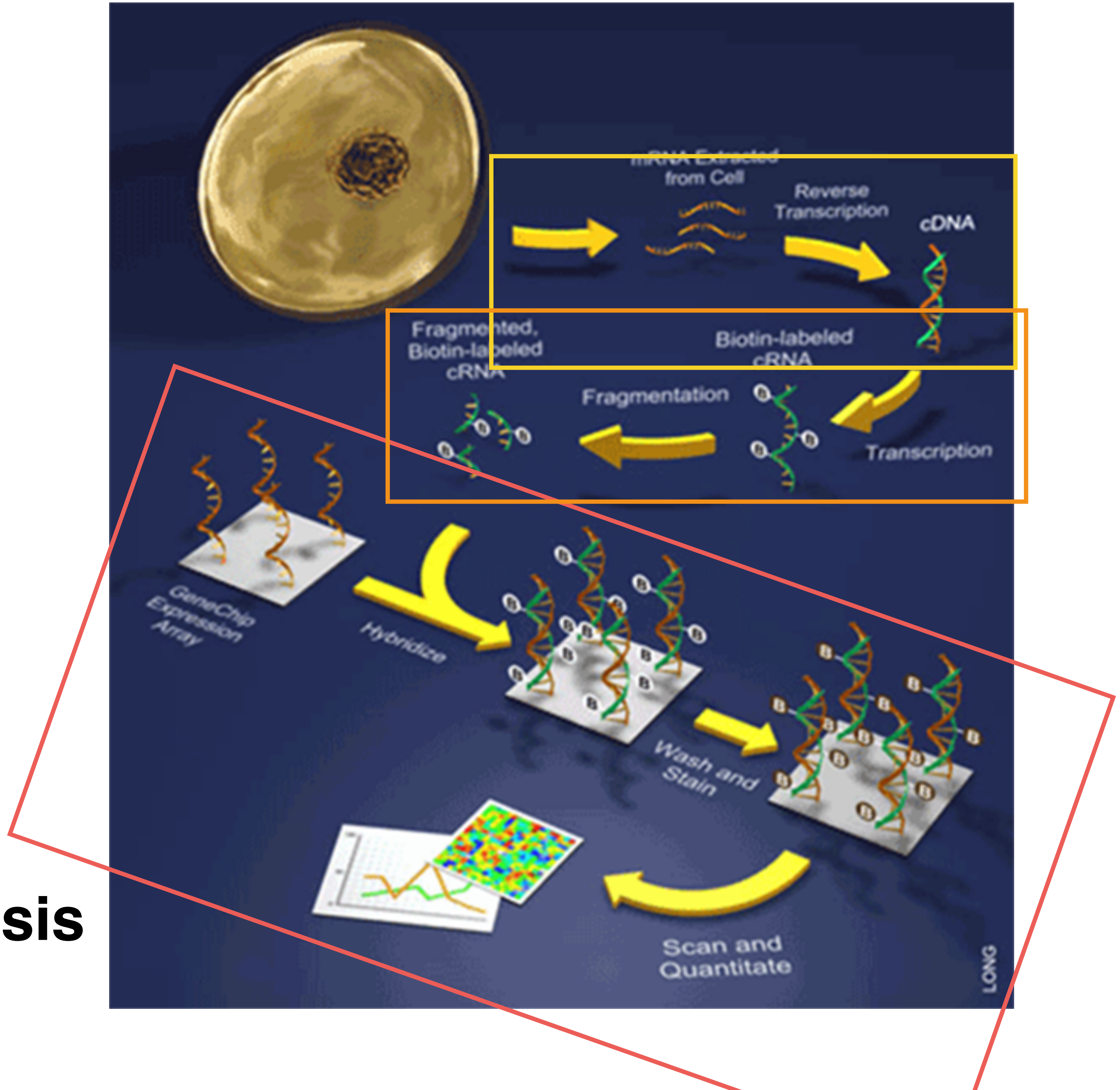
Multiple primers required to be designed for each gene under interrogation

RT-qPCR -Potential pitfalls (difficulty in reproducibility)

- Requires PCR
  - Primer design
    - Primer annealing temperature
    - Loss of RNA due to faulty primer design
  - Protocol optimization for multiple expression products
  - Researcher affects data output
    - Different concentration added (template, dNTP, polymerase)
    - Affinities of primers, differences in melting temperatures, and different polymerases can affect cDNA amplification

- Must choose appropriate normalization before PCR
  - Difficult to quantitate
  - Affects analysis

Alternative Methods



GeneChip® Expression Analysis

Hybridization and Staining

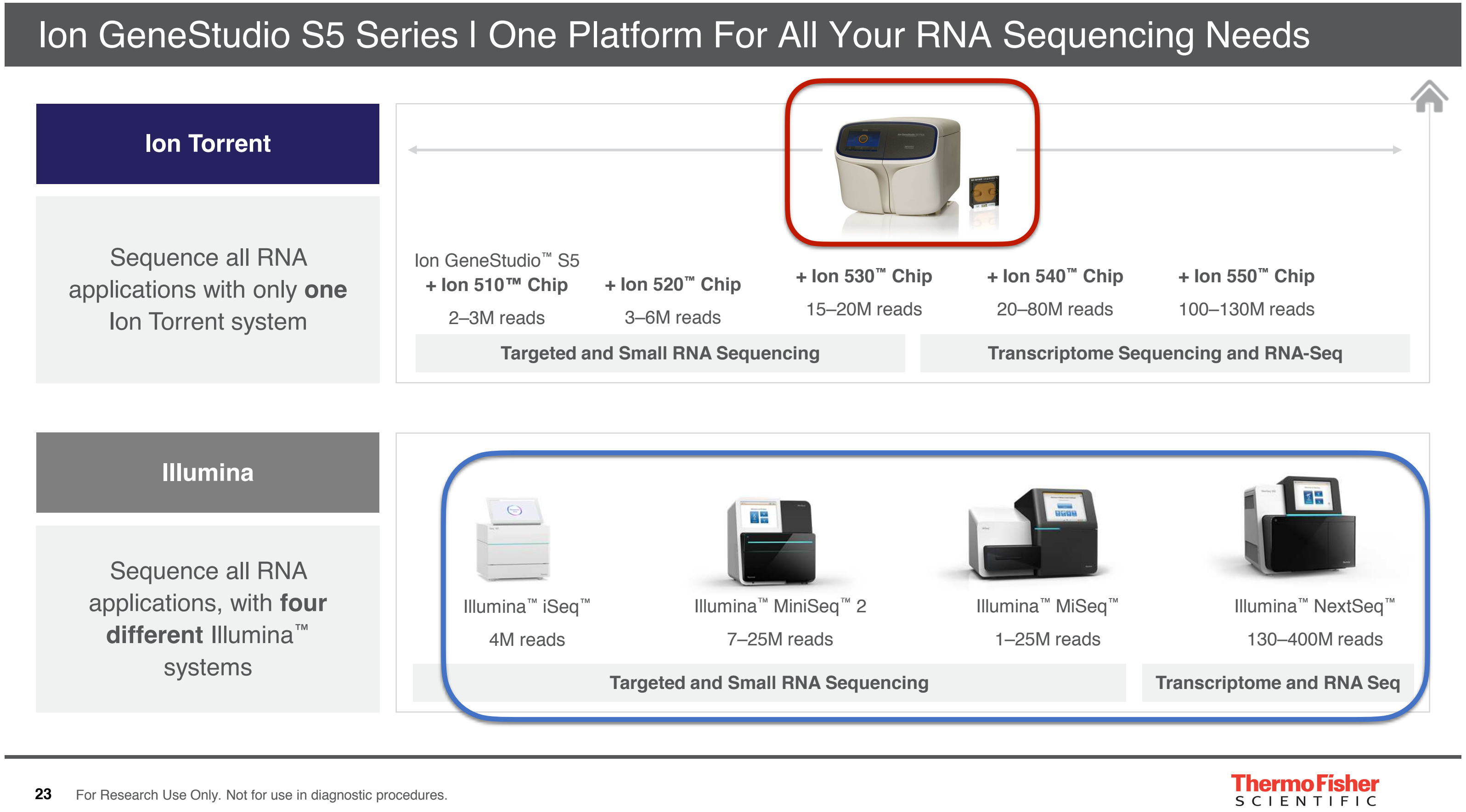
Microarray Potential pitfalls

- Requires Reverse Transcription
  - Primer design
    - Primer annealing temperature
  - Protocol optimization for multiple expression products

- Requires Transcription -additional transcription to label RNA

- Chips are expensive…
  - Little to no flexibility in Chip design

Alternative Methods



Ion GeneStudio S5 Series I One Platform For All Your RNA Sequencing Needs

Ion Torrent

Sequence all RNA applications with only **one** Ion Torrent system

Ion GeneStudio™ S5
+ Ion 510™ Chip
2–3M reads

+ Ion 520™ Chip
3–6M reads

+ Ion 530™ Chip
15–20M reads

+ Ion 540™ Chip
20–80M reads

+ Ion 550™ Chip
100–130M reads

Targeted and Small RNA Sequencing

Transcriptome Sequencing and RNA-Seq

Illumina

Sequence all RNA applications, with **four different** Illumina™ systems

Illumina™ iSeq™
4M reads

Illumina™ MiniSeq™ 2
7–25M reads

Illumina™ MiSeq™
1–25M reads

Illumina™ NextSeq™
130–400M reads

Targeted and Small RNA Sequencing

Transcriptome and RNA Seq

23

For Research Use Only. Not for use in diagnostic procedures.

