Luminex Based Assay Development

Stephen Angeloni, Ph.D.
Sr. Field Application Scientist
Luminex Corporation

Luminex Technology

A Broad Range of Applications

Research

Human Diagnostics

Animal Health

Ag Sciences

Food Safety

Biodefense

Environmental Monitoring

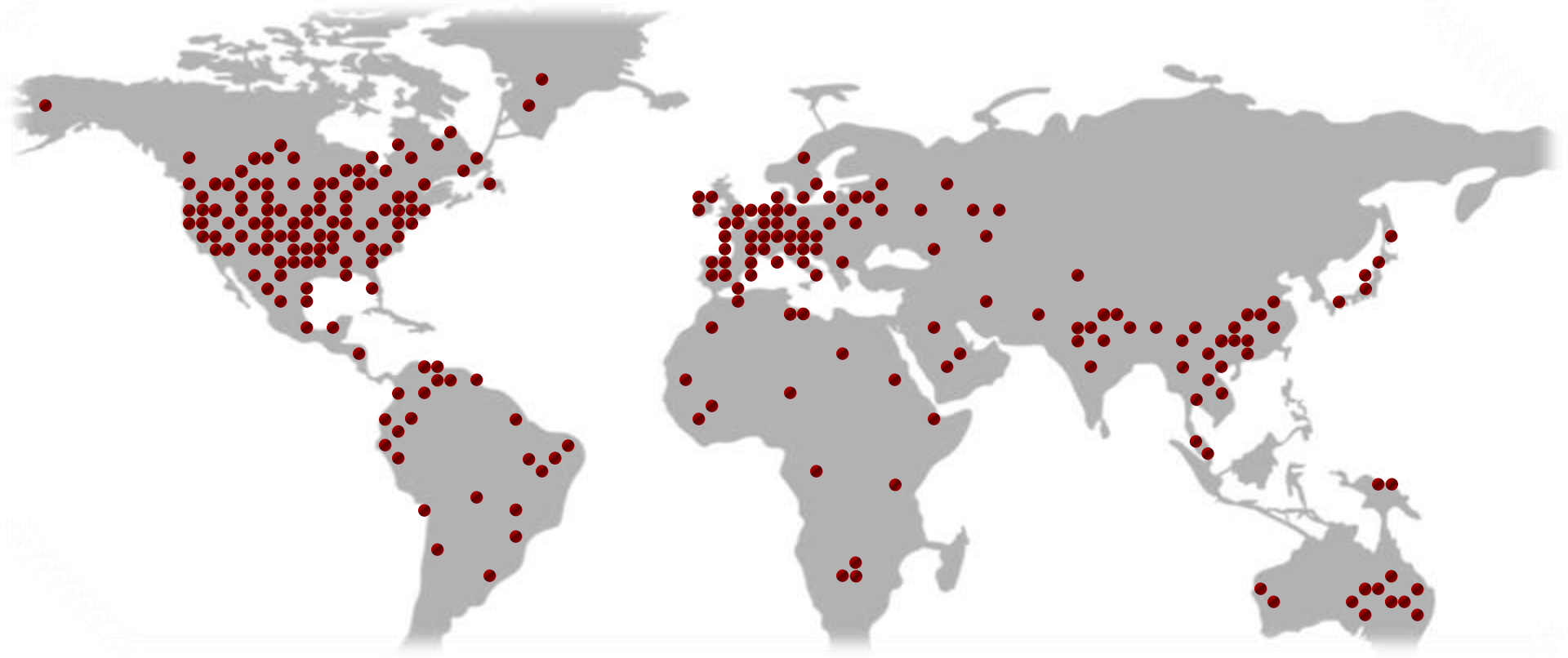
Escondido's

Luminex Corporate Offices

Global Presence

Luminex

Global Presence: Over 11,000 instruments shipped globally

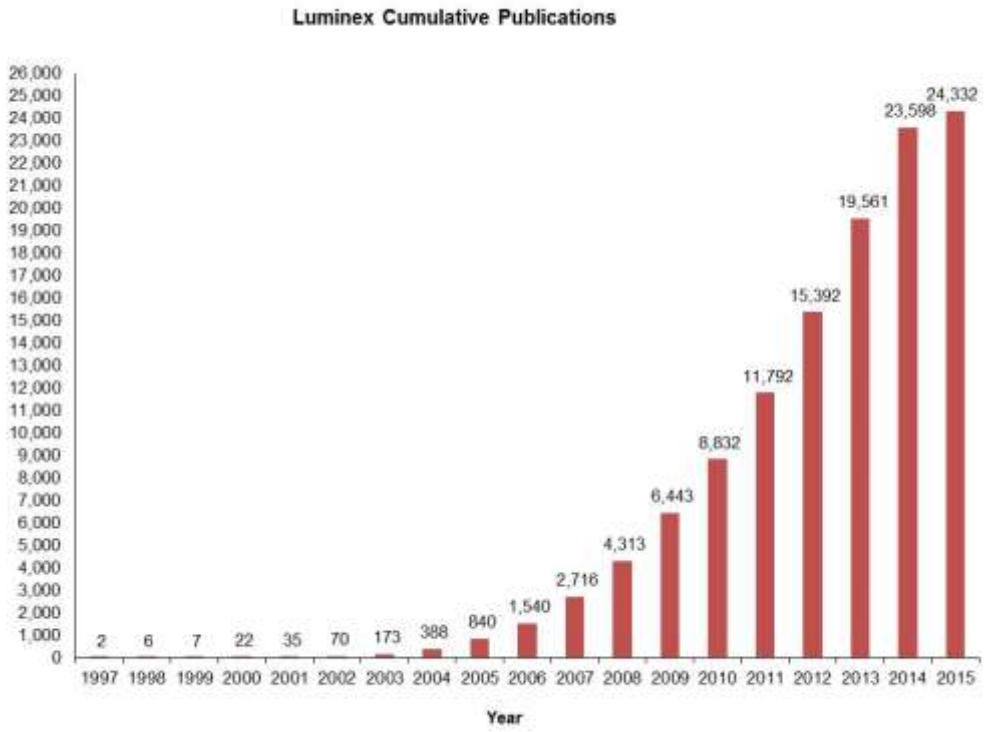


Luminex

Propelling Knowledge

Over 24,000 peer-reviewed scientific studies and growing

For complete listing see www.luminexcorp.com/publications



CVI CLINICAL AND VACCINE IMMUNOLOGY

Neurology®

Clinical Cancer Research

Molecular & Cellular PROTEOMICS

JOURNAL OF CLINICAL VIROLOGY

Journal of Clinical Microbiology

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Luminex Partners

Over 60 partners worldwide

affymetrix Panomics

ORIGENE

ONE LAMBDA
Advancing Transplant Diagnostics

life technologies™

BIO-RAD

R&D SYSTEMS®

affymetrix eBioscience

MERCK MILLIPORE

ACTIVE MOTIF®

MAYO CLINIC

Fisher HealthCare

Luminex Diagnostics

More than 60 US FDA 510(k) Cleared Platforms

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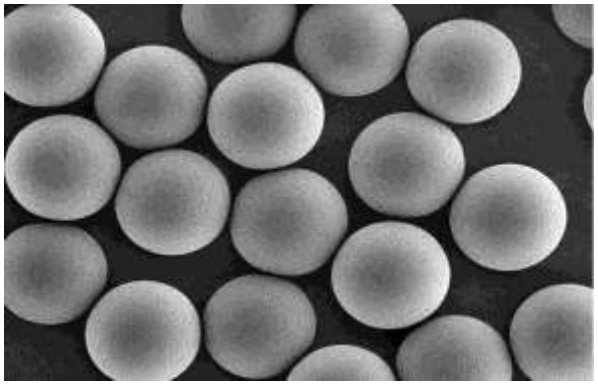
xMAP Technology: An Overview

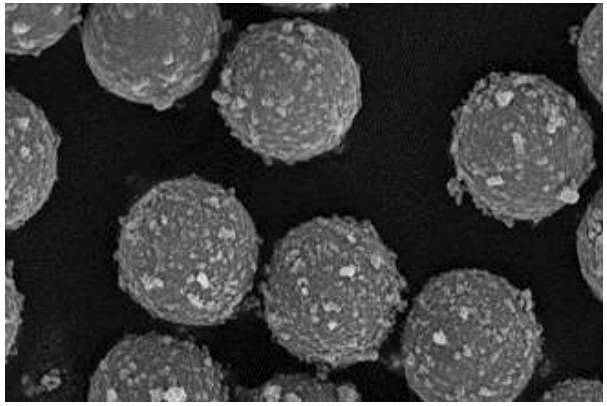
xMAP Technology

Microsphere-based Arrays

MicroPlex™
Microspheres 5.6 um



MagPlex™
Superparamagnetic microspheres 6.5 um



Microsphere Advantages

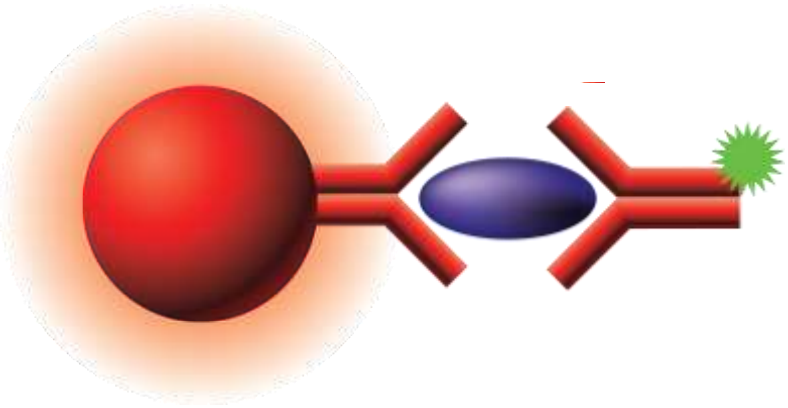
- **Fast liquid phase kinetics**
- **Proprietary dyeing process**
- **Unique bead signatures**
- **High surface-to-volume ratio**
- **Surface carboxyl groups**

xMAP Applications

An Incredibly Flexible Platform

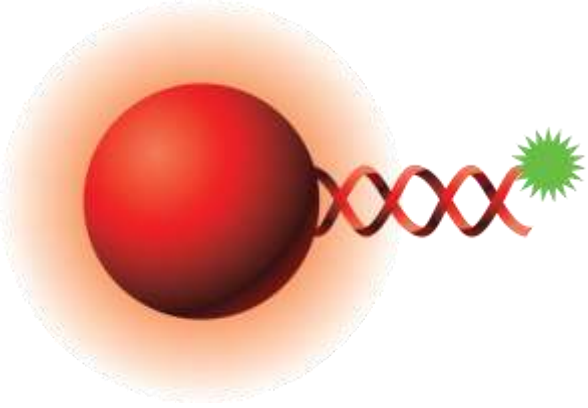
Bead sets can be coated with reagents specific to many bioassays:

Proteins



- Immunoassays
  - » Sandwich Capture
  - » Multiplex ELISA
- Receptor-Ligand/Protein interaction Assays
- Enzyme Substrate

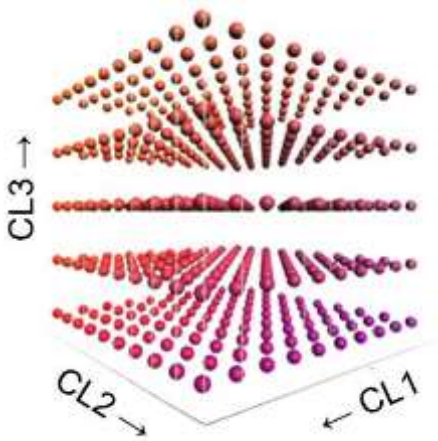
Nucleic Acids

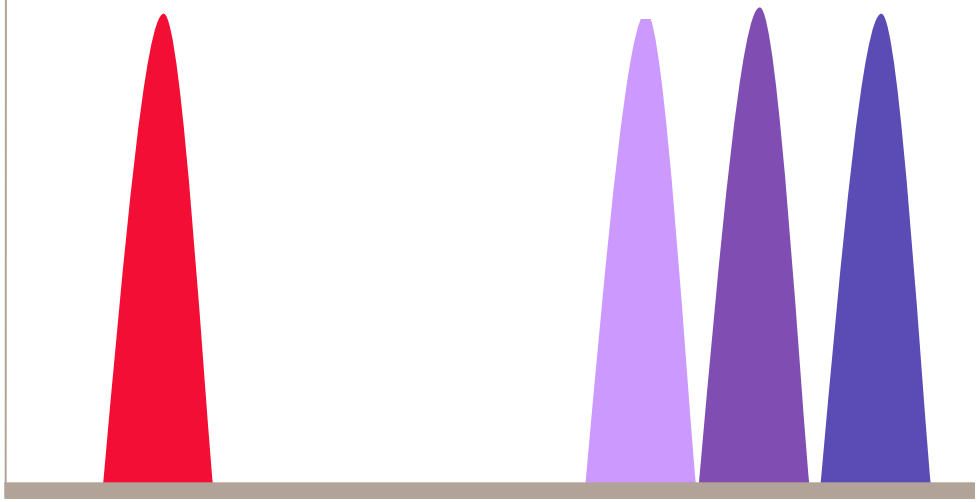


- Gene Expression
- microRNA Profiling
- Genotyping
  - »SNP
  - »CNV
  - »Sequence Detection

Spectral Addressing

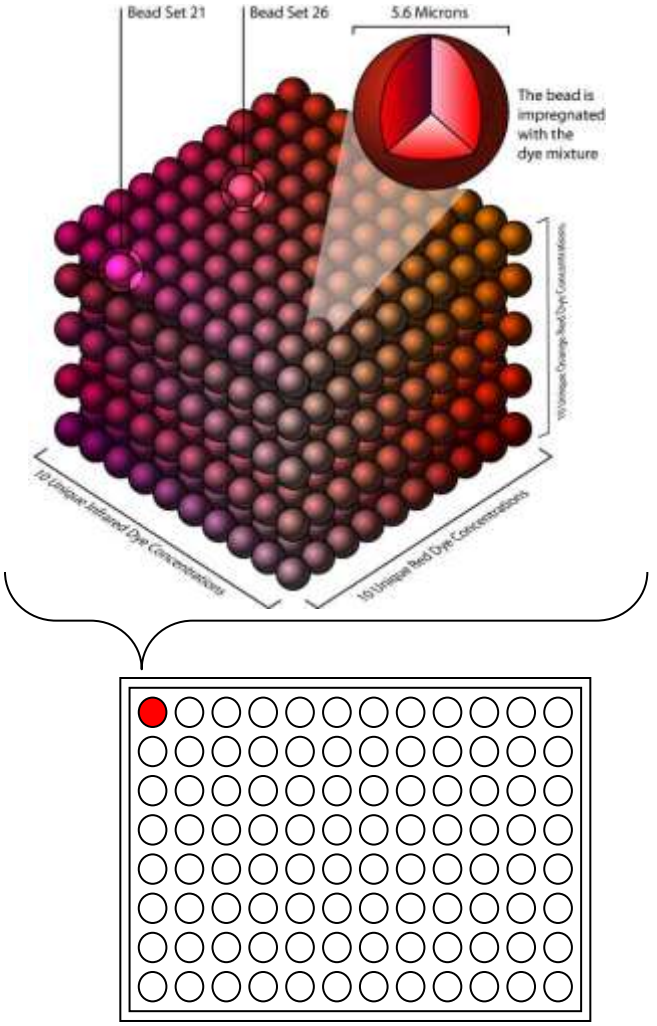
Multiplexing to 500 Analytes/Well with MagPlex beads





Excite One Excitation Wavelength

Observe Three Emission Wavelengths



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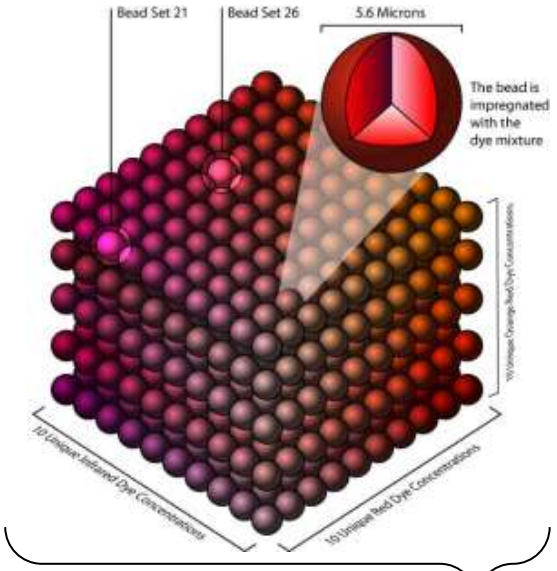
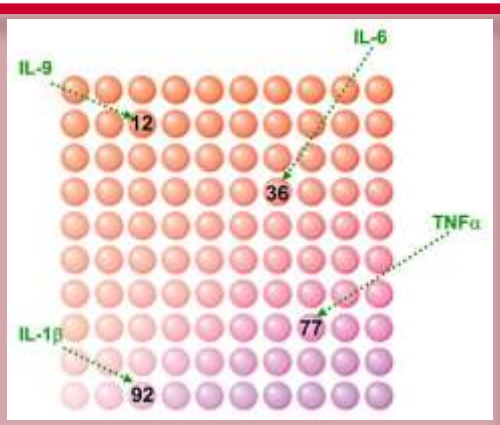
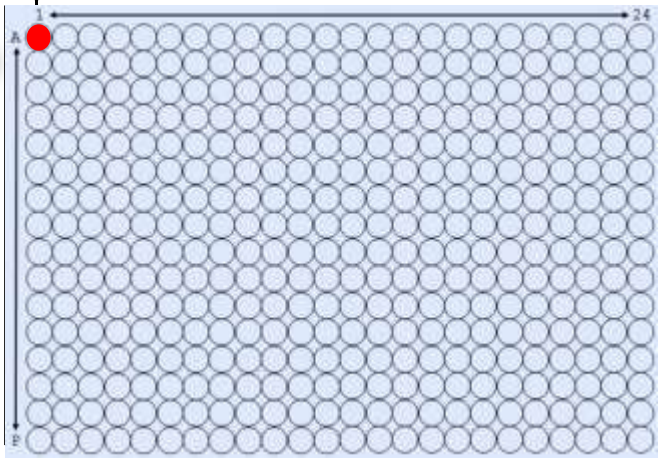
Spectral Addressing = Multiplexing

Each Bead Associated with a Specific Analyte in each reaction



With each bead color assigned an analyte each reaction measures multiple targets for any genomic or proteomic assay





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Several Advantages to Multiplexing

Use less sample, less reagents, get more data faster, get more accurate data

Real Examples

A 107 plex Gene Expression assay.

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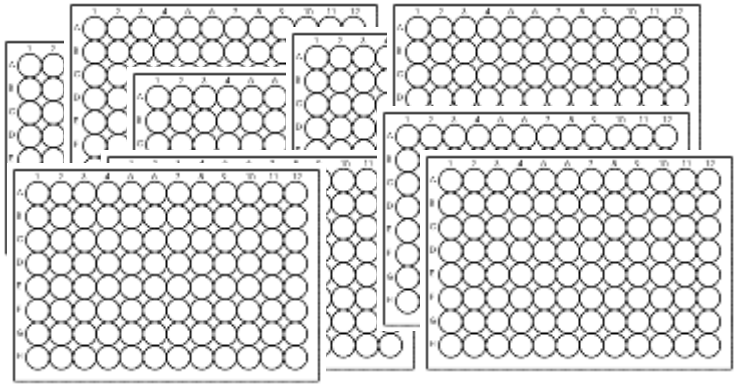
14 samples in triplicate. Done in 42 wells of a 96 well plate.
Single plex chemistry like TaqMan or another real time PCR chemistry would required 4,494 wells = 46.8 96 well plates.

24 plex Protein assay.

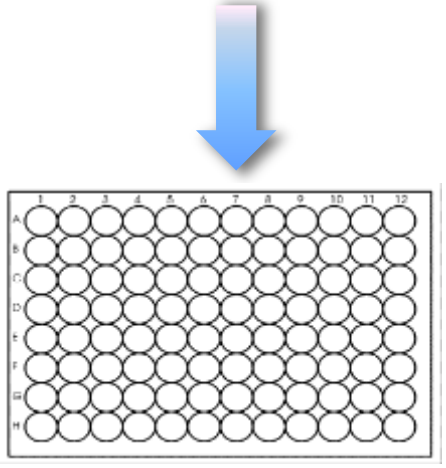
Our partners make protein assays that can analyze as many as 43 proteins or more with some kits. Running a 24 plex assay on 27 samples in triplicate required 81 wells of a 96 well plate.

A standard single plex ELISA chemistry would require 1,944 wells and more than 20 96 well plates. If 43 marker assay, single plex would need 3,483 reactions or 36.3 96 well plates

Single Plex Reactions

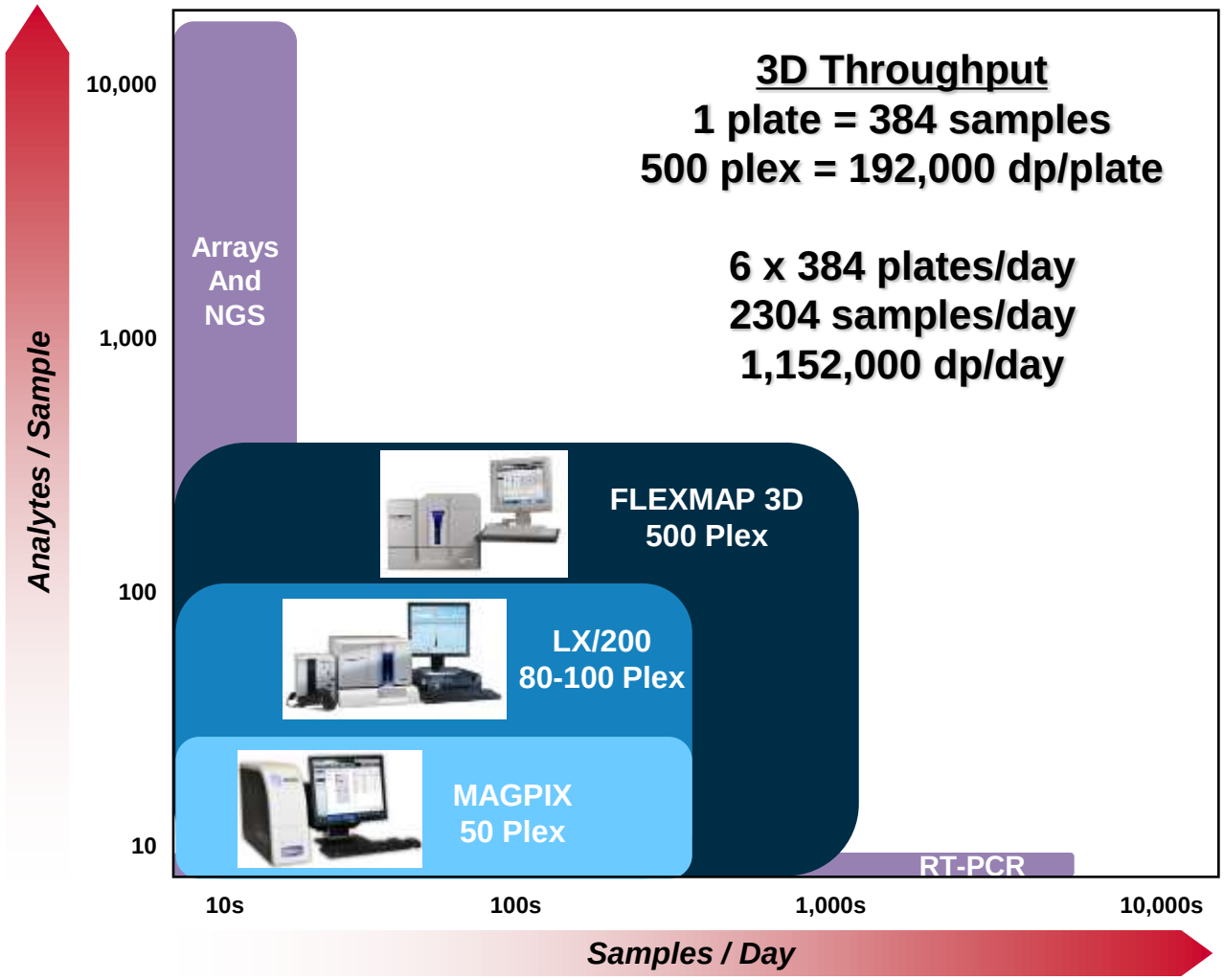


Luminex Multiplexing



Luminex Instruments

Unique Combination of Flexibility, Multiplexing and Throughput



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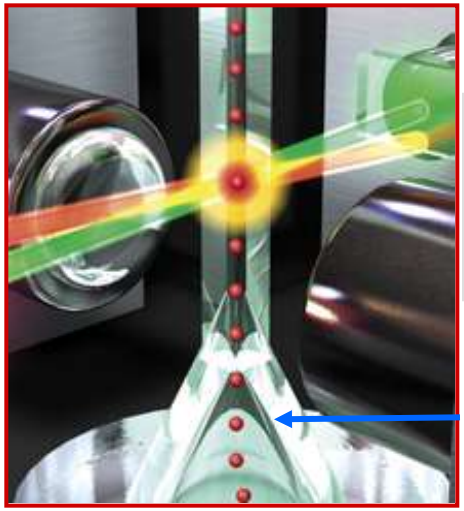
Flow Cytometry and Imaging Comparison

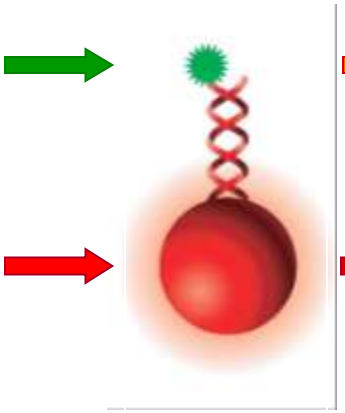
Flow Cytometry-Based Analysis – Luminex 200 & FLEXMAP 3D



Sheath Fluid

Interrogate label with green laser (525 nm)

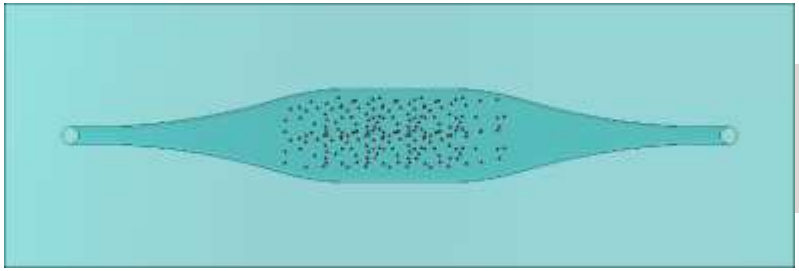
Interrogate bead with red laser (635 nm)



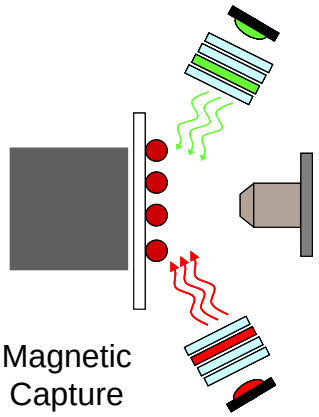
Quantify binding events

Identify bead region based on internal dye concentrations

LED/Image-Based Analysis - MAGPIX



Beads in Chamber



Magnetic Capture

Interrogate label with green LED (525 nm)

Identify and quantify with CCD imager

Interrogate bead with red LED (635 nm)

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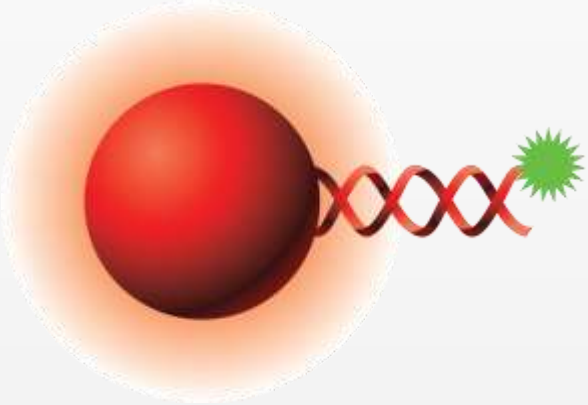
Genomic Assays



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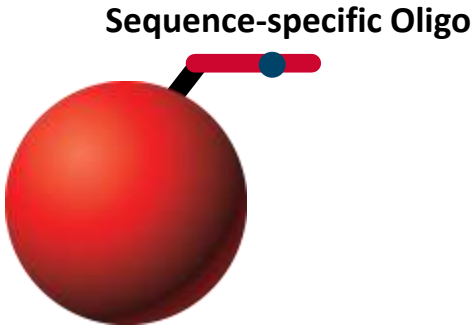
Direct Hybridization

Direct Hybridization

- Couple sequence-specific C18 spacer amine-modified oligo to bead
- Probes are matched for length ~20bp for SNPs, 50-70bp okay for unrelated sequences and mismatches are centered or distributed



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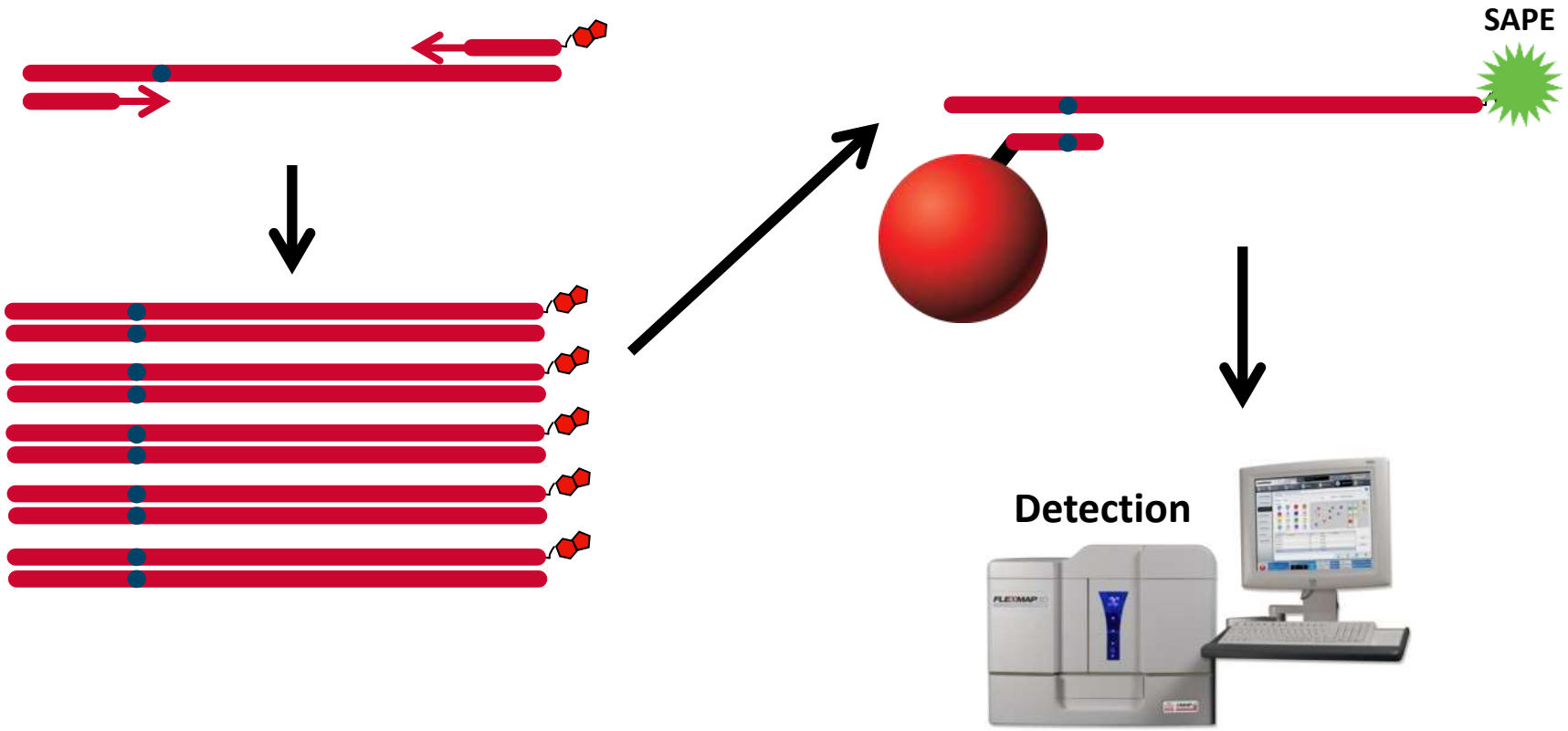
Direct Hybridization

