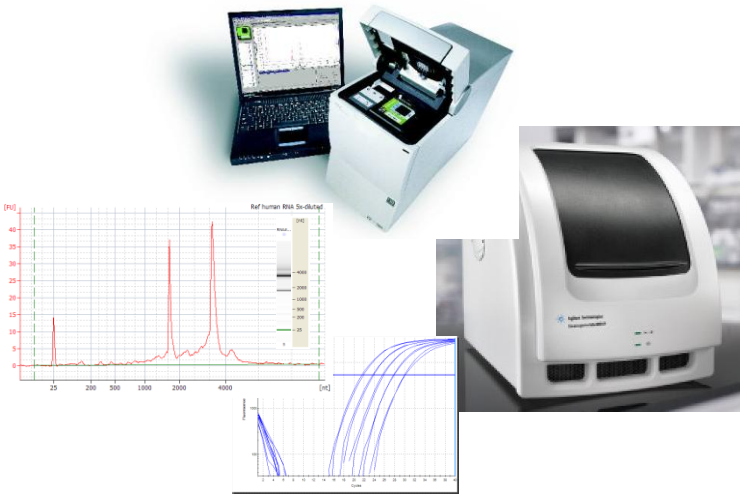
Overview Agilent Micro fluidics



Cathy Cutler
Field Application Scientist
Agilent-Genomics

The Agilent 2100 Bioanalyzer

March 2010: >15,000 citations !

First commercially available Lab-on-a-Chip product

Introduced 1999

Analysis of biomolecules:

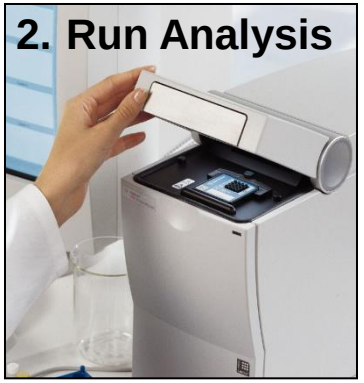
DNA, RNA, Proteins and cells

More than 7000 of instruments sold WW

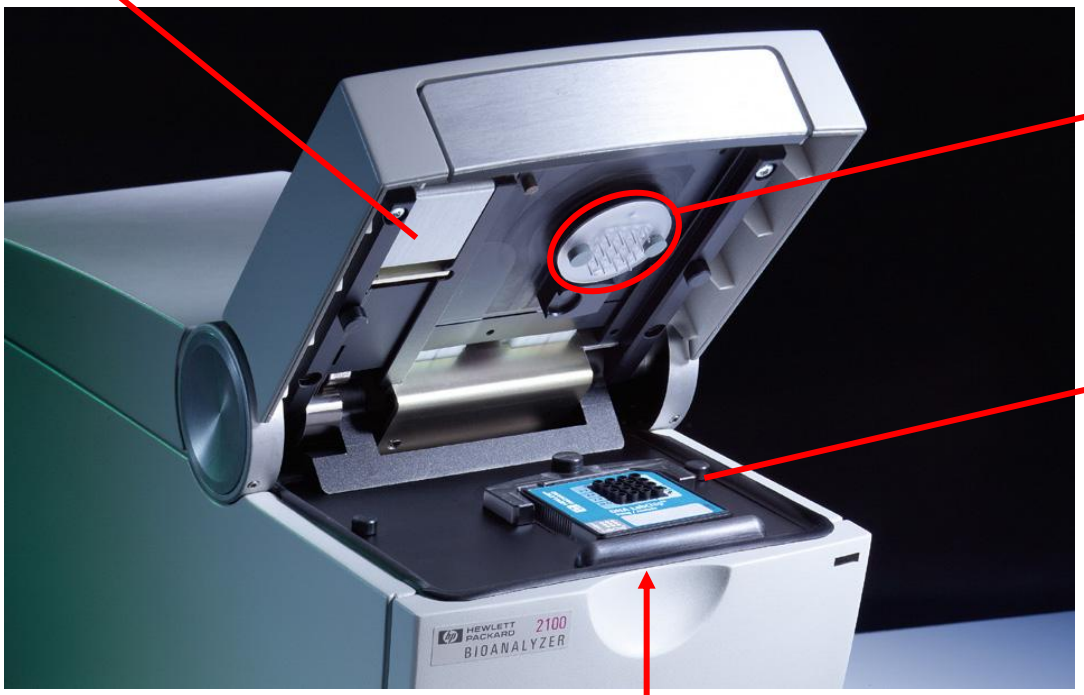
Industry-Standard for the analysis of RNA

