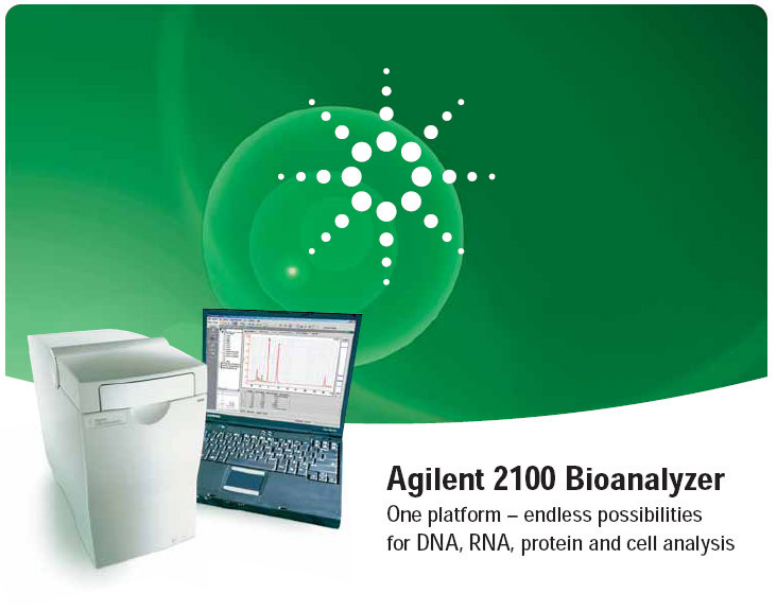
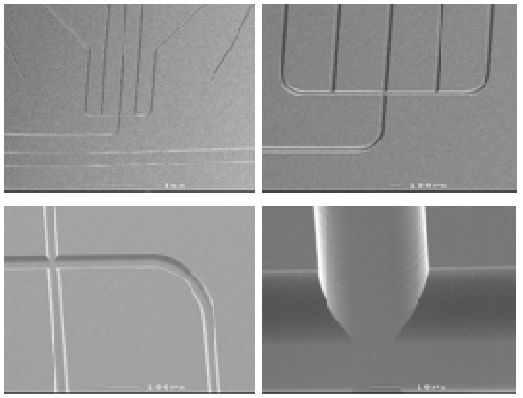
2100 Bioanalyzer

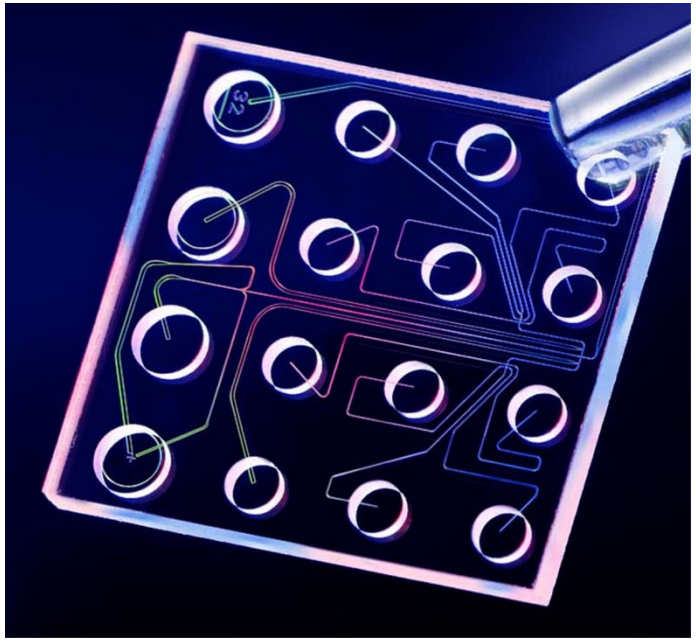
Iman Kishawi, PhD
Senior Application Scientist



The Lab-on-a-Chip Approach



Increasing quality and speed of gel electrophoresis



Sample volumes 1 - 5 µl

10 -12 samples depending on Assay

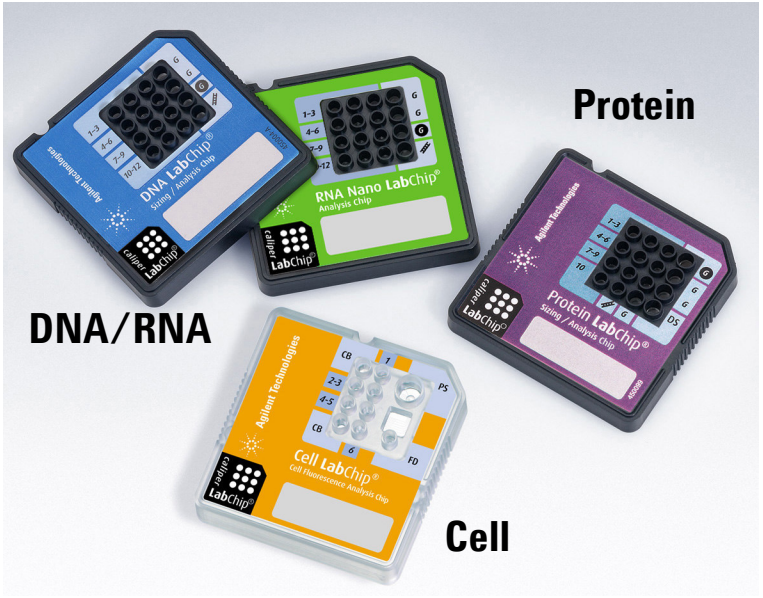
Separation, staining, detection of samples

Results in 5-30 minutes available

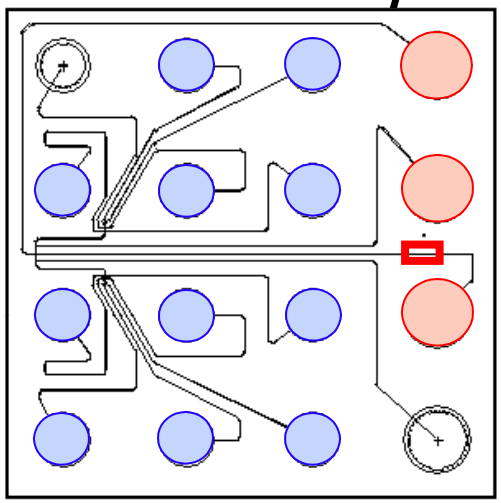
No extra waste removal needed

Disposable Chip, no crosscontamination

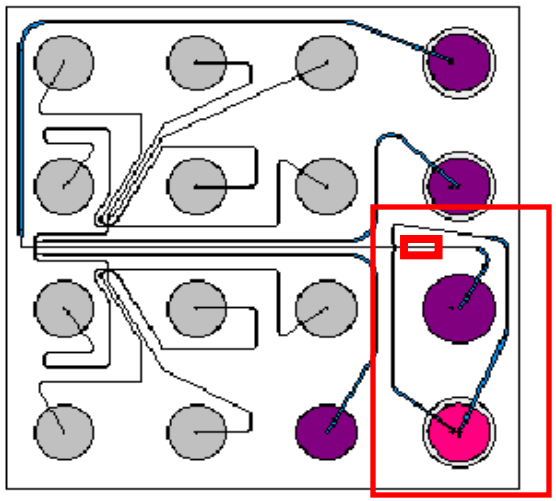
Three Chip Types



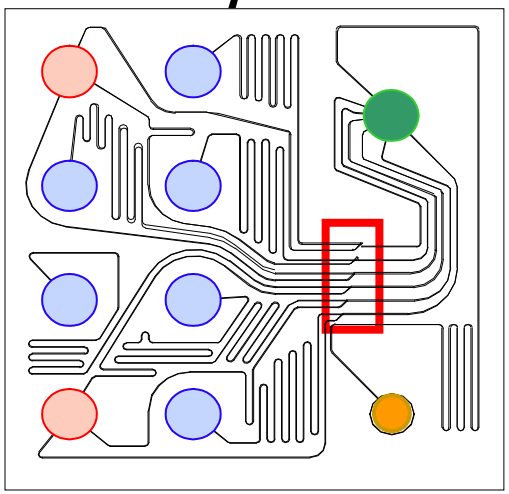
DNA/RNA analysis



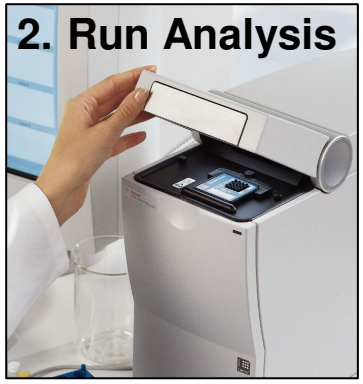
Protein analysis



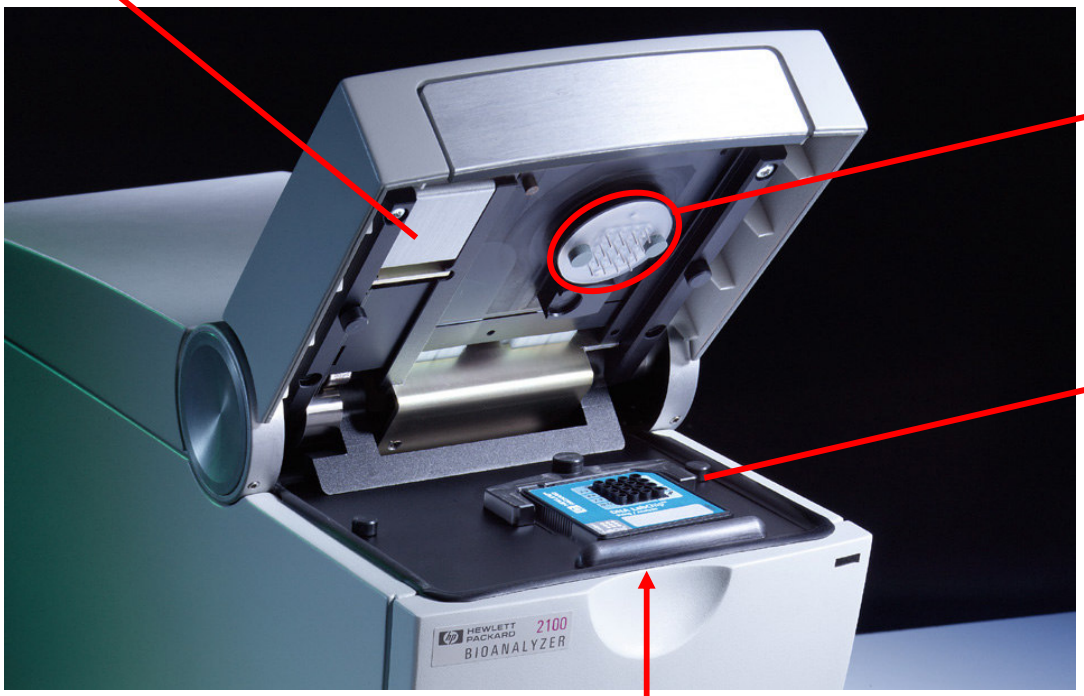
Cell analysis



Agilent 2100 Bioanalyzer



Exchangeable cartridge for different assays



16 pin electrodes connected to HV-sources

Chip holder with heater plate

Optics for detection

Current 2100 Analysis Kits

Electrophoretic Separations

DNA Assays:
1000, 7500, 12000

- Sizing
- Quantitation
- PCR products, digests, larger DNA fragments
- 12 samples in 30 min.

RNA Assays:
nano, pico, Small RNA

- Quantitation (Sizing in Small RNA)
- total RNA, mRNA
- purity & integrity determination
- 10 samples in 30 min.

Protein Assays:
P80, P230, HSP-250

- Sizing
- Quantitation
- cell lysates, column fractions, purified proteins, antibodies etc.
- 10 samples in 40 min.

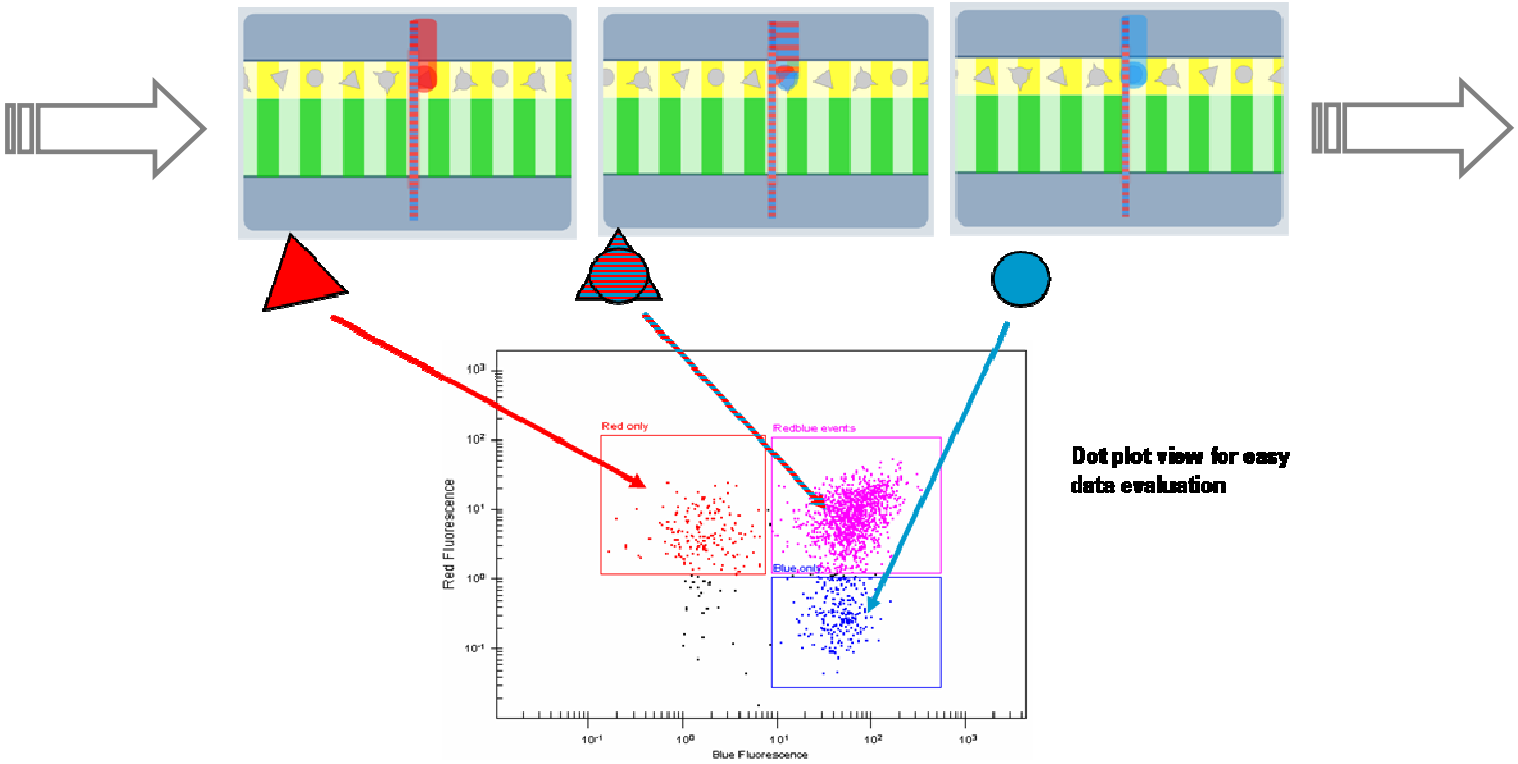
Flow Cytometry

Cell Assays:
Flexible use

- Analysis of 6 samples
- Two color detection
- Analysis of protein expression in cells

Cell Applications

Protein Expression | Apoptosis Detection | Gene Silencing | Transfection Monitoring | Cell Staining Inside / outside



Video

Cell Assay

Apoptosis

Transfection Efficiency Monitoring

- Detection of GFP-transfected cells
- Antibody staining: Detection of transfected cells expressing the encoded protein

Protein Expression Monitoring

- Extracellular and Intracellular Antibody staining for detection of protein expressed on the cell surface, in the cytoplasm, or in the nucleus

Gene silencing

Principle of Pressure-Driven Flow

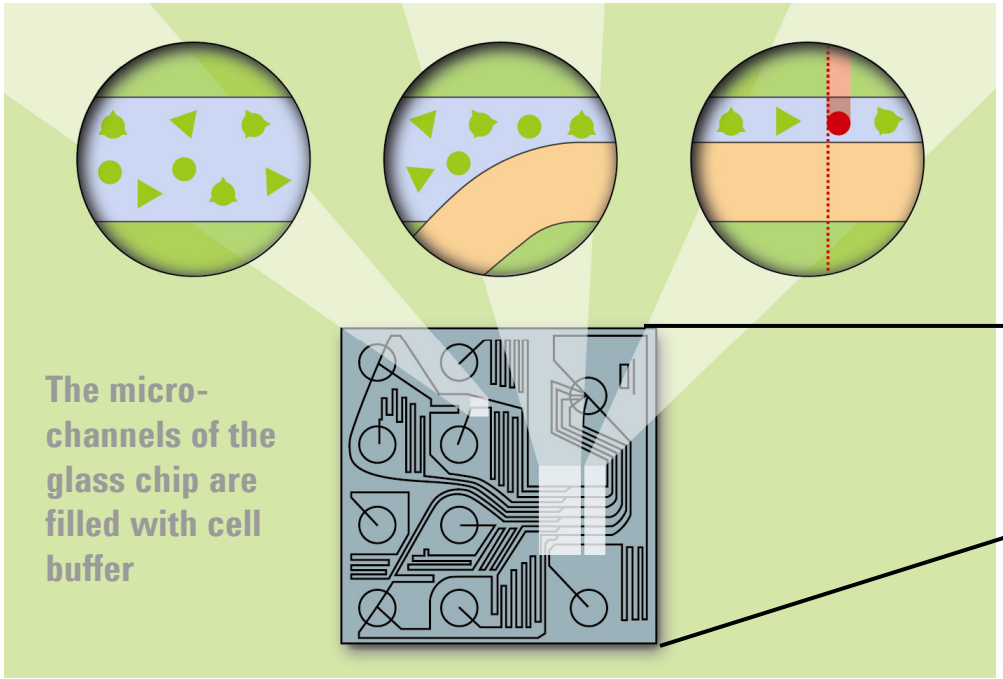
For cell assays (analysis of cell fluorescence parameters)

On-chip simple flow cytometric studies

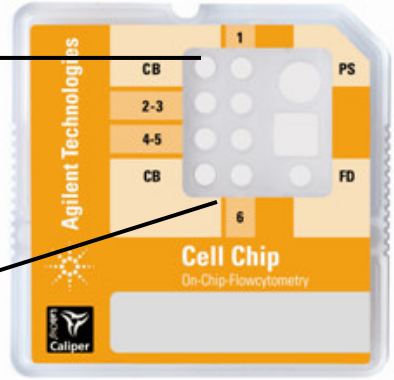
Pressure driven flow is used to move cells in a controlled manner through the micro-channels

Cells are hydrodynamically focused to a portion of the channel by a side stream of buffer

Cells pass the fluo-rescence detector in single file and each event is monitored in a histogram or dot plot



The micro-channels of the glass chip are filled with cell buffer



The Bioanalyzer Lab-on-a-Chip Approach

Separation on disposable, µ-fabricated glass chips

- made of two glass layers:
  - one with micro-channels (x10µm, etched),
  - one with through-holes
- glued into a plastic caddy which accommodates wells for gel, sample, standard (ladder), buffer and other reagents
- for handling nl-amounts of liquids
- one separation channel for ladder and sample
- microfluidic sample movement with fluorescence detection

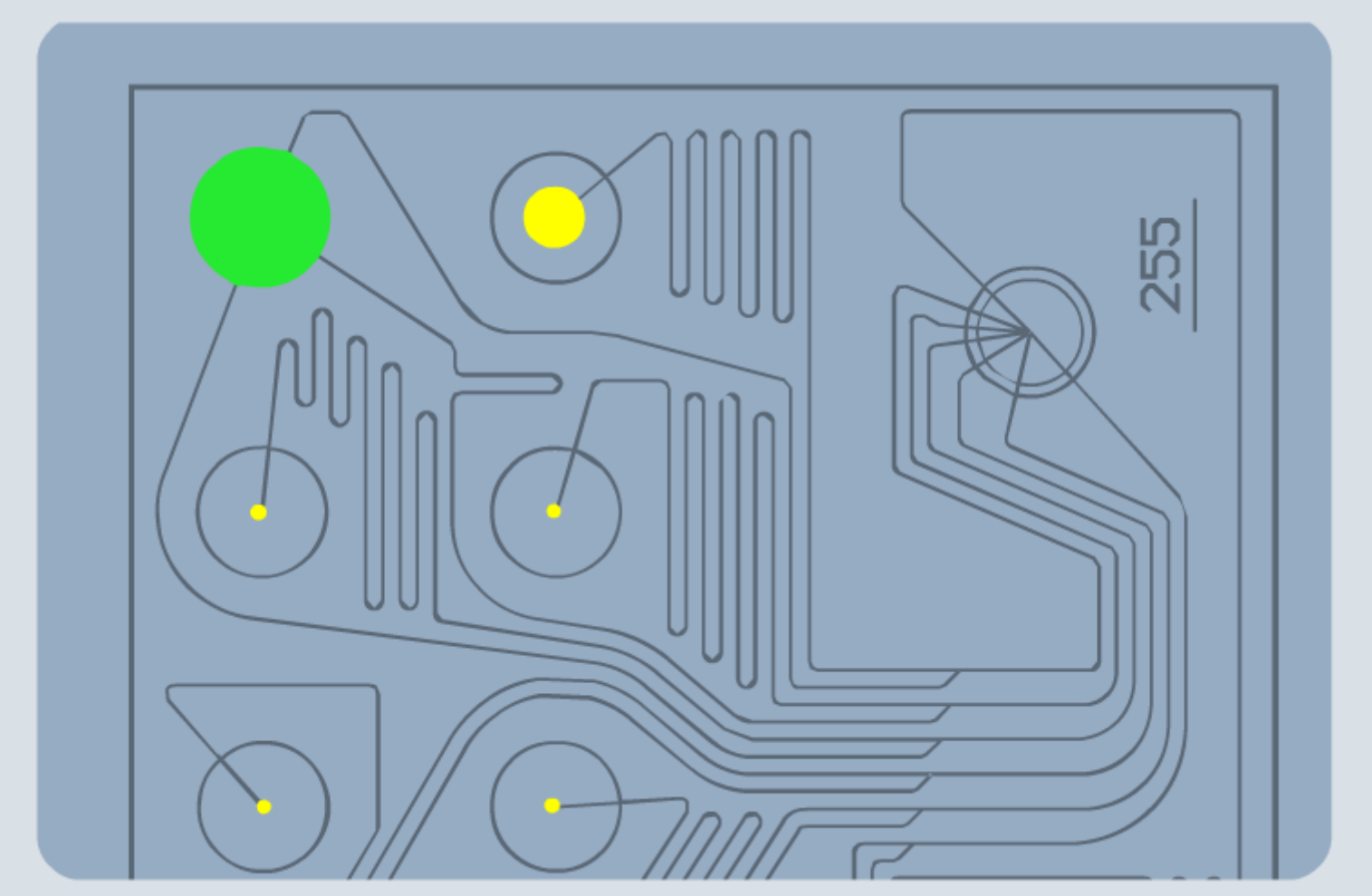
Setup

- micro-channels are filled with gel or buffer
- sample, ladder and reagents are filled into the respective wells
- chip preparation in less than 5 minutes

Benefits

- convenient handling
- minimized risk of cross-contamination
- versatile design for multiple experiments on one platform

Flow cytometry on the chip - Detection Animation



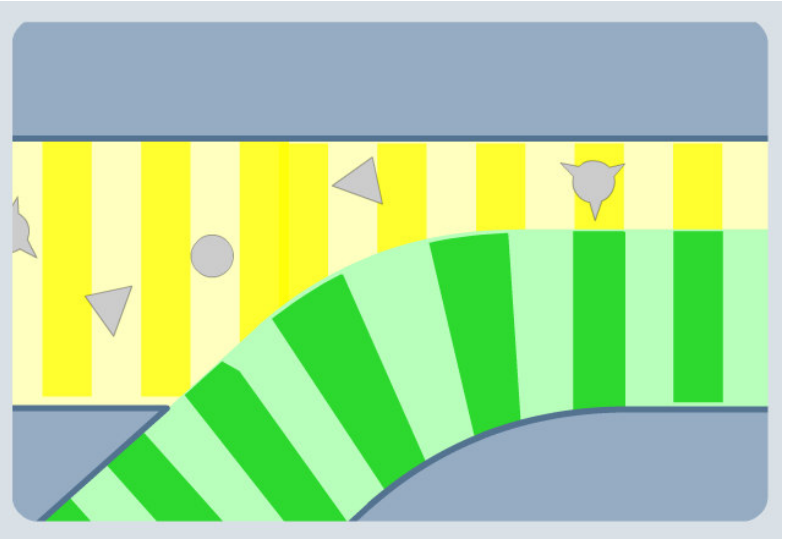
Flow Cytometry on a Chip - Hydrodynamic Focusing

All six cell samples are hydrodynamicly focused to one side of the micro channel

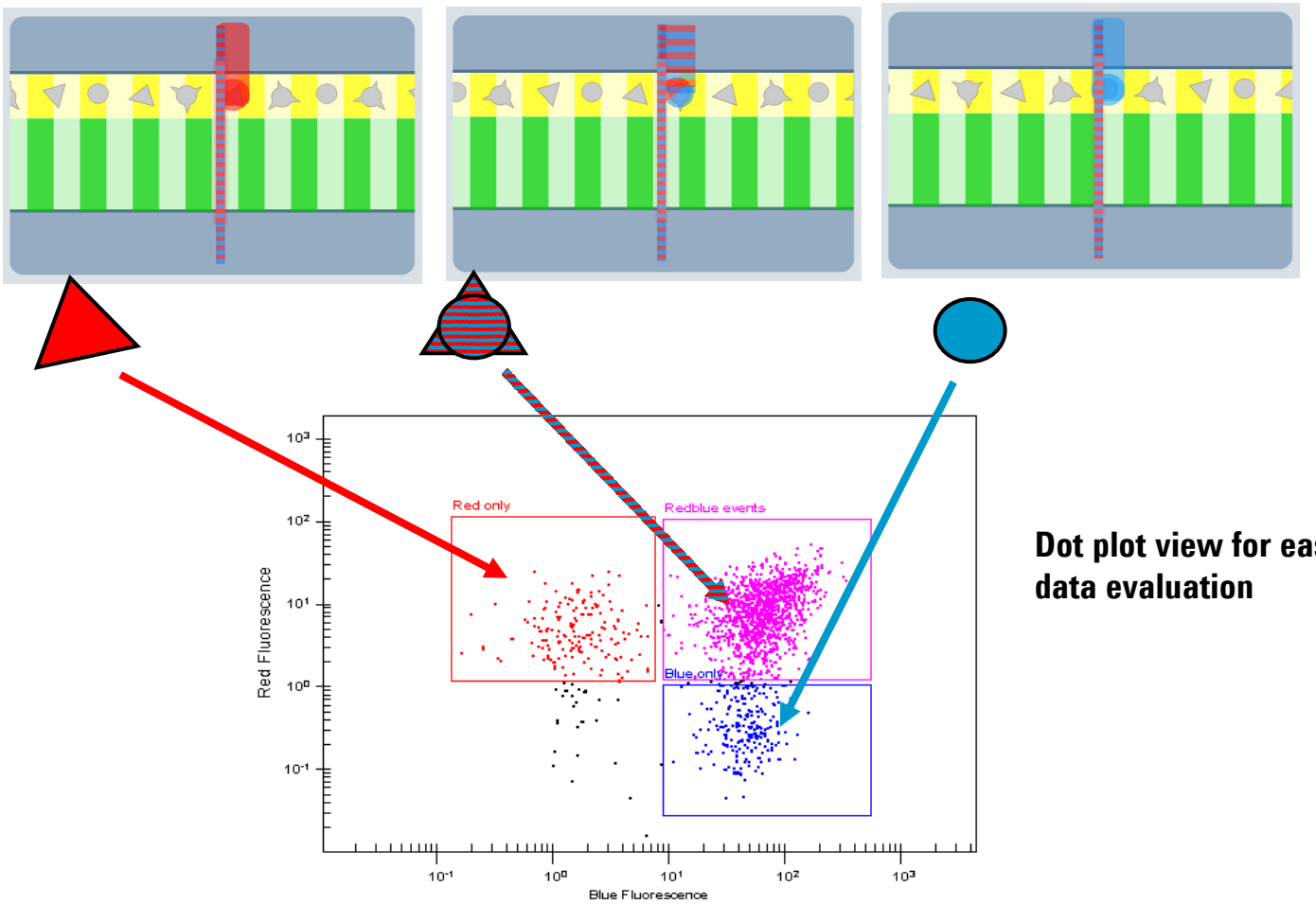
At each of the six pinch points the cell stream is joined by a buffer stream from one of the two buffer wells