- Label target nucleic acid sequence to be detected with biotin PCR primer
- Amplicons should be ≤ 300bp if possible
- Denature, hybridize to beads, incubate with SAPE and detect



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DDL/GSK HPV Genotyping Assay

JCM
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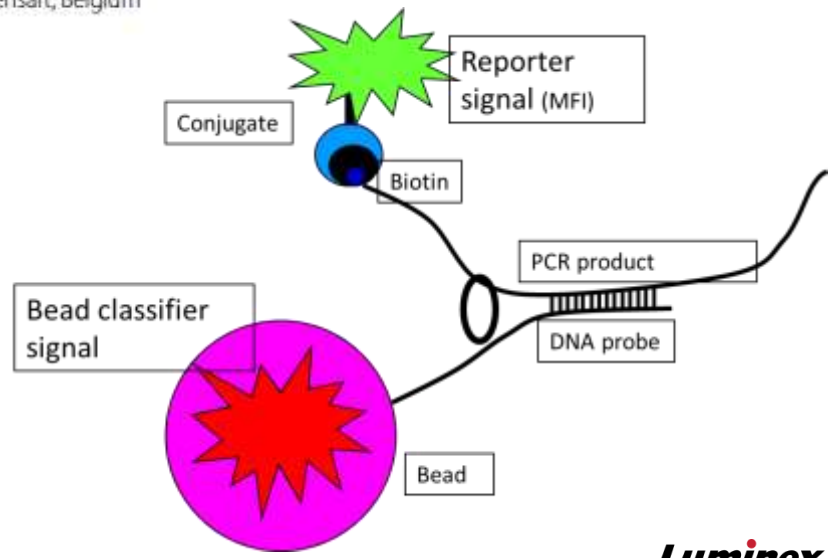
A Human Papilloma Virus Testing Algorithm Comprising a Combination of the L1 Broad-Spectrum SPF_{10} PCR Assay and a Novel E6 High-Risk Multiplex Type-Specific Genotyping PCR Assay

Dirk van Alewijk,[a] Bernhard Kleter,[a] Maarten Vent,[a] Jean-Marc Delroisse,[b] Maurits de Koning,[a] Leen-Jan van Doorn,[a] Wim Quint,[a] Brigitte Colau[b]

DDL Diagnostic Laboratory, Rijswijk, The Netherlands[a]; GlaxoSmithKline Vaccines, Rixensart, Belgium[b]

DDL DIAGNOSTIC LABORATORY

Partner in advanced diagnostic testing



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Luminex assays Developed and Published by DDL

DDL is a contract research organization and is available for collaborations and service

Target	Multiplex	Intended use	Reference
HPV (high-risk mucosal / anogenital)	21-plex	Research	Geraets *et al*, 2009
Chlamydia trachomatis	16-plex	Research & Epidemiology	Quint *et al*, 2009
HPV (wart)	25-plex	Research & Epidemiology	De Koning *et al*, 2010
HPV (high-risk mucosal / anogenital)	19-plex	Research & Epidemiology	Van Alewijk *et al*, 2013
HPV (mucosal / anogenital)	36-plex	Research & Epidemiology	Kleter *et al*, to be submitted

DDL DIAGNOSTIC LABORATORY

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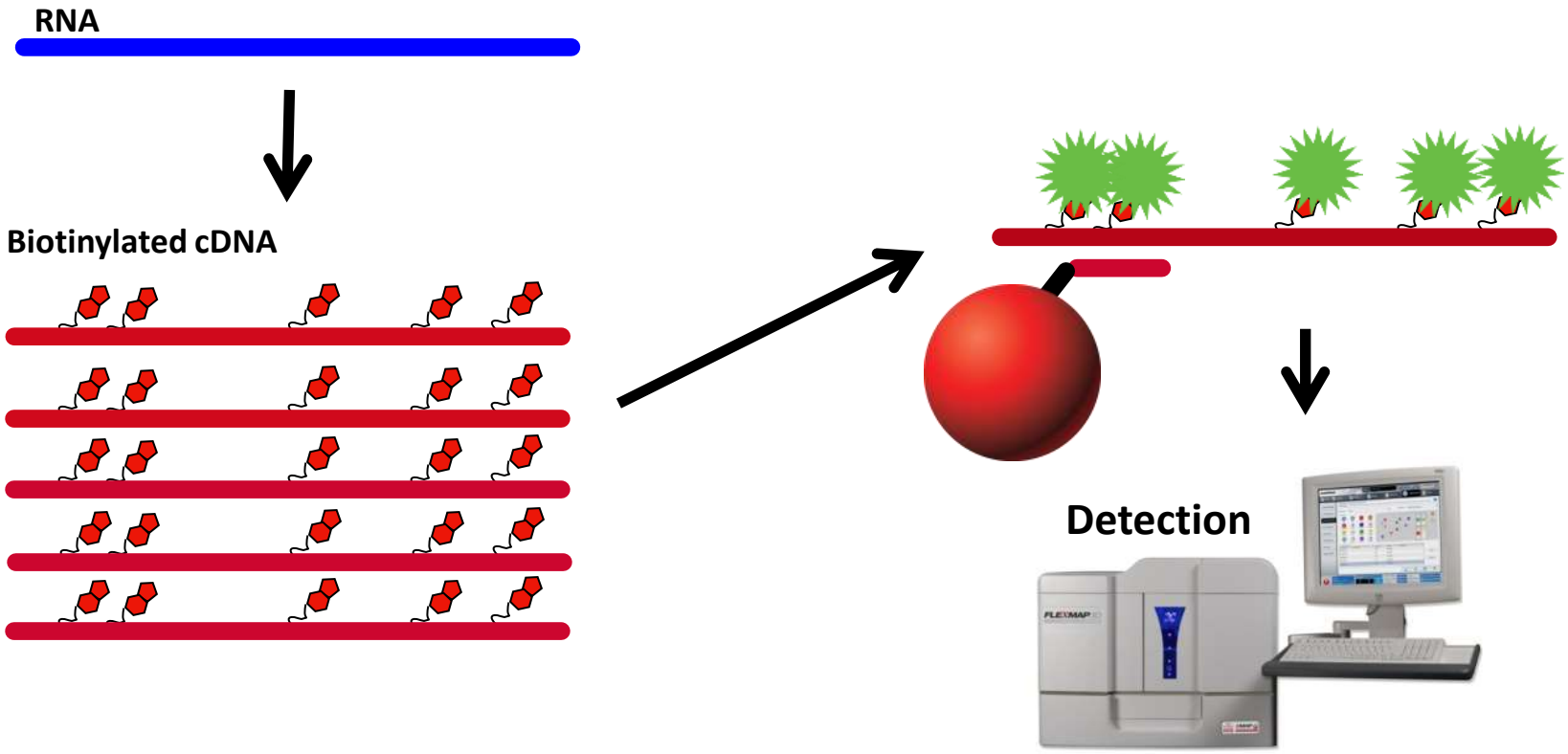
xMAP® Salmonella Serotyping Assay

RUO Kit Training

Direct Hybridization

BADGE Gene Expression Assay

- BADGE, Beads Array for the Detection of Gene Expression, a high-throughput diagnostic bioassay. Yang, L., D.K. Tran, and X. Wang, *Genome Res*, 2001. 11(11): p. 1888-98.



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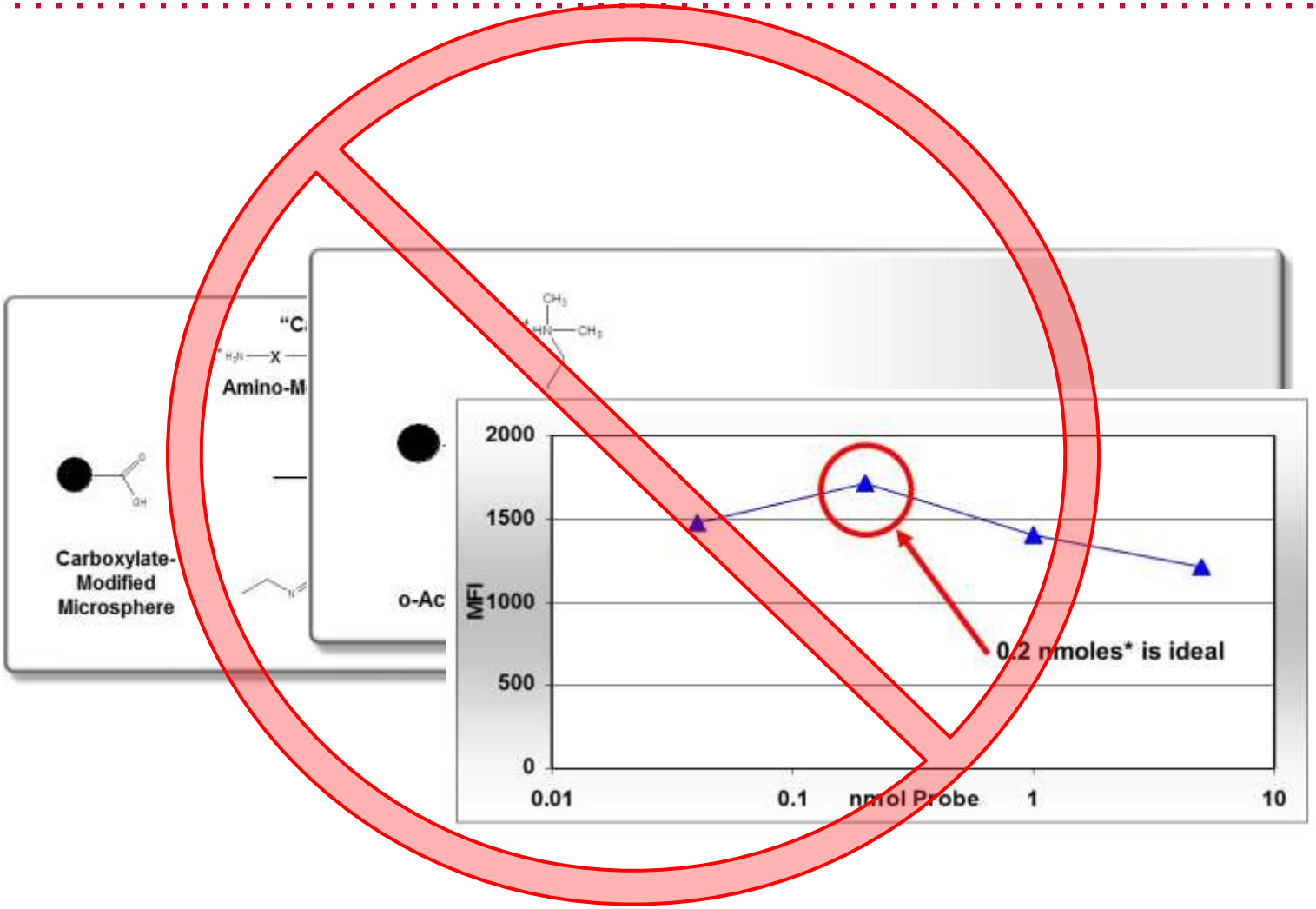
Direct Hybridization

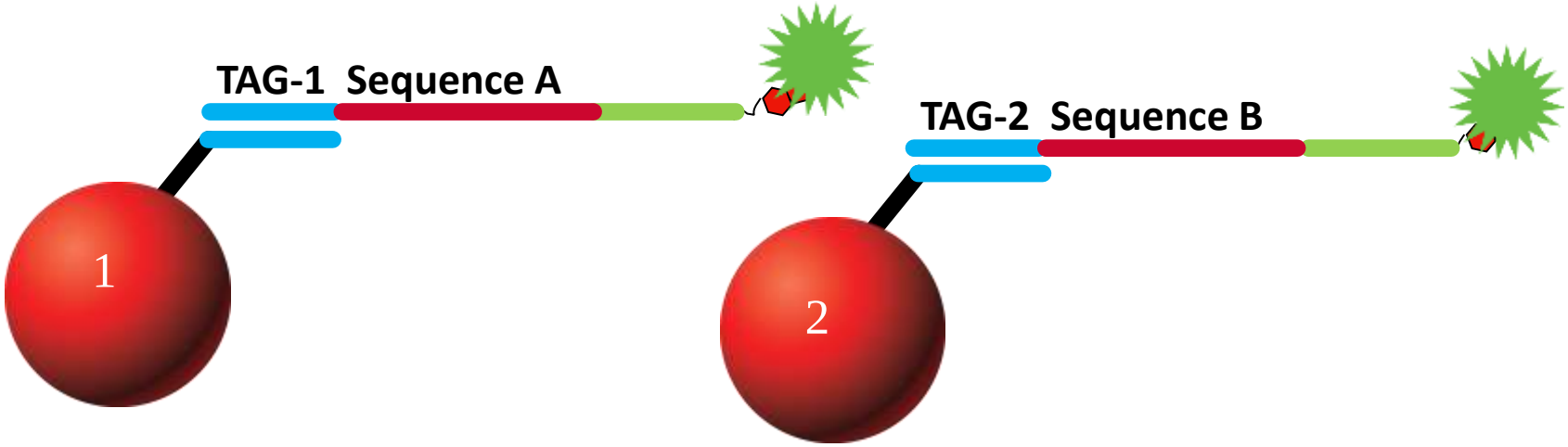
Requires optimization of coupled capture oligonucleotides



xTAG Technology

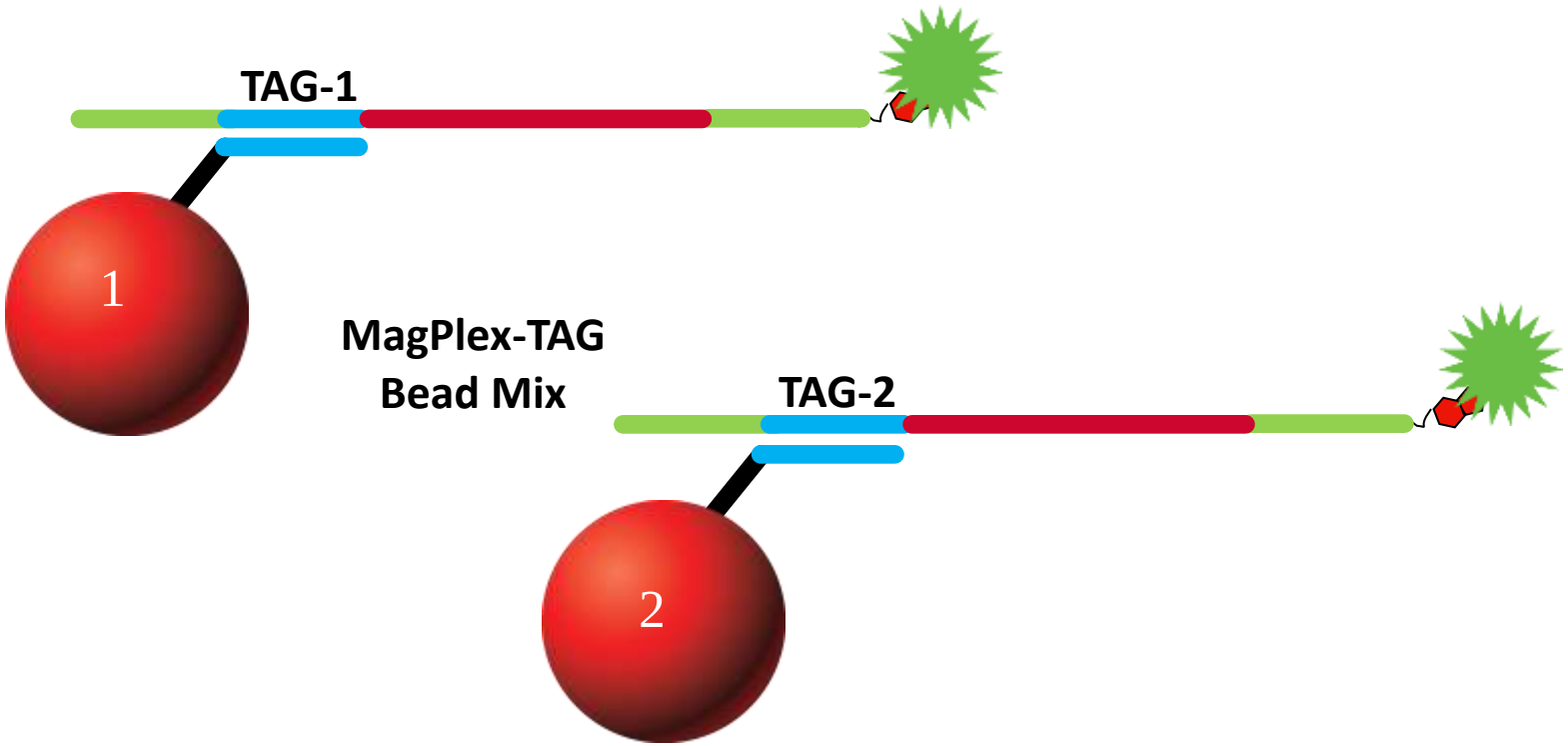
Simplifying Nucleic Acid Assays

- Proprietary sets of 24 base oligonucleotides are coupled to xTAG beads
- Each xTAG bead region's tag is complementary to a specific tag sequence engineered onto reporter molecules
- This approach as been validated and used in multiplexed clinical genetic tests



Key to using xTAG Technology

For Simplifying Nucleic Acid Assays



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Using xTAG Technology

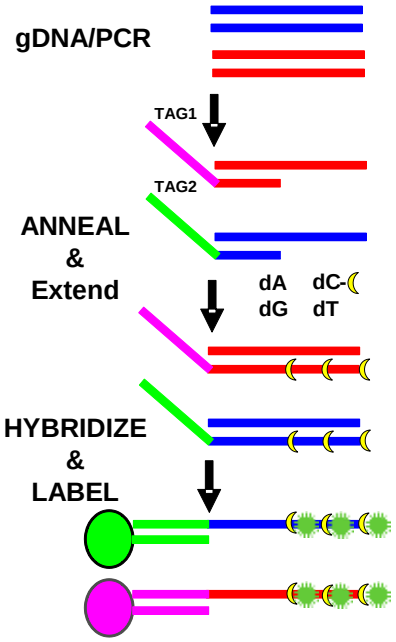
For Different types of Nucleic Acid Assays

- Gene expression
- SNP typing, Specific sequence detection
- Copy Number Variation Analysis (CNV)
- miRNA analysis
- And variations of these chemistries for a number of applications

xTAG Chemistries

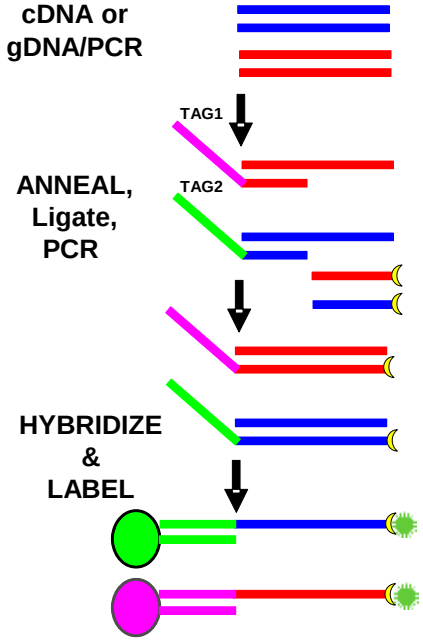
For Different DNA Analysis Assays

Allele Specific Primer Extension



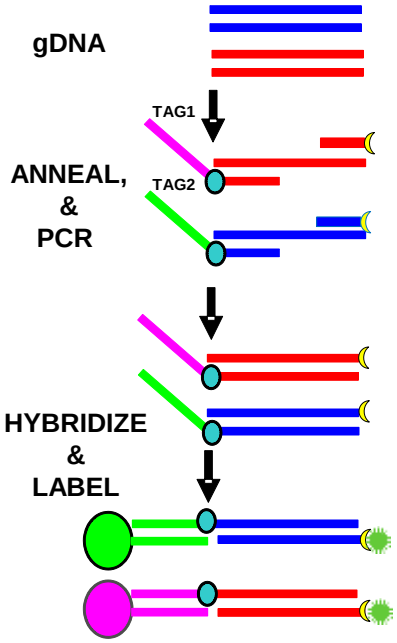
SNP typing, CF, CYP 450, RVP

LMA (OLA)



Gene expression, SNP typing, CNV, etc

Target Specific PCR



RVP FAST, GPP FAST

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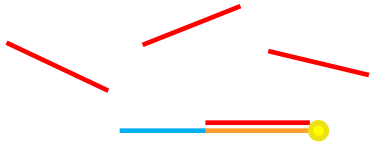
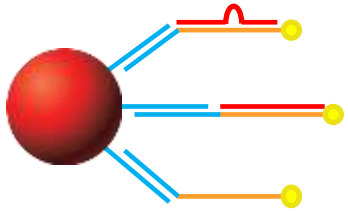
miRNA Analysis:

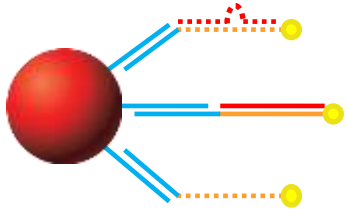
Nuclease Protection Approach



Step-down Probe Hybridization – DNA/RNA chimeric probes hybridize to target miRNAs during incremental reductions in annealing temperature.
2 hours



Microsphere Hybridization – miRNA-chimeric probe complexes are hybridized to microspheres.
30 minutes



RNase Digestion – Excess probes, single-stranded RNAs and mismatched probes are digested. Only perfectly-matched probes are protected.
30 minutes

SAPE Incubation – A brief incubation with streptavidin-conjugated R-Phycoerythrin (SAPE) incorporates reporter molecules.
30 minutes

Detection –Targets of interest are quantified on a Luminex instrument.
< 5 hours total to results

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LMA Multiplex Gene Expression Assays

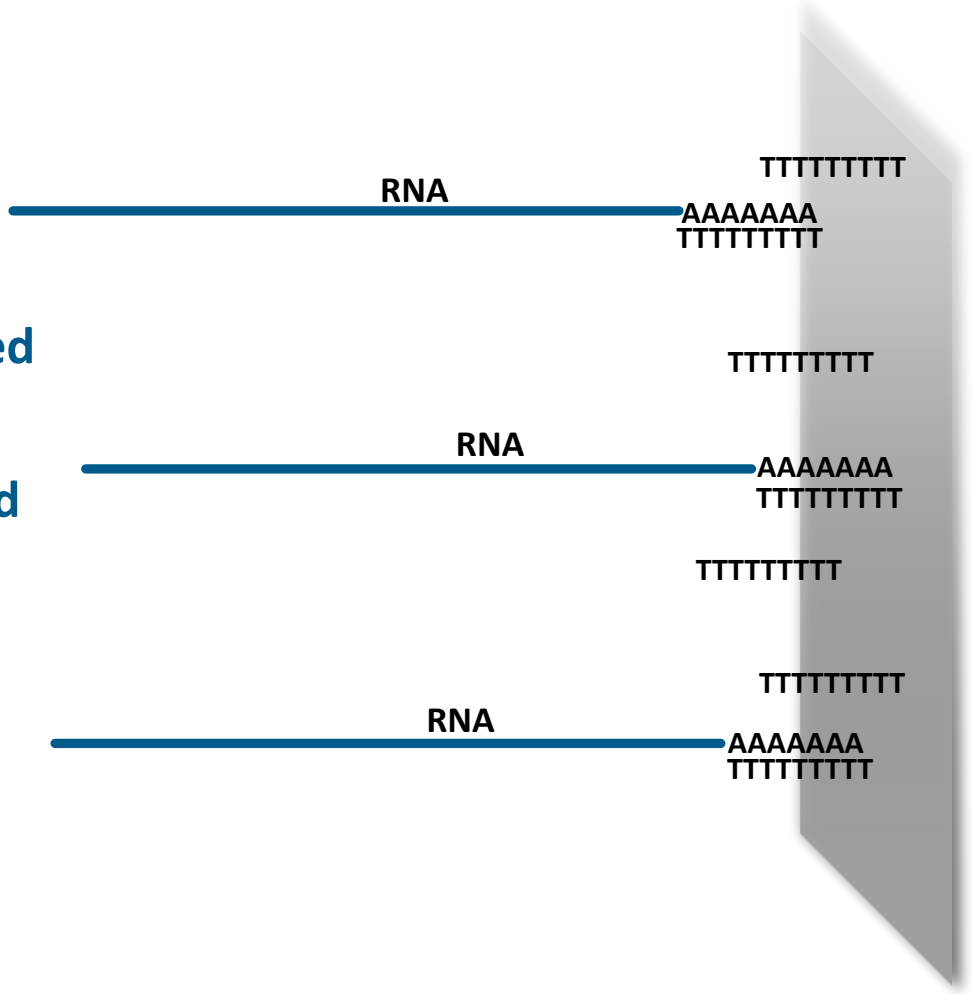
cDNA required for LMA Gene Expression Assay

LMA = Ligation Mediated Amplification

- Oligo-dT primed cDNA synthesis.
  - ✓Can generate cDNA only from poly-A tailed mRNAs
  - ✓Can be optimized with poly-A capture methods
  - ✓Can be more sensitive than TaqMan and other PCR methods
- Random primed cDNA synthesis.
  - ✓Can generate cDNA from all RNA species
  - ✓Can also detect non-ploy-A transcripts
  - ✓Slightly shorter protocol than poly-A capture method

LMA Poly-A mRNA Capture Approach

- **Add sample**
  - » **Cell lysates**
  - » **Purified Total RNA**
- **Polyadenylated RNA is captured with immobilized poly-T**
- **Unbound materials are washed away**



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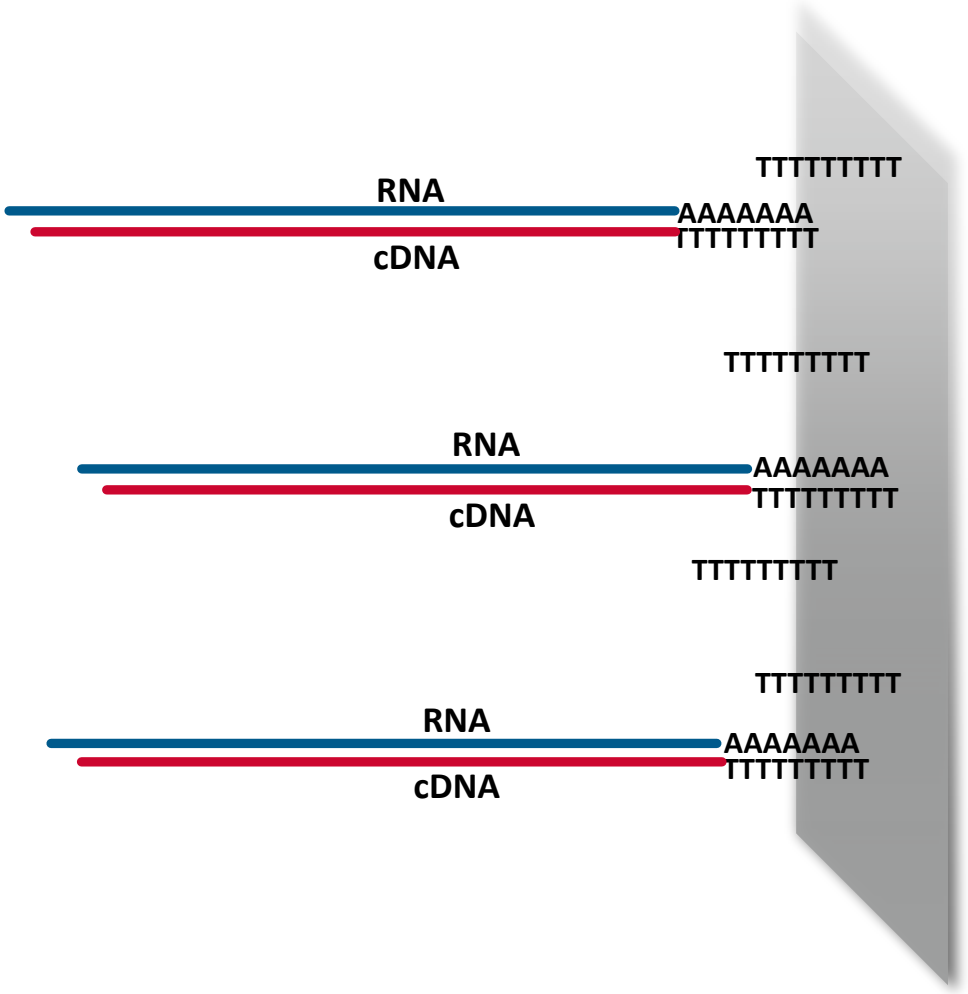
LMA Poly-A mRNA Capture Approach

cDNA Synthesis

- **RT Synthesis of 1st strand cDNA**



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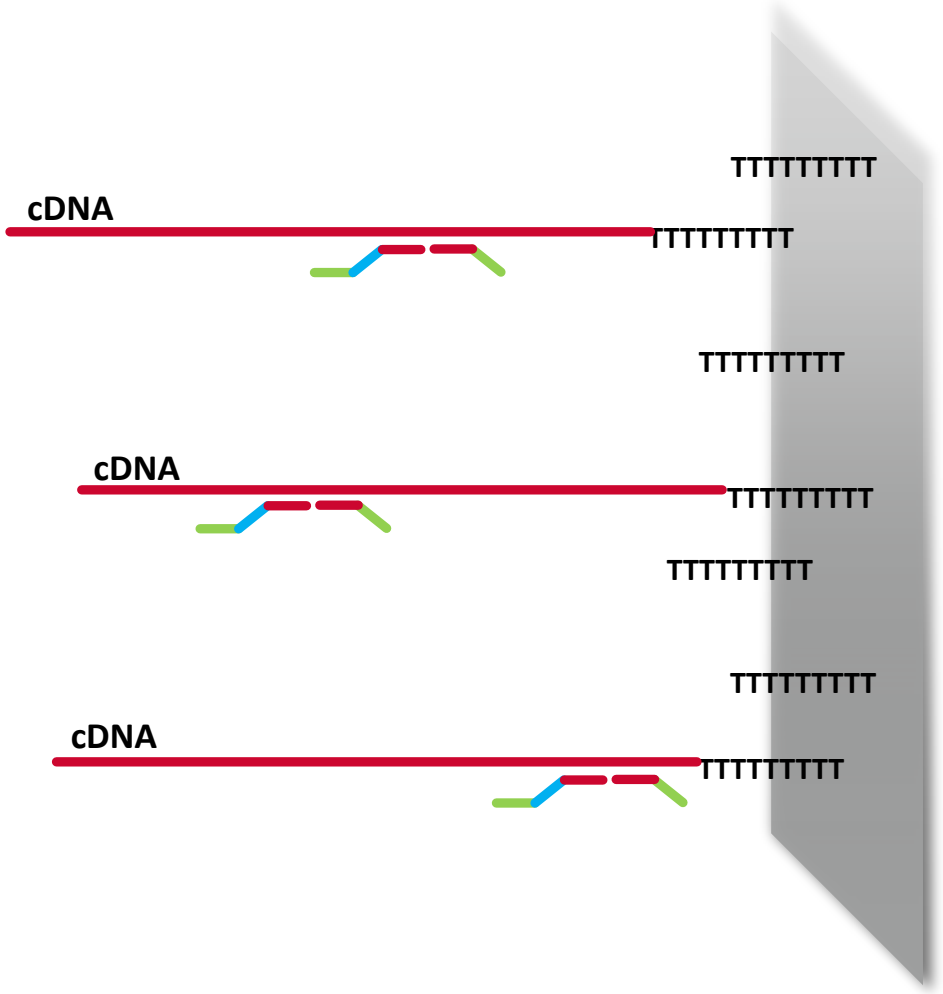
LMA Poly-A mRNA Capture Approach