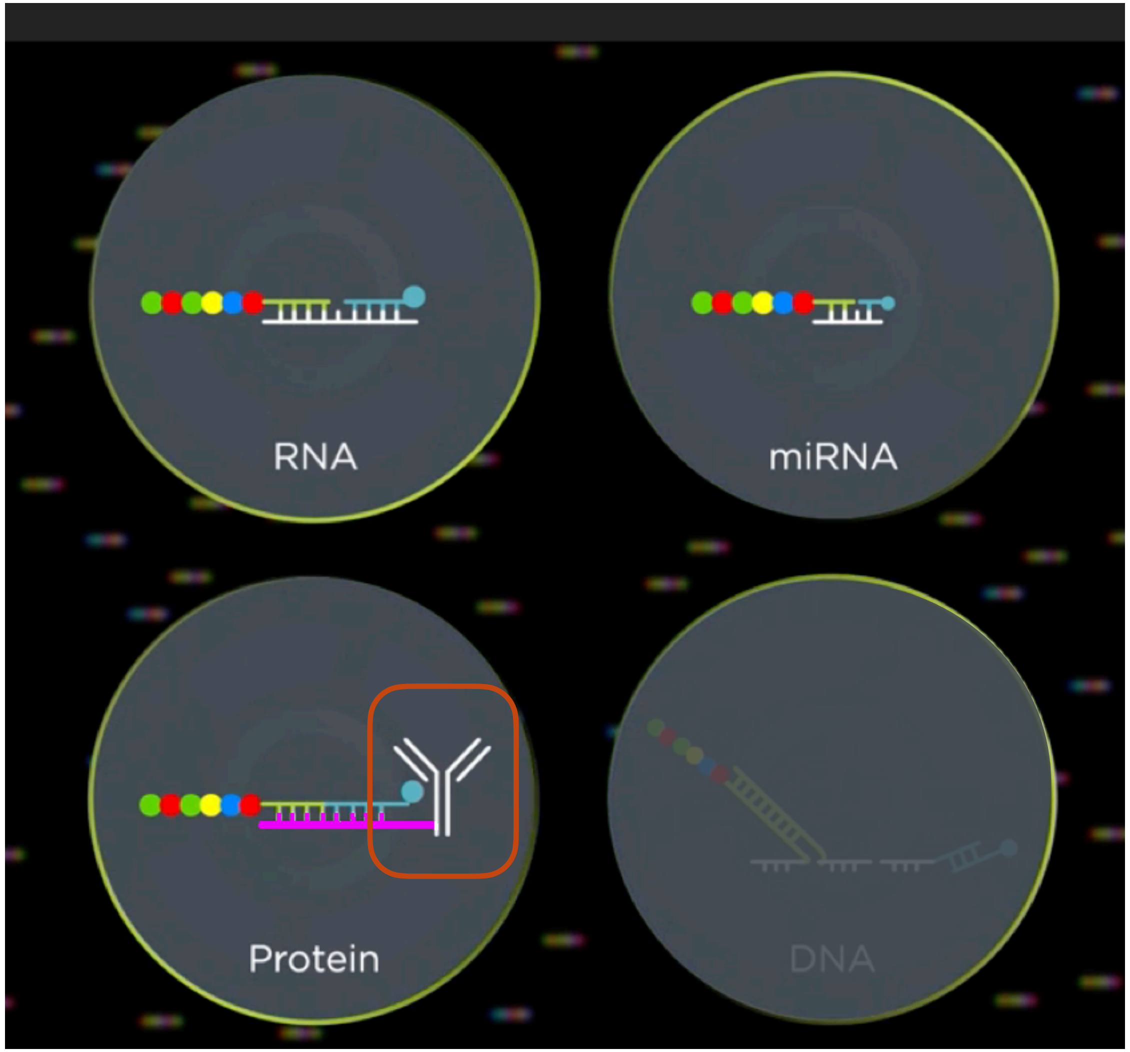
ThermoFisher
SCIENTIFIC

NGS Transcriptome Analysis -Potential pitfalls

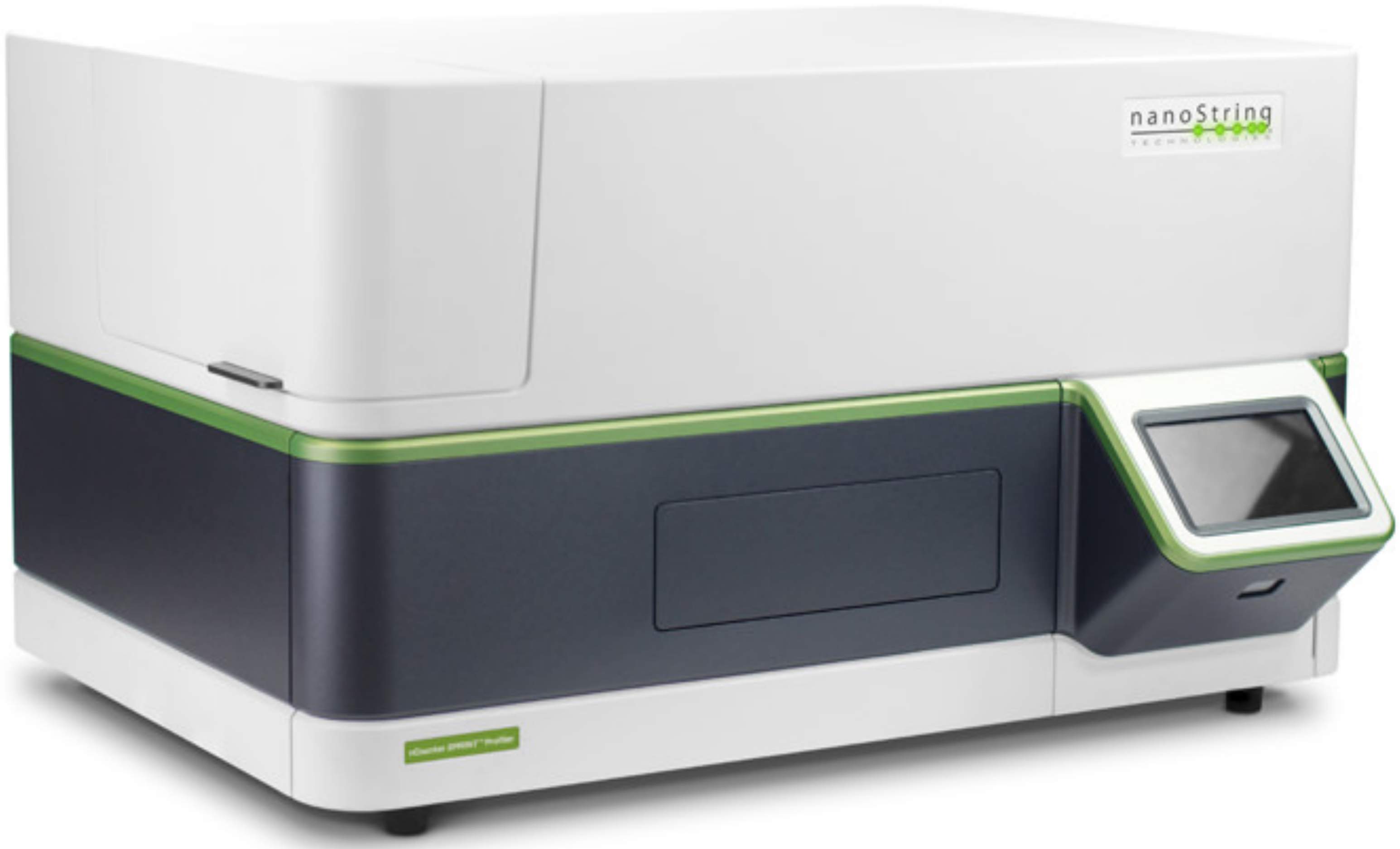
- Requires PCR -yes, but multiplex effectively rules out mutation
  - Primer design
    - Primer annealing temperature
    - Loss of RNA due to faulty primer design
    - low level RNA species might not be amplified proportionally..
- Requires Reverse Transcription
  - Primer design
    - Primer annealing temperature
  - Protocol optimization for multiple expression products
- Set-up is relatively cumbersome for few genes…
- Chips are EXPENSIVE

nanoString™

nanoString (multi target-rich analyses)

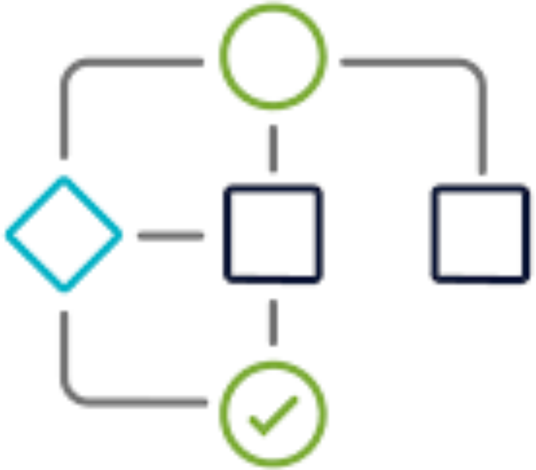


nanoString (PCR Free Expression Assay)



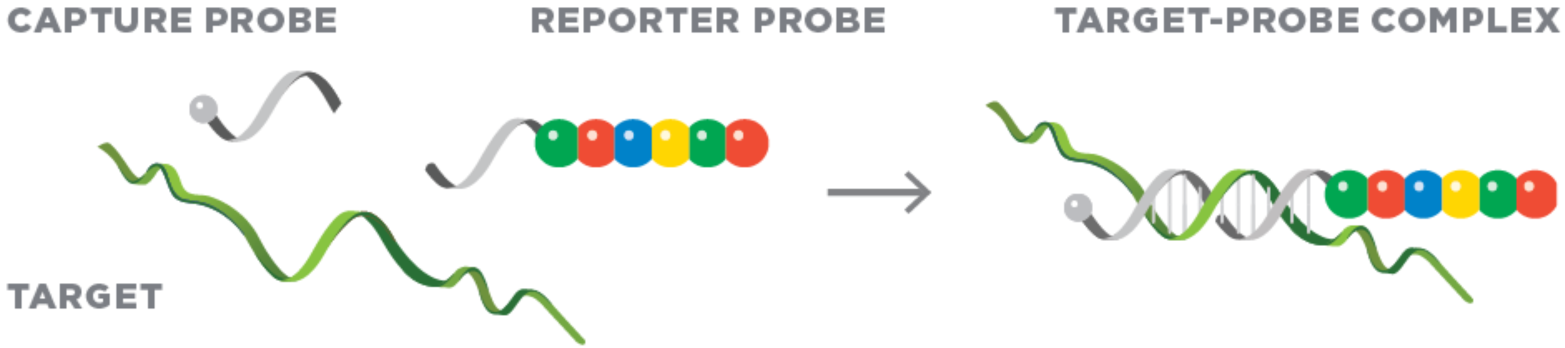
Work Flow

- Decide target genes and order probe-set
  - Prebuilt panels
  - Custom panels
- Hybridize probes to RNA (16 hr)
- Load onto nanoString fluidics chip
  - 12 simultaneous samples
  - Magnetic bead technology
- Run Protocol (6 - 7 hr)
- Analyze data

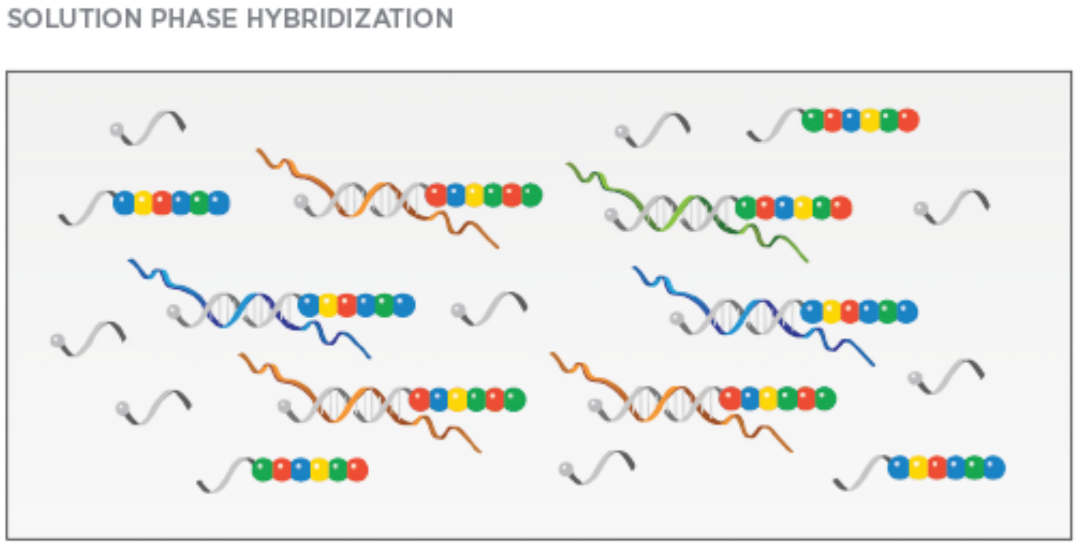


nanoString Hybridization Probe Set

- Capture and reporter probe are designed for each target gene… by Nanostring Inc.
  - Capture ~50 nt compliment to target and biotin
  - Reporter ~50 nt compliment to target and a 6-sequence color “bar-code”
    - 4 colors and 6 (6^4 = 1,296) positions allows for 800 unique genes assayed simultaneously with appropriate controls
    - Some color combinations are unusable due to equipment sensitivity and a subset is retained for the controls



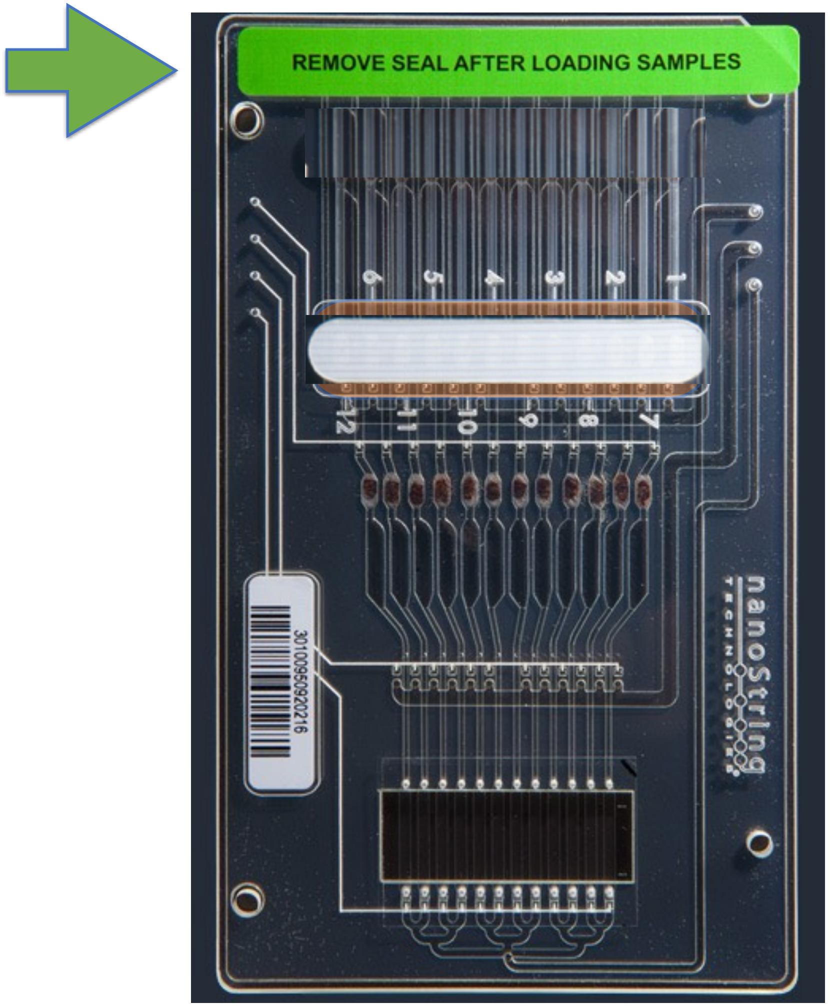
nanoString Hybridize



- Single-step hybridization
  - Template + Probes →Thermocycler
- 16-hour incubation at 65^{o}C
- High specificity
  - Separate capture and reporter probe decrease likeliness of false positives (both must bind to show up at final analysis)
- Hybridized sample will hold at 4^{o}C for 20 hours after completion
- Also contains technical positive and negative control probes

