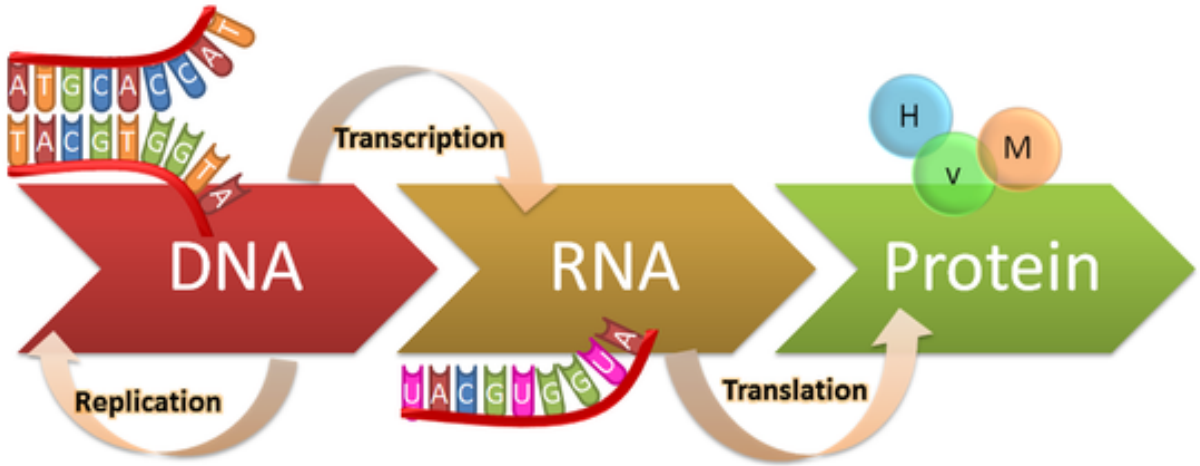
SUMMER INSTITUTE CALENDAR 2022

SUN	MON	TUE	WED	THU	FRI	SAT
						July 02 Early Arrival Airport Arrivals and Check-in
July 03 Early Arrival Airport Arrivals and Check-in	04 Airport Arrivals and Check-in 6:00pm: 4th of July Celebrations	05 9:30am-12pm: Campus tour, Panther ID & ISSS Check-in 12-2pm Lunch 2:00-6:00pm, Shuttle to local grocery store	06 9:30am-11:30am ISSS, OII, & Housing Orientation & Presentation **2:30-4:30pm:-Welcome Reception and Buddy Meet & Greet Event**	07 **Classes begin!** 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 INTRO - TRAINING**	08 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 DNA PREPARATION**	09 Free Day
10 12:00-4:00pm: The World Coca-Cola and Georgia Aquarium	11 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS I**	12 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS II**	13 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm:BIOL4905 PROTEOMICS III** 6:00-10:00pm: Atlantic Station Shopping & Movie (Sign-up)	14 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 PROTEOMICS IV ?**	15 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 RNA PREPARATION**	16 6:00-9:00pm: Dinner in America (Sign-up)
17 Free Day	18 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 qPCR & AUTOMATION**	19 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 DNA SEQUENCING**	20 **MINI BREAK**	21 9-11:20am: Morning course **CDC TRIP** **1:30-4:30pm: BIOL4905 MICROSCOPY / AFM**	22 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30 - 4:30pm: BIOL4905 NEXT GEN SEQ.** 5:30-7:30pm: Meet & Greet BBQ event @ The Commons	23 9:00am - 6:00pm: Outlet Mall
24 Free Day	25 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 MICROARRAY I**	26 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 NANOSTRING**	27 9-11:20am: Morning course 11:20am-2:00pm: Lunch **Notebook Day** 1:30-4:30pm: BIOL4905 NANOSTRING	28 **Last day of classes** 9-11:20am: Morning course 11:20am-2:00pm: Lunch break **1:30-4:30pm: BIOL4905 FLOW CYTOMETRY**	29 **FINALS**	30 Free Day
31 Free Day	August 01 Activity Day at the Recreation Center (Sign-up)	02 Free Day	03 9:30-11:00am: Georgia Capitol Tour (Sign-up) 2:00-4:00pm: Closing Reception	04 Departures (check-out at 12:00pm)		

Note: Students may arrive prior to the program date with an extra charge of $35 per night. Earliest day to check-in to University Commons is July 2.

Legend:

Orange: Courses Blue: Lunch Break. Red: Sign-up events



nanoString

Direct Expression Profiling

Adapted from

Jesse Gardner's PPT

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Supporting Life Sciences at GSU

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

Georgia State University

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Profiling Cells

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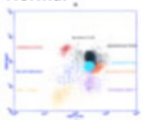
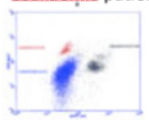
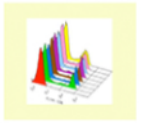


Leukaemia patient





See effects of different drugs on Cell cycle

Apoptotic cells

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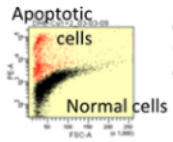

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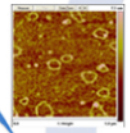

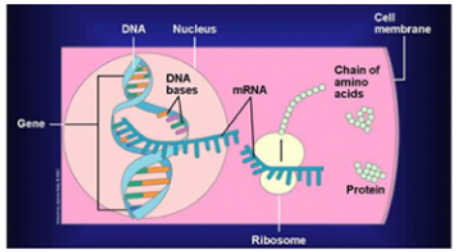

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Replication

Cellular Functions

RNA

Expression

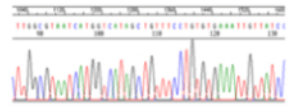
Protein

Expression

DNA Sequence Analysis:

Profiling DNA

Sanger Sequencing – >800 base pairs/run



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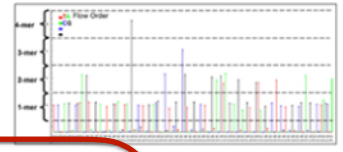

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Colour of pin-point dots demonstrates the presence / absence of gene sequences

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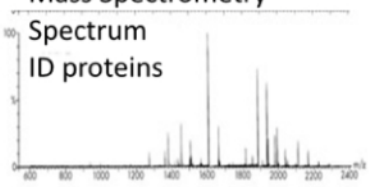

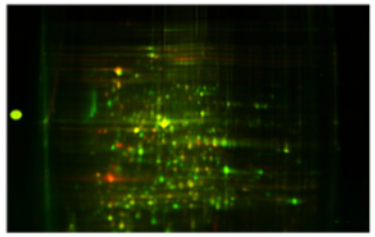

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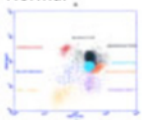

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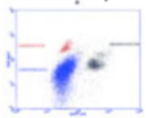

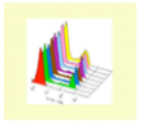

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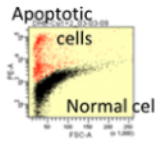

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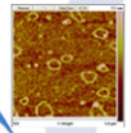

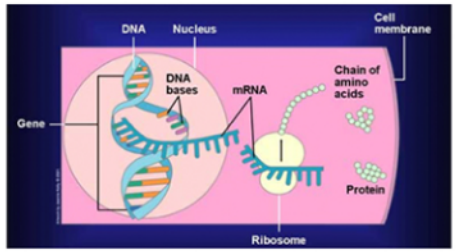

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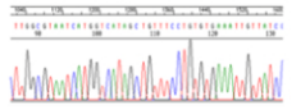

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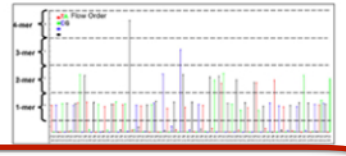

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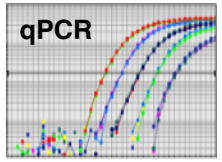

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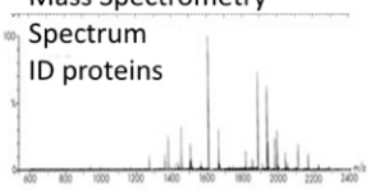

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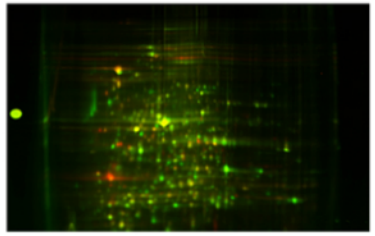

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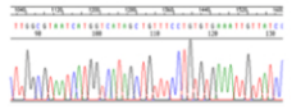

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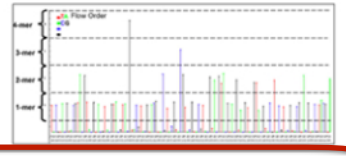

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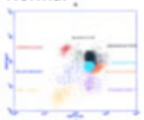

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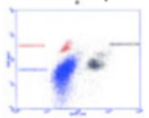

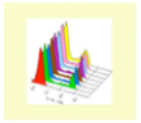

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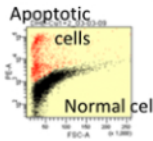

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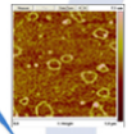

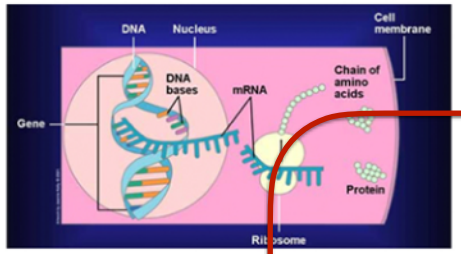

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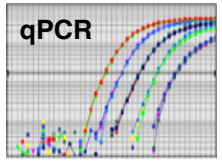

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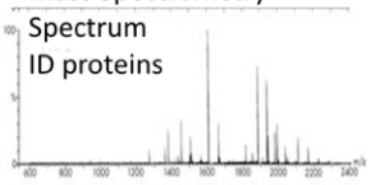

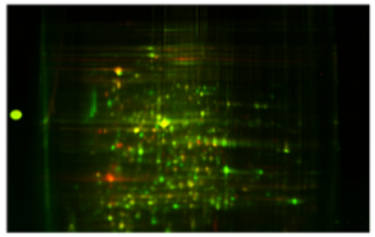

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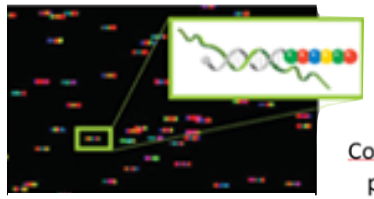


Nanostring

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

UNIVERSITY of WASHINGTON Students

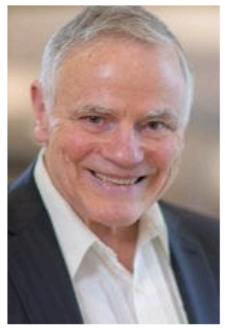
ABOUT IMMUNOLOGY / GRADUATE PROGRAM / CIIID / FACULTY / RESEARCH /

Department of Immunology

Department of Immunology › Faculty › Affiliate Faculty › Leroy Hood, M.D., Ph.D.

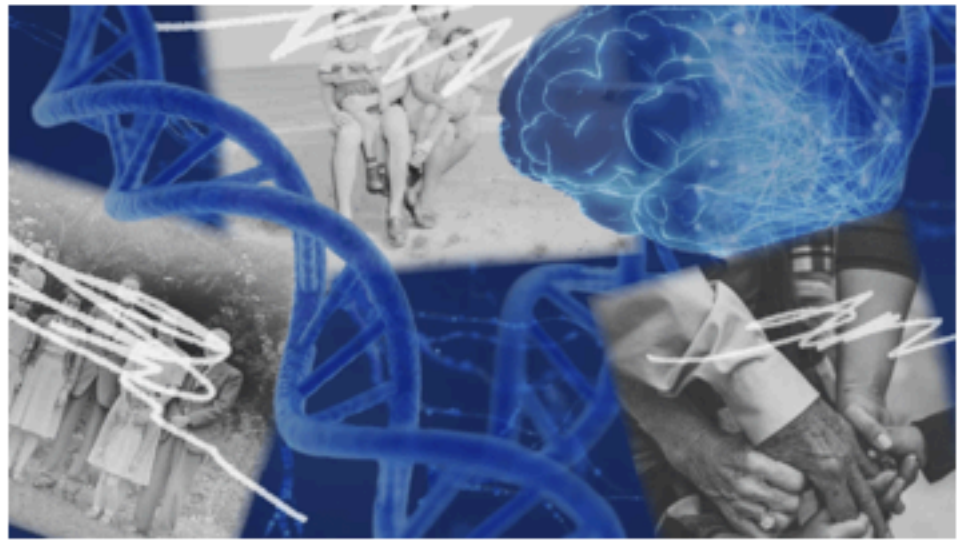
Leroy Hood, M.D., Ph.D.

Like 0 Tweet Share



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.



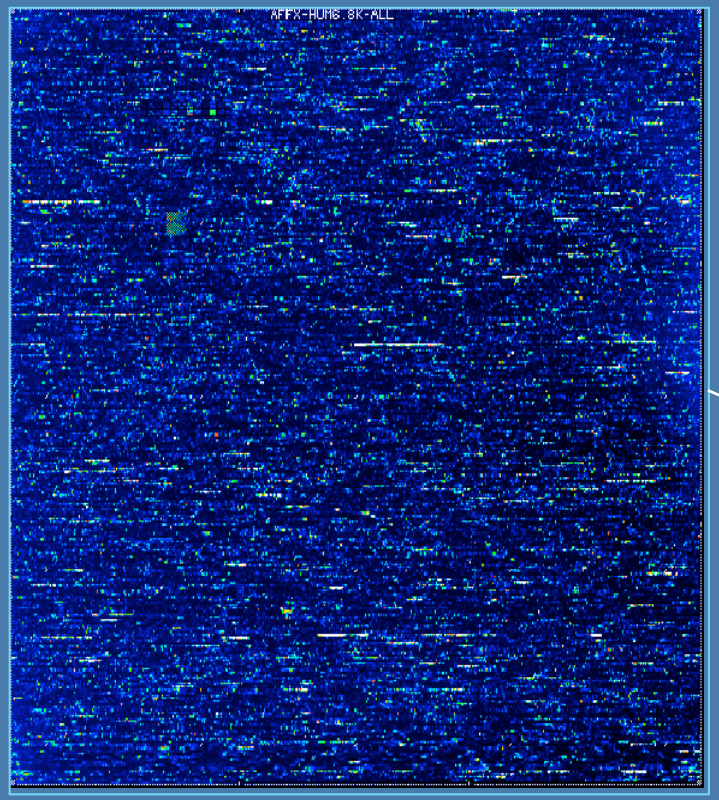
DR. LEE HOOD WRITES 'SECOND OPINION' COLUMNS FOR LOS ANGELES TIMES

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

1.28cm



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

Image of Hybridized Probe Array

GeneChip® Expression Analysis
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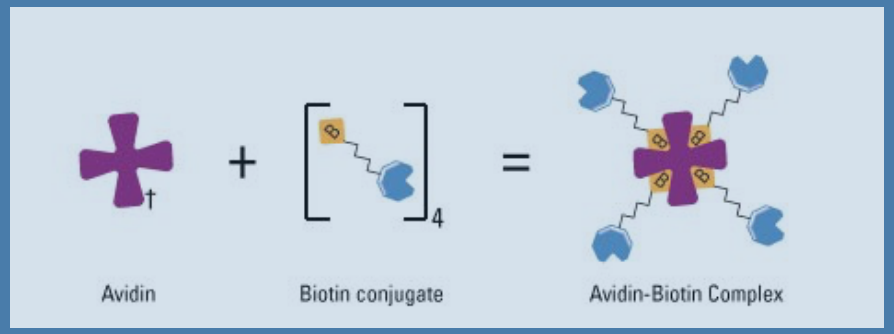