Standard for sample QC in Next-Generation Sequencing workflows

Agilent 2100 Bioanalyzer



Exchangeable cartridge for different assays

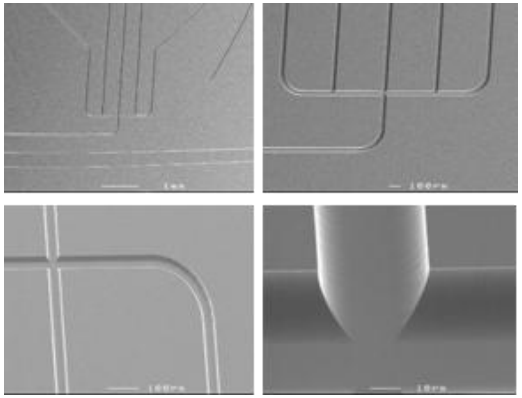


16 pin electrodes connected to HV-sources

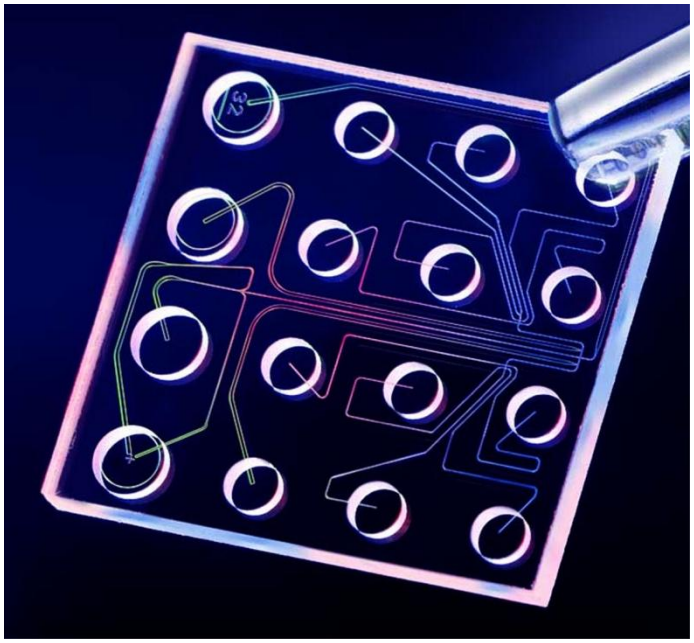
Chip holder with heater plate

Optics for detection

The Lab-on-a-Chip Approach



Increasing quality and speed of gel electrophoresis



Sample volumes 1 - 5 µl

10 -12 samples depending on Assay

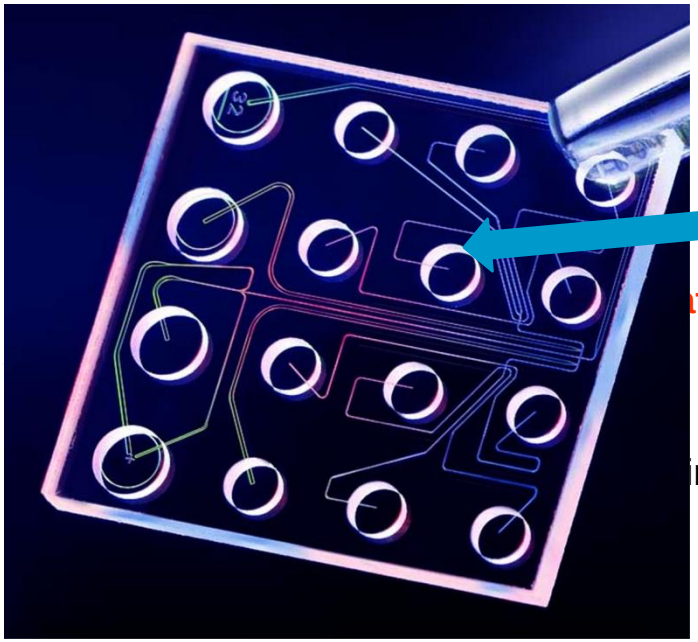
Separation, staining, detection of samples

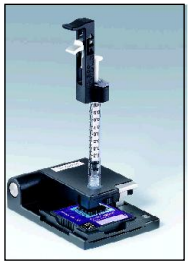
Results in 5-30 minutes available

No extra waste removal needed

Disposable Chip, no crosscontamination

Chip Architecture



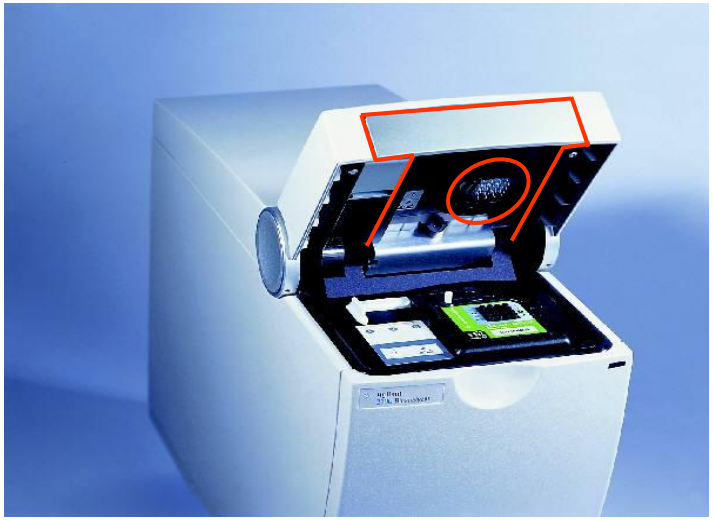


Gel loading device

ation channel and point of detection

ing

- Chip accommodates **sample** wells, **gel** and **conditioning/destaining** wells, and a well for a **standard (ladder)**
- Sieving gel/dye matrix is forced into capillaries
- 16 pin electrodes apply voltage
- Capillary fluidics become “lanes”



Principle of Electrodriven Flow

Lab-on-a-Chip - General Features and Benefits

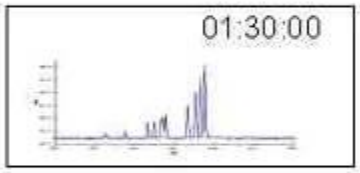
Miniaturization (Scale)

- small sample volumes
- reduced reagent usage
- reduced bench space

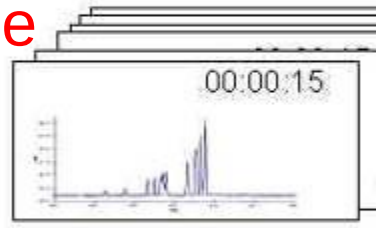
Handling

Miniaturization (Speed)

- fast analysis



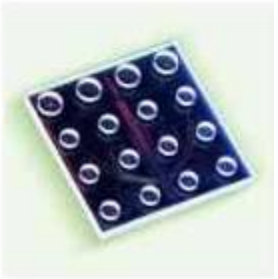
Surface



Automation

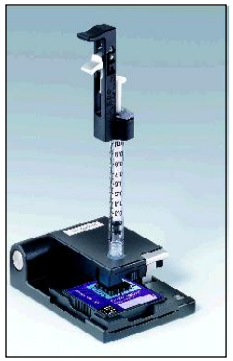
- improved accuracy
- improved precision
- improved productivity