The two liquids do not mix immediately

The cells then move towards the detector in single file



Flow Cytometry on a Chip
- Two Color Detection- Three Types of Events



Dot plot view for easy data evaluation

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Some Target applications

Apoptosis:

Annexin V	**Detection of phosphatidylserine on the cell surface**
Caspase-3	**Detection of activated caspase-3 in the cytoplasm**

Transfection Efficiency Monitoring:

GFP:	**Detection of GFP-transfected cells**
Antibody staining:	**Detection of transfected cells expressing the encoded protein**

Protein Expression Monitoring:

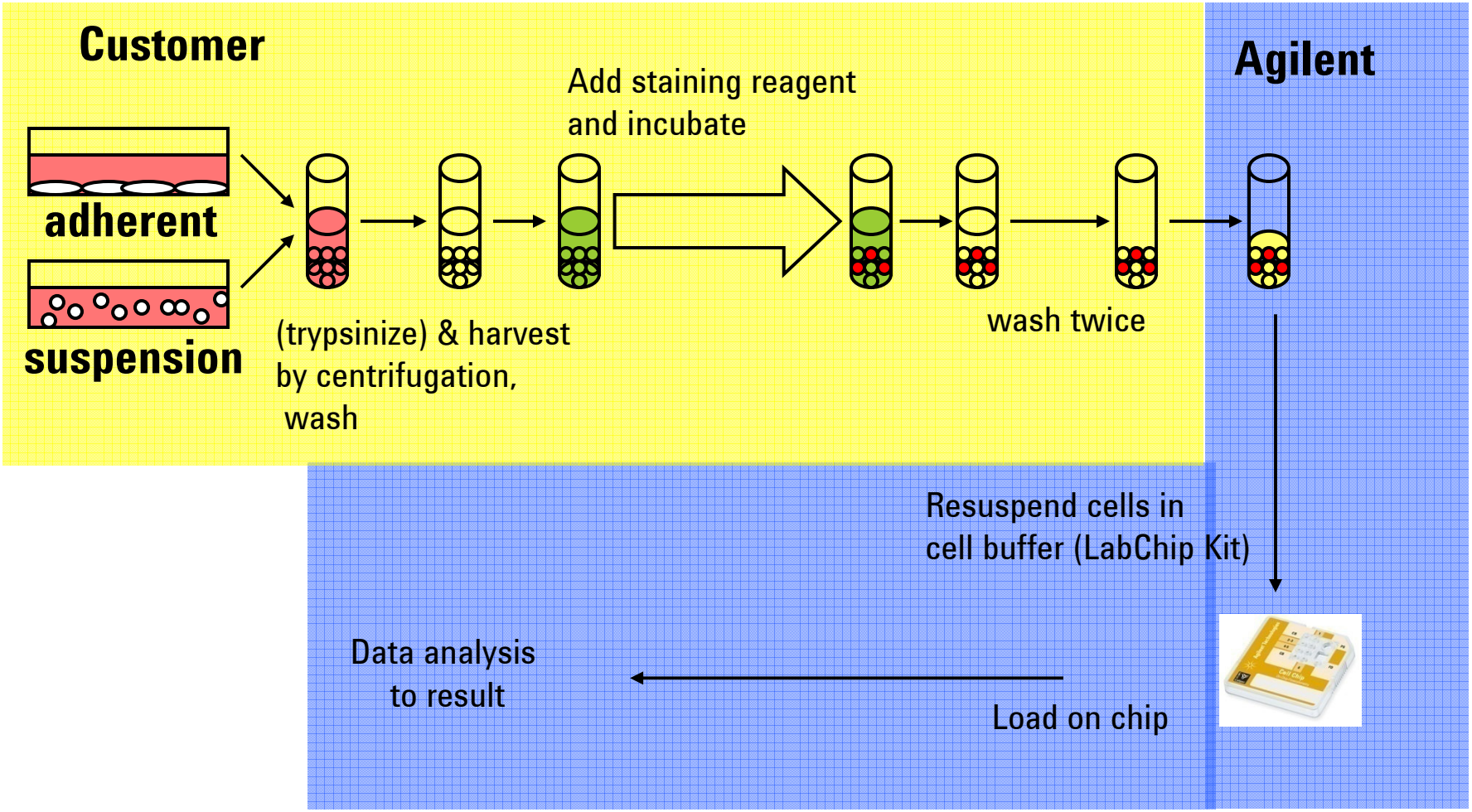
Extracellular and Intracellular Antibody staining for detection of protein expressed on the cell surface, in the cytoplasm, or in the nucleus

Gene silencing:

Optimization of siRNA transfection procedure
Verify silencing by cellular protein expression measurement
Correlation of siRNA uptake and gene knockdown

Cell assays: sample preparation

Typical workflow:

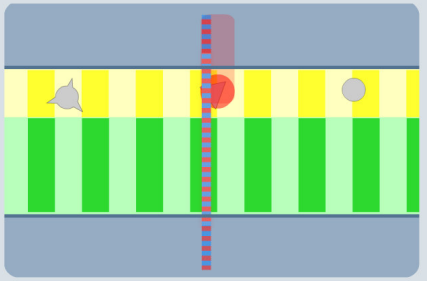


Flow Cytometry on a Chip - Optics & Detection

2100 Bioanalyzer

Red detection channel:

- 620-645 nm excitation with Laser (Maximum 630 nm
- 674-696 nm detection range (Maximum 680 nm)

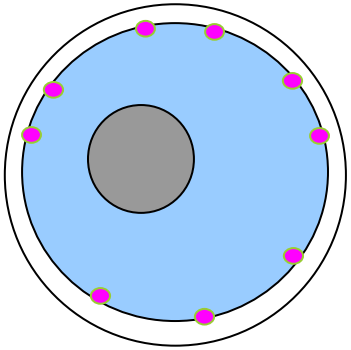


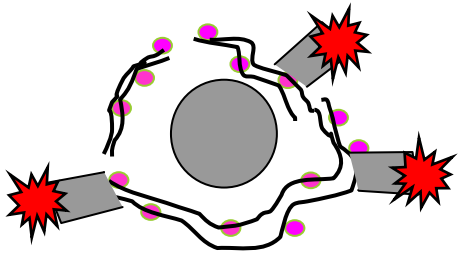
Blue detection channel:

- 458-482 nm excitation with LED (Maximum 470 nm)
- 510-540 nm detection range (Maximum 525 nm)

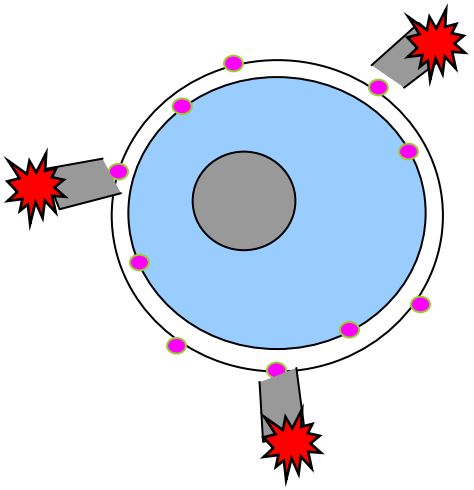
Cell Assays - Applications: Apoptosis Annexin Binding

Healthy cell





Dead cell



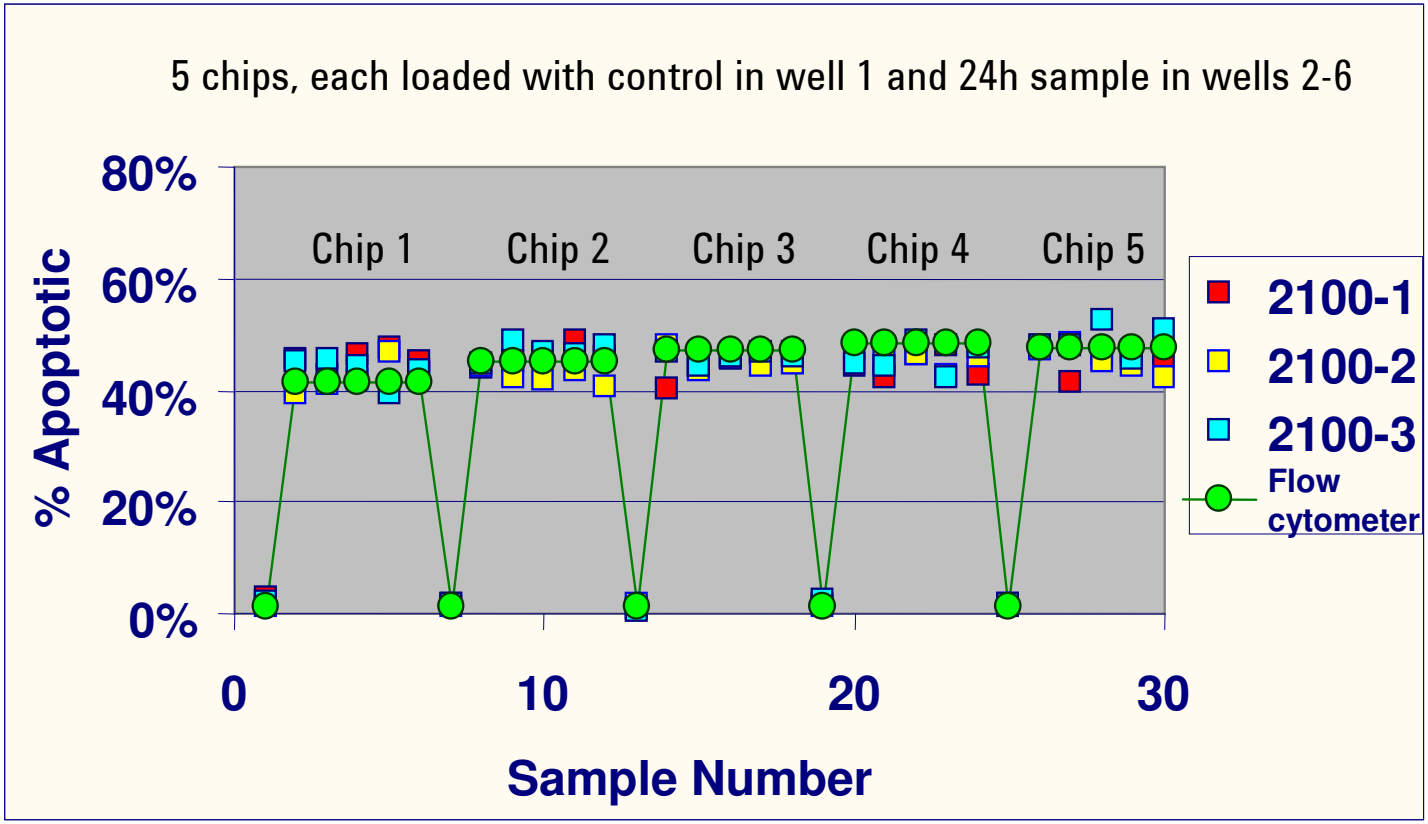
“Live” apoptotic cell

Live dye: Calcein
biotin-Annexin+ Cy5-streptavidin

Phosphatidyl-serine from inner leaflet flips to outer membrane during apoptosis and can be labeled by Annexin V

Annexin V Assay (24h Induction)

Three Bioanalyzer instruments vs a flow cytometer reference instrument



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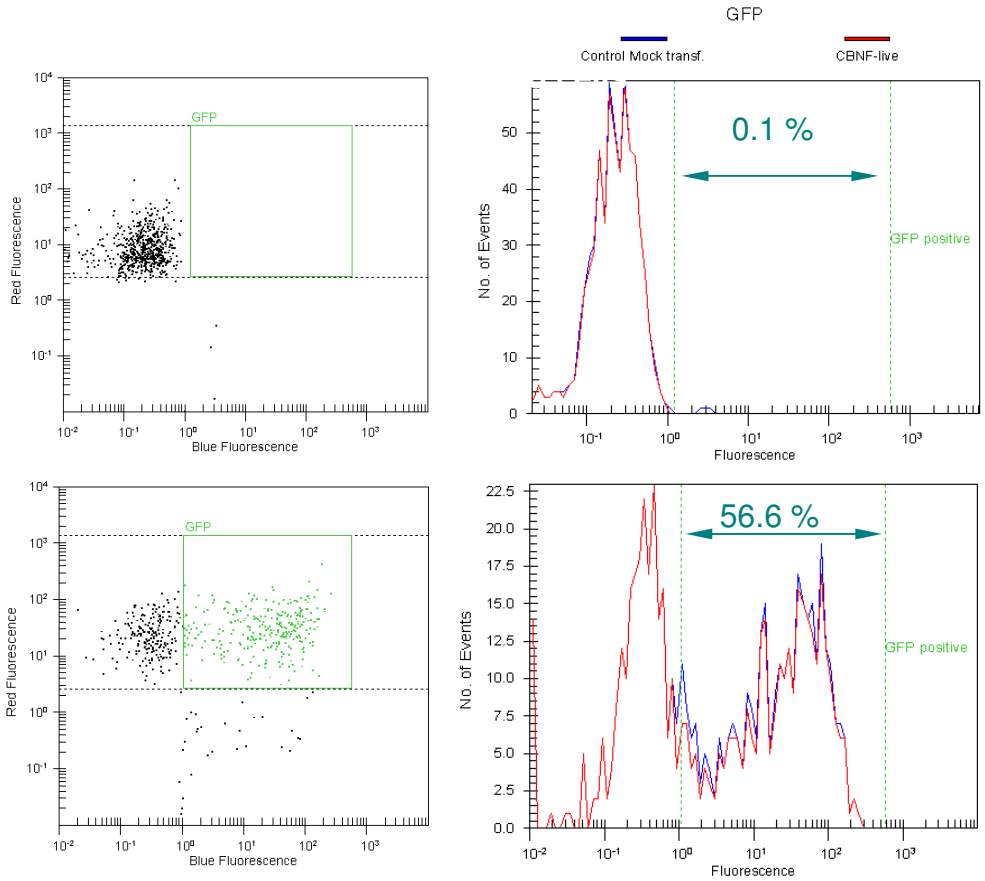
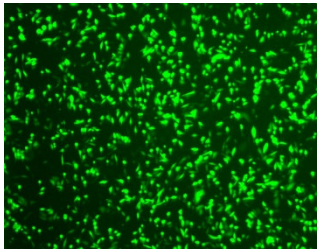
Applications: Protein Expression Analysis

GFP Transfection Efficiency Control



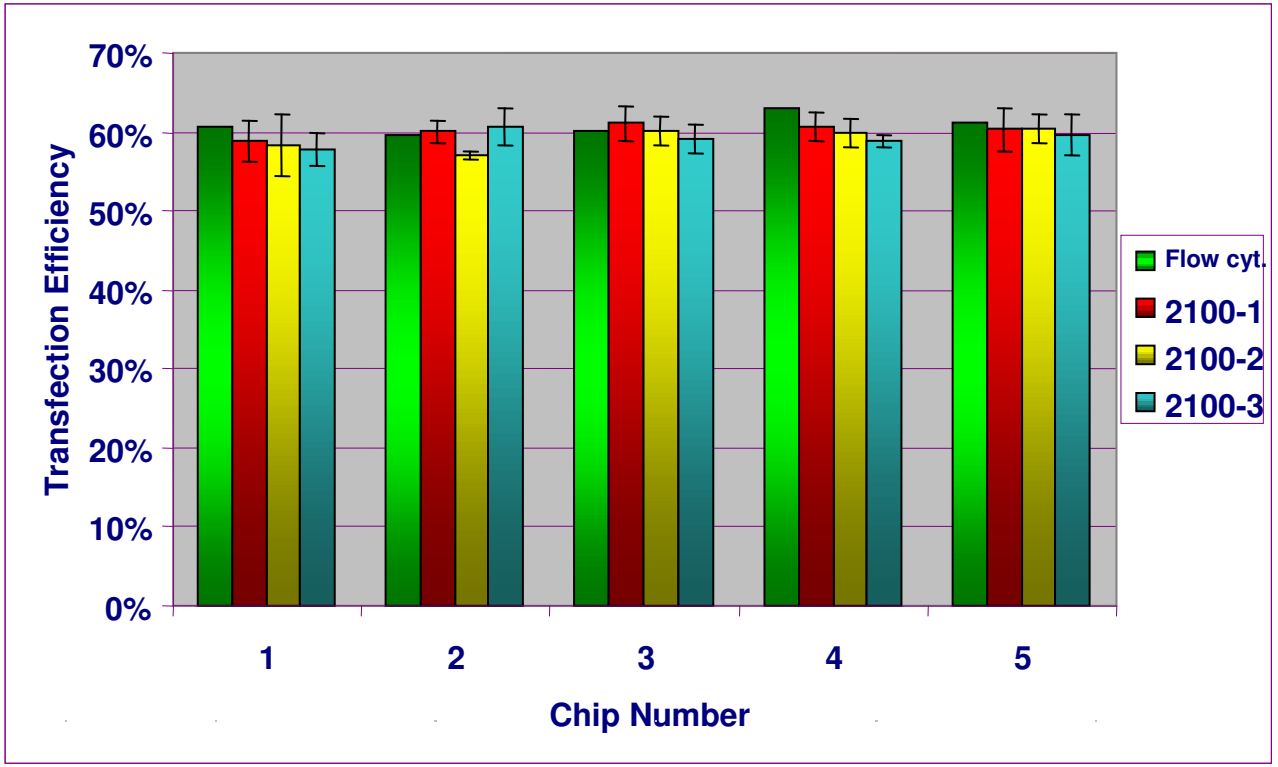
CHO-K1 cells were transfected with EGFP DNA and Lipofectamine.

Control



EGFP transfected

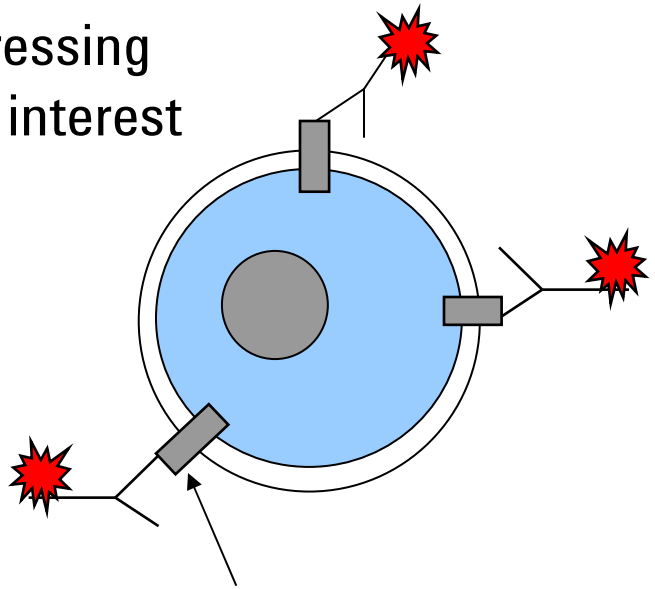
GFP Transfection Efficiency



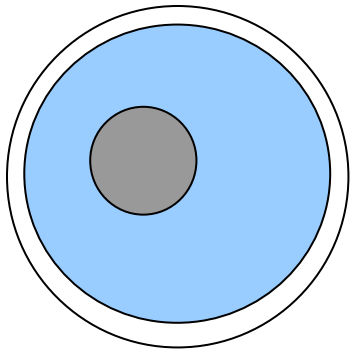
		2100-1	2100-2	2100-3	All	Flow cyt.
ctrl	mean	0.46	0.31	0.47	0.40	0.16
	SD	0.08	0.29	0.43	0.29	0.12
GFP	mean	60.19	59.15	59.26	59.53	60.90
	SD	2.13	2.48	2.10	2.26	1.22
	%CV	3.54	4.19	3.54	3.80	2.01

Flow Cytometry Assays Applications - Cell surface Antibody staining

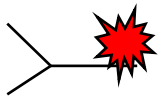
Cell expressing protein of interest



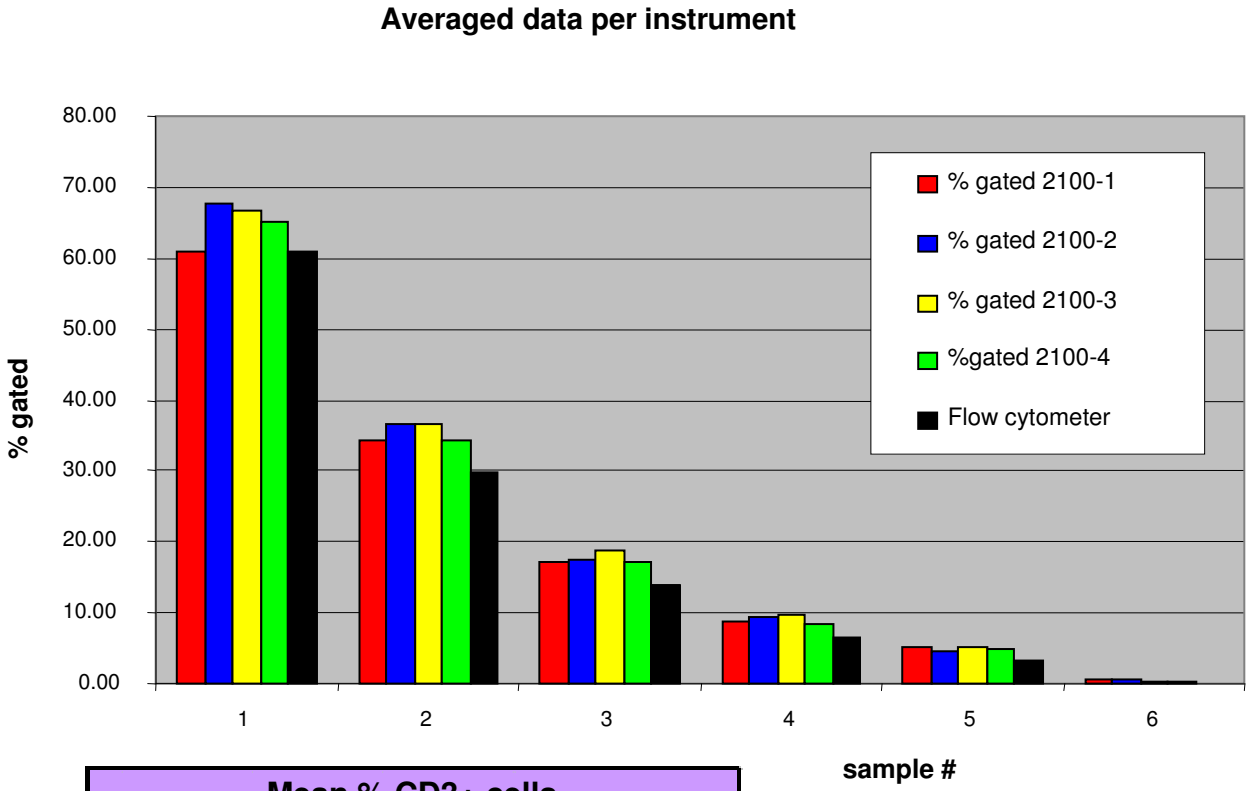
Cell not expressing protein of interest



Live dye: Calcein
Cy5 or APC-labeled Antibody



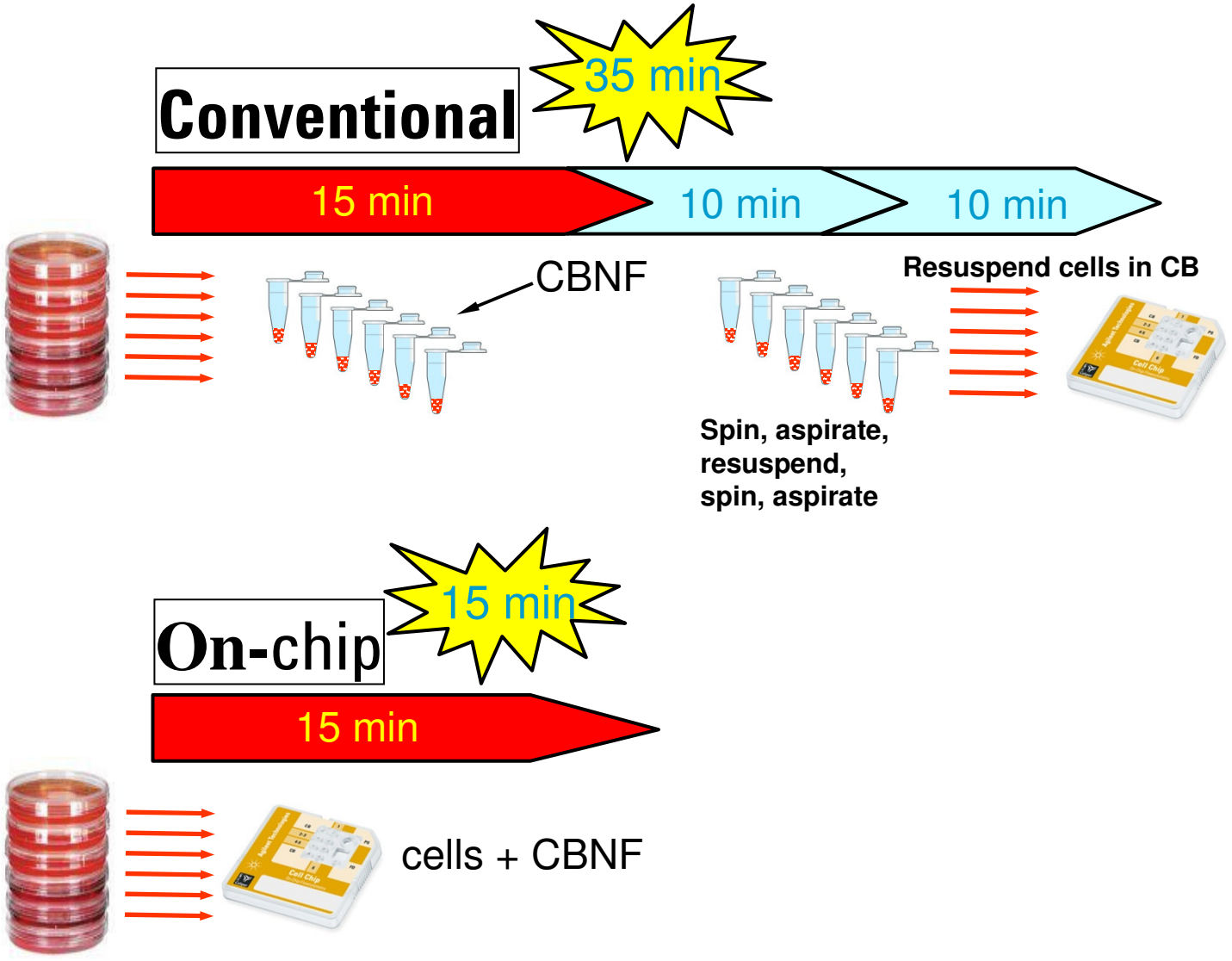
Extracellular Antibody Staining



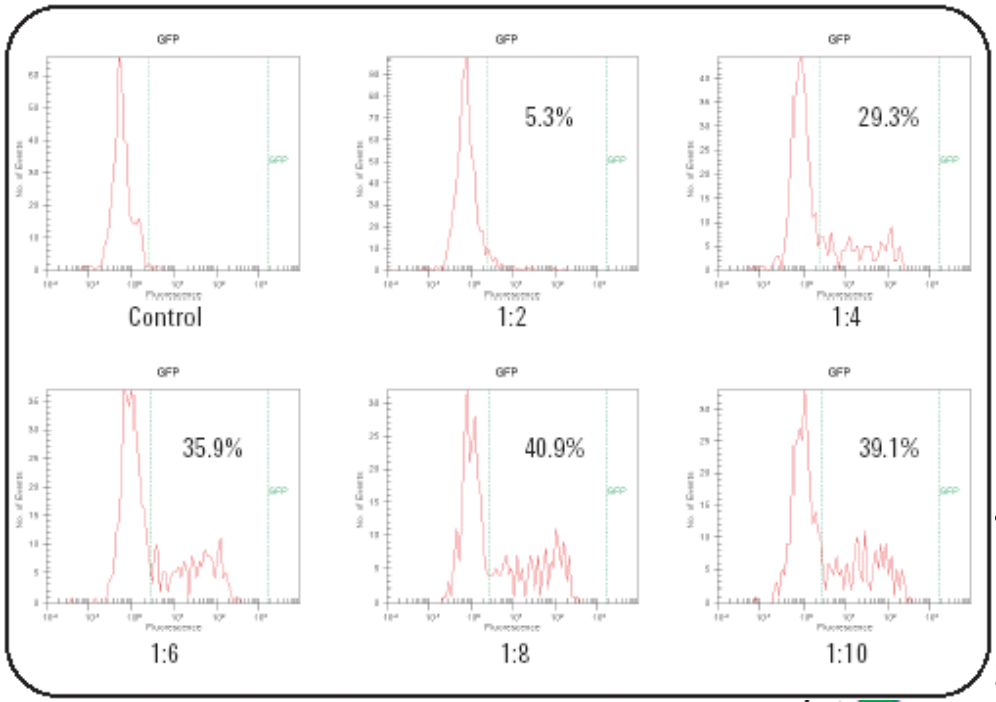
Mean % CD3+ cells				
2100-1	2100-2	2100-3	2100-4	Flow cyt.
60.9	67.8	66.6	65.0	60.9
34.4	36.7	36.7	34.3	29.8
17.3	17.6	18.7	17.2	13.8
8.9	9.4	9.9	8.3	6.5
5.1	4.4	5.3	4.9	3.2
0.8	0.6	0.3	0.3	0.0