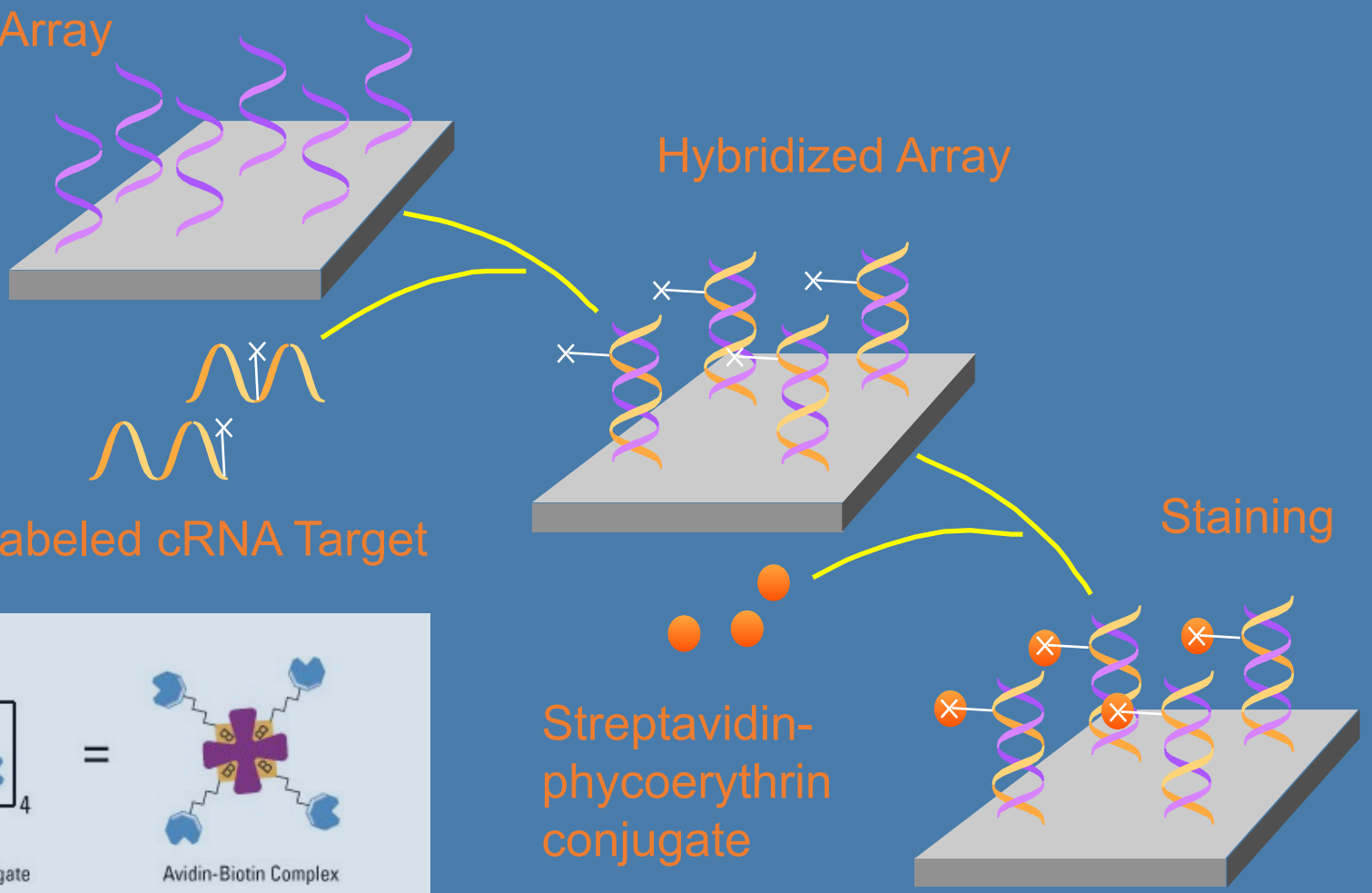

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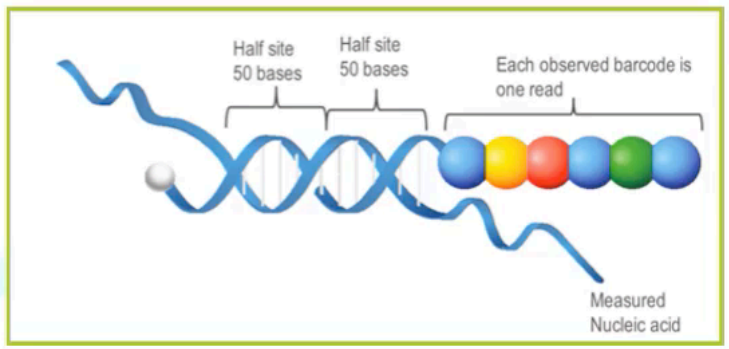
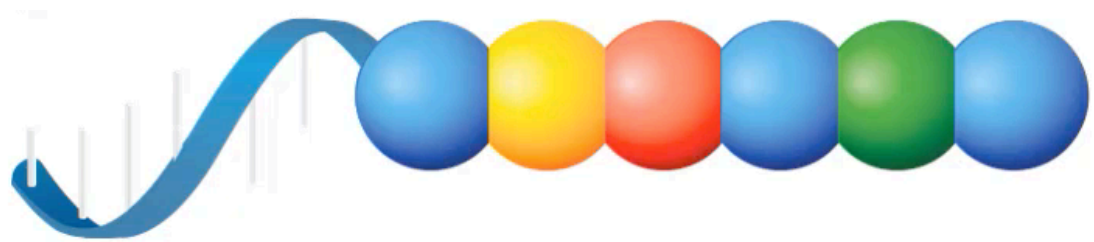
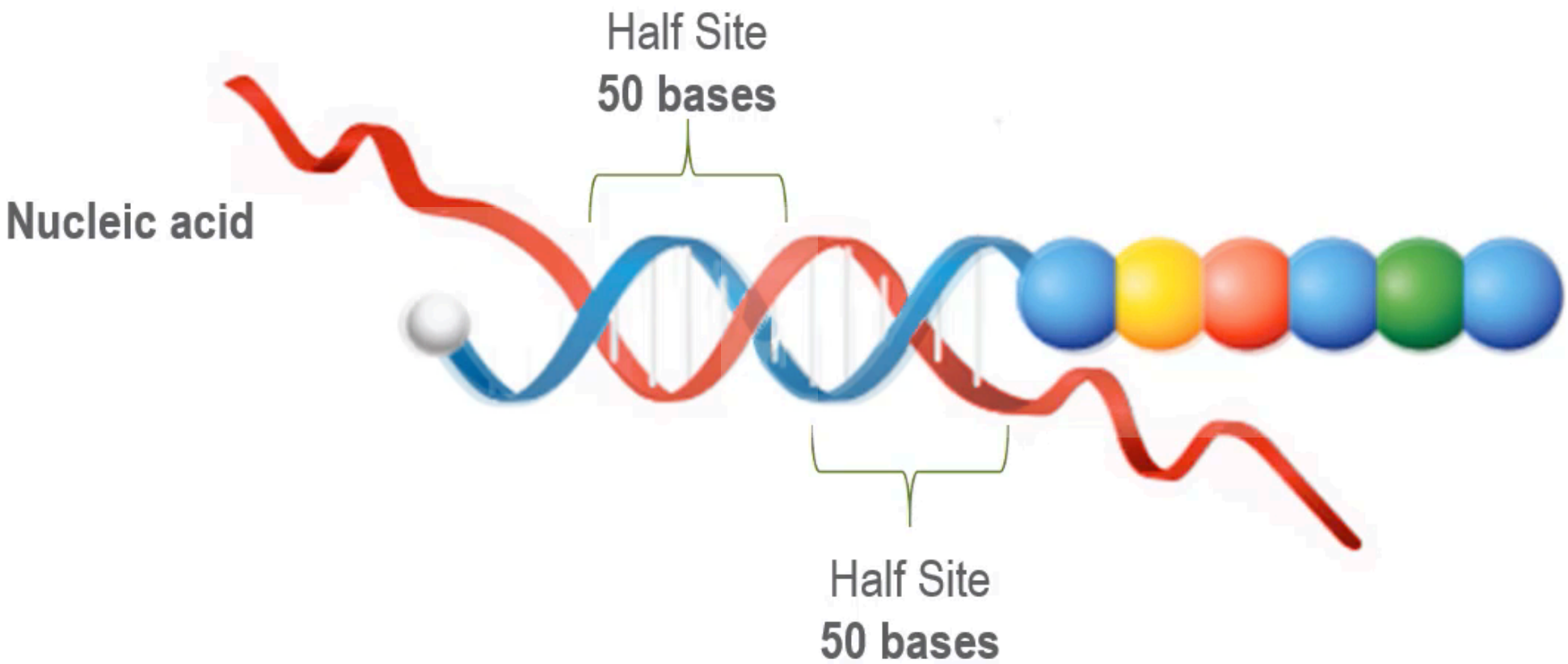
- **Gene Expression is quantified by directly counting each barcode bound on the slide surface**



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




Half Site
50 bases
Nucleic acid
Half Site
50 bases

Automated instrumentation

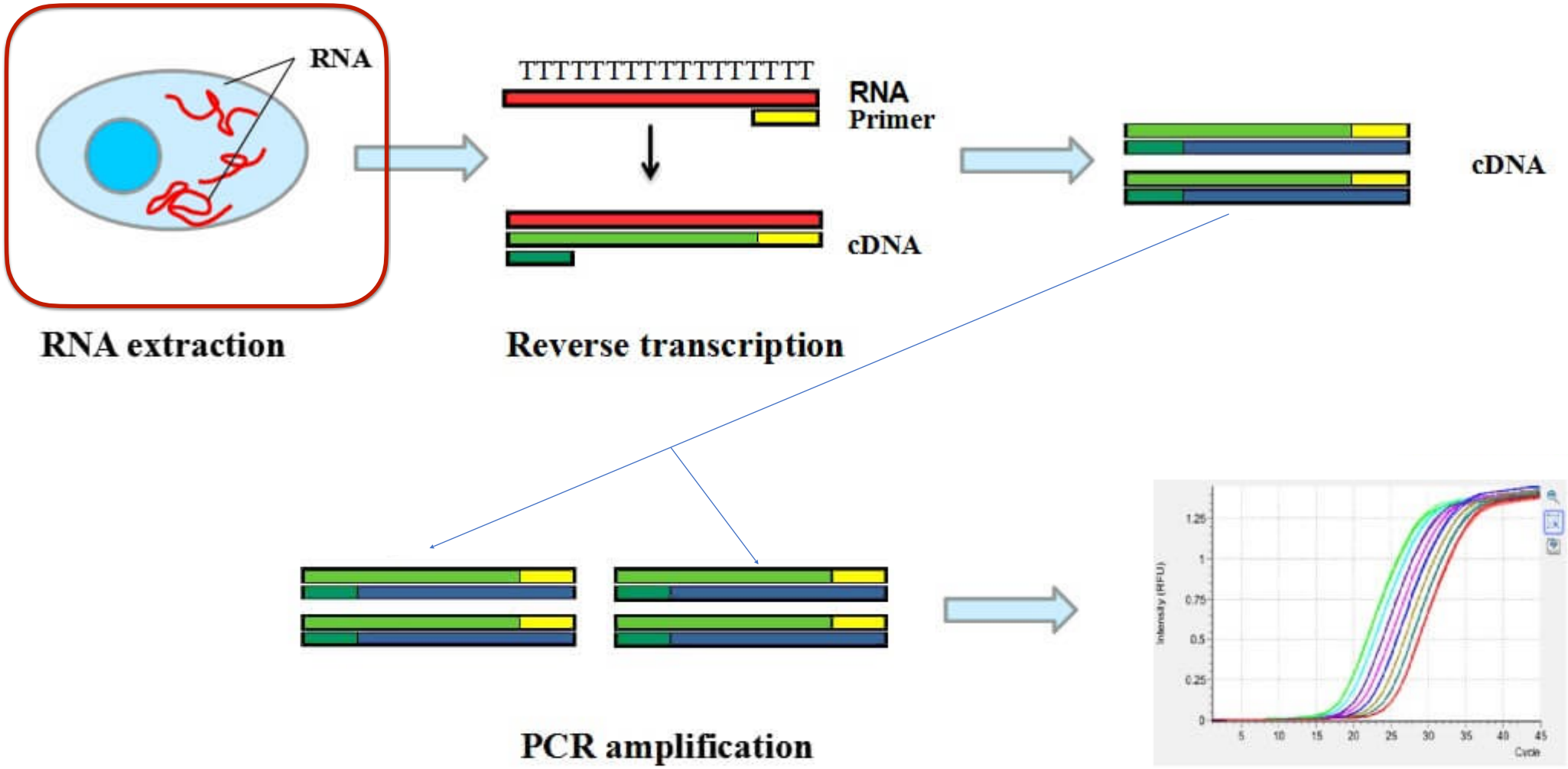
Purification

Alternative Methods

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

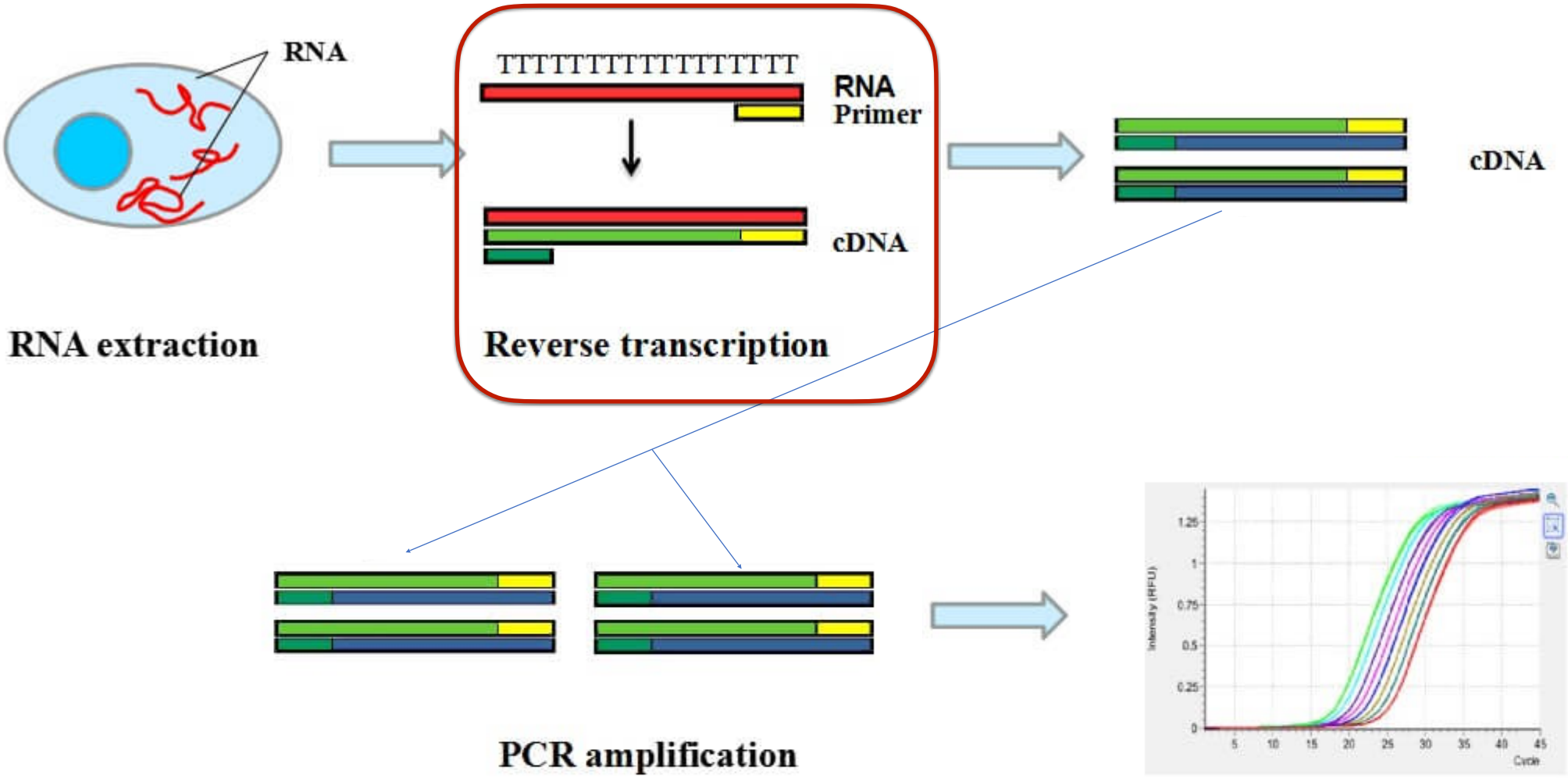
- RT-qPCR (Polymerase)
  - cDNA
  - qPCR
  - Pitfalls
- nanoString (no Polymerase)
  - Bar-codes
  - Hybridization
  - Analysis