Software

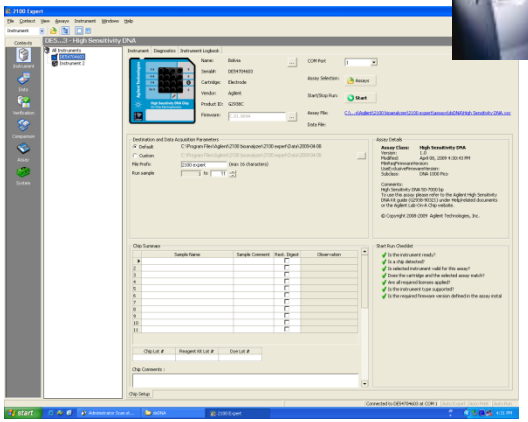


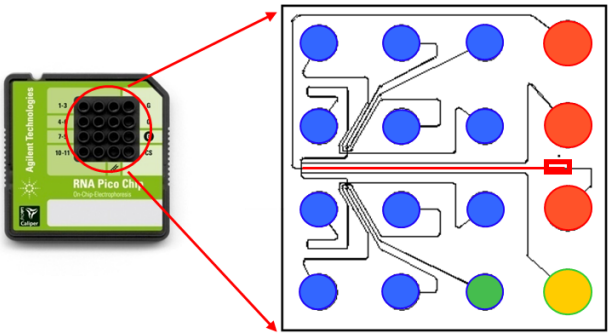
Smaller - Faster - Smarter

The Kits




Agilent Technologies
DNA Chips
LAB-ON-A-CHIP
Agilent DNA 1000 Reagents



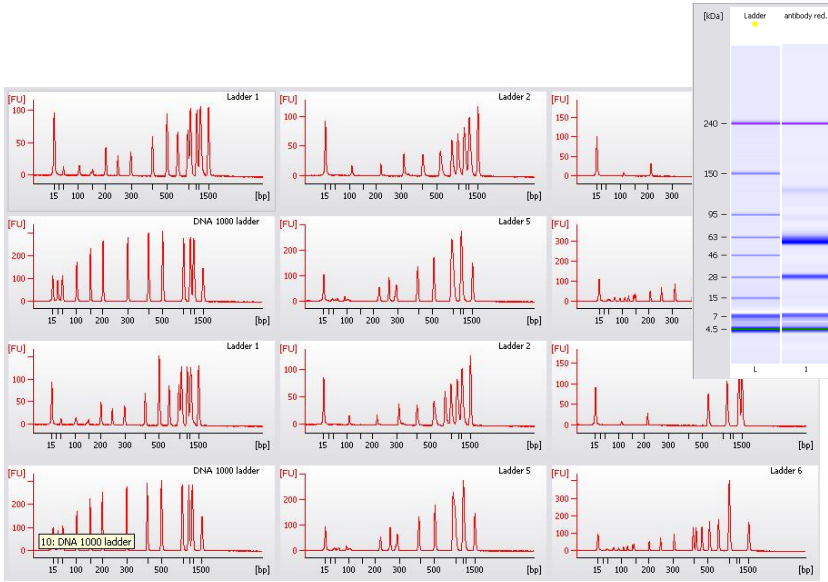

Agilent Technologies
RNA Pico Chip

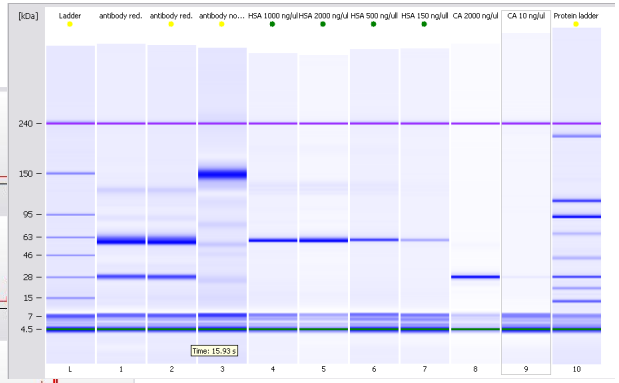
2100 Expert Software
Version B.02.08

- **Easy to use for Instrument control, Data Analysis, Data comparison and Reporting**
- **Patended RIN (RNA Integrity Number)**
- **Color coded Result Flagging**
- **Easy comparison context (multi samples from various files)**
- **Customizable result tables for printing and reports**
- **Optional security pack software for 21 CFR part 11 compliance requirements**

NEW with B.02.08:

- High Sensitivity DNA Assay
- Plant RIN Application
- Windows 7 Compatibility





		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
▶	◀	15	4.20	424.2	Lower Marker
2		111	0.25	3.4	
3		214	0.60	4.3	
4		556	1.34	3.7	
5		782	2.02	3.9	
6		1,322	3.02	3.5	
7	▶	1,500	2.10	2.1	Upper Marker