Probe Hybridization

- **Probes added**
  - » **2 probes per transcript**
  - » **~25 bases of target-specific sequence per probe**
- **Overnight or shorter Hybridization**



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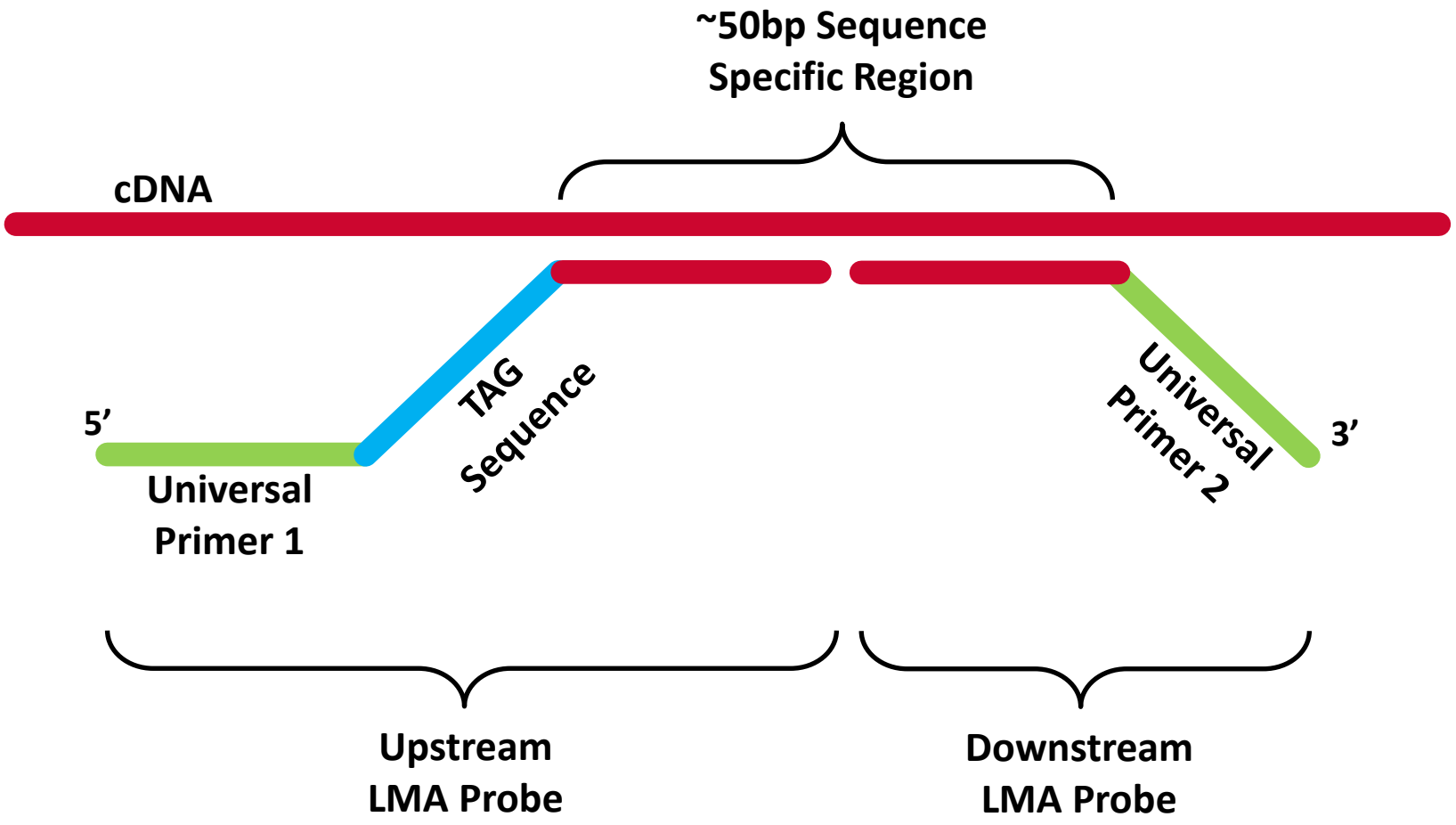
LMA Probe Design



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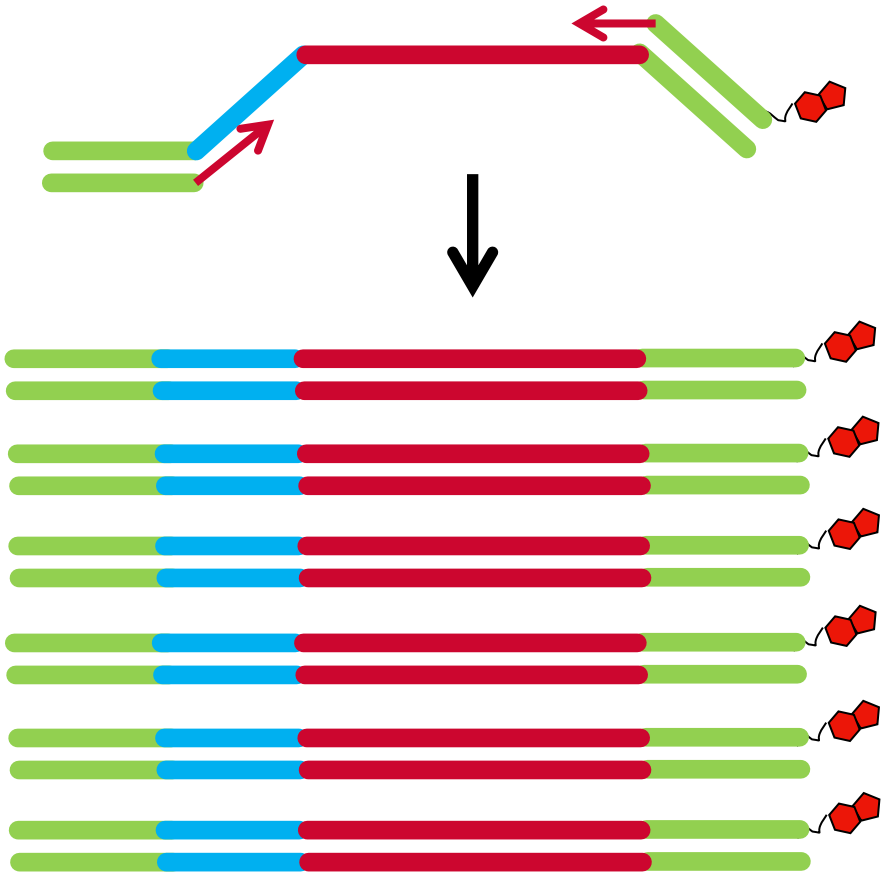
LMA Poly-A mRNA Capture Approach

PCR Amplification

- **Same universal primers used for all probe sets**
- **No size bias: all PCR products are same size**
- **Downstream primer is biotinylated**



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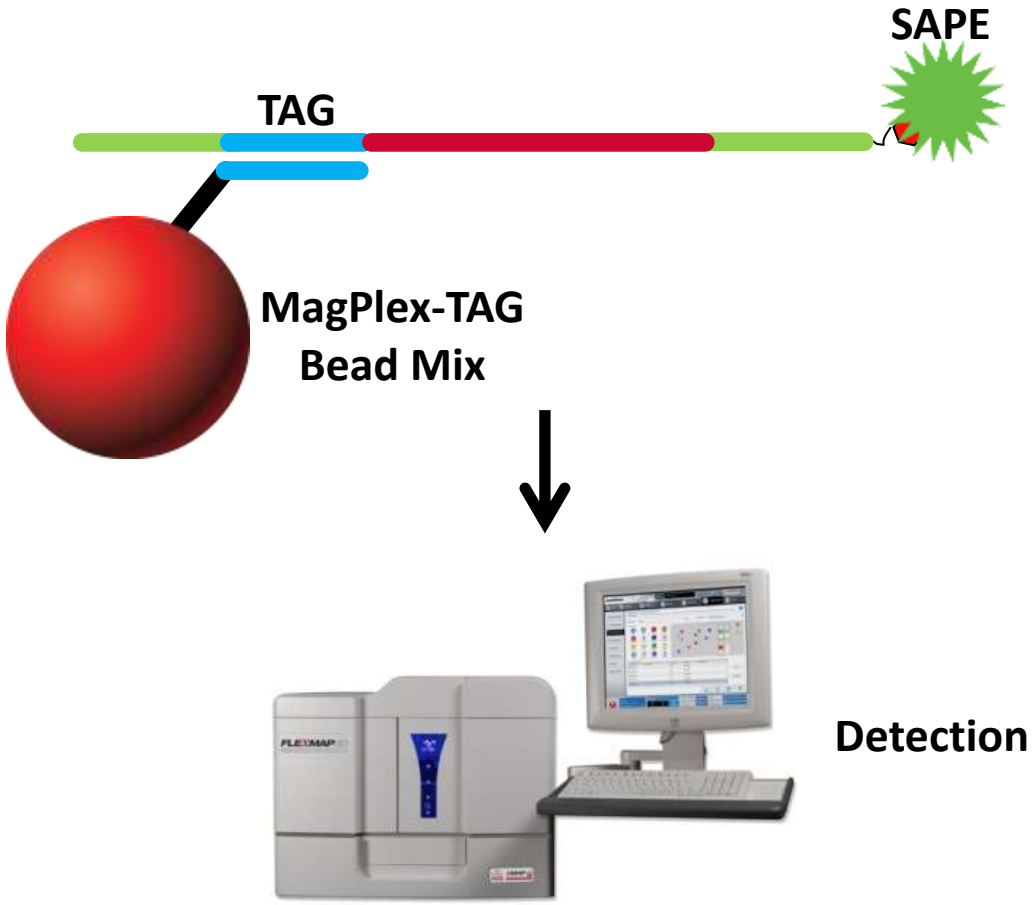
LMA Poly-A mRNA Capture Approach

Bead Hybridization and Detection

- **PCR products are λExo treated and hybridized to MagPlex-TAG beads**
- **Biotinylated products incubated with Streptavidin-Phycoerythrin (SAPE)**
- **Detection on Luminex Instruments**



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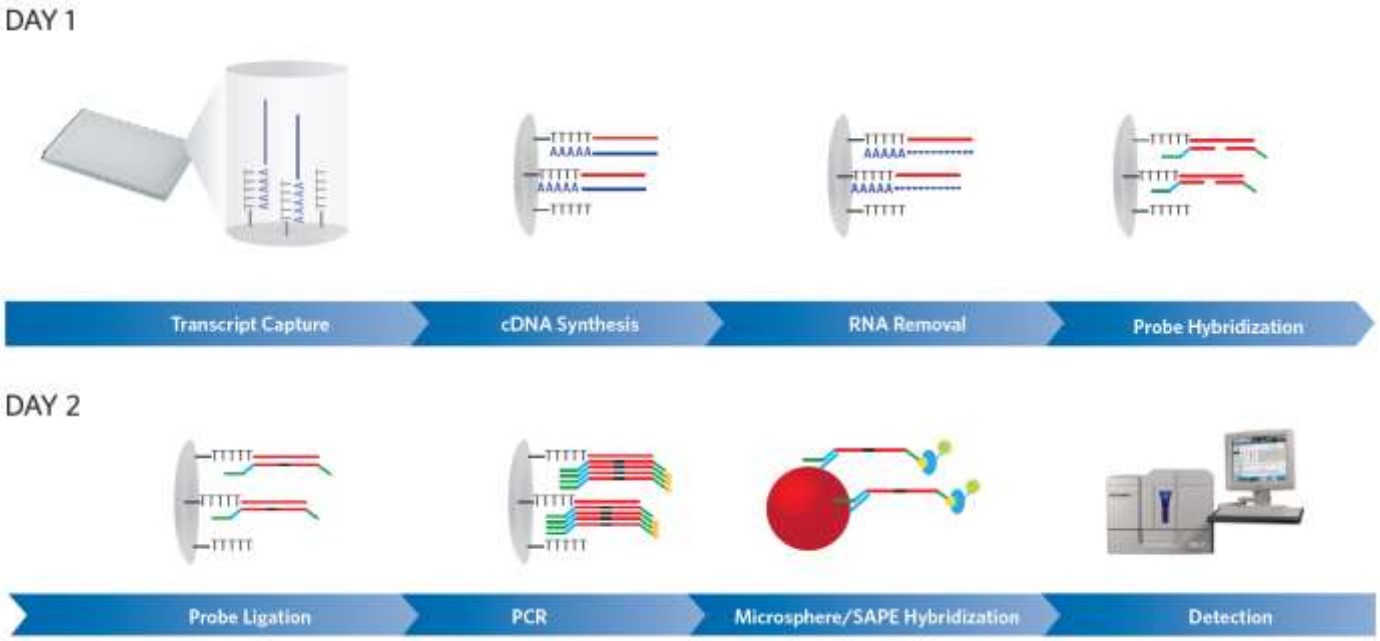
LMA Poly-A mRNA Capture Approach

Workflow



<3 Hours Setup

<7 Hours to Detection

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Literature References

Peck et al. 2006; A method for high-throughput gene expression signature analysis.

Haining et al. 2008; High-throughput gene expression profiling of memory differentiation in primary human T cells.

Shao, X. J., et al. 2011; (Chinese) Development of a Bead-based Liquid Array for Analysis of Gene Expression Profiling.

Reijans, M., et al. 2008; RespiFinder: a new multi parameter test to differentially identify fifteen respiratory viruses.

Used for L1000 platform by GENOMETRY, INC

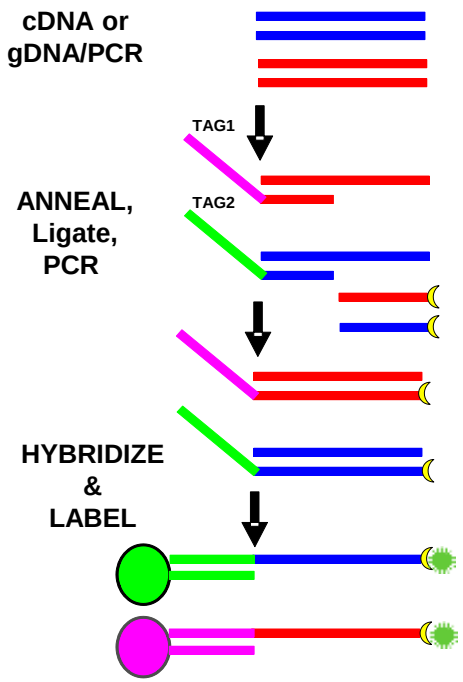
Luminex Partner Options for Genomic assays

Luminex

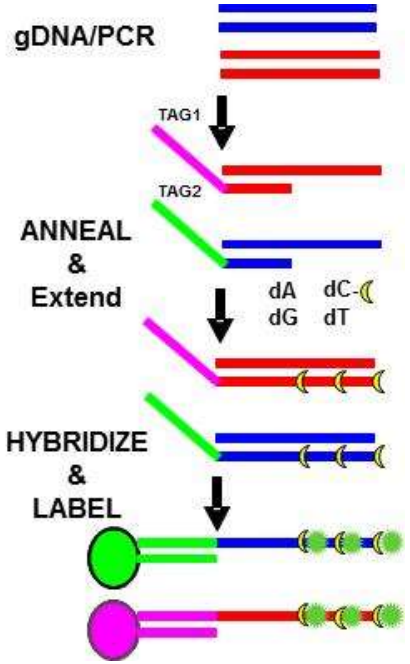
Luminex Based Multiplex SNP Genotyping Assays

Different SNP Chemistries

LMA (OLA)



Allele Specific Primer Extension



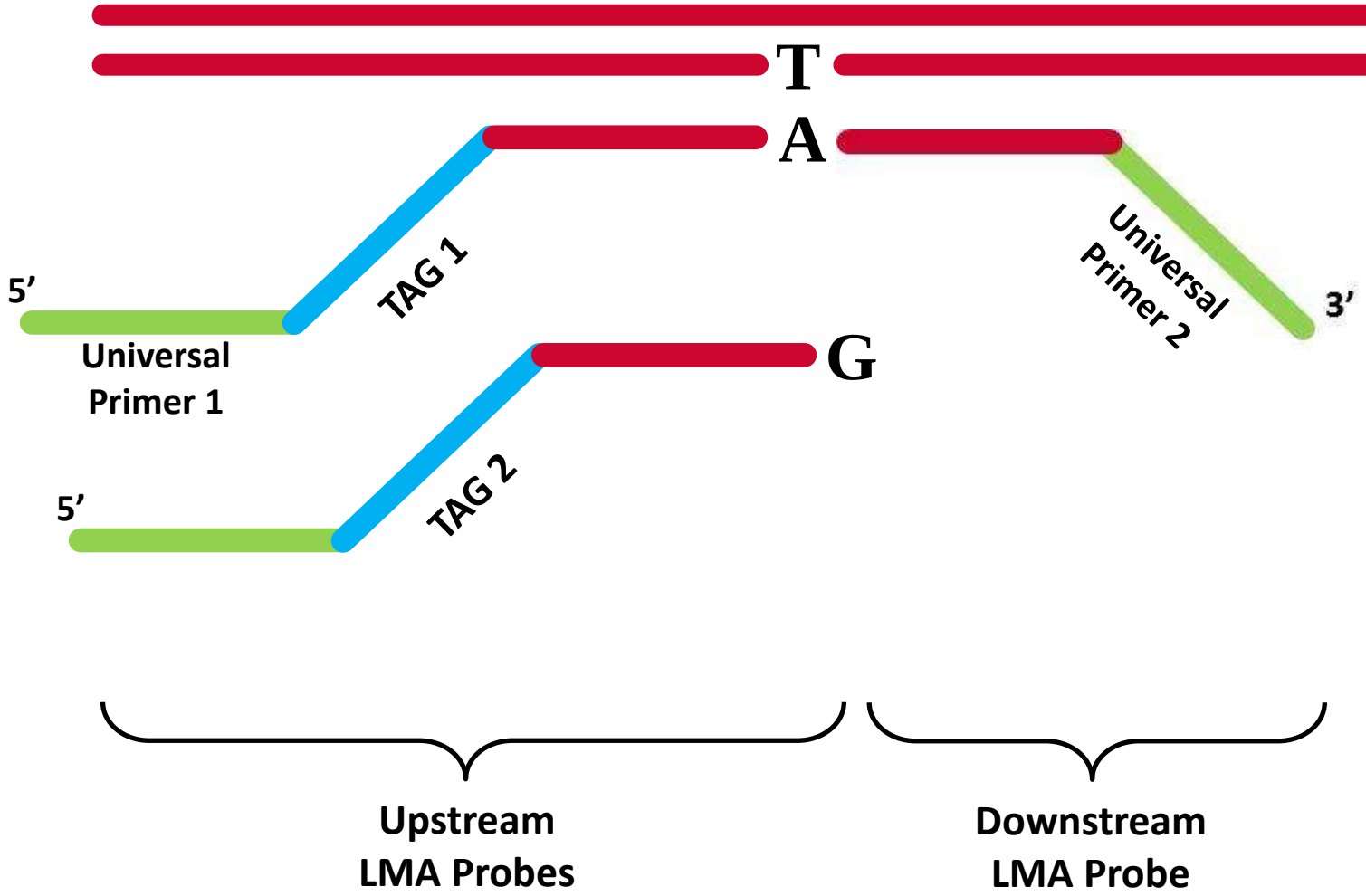
Luminex

Luminex

LMA Genotyping Assay

LMA Probe Design

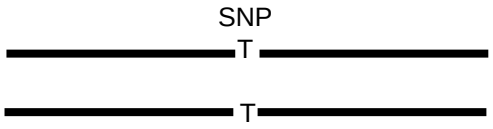
aka… LMA, MLPA, MolPCR



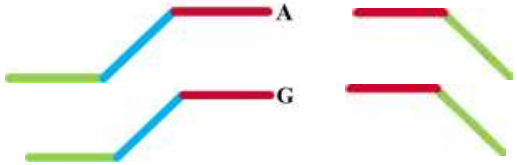
Luminex

LMA Single Nucleotide Analysis

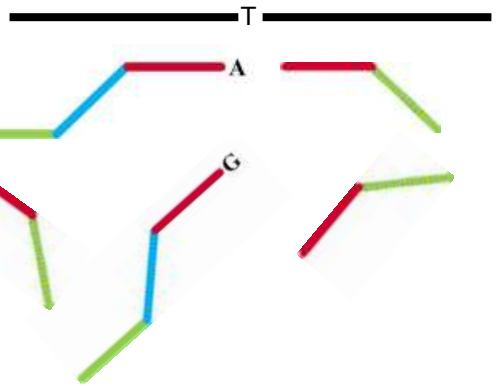
Purified Genomic DNA



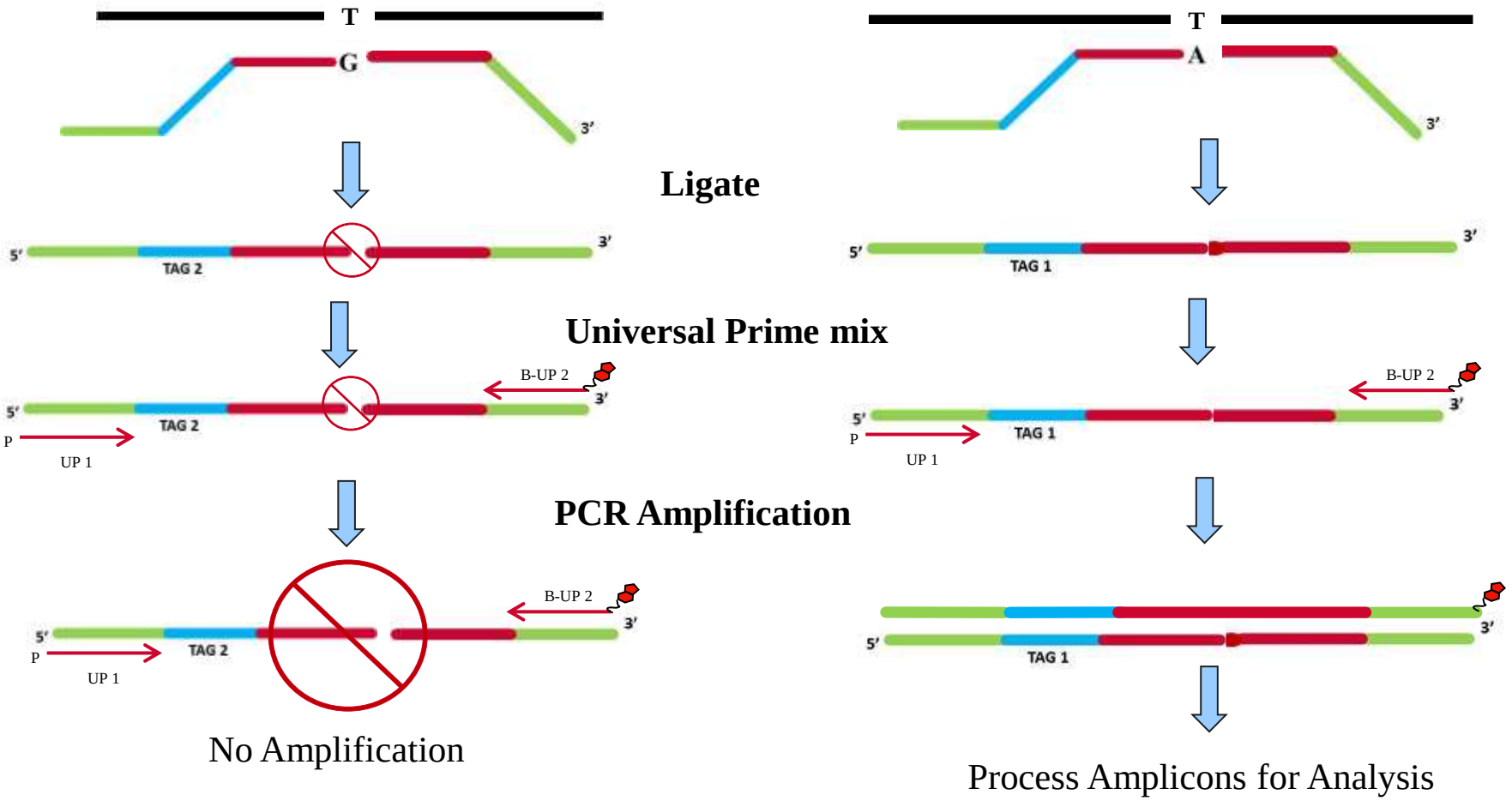
Ligation Probe Mix



Hybridize Multiplex Ligation Probes

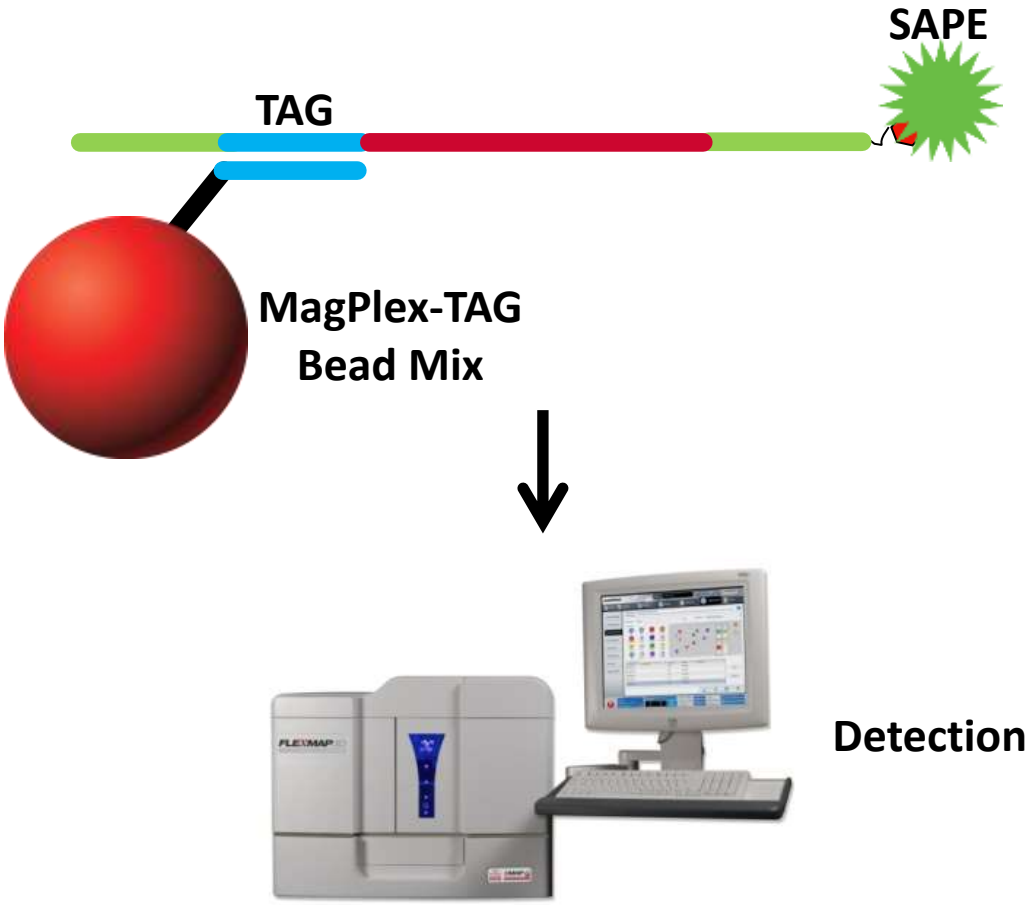


LMA Single Nucleotide Analysis



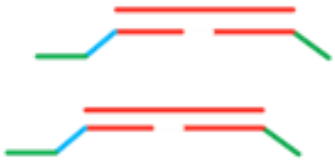
Bead Hybridization and Detection

- **PCR products are λExo treated, hybridized to MagPlex-TAG beads**
- **Biotinylated products incubated with Streptavidin-Phycoerythrin (SAPE)**
- **Detection on Luminex Instruments**



Luminex

LMA Genotyping Workflow



Probe Hybridization

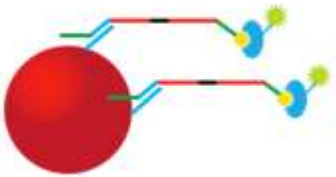
Overnight or less Incubation

Probe Ligation

70 minutes

PCR Signal Amp

2.5 hours



Bead Hyb

80 minutes

Detection

20-75 Minutes

- **Overnight or shorter Incubation Followed by Data Collection**
- **Automation-Friendly Workflow**
- **PCR Uses Universal Primers for Unbiased Signal Amplification**
- **Optimized for Fast Read Times**

Luminex

Literature References

Kaderali et al 2003; Primer-design for multiplexed genotyping

Zhi, J. and E. Hatchwell 2008; Human MLPA Probe Design (H-MAPD): a probe design tool for bothelectrophoresis-based and bead-coupled human multiplexligation-dependent probe amplification assays

Milosevic et al. 2010 Development and validation of a comprehensive mutation and deletion detection assay for SDHB, SDHC, and SDHD

Deshpande et al. 2010; A rapid multiplex assay for nucleic acid-based diagnostics

Song et al. 2010; Simultaneous Pathogen Detection and Antibiotic Resistance Characterization Using SNP-Based Multiplexed Oligonucleotide Ligation-PCR (MOL-PCR)

Stucki et al. 2012; Two new rapid SNP-typing methods for classifying *Mycobacterium tuberculosis* complex into the main phylogenetic lineages

Deshpande & White 2012; Multiplexed nucleic acid-based assays for molecular diagnostics of human disease

Luminex

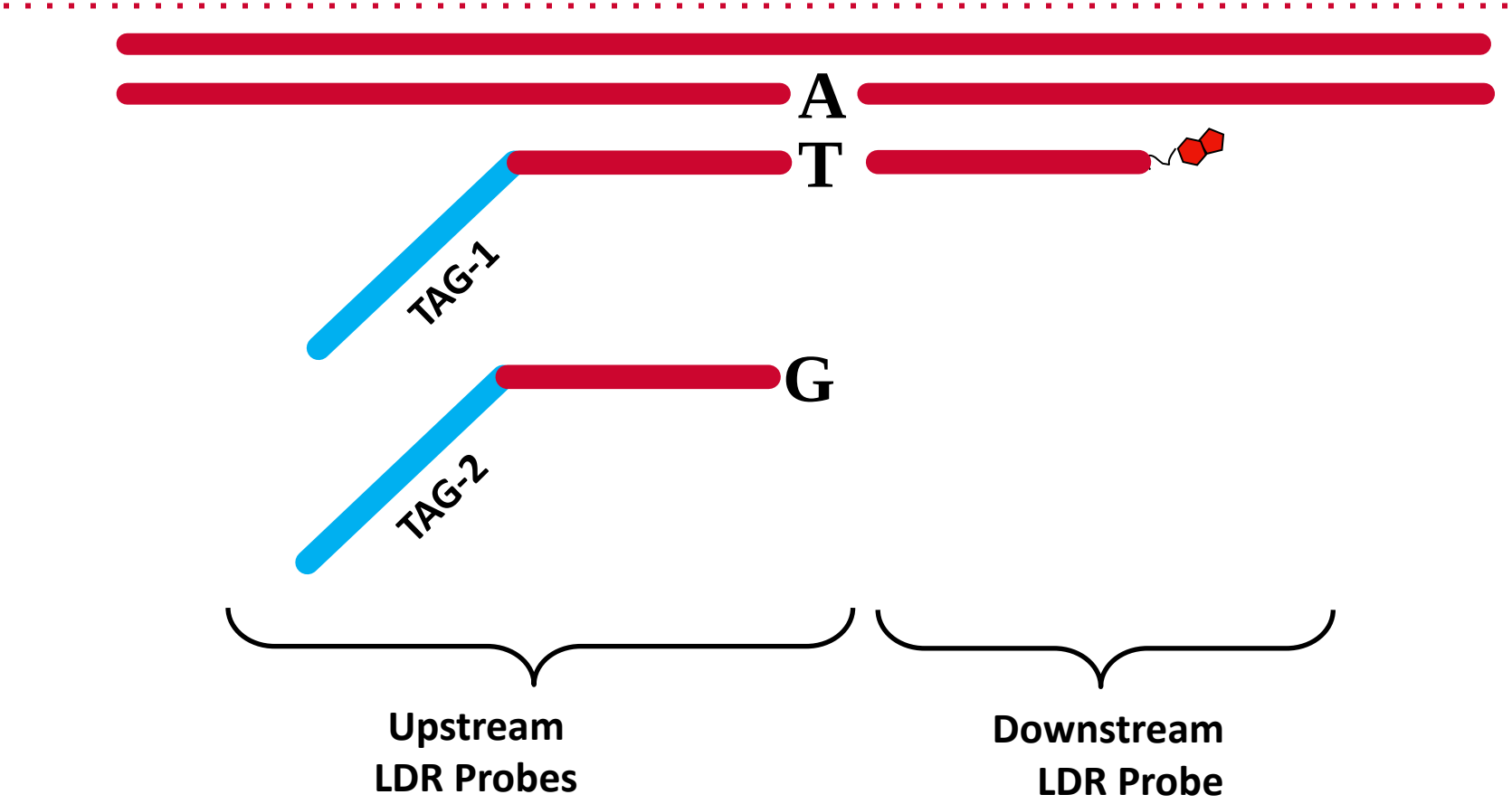
OLA (LDR-FMA) Genotyping Assay

OLA (LDR-FMA) Genotyping

OLA = Oligo Ligation Amplification
LDR-FMA = ligase detection reaction - fluorescent microsphere assay