Jurkat cells were stained with calcein alone or with calcein and APC-labeled anti-CD3 antibody.
Mixtures of both populations were prepared at various ratios.
Samples were analyzed with four 2100 instruments on 5 chips and compared to a flow cytometer reference instrument

GFP On-Chip Staining - Workflow

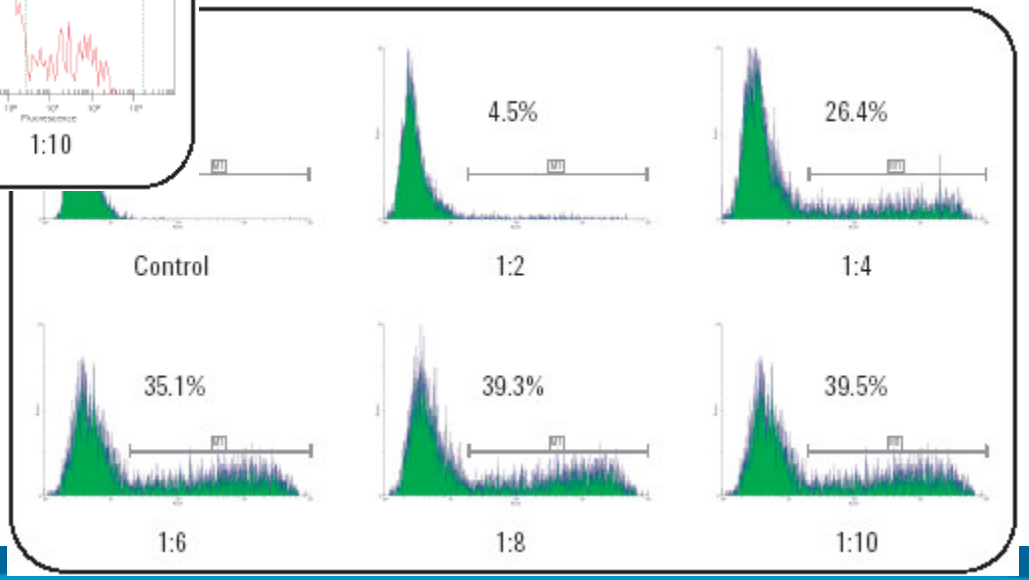


GFP On-Chip Staining - Histogram Quality



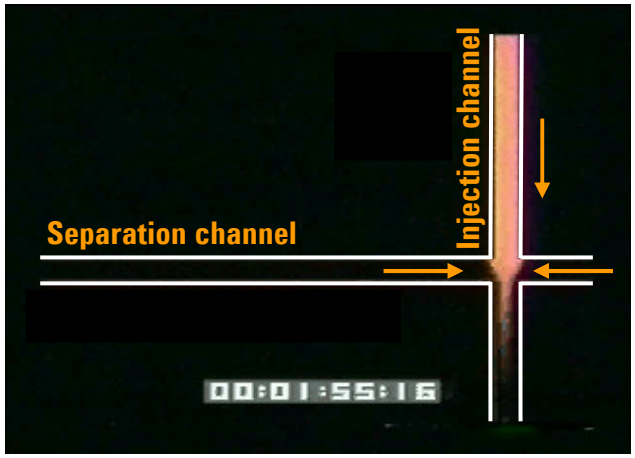
2100 bioanalyzer

Flow Cytometer



Lab-on-a-Chip - Principle of Injection & Separation

1



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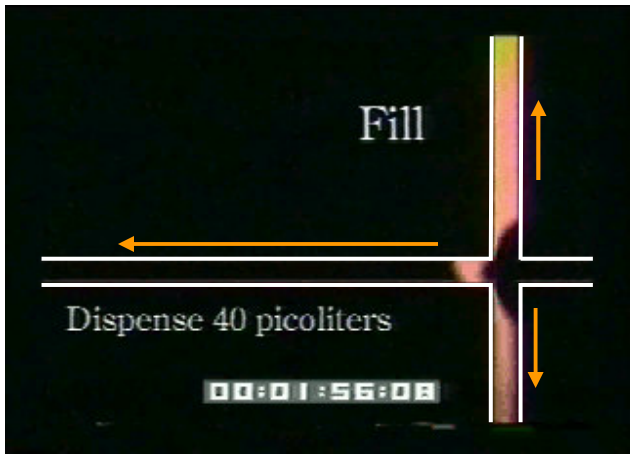

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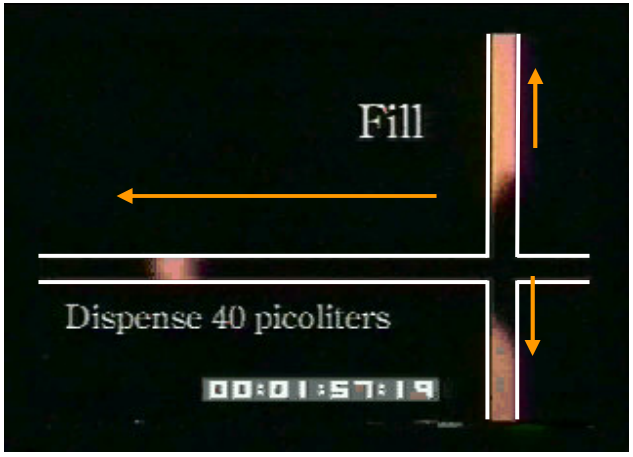
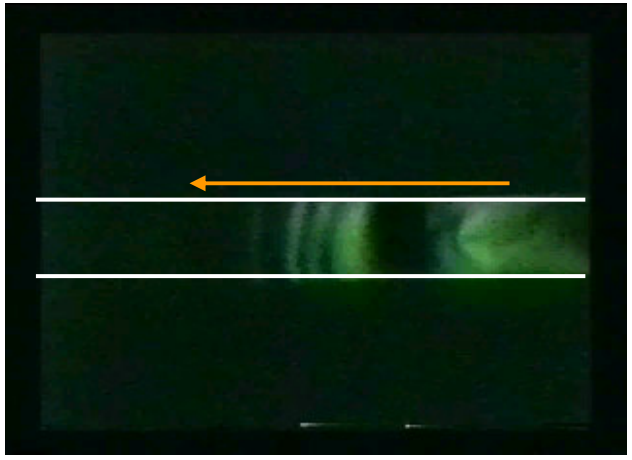


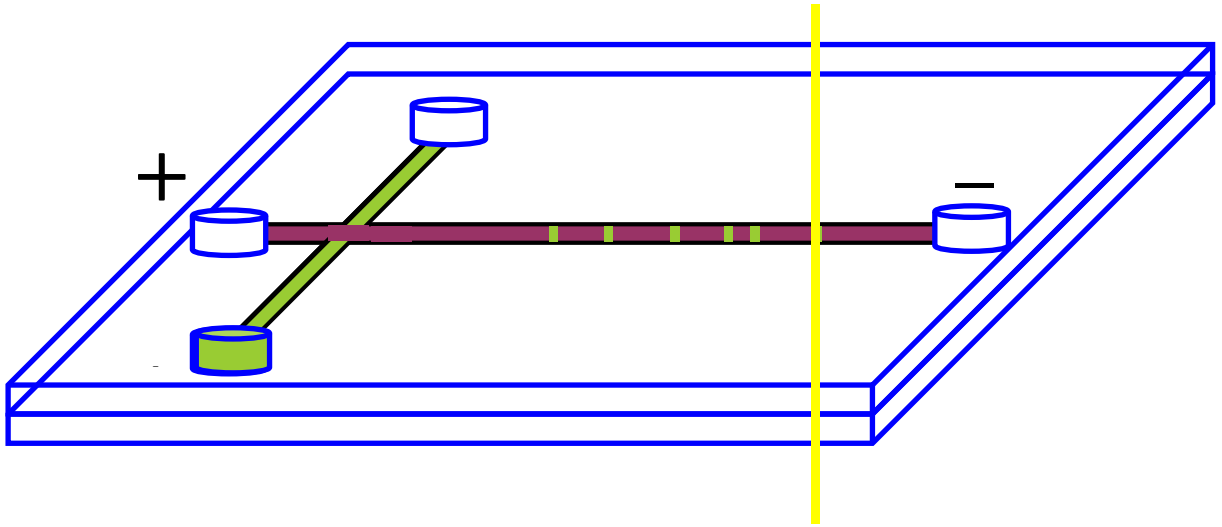
4



← Direction of electrodriven movement of liquids and molecules within liquids

The LabChip® Approach - Simplified Model

(see chip animation.ppt)



DNA Applications

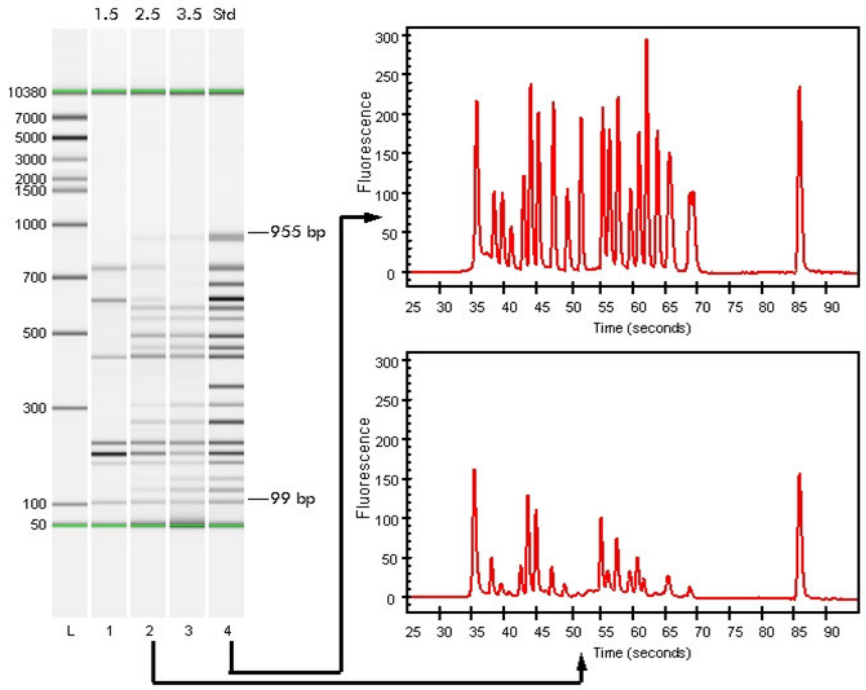
mPCR validation, impurity check

Gene Expression

Restriction Digest Analysis

Food Analysis

Forensic Testing



Application Areas for the DNA Assays

PCR product purity

Multiplex PCR Applications

Gene expression analysis via RT-PCR (target validation)

GMO testing

Pathogen detection (homeland defense, hospitals, environmental)

Genotyping applications

- **Duplications/ deletions**
- **Allele frequency**
- **Bacterial sub-typing**
- **Forensics**

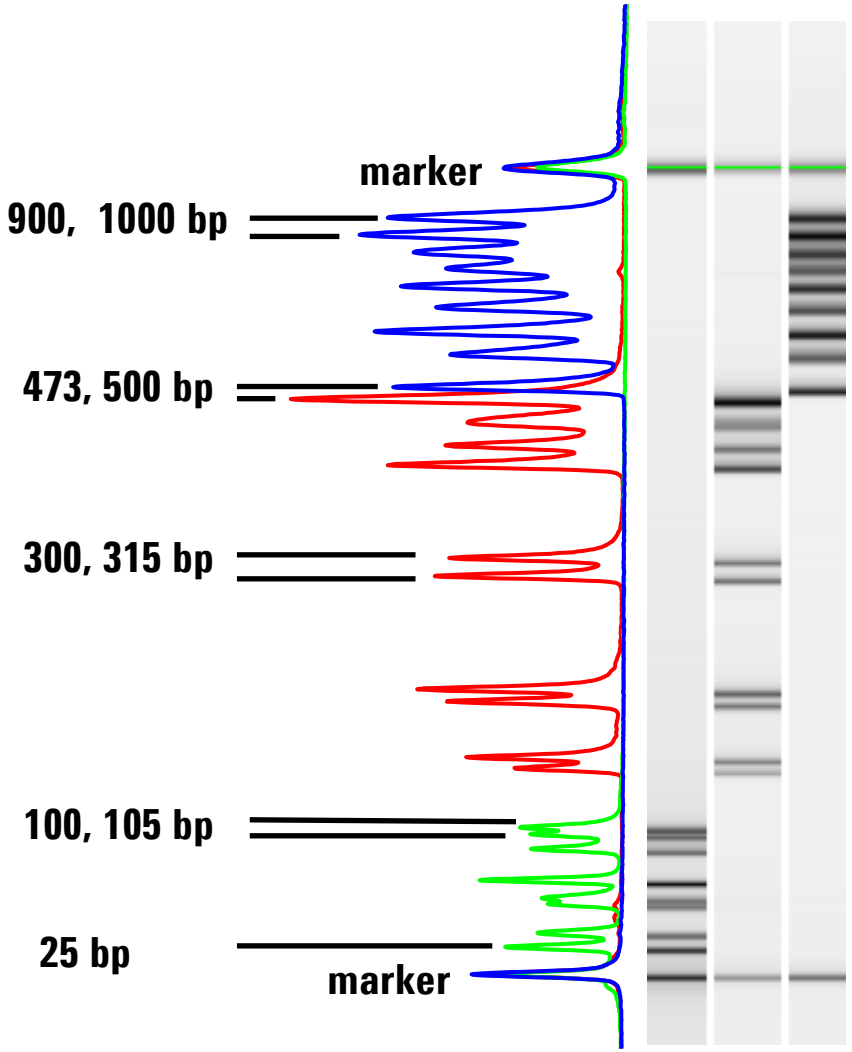
Cancer diagnostics

DNA Kit Specification

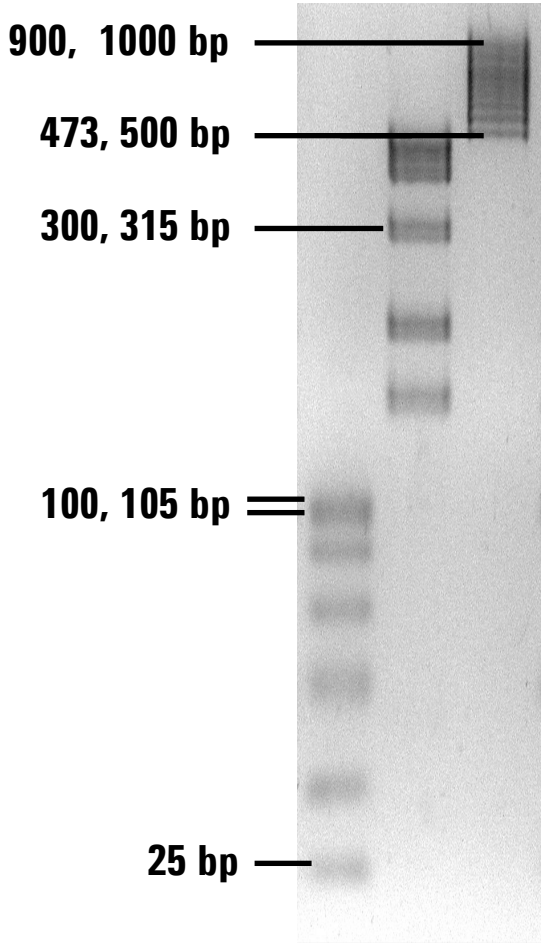
	DNA 1000 Assay	DNA 7500 Assay	DNA 12000 Assay
Sizing range	25–1000 bp	100–7500 bp	100–12000 bp
Sizing resolution	25–100 bp: 5 bp 100–500 bp: 5 % CV 500–1000 bp: 10 % CV	100–1000 bp: 5 % 1000–7500 bp: 15 %	100–1000 bp: 5 % 1000–12000 bp: 10 %
Sizing accuracy	± 10 %	± 10 % CV *	±15 %
Sizing reproducibility	5% CV *	5% CV *	5% CV *
Quantitation accuracy	20% CV *	20% CV *	25% CV *
Quantitation reproducibility	25–500 bp: 15 % CV * 500-1000 bp: 5 % CV *	100-1000 bp:10 % CV * 1000-7500 bp: 5 % CV *	100-1000 bp: 15 % CV * 1000-12000 bp: 10 % CV *
Quantitative range	0.1 - 50 ng/µL *	0.1 - 50 ng/µL *	0.1 - 50 ng/µL *
Maximum salt concentration in sample	250 mM for KCl, 15 mM for MgCl2 250 mM NaCl	250 mM for KCl 15 mM for MgCl2 250 mM NaCl	250 mM for KCl 15 mM for MgCl2 250 mM NaCl
25 chips per kit	DNA 12/chip = 300 samples/kit		