Agilent Technologies

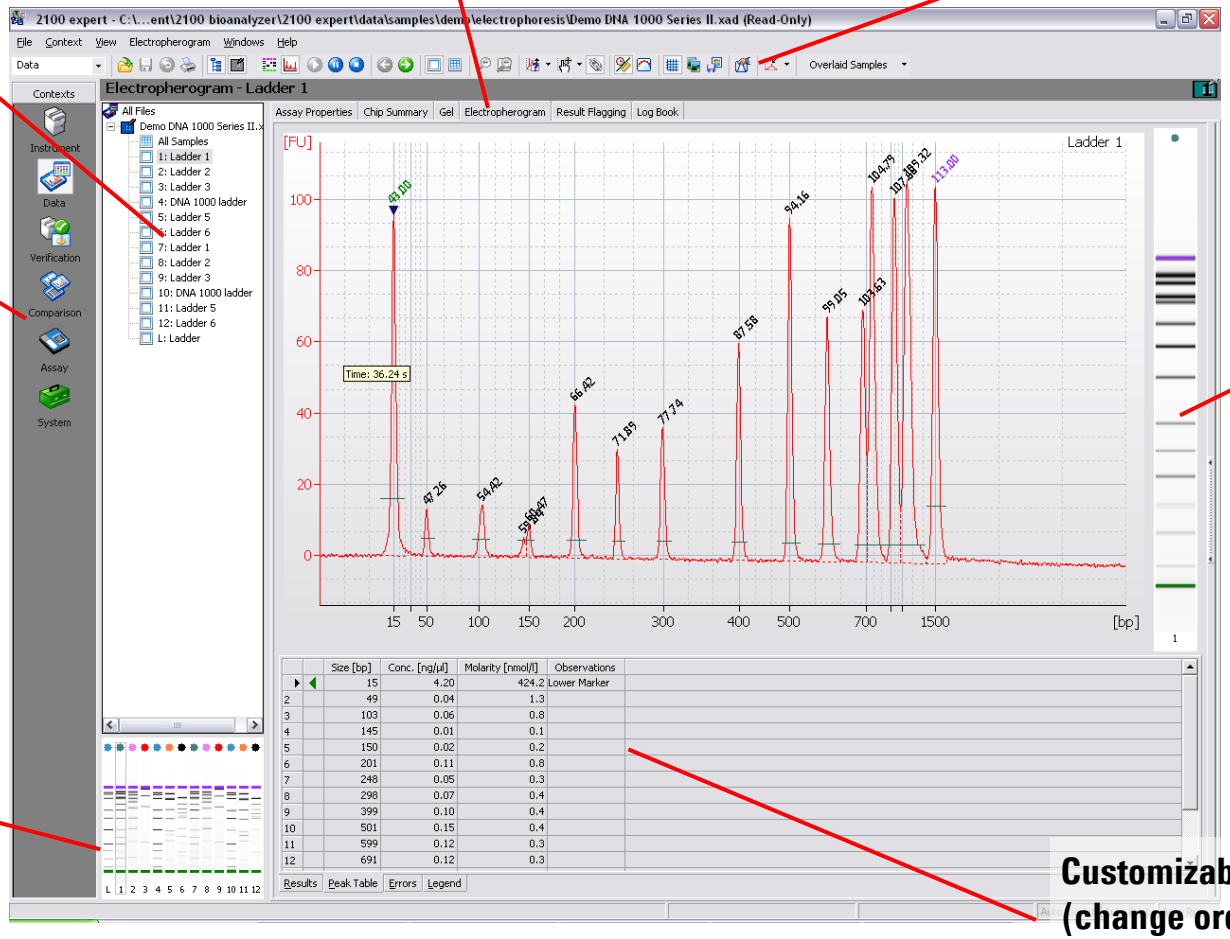
Analyze Data (Peak based view)