Generate ligation probe target regions of interest with PCR

OLA (LDR-FMA) Probe Design



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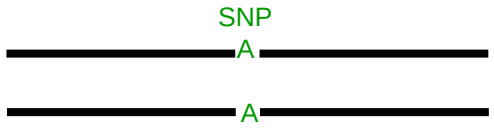
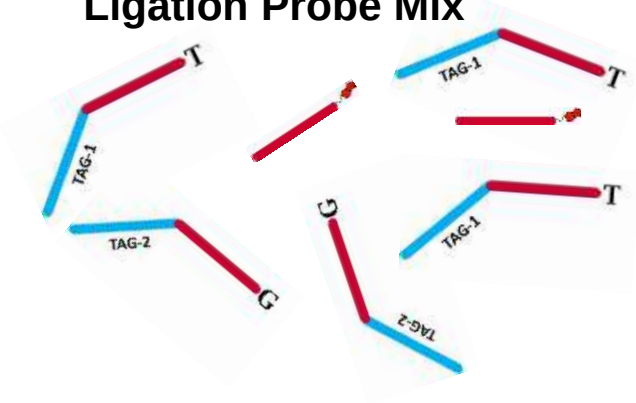
OLA Single Nucleotide Analysis

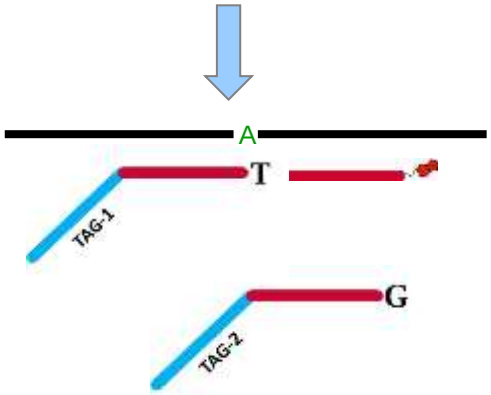
Amplified Genomic Target



Ligation Probe Mix

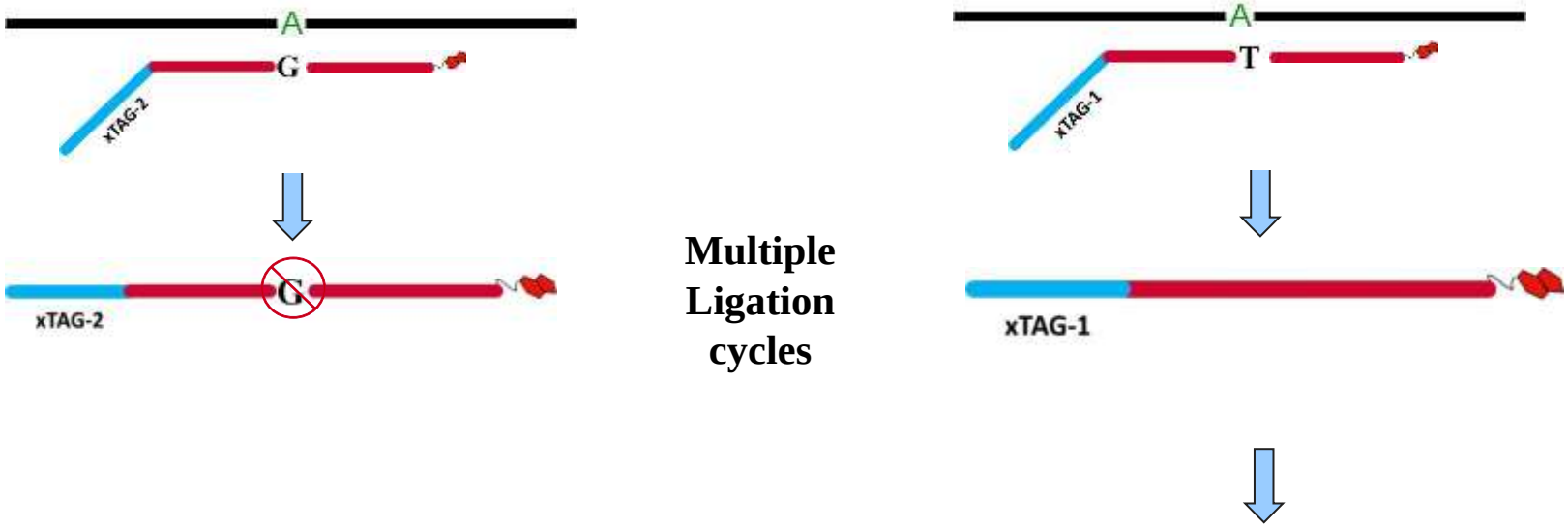


Hybridize Multiplex Ligation Probes



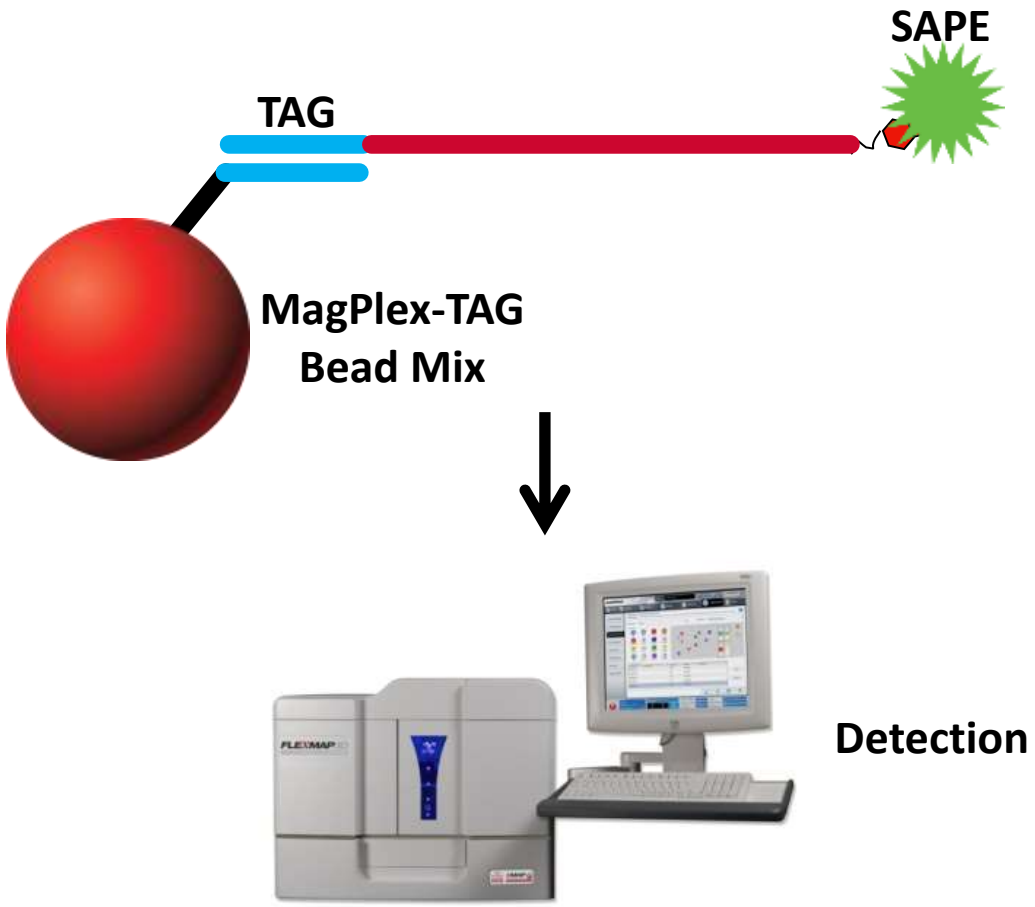
Luminex

OLA Single Nucleotide Analysis



Bead Hybridization and Detection

- **hybridized to MagPlex-TAG beads**
- **Biotinylated products incubated with Streptavidin-Phycoerythrin (SAPE)**
- **Detection on Luminex Instruments**



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Literature References

Carnevale et al. 2007; A Multiplex Ligase Detection Reaction-Fluorescent Microsphere Assay for Simultaneous Detection of Single Nucleotide Polymorphisms Associated with *Plasmodium falciparum* Drug Resistance

Bruse et al. 2008; Improvements to bead-based oligonucleotide ligation SNP genotyping assays

Mehlotra et al. 2011; Chemokine (C-C motif) receptor 5 -2459 genotype in patients receiving highly active antiretroviral therapy race-specific influence on virologic success

Henry-Halldin et al. 2012; Multiplex Assay for Species Identification and Monitoring of Insecticide Resistance in *Anopheles punctulatus* Group Populations of Papua New Guinea

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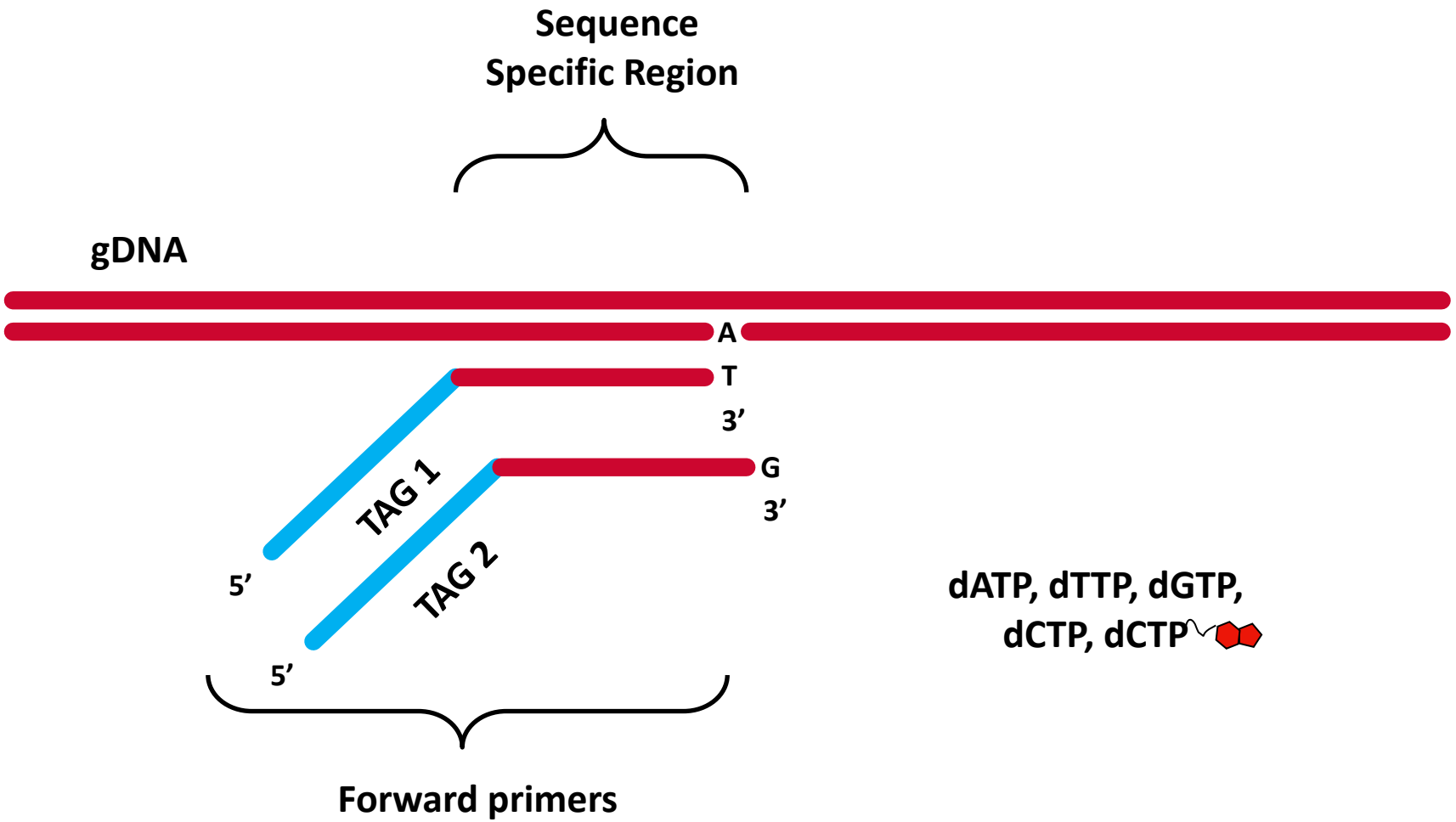
ASPE SNP Typing Assays

ASPE Probe Design

With one Biotinylated-Nucleotide Chemistry



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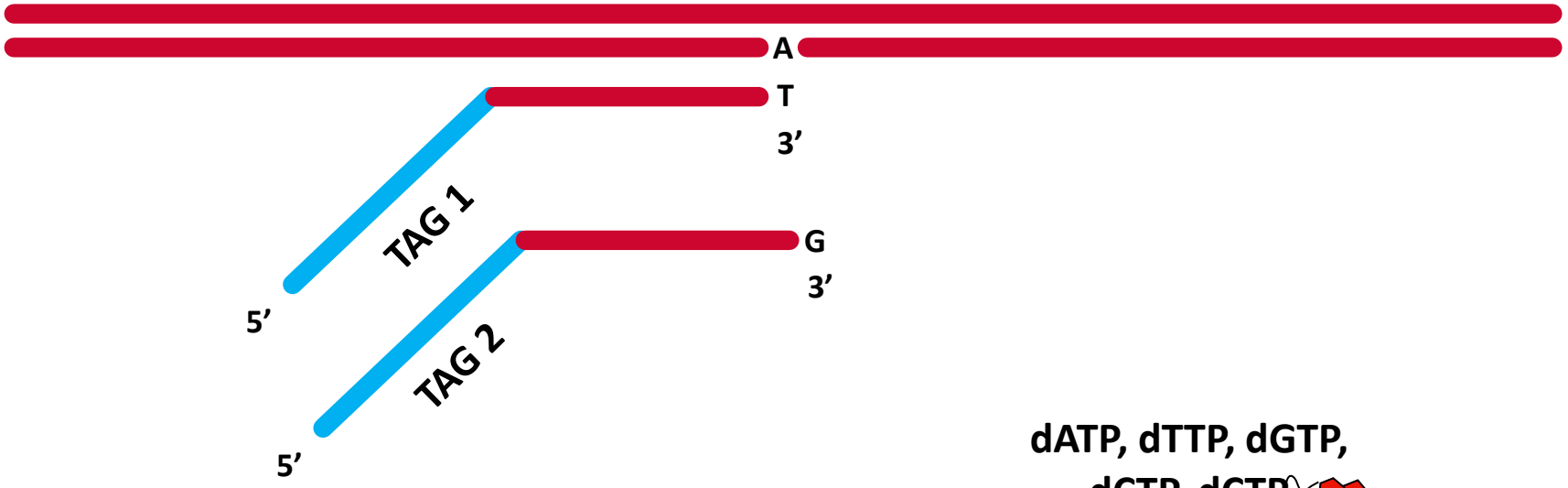
ASPE SNP Genotyping

gDNA Target Amplification

Amplify probe target regions of interest with PCR

ASPE SNP Detection

Primer Extension on amplified regions



dATP, dTTP, dGTP, dCTP, dCTP

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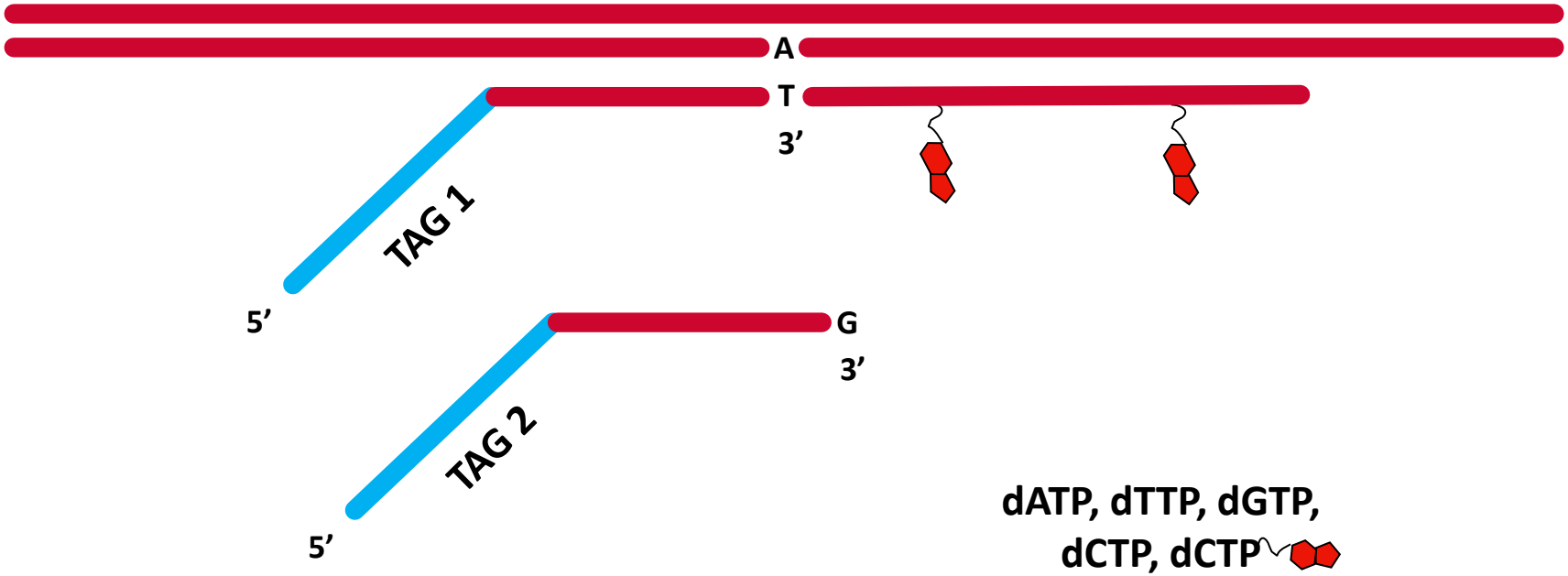
ASPE SNP Detection

Primer Extension on amplified regions



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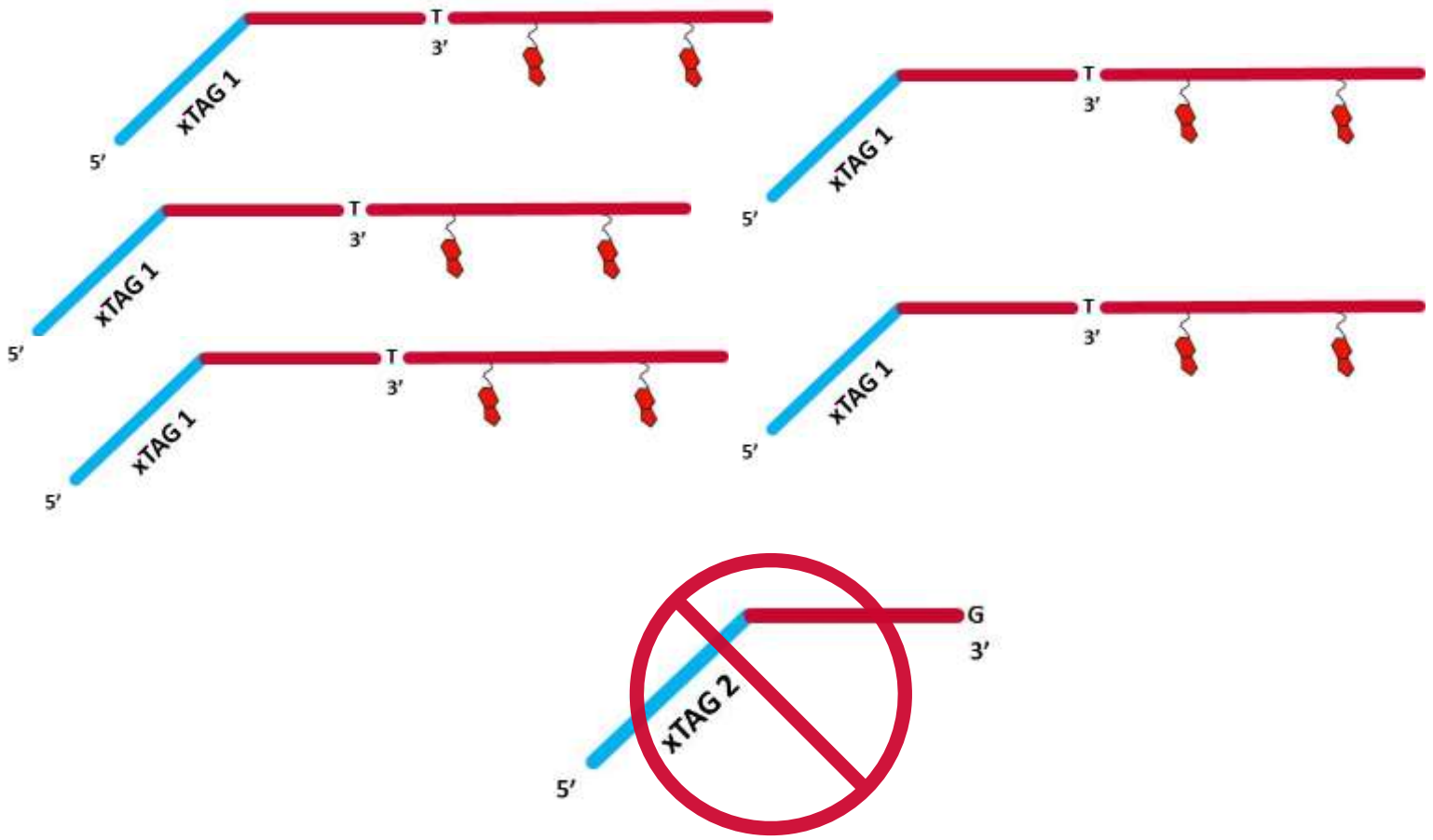
ASPE SNP Detection

Primer with complimentary base is extended and biotynalated



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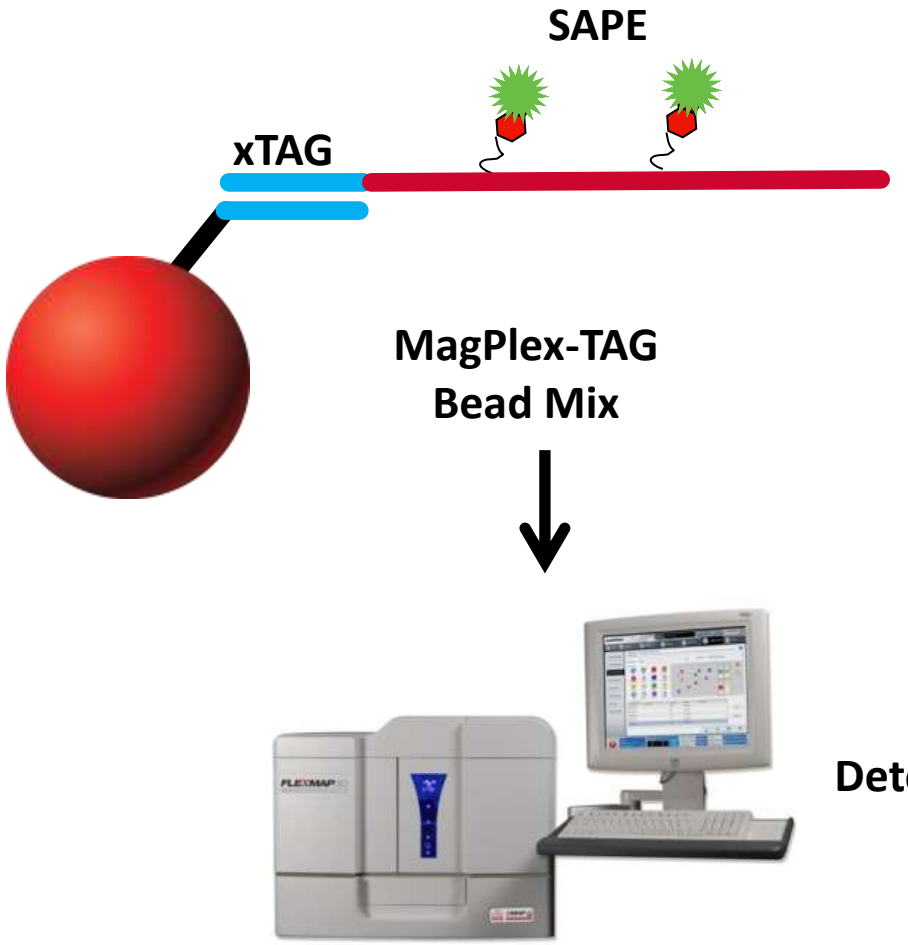
ASPE SNP Detection

With Biotinylated-nucleotide.

- **PCR products hybridized to MagPlex-TAG beads**
- **Biotinylated products incubated with Streptavidin-Phycoerythrin (SAPE)**
- **Detection on Luminex Instruments**



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Literature References

Strom et al. 2005; Technical validation of a multiplex platform to detect thirty mutations in eight genetic diseases prevalent in individuals of Ashkenazi Jewish descent

Koo et al. 2007; Multiplexed genotyping of ABC transporter polymorphisms with the Bioplex suspension array

Li et al. 2011; A novel liquidchip platform for simultaneous detection of 70 alleles of DNA somatic mutations on EGFR, KRAS, BRAF and PIK3CA from formalin-fixed and paraffin-embedded slides containing tumor tissue

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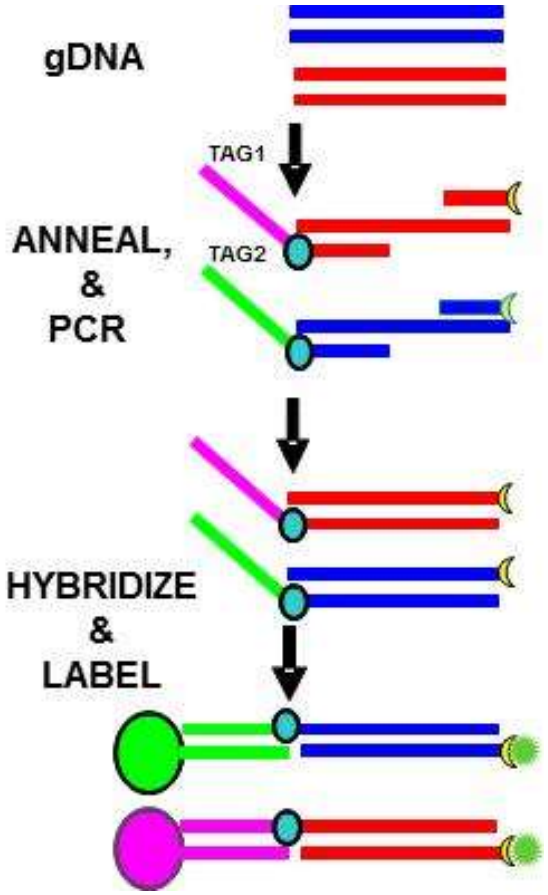
Target Specific PCR Chemistry