RT-qPCR



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

RT-qPCR



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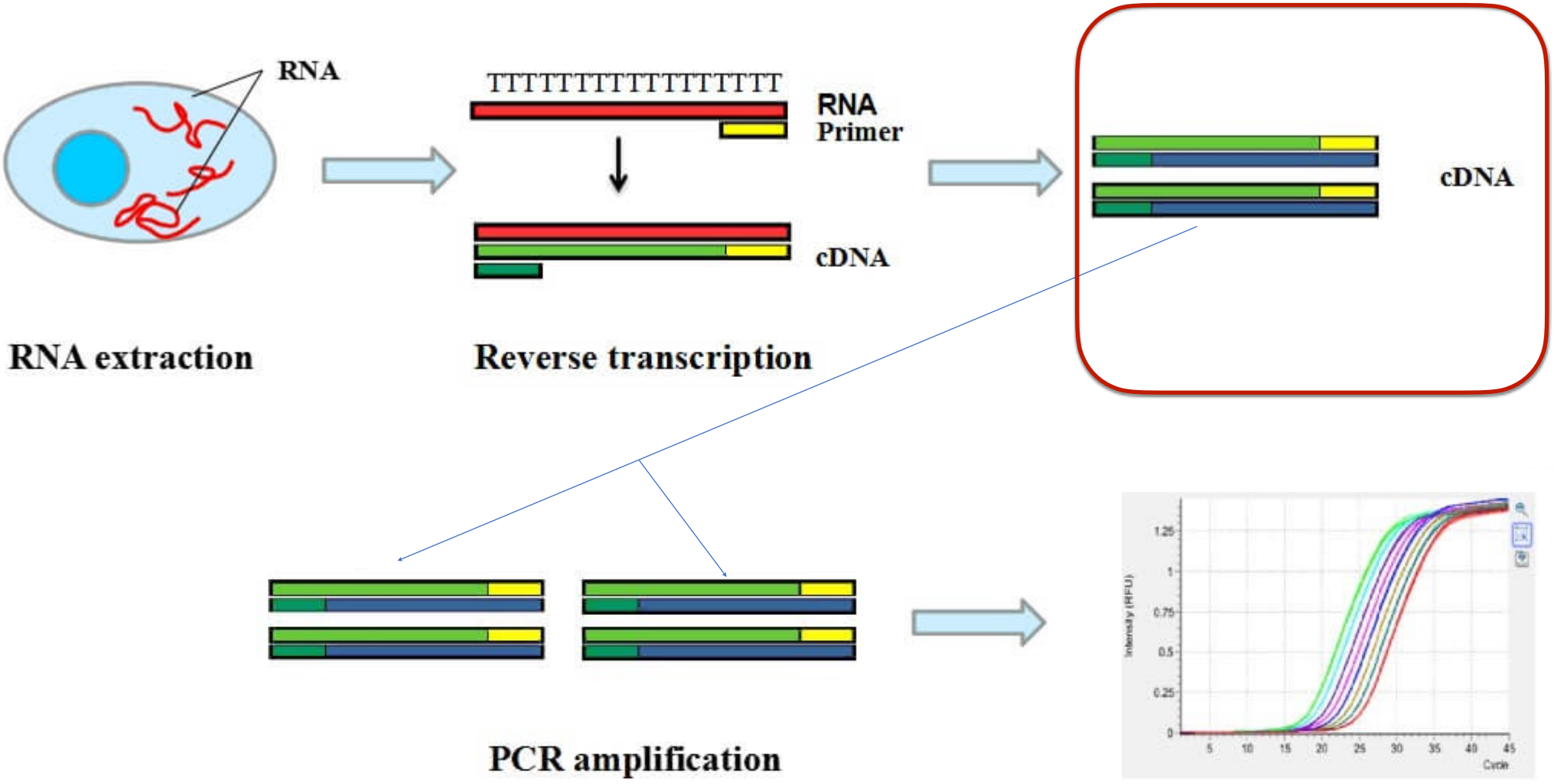

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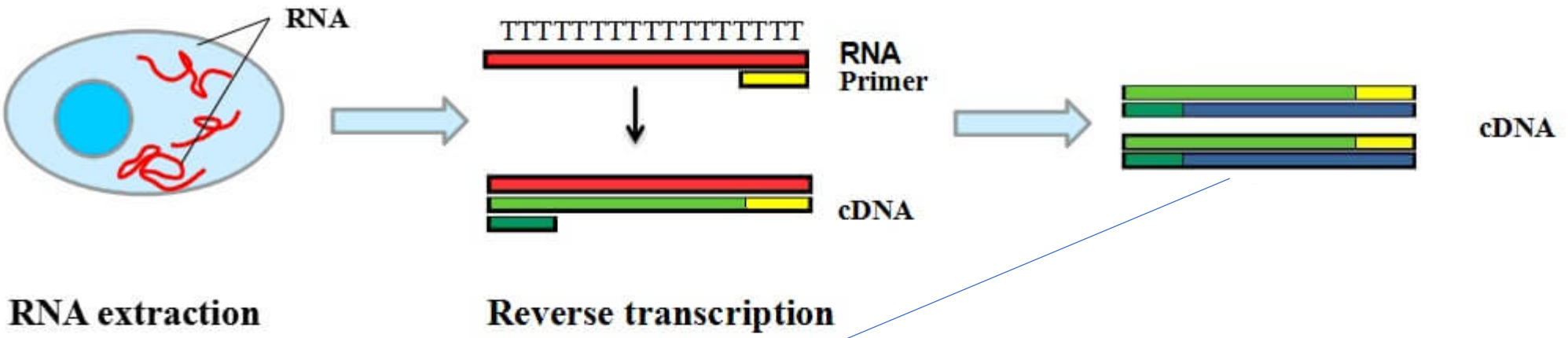

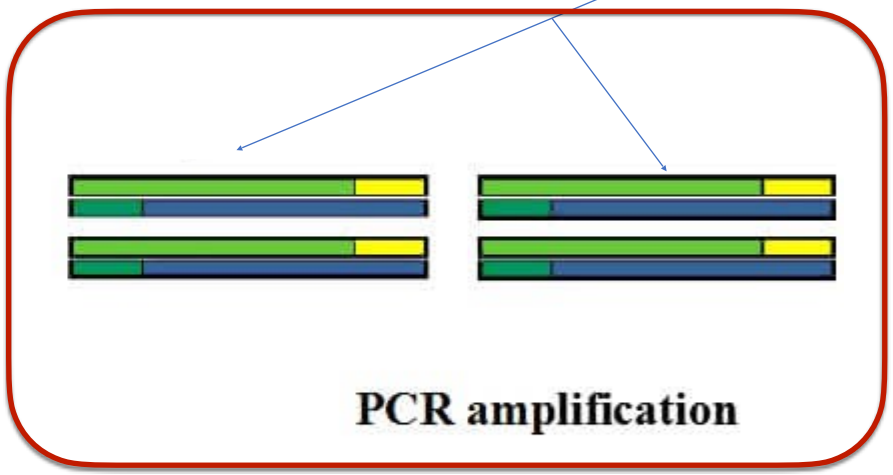

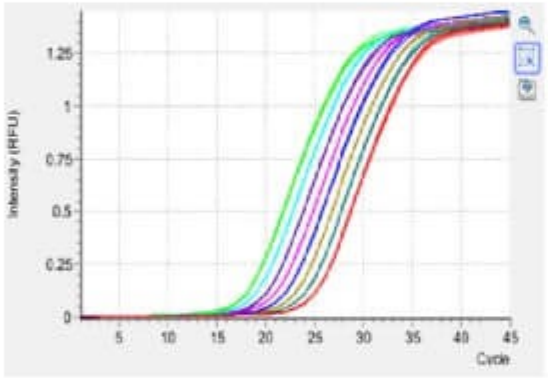

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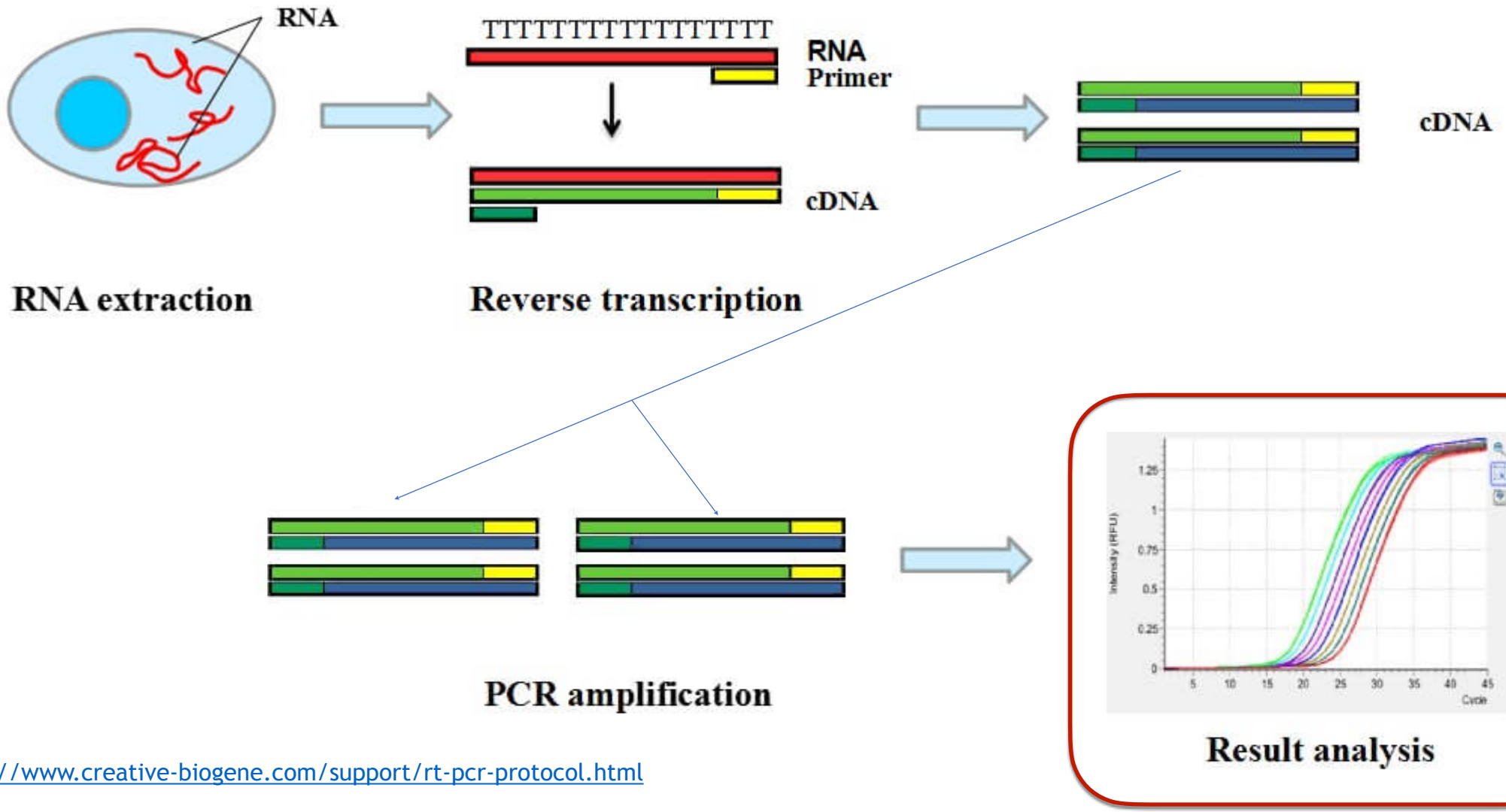
RT-qPCR



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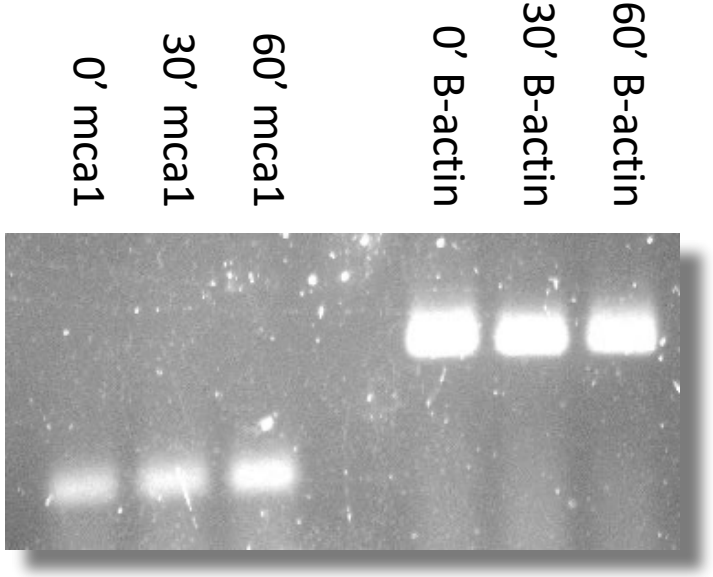
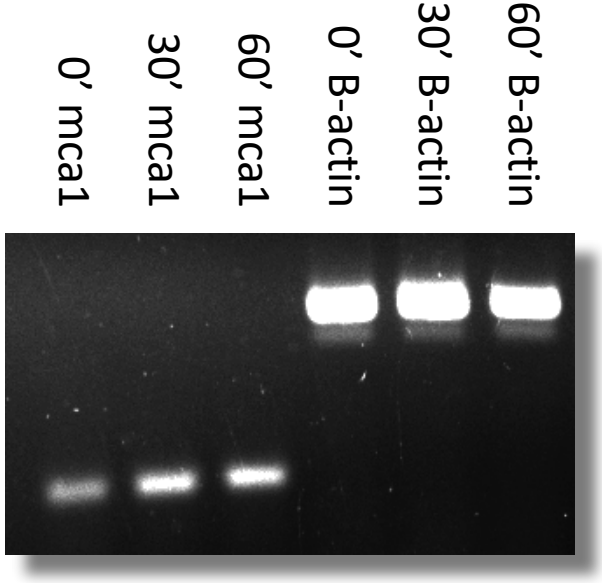
Metacaspase-1 (mca1) was induced by yeast acute exposure to the heavy metals chromium and cadmium