Tree view for navigation between samples and files

Tabs for different data analysis functions

Task bar with context sensitive icons for different actions

Context menu bar

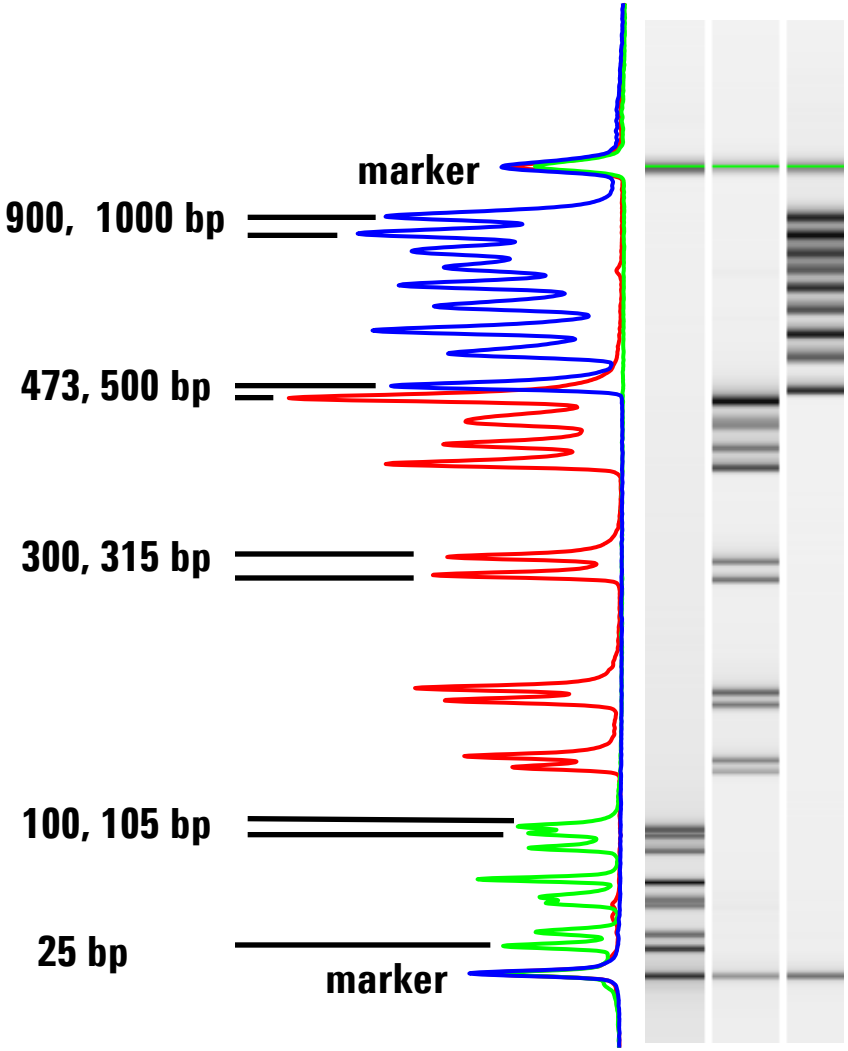


Single gel lane for selected E-gram

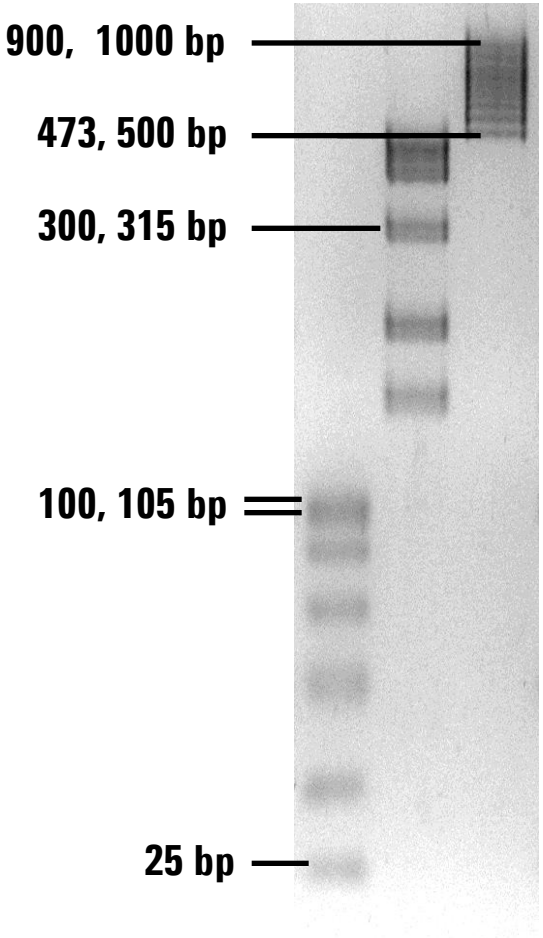
Customizable gel-like image (change order)

Customizable result table (change order and add additional columns)

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



2100 bioanalyzer data
Gel-like image



2 % agarose gel stained
with Ethidiumbromide

Agilent Technologies

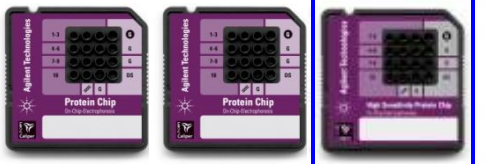
Current Assays (including anything required for analysis)

DNA1000 DNA7500 DNA12000 High Sensitivity DNA

DNA Assays:

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

NEW



P230 P80 **HSP 250**

Protein Assays:

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

6000 Nano 6000 Pico Small RNA

RNA Assays:

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

Flow Cytometry

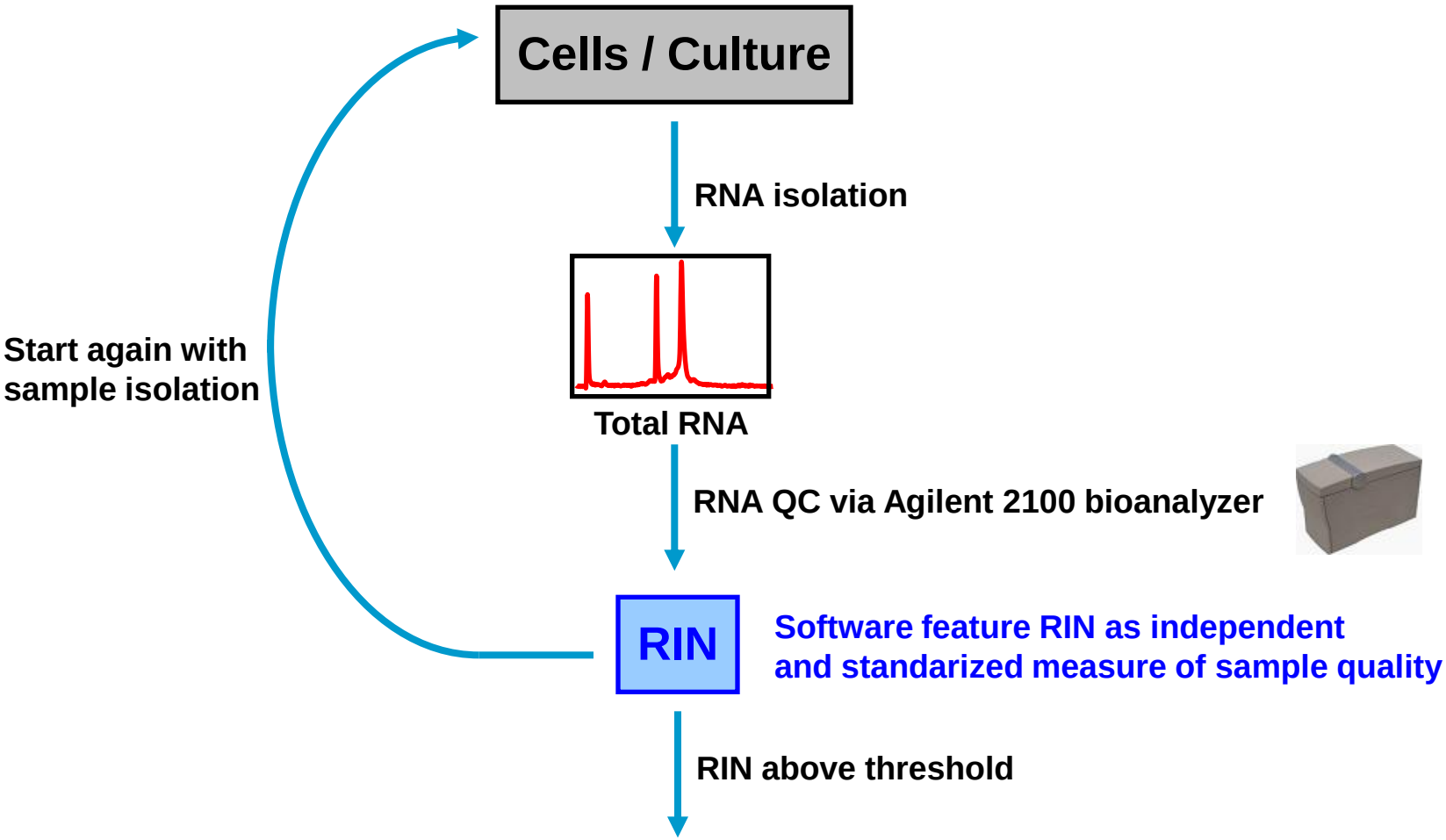
Cell Assays:

