

# NanoString nCounter Technology in the Human DC Lab

Ana Resteu

nanoString  
TECHNOLOGIES®

[www.nanostring.com](http://www.nanostring.com)



# nCounter Analysis System

The first and only technology platform to deliver **highly multiplexed, direct profiling** of individual molecules in a single reaction **without amplification**.



# Technological Advantages

- Analysis of:
  - mRNA
  - cDNA
  - miRNA
  - smRNA
  - Fusion transcripts
  - CNV assay
  - Protein
- Digital and automated: 1 probe to 1 molecule - no amplification
- Sensitive and reproducible
- 20-800 genes
- Custom or prebuilt panels
- Alternative is RNA-Seq but still technically challenging for small numbers of cells, **not possible with FFPE material** and more costly



# Samples



- Total RNA (30-100ng/sample)
- Whole cell lysates (minimum 10k cells)
- Total RNA extracted from FFPE (short-length RNA works well)
- Crude extracts from FFPE
- Single cell with CodeSet-specific amplification – including micro-dissection of tissue

# Gene Expression panels

PanCancer IO 360™ Panel

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nCounter® PanCancer Pathways Panels

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nCounter® PanCancer Immune Profiling Panels

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nCounter® PanCancer Progression Panel

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nCounter® Immunology Panels

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nCounter® Inflammation Panels

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nCounter® Myeloid Innate Immunity Panel

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nCounter® Neuropathology Panels

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nCounter® Vantage 3D™ RNA Panels for 3D Biology™ Technology

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nCounter® Vantage 3D™ Gene Fusion Panels

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nCounter® Stem Cell Panel

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nCounter® Kinase Panel

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nCounter® Reference Gene Selection Panel

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nCounter® Customer Assay Evaluation (CAE)

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# Technology – probe selection

>gi|168480144|ref|NM\_001101.3| Homo sapiens actin, beta (ACTB), mRNA

ACCGCCGAGACCGCGTCCGCCCCGCGAGCACAGAGCCTCGCCTTTGCCGATCCGCCGC

CCGTCCACACCCGCCGCCAGCTCA  
CCATGGATGATGATATCGCCGCGCTCGTCGTGACAAACGGCTCCGGCATGTGCAAGGCCGGCTTCGCGGGCGACGATGCCCC  
CCGGGCCGTCTTCCCCTCCATCGTGGGGCGCCCCAGGCACCAGGGCGTGATGGTGGGCATGGGTGAGAAGGATTCCTATGTG  
GGCGACGAGGCCAGAGCAAGAGAGGCATCCTCACCTGAAGTACCCCATCGAGCACGGCATCGTCACCAACTGGGACGACA  
TGGAGAAAATCTGGCACCACACCTTCTACAATGAGCTGCGTGTGGCTCCCGAGGAGCACCCCGTGCTGCTGACCGAGGCCCC  
CCTGAACCCCAAGGCCAACCGCGAGAAGATGACCCAGATCATGTTTGAGACCTTCAACACCCAGCCATGTACGTTGCTATC  
CAGGCTGTGCTATCCCTGTACGCCTCTGGCCGTACCACTGGCATCGTGATGGACTCCGGTGACGGGGTCACCCACACTGTGC  
CCATCTACGAGGGGTATGCCCTCCCCATGCCATCCTGCGTCTGGACCTGGCTGGCCGGGACCTGACTGACTACCTCATGAA  
GATCCTCACCGAGCGCGGCTACAGCTTACCACCACGGCCGAGCGGGAAATCGTGCGTGACATTAAGGAGAAGCTGTGCTAC  
GTGCGCCTGGACTTCGAGCAAGAGATGGCCACGGCTGCTTCCAGCTCCTCCCTGGAGAAGAGCTACGAGCTGCCTGACGGCC  
AGGTCATCACCAATTGGCAATGAGCGGTTCCGCTGCCCTGAGGCACTCTTCCAGCCTTCTTCCCTGGGCATGGAGTCCGTGG  
CATCCACGAAACTACCTTCAACTCCATCATGAAGTGTGACGTGGACATCCGCAAAGACCTGTACGCCAACACAGTGCTGTCT  
GGCGGCACCACCATGTACCCTGGCATTGCCGACAGGATGCAGAAGGAGATCACTGCCCTGGCACCCAGCACAAATGAAGATCA

100 base region

Check for:

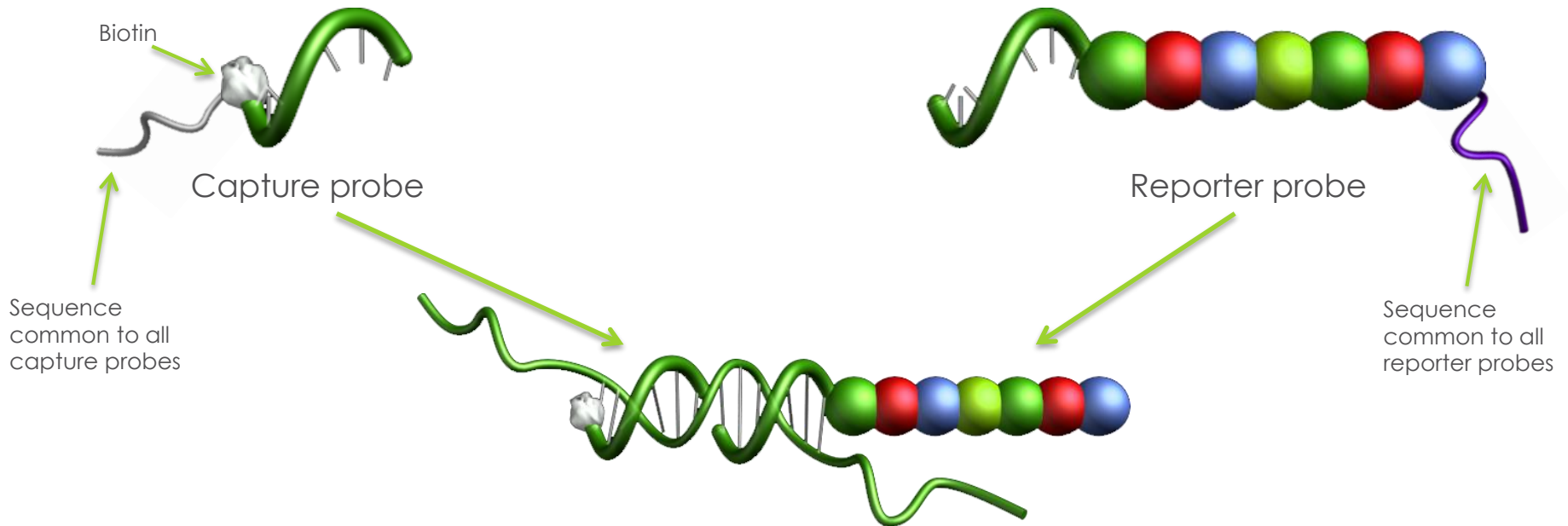
- Secondary Structure
- Synthesis Issues
- Uniqueness (BLAST against transcriptome and system controls)
- Melting Temperature