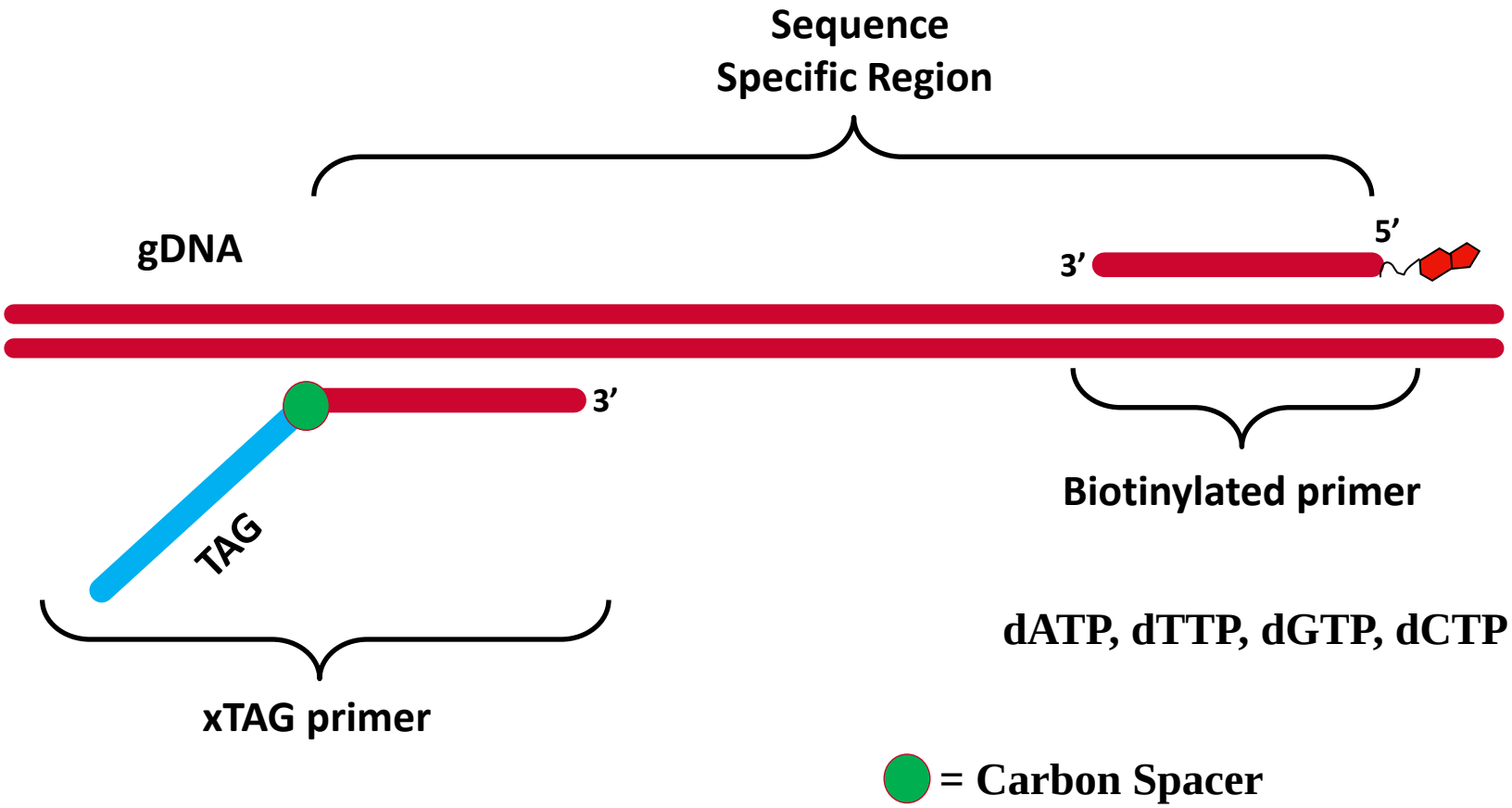
Target Specific PCR

TS-PCR Chemistry



TS PCR Probe Design

With Biotinylated primer and standard nucleotides



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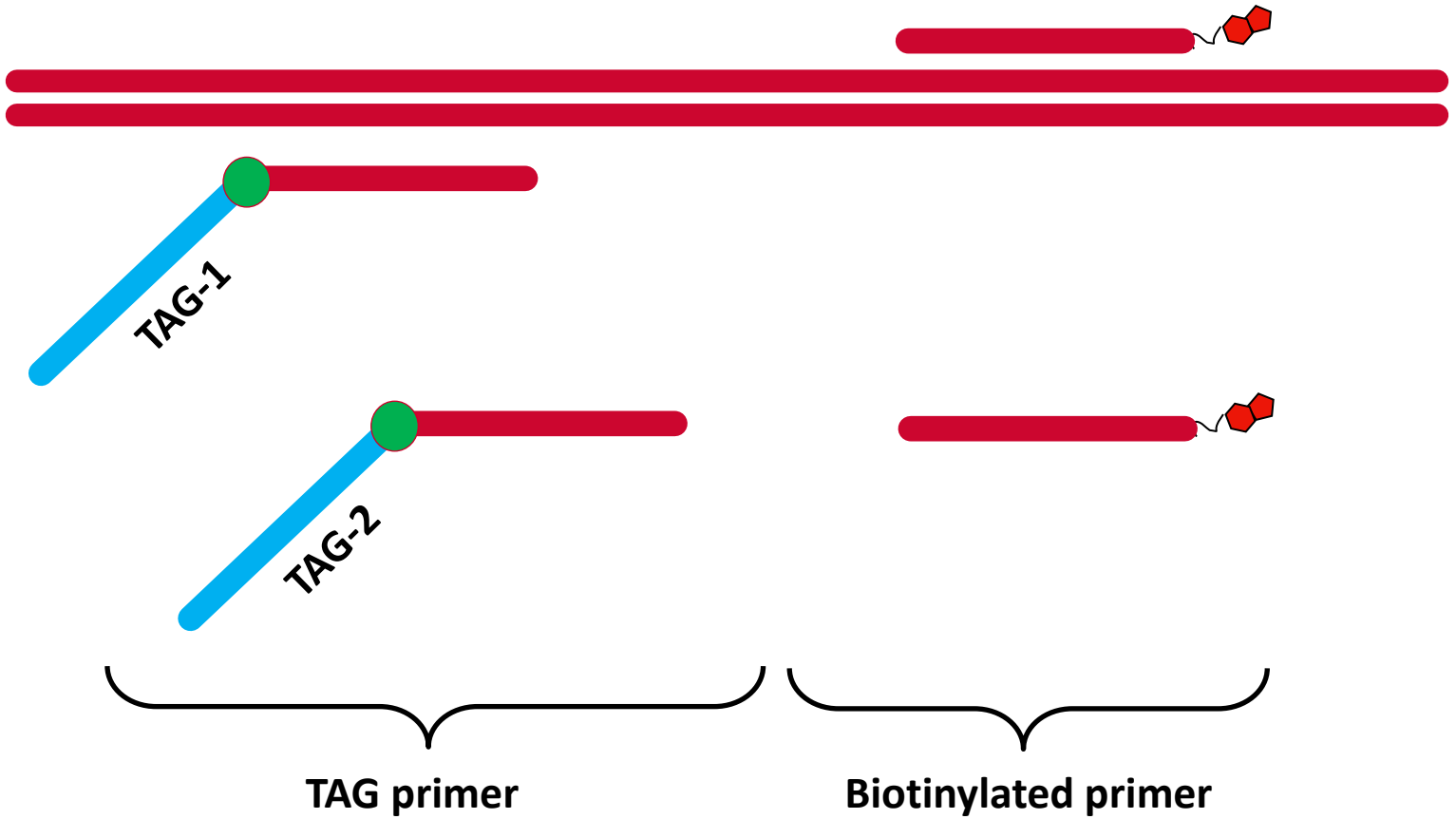
TS PCR Probe Design

With Multiplex Probe mix



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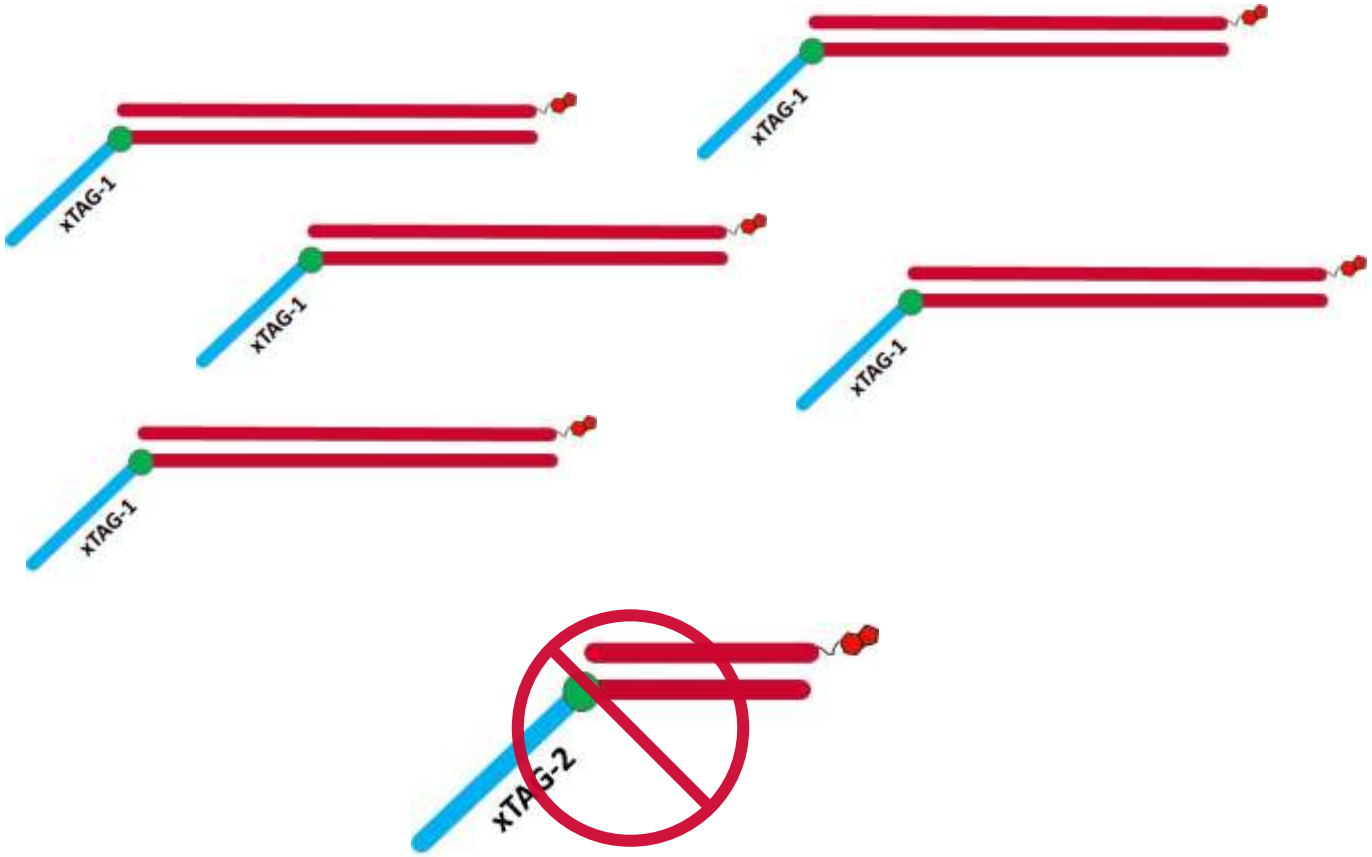
Luminex

Multiplex Amplification Products



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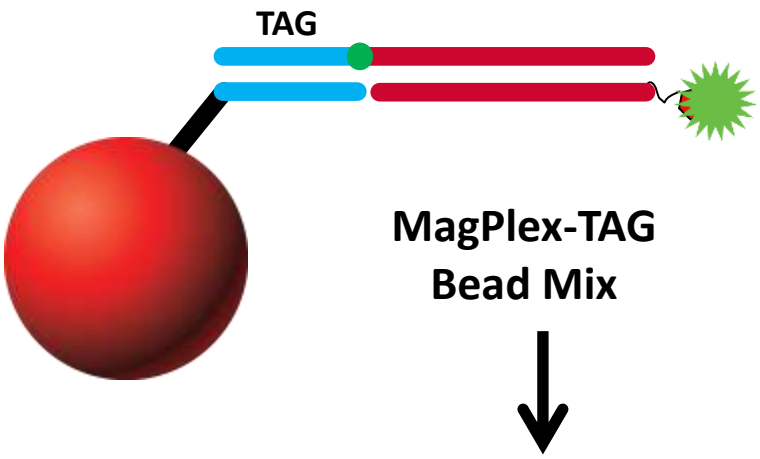
Sequence Detection

- PCR products hybridized to MagPlex-TAG beads
- Biotinylated products incubated with Streptavidin-Phycoerythrin (SAPE)
- Detection on Luminex Instruments



Detection

Luminex

FAST PCR Assay Characteristics

Summary

- **Easy and Fast**
  - Simple sample processing from a number of different sample types such as swabs, stool, cultures, etc.
  - Results in a few hours.
- **Used in Diagnostics for;**
  - Detection of 8 viruses and subtypes of Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus (RSV), Human Metapneumovirus (hMPV), Rhinovirus, and Adenovirus.
  - Detection of 11 gastrointestinal pathogens and subtypes of bacteria, viruses, and parasites of *C. difficile,* norovirus, *E. coli, Salmonella,* rotavirus A, *Campylobacter*, and *Shigella*.

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Multiplex microRNA Assay: *Nuclease protection Approach*

Multiplex miRNA Nuclease protection Assay Characteristics

- **Extremely Specific Results:**
  - »Can discriminate single base mismatches anywhere in mature miRNA sequences
- **Fast Results:**
  - »From total RNA to results in less than 5 hours with less than 30 minutes of handling
- **Unbiased Sensitivity:**
  - »No amplification or sample labeling required, yet data from 50-500ng total RNA with single nucleotide difference resolution

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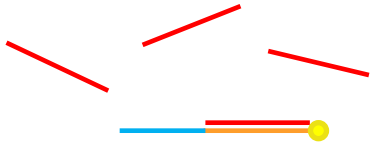
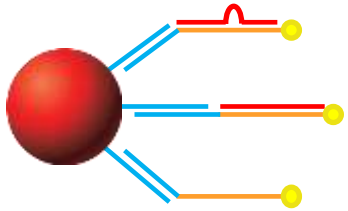
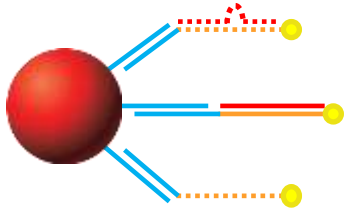
Workflow



Step-down Probe Hybridization – DNA/RNA chimeric probes hybridize to target miRNAs during incremental reductions in annealing temperature.
2 hours



Microsphere Hybridization – miRNA-chimeric probe complexes are hybridized to microspheres.
30 minutes



RNase Digestion – Excess probes, single-stranded RNAs and mismatched probes are digested. Only perfectly-matched probes are protected.
30 minutes

SAPE Incubation – A brief incubation with streptavidin-conjugated R-Phycoerythrin (SAPE) incorporates reporter molecules.
30 minutes

Detection –Targets of interest are quantified on a Luminex instrument.
< 5 hours total to results

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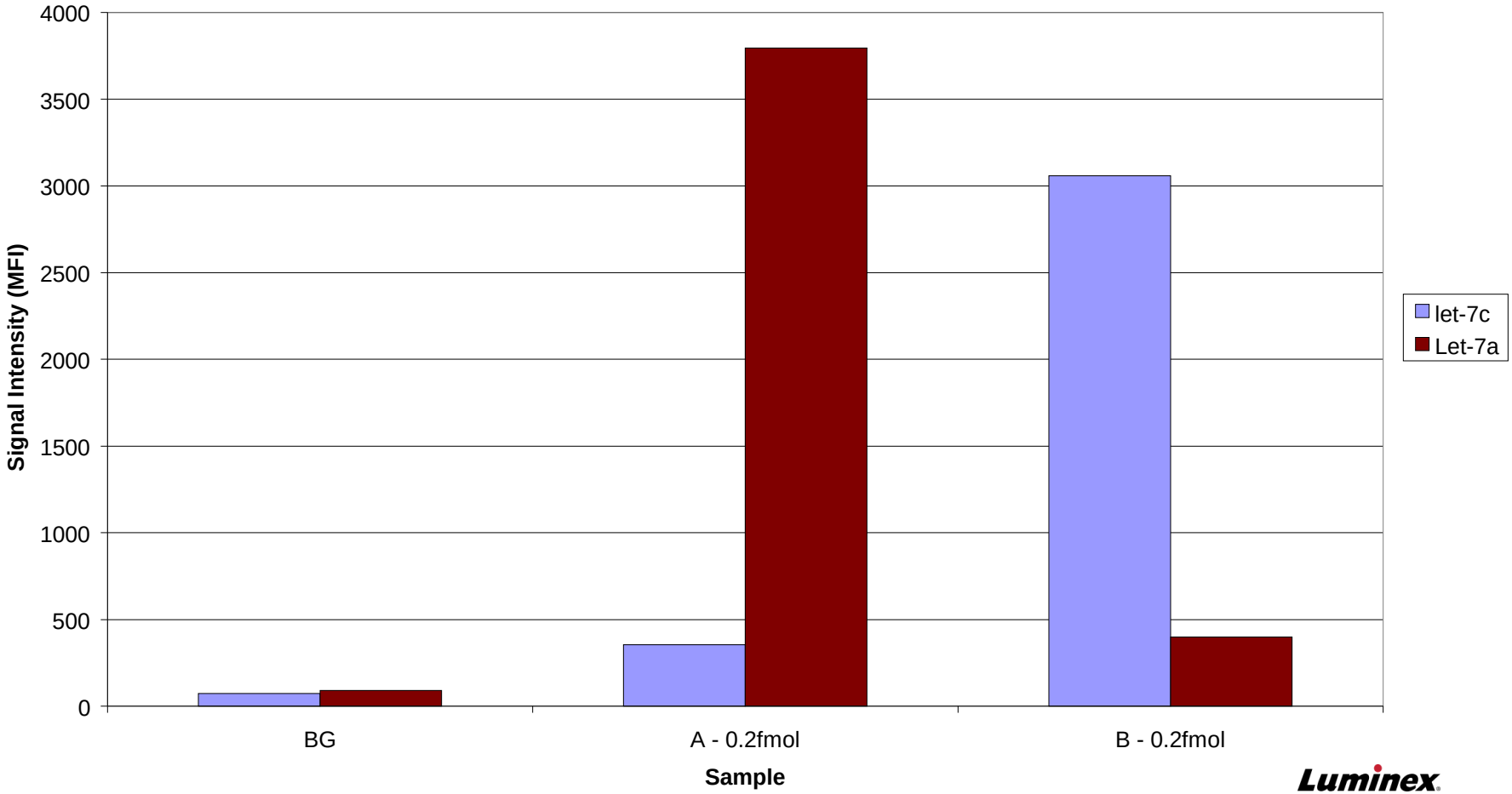
Specificity

Single Base Mismatch Discrimination

hsa-let-7a UGAGGUAGUAGGUUGUAUAGUU
hsa-let-7c UGAGGUAGUAGGUUGUAUGGUU

Discrimination of single base mismatch near ends!

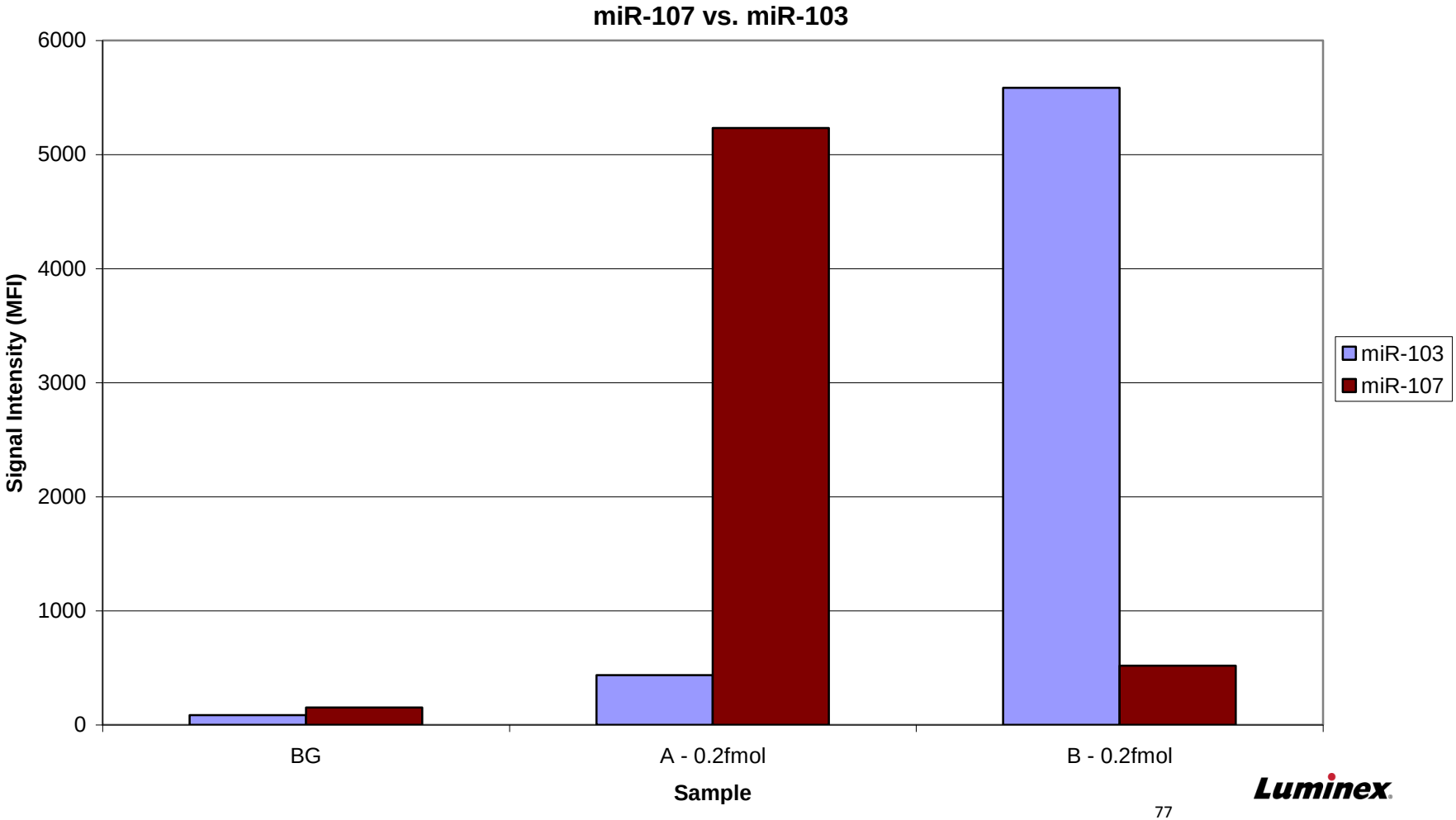


Specificity

Single Base Mismatch Discrimination

hsa-miR-107 AGCAGCAUUGUACAGGGCUAUCA
hsa-miR-103 AGCAGCAUUGUACAGGGCUAUGA

Discrimination of single base mismatch near ends!

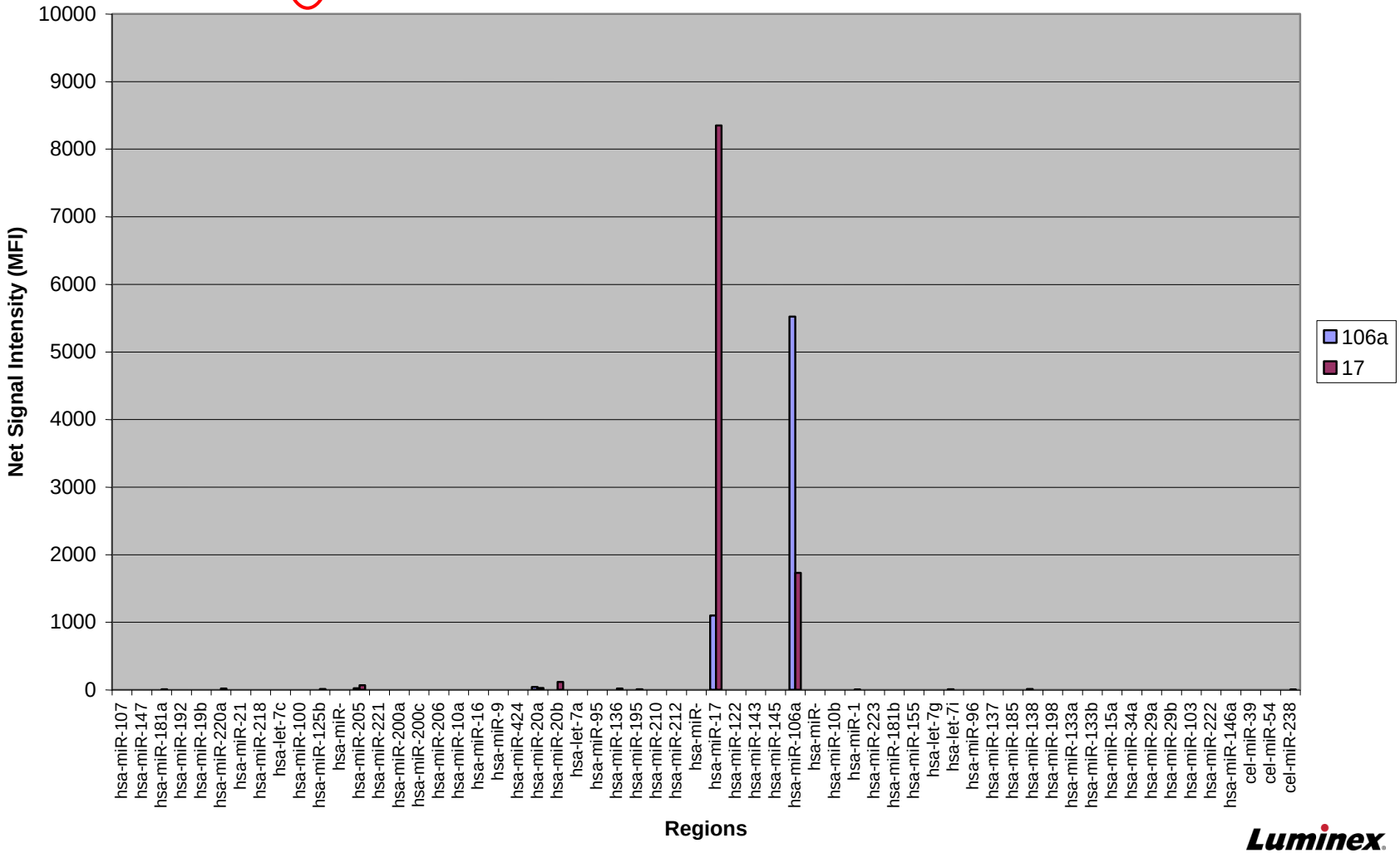


Specificity for Targets in Complex mix

Closely-Related miRNAs in 60 Plex

miR-106a CAAAGUGCUAACAGUGCAGGUAG
miR-17 CAAAGUGCUUACAGUGCAGGUAG

1.0 fmol input



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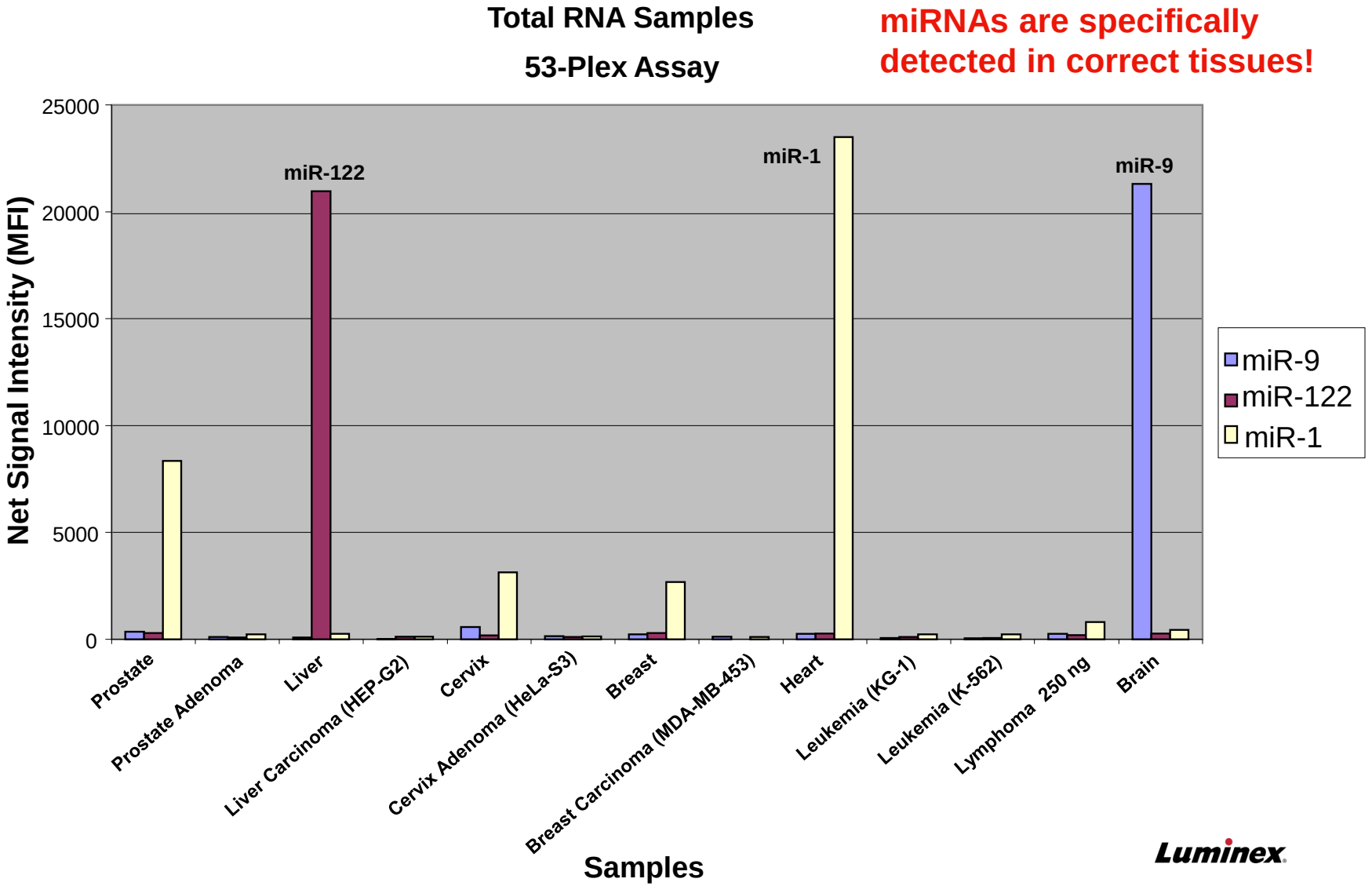
Specificity in Tissue Samples

Correct Detection of Tissue-Specific miRNAs



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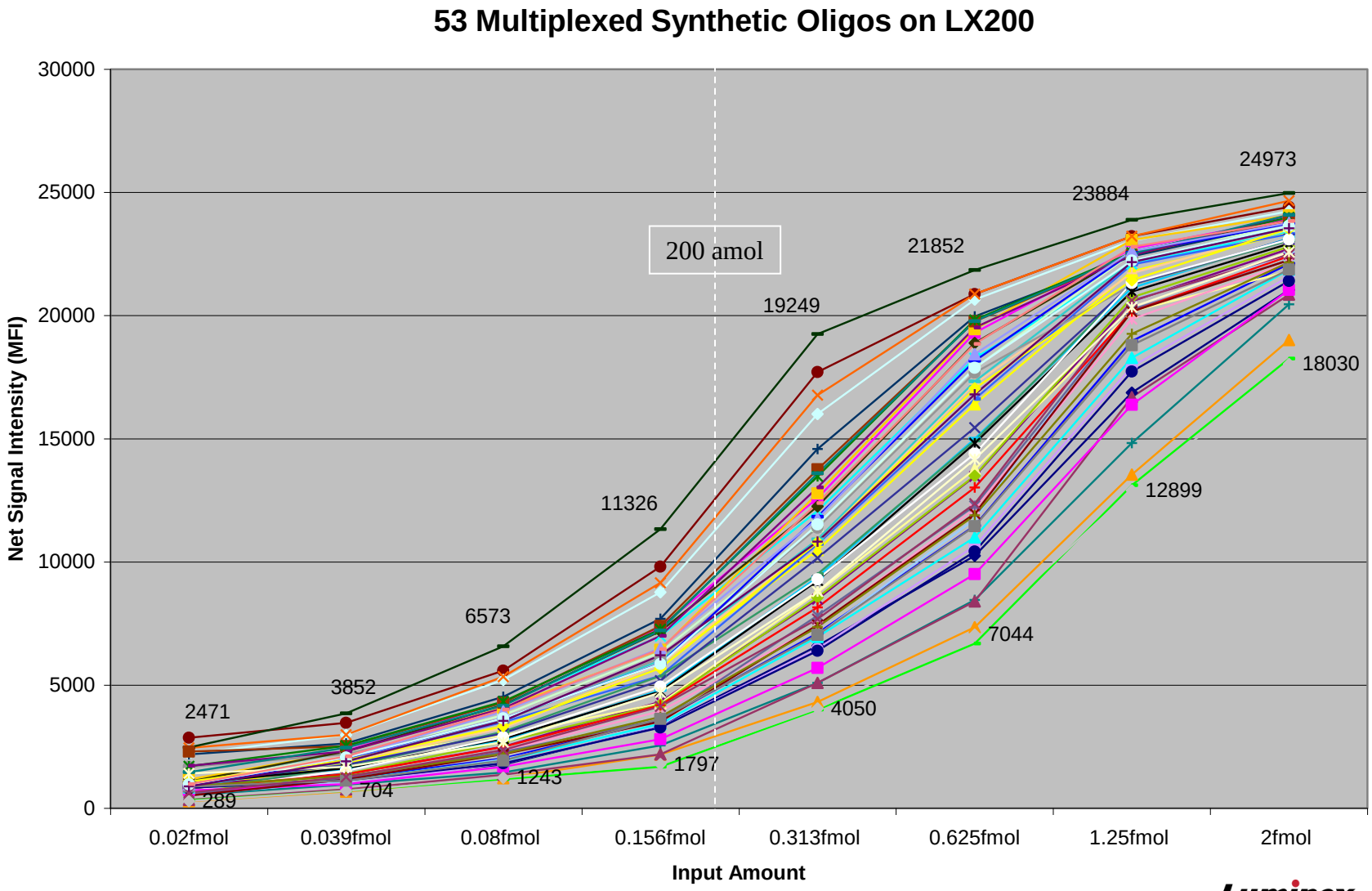
Sensitivity in Complex mix

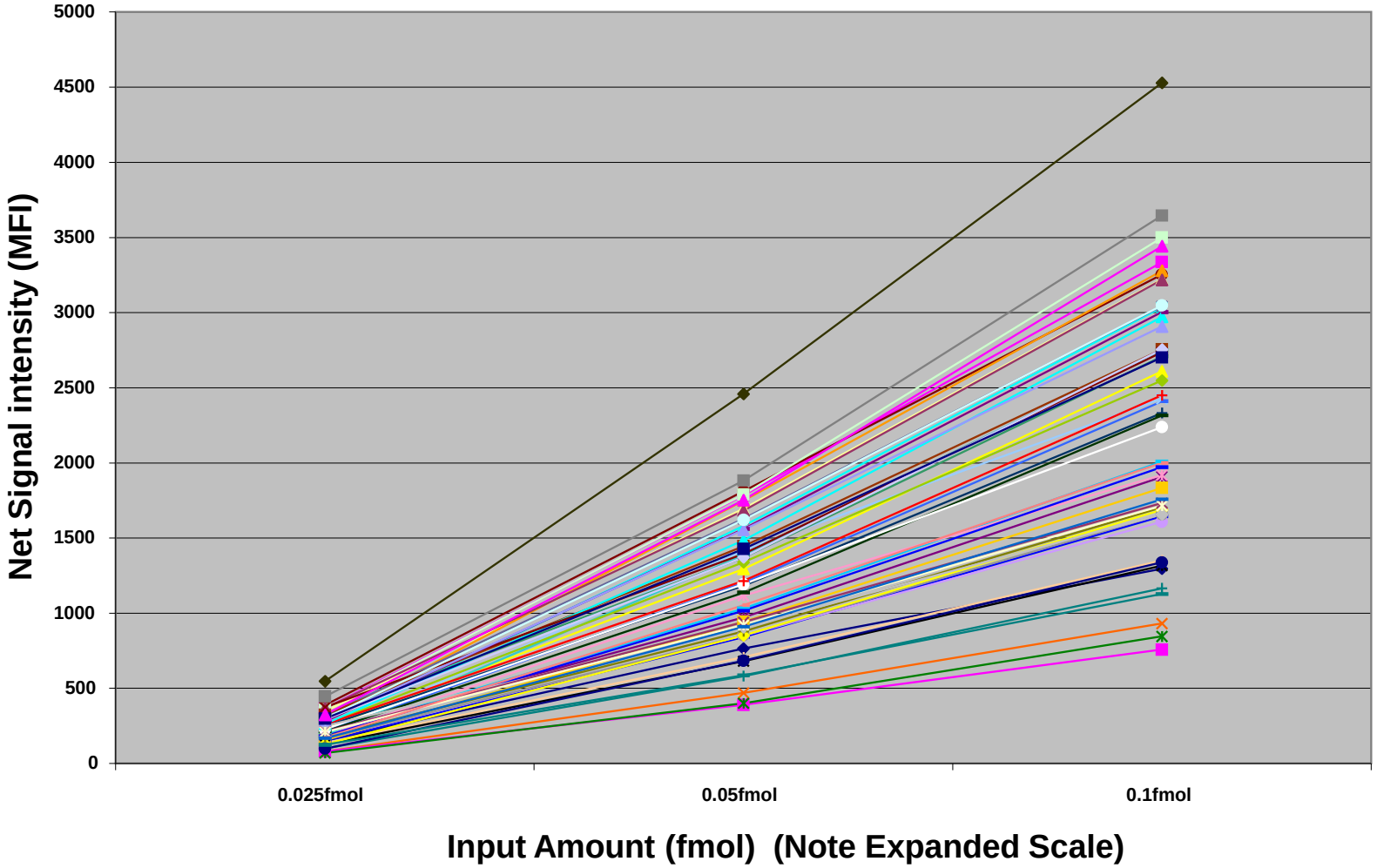
200 amol Molecules or Less – Unamplified!