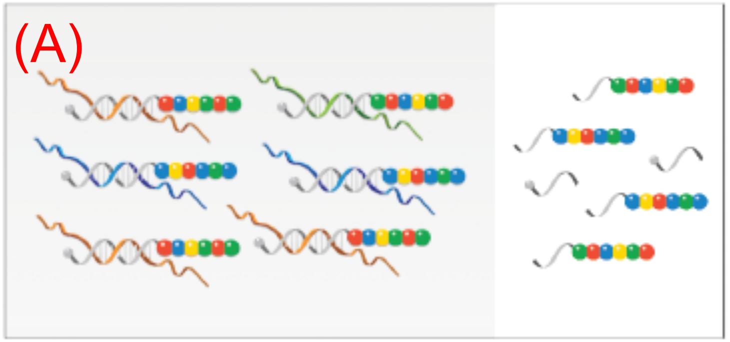
Load nanoString Fluidics Chip

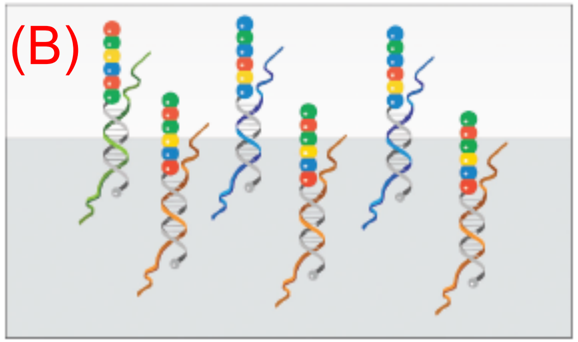
- Hybridized sample volumes are equalized to 35 µL and loaded into separate wells
- Place protection sticker over loading ports
- Remove fluidics ports protector (green sticker)
- Place into **nCounter** and start protocol

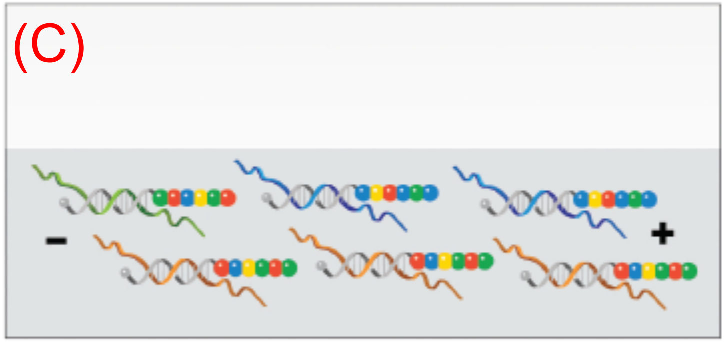


Running Protocol (inside the box)

- Chip contains magnetic beads containing short oligo sequences
  - One sequence compliments capture probe and the other sequence compliments reporter probe
- Sequential hybridization, washing, and melting of sample to magnetic beads allows for cleaning of unbound and non-specifically bound probes (A)

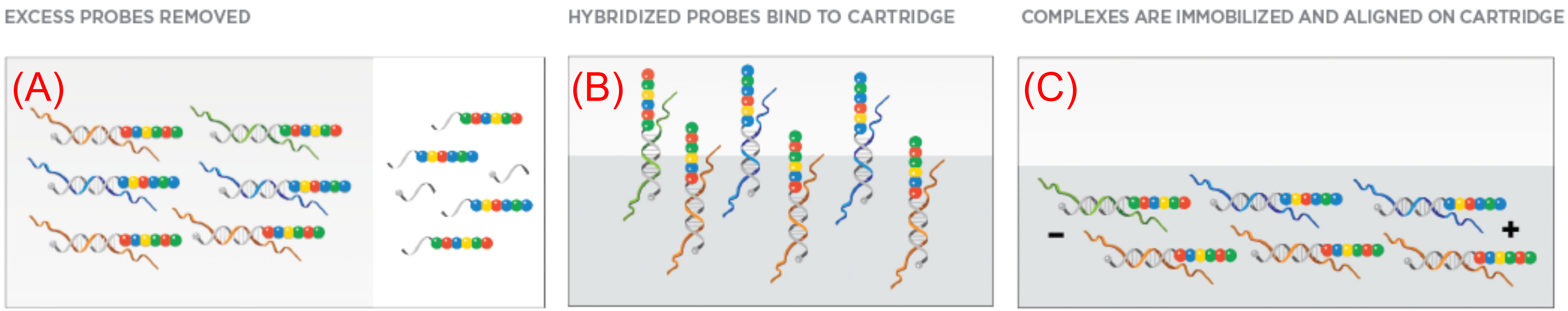

EXCESS PROBES REMOVED


HYBRIDIZED PROBES BIND TO CARTRIDGE


COMPLEXES ARE IMMOBILIZED AND ALIGNED ON CARTRIDGE

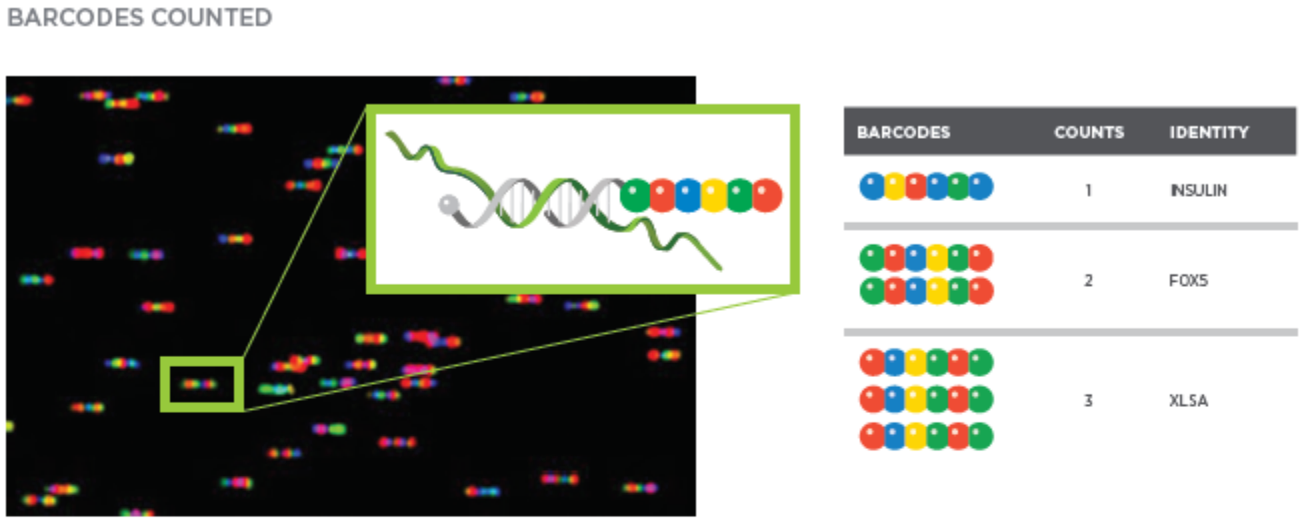
Running Protocol (inside the box)

- After wash beads are moved into viewing area where ubiquitin tags on the capture probe bind to cartridges (B)
- Reporter oligo’s are melted from bead and an electric field is applied to the sample which align the samples and allow ubiquitin tag on reporter tags to bind cartridge (C)



Running Protocol (inside the box)

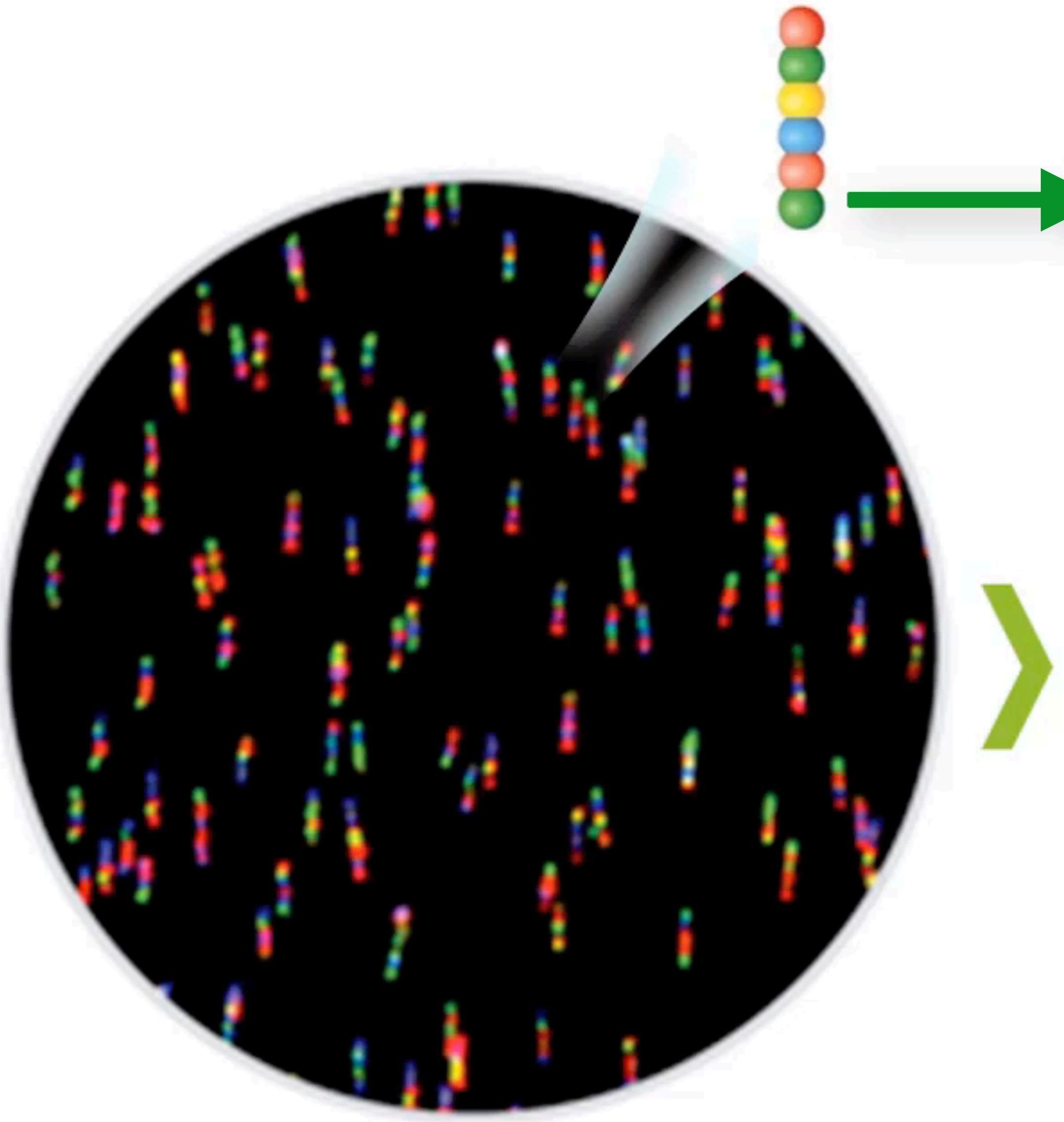
- High quality imaging allows a computer to analyze the thousands of images captured.
  - About 700 images are taken per sample
- Running time is about 8 hours.



Analyze Data

- Technical controls allow for normalization regardless of input concentration
- Built in quality control flags allow for confidence of data
- nCounter freeware provided by nanoString does hard analysis
    - Heat maps
    - Box-whisker plots
    - Fold change/significance plots
    - etc.

33		30102260481220...	9	Mar 31, 2019 11:29	mRNA	NS_IMMUNOLOG...					
34		30102260481220...	10	Mar 31, 2019 11:29	mRNA	NS_IMMUNOLOG...					
35		30102260481220...	11	Mar 31, 2019 11:29	mRNA	NS_IMMUNOLOG...	⚑		⚑		
36		30102260481220...	12	Mar 31, 2019 11:29	mRNA	NS_IMMUNOLOG...					