# Technology – reporter and capture probes

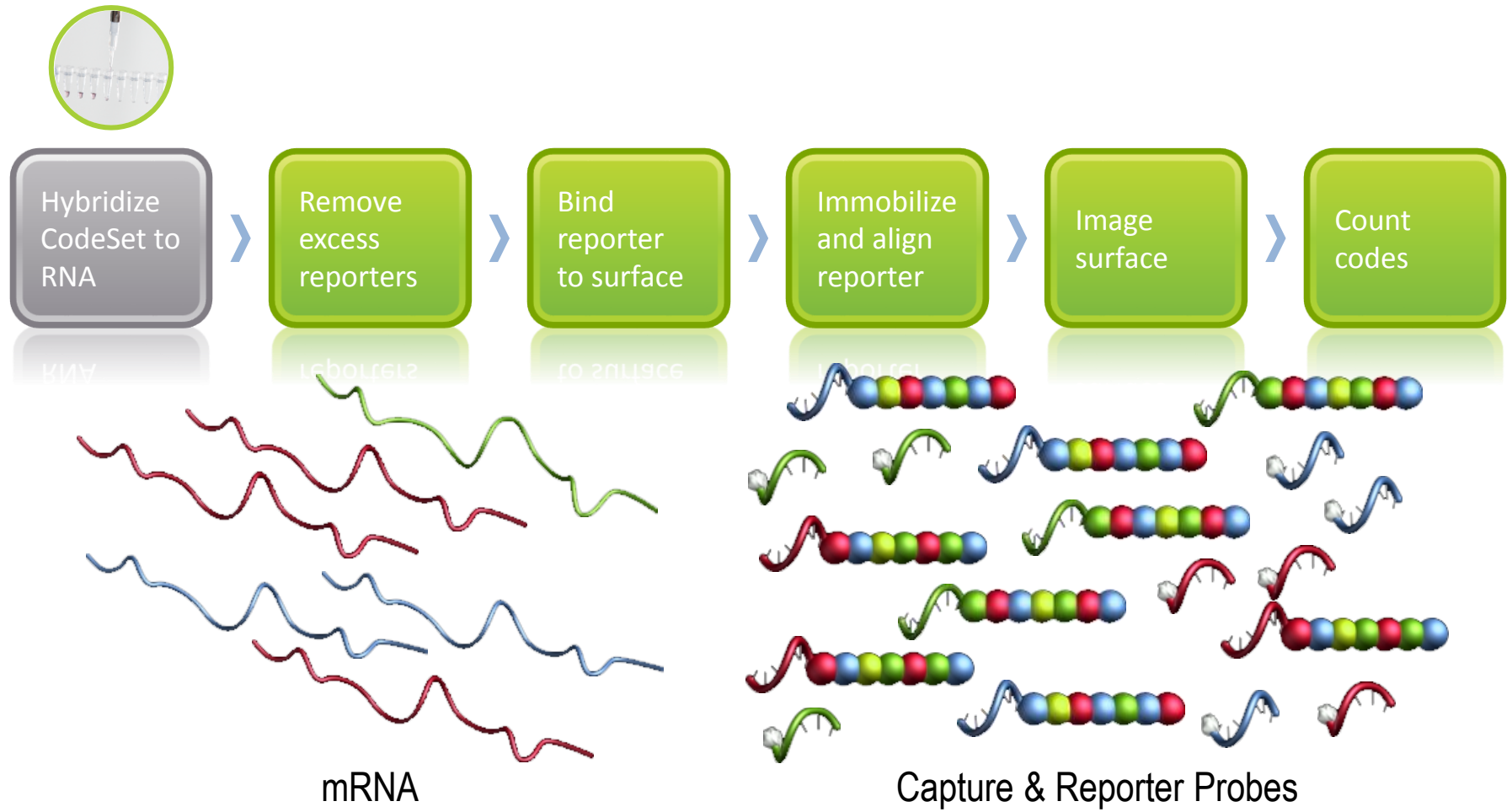
CGCGTCCGCCCCGCGAGCACAGAGCCTCGCCTTTGCCGATCCGCCGCCCCGTCCACACCCGC

CGCGTCCGCCCCGCGAGCACAGAGCCTC

GCCTTTGCCGATCCGCCGCCCCGTCCACACCCGC

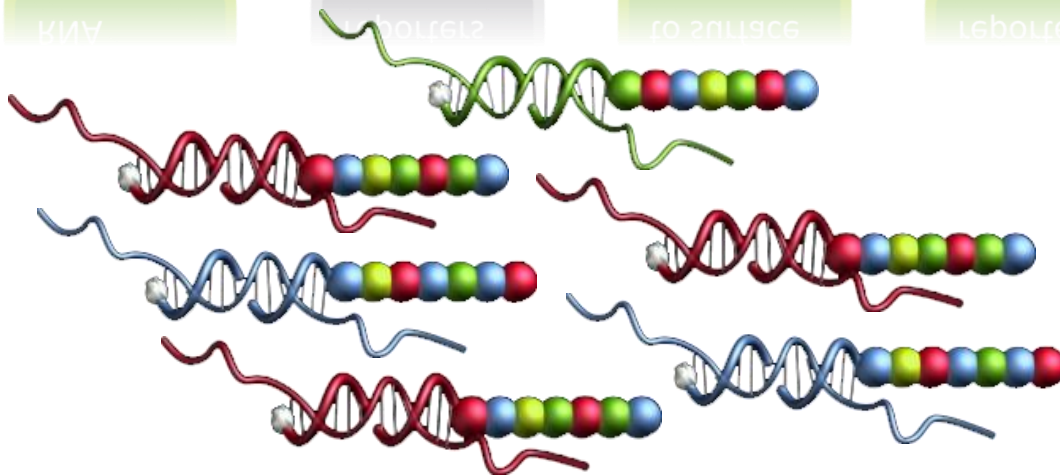


# Work flow - assay

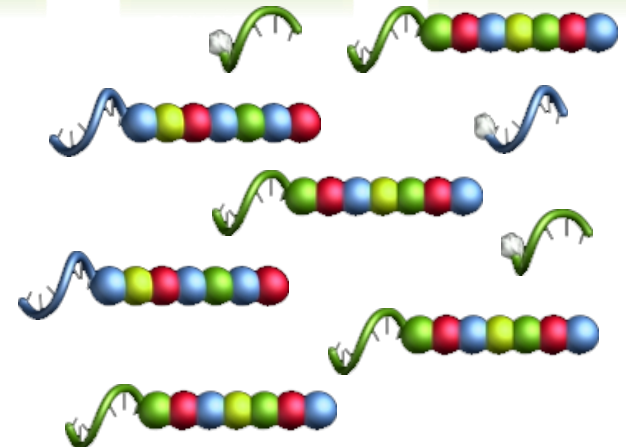




# Work Flow - assay

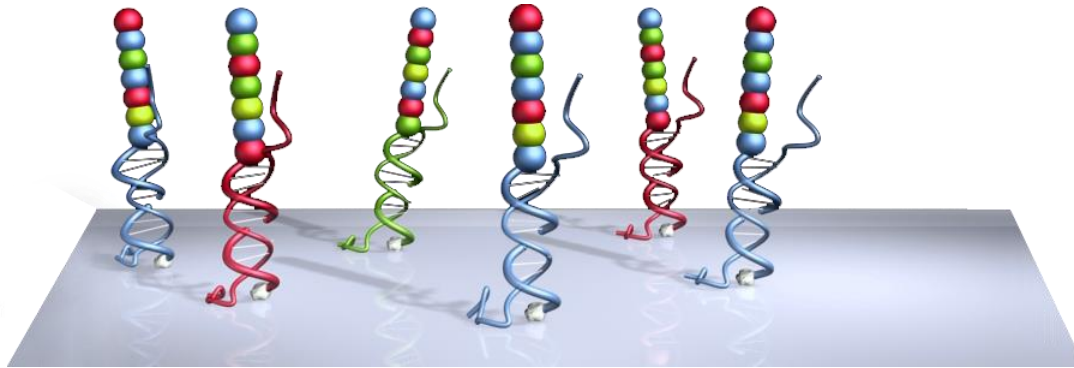


Hybridized mRNA



Excess Probes

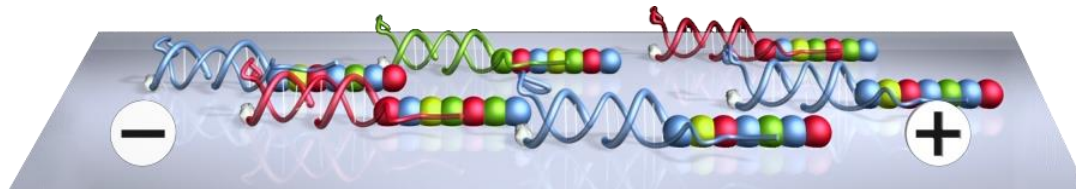
# Work Flow - assay



Surface of cartridge is coated with streptavidin

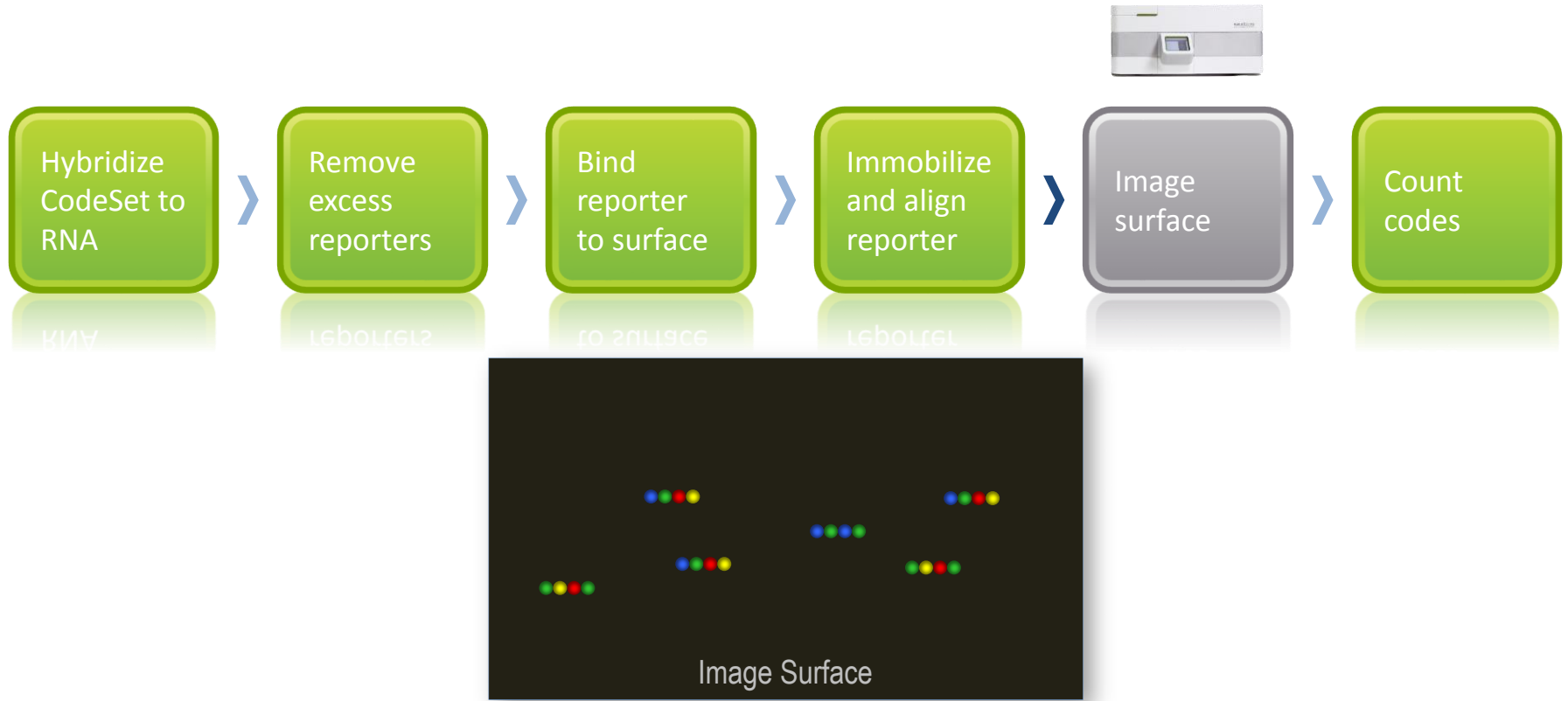