* Respective DNA ladder as sample

Data Format - Gel-Like Image c/w Agarose Gel

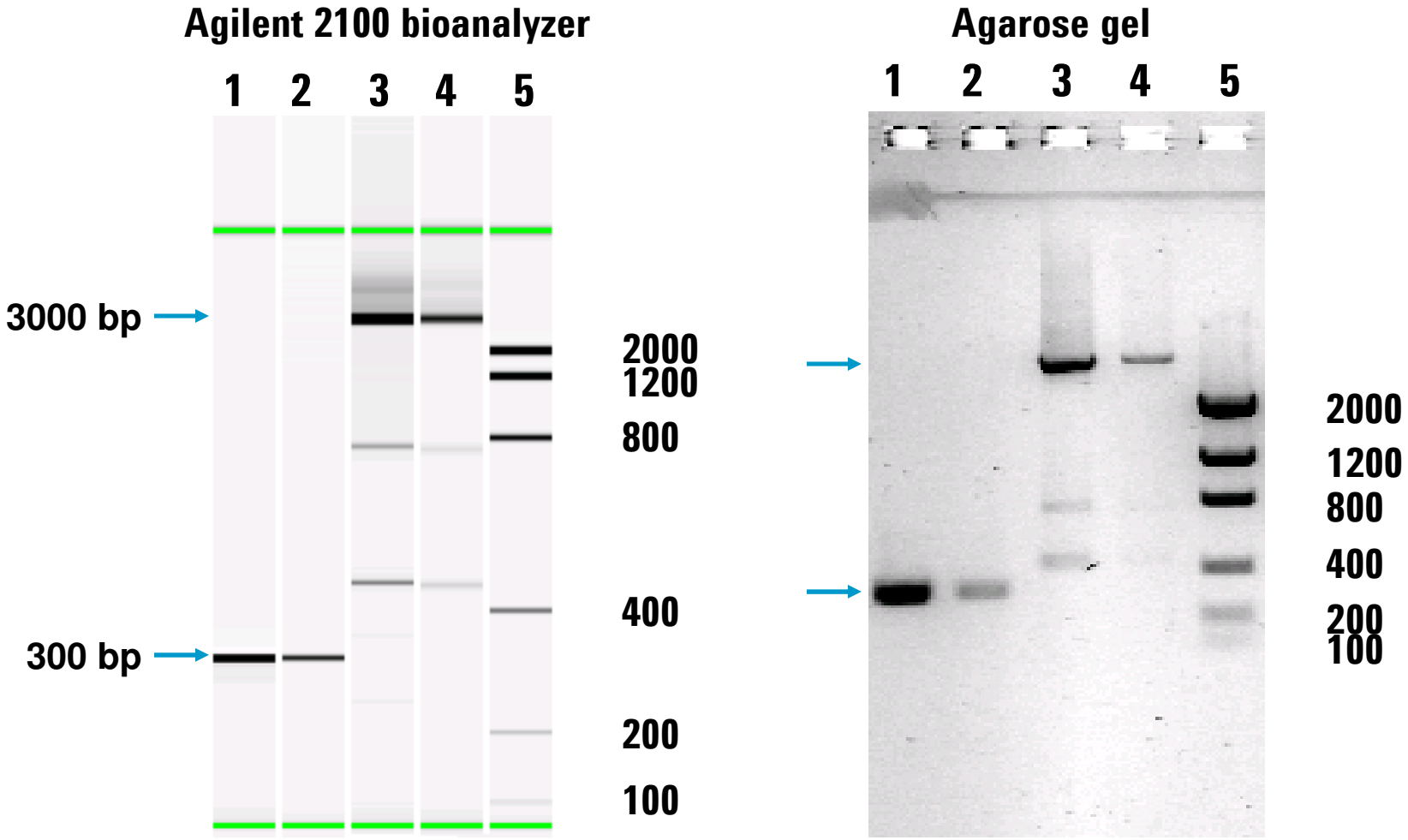


2100 bioanalyzer data
Gel-like image

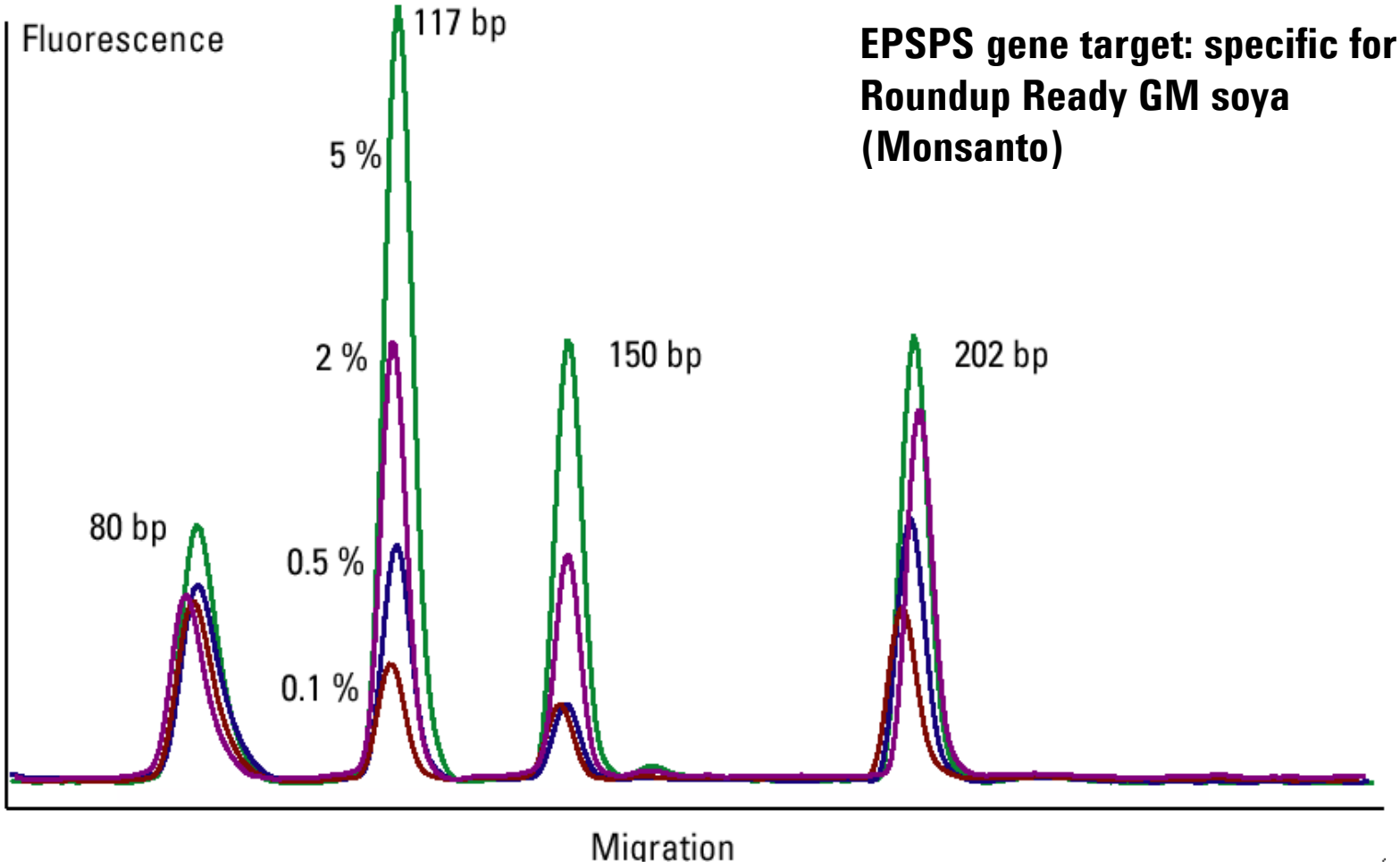


2 % agarose gel stained
with Ethidiumbromide

Determination of PCR Product Impurity

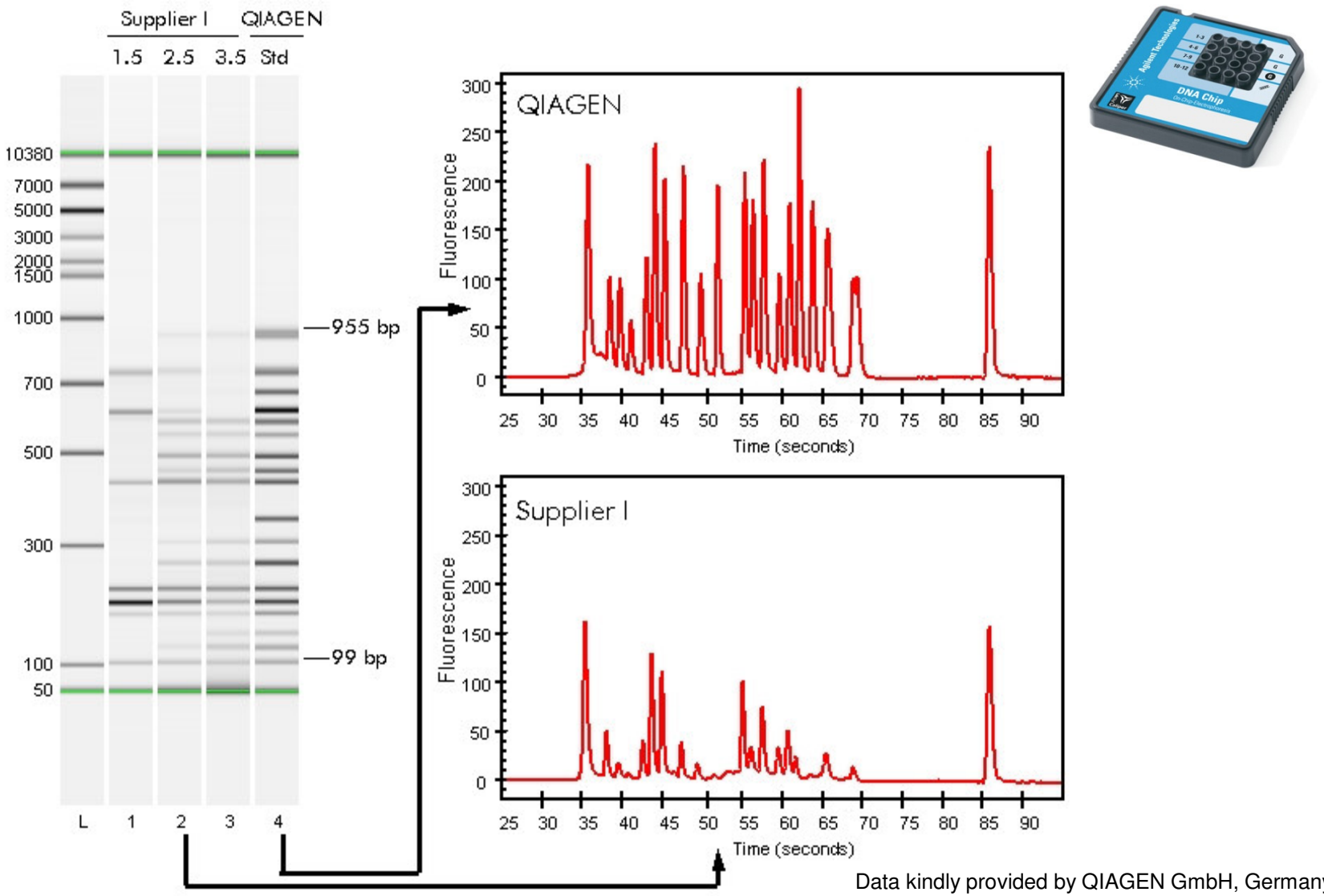


GMO Detection: Determination of GM Soya Percentage



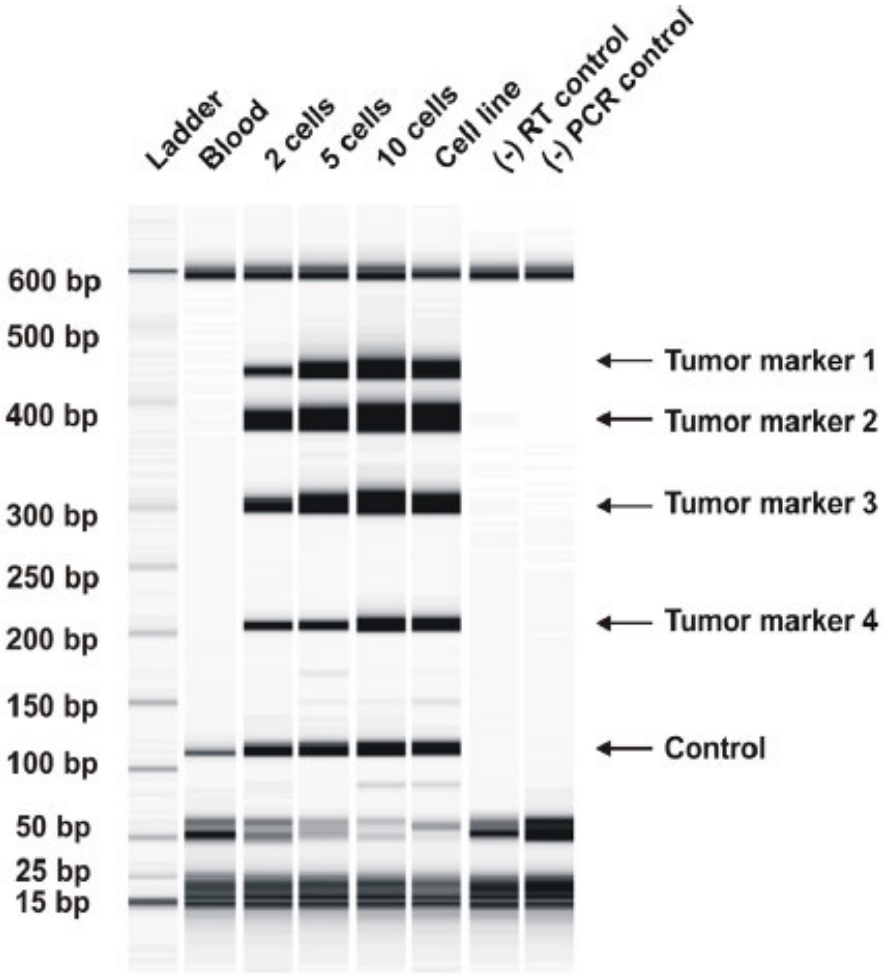
EPSPS gene target: specific for Roundup Ready GM soya (Monsanto)

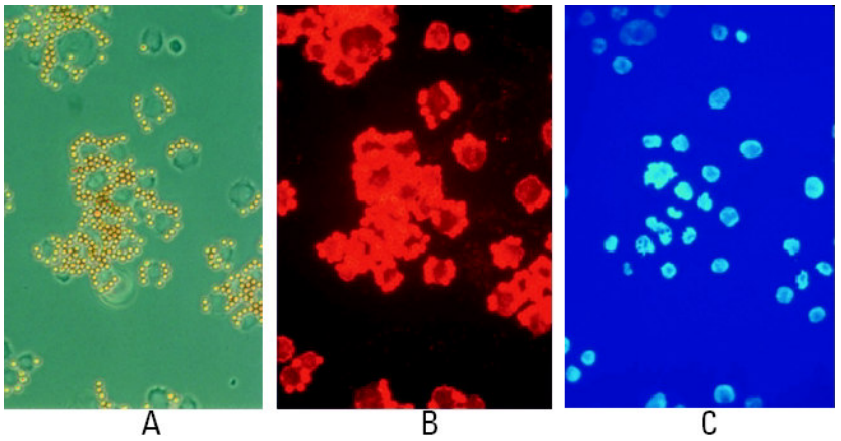
Optimization of Multiplex PCR on a 19-plex PCR



Data kindly provided by QIAGEN GmbH, Germany

Tumor Diagnostics



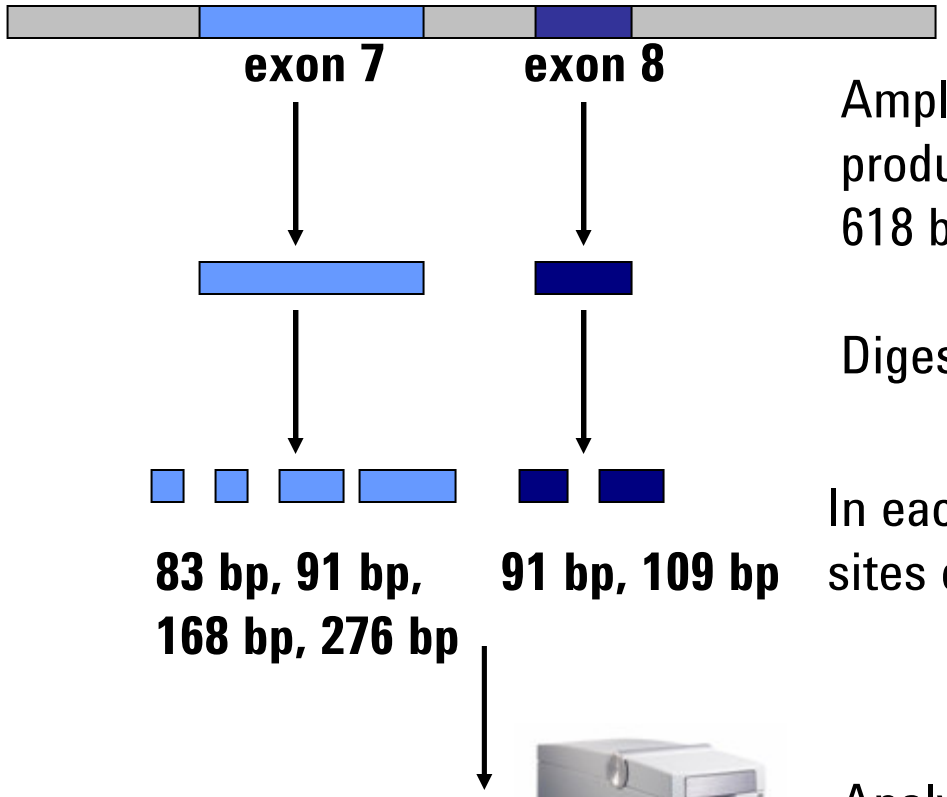


1. Spiking experiment with given amount of cancer cells
2. Enrichment with AdneGen Cancer Select kit (antibody based immunomagnetic enrichment.)
3. Multiplex Amplification with AdnaGen CancerDetect kit
4. Detection with Agilent 2100 Bioanalyzer and DNA 500 LabChip kit

Data kindly provided by Adnagen

Detection of Single Base Mutations (1)

in Exons 7 and 8 of the Human p53 Gene by RFLP Mapping using the DNA 7500 kit



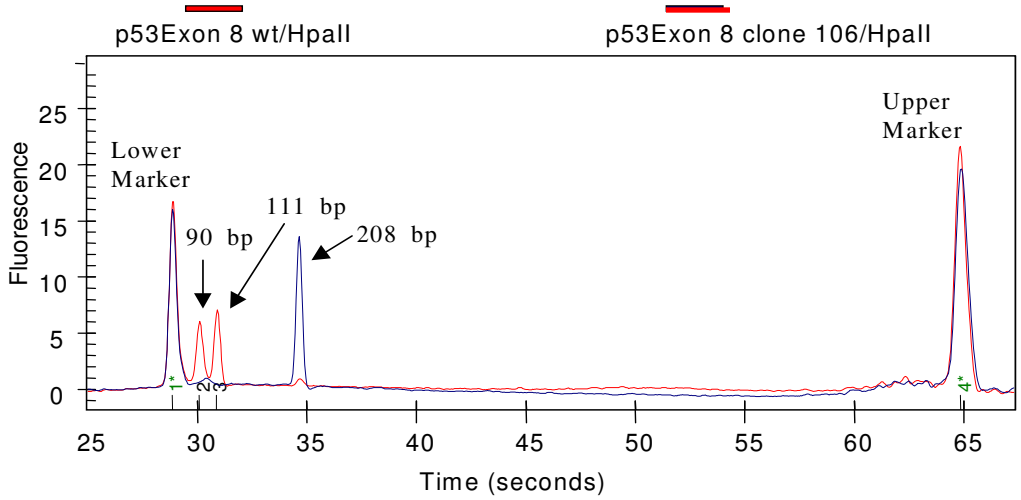
Amplify exons 7 and 8 (resulting products:
618 bp fragment and 200 bp fragment)

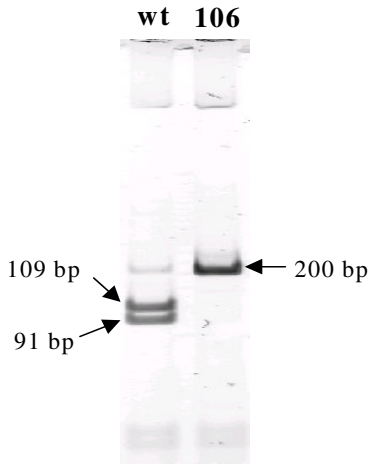
Digest with Hpa II

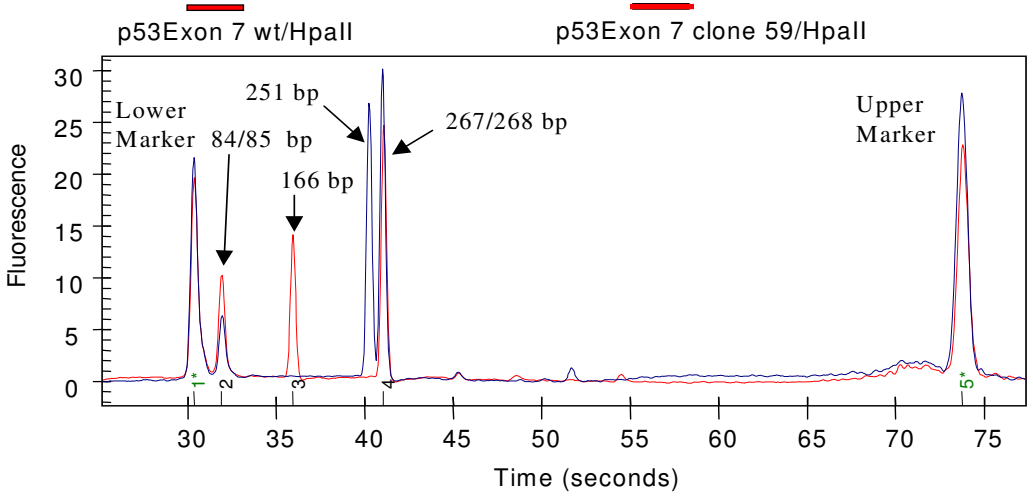
In each example one of the restriction sites can be deleted by a point mutation

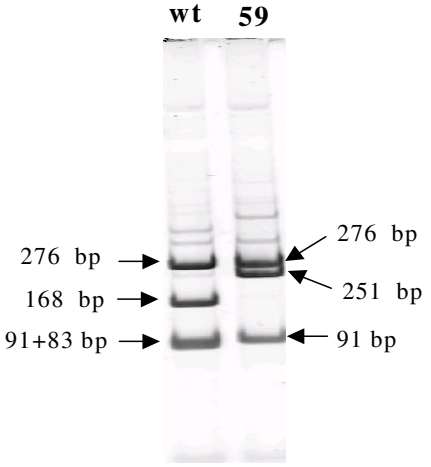
Analyze using Agilent 2100 bioanalyzer and 4-20 % acrylamide gel

Detection of Single Base Mutations (2)









Label free Analysis of Microsatellite Instabilities

Clinical Diagnostics and Molecular Diagnostics of Cancer

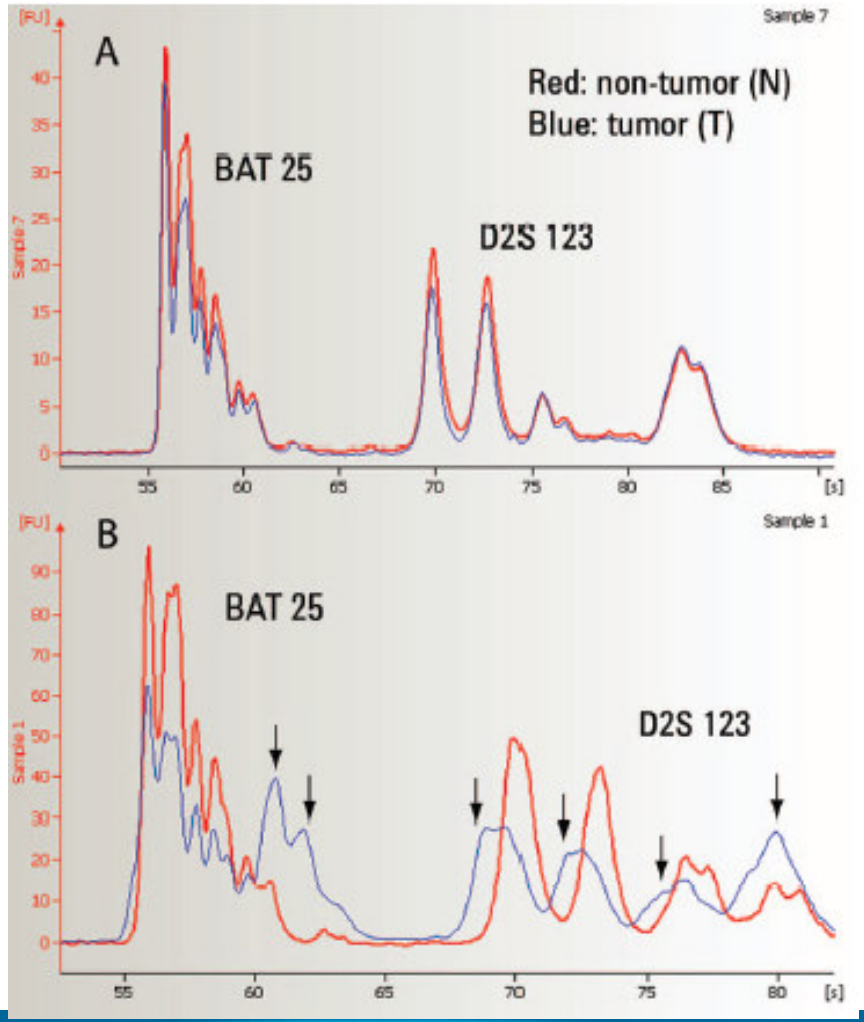
Microsatellite instabilities present in 10-15% of colon and gastric carcinomas

Study: 40 cases of colon carcinoma

5 microsatellite loci investigated

Results compared with traditional PAGE:

95% concordance rate



Agilent Technologies

RNA Applications

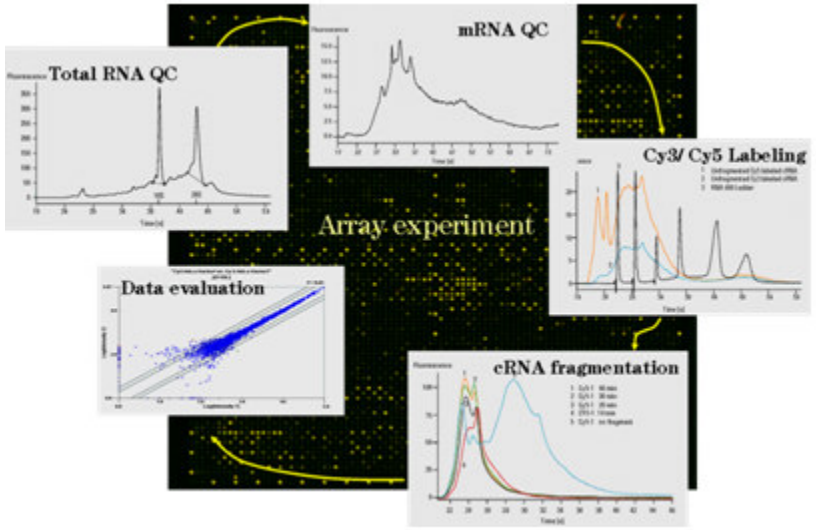
RNA QA/QC for Microarrays

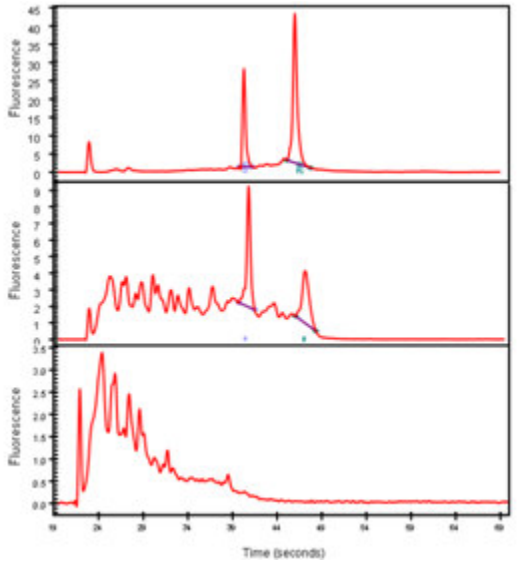
Gene Expression

RNA QA/QC for qPCR

RNA QA/QC for mPCR

smallRNA QA/QC





Intact RNA: RIN 10

Partially degraded RNA: RIN 5

Strongly Degraded RNA: RIN 3

Agilent 2100 bioanalyzer: the industry standard in RNA QC

Electrophoretic sizing, quantitation and QC of XNA and Proteins on a small glas Chip as done traditionally on slab gels (Agarose or SDS-PAGE)

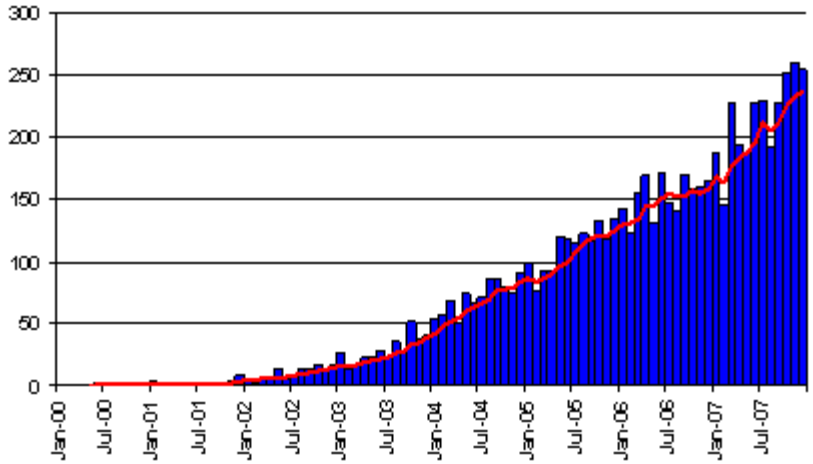
First commercially available Lab-on-a-Chip product (since October 1999)

Analysis of totalRNA, mRNA and small RNA samples in ng and pg concentration range

Standardized RNA integrity assessment with **RIN*** algorithm

Multi-analysis capabilities: DNA, RNA, Proteins and Flow Cytometry

Number of BioA publications/month



January 2008: ~ 7200 citations

RIN = RNA **I**ntegrity **N**umber, an Agilent patented algorithm to Determine RNA quality in a normalized way

RNA Kit Specifications

	RNA 6000 Nano total RNA	RNA 6000 Nano mRNA	RNA 6000 Pico total RNA	RNA 6000 Pico mRNA	Small RNA Assay
Analytical Specifications					
Quantitative range	25–500 ng/µL	25–250 ng/µL			50-2000 pg/µL of purified miRNA in water
Qualitative range	5–500 ng/µL	25–250 ng/µL	50–5000 pg/µL in water	250–5000 pg/µL in water	50-2000 pg/µL of purified miRNA in water
Sensitivity (S/N>3)	5 ng/µL in water	25 ng/µL in water	50 pg/µL in water 200 pg/µL in TE	250 pg/µL in water 500 pg/µL in TE	50 pg/µL in water**
Quantitation reproducibility	10% CV (within a chip)	10% CV (within a chip)	20 % CV (within a chip)	20 % CV (within a chip)	25 % CV (within a chip)
Quantitation accuracy	20 % CV*	20 % CV*	30 % CV*	30 % CV*	
Maximum sample buffer strength	100 mM Tris, 0.1 mM EDTA or 125 mM NaCl or 15 mM $MgCl_2$	100 mM Tris, 0.1 mM EDTA or 125 mM NaCl or 15 mM $MgCl_2$	50 mM Tris, 0.1 mM EDTA or 50 mM NaCl or 15 mM $MgCl_2$	50 mM Tris, 0.1 mM EDTA or 50 mM NaCl or 15 mM $MgCl_2$	10 mM Tris, 0.1 mM EDTA
Physical Specifications					
Analysis time	30 minutes	30 minutes	30 minutes	30 minutes	30 minutes
Samples per chip	12	12	11	11	11
Sample volume	1 µL	1 µL	1 µL	1 µL	1 µL
Kit stability	≥4 months at 4 °C	≥4 months at 4 °C	≥4 months at 4 °C	≥4 months at 4 °C	≥4 months at 4 °C
25 chips per kit	RNA Nano 12/chip = 300 samples/kit		RNA Pico 11/chip = 275 samples/kit		

* Determined analyzing the RNA ladder as sample

** Measeured for the 40 nt fragment of the Small RNA ladder

Features of the RNA 6000 Assays

total RNA

determine integrity and quality of total RNA

determination of RNA concentration

identify ribosomal peaks

calculate the ratio of ribosomal peaks (18S/28S or 16S/23S)

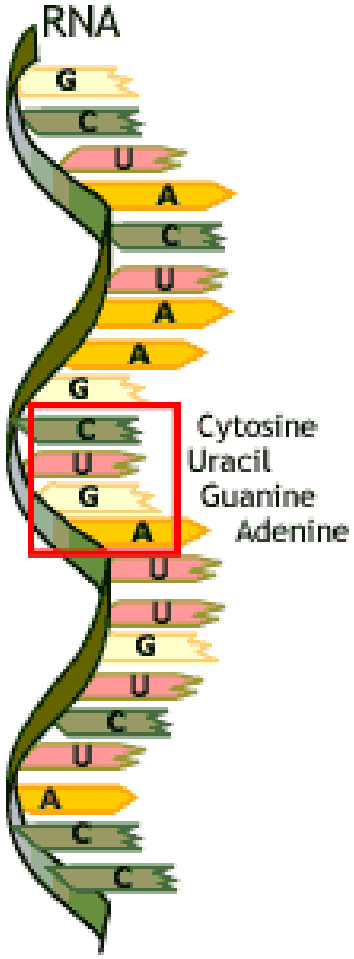
RNA integrity number (RIN)

mRNA

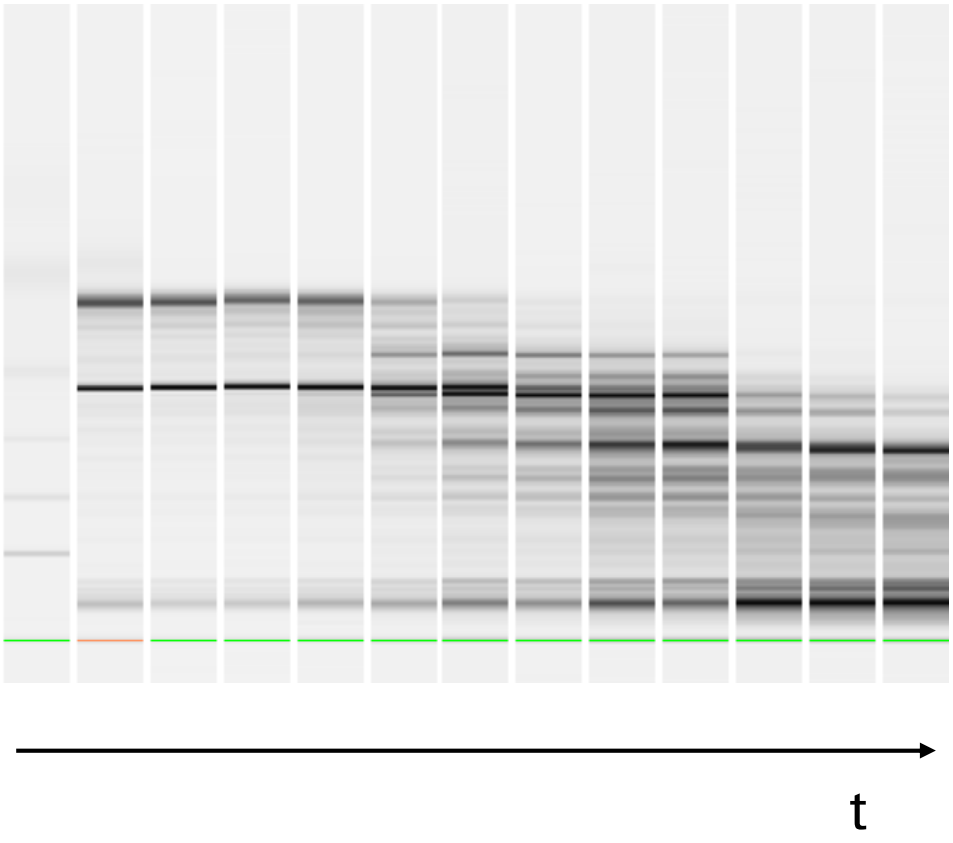
determine integrity and quality of mRNA samples