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

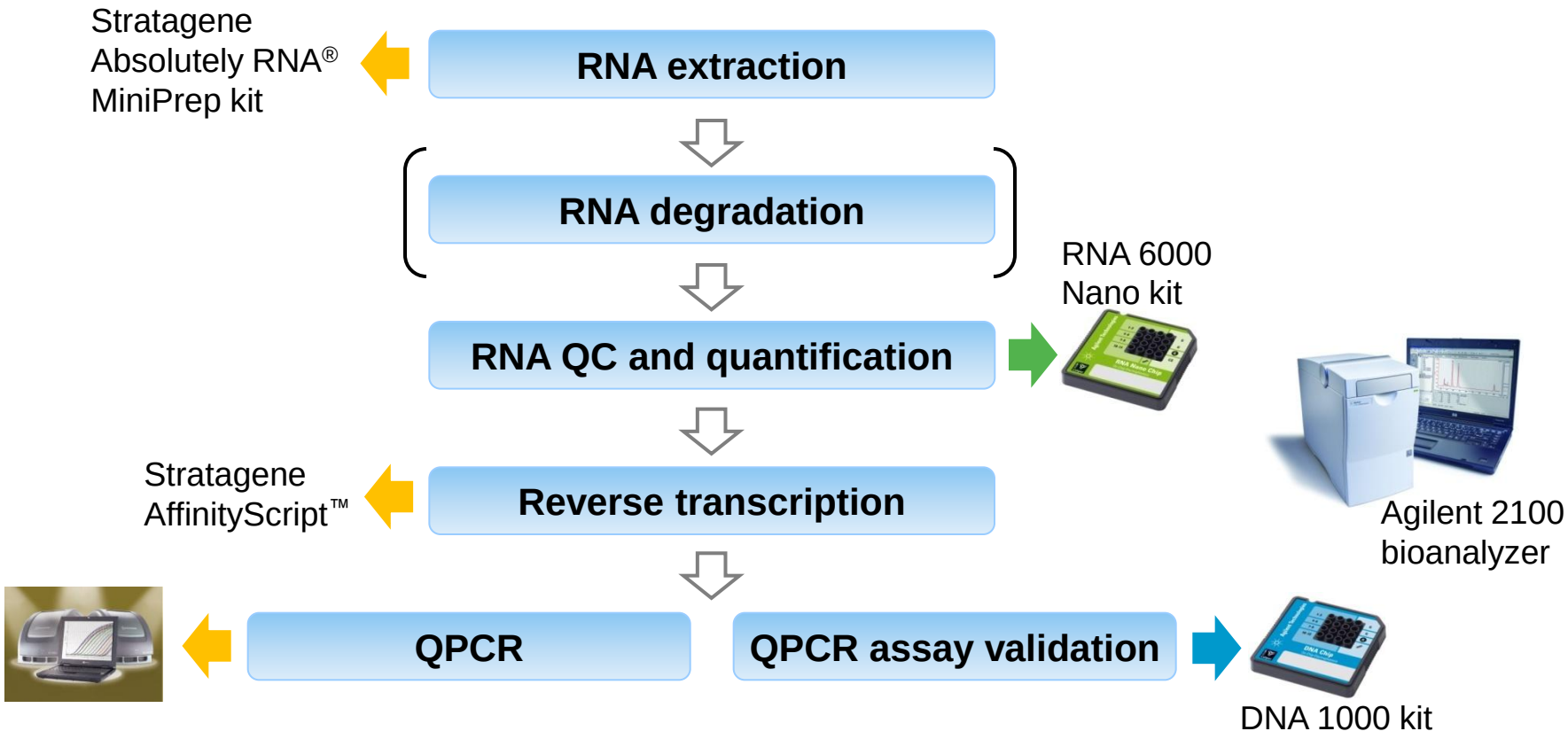
RNA and Small RNA Assays

Agilent Technologies

RNA QC in Routine Gene Expression Workflow



Experimental workflow



Successful QPCR with the Agilent 2100 bioanalyzer

RNA quality - Effects of degraded RNA

Not knowing the extend of possible degradation might lead to false negative results or misinterpretation of the data if the amplicon falls into a degraded region

Knowing RNA quality allows to accommodate the design and set expectations avoiding wrong interpretation of results

Assay validation - Limitations of SYBR Green melt curves

Restricted resolution, which can make it difficult to determine specificity

T_m depends on dye/template ratio

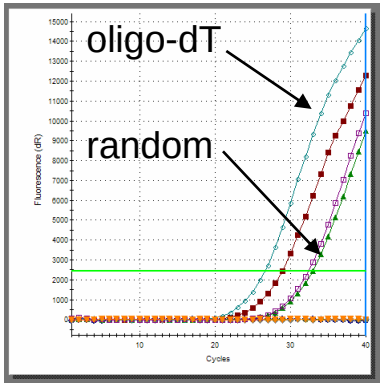
SYBR Green is a non-saturating dye with non-uniform distribution along the double-strand

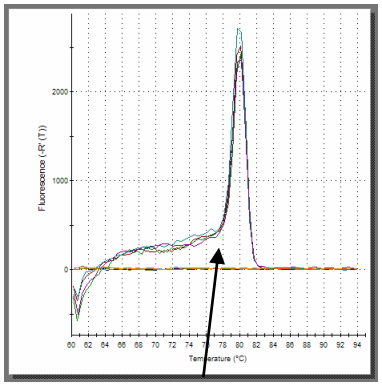
Melt curve provides no info on the size of the generated amplicon