Hybridized Probes Bind to Cartridge

# Work Flow - assay



Immobilize and align reporter for image collecting and barcode counting

# Work Flow - assay

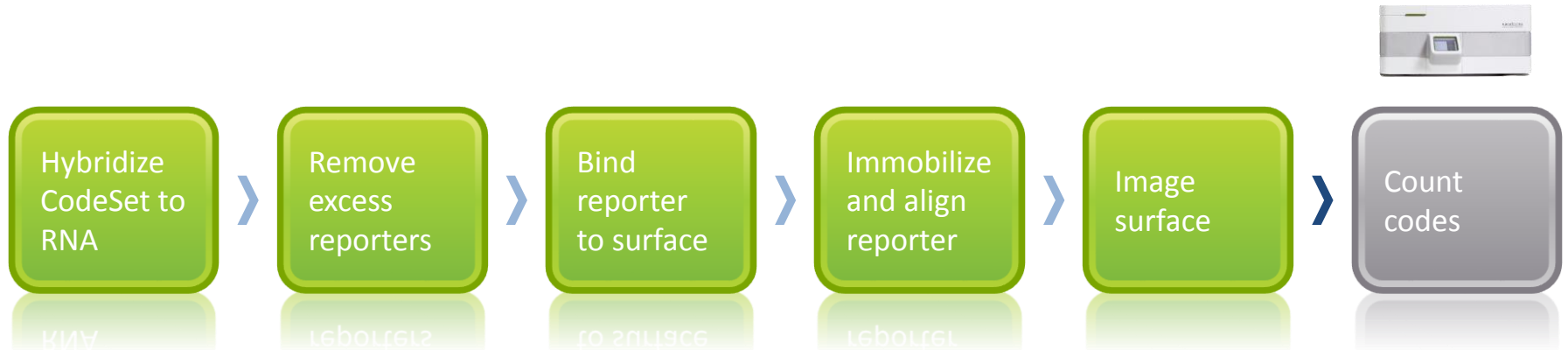


One reporter code = 1 mRNA

# Work Flow - assay



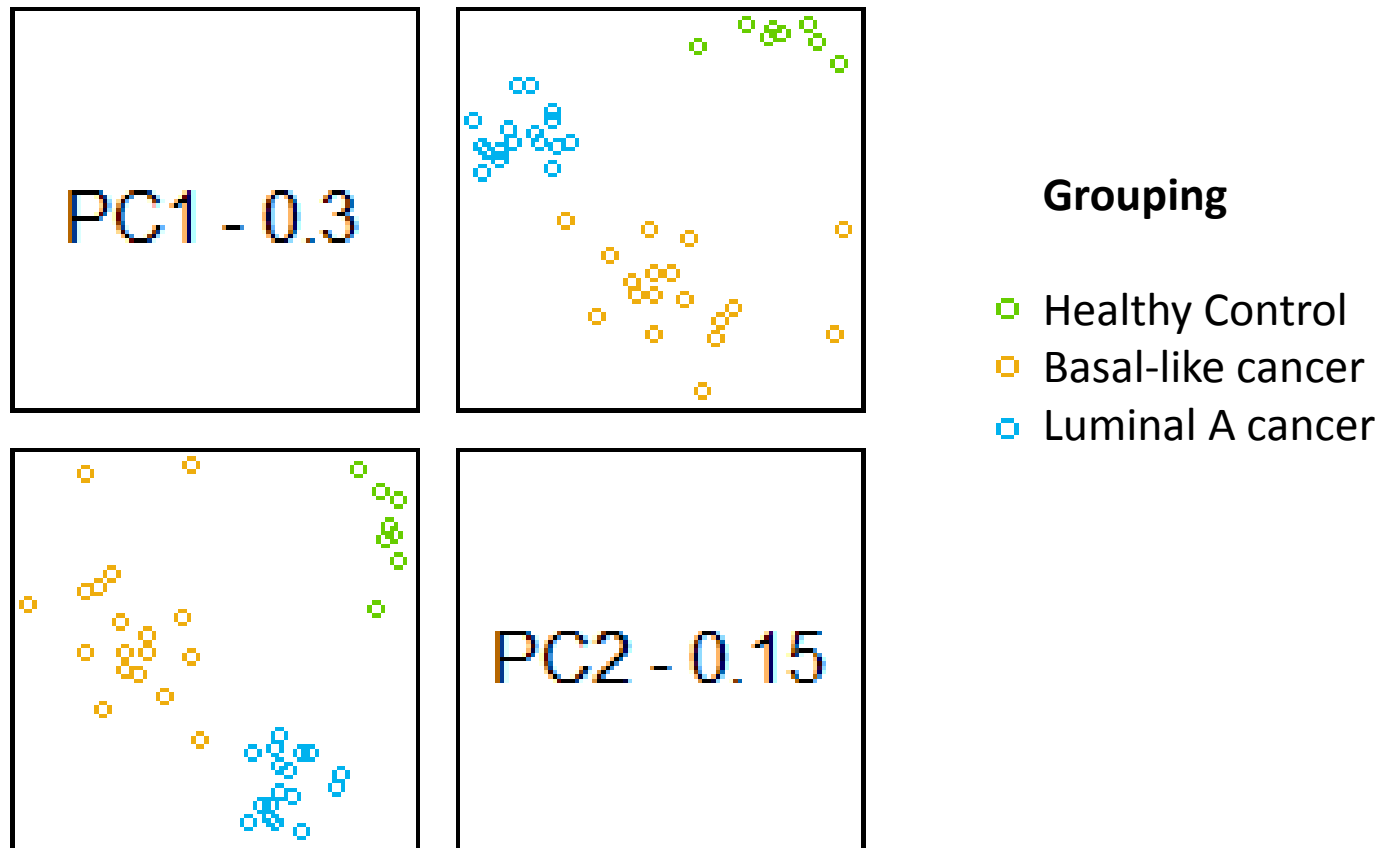
# Work Flow - assay



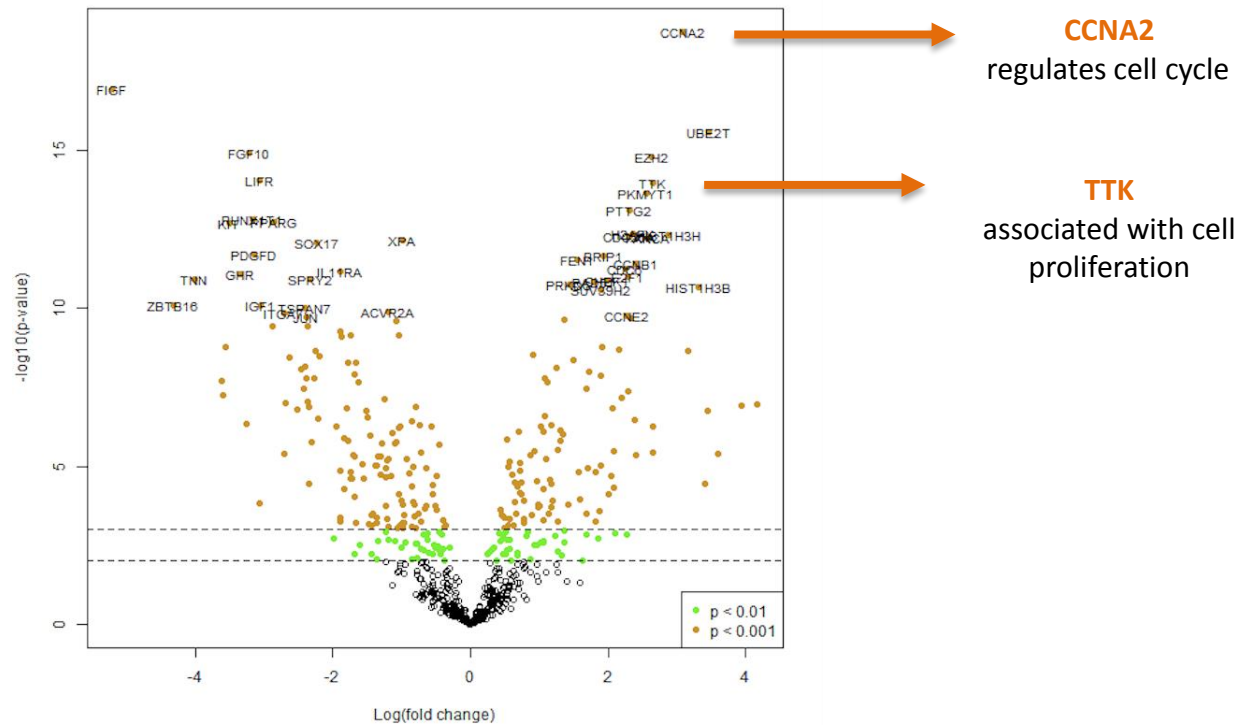
Code	Gene	Count
	X	3
	y	1
	z	2

Codes are counted and tabulated

# PCA of cancer and healthy control samples



# Healthy vs basal-like cancer comparison





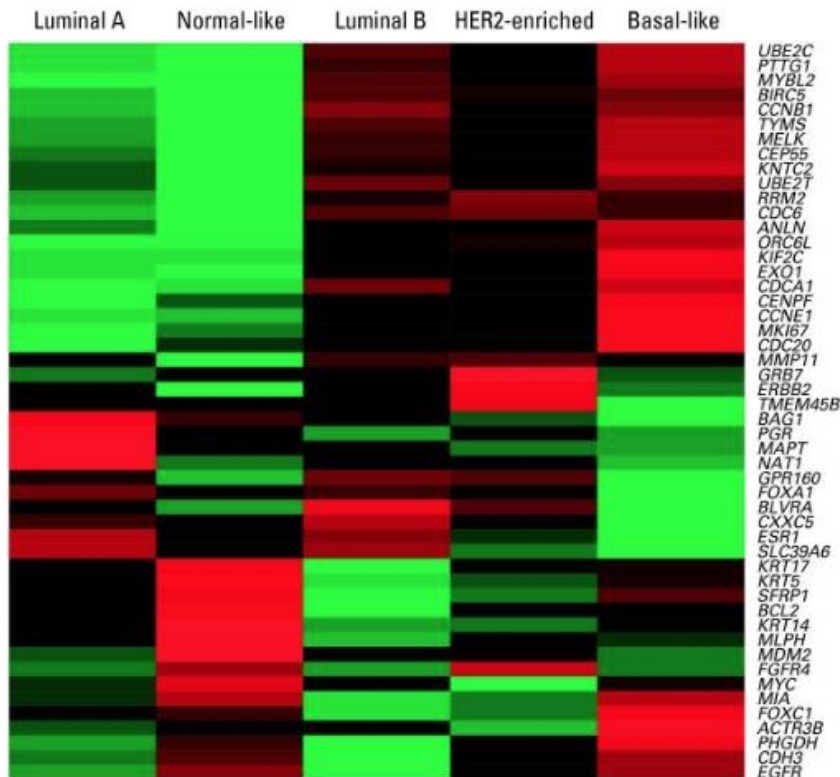