Sensitivity limits

Titration below 100 amols – Unamplified!

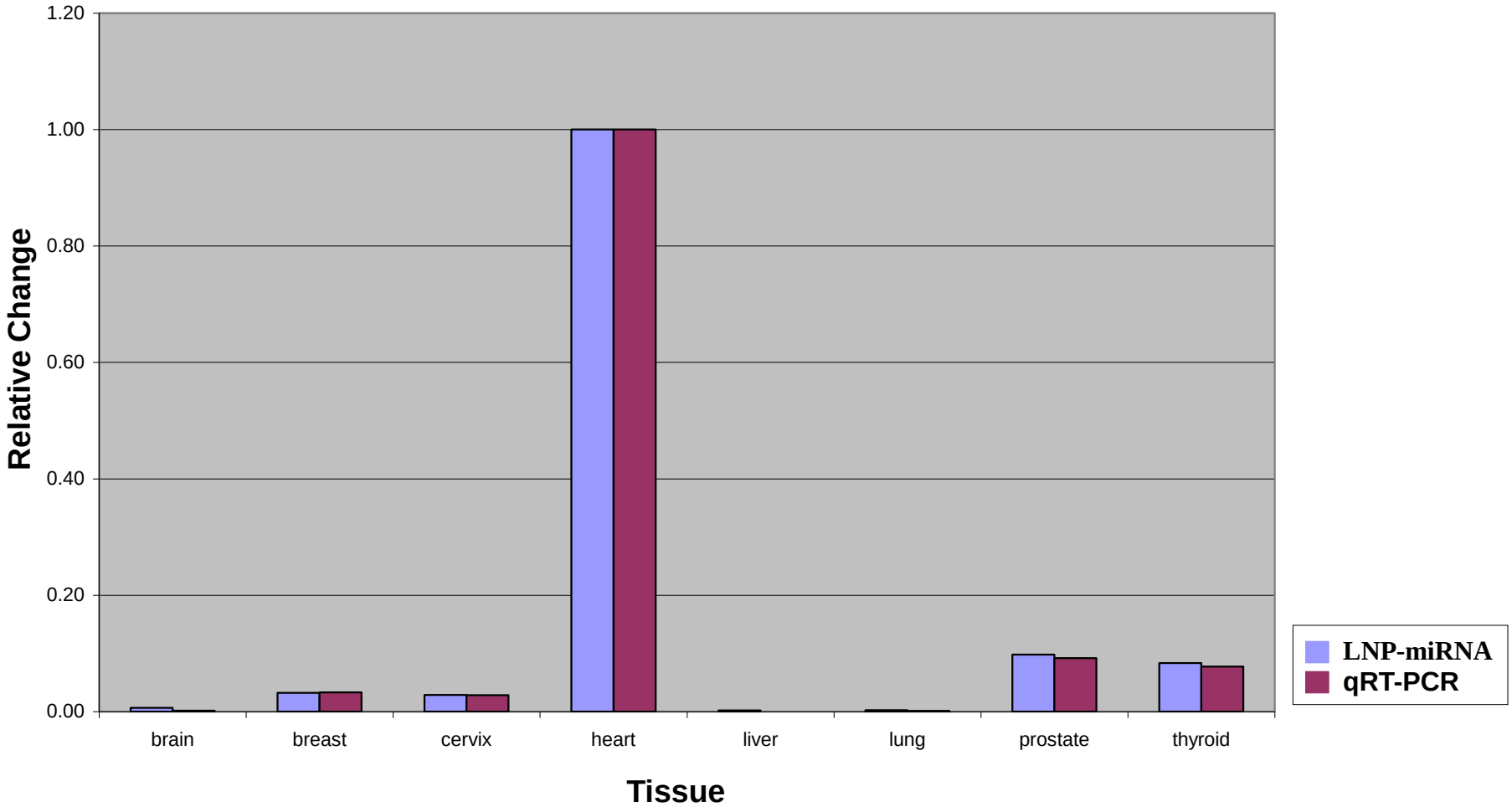
53 plex Synthetic Oligos on FlexMAP 3D



Luminex

Accuracy vs. qPCR

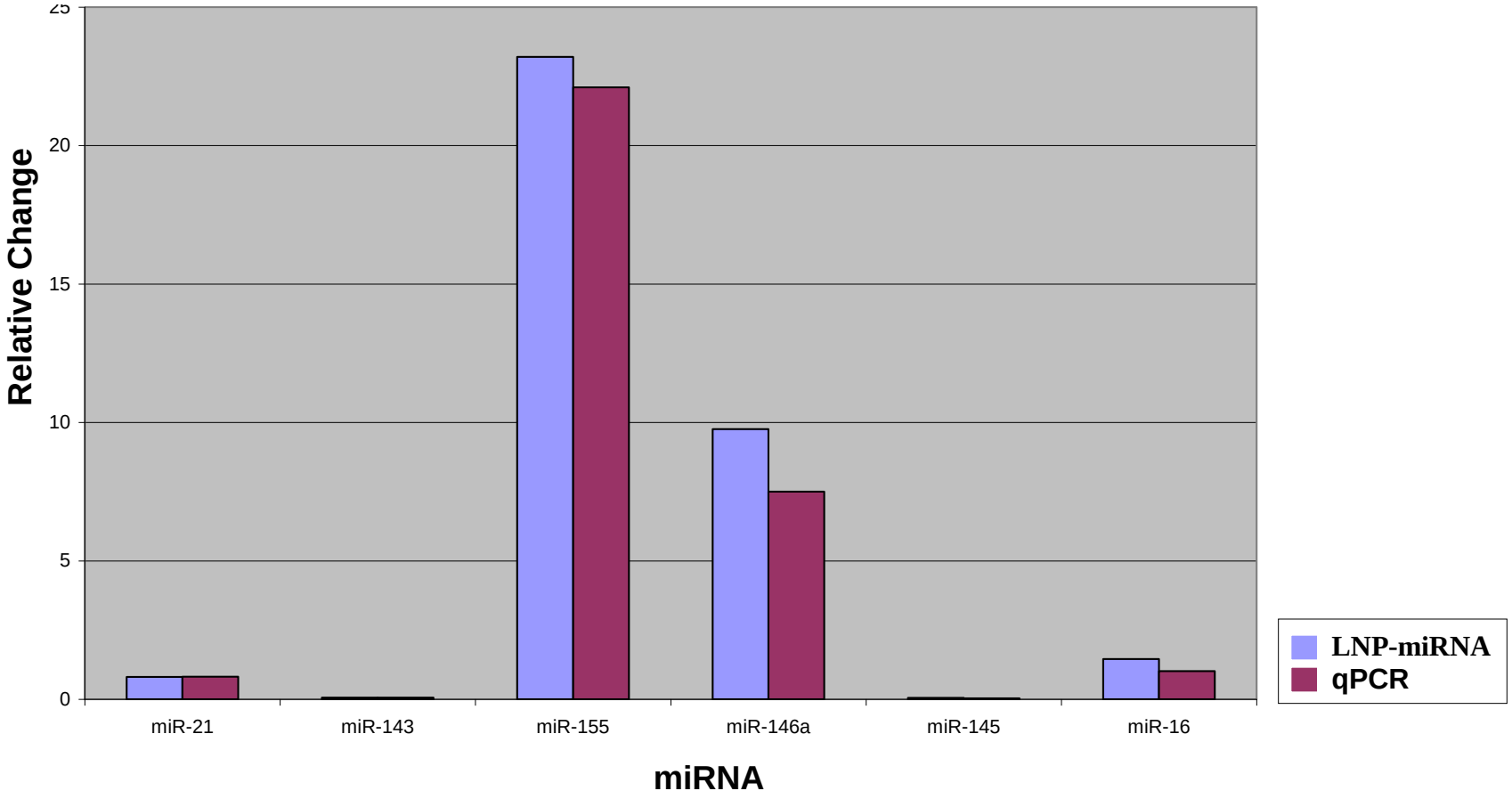
Correlation to qRT-PCR for miR-133a



Accuracy vs. qPCR

Correlation to qPCR of 6 microRNAs

Fold Change of Tumor vs. NAT - Lymphoma Samples



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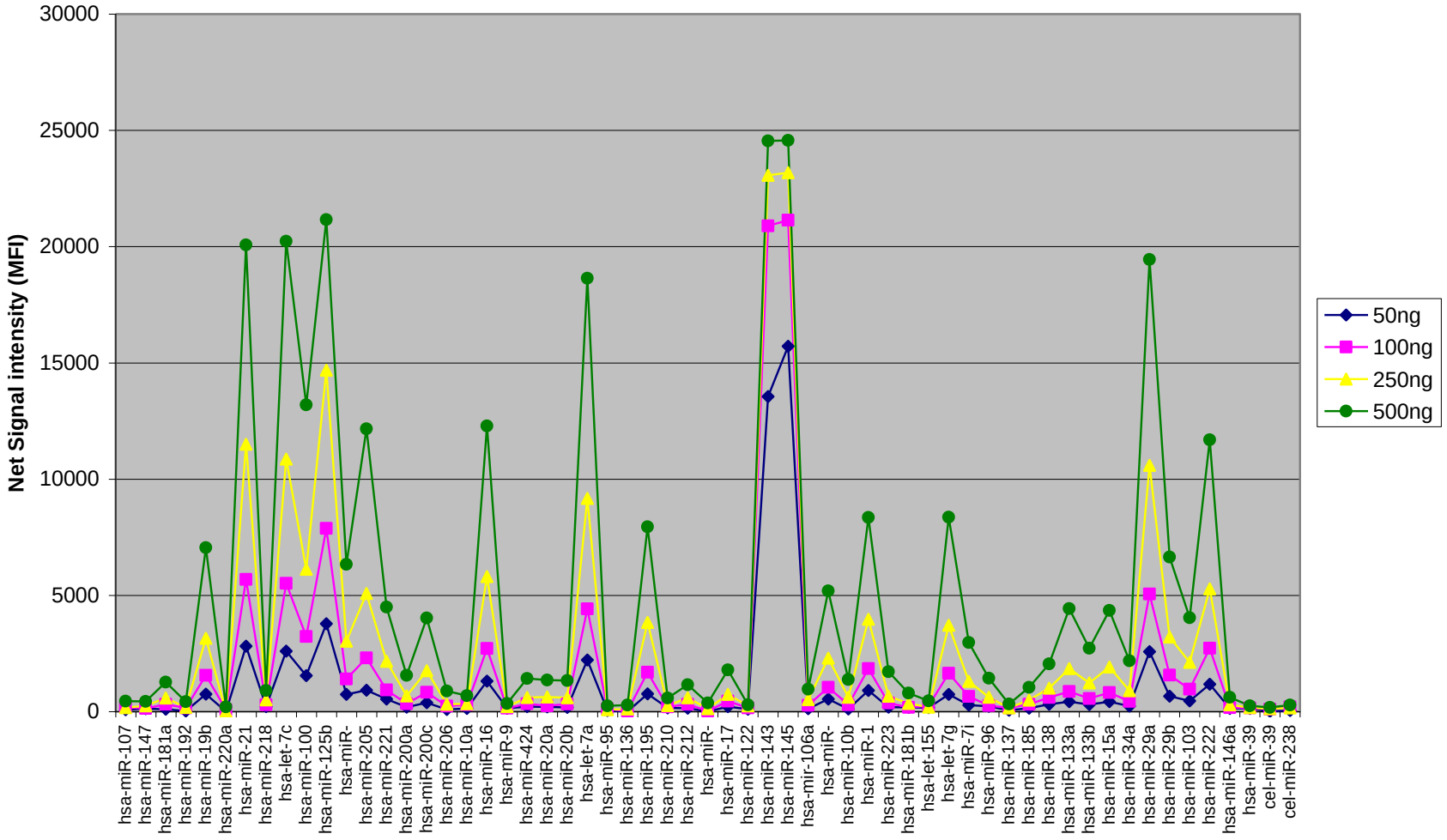
Accuracy at Different RNA Inputs

Consistent Results with Varying Total Prostate RNA



Luminex

miRNA Assay Characteristics

Summary

- **Fast, Easy and Unbiased**
  - »Only 30 Minutes of Handling
  - »No Sample Labeling or Amplification
  - »Time to Results <5 hours
- **Solid Performance**
  - »Reproducible: CVs <15%
  - »Sensitive: 50-500ng Total RNA Input
  - »Highly Specific: Discriminates Single Base Mismatches
- **Flexible**
  - »Multiplexing of >80 miRNAs/well possible
  - »Design your own custom probes

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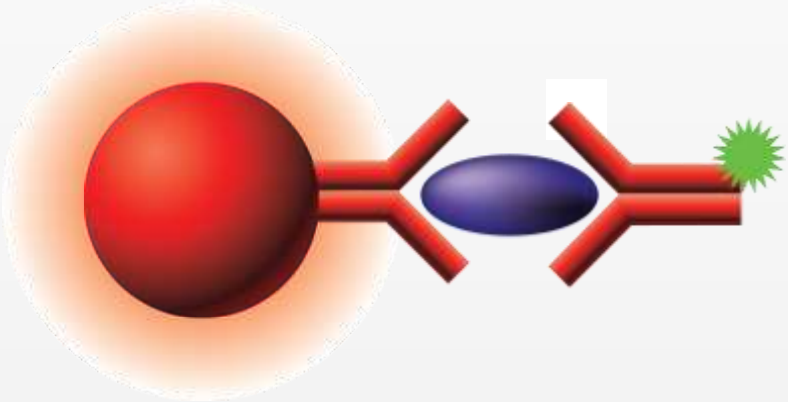
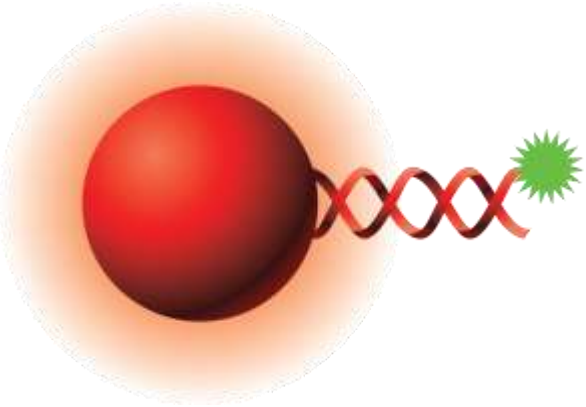
Proteomic Assays



xMAP Applications

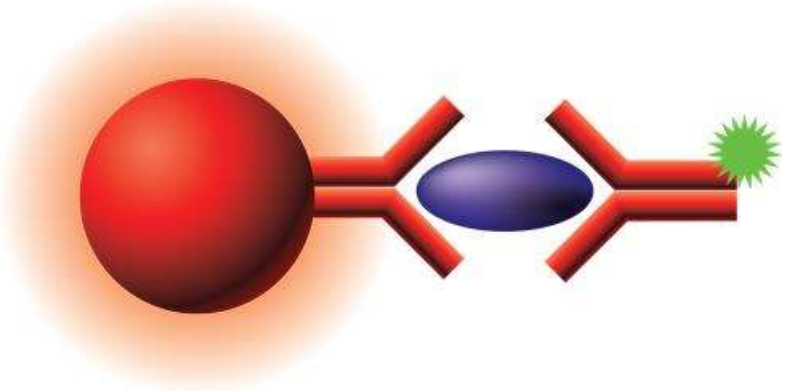
An Incredibly Flexible Platform

Nucleic Acids



- Gene Expression
- microRNA Profiling
- Genotyping
  - »SNP
  - »CNV
  - »Sequence Detection

Proteins



- Immunoassays
  - » Sandwich Capture
  - » Multiplex ELISA
- Receptor-Ligand/Protein interaction Assays
- Enzyme Substrate

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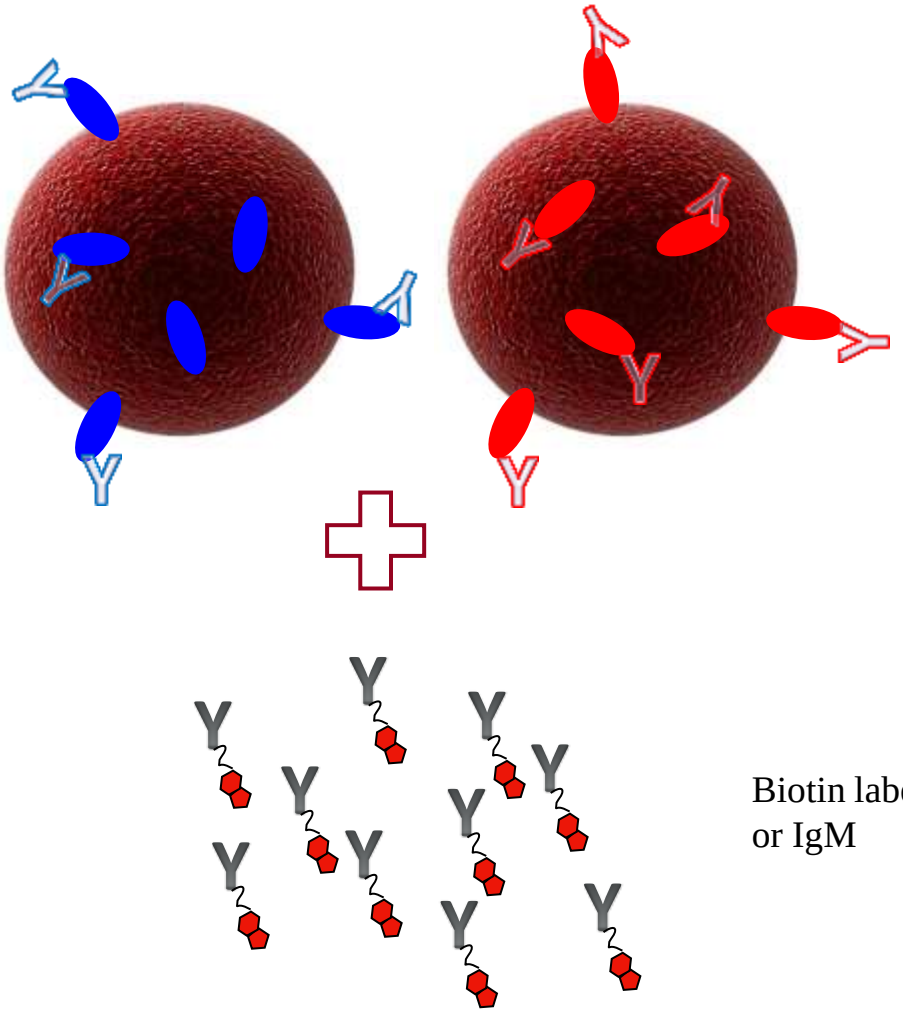
Multiplex Serological (ELISA) Assays

Multiplex ELISA

Mix beads with sera

Multiplex ELISA

Mix Beads with Biotinylated Detection Antibody mix



Biotin labeled anti IgG (1 to 4), IgA or IgM

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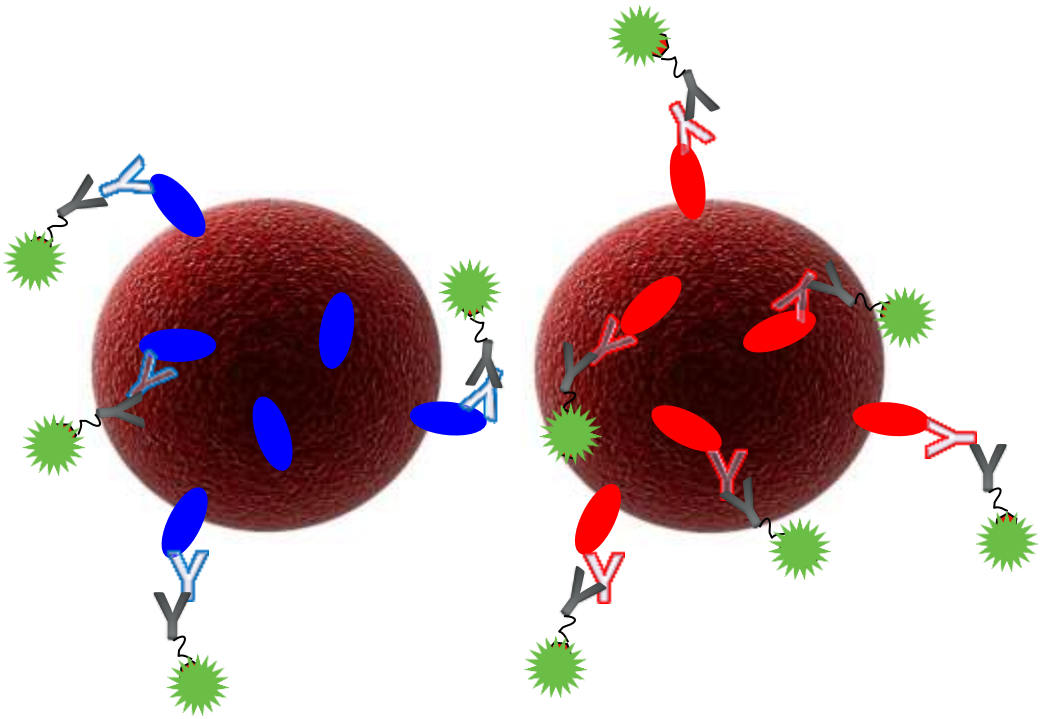
Multiplex ELISA

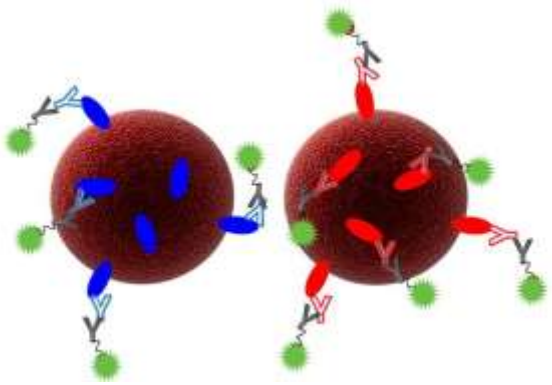
Mix Beads with SA-PE

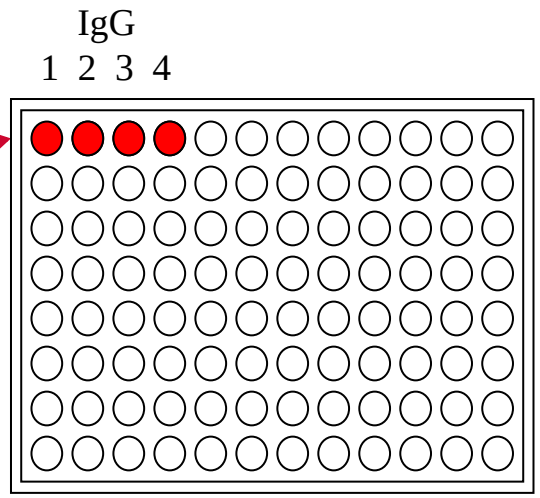


Can now determine if antibody response to antigen is IgG1, 2, 3, 4, or IgA or IgM

Luminex

Multiplex Detection





- **Each well can be plexed to measure as many antigens as reagents will allow up to 500 maximum.**

Detection

Luminex

Literature References

Anderson, S., P. Wakeley, G. Wibberley, K. Webster and J. Sawyer (2011). "Development and evaluation of a Luminex multiplex serology assay to detect antibodies to bovine herpes virus 1, parainfluenza 3 virus, bovine viral diarrhoea virus, and bovine respiratory syncytial virus, with comparison to existing ELISA detection methods." Journal Of Immunological Methods 366: 79-88.

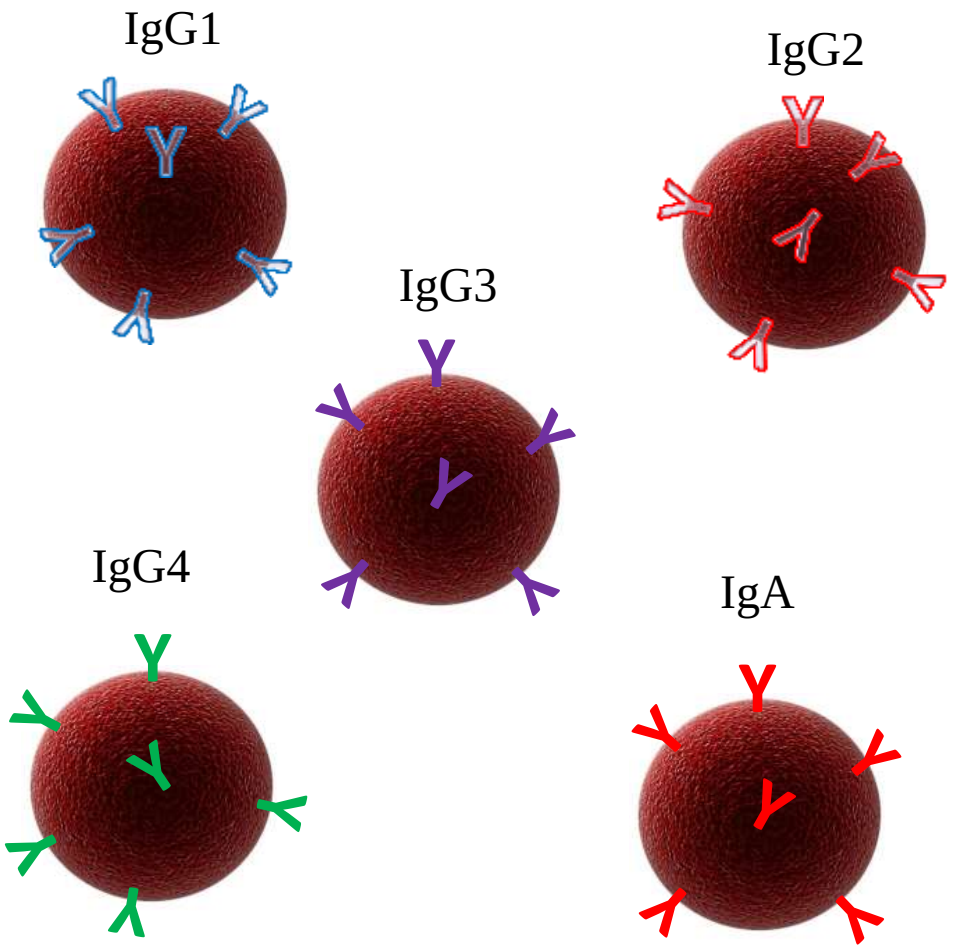
Gimenez-Lirola, L. G., Y.-H. Jiang, D. Sun, H. Hoang, K.-J. Yoon, P. G. Halbur and T. Opriessnig (2014). "Simultaneous Detection of Antibodies against Apx Toxins ApxI, ApxII, ApxIII, and ApxIV in Pigs with Known and Unknown Actinobacillus pleuropneumoniae Exposure Using a Multiplexing Liquid Array Platform." Clinical and Vaccine Immunology 21(1): 85 - 95.

Langenhorst, R. J., S. Lawson, A. Kittawornrat, J. J. Zimmerman, Z. Sun, Y. Li, J. Christopher-Hennings, E. A. Nelson and Y. Fang (2012). "Development of a fluorescent microsphere immunoassay for detection of antibodies against porcine reproductive and respiratory syndrome virus using oral fluid samples as an alternative to serum-based assays." Clin Vaccine Immunol 19(2): 180-189.

Smits, G. P., P. G. van Gageldonk, L. M. Schouls, F. R. M. van der Klis and G. A. M. Berbers (2012). "Development of a Bead-Based Multiplex Immunoassay for Simultaneous Quantitative Detection of IgG Serum Antibodies against Measles, Mumps, Rubella, and Varicella-Zoster Virus." Clinical And Vaccine Immunology 19(3): 396-400.