QPCR assay validation

– amplification plots, melt curve and bioanalyzer analysis

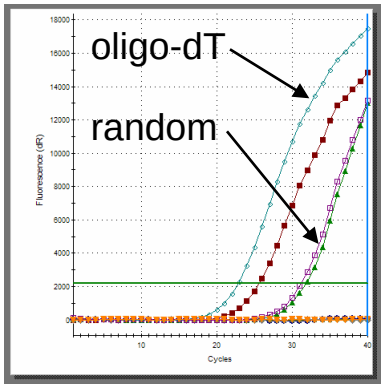
GAPDH 5‘ assay: Expected size 118 bp

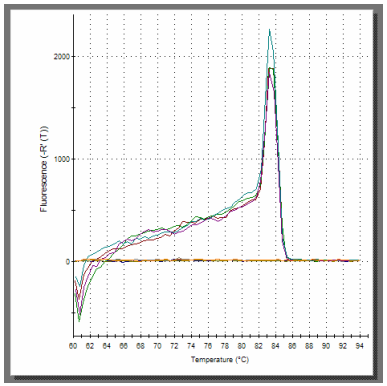


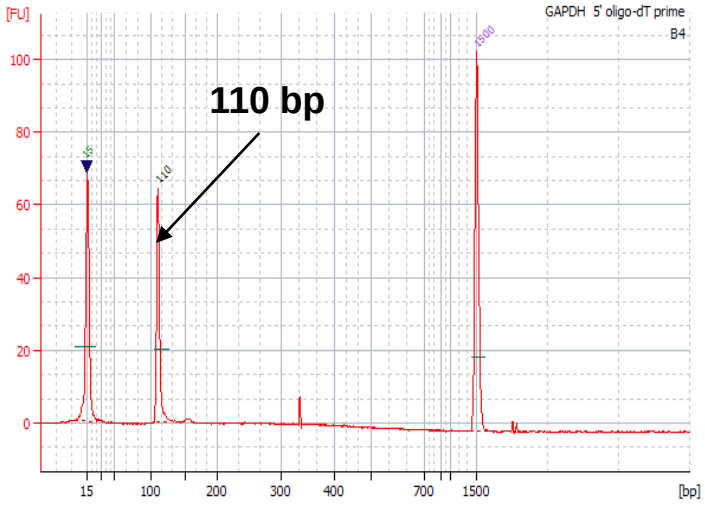


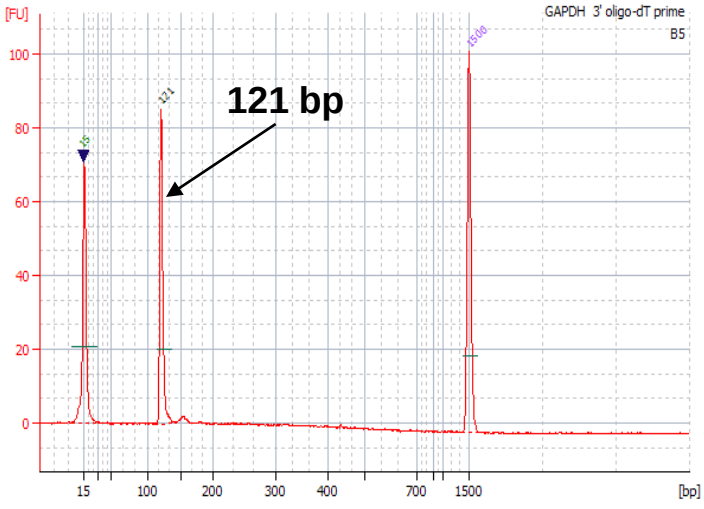
NoRT

GAPDH 3‘ assay: Expected size 126 bp

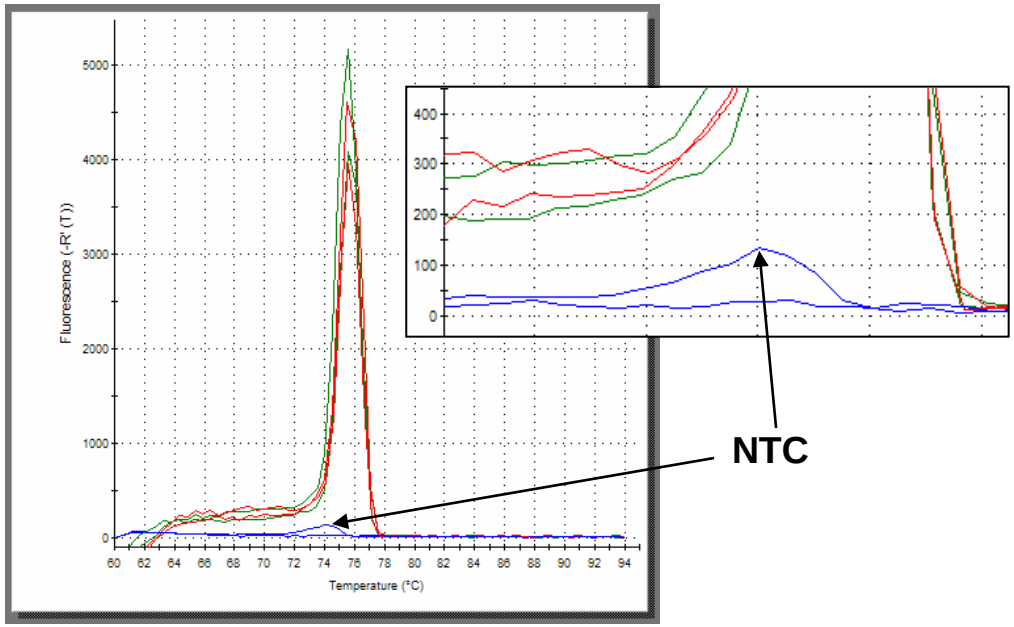


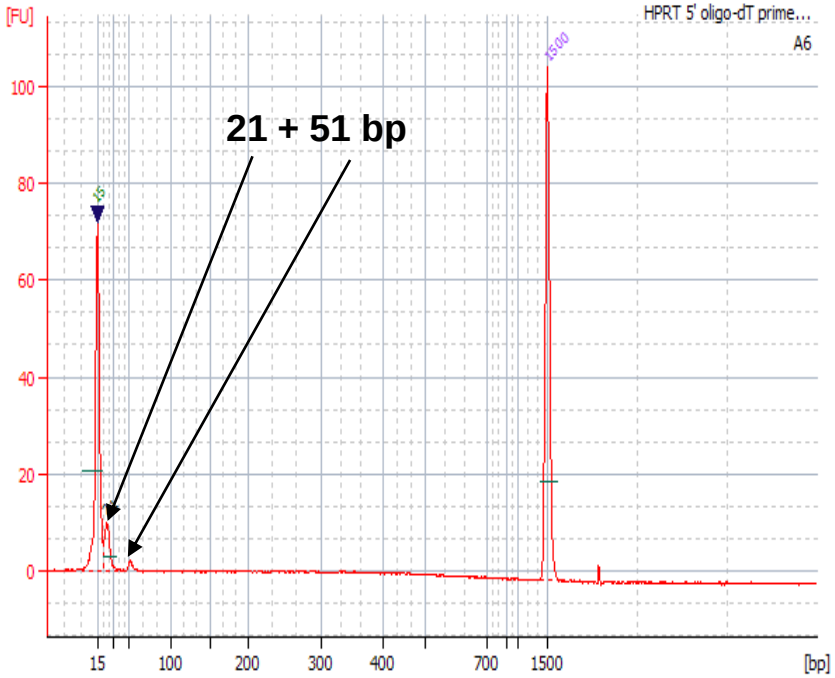






QPCR assay validation
– No-template controls (NTC)





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)

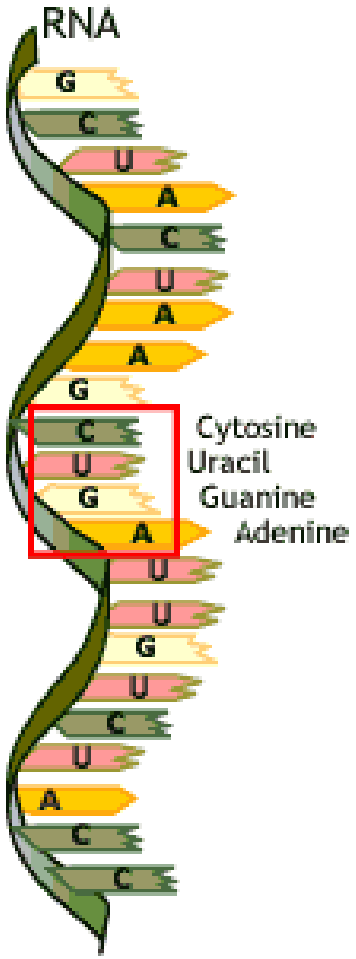