# Applications

- Diagnostic
- Life Sciences
- Translation

# Diagnostics - Prosigna

## Breast Cancer

Parker et al., J Clin Oncol, 2009



50-gene subtype predictor was developed using microarray and qRT-PCR data from 189 prototype samples diagnosed with invasive breast carcinoma

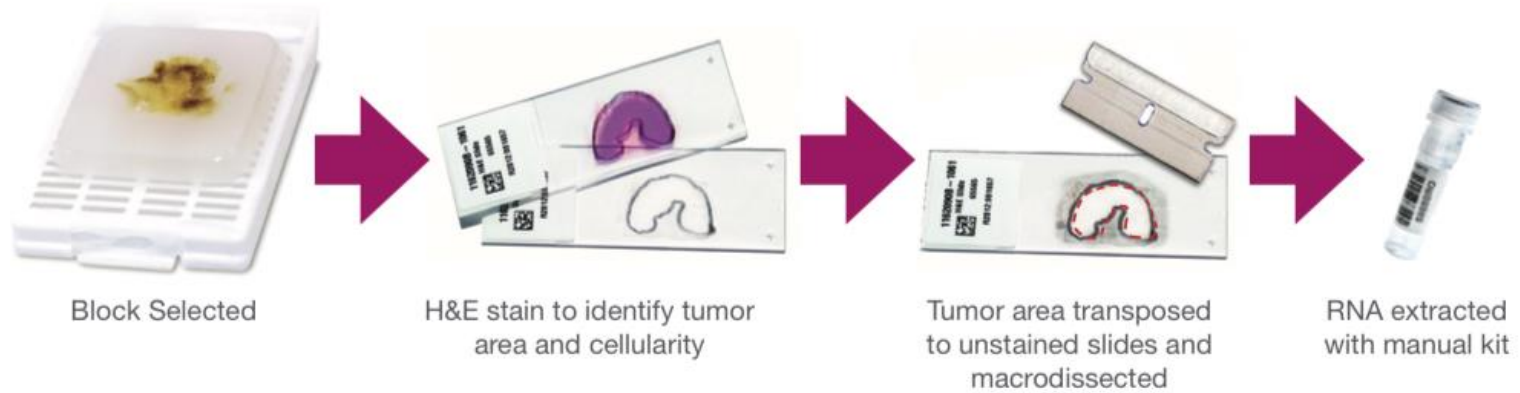
# Diagnostics - Prosigna

## Gene signature was tested for Prosigna

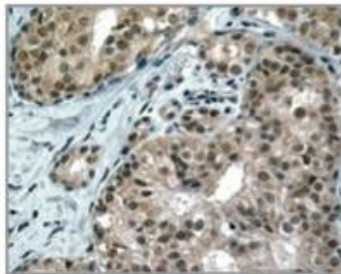
- Precision was assessed by testing 5 pooled FFPE breast tumor RNA samples >100 times each