Chromium Exposure

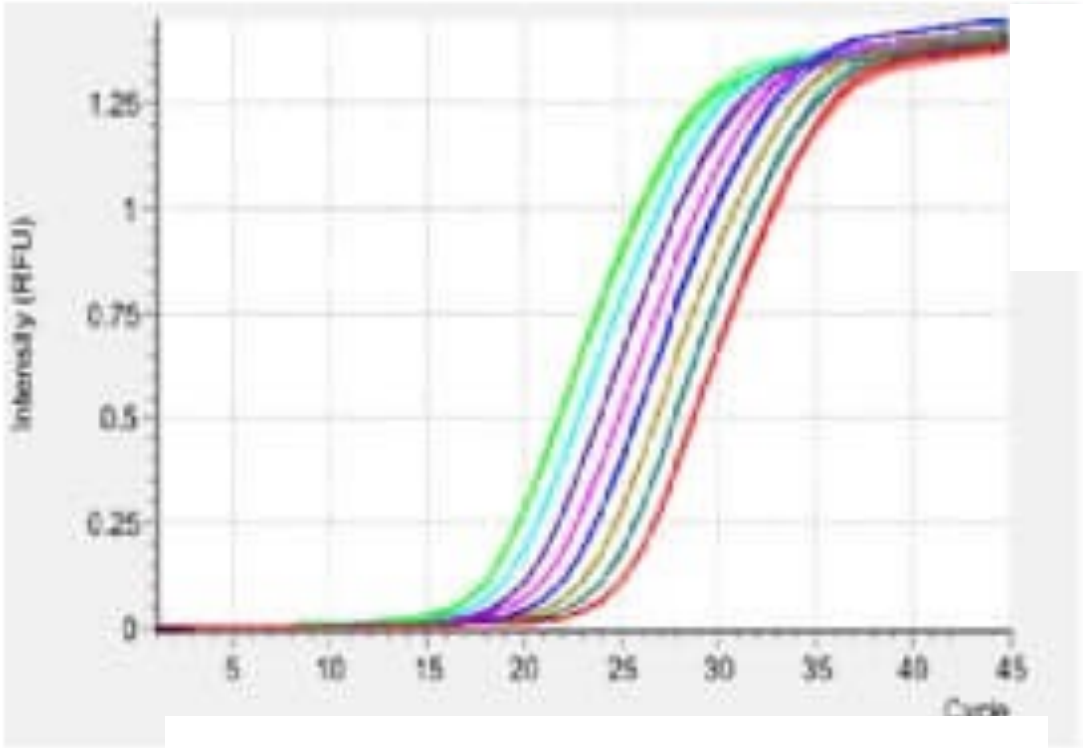


Cadmium Exposure



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

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



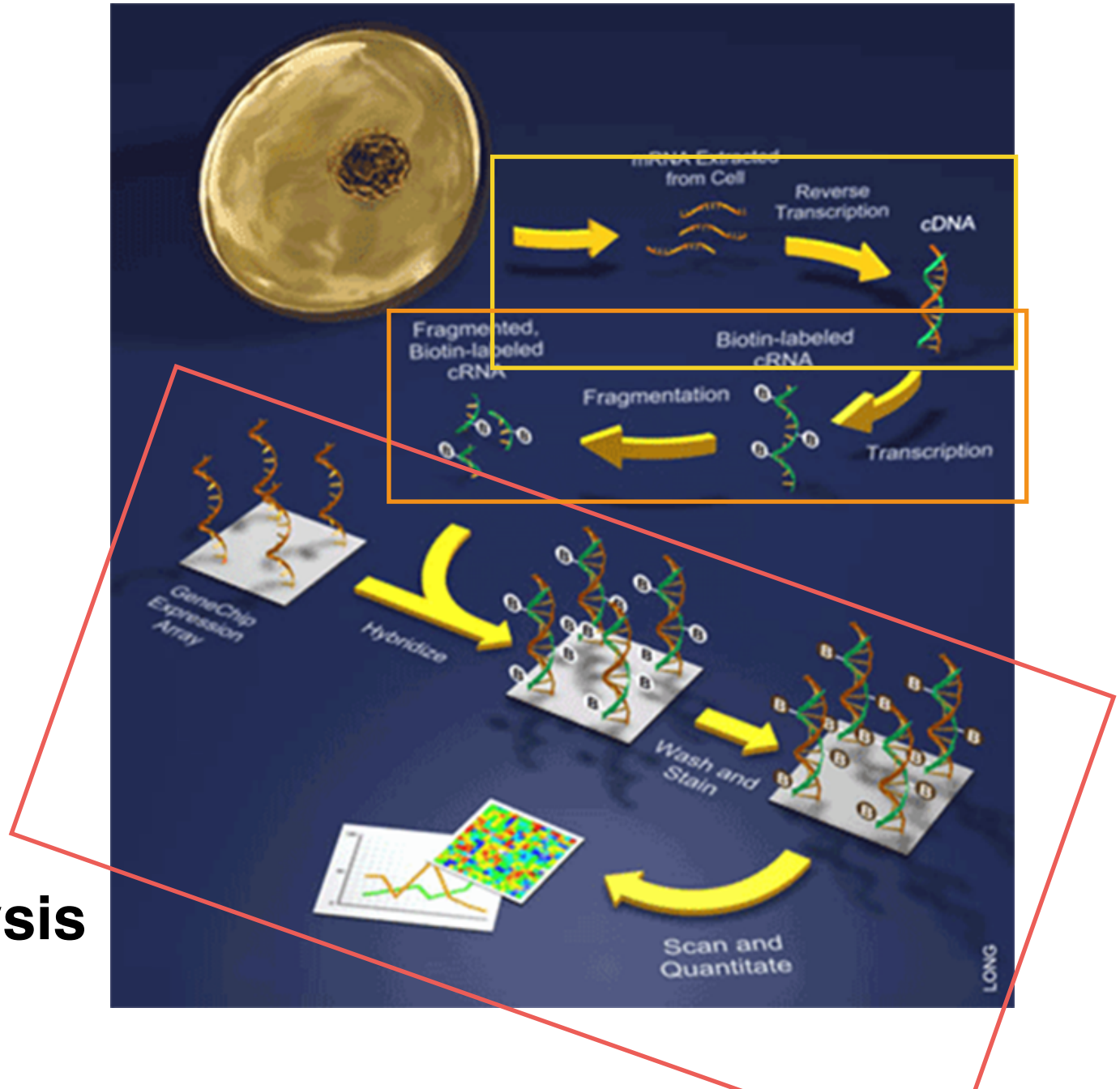
qPCR does provide for multiplex analysis

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

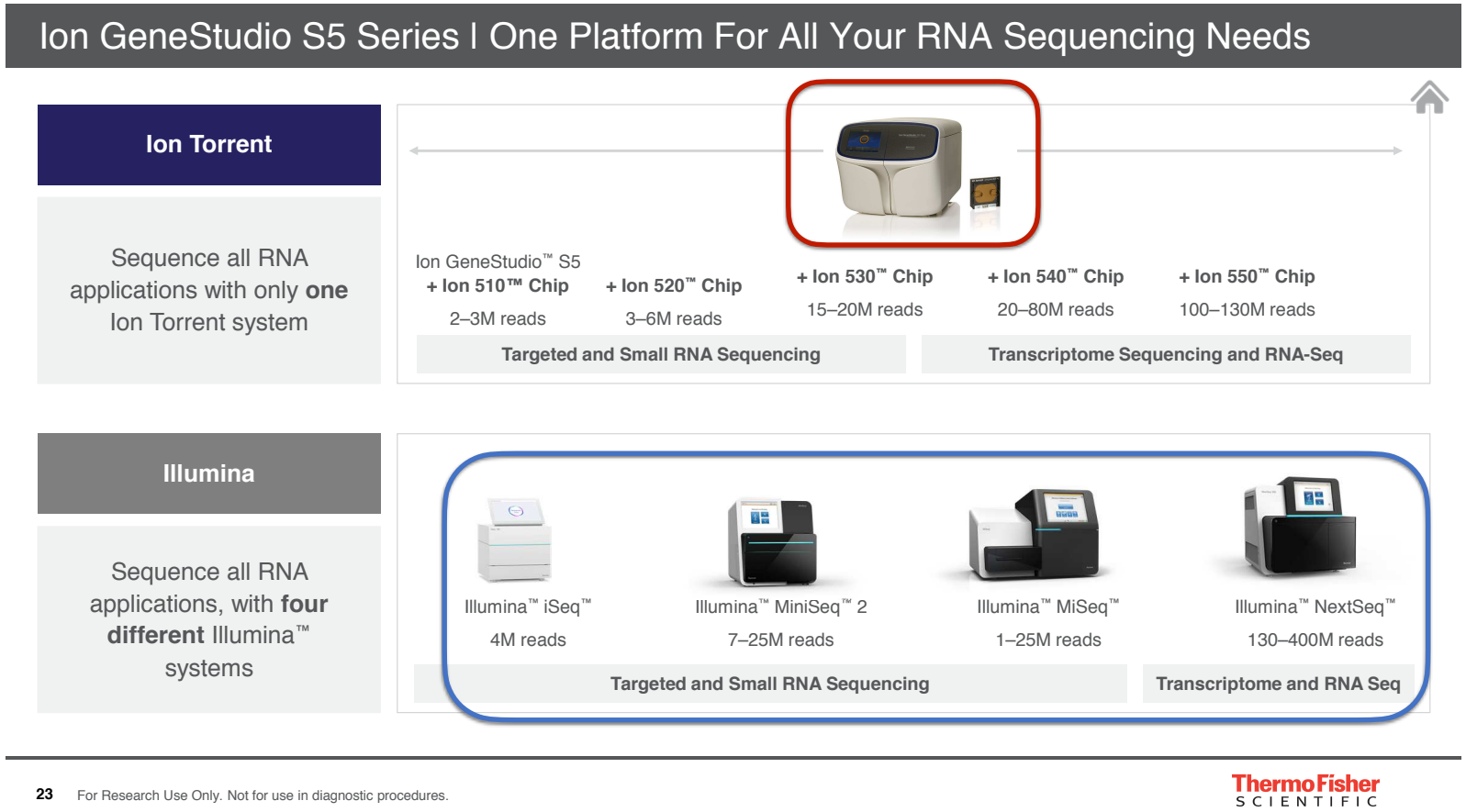


GeneChip® Expression Analysis
Hybridization and Staining

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



Next Generation Transcriptome Analysis

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..

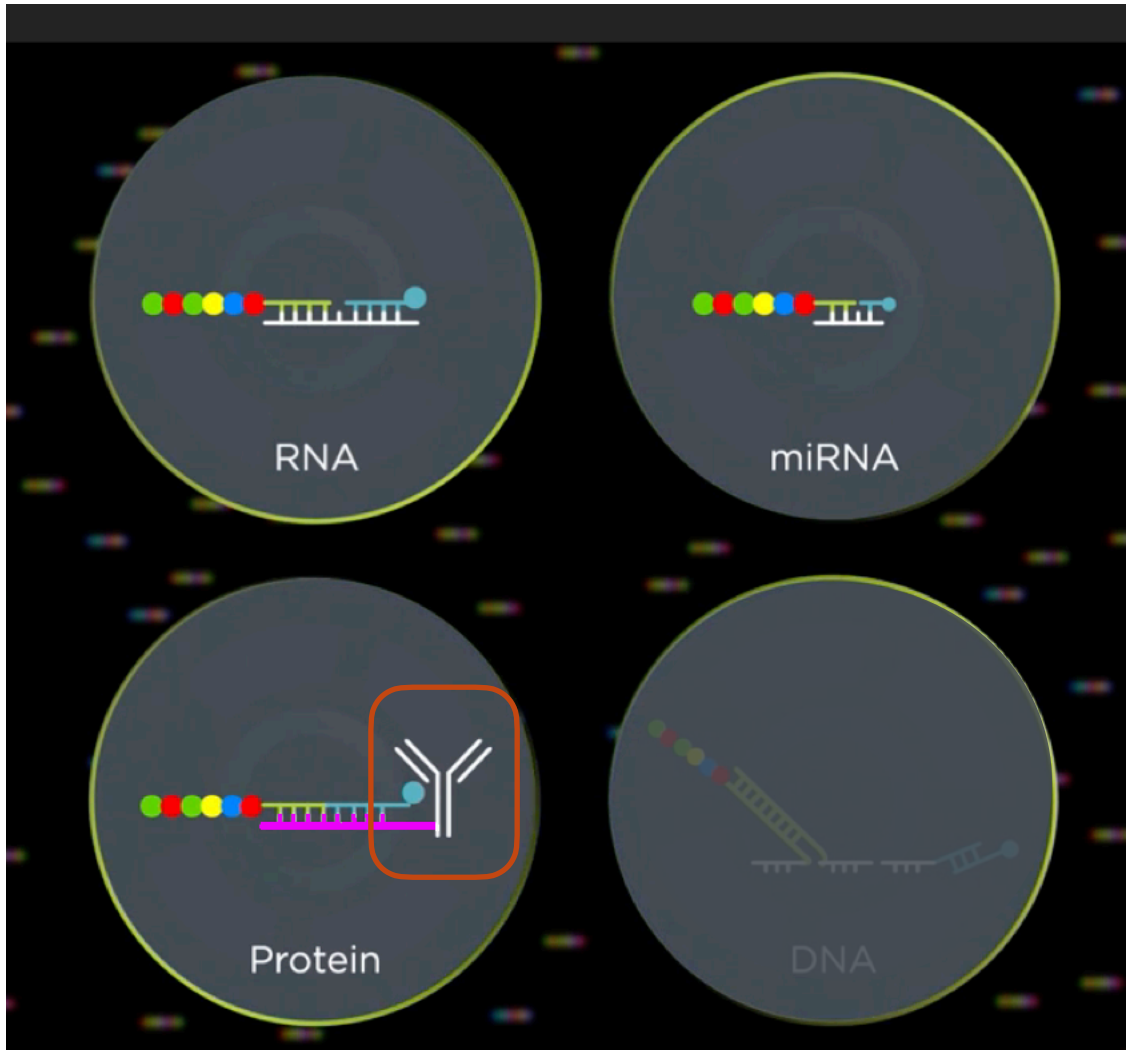
- Requires Reverse Transcription
  - Primer design
    - Primer annealing temperature
  - Protocol optimization for multiple expression products