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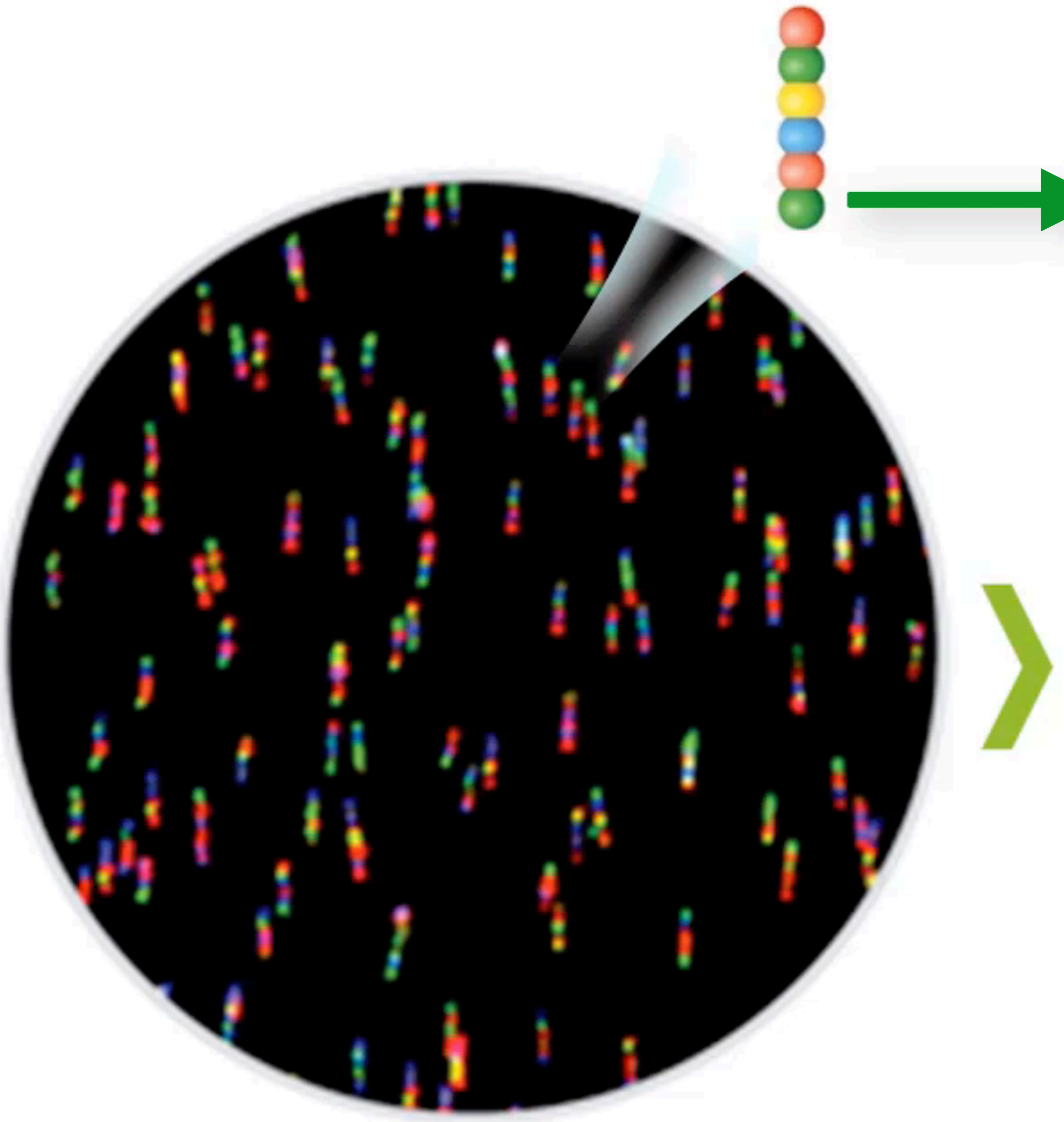

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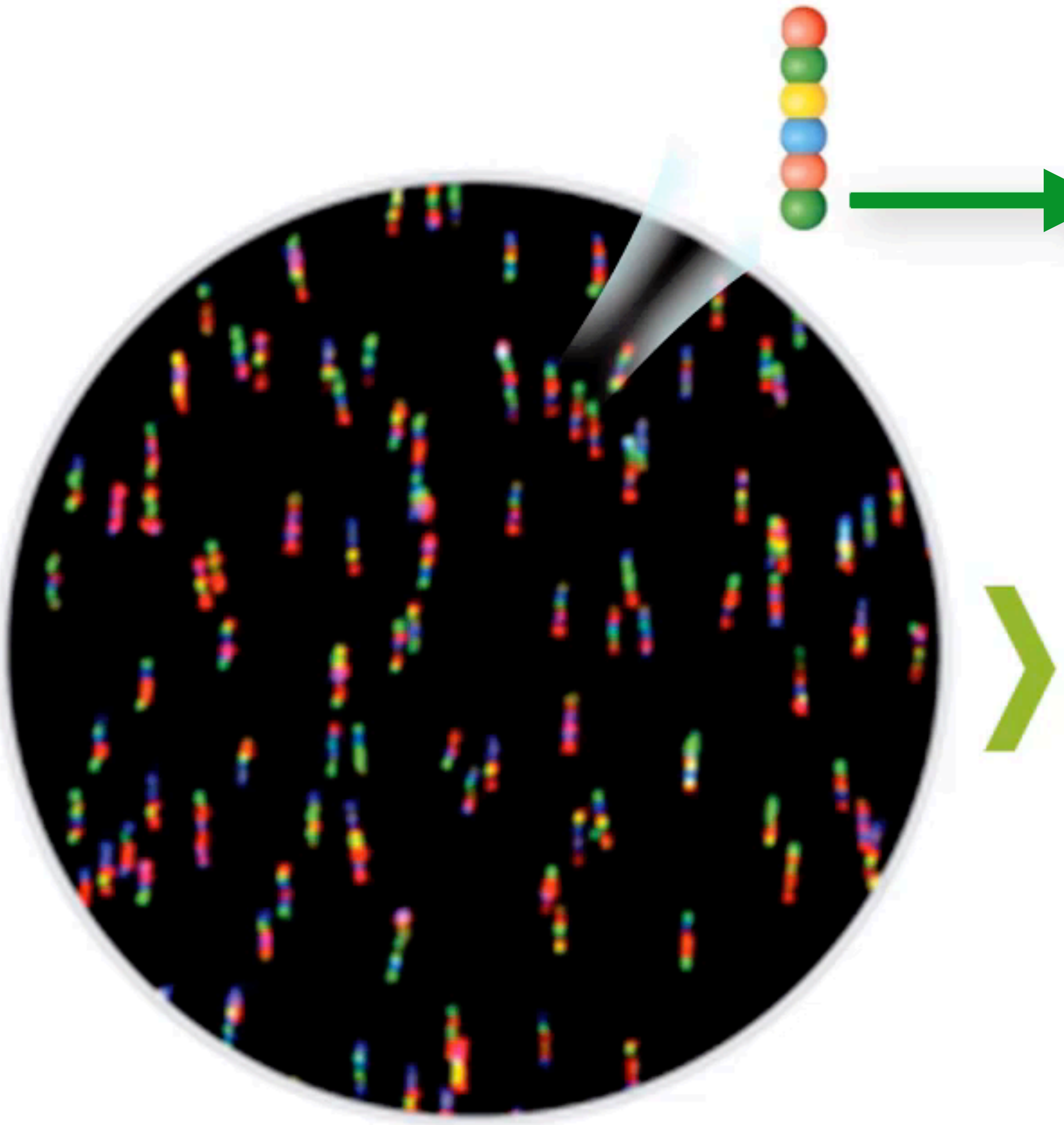

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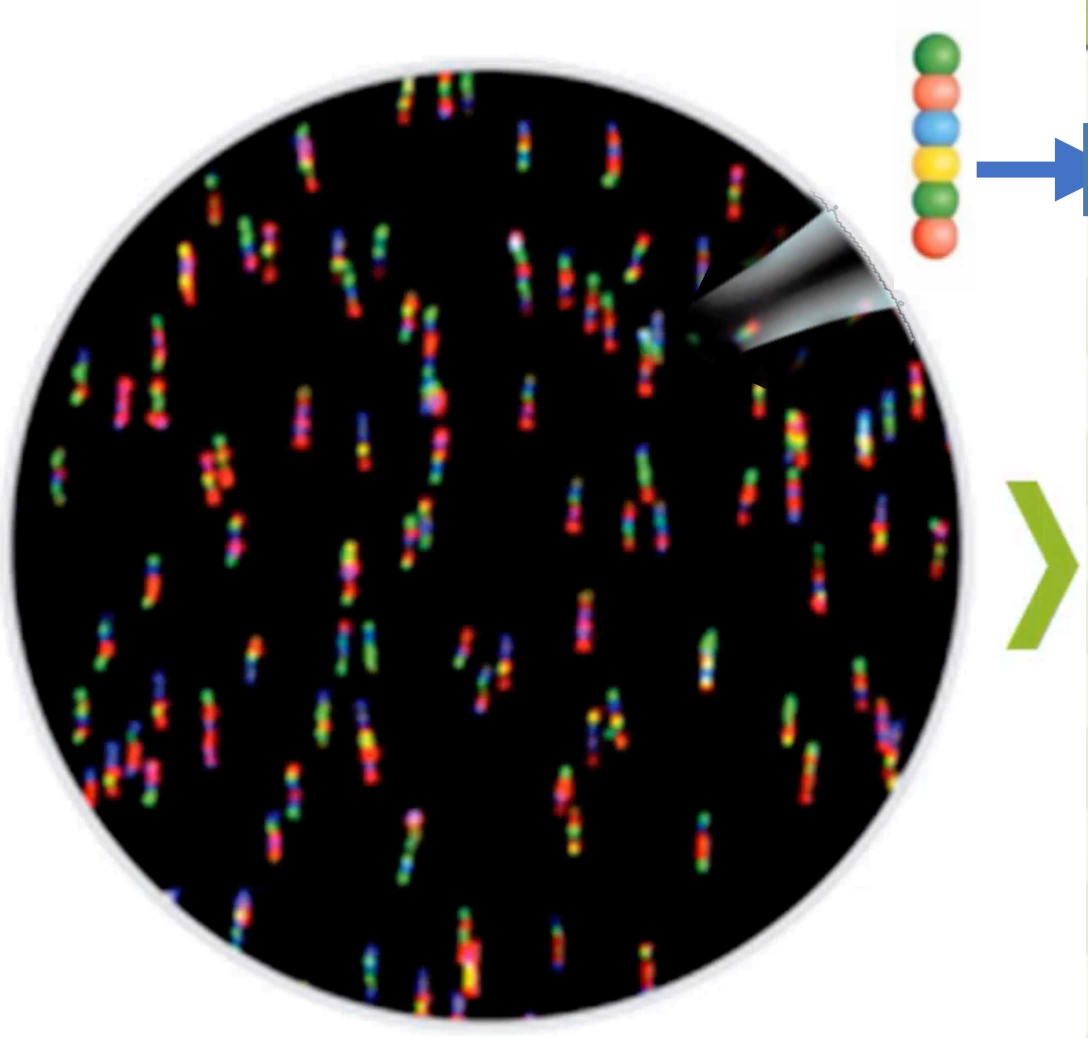