- RNA samples were run independently at 3 different testing sites by a total of 6 different operators using 3 different reagent lots
- The range of ROR scores for 108 independent measurements was  $\leq 4$  units for each of the 5 sample pools
- 100% concordance was shown between measured and expected subtype result and risk group



# Prosigna workflow



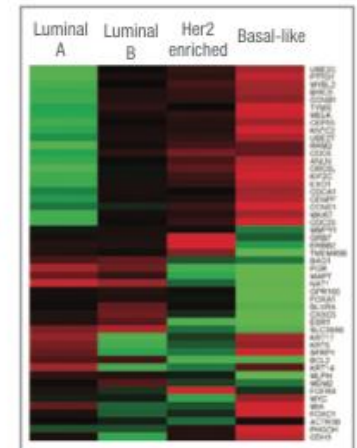
Extract RNA from FFPE tumor sample



Run RNA and Prosigna CodeSet on nCounter Analysis System



Patient specific expression profile



# Diagnosics - Prosigna

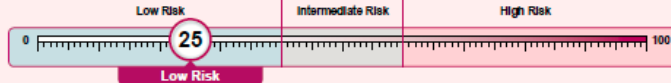


Patient	Specimen	Comments
Tumor Size: ≤ 2cm Lymph Nodes: Node-negative	ID #: Date Reported:	

## Assay Description:

The Prosigna™ breast cancer gene signature assay measures the expression of 58 different genes to report the Prosigna Score, which is used along with the patient's nodal status to assign a risk classification. The Prosigna Score is derived from a proprietary algorithm based on the PAM50 gene signature, and includes information on the tumor's molecular subtype and proliferation as well as the pathologic tumor size.