- Set-up is relatively cumbersome for few genes…

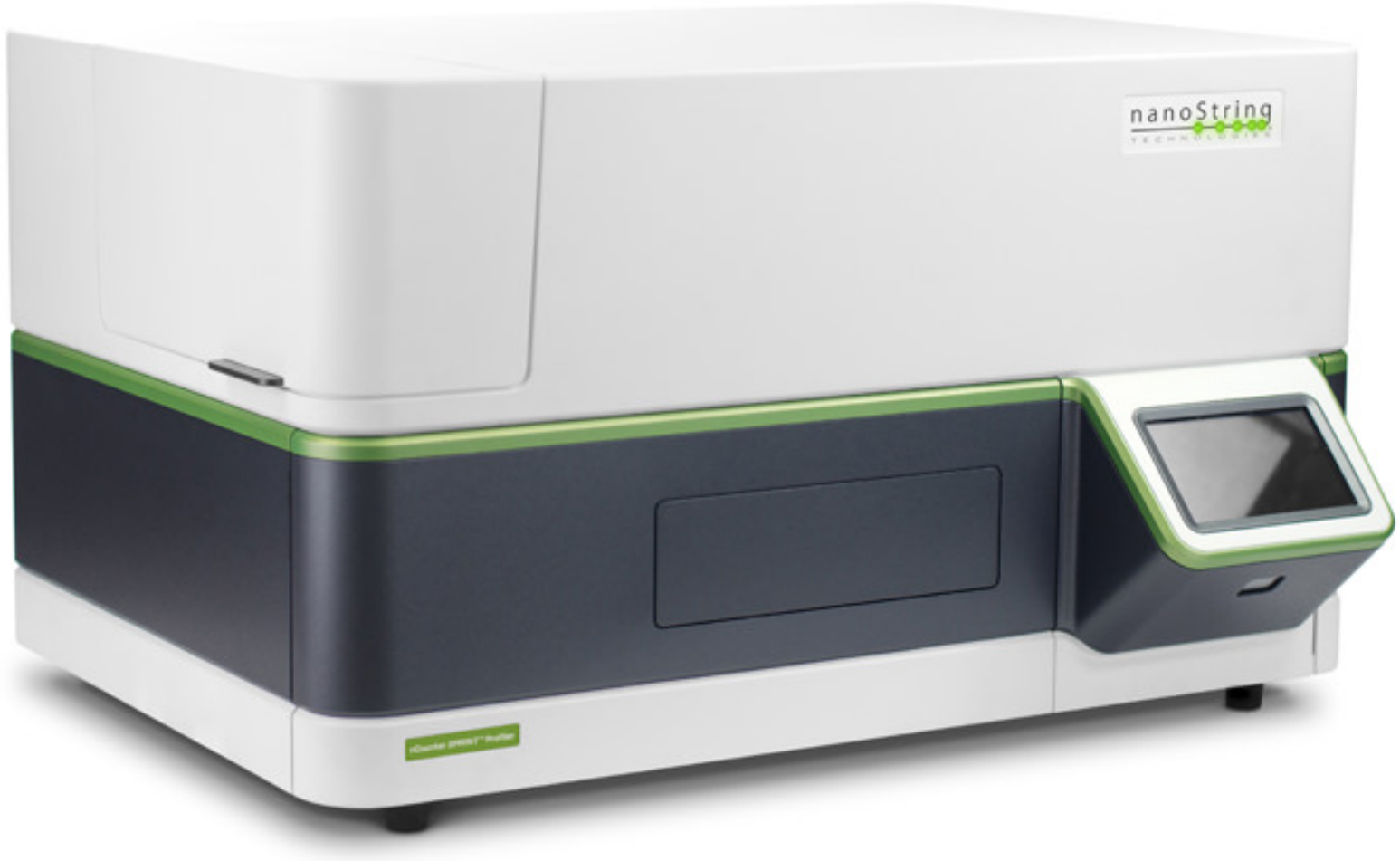
- Chips are EXPENSIVE

nanoString
TM

nanoString (multi target-rich analyses)

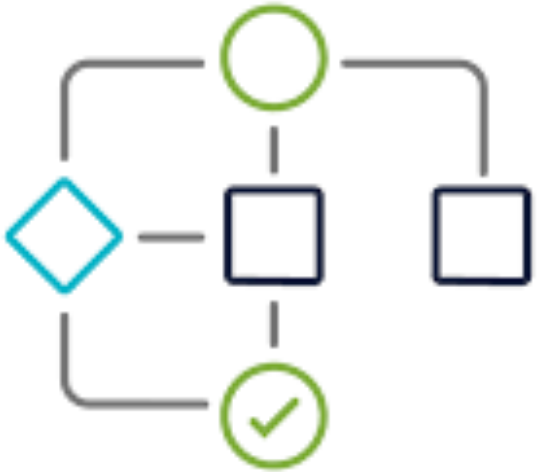


nanoString (PCR Free Expression Assay)



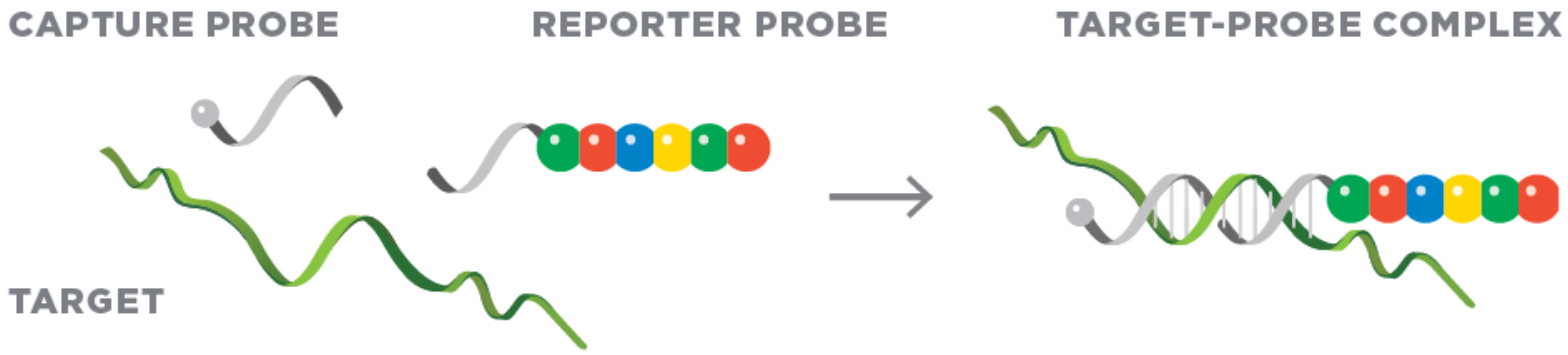
Work Flow

- 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

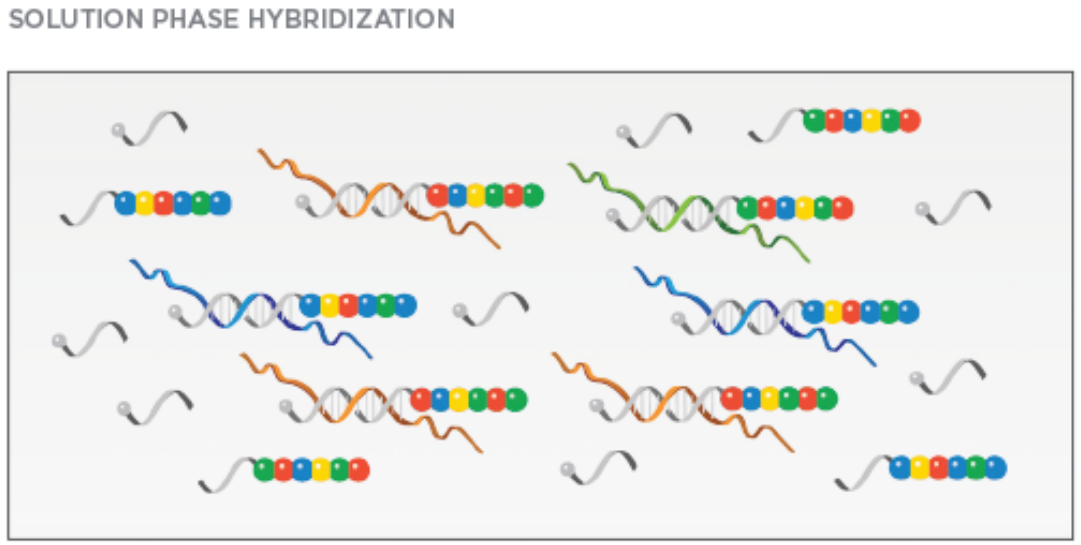


nanoString Hybridization Probe Set

- 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



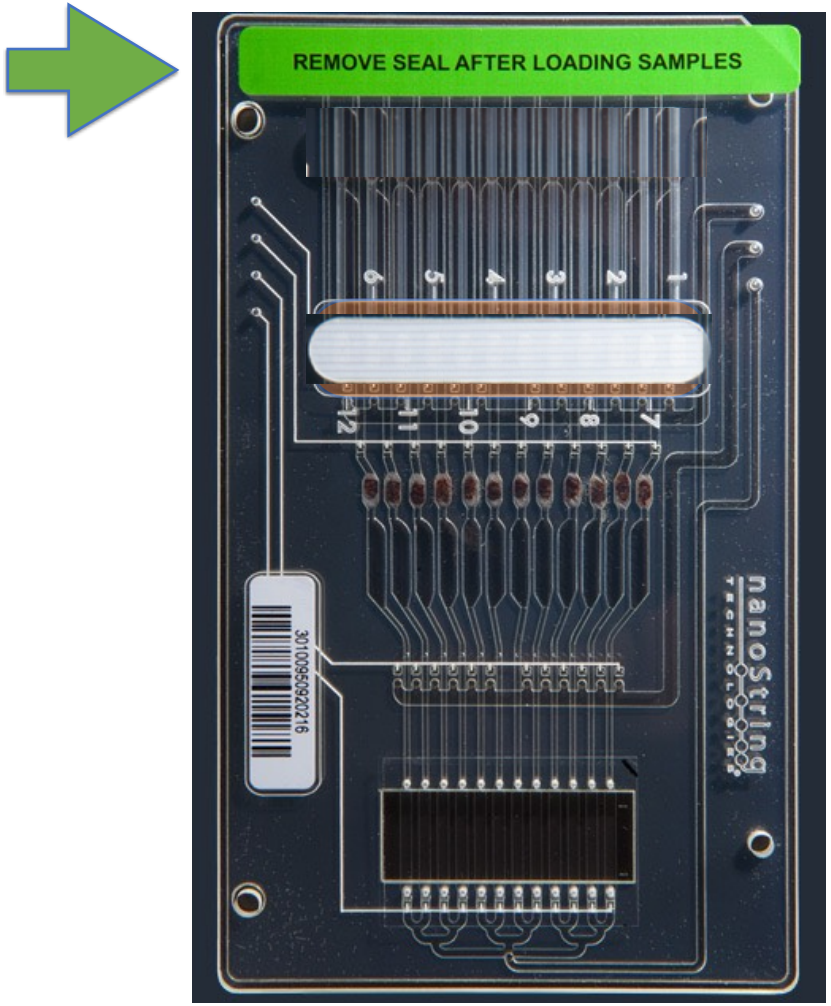
nanoString Hybridize



- Single-step hybridization
  - Template + Probes →Thermocycler
- 16-hour incubation at 65°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°C for 20 hours after completion
- Also contains technical positive and negative control probes

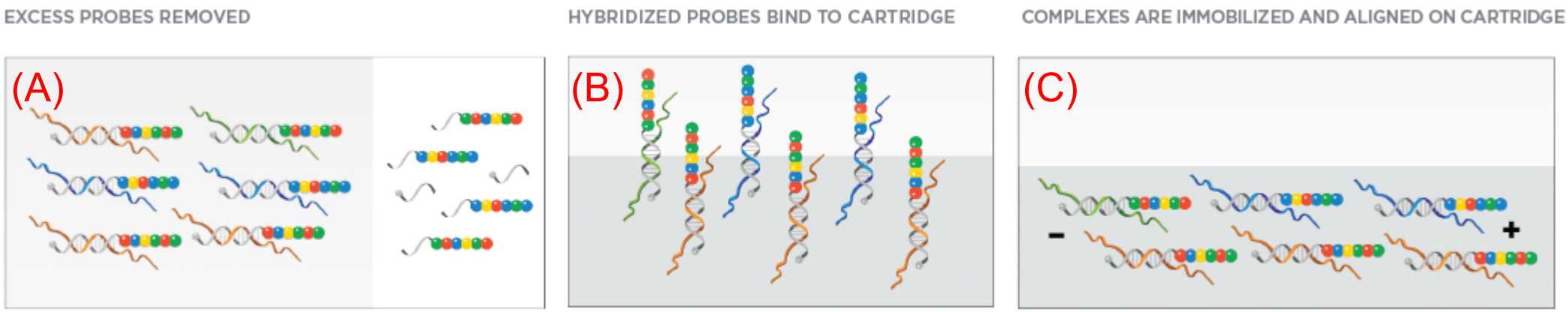
Load nanoString Fluidics Chip

- 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



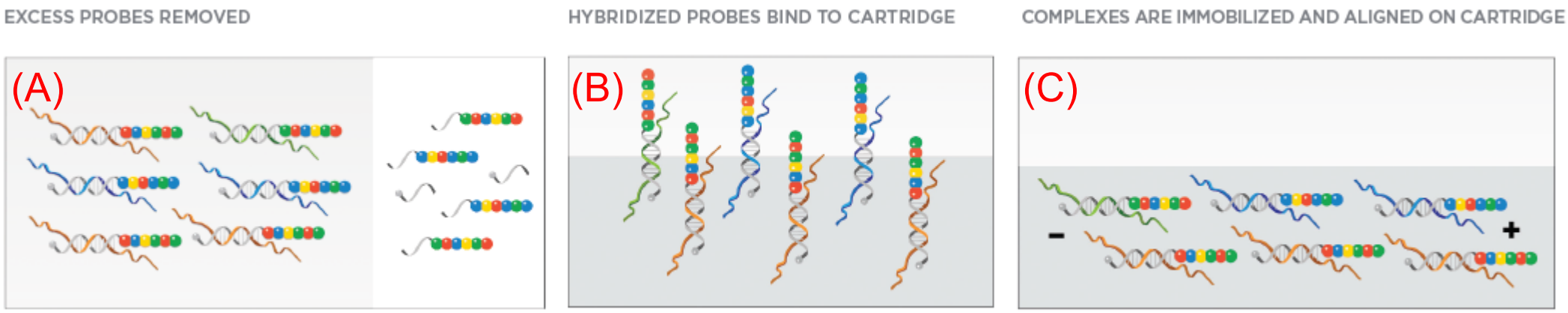
Running Protocol (inside the box)

- 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)



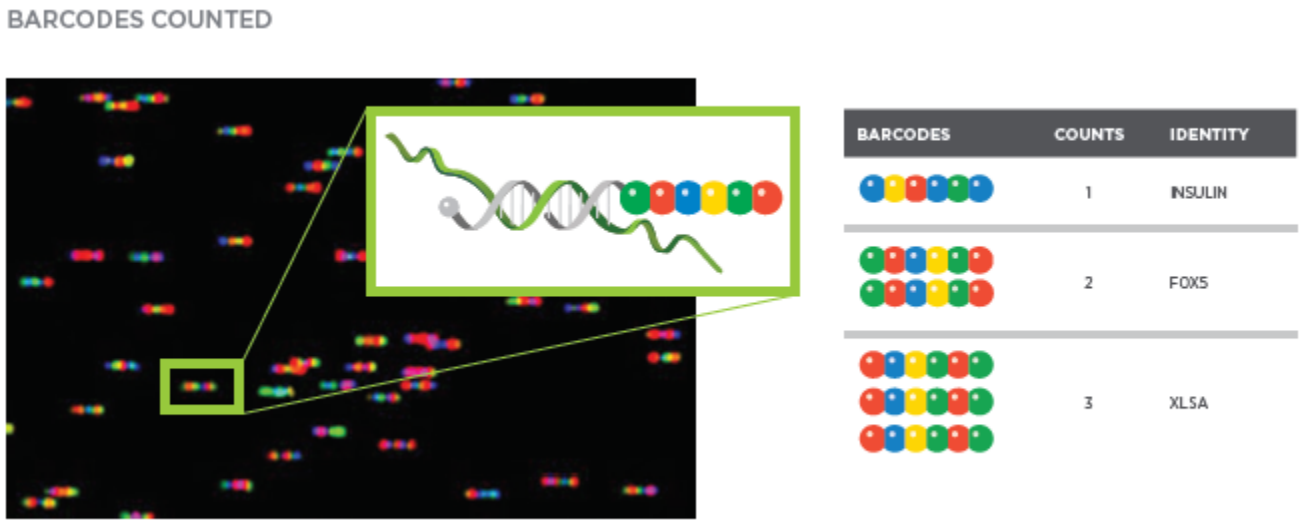
Running Protocol (inside the box)

- 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)