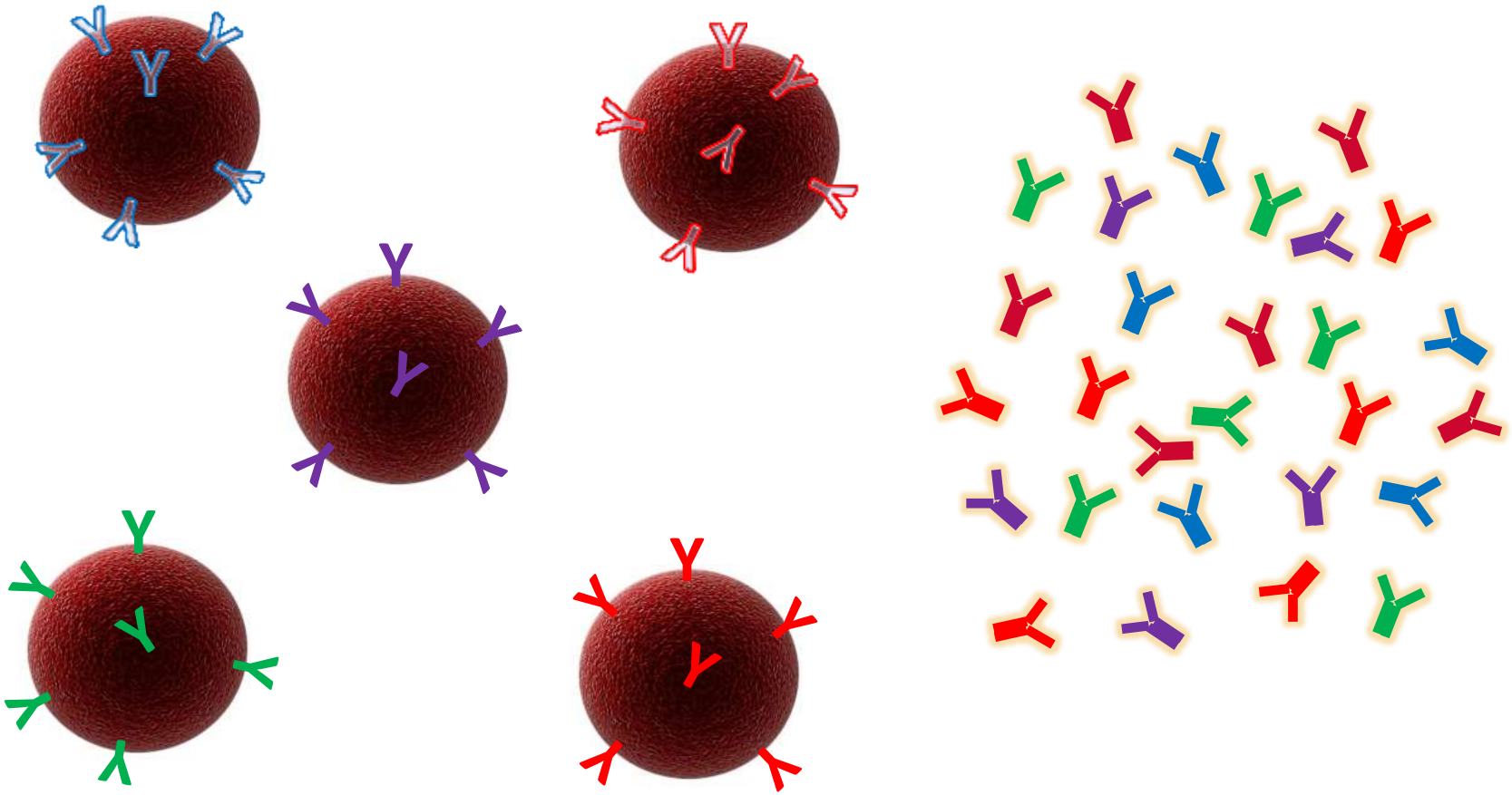
Multiplex Antigen Specific Ig ELISA assay

Beads Coated with Capture Antibodies for Different Ig types



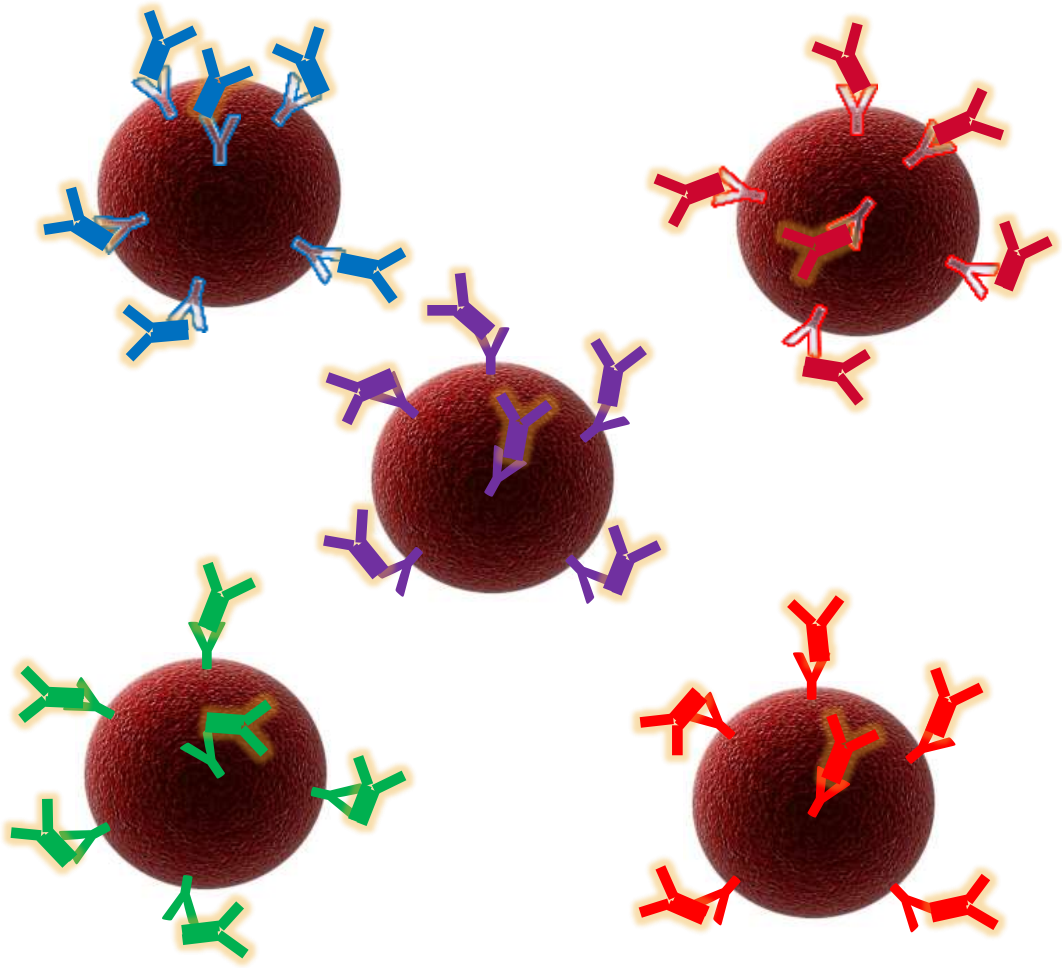
Multiplex Antigen Specific Ig ELISA assay

Beads with Capture Antibodies incubated with serum sample



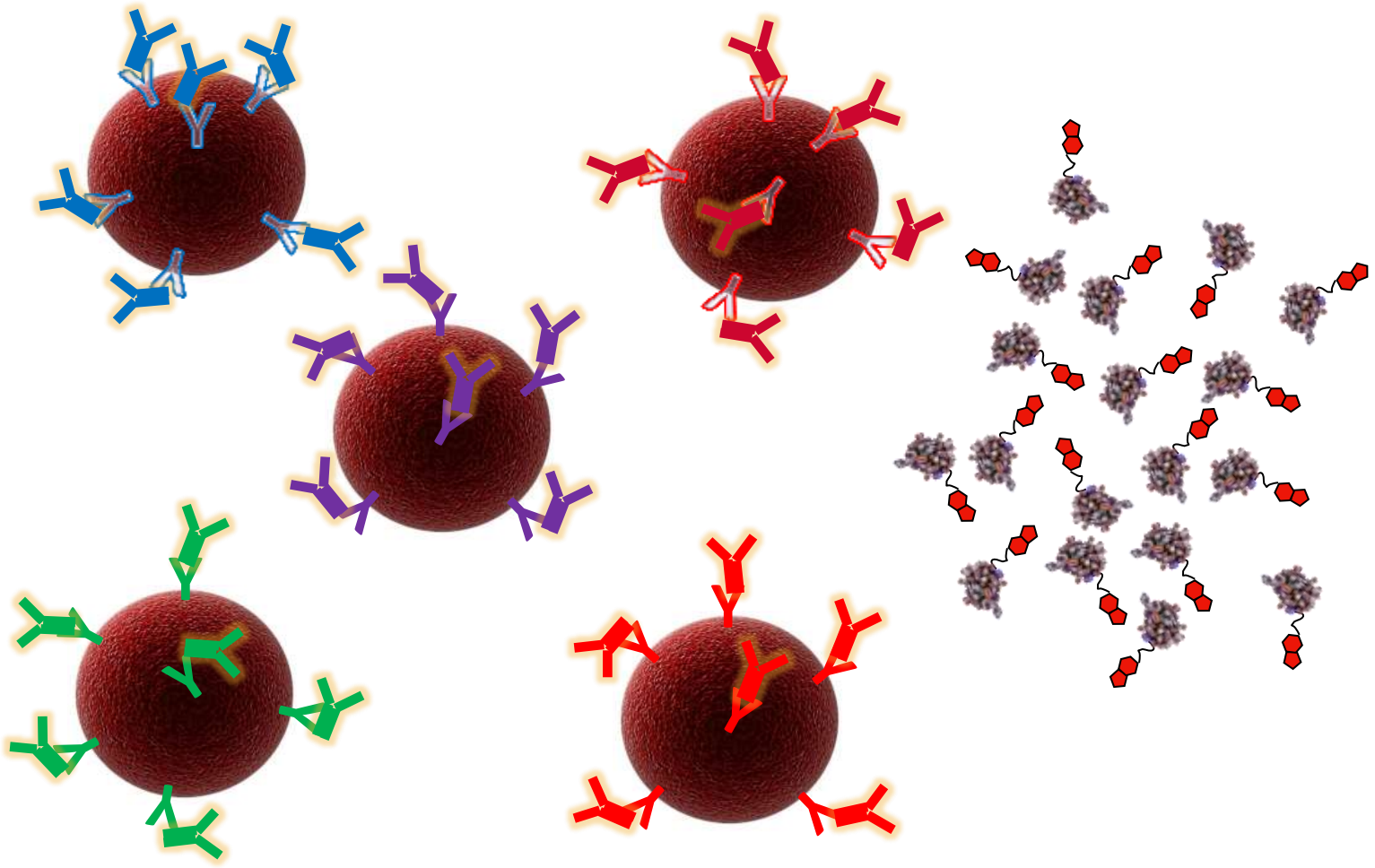
Multiplex Antigen Specific Ig ELISA assay

Beads Capture Antibodies of Different Ig types



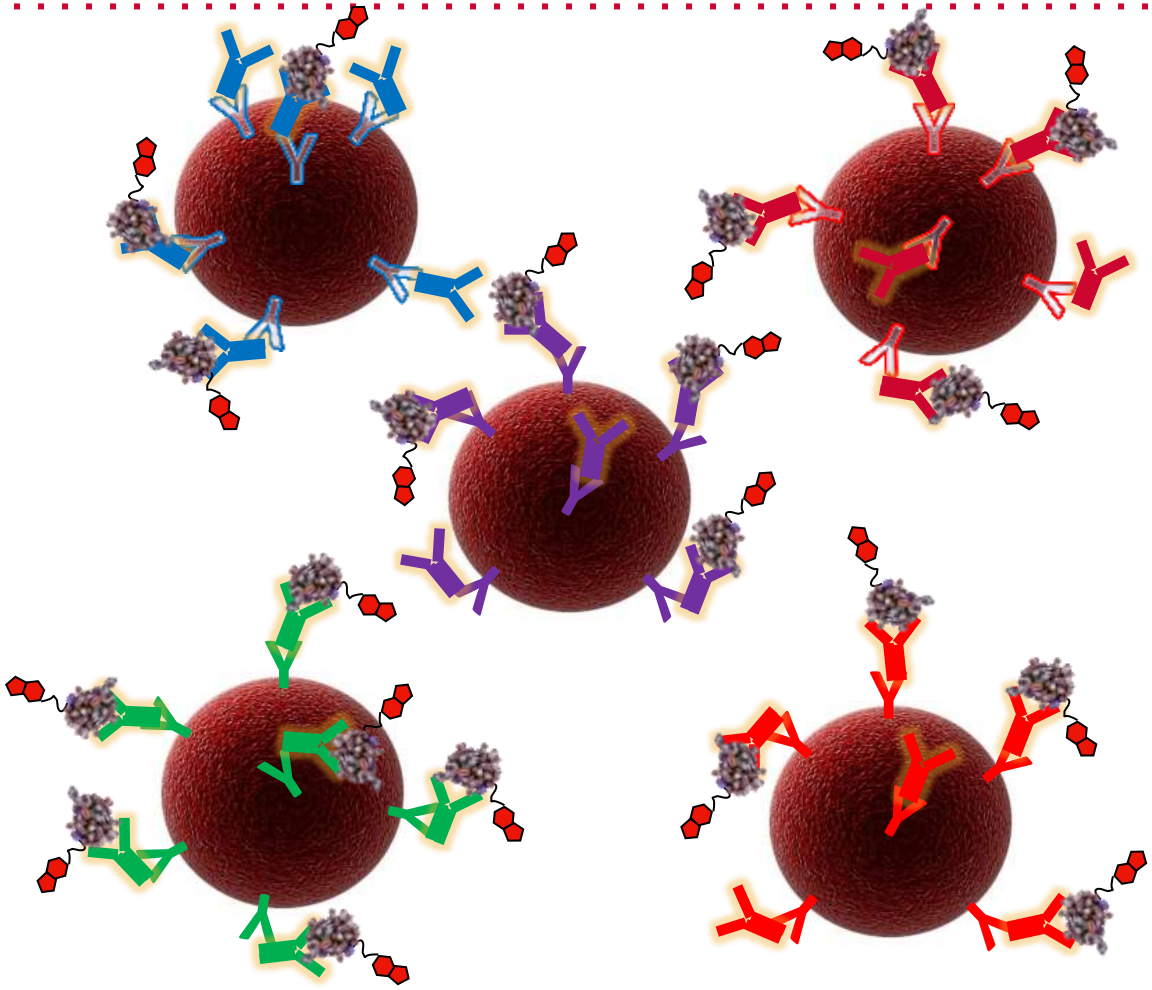
Multiplex Antigen Specific Ig ELISA assay

Beads Incubated with Biotin Labeled Antigen



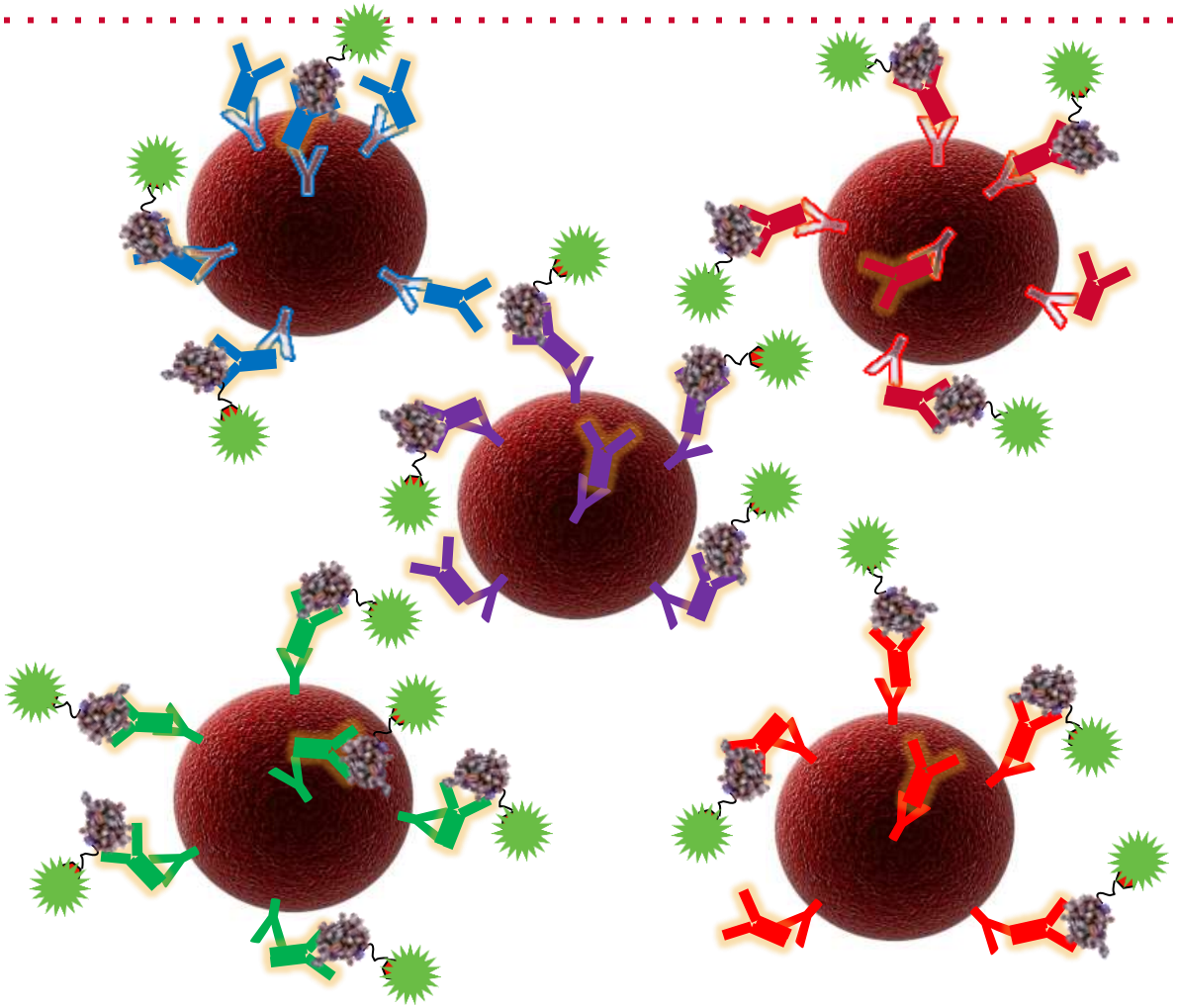
Multiplex Antigen Specific Ig ELISA assay

Different Ig types will bind Antigen



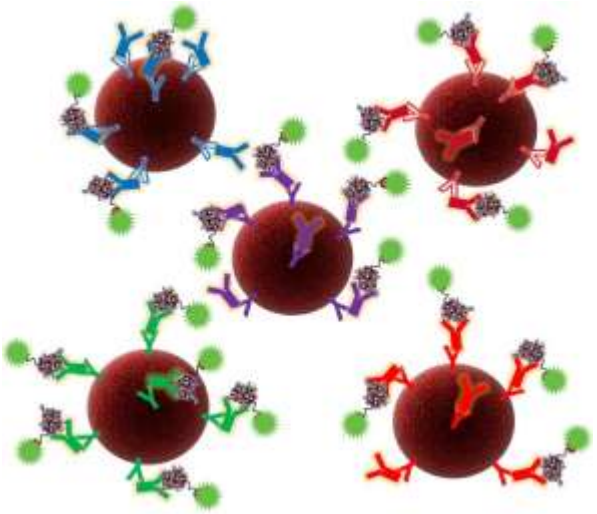
Multiplex Antigen Specific Ig ELISA assay

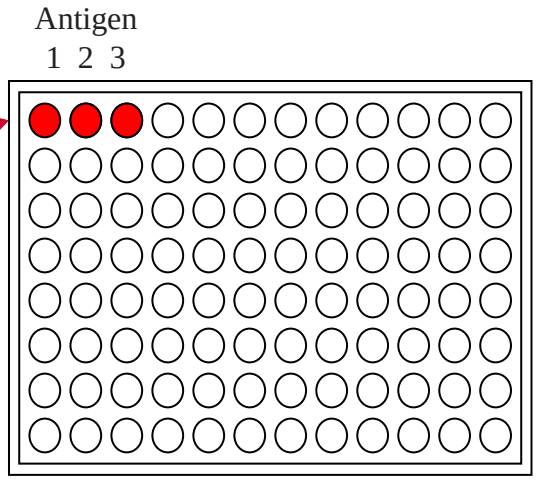
Incubate with SAPE (Streptavidin-R-Phycoerythrin)



Multiplex Antigen Specific Ig ELISA assay

Detection





- **Each well can be plexed to measure as many antigen specific Ig subtypes as needed**

- **Allows more rapid analysis of Ig type responses to multiple antigens in large numbers of samples.**

Detection

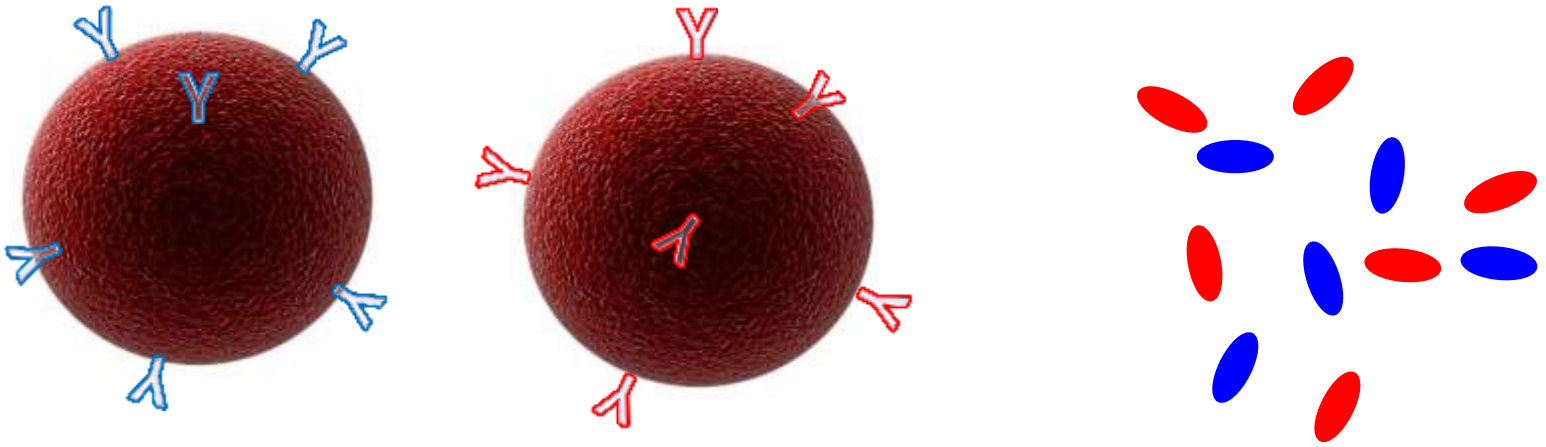
Luminex

Luminex

Multiplex Sandwich Assays

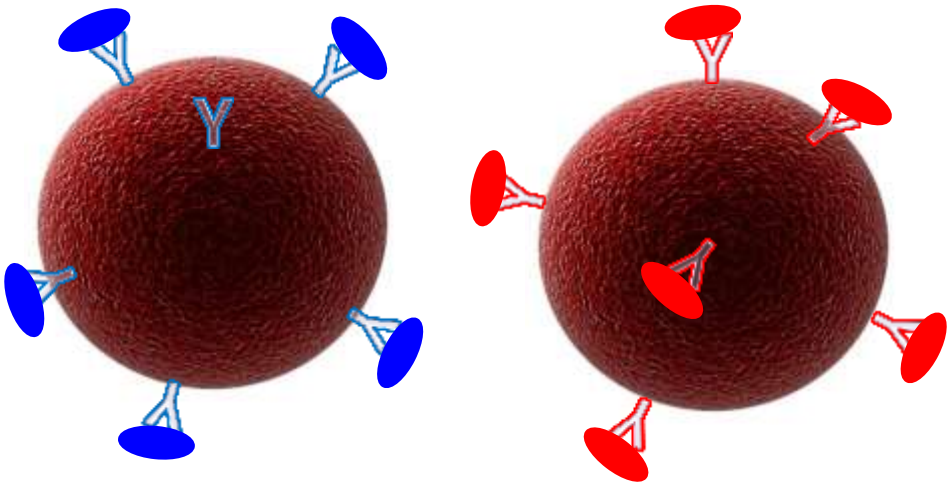
Multiplex Sandwich Assays

Beads Coated with Capture Antibodies Mixed with Sample



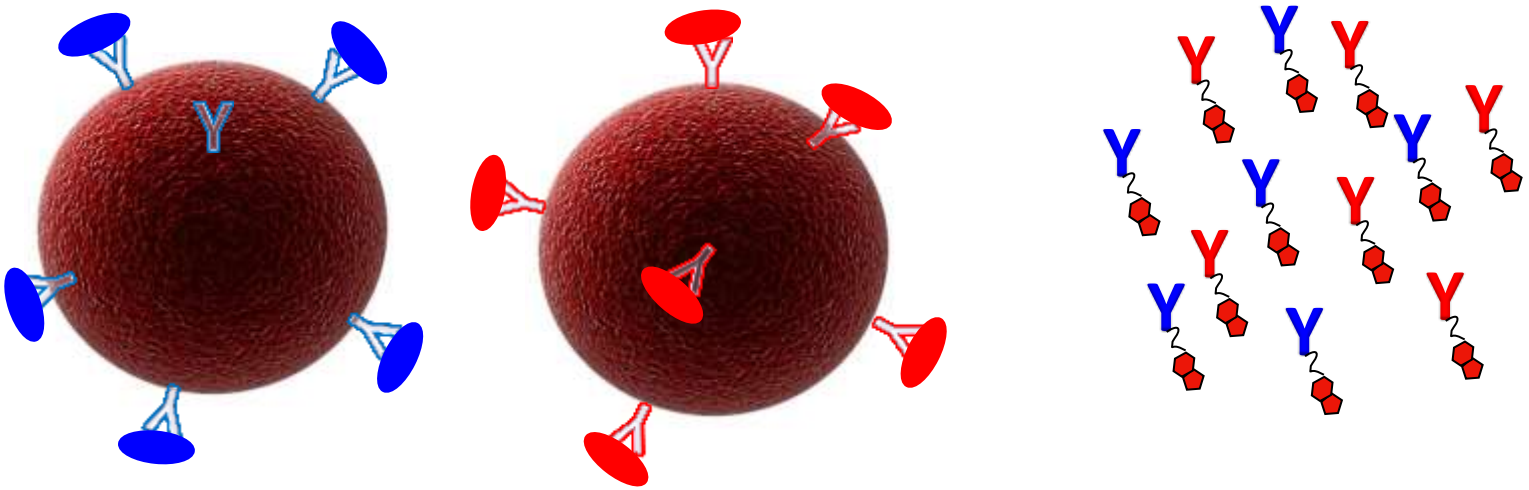
Multiplex Sandwich Assays

Targets Bind to Capture Antibodies



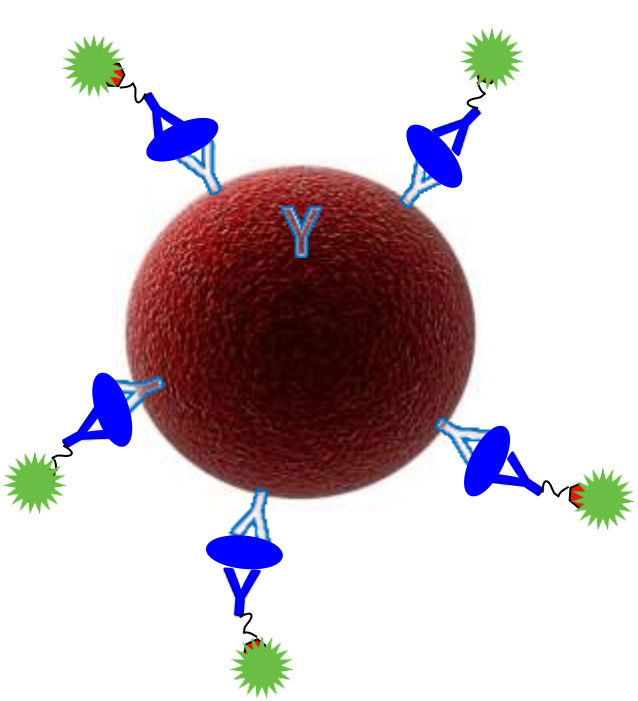
Multiplex Sandwich Assays

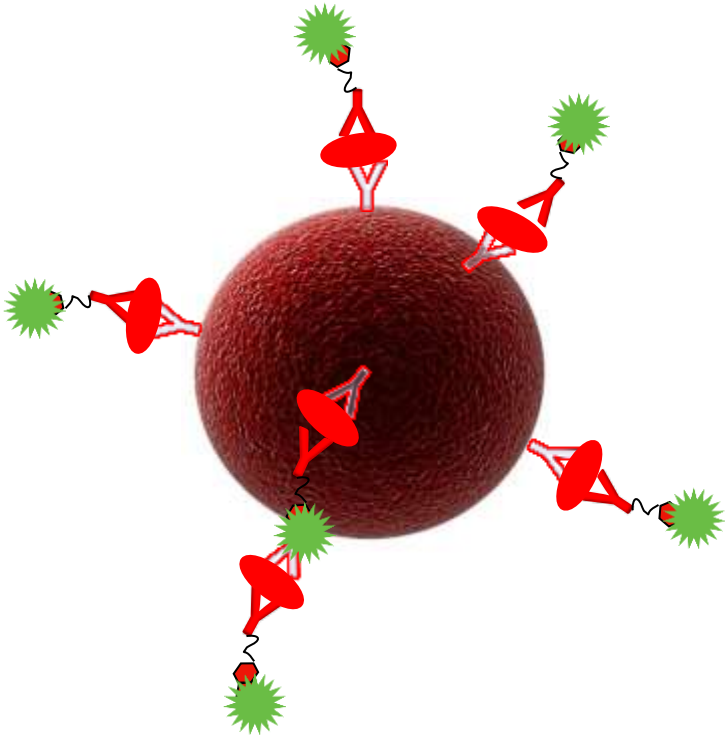
Mix with Labeled Target Specific Antibodies



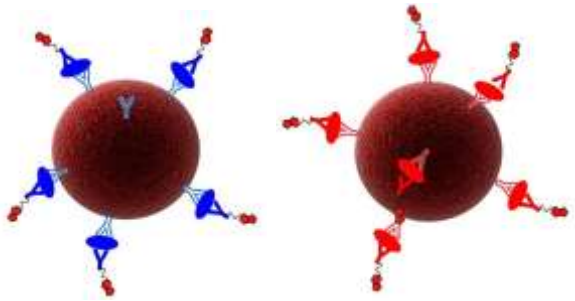
Multiplex Sandwich Assays

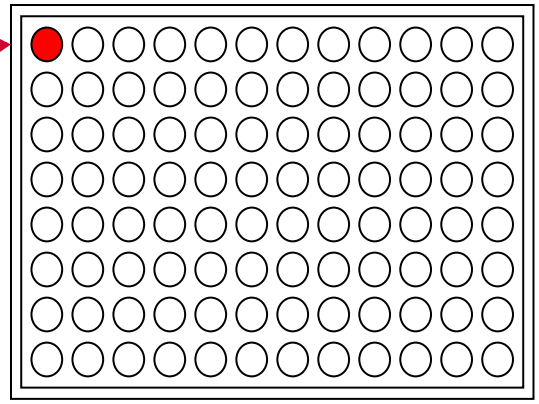
Mix with Labeled Target Specific Antibodies





Multiplex Detection





- **Each well can be plexed to measure as many antigens as reagents will allow up to 500 maximum.**

Detection

Luminex

Literature References

Bjerre, M., T. Hansen, A. Flyvbjerg and E. Tonnesen (2009). "Simultaneous detection of porcine cytokines by multiplex analysis: Development of magnetic bioplex assay." Veterinary Immunology And Immunopathology **130**: 53-58.

Sun, K., Q. Wang, X. Huang, M. Zhen, W. Li and L. Zhang (2007). "Establishment of multiplexed, microsphere-based flow cytometric assay for multiple human tumor markers." Acta Pharmacologica Sinica **28**(12): 2011-2018.