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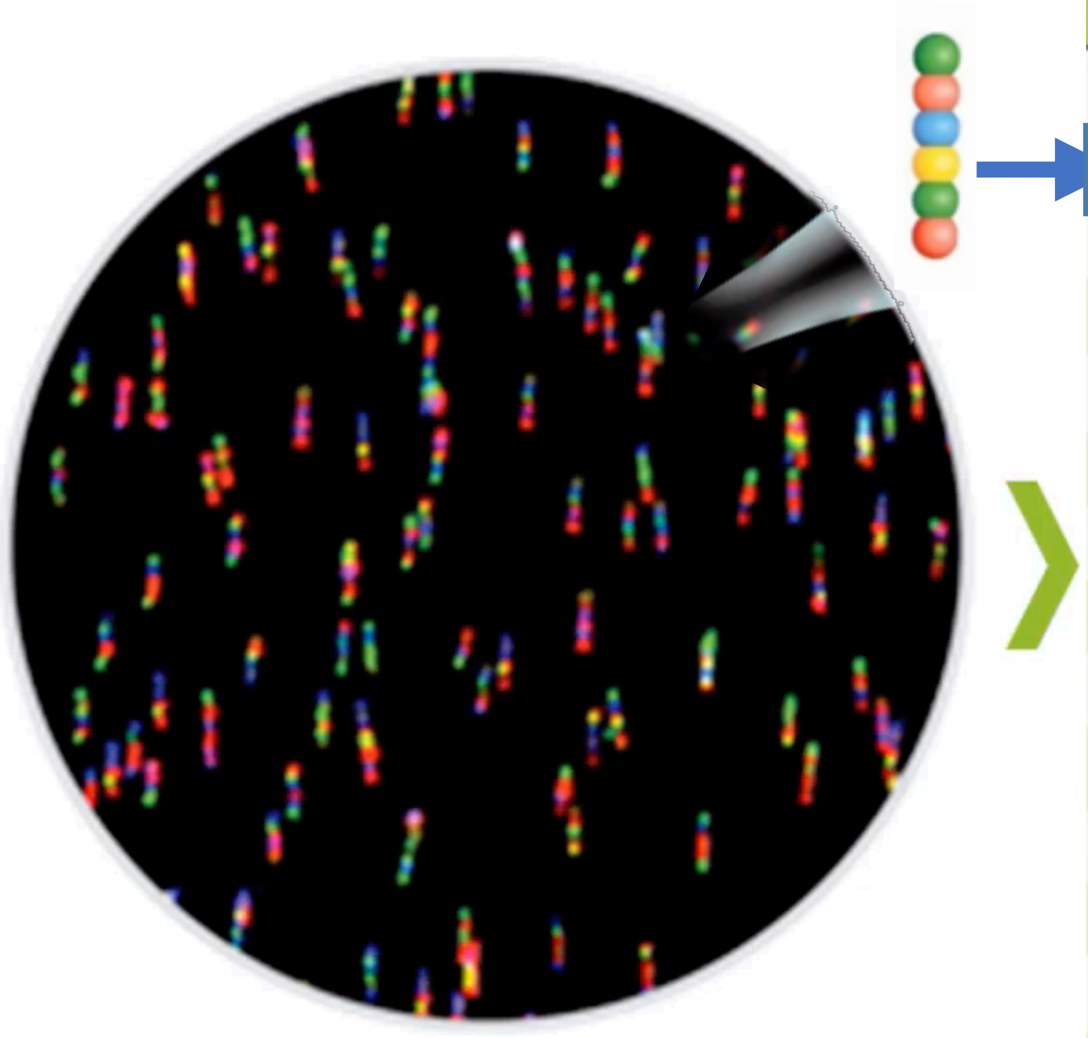

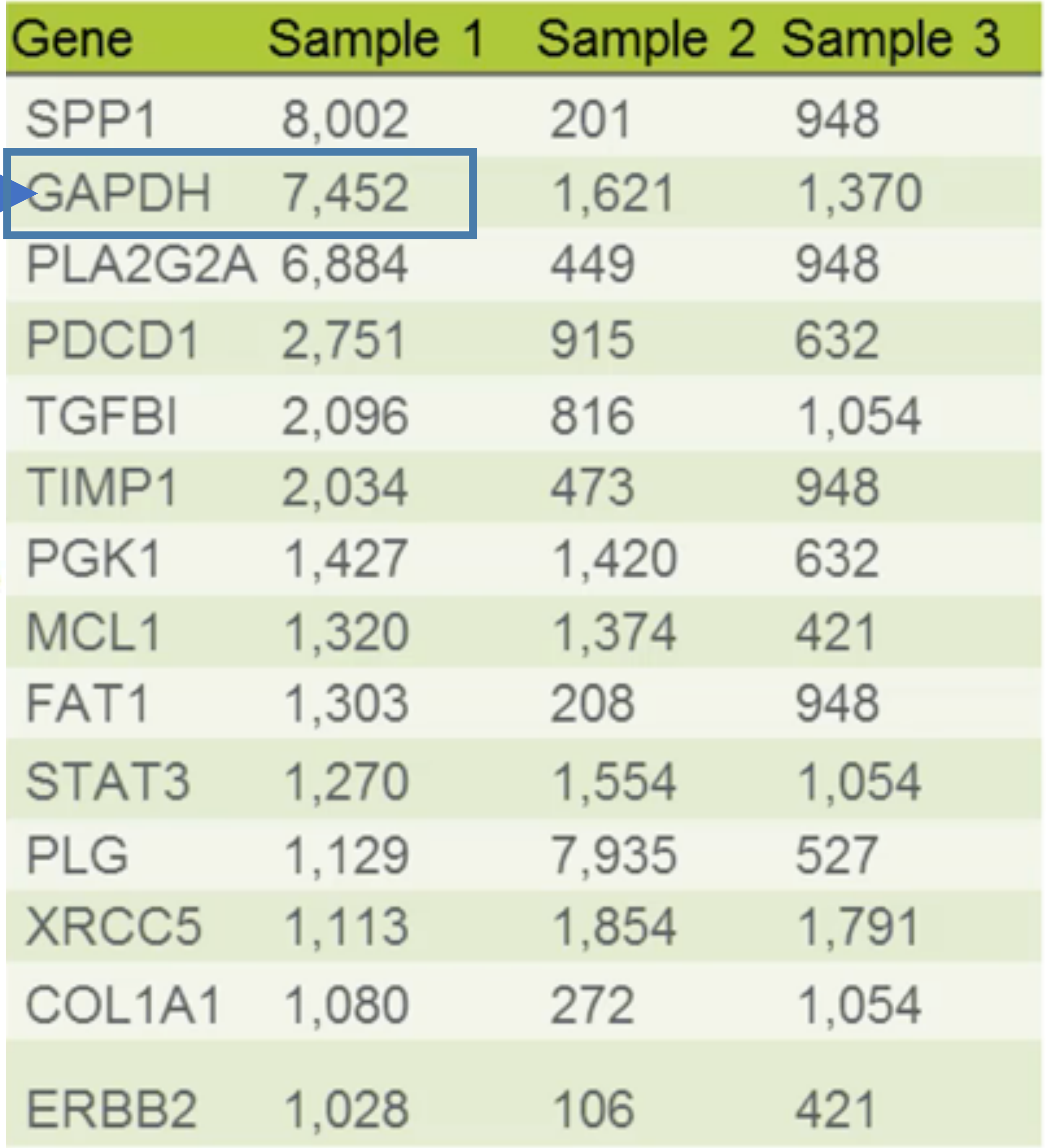

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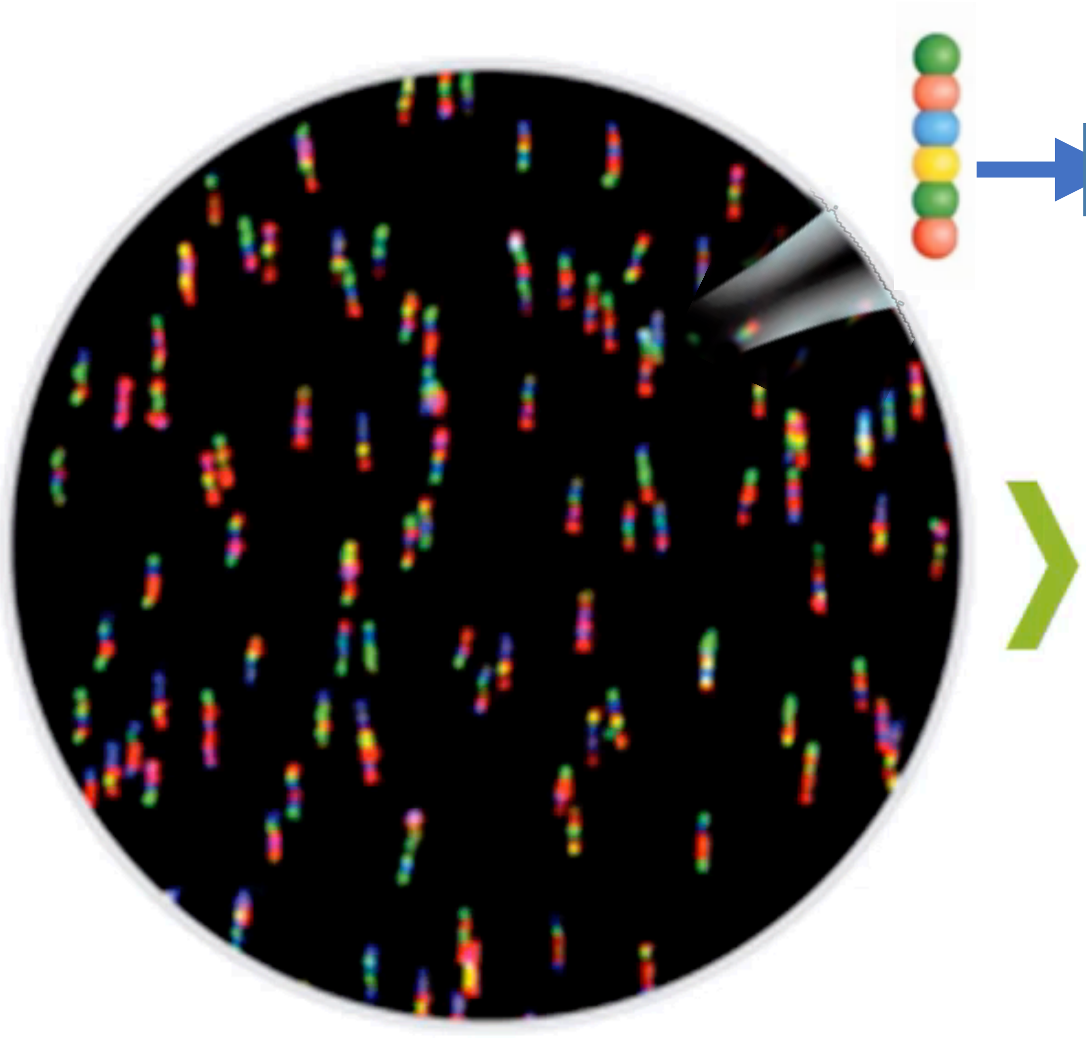