Determination of mRNA concentration

calculate % ribosomal RNA in mRNA samples

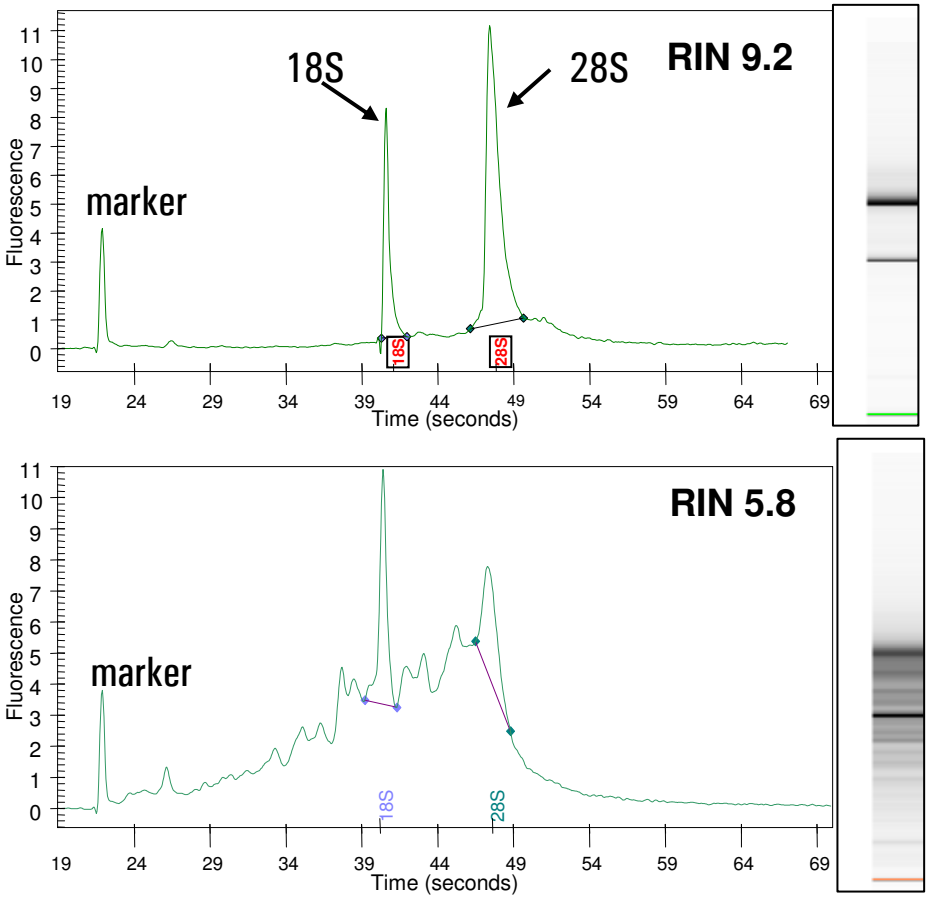


Problem Description



- The ratio of ribosomal bands is not sufficient to describe RNA integrity!
- RNA degradation is a gradual process.
- Results have to be interpreted by visual inspection.
- Overlay of electropherograms only works well for samples with the same concentration.
- Instrument dependency in signal height

RNA Quality Control: Assessing Total RNA Integrity

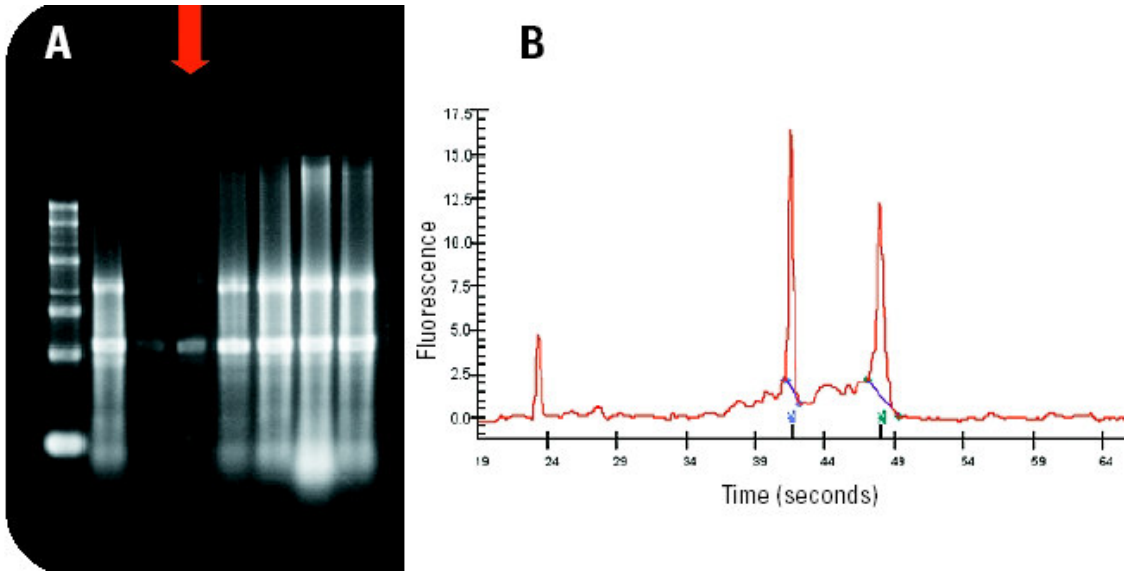


Typical first QC step after RNA sample prep prior to microarrays or real-time PCR

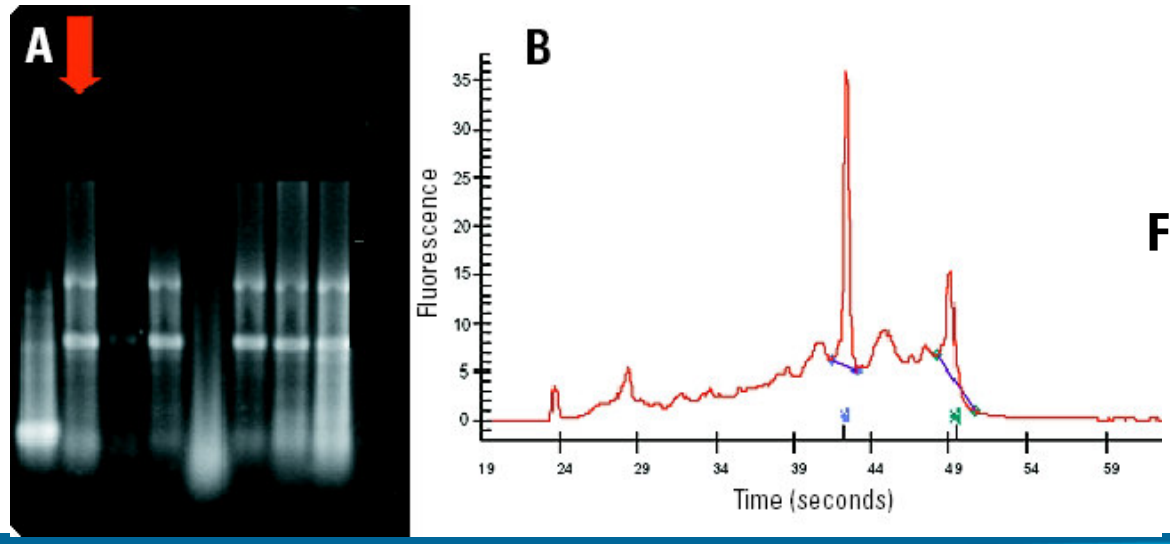
High quality total RNA

Partially degraded total RNA

Gel Chip Comparison



False Negative

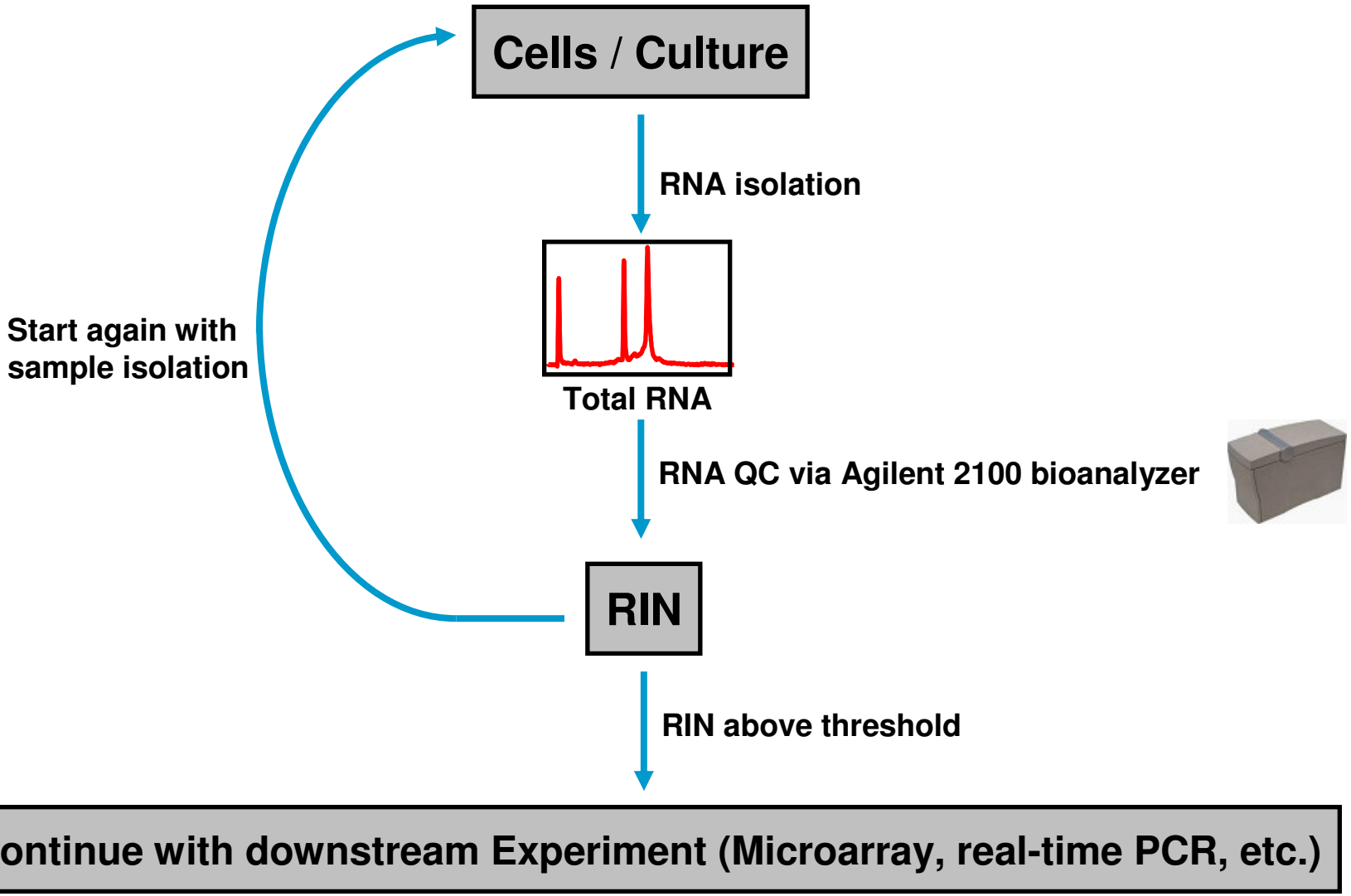


False Positive

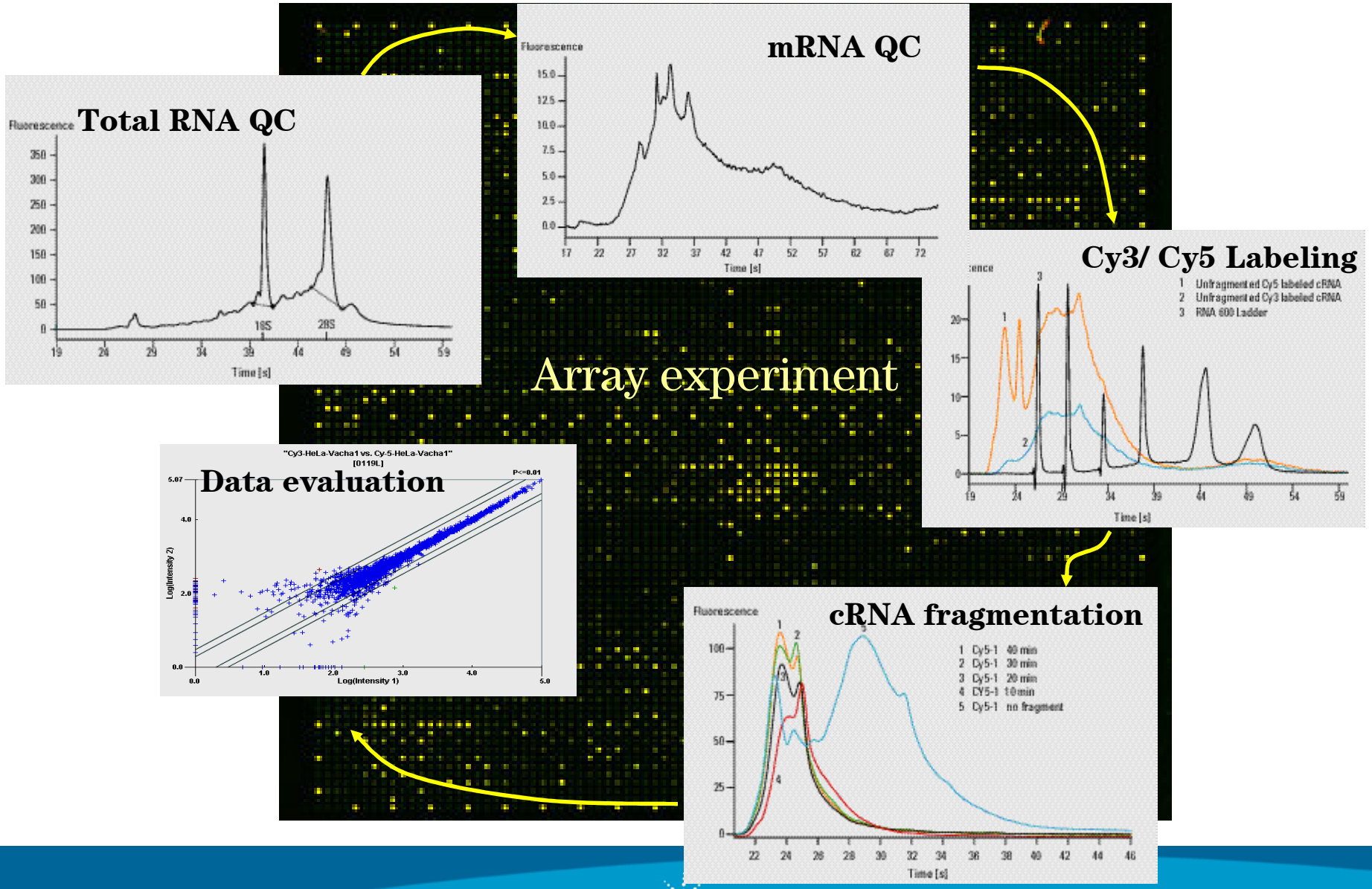
Agilent Technologies

Data kindly provided by Gene Logic Inc.

RNA QC in Routine Gene Expression Workflow

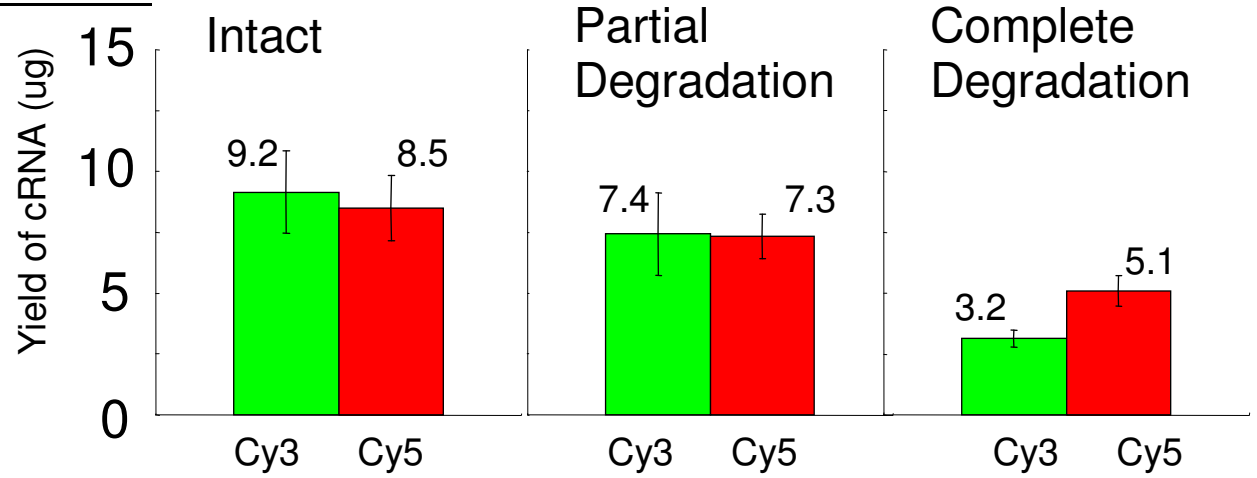


cRNA Hybridization - Workflow

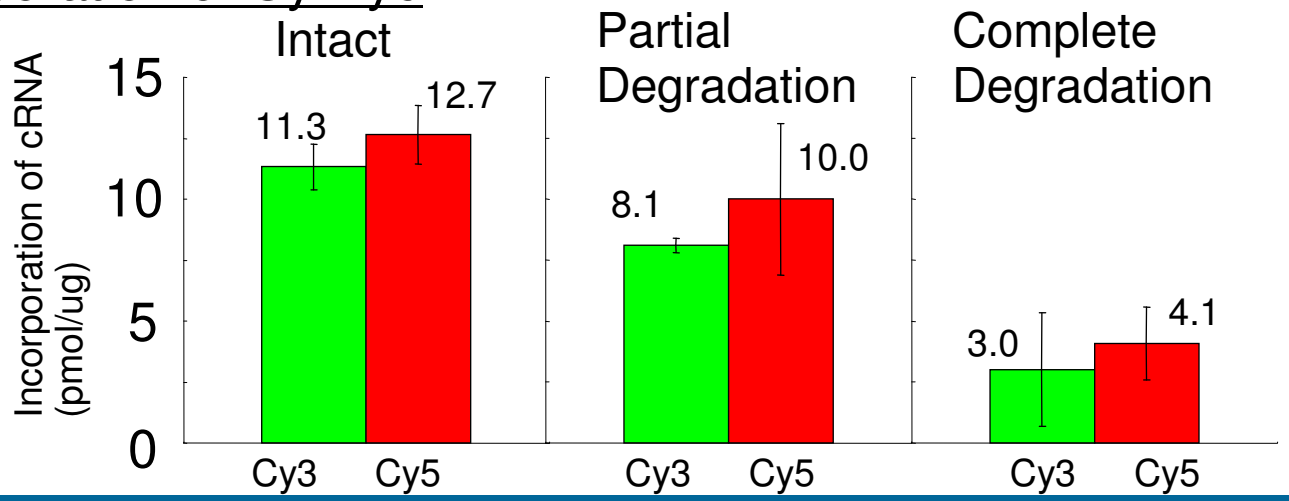


Labeled cRNA Quality Check by NanoDrop

Yield of cRNA

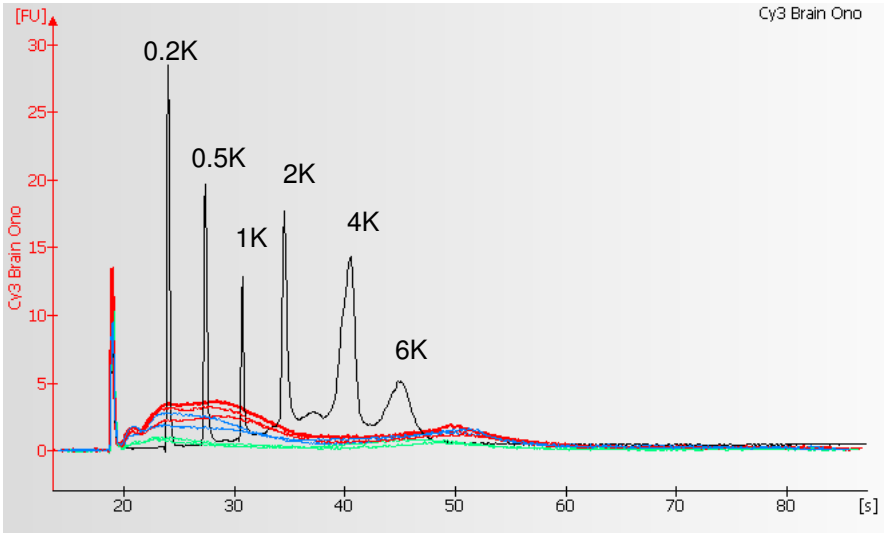


Incorporation of Cy Dye

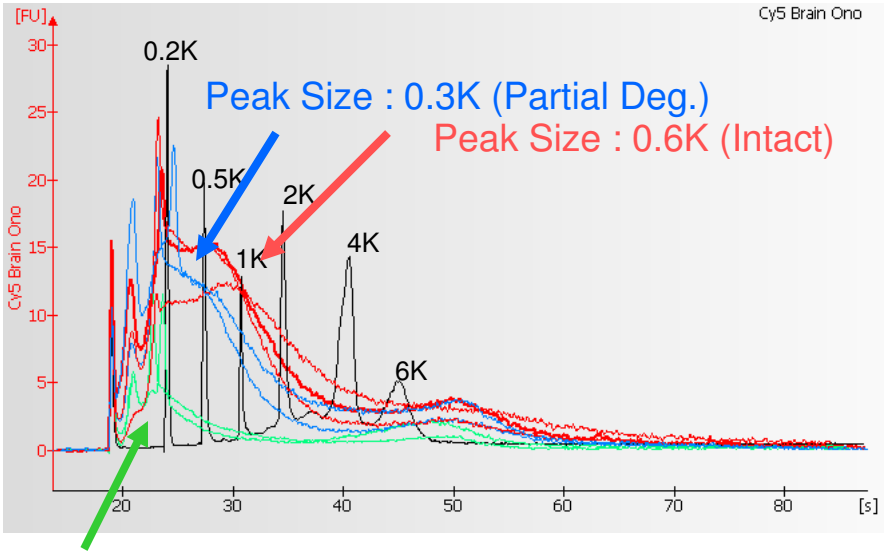


Labeled cRNA Quality Check by BioAnalyzer

Cy3 labeled cRNA

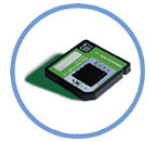


Cy5 labeled cRNA

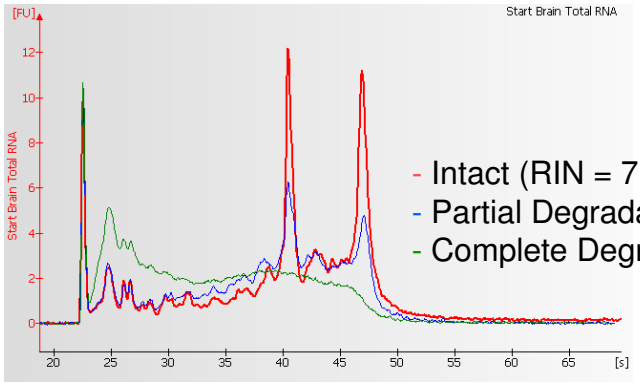


Peak Size : 0.1K (Complete Deg.)

- Ladder
- Intact (RIN = 7.3)
- Partial Degradation (RIN = 5.2)
- Complete Degradation (RIN = 2.4)

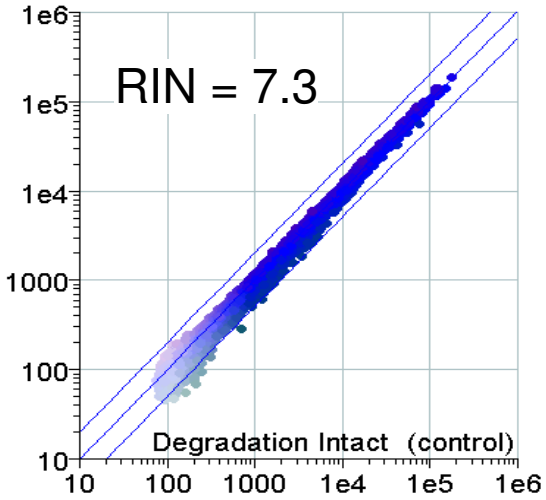


Scatter Plots : Self vs. Self

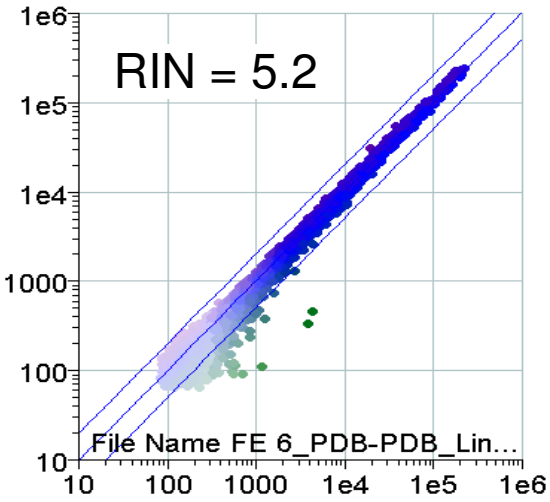


- Intact (RIN = 7.3)
- Partial Degradation (RIN = 5.2)
- Complete Degradation (RIN = 2.4)

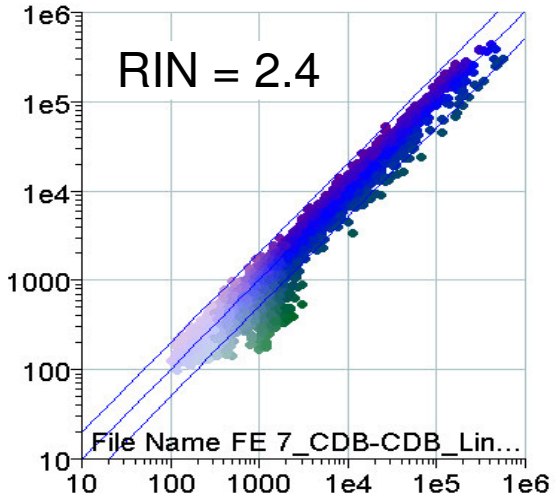
Intact



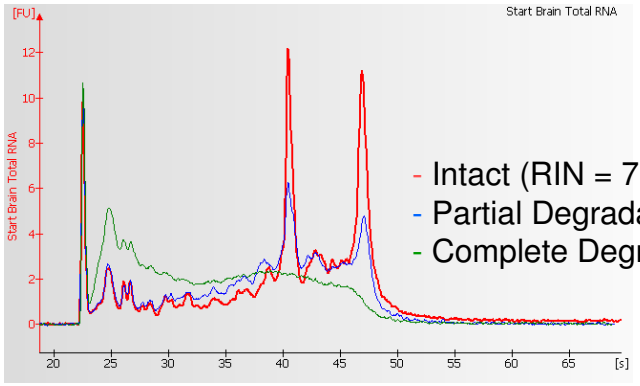
Partial Degradation



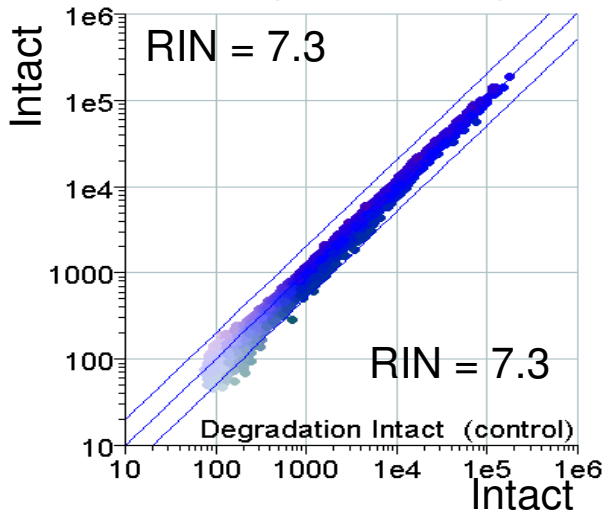
Complete Degradation



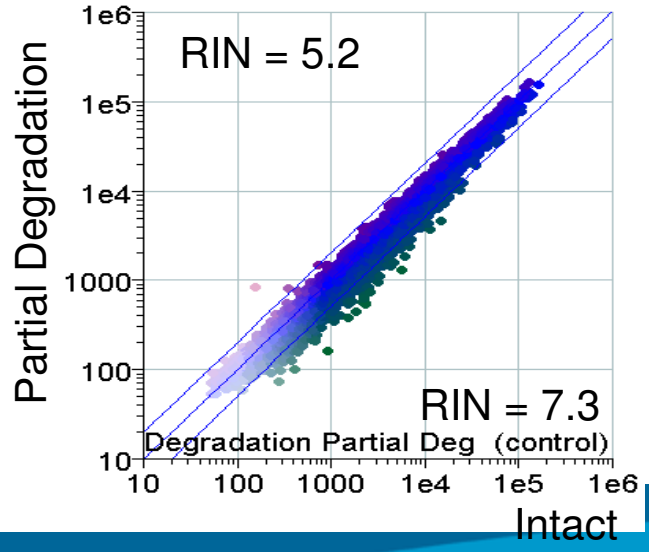
Scatter Plots : Intact vs. Degradation



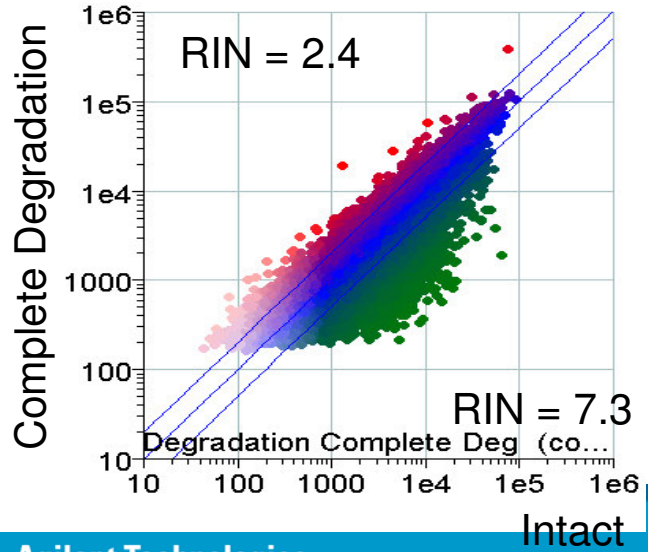
Intact (self to self)



