nCounter™ Analysis System

Molecules That Count™
Molecules that Count™
nCounter Analysis System

The first and only technology platform to deliver highly multiplexed, direct profiling of individual molecules in a single reaction without amplification.
NanoString enables sensitive analysis of hundreds of genes in a single-tube assay.
nCounter Analysis System

- Simultaneously measure 20 to over 500 genes per single reaction
- Digital Detection and Analysis
- No Polymerases or Signal Amplification Required
- Easy-to-use System
- Fully Automated
- Highly Sensitive, Reproducible Data
- Flexibility in Sample Type
- Over 41,000 Data Points in 24 hours
Probe Architecture

Target Specific Capture Probe

Target Specific Reporter Probe

Biotin
Probe Architecture

Target Specific Capture Probe

Target Specific Reporter Probe
nCounter CodeSet

Sequence-Specific Capture Probes, Reporter Probes and System Controls are Combined to Create Your Custom CodeSet
Simple Protocol

Day 1
5 min HANDS-ON
1. Hybridize

Day 2
5 min AUTOMATED
2. Purify
5 min HANDS-ON
3. Count
Flexibility with Sample Input

- Total RNA (100ng/sample)
- Amplified RNA from Small Amount of Sample
- Whole Cell Lysates
- PaxGene Lysed Whole Blood
- Total RNA Extracted from FFPE Samples
- Crude Extracts from FFPE samples
nCounter Assay

1. Hybridize CodeSet to RNA
2. Remove excess reporters
3. Bind reporter to surface
4. Immobilize and align reporter
5. Image surface
6. Count codes
nCounter Assay

Hybridize CodeSet to RNA → Remove excess reporters → Bind reporter to surface → Immobilize and align reporter → Image surface → Count codes

Hybridized mRNA → Excess Reporters
nCounter Assay

Hybridize CodeSet to RNA

Remove excess reporters

Bind reporter to surface

Immobilize and align reporter

Image surface

Count codes

Hybridized Probes Bind to Cartridge

Surface of cartridge is coated with streptavidin
Hybridize CodeSet to RNA

Remove excess reporters

Bind reporter to surface

Immobilize and align reporter

Image surface

Count codes

Immobilize and align reporter for image collecting and barcode counting

nCounter Assay
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1. Hybridize CodeSet to RNA
2. Remove excess reporters
3. Bind reporter to surface
4. Immobilize and align reporter
5. Image surface
6. Count codes

One reporter code = 1 mRNA
nCounter Assay

Hybridize CodeSet to RNA ➔ Remove excess reporters ➔ Bind reporter to surface ➔ Immobilize and align reporter ➔ Image surface ➔ Count codes

<table>
<thead>
<tr>
<th>Code</th>
<th>Gene</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>y</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>z</td>
<td>2</td>
</tr>
</tbody>
</table>

Codes are counted and tabulated
Data Output

Simple enough to analyze in Excel™
miRNA Sample Preparation Basics
miRNA Sample Preparation Basics

Bridge oligo specifically anneals to each miRNA target

miRNAs
miRNA Sample Preparation Basics

Bridge oligo specifically anneals to each miRNA target

Unique miRtag for each miRNA species

miRNAs
miRNA Sample Preparation Basics

- miRNAs
- miRNA is covalently linked to miRtag via ligation
- Bridge oligo specifically anneals to each miRNA target
- Unique miRtag for each miRNA species
miRNA Sample Preparation Basics

Excess ligation components are removed via enzymatic digestion, leaving specifically tagged miRNAs to be counted via hybridization with the nCounter Human miRNA Panel CodeSet.
Probe Architecture

Target Specific Capture & Reporter Probes bind to the chimaeric miRNA:miRtag molecule