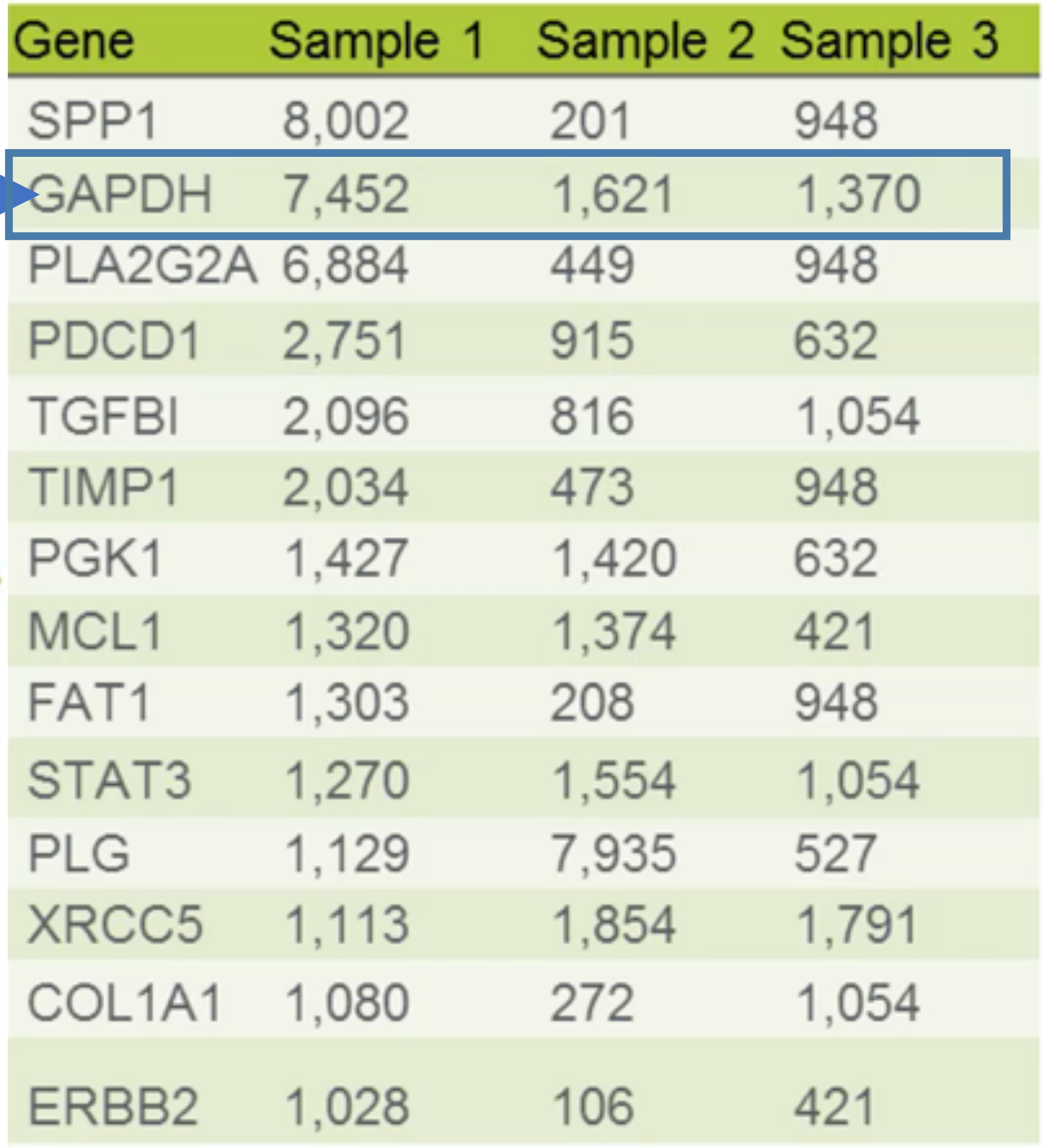

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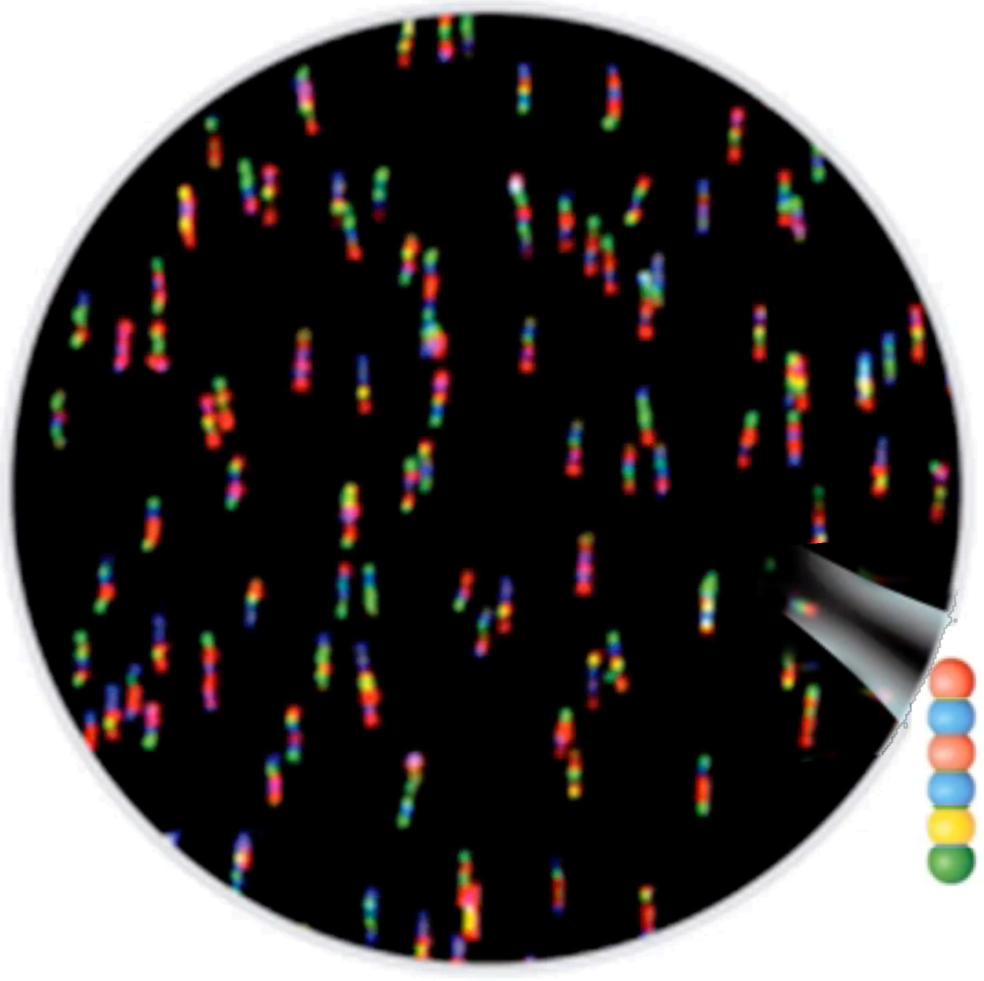

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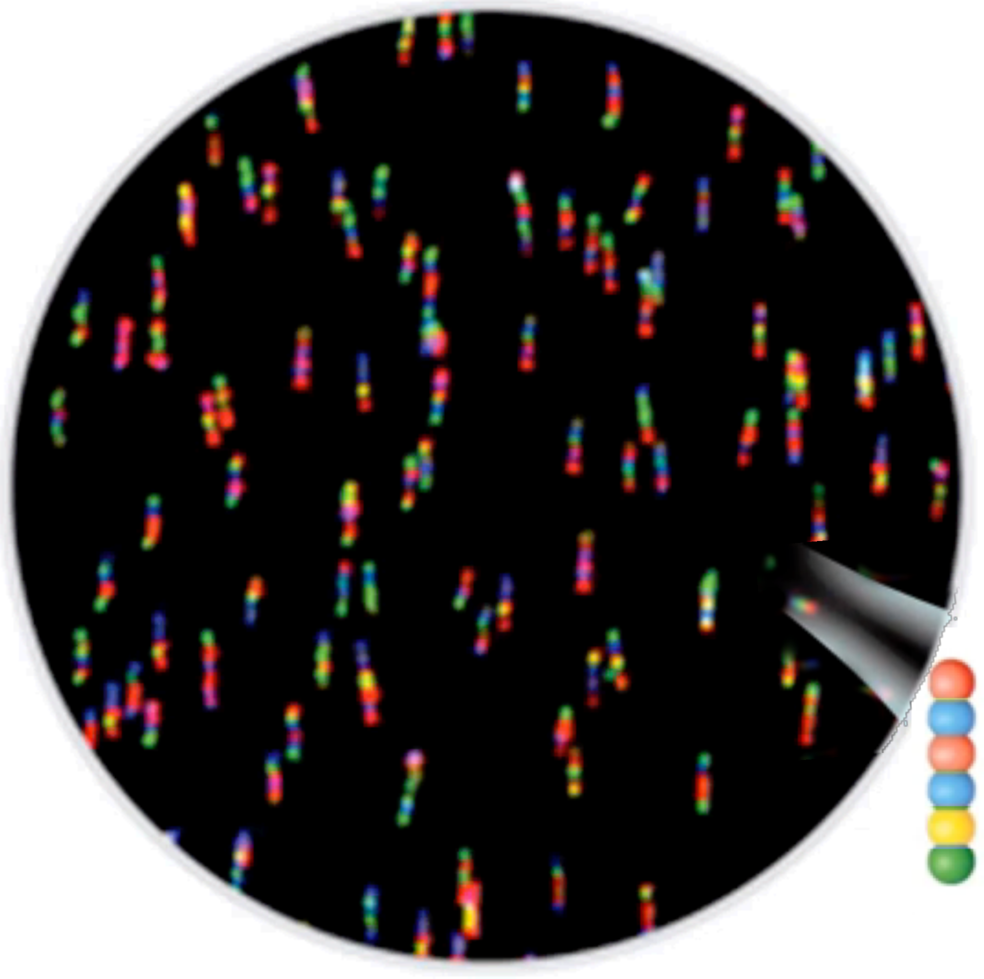

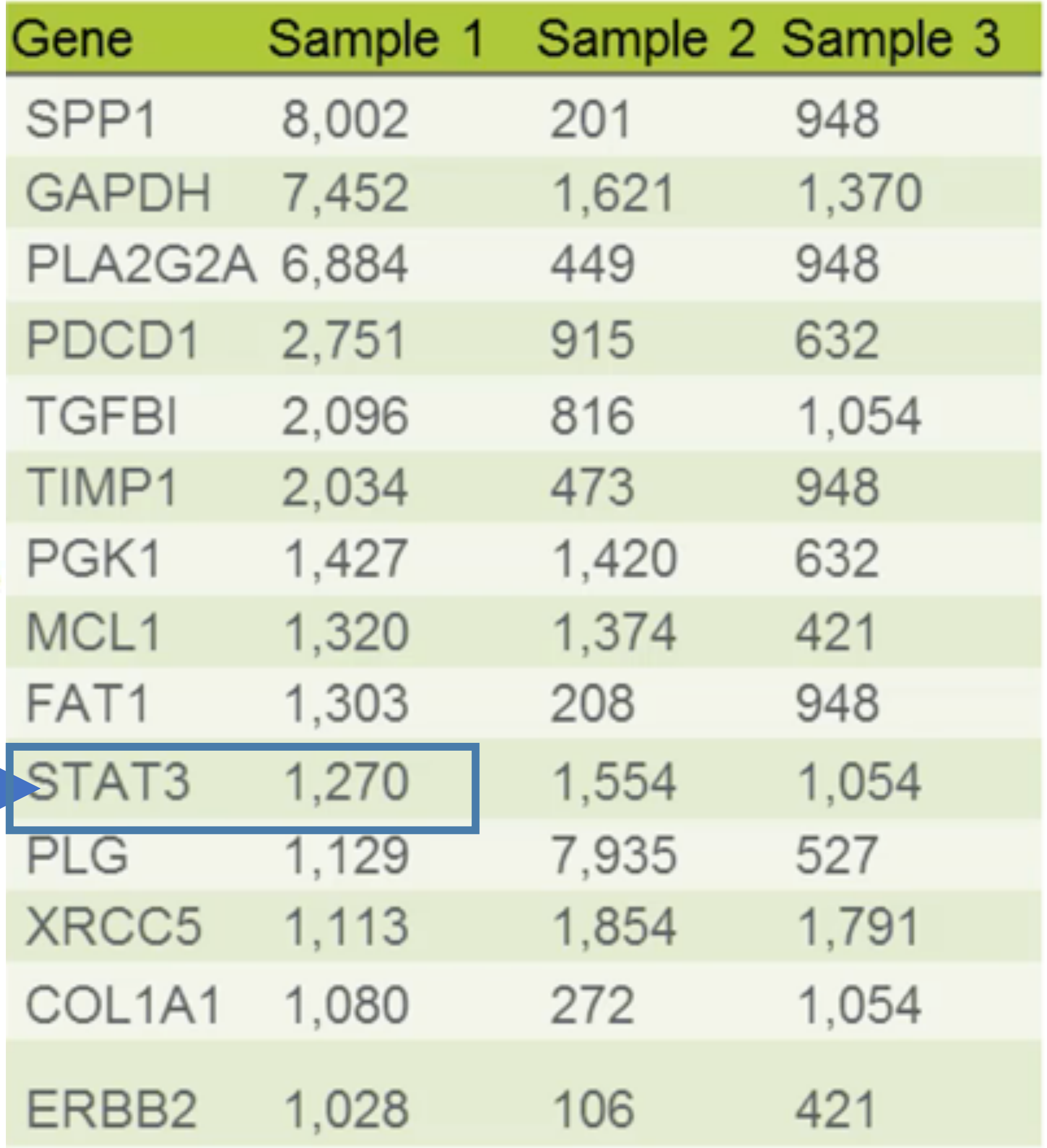

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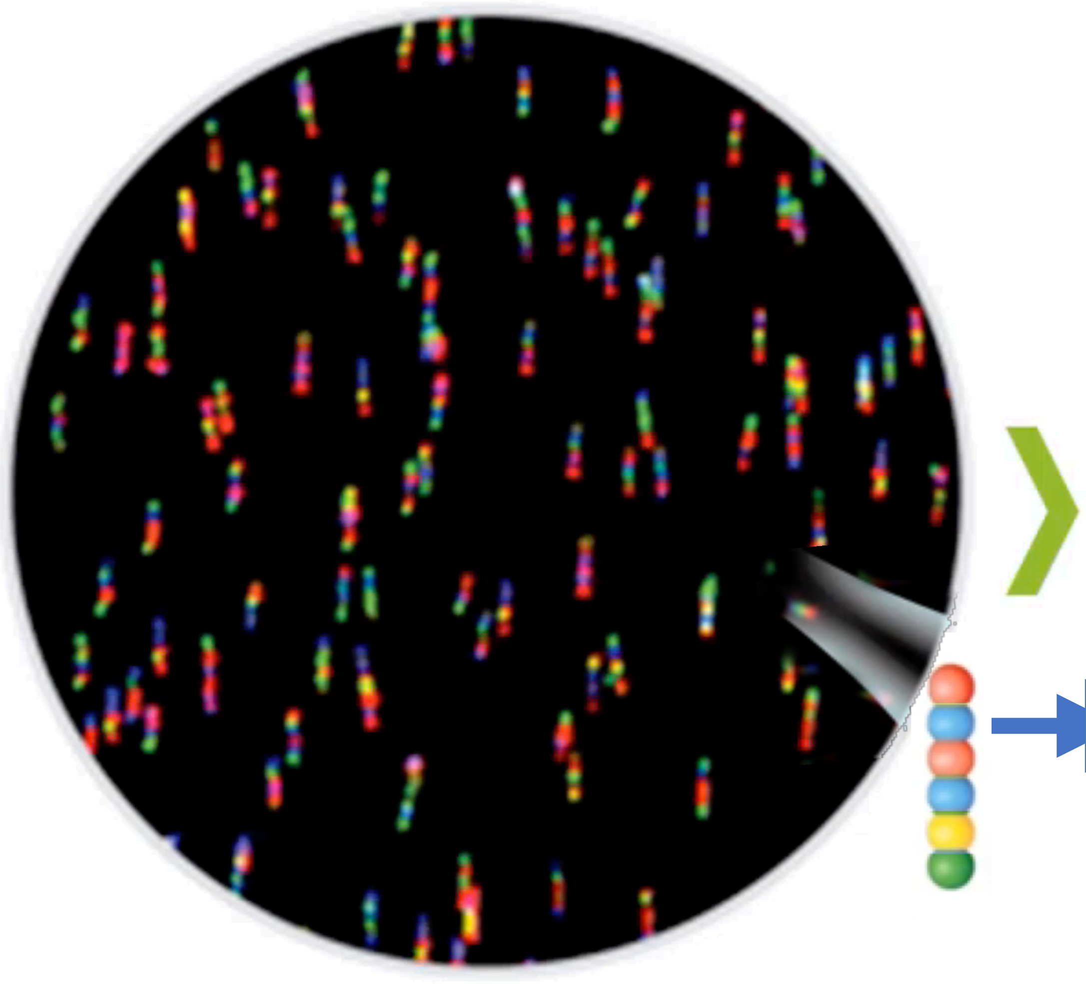