Intact vs. Partial Degradation



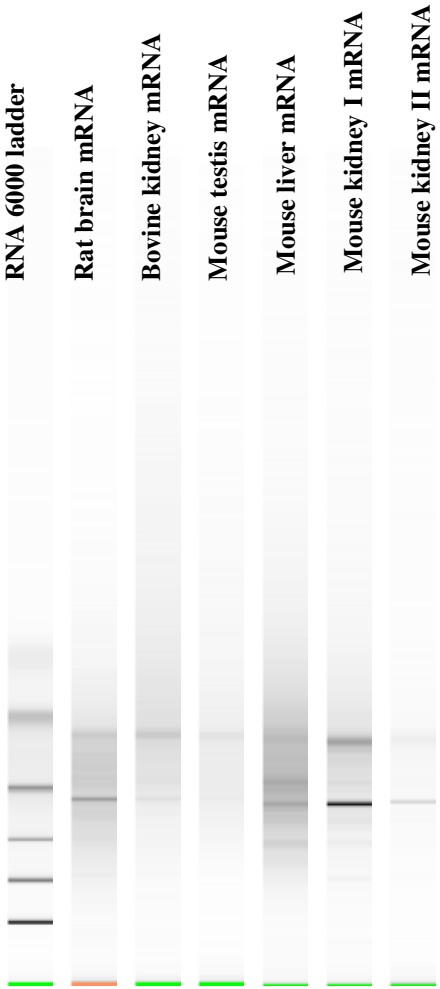
Intact vs. Complete Degradation

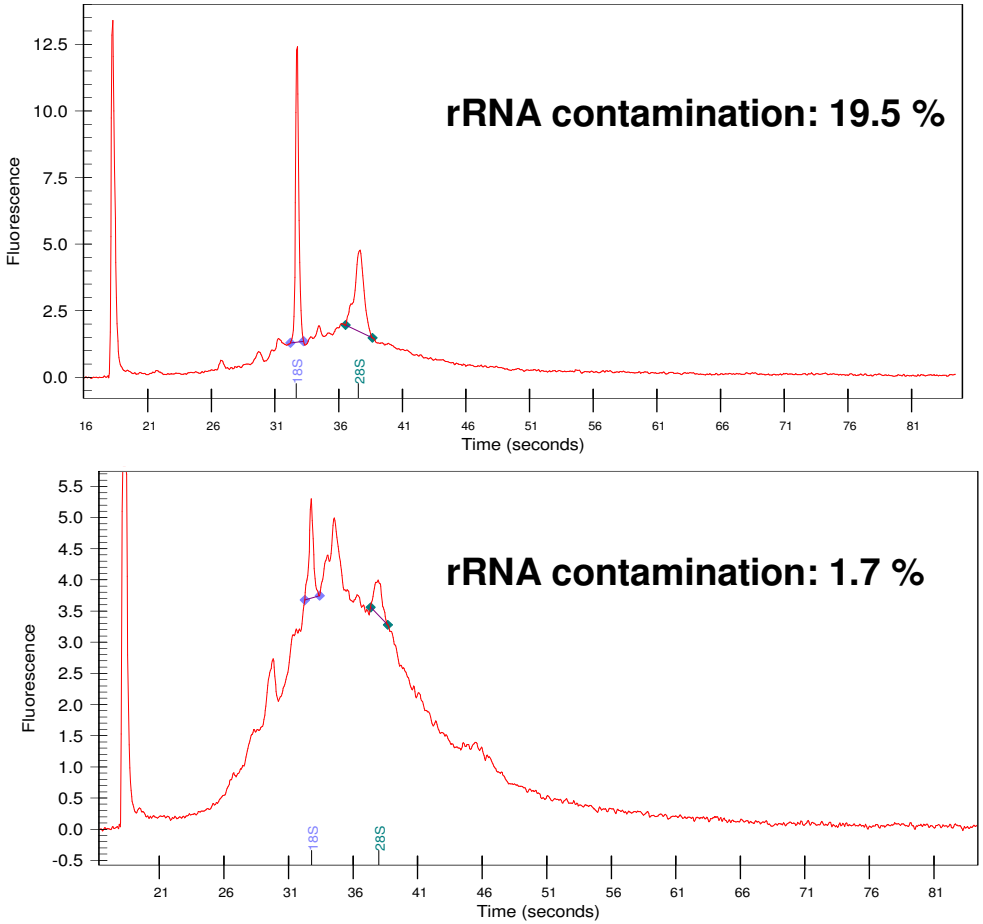


Conclusions

- **Labeled cRNA from different levels of RNA degradation results in low Cy Dye incorporation and low yield of cRNA**
- **RNA integrity levels of starting material had serious impact on downstream gene expression microarray results**
- **The RIN is an effective tool that can be used to evaluate RNA integrity objectively**

Ribosomal RNA contamination in mRNA samples

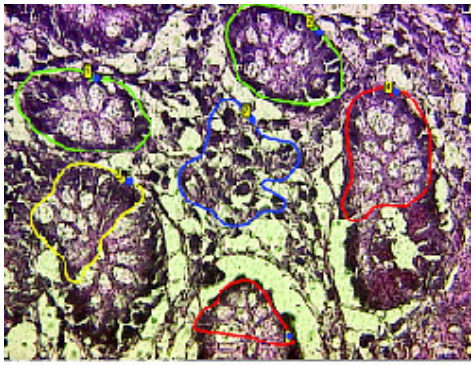




Laser Microdissection – PALM MicroBeam System and RNA Pico kit

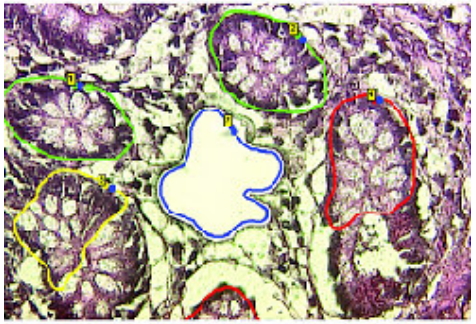
A

Laser microdissection



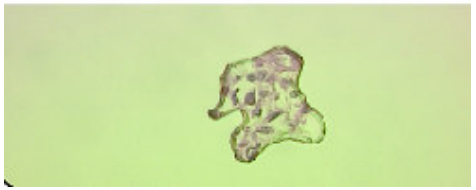
B

Laser pressure catapulting: Section after catapulting of selected area



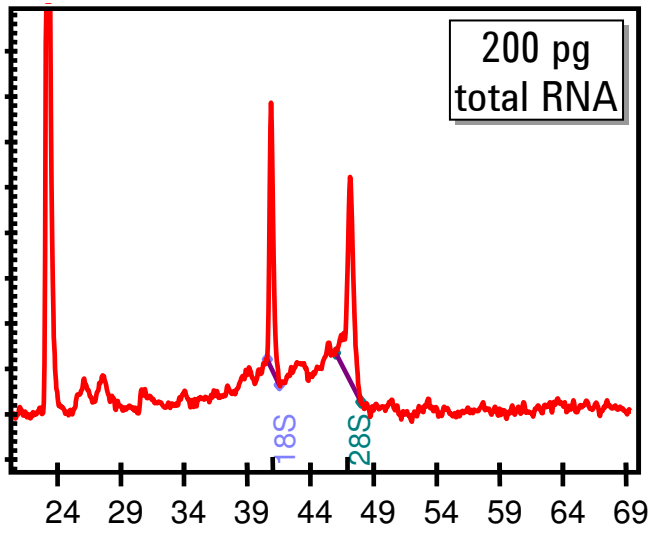
C

Catapulted area in the collection device



Laser Microdissection and Pressure Catapulting (LMPC)

RNA extraction



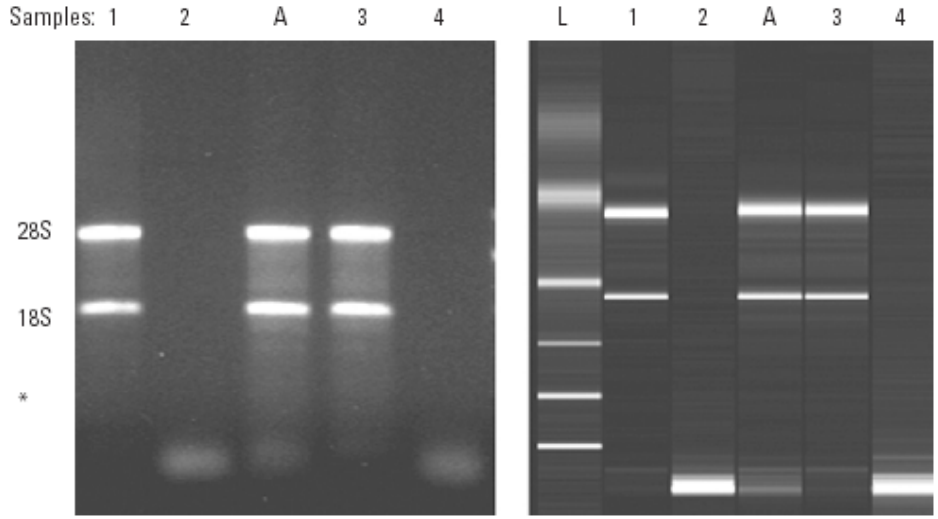
RNA sample QC using the Agilent 2100 bioanalyzer and the RNA 6000 Pico LabChip kit

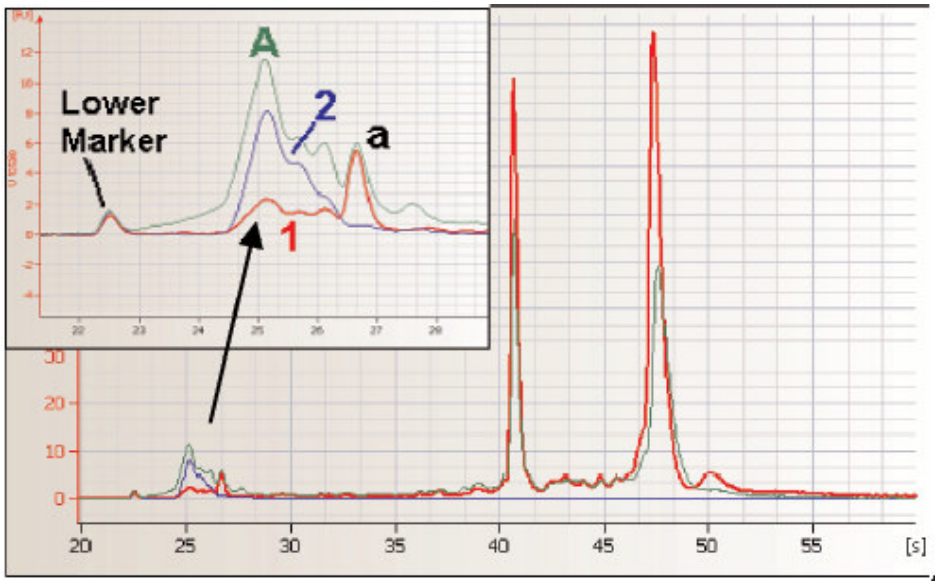
Agilent Technologies

Data kindly provided by P.A.L.M. Microlaser Technologies

Analysis of Small RNA (using RNA 6000 Assay)

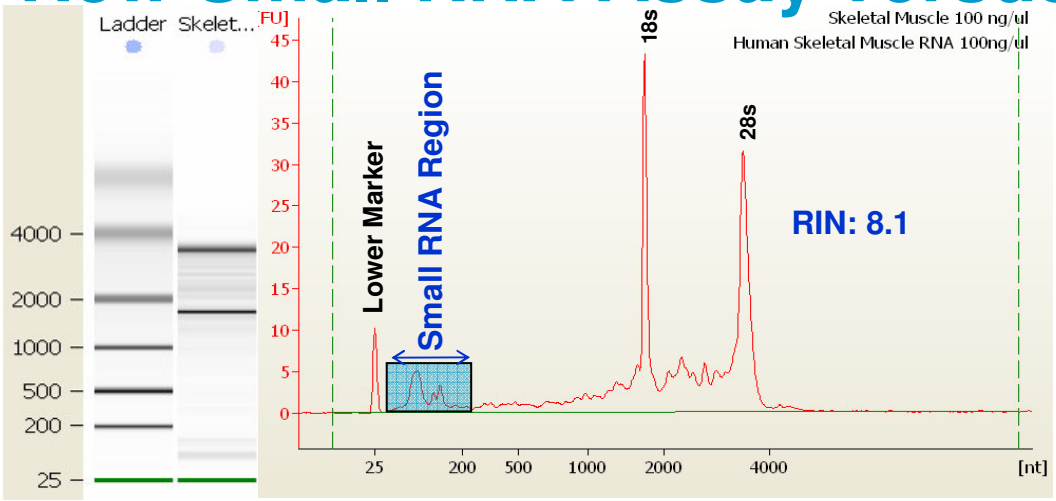
Small RNA fraction: < 200 nts
e.g. miRNA, siRNA, snRNA, tRNA, 5S RNA





Bioanalyzer allows discrimination of different profiles

New Small RNA Assay versus existing RNA Assay



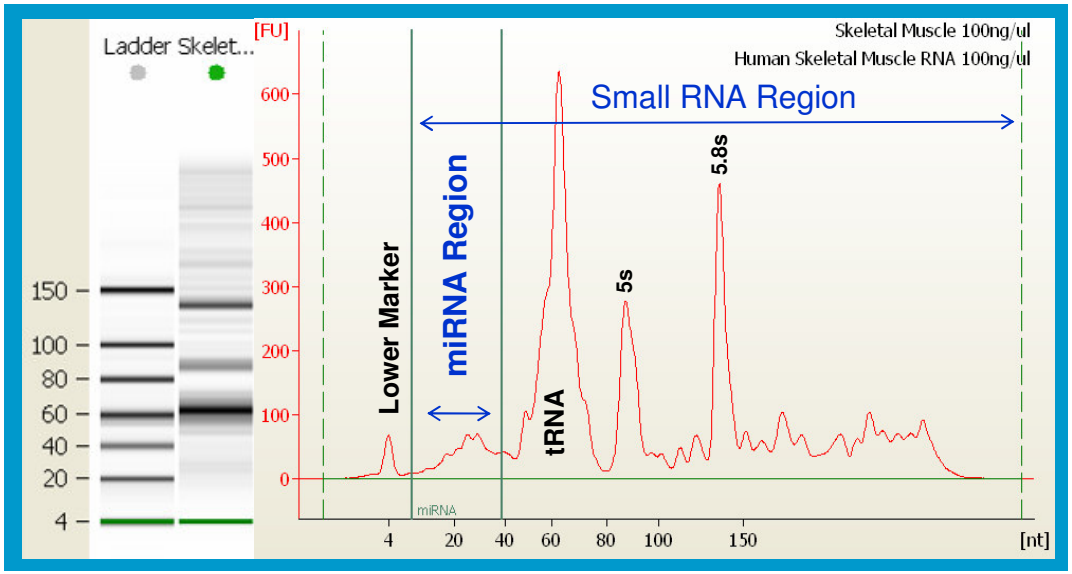
RNA 6000Nano kit

RNA 6000Nano

Size range: 25-6000nt

Results: Integrity, Total RNA amount, gDNA contamination

Small RNA Kit



NEW! Small RNA
Size range: 6-150nt

Results: miRNA amount, Ratio and amount of other Small RNA

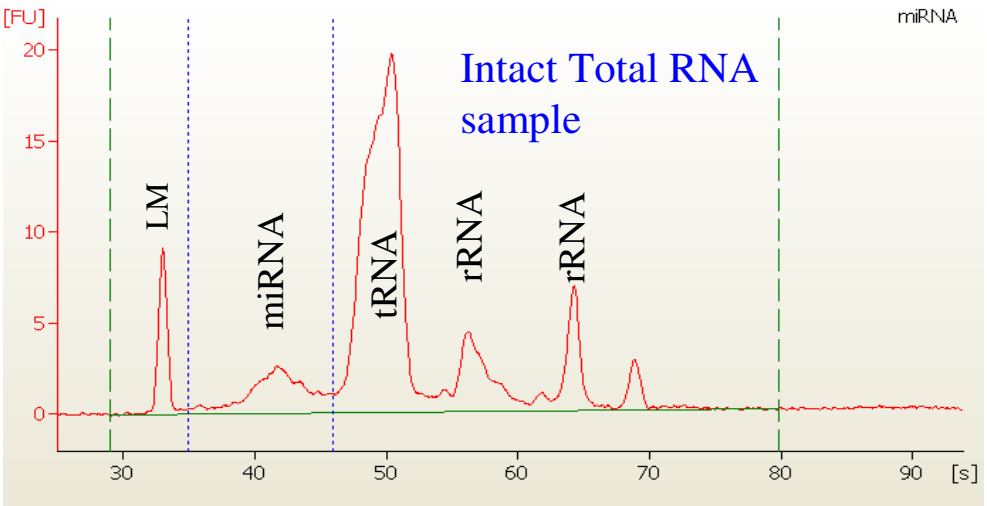
Applications
The new small RNA Assay as a tool for:

Verification, comparison and optimization in the small RNA region:

- High sensitivity to detect low abundant fragments
- High resolution for ss oligos, miRNA, pre-, t-, 5S-RNA's
- compatible with Total RNA samples or purified small RNAs.
- Semi-quantitative for single stranded RNA.
- semi- Denaturing
- Analysis up to 150nt

Plus: Qualitative assessment of dsDNA, siRNA or other hairpin RNA up to 150bp

(size separation and relative amount estimation)

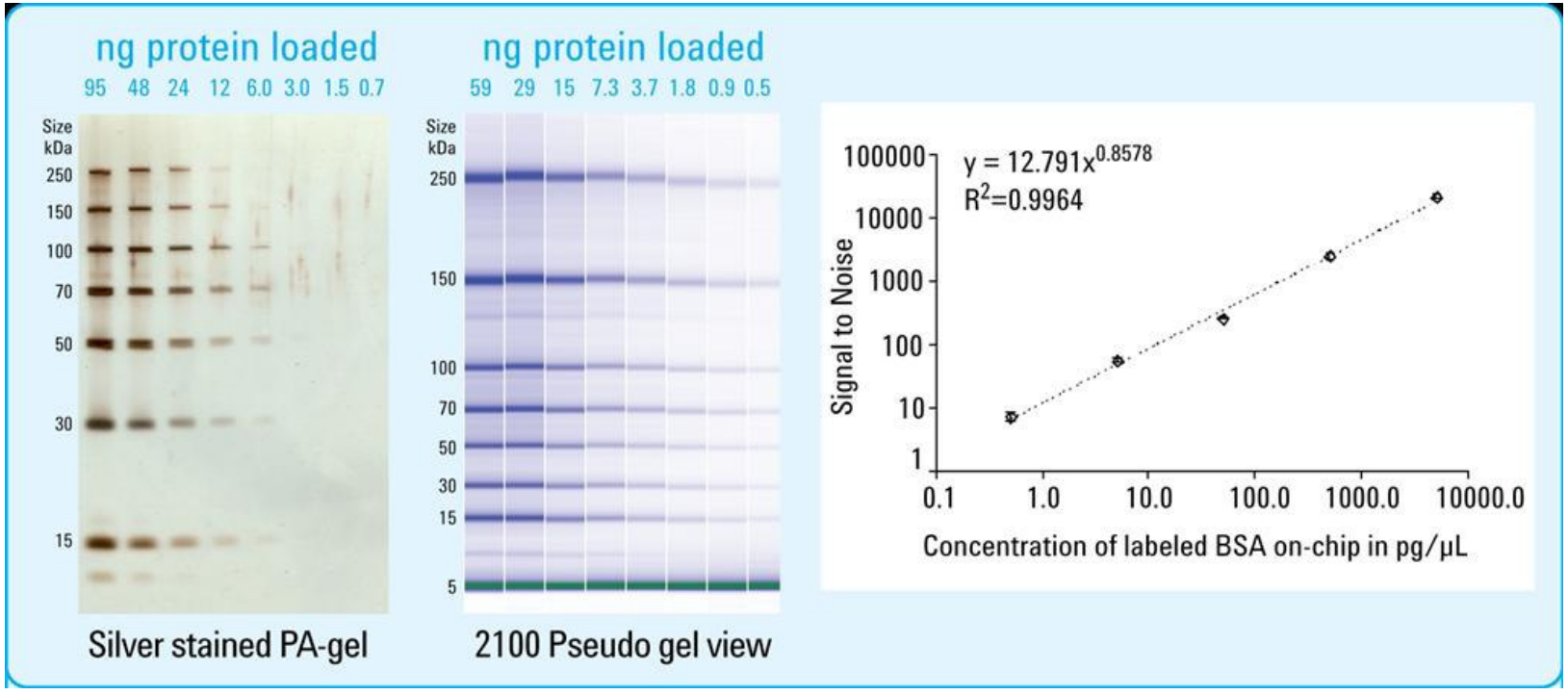


Small RNA Assay specifications

Analytical Range	6 -150 nt (to avoid overlap)
Sensitivity	50 pg/µl
(diluted Ladder - 40 nt fragment; S/N > 3:1)	
Quantitative range	50 pg/µl – 2000 pg/µl
(purified miRNA in water after extraction ~<200nt)	
Quantitation Reproducibility	25 % CV
(defined on Ladder)	
Max amount total RNA	100 ng/µl total RNA
Carryover	Below detection limit

Protein Applications

Protein Purification | Protein Expression | Protein Production | Food Analysis | Purity and QA/QC



Bioanalyzer Protein Kit portfolio

Agilent Protein 80 kit	Prod Number	5067-1515
Agilent Protein 230 kit	Prod Number	5067-1517
Agilent High Sensitivity Protein 250 kit	Prod Number	5067-1575

P 80

Range	5 - 80 kDa
Sensitivity:	Coomassie
Samples:	10

Samples
-Antibodies (reduced)
-Small Proteins

P 230

Range	14 - 230 kDa
Sensitivity:	Coomassie
Samples:	10

Samples
-Antibodies (all types)
-Standard Proteins

Coomassie Range (5 ng/µL BSA)

HSP 250

Range:	10 - 250 kDa
Sensitivity:	1 pg/µl BSA on Chip
Samples #:	10 per Chip
Chips #:	10 per Kit
Labeling Conc:	1 ng – 1 µg /µl

Silver stain Range (200 pg/µL BSA)

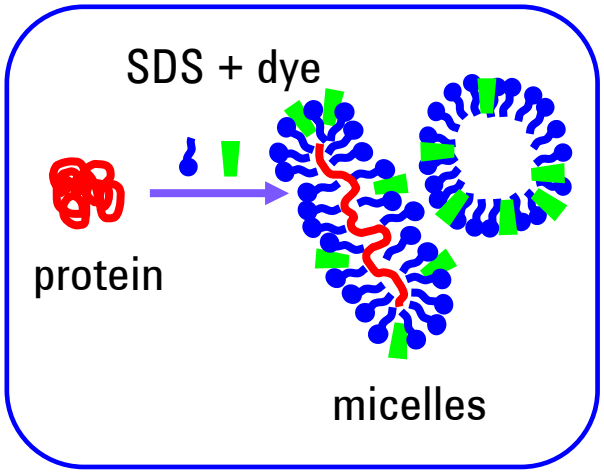
Protein Kit Specifications

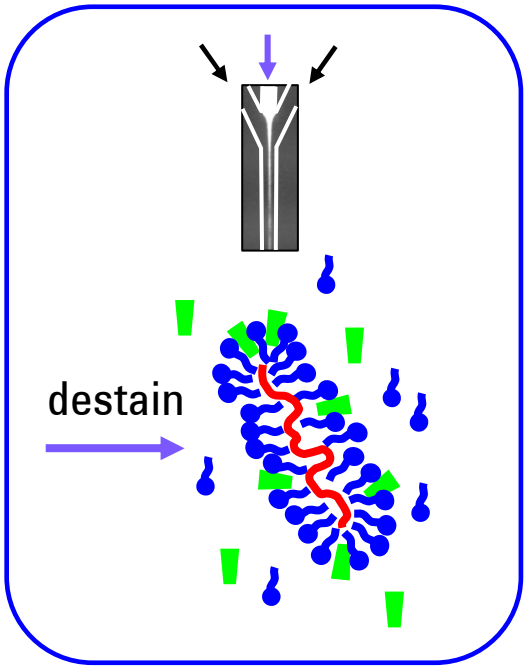
New

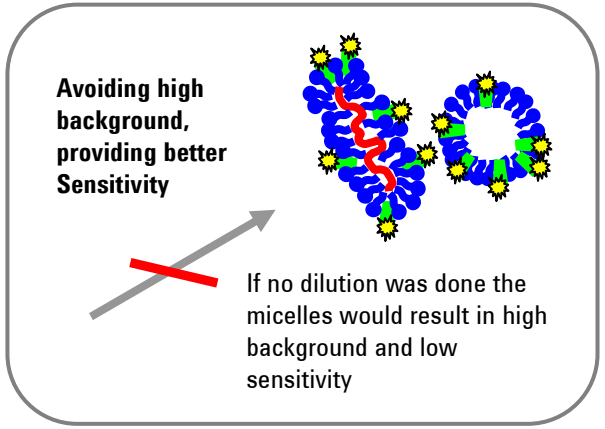
	Product No. 5067-1515	Product No. 5067-1517	Product No. 5067-1575
	Protein 80 Assay	**Protein 230 Assay**	**High Sensitivity Protein 250 Assay**
Analytical specifications			
Sizing range	5-80 kDa	14-230 kDa	10-250 kDa
Typical sizing resolution	10%	10%	10%
Typical sizing accuracy	10% CV (CAII, BLG)	10% CV (BSA, CAII)	10% CV (BSA)
Sizing reproducibility	3% CV (CAII, BLG)	3% CV (BSA, CAII)	3% CV (BSA)
Sensitivity (Signal/Noise > 3)	6 ng/µL CAII (15 ng/µL BSA) in PBS, 10 ng/µL (CAII) in 0.5 M NaCl (30 ng/µL BSA in 0.5 M NaCl)	6 ng/µL CAII (15 ng/µL BSA) in PBS 30 ng/µL (BSA) in 0.5 M NaCl	1 pg/µL (labeled BSA) in water on chip 5 pg/µL (labeled BSA) in PBS on chip Labeling reaction at 1 ng/µL of total protein *
Quantitative range	60-2000 ng/µL CAII in PBS	15-2000 ng/µL CAII, 30-2000 ng/µL BSA in PBS	up to 4 orders of magnitude (0.3 to 3000 ng/µL BSA)
Qualitative range	6-4000 ng/µL CAII and BLG	6-5000 ng/µL CAII, 15-5000 ng/µL BSA in PBS	-
Quantitation reproducibility	20% CV (CAII, BLG)	20% CV (BSA, CAII)	20% CV (BSA)
Physical specifications			
Analysis run time	30 minutes	25 minutes	30 minutes
Number of samples	10 samples/chip	10 samples/chip	10 samples/chip
Sample volume	4 µL	4 µL	5 µL
Kit stability	4 months (for storage temperature see individual box)	4 months (for storage temperature see individual box)	6 months at -20° C
Compatible buffers	List of compatible buffers	List of compatible buffers	List of compatible buffers
CAII = Carbonic Anhydrase, BSA = Bovine Serum Albumin, BLG = beta-Lactoglobulin			

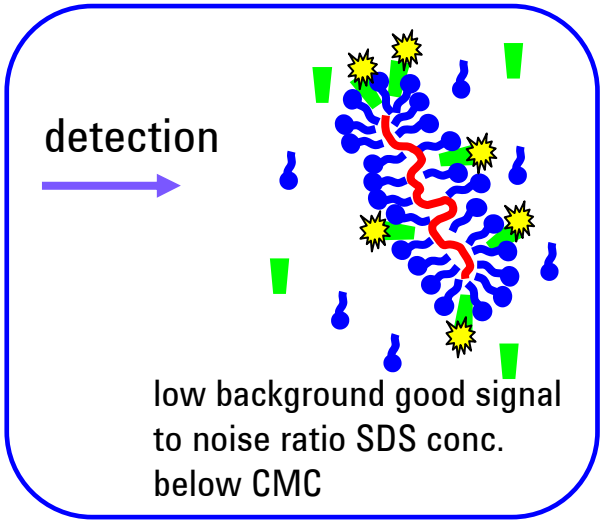
* Prior to measurement on Chip we recommend within the High Sensitivity Protein 250 labeling protocol to dilute the labeled sample by a factor of 200.