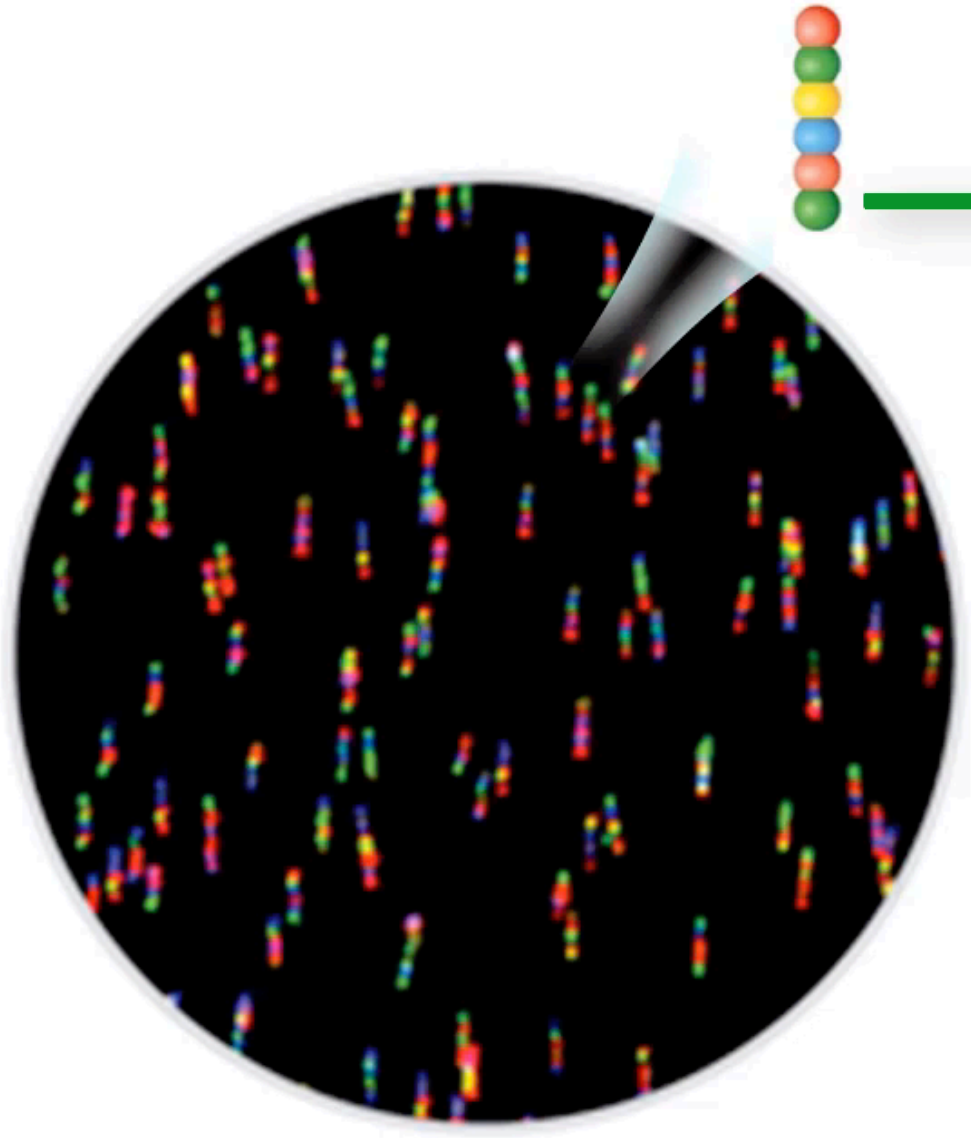
- High quality imaging allows a computer to analyze the thousands of images captured.
  - About 700 images are taken per sample
- Running time is about 8 hours.



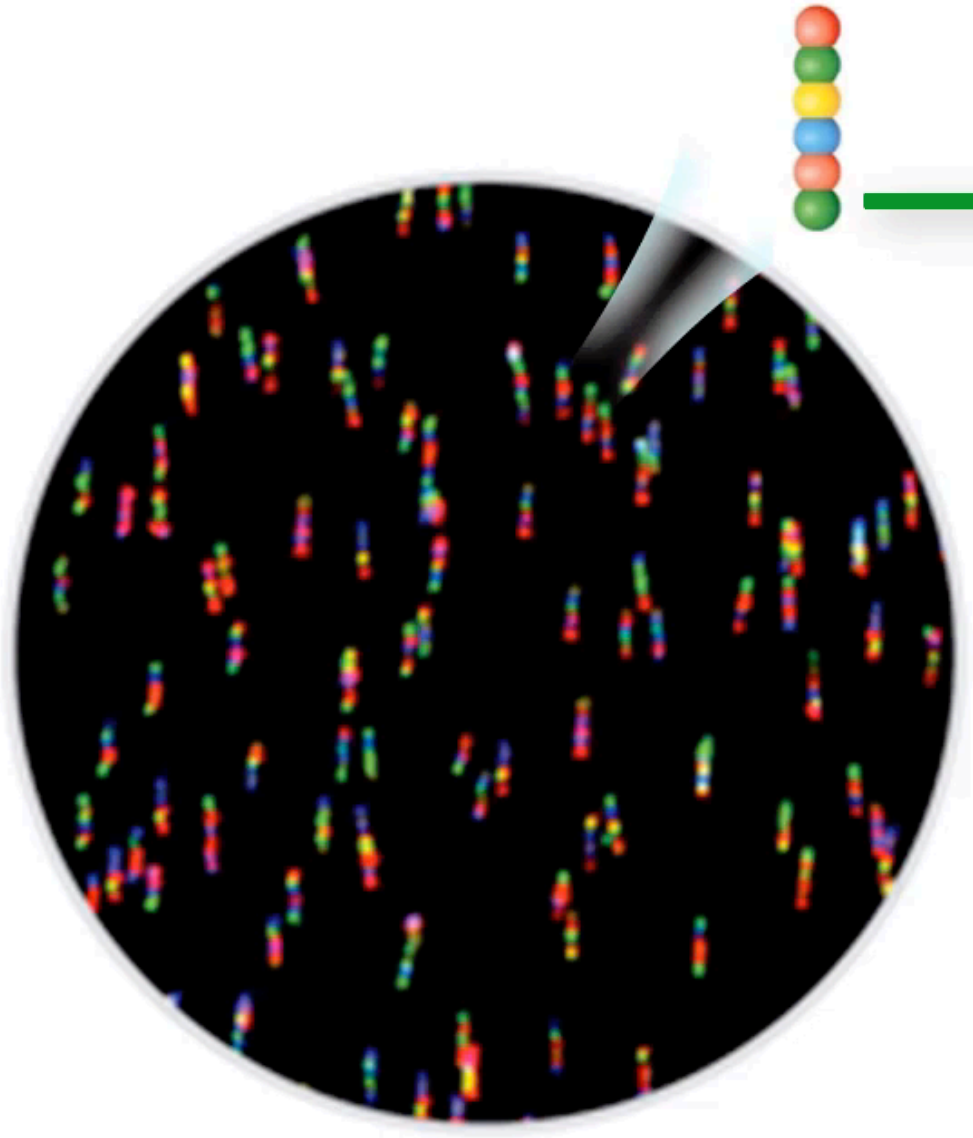
Analyze Data

- 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...					



Gene	Sample 1	Sample 2	Sample 3
SPP1	8,002	201	948
GAPDH	7,452	1,621	1,370
PLA2G2A	6,884	449	948
PDCD1	2,751	915	632
TGFBI	2,096	816	1,054
TIMP1	2,034	473	948
PGK1	1,427	1,420	632
MCL1	1,320	1,374	421
FAT1	1,303	208	948
STAT3	1,270	1,554	1,054
PLG	1,129	7,935	527
XRCC5	1,113	1,854	1,791
COL1A1	1,080	272	1,054
ERBB2	1,028	106	421



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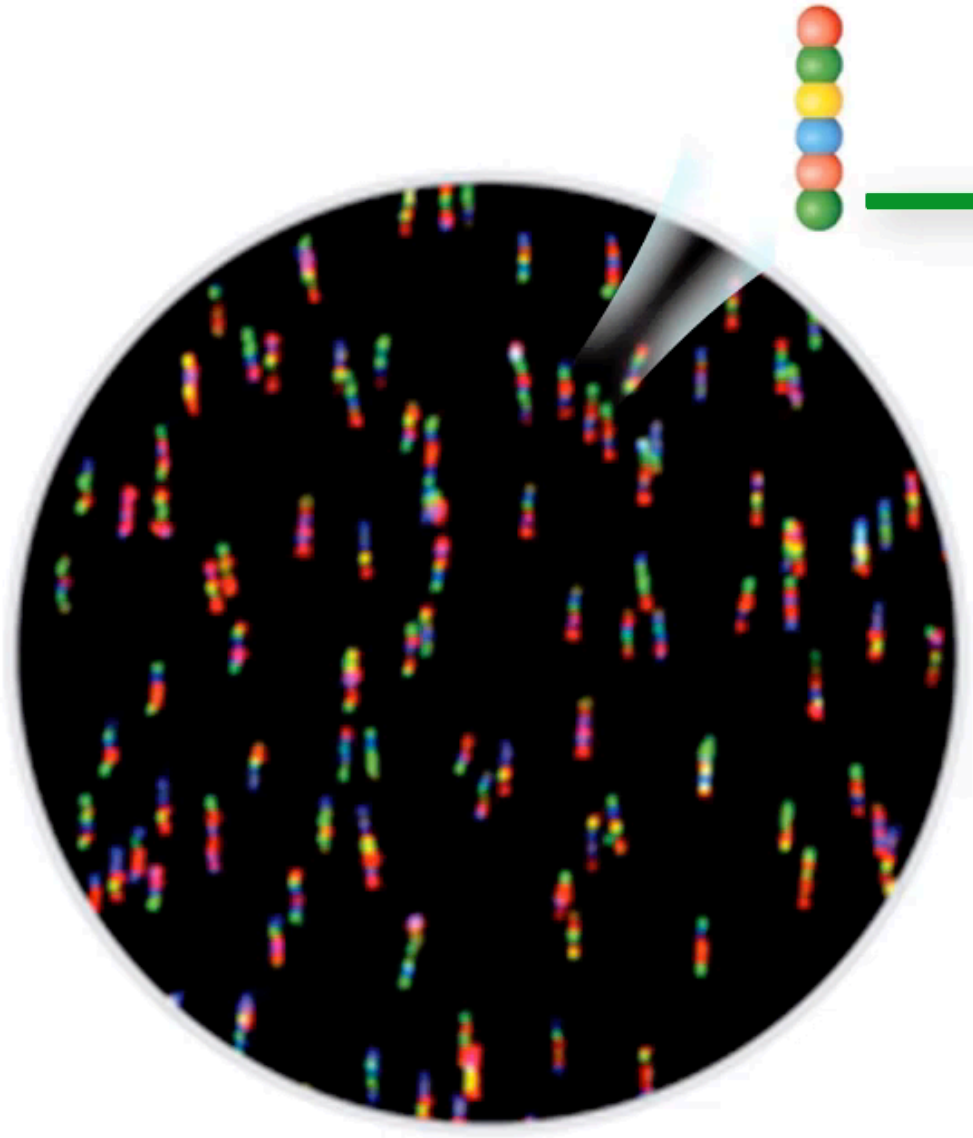

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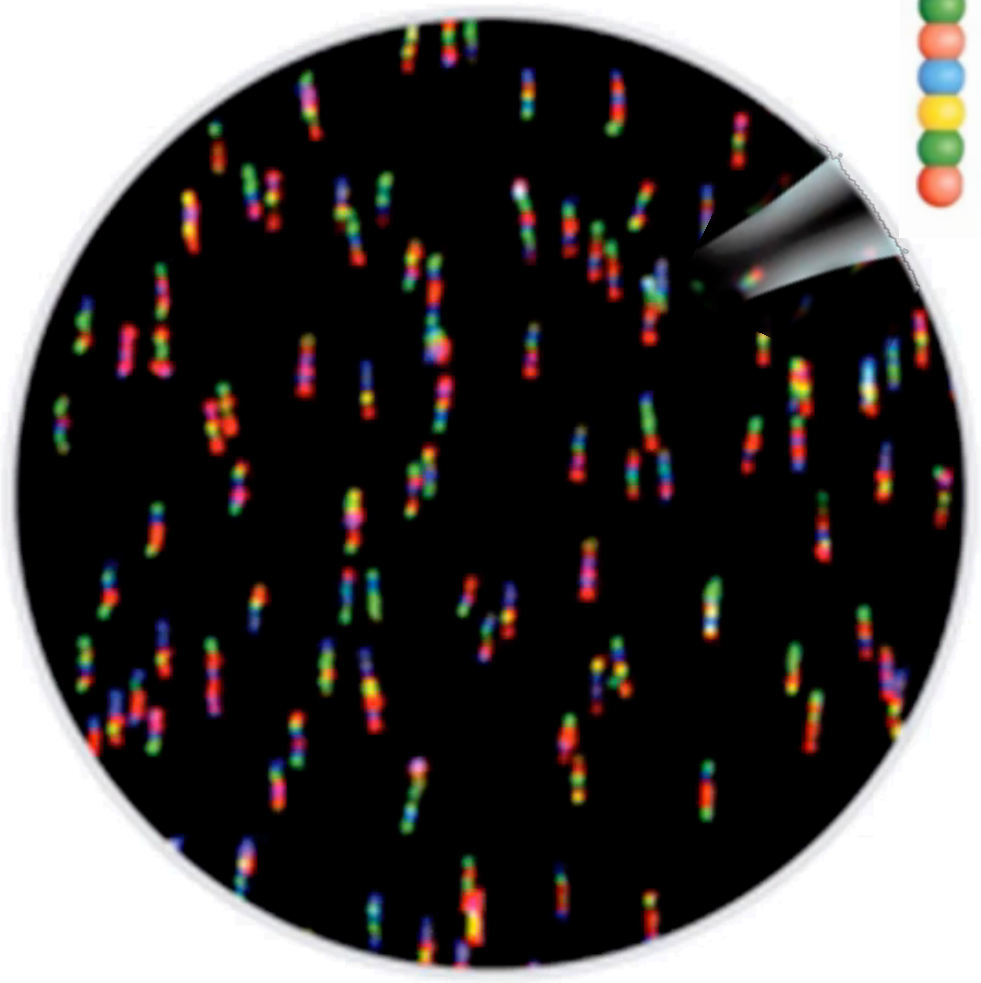

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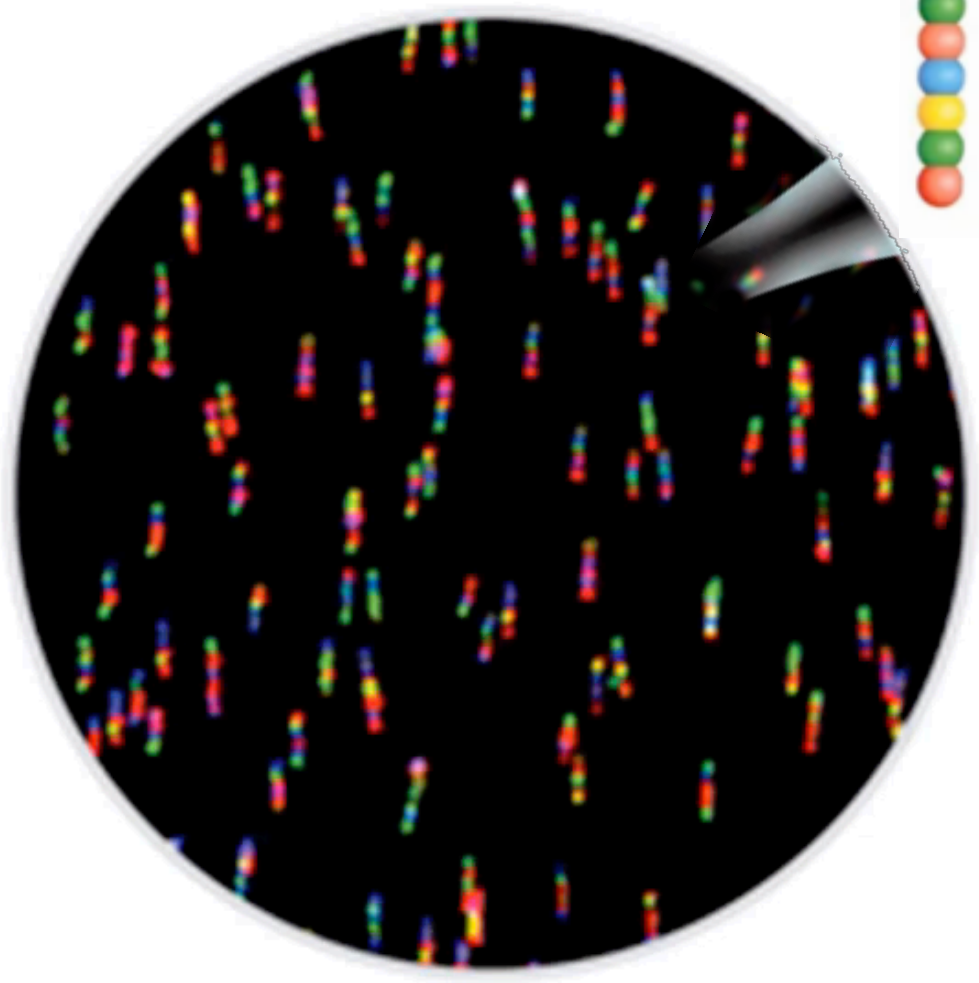