Luminex

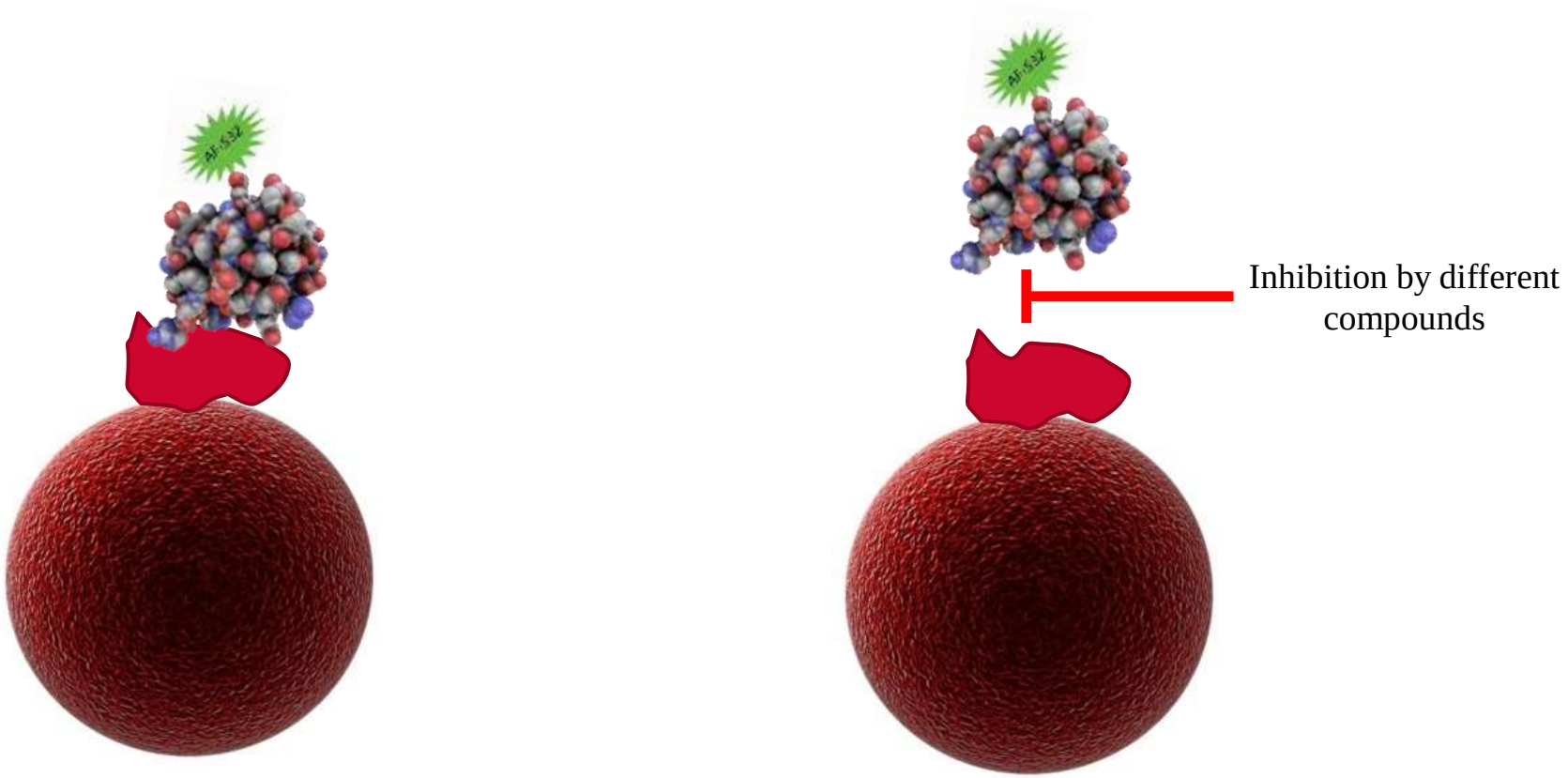
Protein Interaction and Receptor Ligand/Subatrate Assays

Types of Receptor Ligand and Protein Interaction Assays

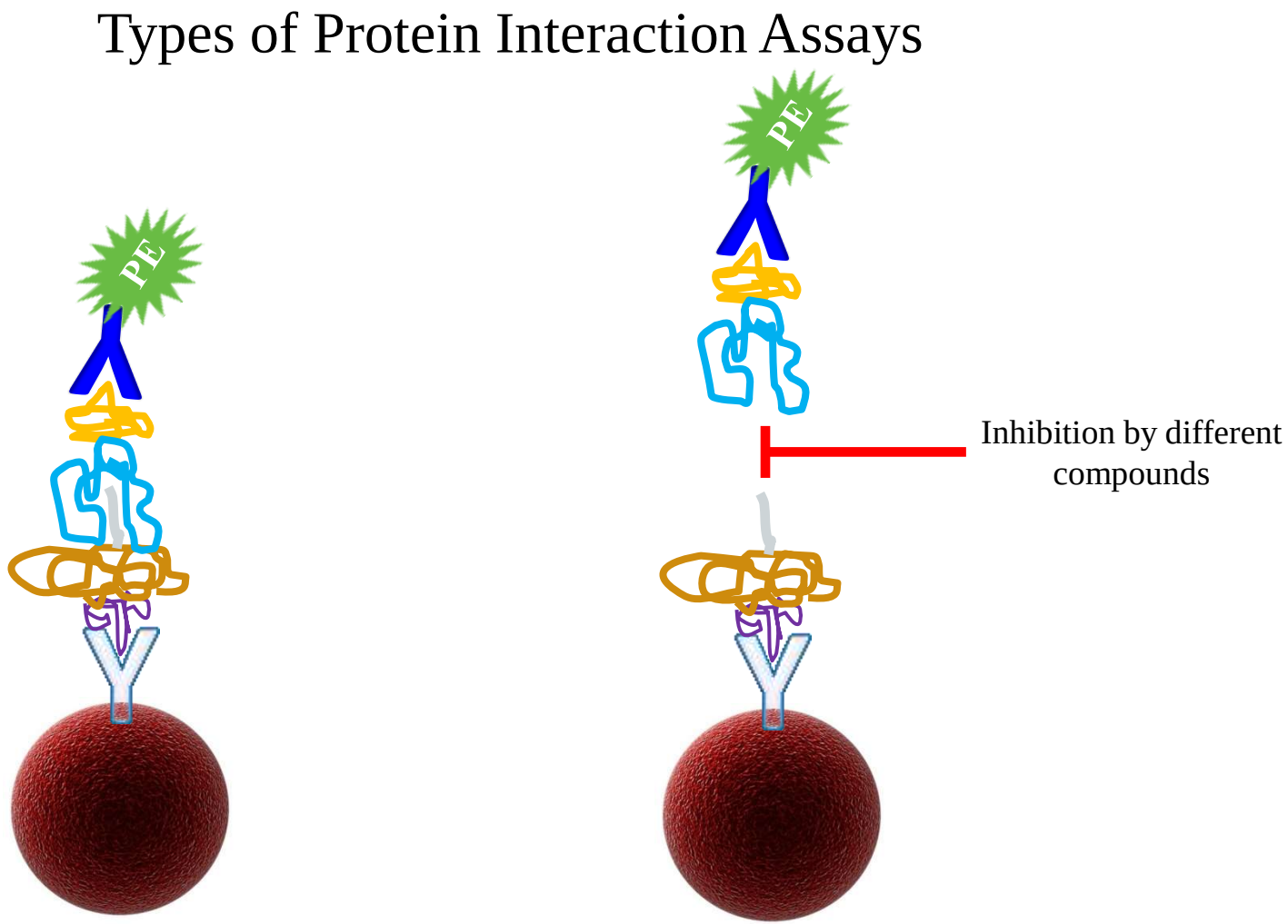
Types of Receptor Ligand Interaction assays

Types of Receptor Ligand and Protein Interaction Assays

Types of Receptor Ligand Interaction assays

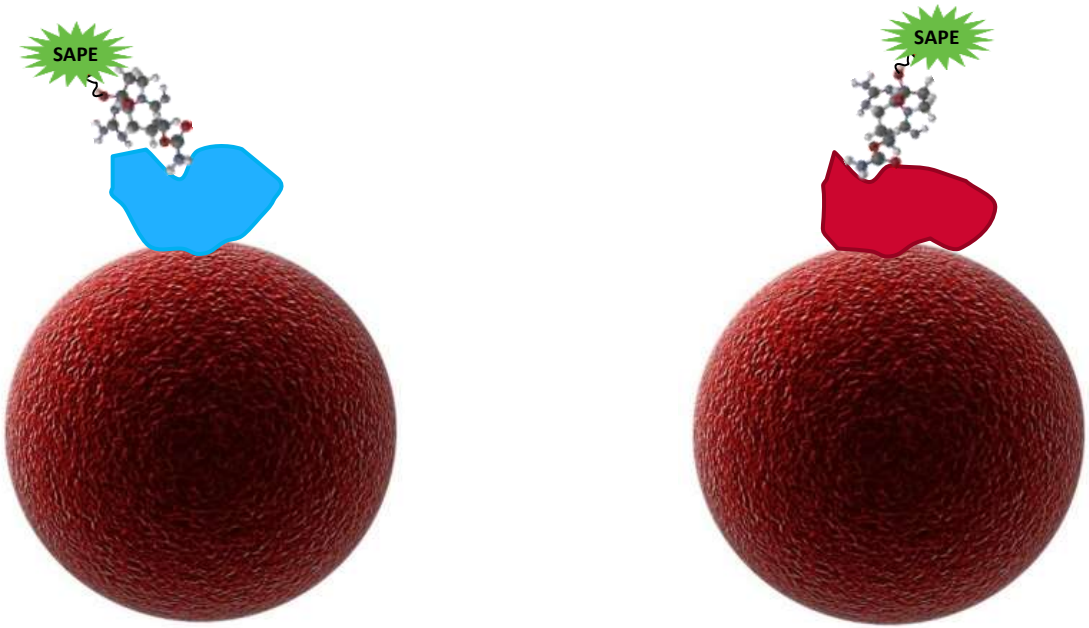


Types of Receptor Ligand and Protein Interaction Assays



Multiplex Enzyme Substrate Interaction Assays

A two plex assay: Labeled Substrate



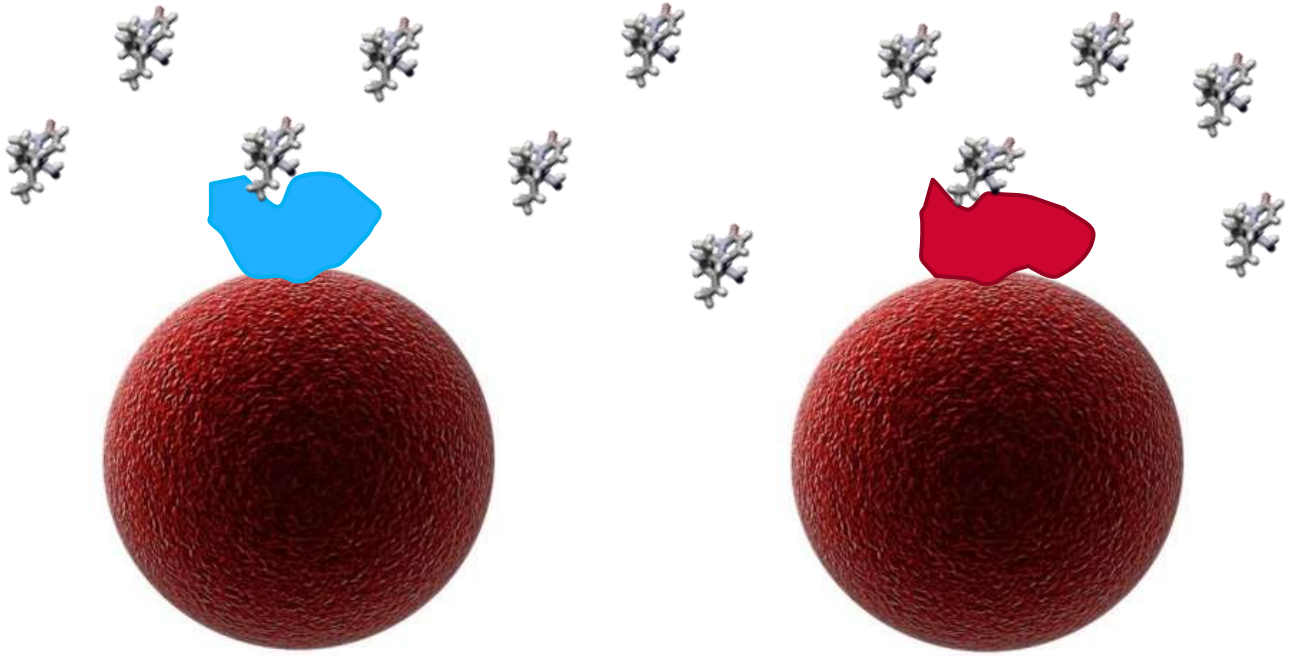
Multiplex Enzyme Substrate Interaction Assays

A two plex assay: Inhibitor Treatment

= inhibiting compound

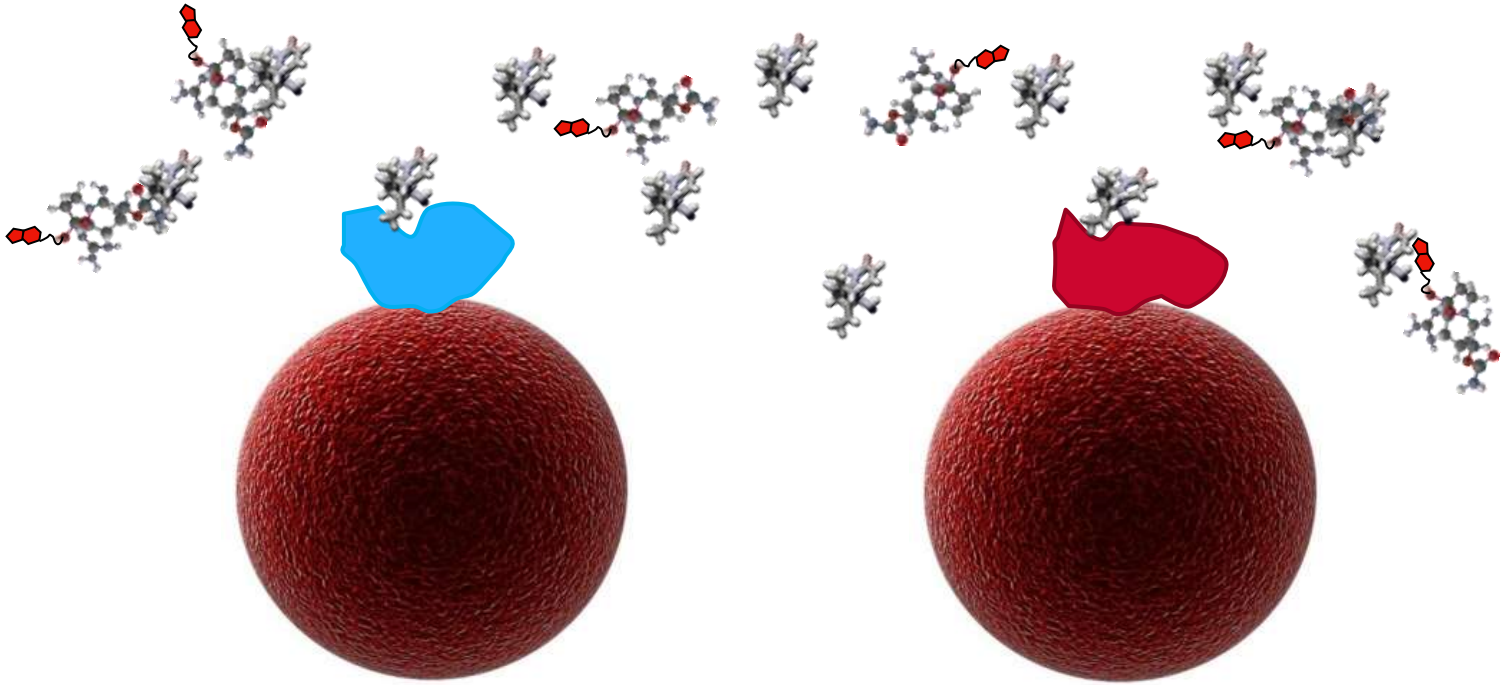
Multiplex Enzyme Substrate Interaction Assays

Inhibitor Treatment at Different Concentrations



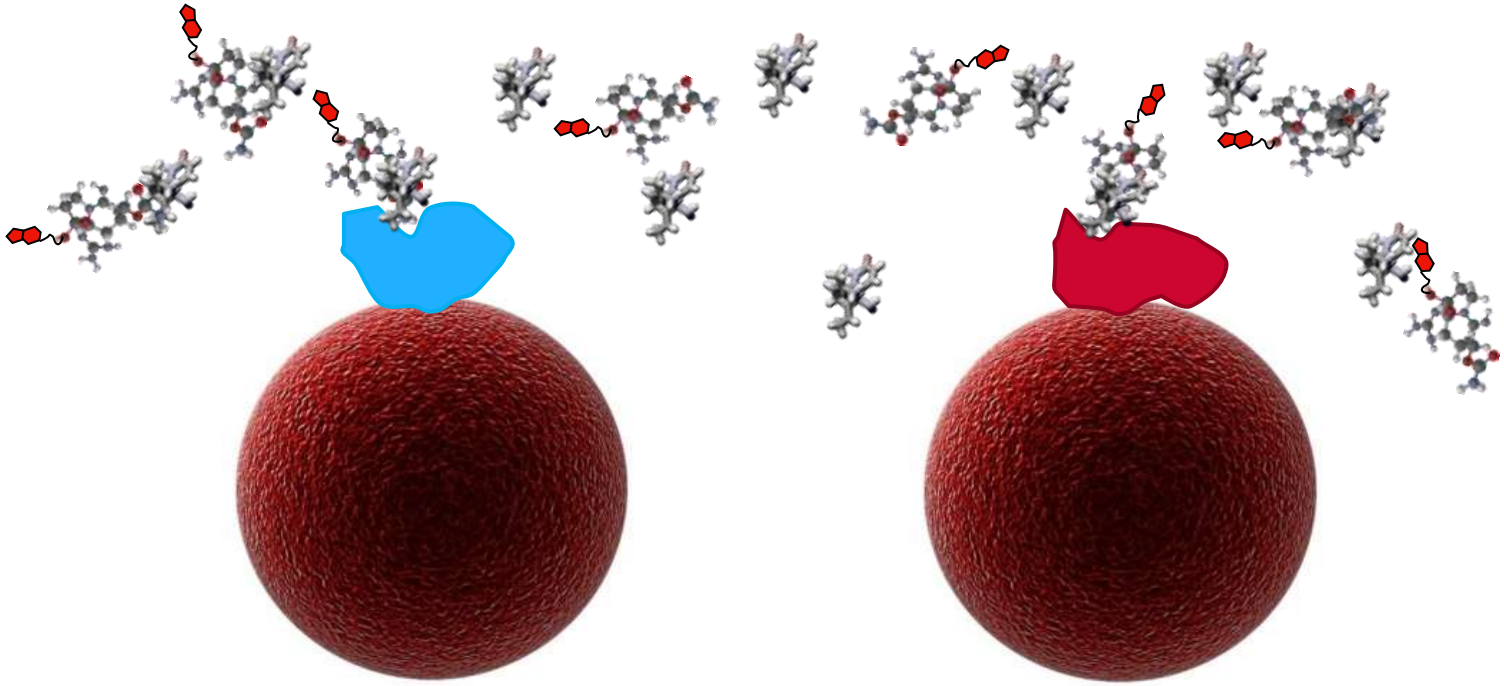
Multiplex Enzyme Substrate Interaction Assays

Add Labeled Substrate



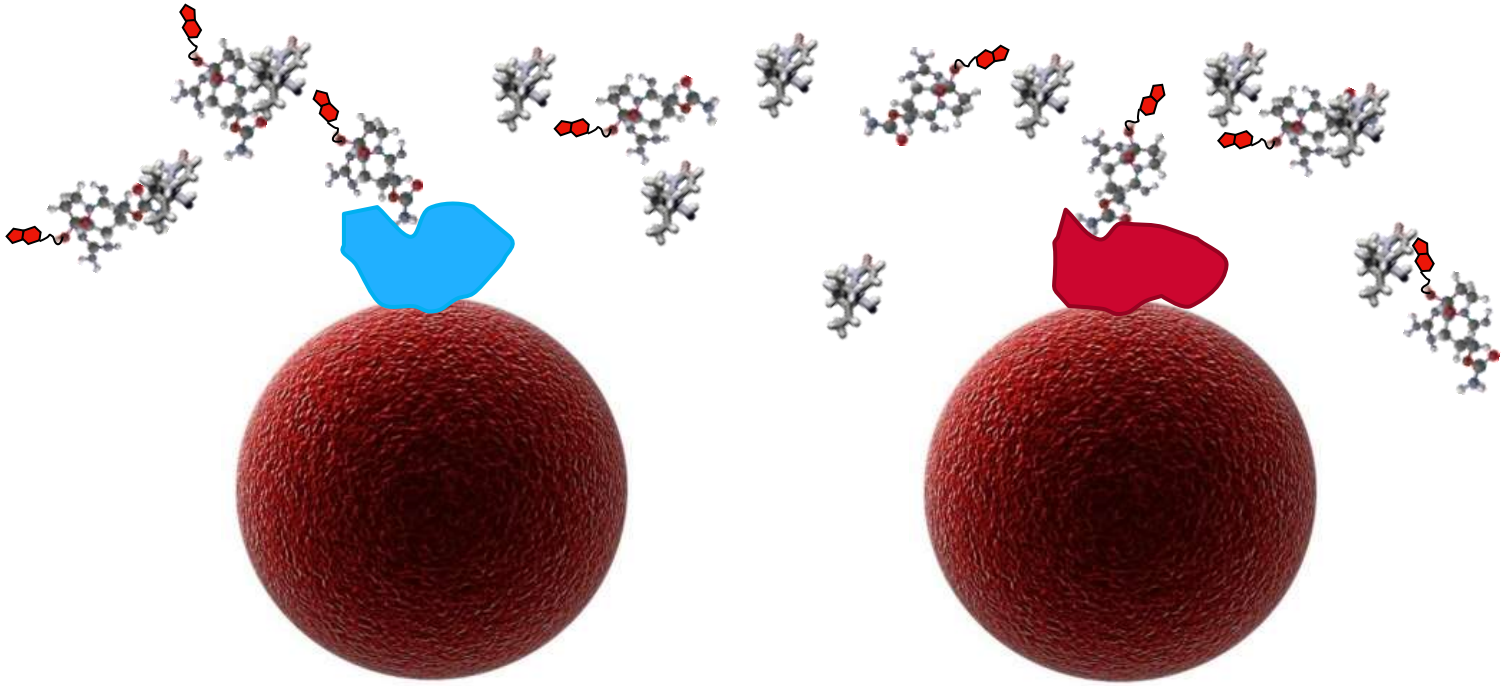
Multiplex Enzyme Substrate Interaction Assays

Labeled Substrate competes with Inhibitor



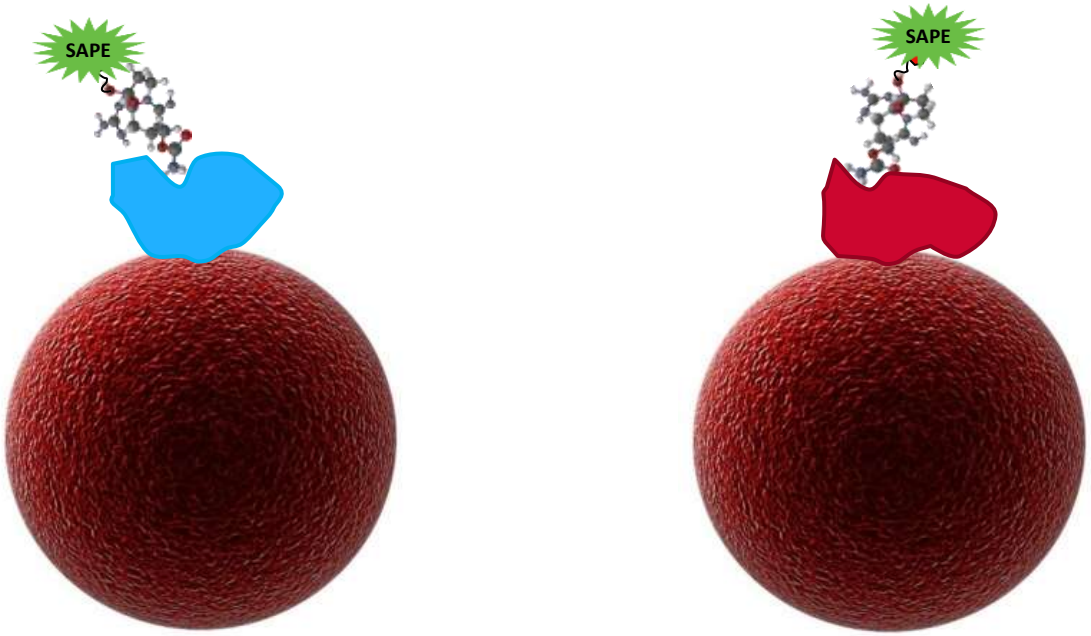
Multiplex Enzyme Substrate Interaction Assays

Wash out Excess Inhibitor and Labeled Substrate

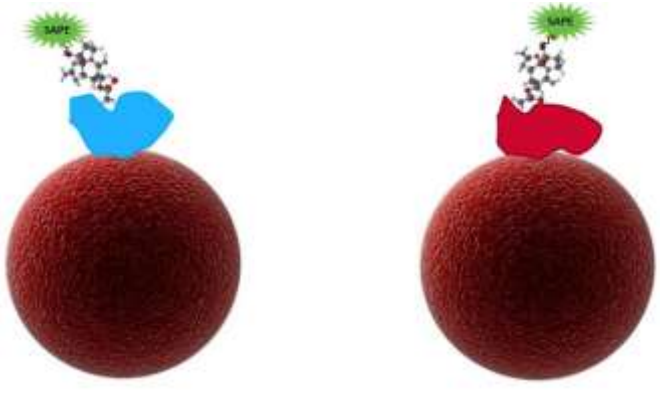


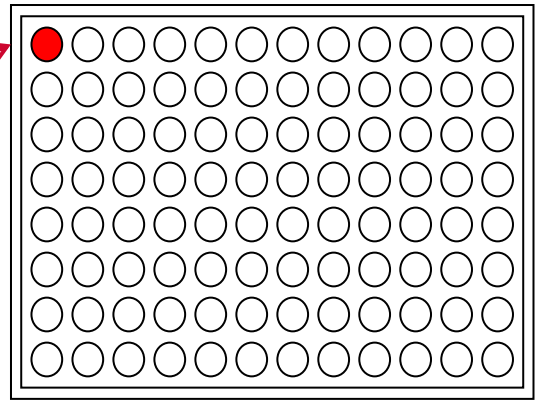
Multiplex Enzyme Substrate Interaction Assays

Hybridize with SAPE



Multiplex Detection





- **Each well can be plexed to measure as many antigens as reagents will allow up to 500 maximum/reaction.**

Detection

Luminex

Literature References

Bachovchin, D. A., et al. (2014). A high-throughput, multiplexed assay for superfamily-wide profiling of enzyme activity. Nature Chemical Biology 10(8): 656 - 666.

Poetz, O., K. Luckert, T. Herget and T. Joos (2009). "Microsphere-based co-immunoprecipitation in multiplex." Analytical Biochemistry 395(2): 244-248.

Blazer, L. L., D. L. Roman, M. R. Muxlow and R. R. Neubig (2010). "Use of flow cytometric methods to quantify protein-protein interactions." Curr Protoc Cytom Chapter 13: Unit 13 11 11-15.

Rimmele, S., P. Gierschik, T. Joos and N. Schneiderhan-Marra (2010). "Bead-based protein-protein interaction assays for the analysis of Rho GTPase signaling." Journal Of Molecular Recognition 23(6): 543-550.

Schmohl, M., S. Rimmele, O. Poetz, Y. Kloog, P. Gierschik, T. Joos and N. Schneiderhan-Marra (2010). "Protein-protein-interactions in a multiplexed, miniaturized format a functional analysis of Rho GTPase activation and inhibition." Proteomics 10(8): 1716-1720.

Schmohl, M., S. Rimmele, P. Gierschik, T. Joos and N. Schneiderhan-Marra (2012). "Functional Analysis of Rho GTPase Activation and Inhibition in a Bead-Based Miniaturized Format." Rho GTPases 827: 271-282.

Luminex

Resources for Developing Multiplex Assays

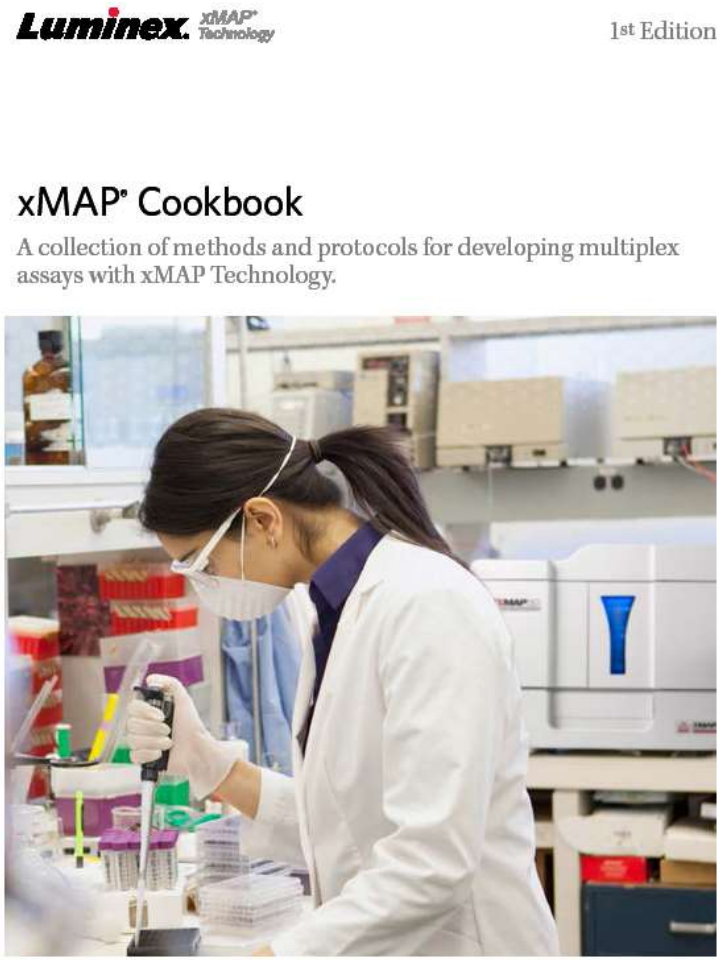
Resources for Developing Multiplex Assays

1. The Kit Finder. Allows users to search for existing kits made by Luminex partners.

2. Papers with protocols of interest. Try the Luminex publication search tool.

3. The xMAP Cookbook. Helps users develop their own assays with all of their own reagents.

Resources for Developing Multiplex Assays



The xMAP Cookbook is a collection of easy to follow protocols with lists of reagents and equipment needed to develop you own Luminex based assay.

Interested in Getting Started?

For Instrument/Reagent Pricing
Josh Jenkins
Business Manager South East USA
jjenkins@luminexcorp.com
Cell: 512.202.9372

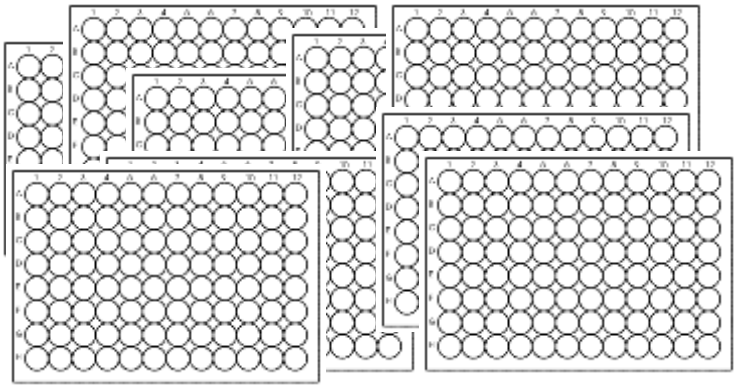
For Technical Information
Stephen Angeloni, PhD
Sr. Field Application Scientist
sangeloni@luminexcorp.com
Cell: 512.348.1535

And visit us on the web at www.luminexcorp.com

Thank you for Attending

I will now take questions about converting your…

Single Plex Assays



To Luminex Multiplex Assays