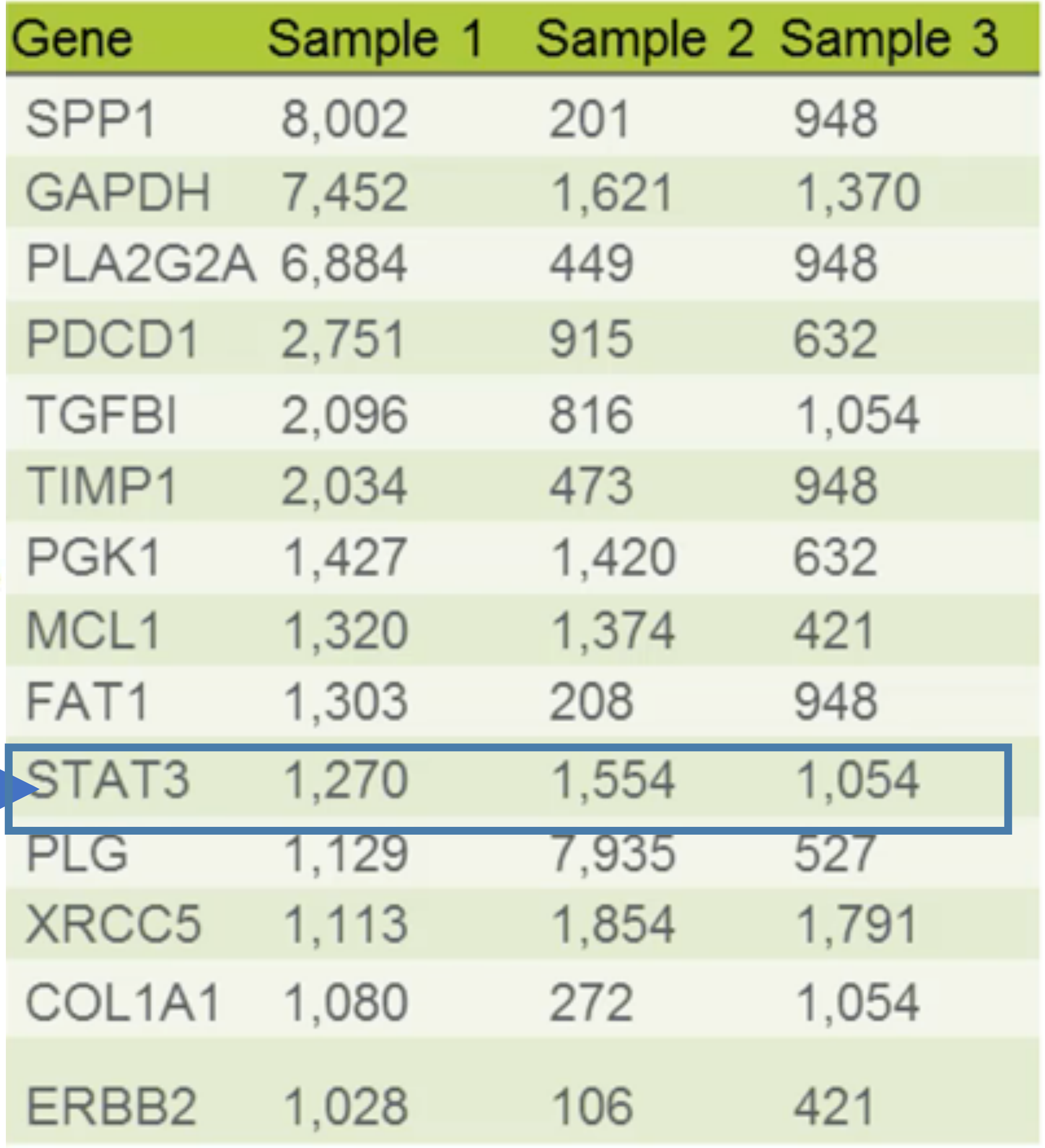

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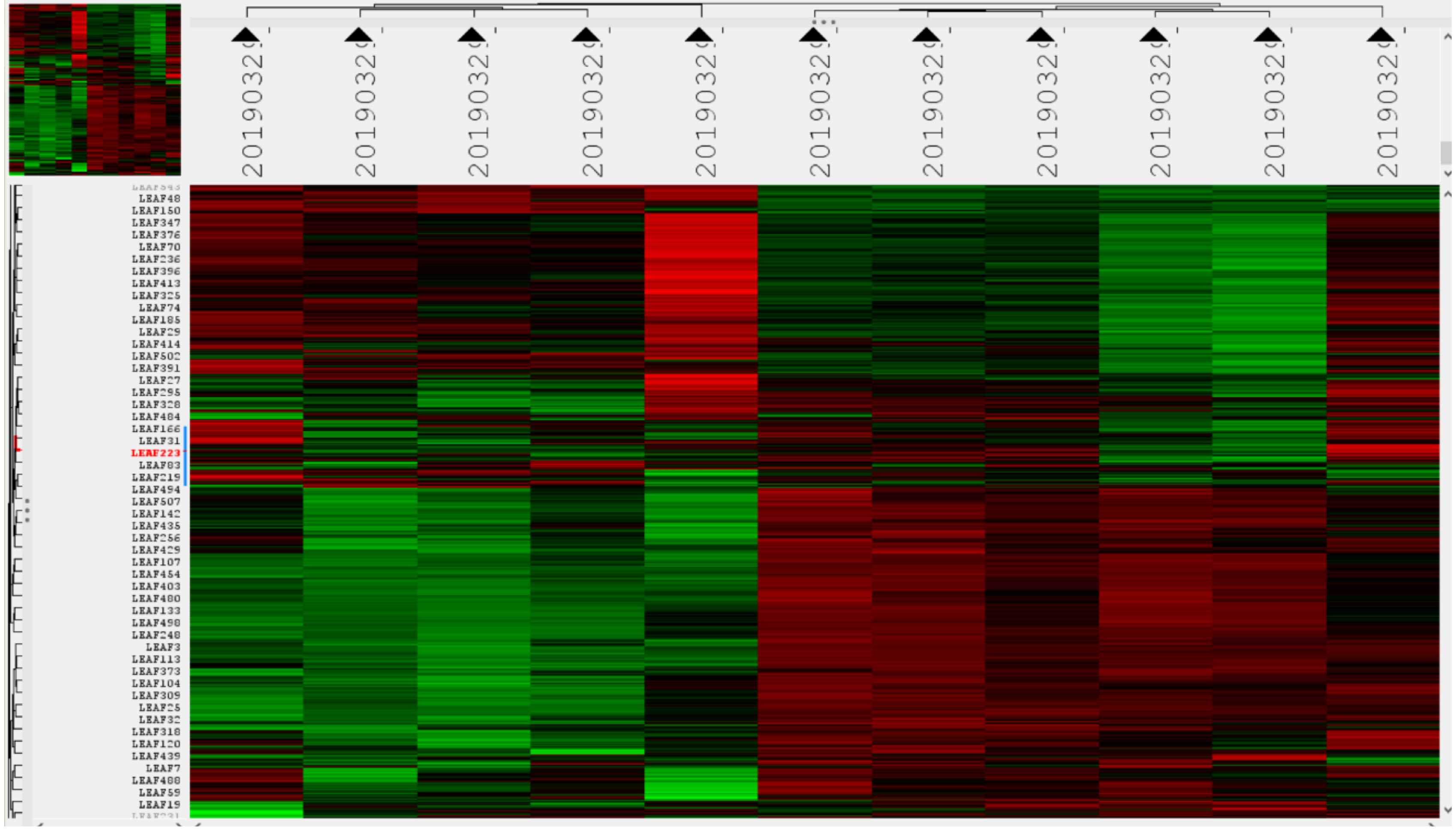
Analyze Data

Increased Expression

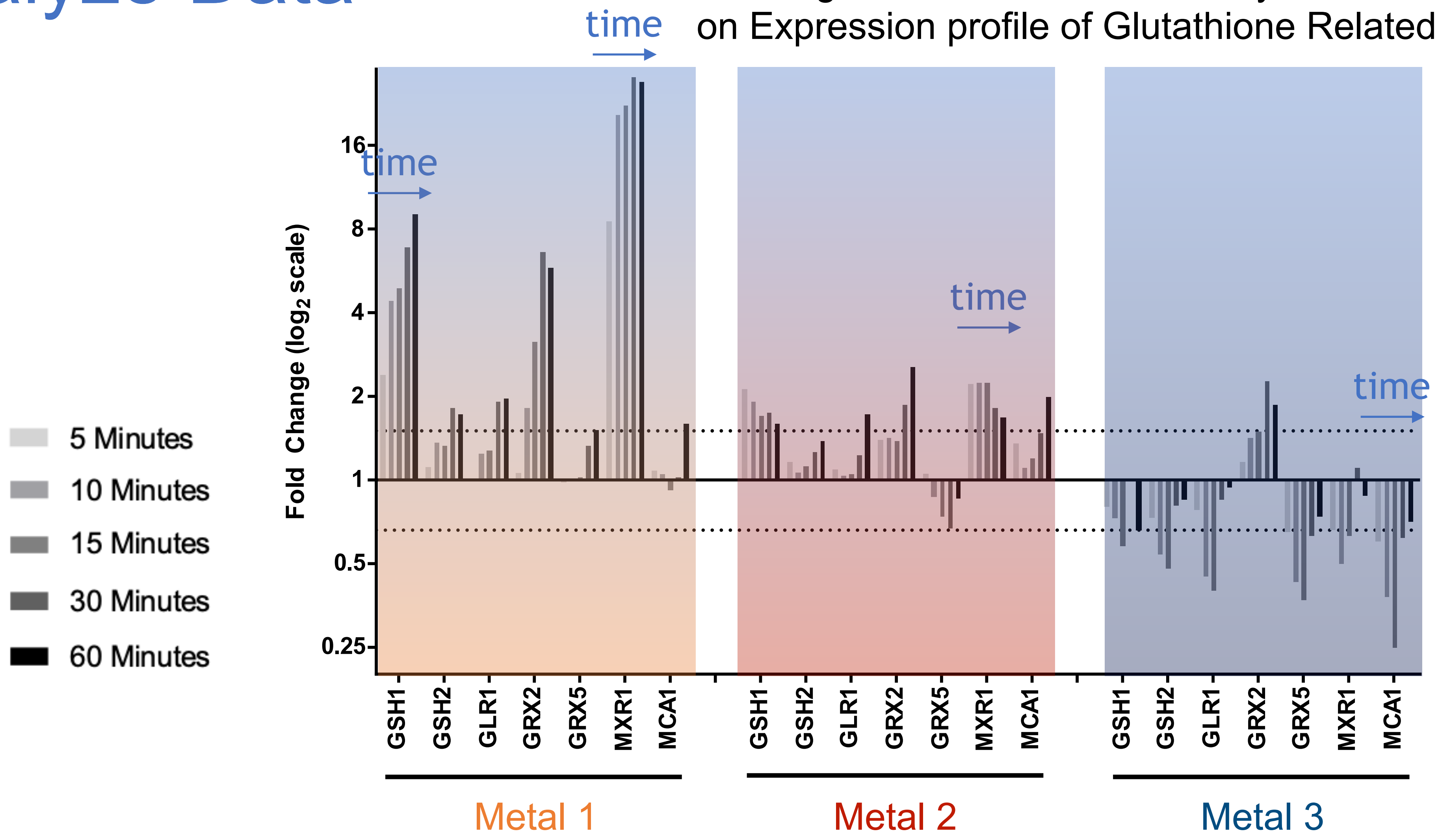


Decreased Expression

- Sample heat-map of mouse immunology assay.
- Mice were treated with an ocular herpes virus and whole eye expression was analyzed