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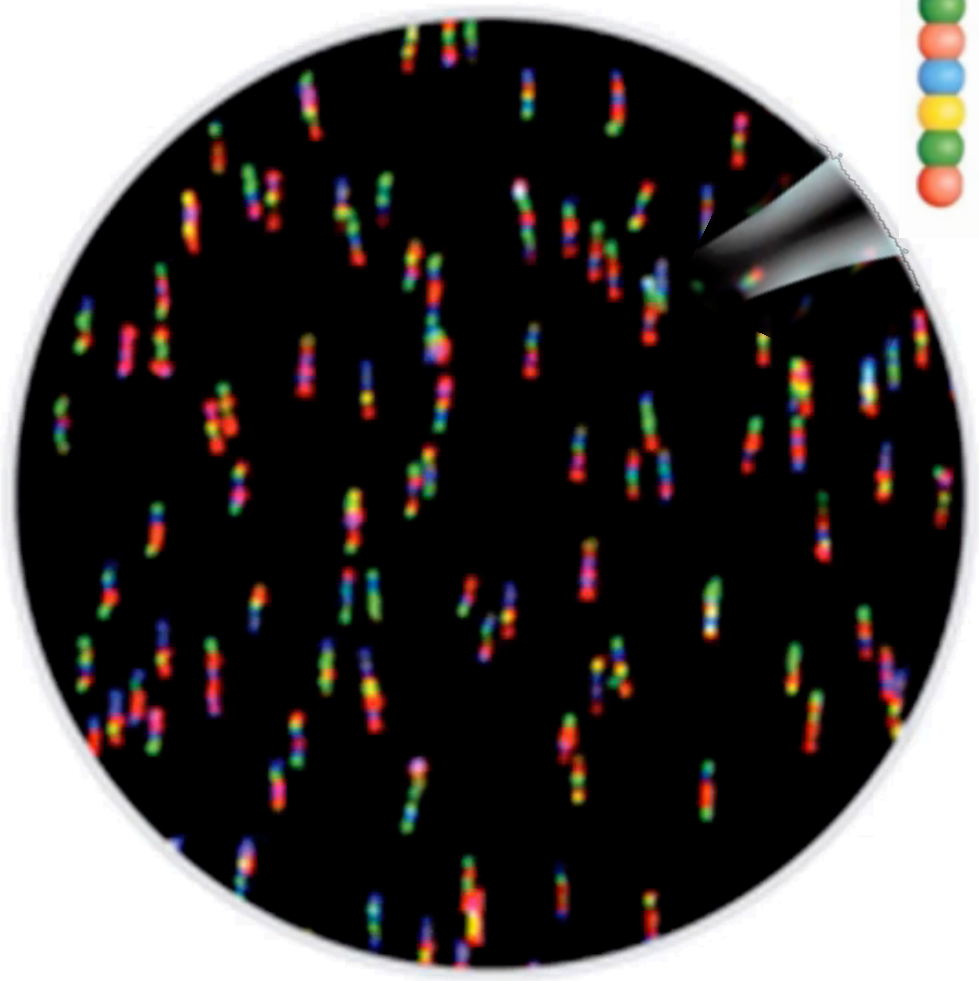

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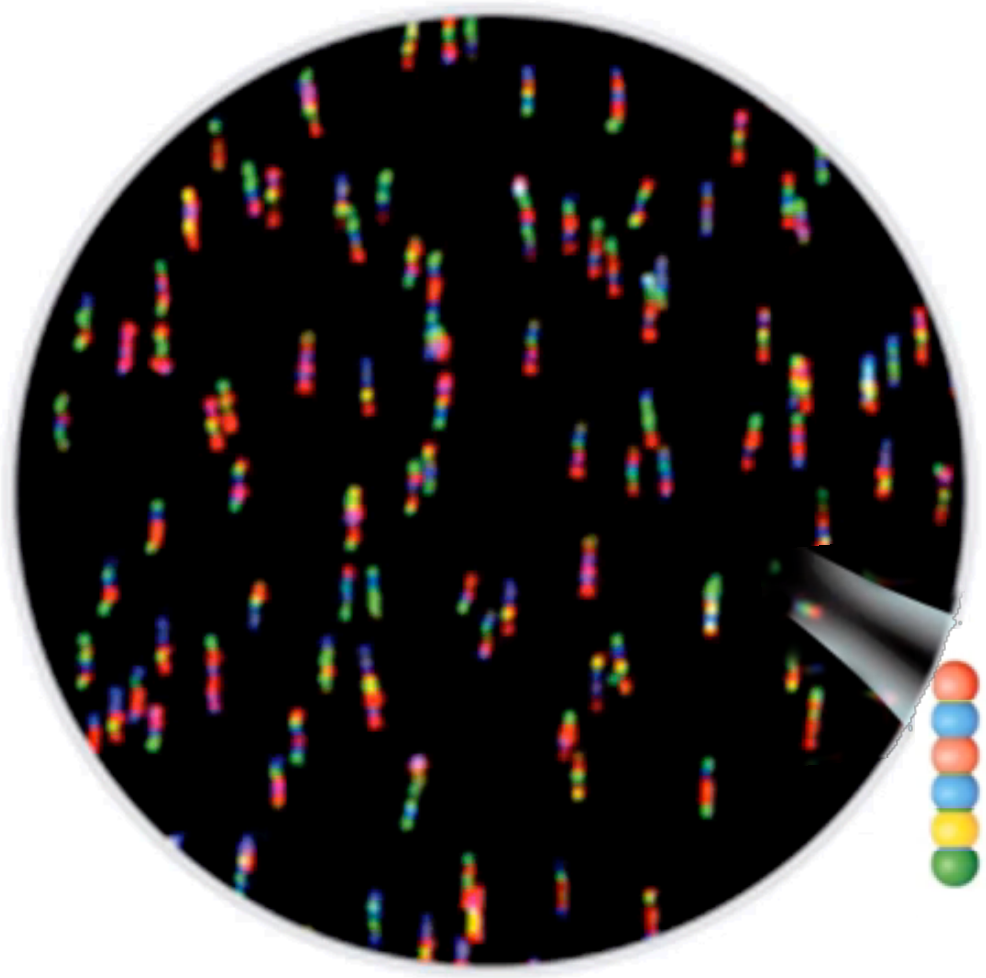

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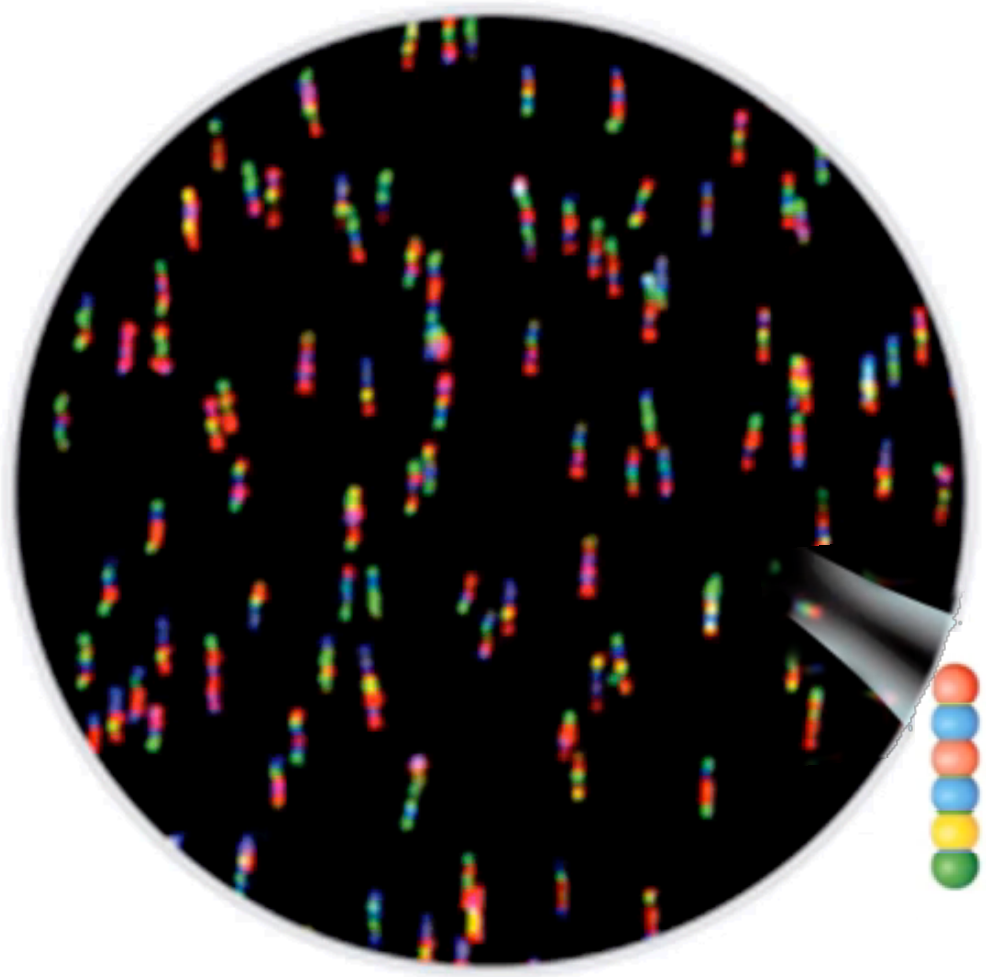

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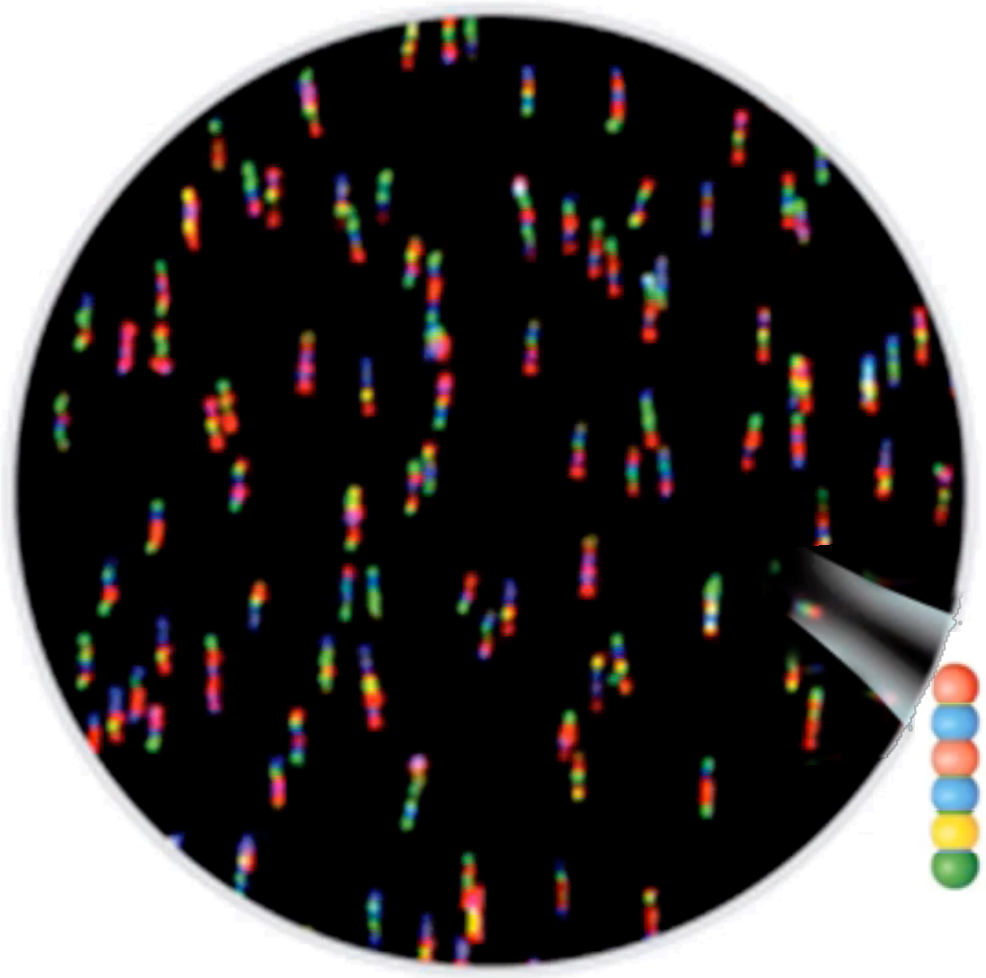

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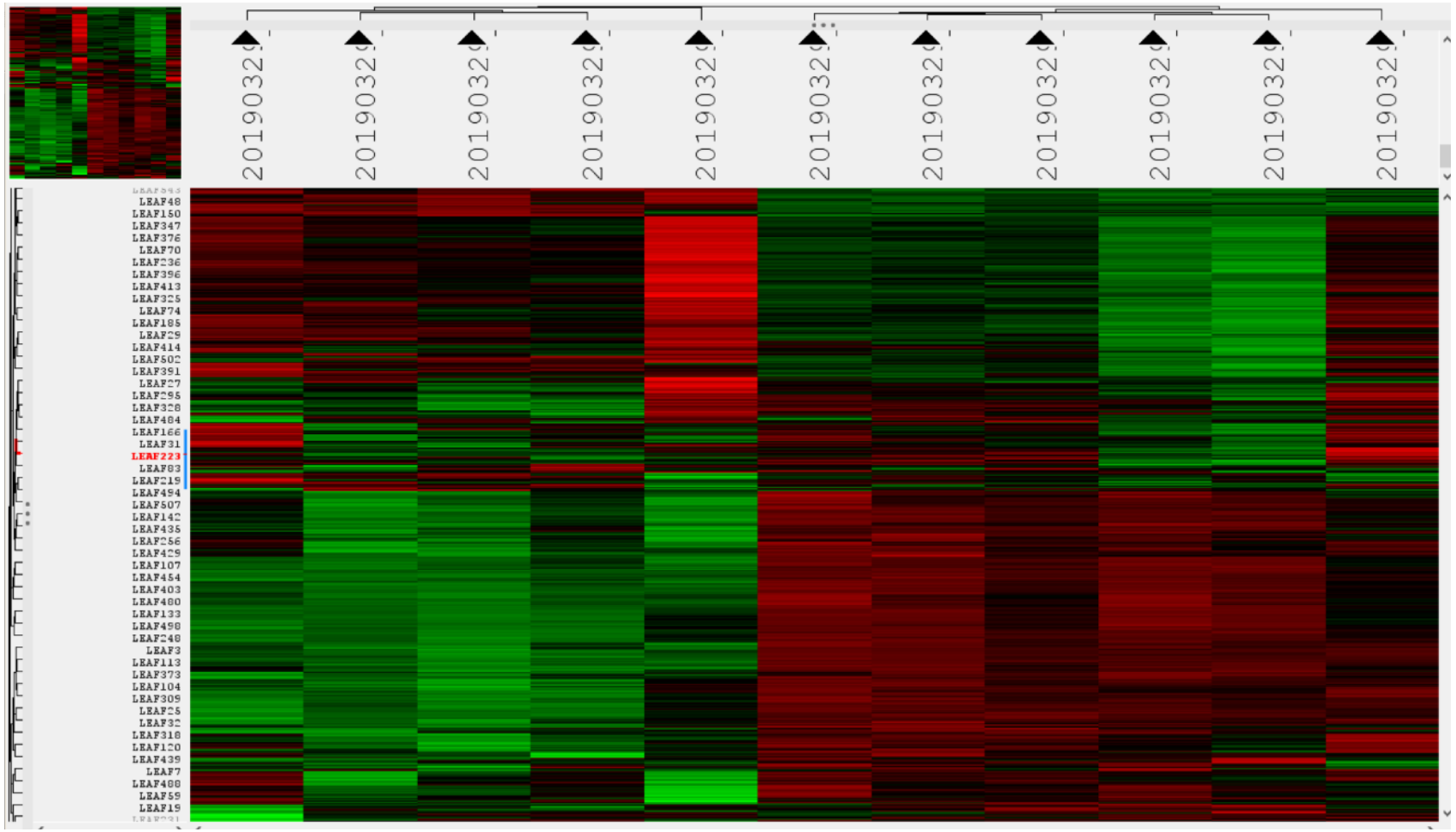
Analyze Data