## Patient Prosigna Score\*:



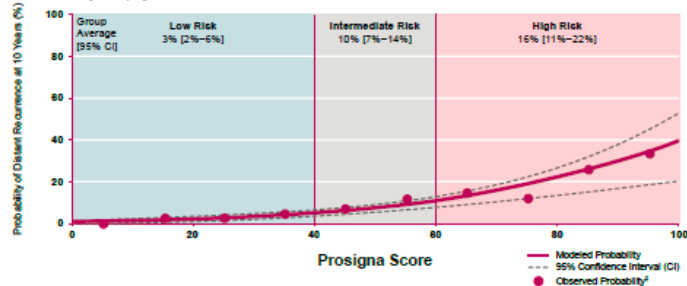
\*The Prosigna Score ranges from 0 through 100 and correlates with the probability of distant recurrence (DR) in the tested population. Risk classification is provided to guide the interpretation of the Prosigna Score using cutoffs related to clinical outcome.

## Clinical Trial Results:

### Probability of Distant Recurrence

In the clinical validation study, patients who were node-negative, with a Prosigna Score of 25 were in the low risk group. The low risk population defined by a prespecified Prosigna Score cutpoint averaged a 3% probability of distant recurrence at 10 years.

The Prosigna algorithm was used in retrospective analysis of the ABCSG-8 clinical trial which included more than 1400 patients with varying risks of distant recurrence. The retrospectively fitted model relating Prosigna Score to 10-year distant recurrence in the ABCSG-8 study is displayed below.<sup>†</sup>



<sup>†</sup>Data apply to patients being treated with endocrine therapy for 5 years as in the tested patient population. See Package Insert for further information on therapeutic regimens and tested patient population. It is unknown whether these findings can be extended to other patient populations or treatment schedules. <sup>†</sup>Average DR rate observed in ABCSG-8 for patients within 10 Prosigna Score units.

NanoString Technologies, Inc. 530 Fairview Avenue N | Suite 2000 | Seattle, Washington 98109 | 1-206-379-6266 | nanostring.com

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For more information, visit PROSIGNA.com or e-mail dxsupport@nanostring.com

The nCounter-PAM50 Gene Signature Assay is intended for use as a prognostic indicator for distant recurrence-free survival at 10 years

## Risk of Recurrence (ROR Score)

- Zero to 100
- Relates to recurrence rate at 10 years on 5 years of endocrine therapy alone

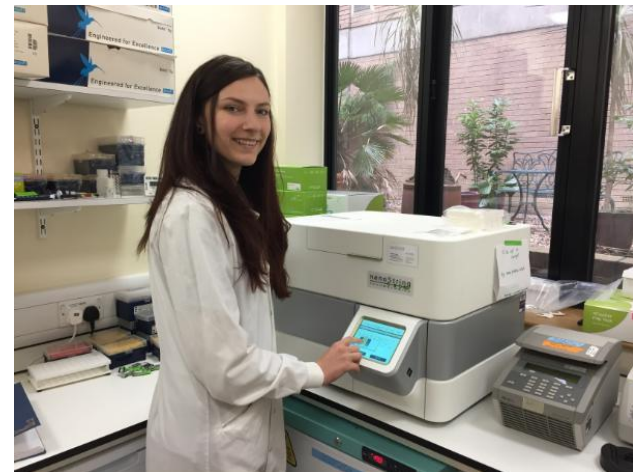
## Risk Groups

- Node Negative
  - Low, Intermediate, or High risk
  - Based on ROR Score and nodal status
- Node Positive
  - Low or High risk
  - Based on ROR Score and nodal status

Prosigna is now included in international clinical practice guidelines

# NanoString in HuDC lab

- Technician lead service
- University Category B Facility Feb 2017
- Internal and external users (academia)
  - UK universities
  - Europe (Norway)



# NanoString in HuDC lab

- nCounter analysis system
- Current capacity 8 plates (96 samples) a week
- Annual capacity approx 2880 samples/year

## Dr Venetia Bigley

- **Wellcome Intermediate Clinical Fellow**
- Email: [venetia.bigley@ncl.ac.uk](mailto:venetia.bigley@ncl.ac.uk)
- Telephone: 0191 282 1570



## Anastasia Resteu

- **Laboratory Technician**
- Email: [anastasia.resteu@ncl.ac.uk](mailto:anastasia.resteu@ncl.ac.uk)



<http://research.ncl.ac.uk/nanostring/>