Analyze Data

Differing Effects of Different Heavy Metals on Expression profile of Glutathione Related Genes



Strengths of nanoString

- No PCR
  - Reduces work time
  - Reduces sources of error

- Built in QC
  - Removes need for technical repeats/researcher artifacts in data
  - Allows for high confidence in data
  - Provides route for analysis of very low transcribed or completely untranscribed products under treatment conditions

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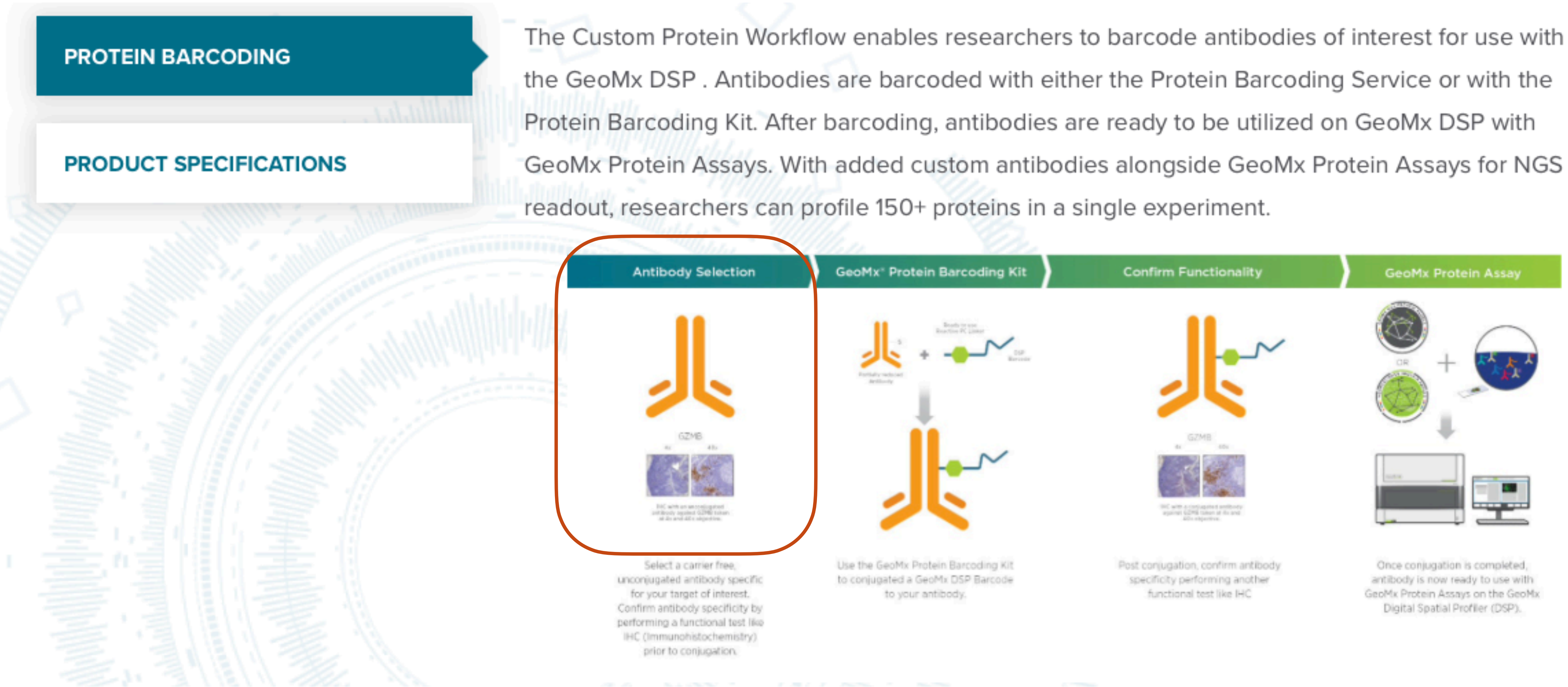
nanoString (Future purchase?)



PROTEIN BARCODING

PRODUCT SPECIFICATIONS

The Custom Protein Workflow enables researchers to barcode antibodies of interest for use with the GeoMx DSP . Antibodies are barcoded with either the Protein Barcoding Service or with the Protein Barcoding Kit. After barcoding, antibodies are ready to be utilized on GeoMx DSP with GeoMx Protein Assays. With added custom antibodies alongside GeoMx Protein Assays for NGS readout, researchers can profile 150+ proteins in a single experiment.

Antibody Selection

Select a carrier free, unconjugated antibody specific for your target of interest. Confirm antibody specificity by performing a functional test like IHC (Immunohistochemistry) prior to conjugation.

GeoMx® Protein Barcoding Kit

Use the GeoMx Protein Barcoding Kit to conjugated a GeoMx DSP Barcode to your antibody.

Confirm Functionality

Post conjugation, confirm antibody specificity performing another functional test like IHC

GeoMx Protein Assay

Once conjugation is completed, antibody is now ready to use with GeoMx Protein Assays on the GeoMx Digital Spatial Profiler (DSP).

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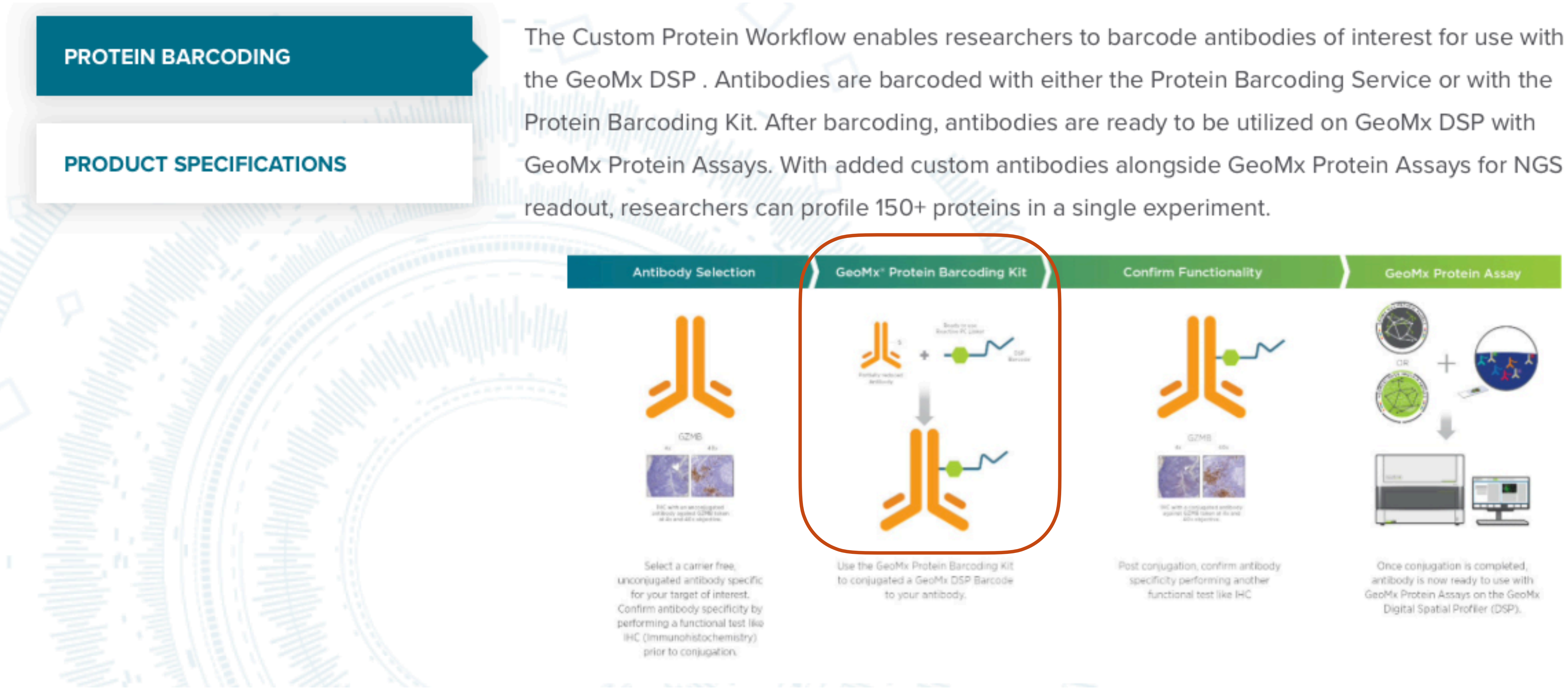

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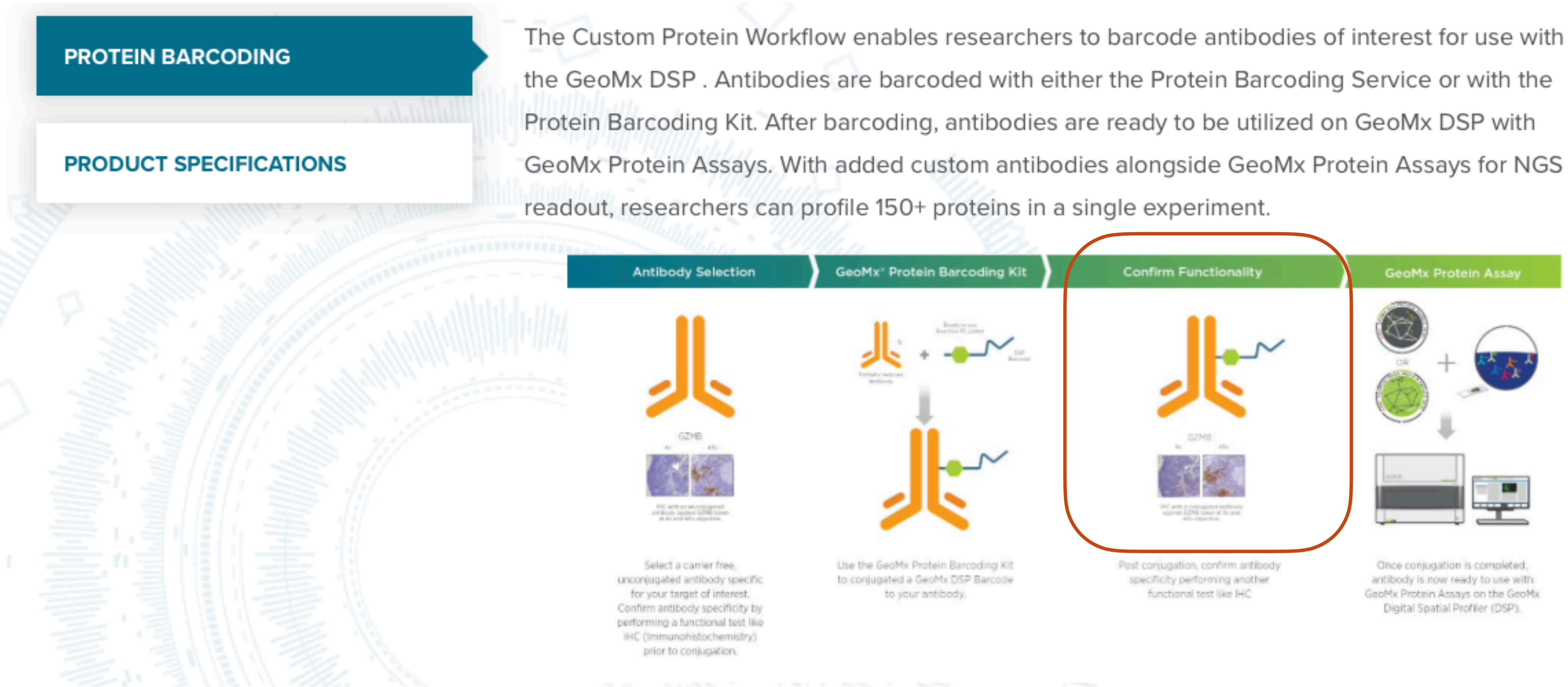

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