Increased Expression

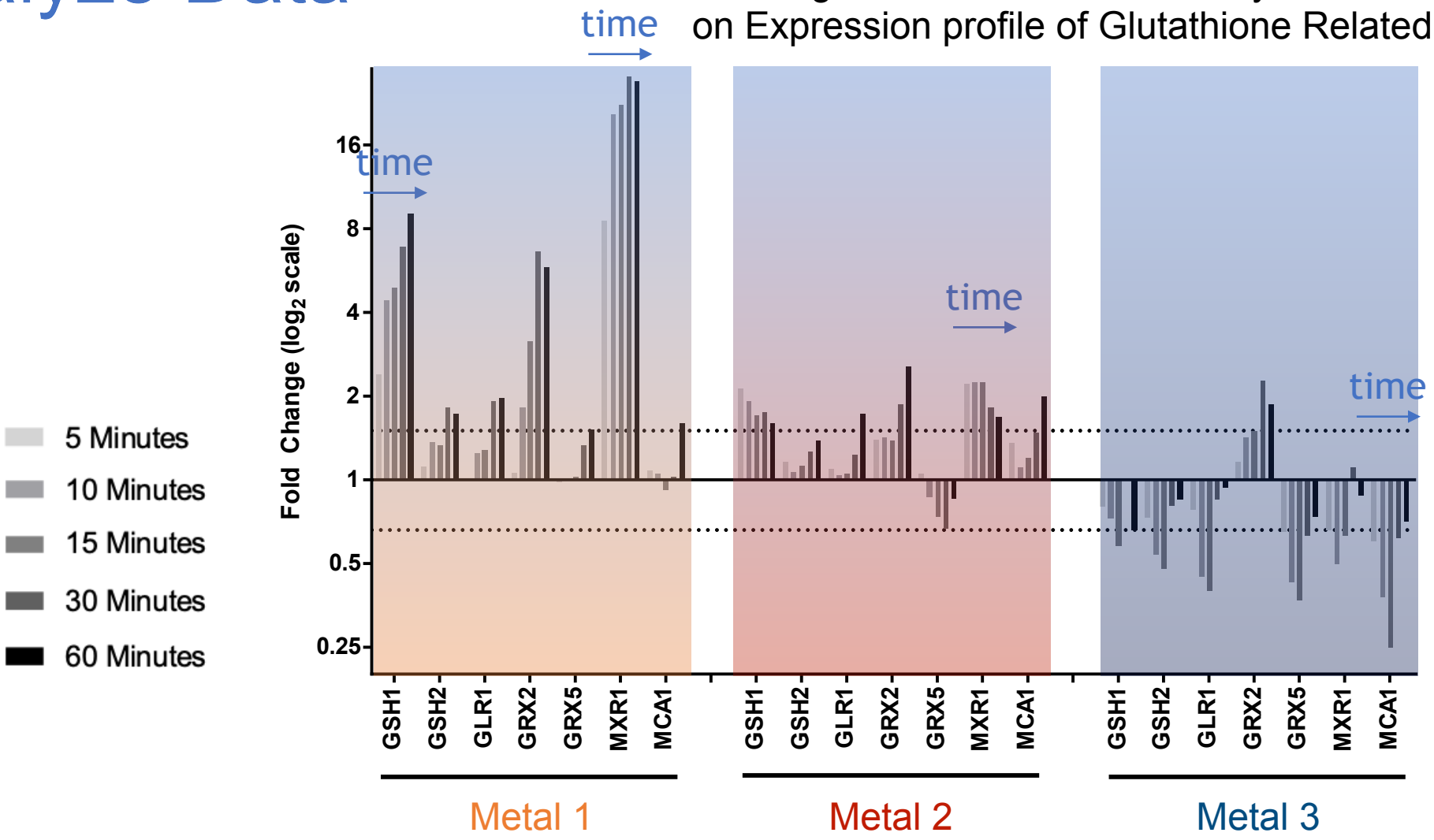


Decreased Expression

- 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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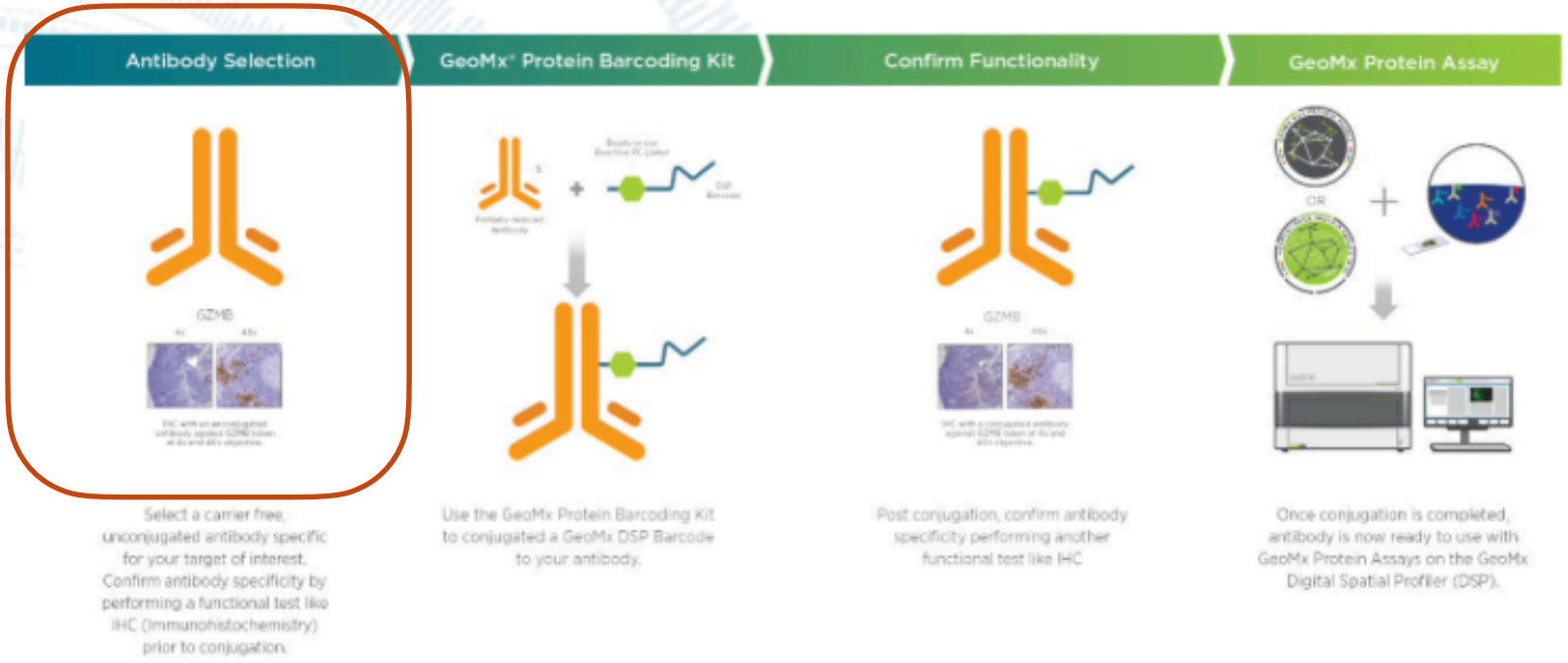
nanoString (Future purchase?)

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.



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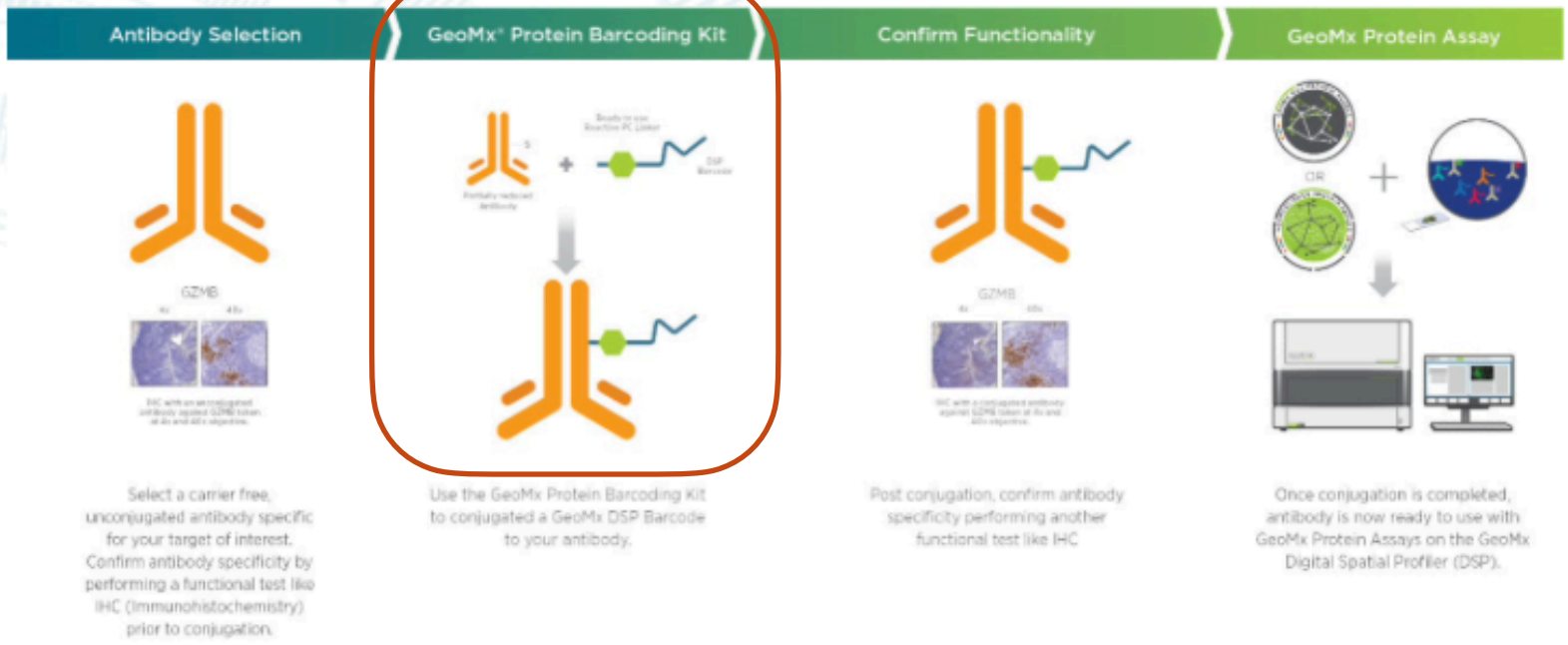

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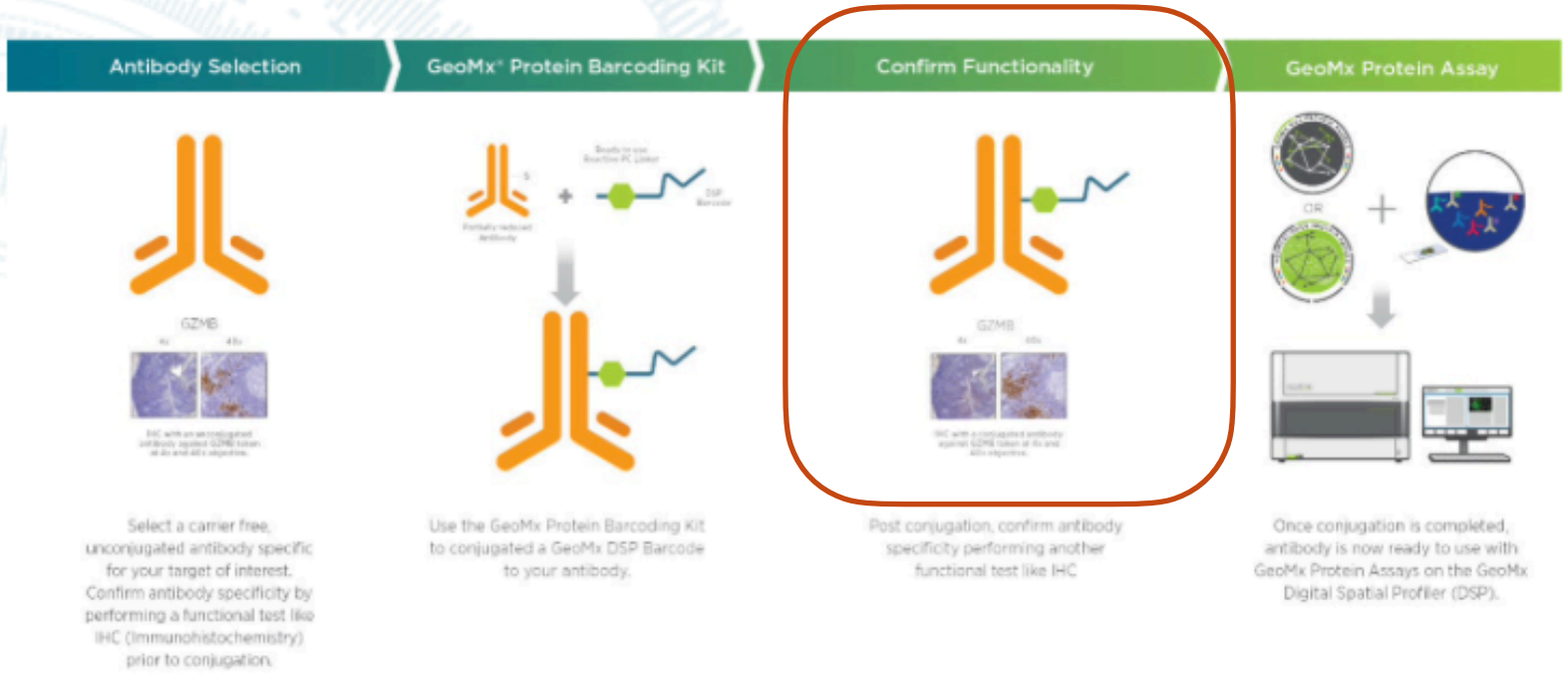
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