Staining, Destaining and Detection (P-80 and P-230)



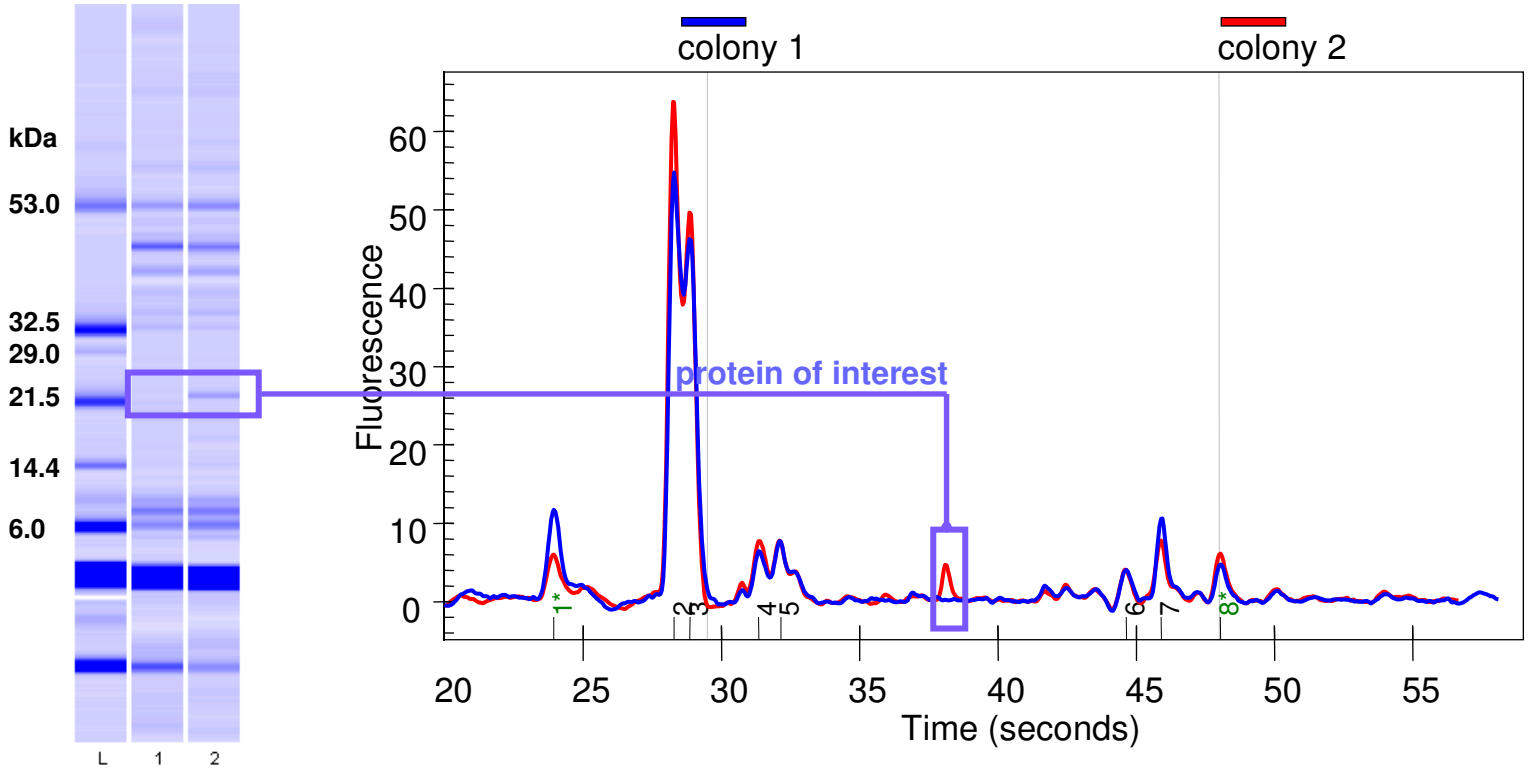






Clone Selection based on Protein Expression

Example measured with
Protein 50 kit



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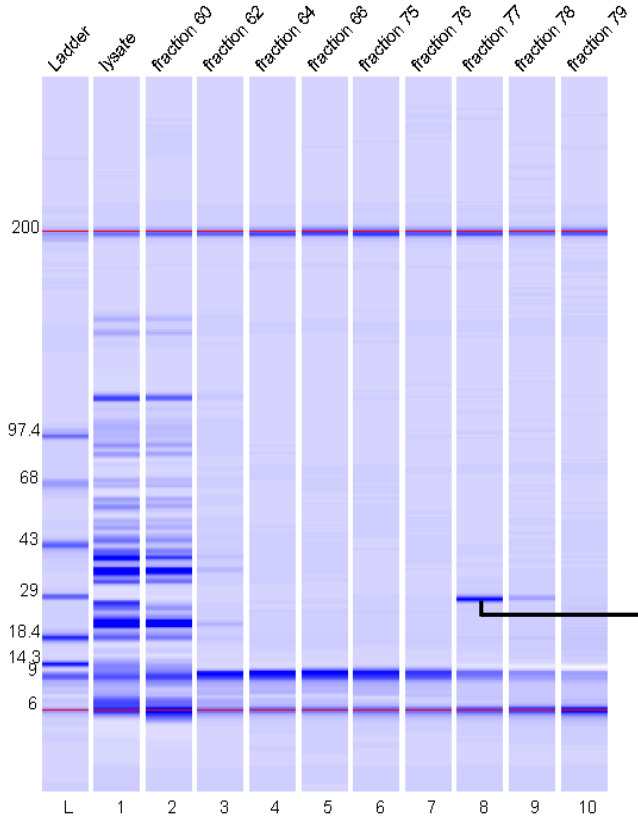
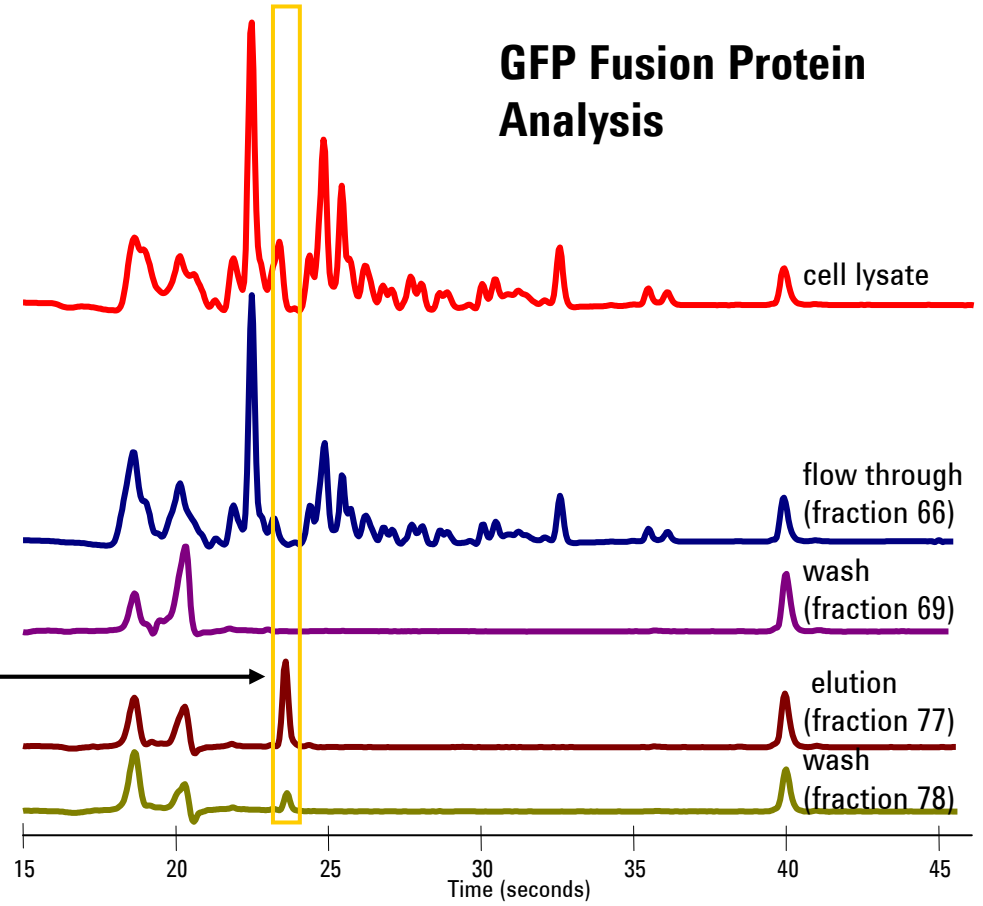
Monitoring of Protein Purification Process

Example measured with Protein 200 plus kit



2100 bioanalyzer: gel-like image

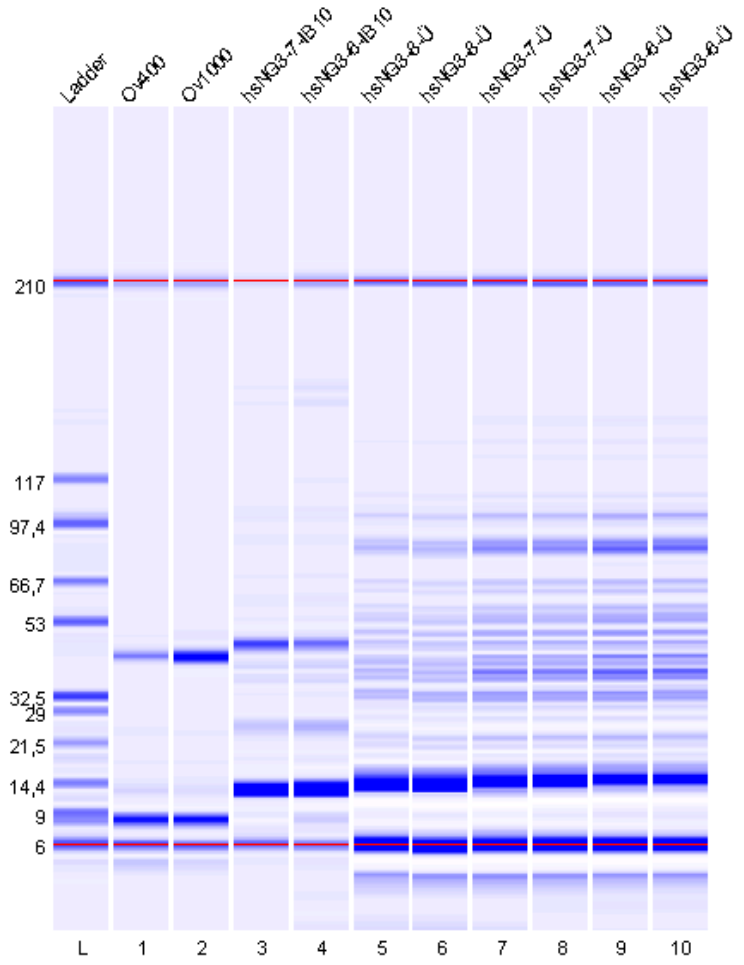


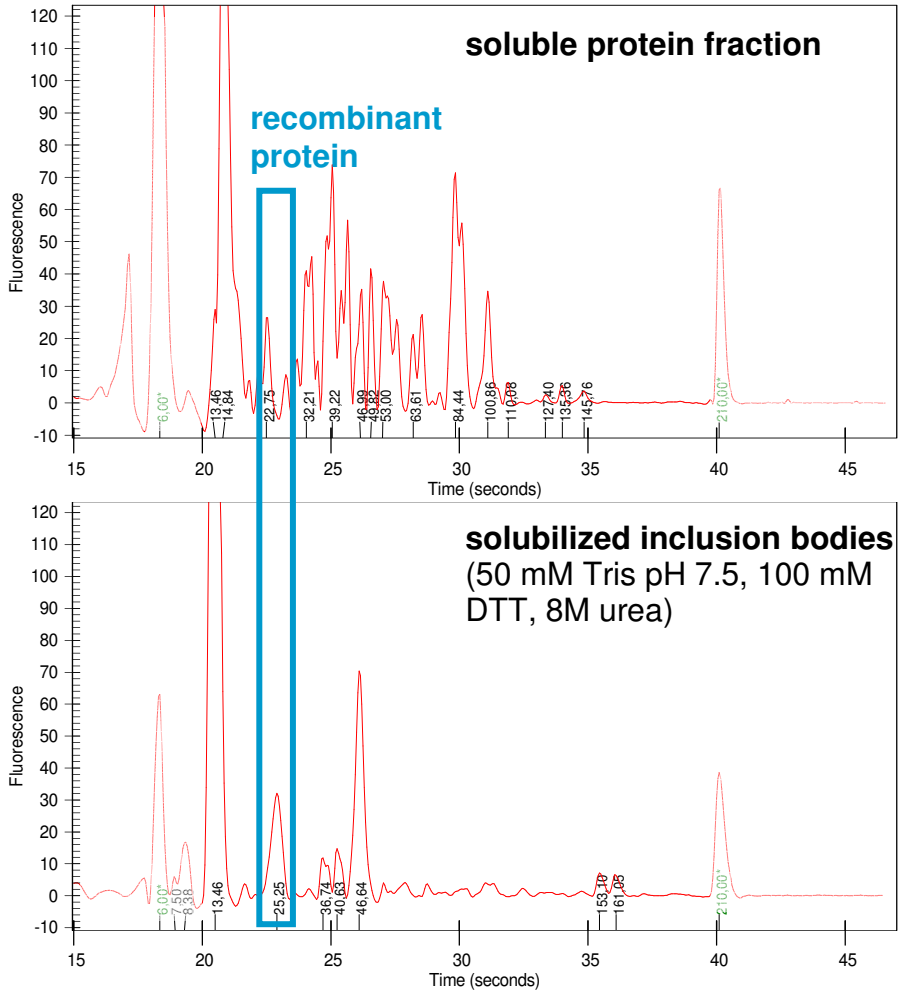
2100 bioanalyzer: electropherogram

Courtesy of P. Sebastian and S.R. Schmidt
GPC-Biotech AG, Martinsried, Germany

Expression of a Recombinant Protein in *E.coli*

- Optimization of Fermentation and Induction Conditions

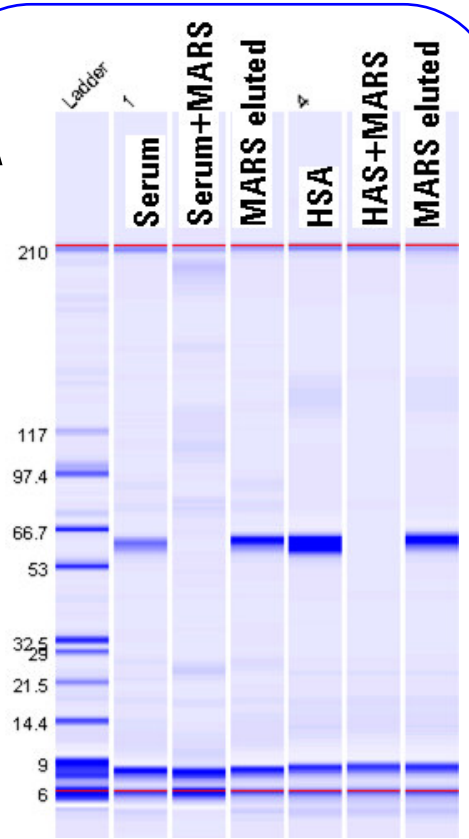




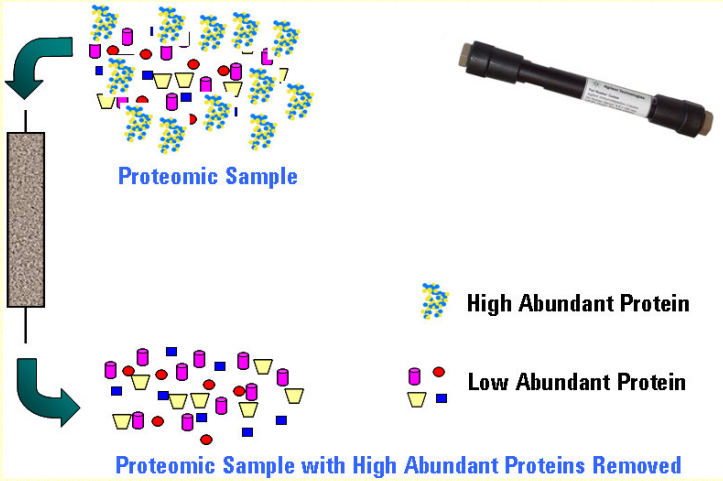
Agilent Technologies

Quality control of the Depletion of High Abundance Proteins in Human Serum

A

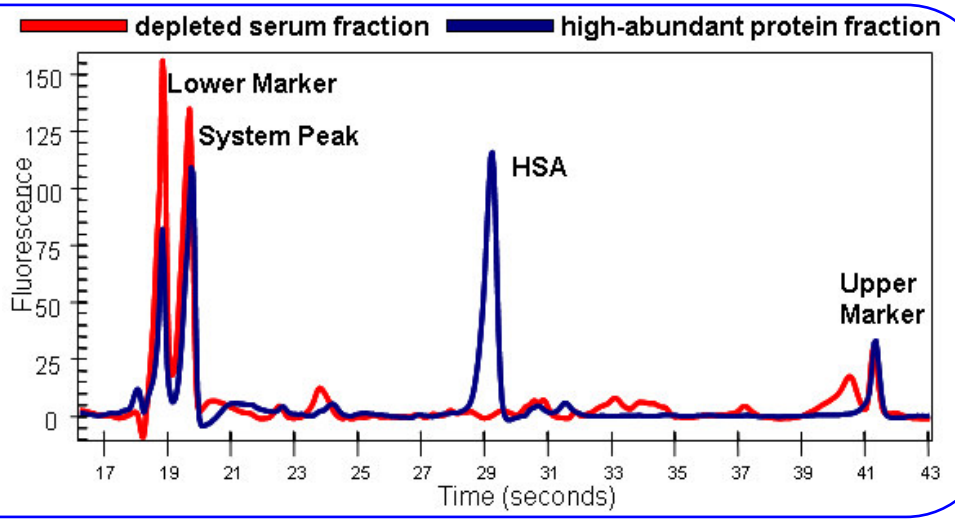


Depletion of High Abundance Proteins by Agilent MARS HPLC columns



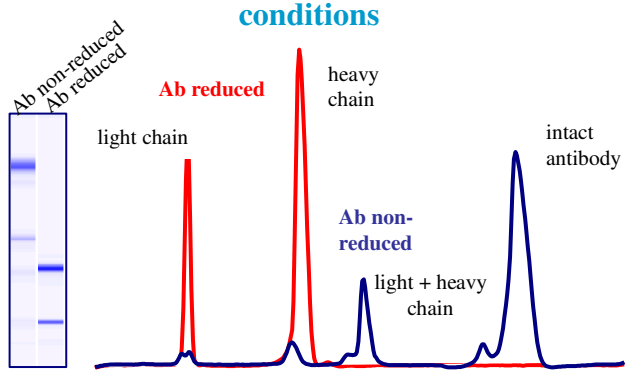
B

Fractions checked by 2100

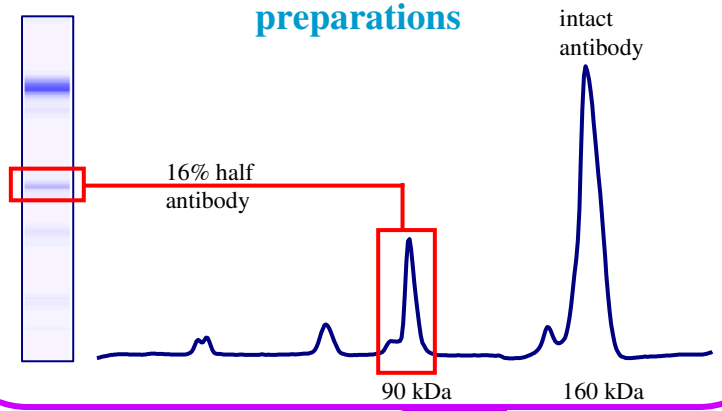


Quality Control of Antibodies

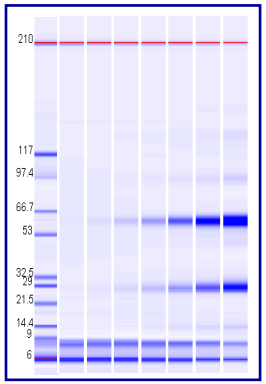
Antibody analysis under reducing and non-reducing conditions

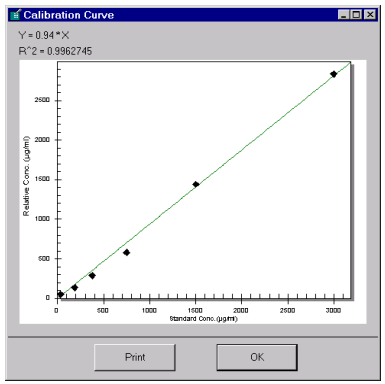


Determine the half antibody content in IgG preparations

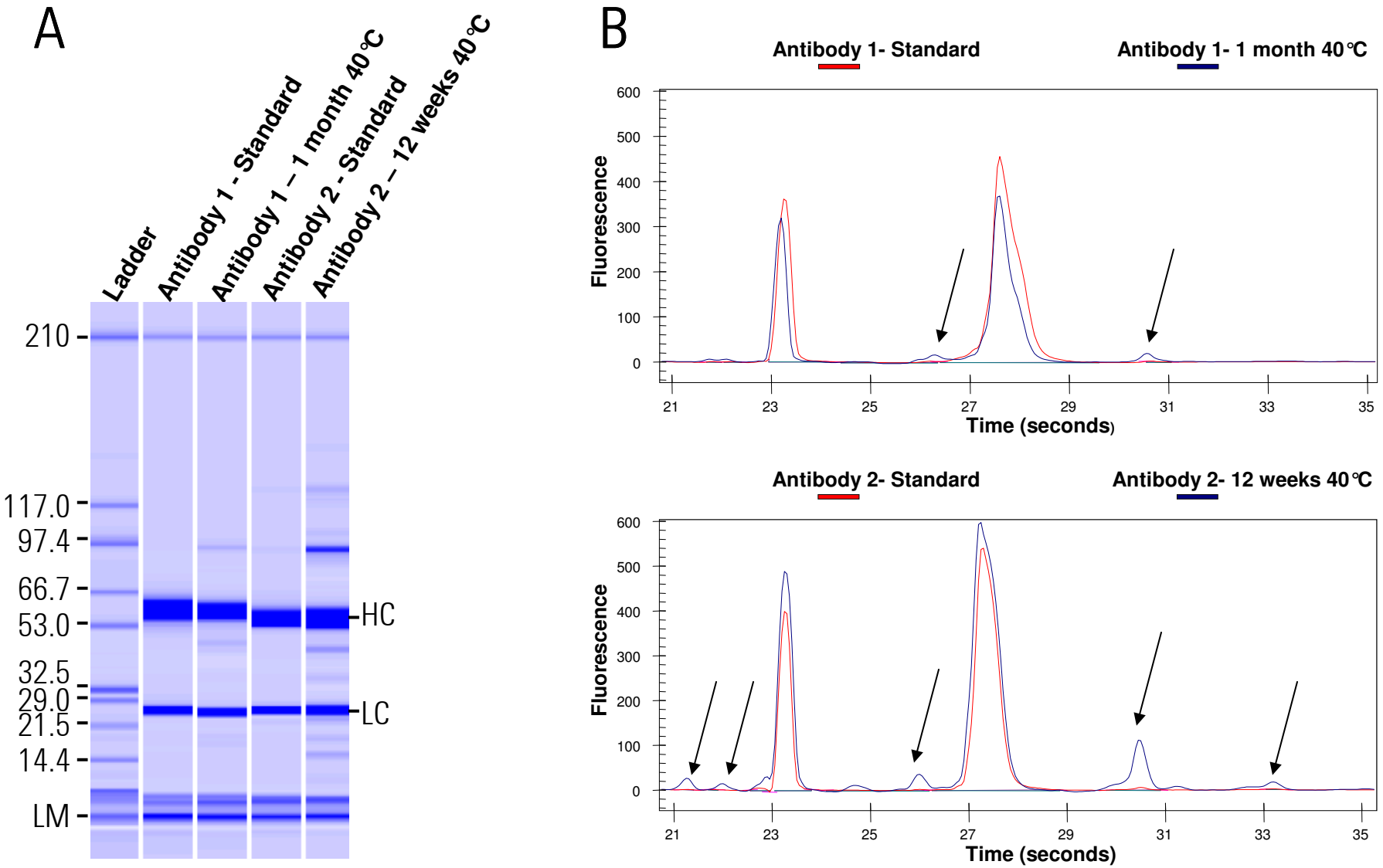


Absolute Quantitation of IgG samples





Analysis of Antibody Stability – stress test



Combination of IEF with SDS-PAGE

Agilent 3100 OFFGel Fractionator + 2100 bioanalyzer

Complex protein mixture

↓

Sample clean up

(chemicals are removed that interfere with labeling reaction)

↓

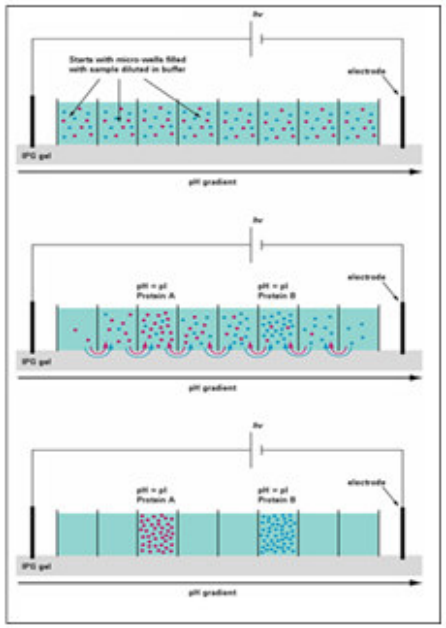
Labeling reaction with fluorescent dye

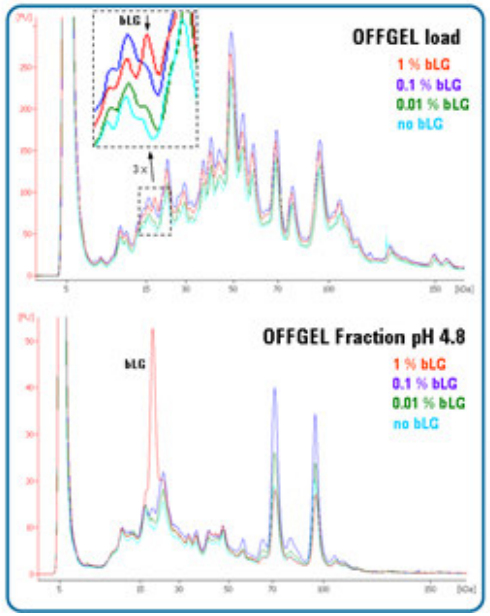
↓

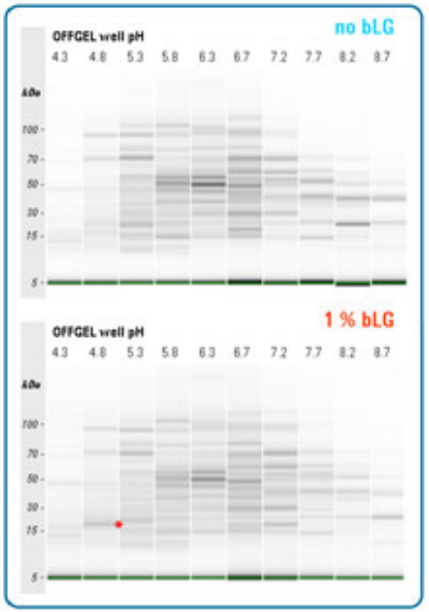
OFFGEL electrophoresis

↓

High sensitivity protein sizing on the Bioanalyzer







Description of the new HSP-250 Assay
(Direct labeling reaction, silver stain sensitivity)

- Reach and beat traditional „silver stain sensitivity“
- Offer solid quantitation for a large dynamic range

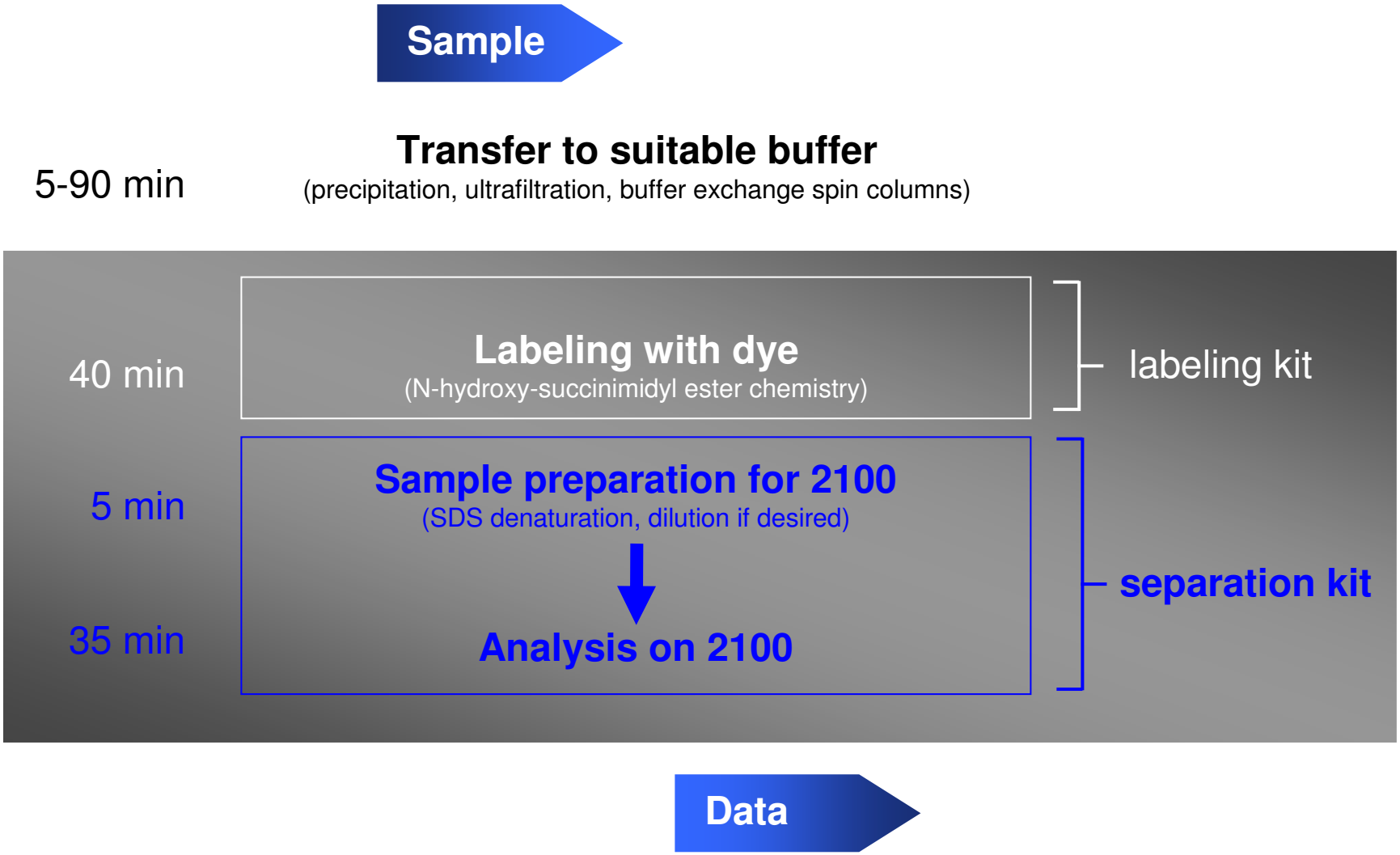
Target Applications:

- Protein QA/QC
  reliable quantitation of main compound besides minor impurities
- Protein detection at lowest concentrations in research

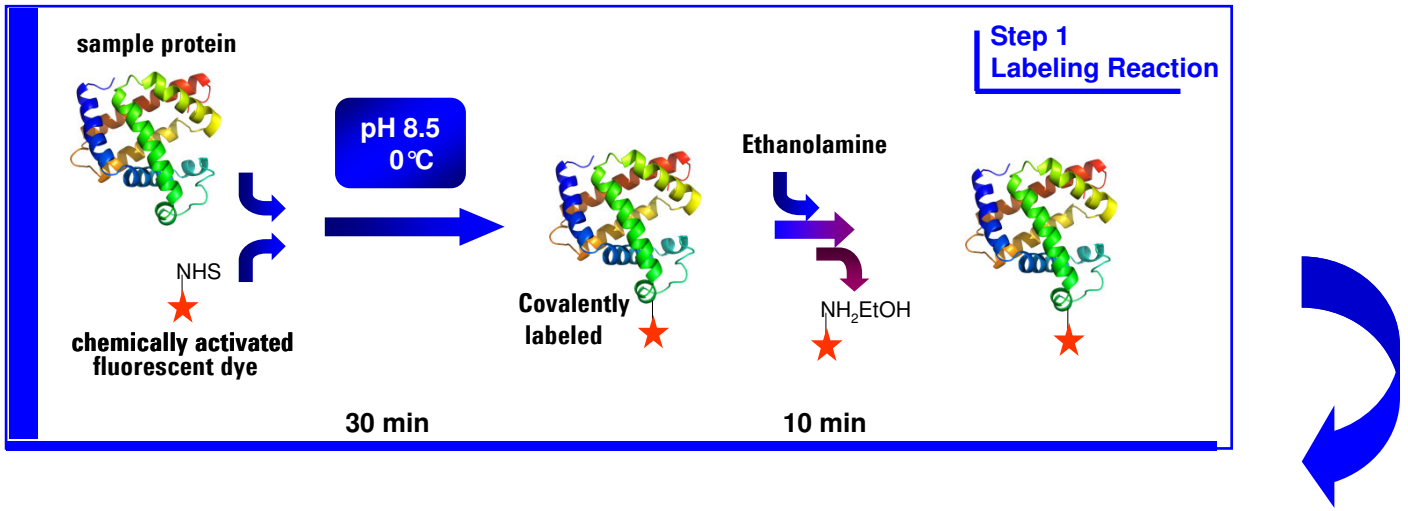
High Sensitivity Protein 250 Kit 5067-1575 content is:

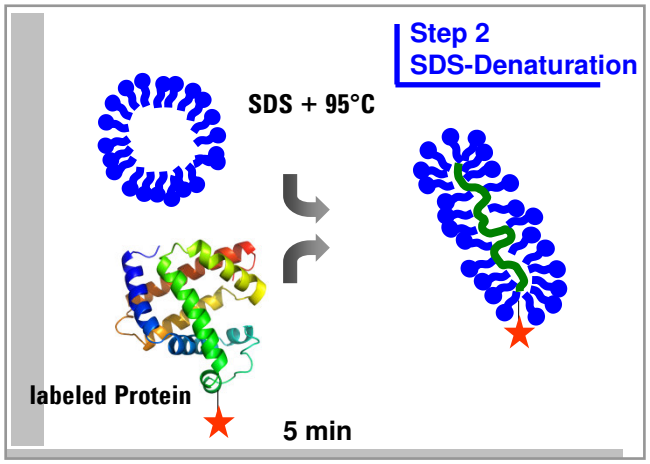
- 10 Chips	(100 samples)
- Labeling Kit	(Dye and Reagents)
- 2100 Separation Kit	(Gel, Marker, Ladder, Buffer)
- User Documentation	(Quick Start Guide & Labeling Protocol)

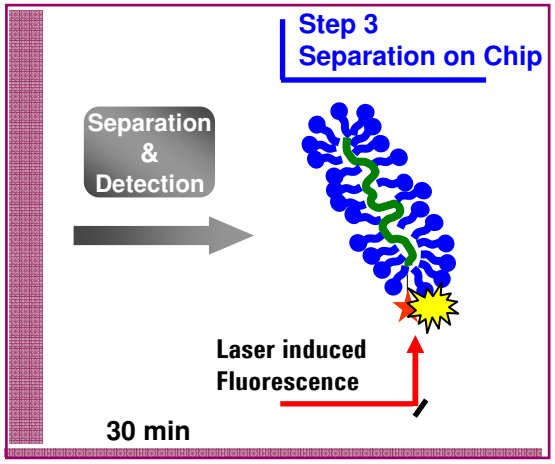
Extended experimental workflow



Principle of High Sensitivity 250 Protein Staining





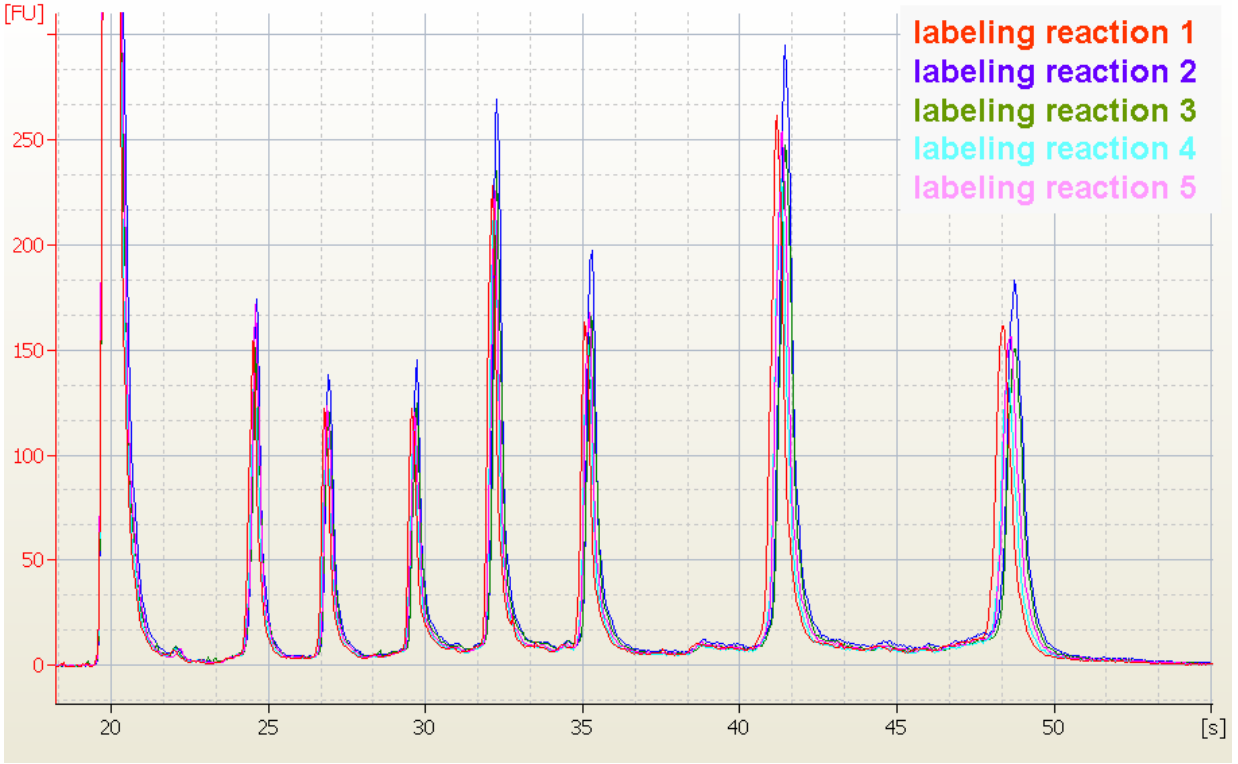


Reproducibility of Labeling Reaction: Ladder

Rugged Labeling reaction:

Reproducible reaction provides comparable signal intensities.
Homogenous labeling without extra bandbroadening

No deviation in peak width is indicating a constant number of dye per protein molecule and proves a stable protocol

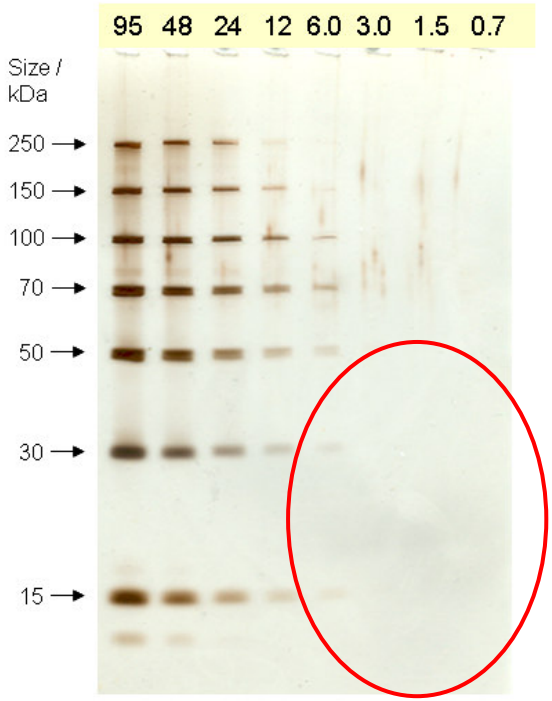


Sensitivity: Silver Staining vs. Bioanalyzer

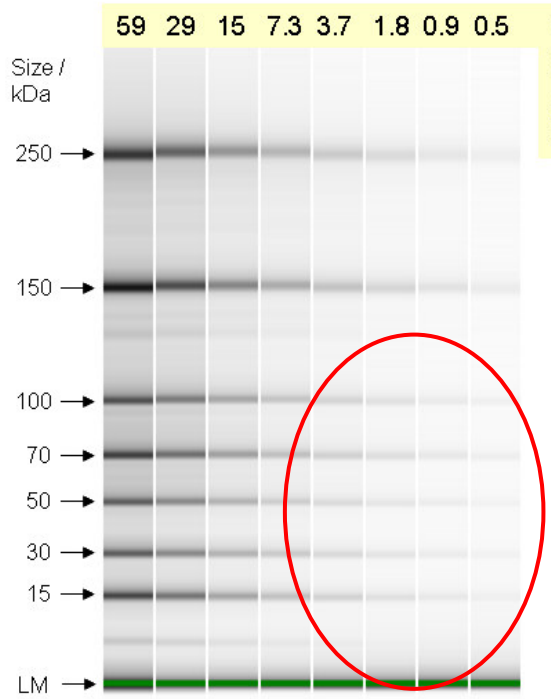
Highest sensitivity:
Labeled proteins can be measured down to pg/µL concentrations loaded on Chip

Direct comparison of samples run on SDS-PAGE with Silver staining and on 2100 Bioanalyzer.

Concentrations are given per lane (as total concentration of 7 different proteins)



Silver stained PA-gel
(4-12% BisTris minigel)



2100 Pseudo gel view

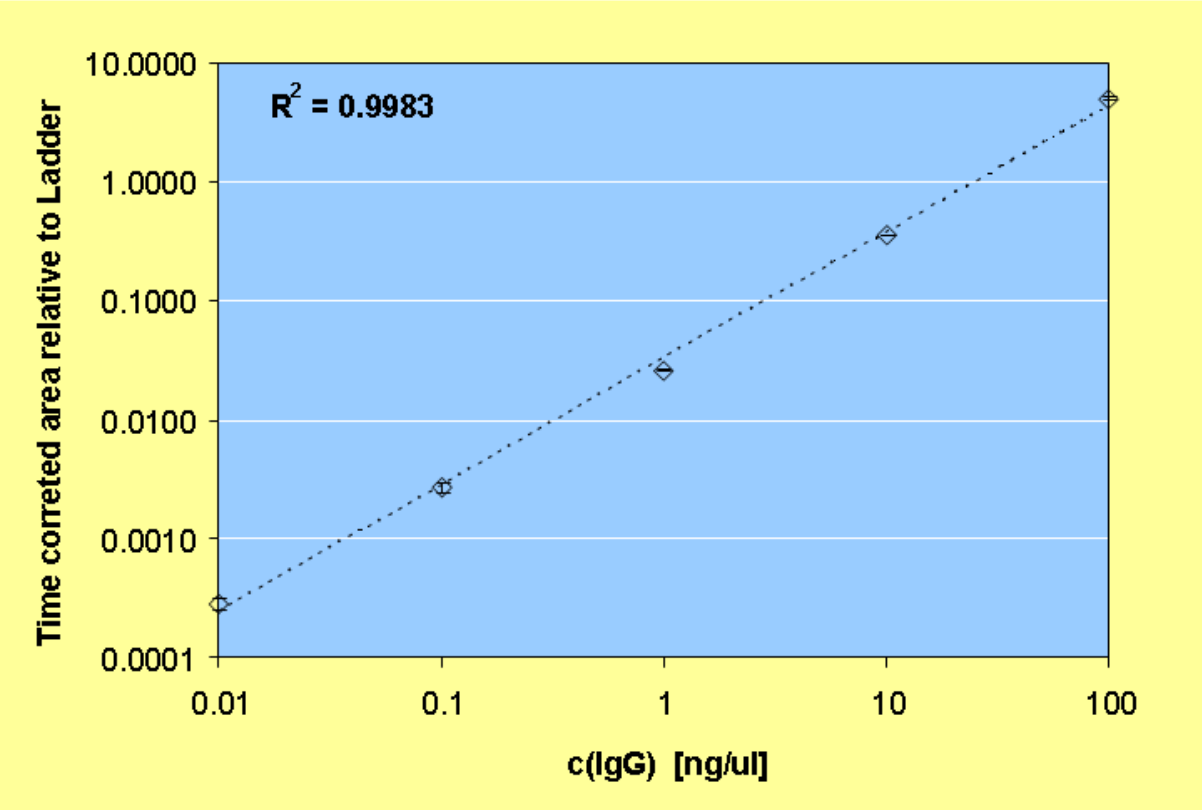
Linear Dynamic Range Test: IgG

Linear dynamic range:

Quantification of labeled IgG from 10 pg/µL to 100 ng/µL

averages ± SD of 7 measurements (7 chips, 1 chip lots, 4 instruments)

4 orders of linear dynamic range allows to quantify an 0.05% impurity besides the main peak in a single run



2100 Bioanalyzer Compliance

2100 expert software
- One version for all assays
- Declaration of system validation

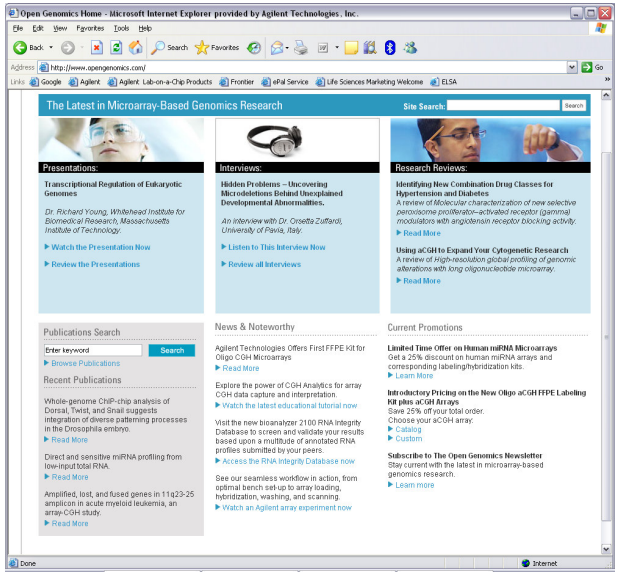
2100 expert security pack
- 21 CFR part 11compliance
- Electronic records
- Electronic signatures
- Audit trails

2100 bioanalyzer
- IQ and OQ/PV services
- Declaration of conformity

Chips and reagents
- Declaration of conformity

Agilent Web pages with 2100 content

www.agilent.com/chem/labonachip



www.opengenomics.com

2100 kits for Protein applications

Protein Kits – Coomassie stain sensitivity

Number	Kit	Max # of samples
5067-1517	**Agilent Protein 230 Kit**	250
5067-1518	Agilent Protein 230 Reagents	
5067-1515	**Agilent Protein 80 Kit**	250
5067-1516	Agilent Protein 80 Reagents	

Protein Kits – Silver stain Sensitivity

Number	Kit	Max # of samples
5067-1575	**High sensitivity Protein 250 Kit**	100
5067-1576	High sensitivity Protein 250 Reagents	
5067-1577	High sensitivity Protein 250 Labeling Reagents	
5067-1578	High sensitivity Protein 250 Ladder	

2100 kits for Cell Assay and DNA applications

Cell Fluorescence Kits

Number	Kit	Max # of samples
5067-1519	**Agilent Cell Kit**	150
5067-1520	Cell Checkout Kit	

DNA Kits

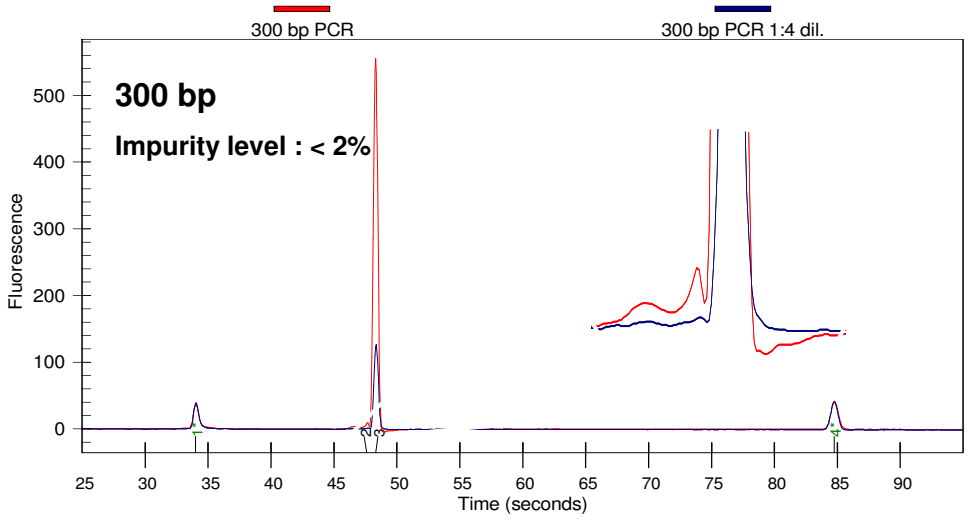
Number	Kit	Max # of samples
5067-1504	**Agilent DNA 1000 Kit**	300
5067-1505	Agilent DNA 1000 Reagents	
5067-1506	**Agilent DNA 7500 Kit**	300
5067-1507	Agilent DNA 7500 Reagents	
5067-1508	**Agilent DNA 12000 Kit**	300
5067-1509	Agilent DNA 12000 Reagents	

2100 kits for RNA applications

RNA Kits

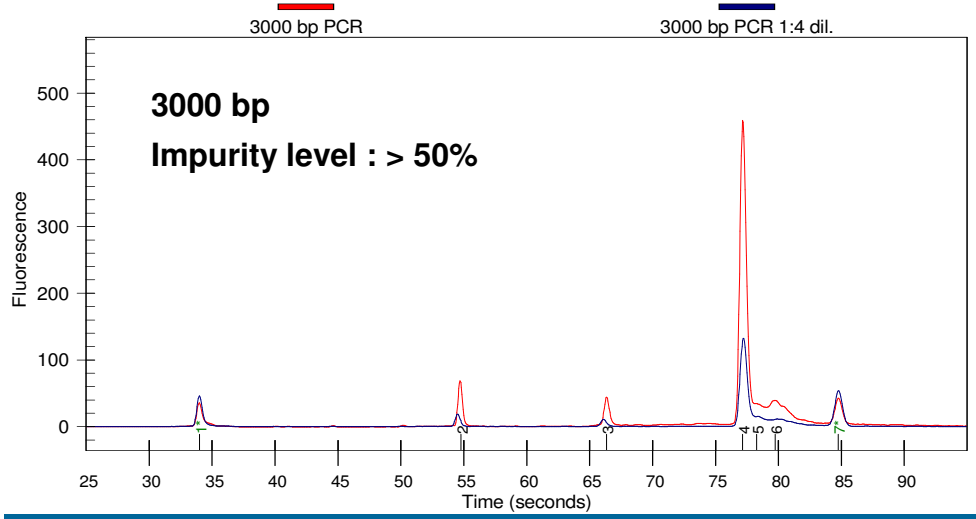
Number	Kit	Max # of samples
5067-1511	**Agilent RNA 6000 Nano Kit**	300
5067-1512	Agilent RNA 6000 Nano Reagents	
5067-1529	Agilent RNA 6000 Nano Ladder	
5067-1513	**Agilent RNA 6000 Pico Kit**	275
5067-1514	Agilent RNA 6000 Nano Reagents	
5067-1535	Agilent RNA 6000 Nano Ladder	
5067-1548	**Agilent Small RNA Kit**	275
5067-1549	Agilent Small RNA Reagents	
5067-1550	Agilent Small RNA Ladder	

Determination of PCR Product Impurity



Quantitative data from Agilent 2100 bioanalyzer

Sample	c (DNA)	main peak
300 bp PCR	41.4 ng/ul	40.7 ng/ul
300 bp PCR 1:4	9.6 ng/ul	9.6 ng/ul



Quantitative data from Agilent 2100 bioanalyzer

Sample	c (DNA)	main peak
3000 bp PCR	61.9 ng/ul	40.7 ng/ul
3000 bp PCR 1:4	14.8 ng/ul	9.8 ng/ul