RNA integrity number (RIN)

mRNA

determine integrity and quality of mRNA samples

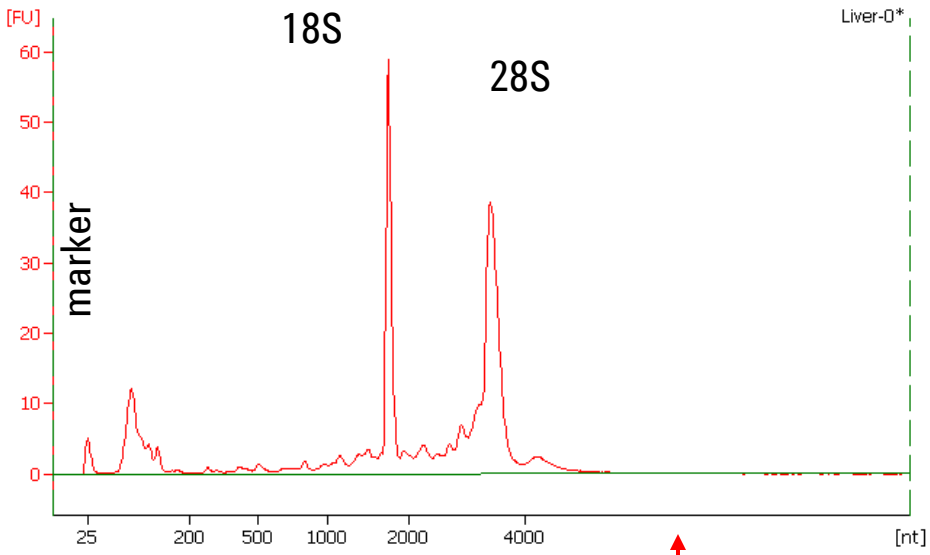
Determination of mRNA concentration

calculate % ribosomal RNA in mRNA samples



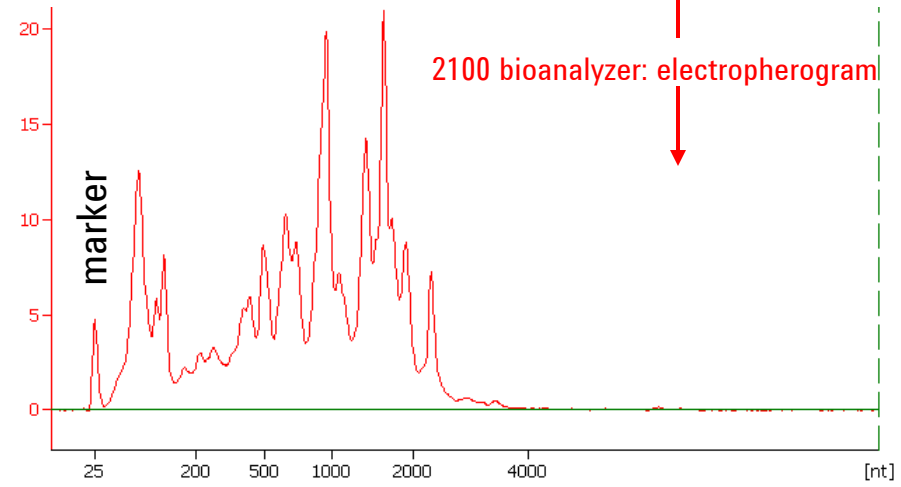
Assesment of RNA Integrity

RNA 6000 Nano LabChip kit



2100 bioanalyzer: single lane gel-like image

2100 bioanalyzer: electropherogram



Typical first QC step during cDNA or cRNA sample prep for QPCR

High quality total RNA (RIN 8.8)

Partially degraded total RNA (RIN 3.7)

DNA Applications

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**

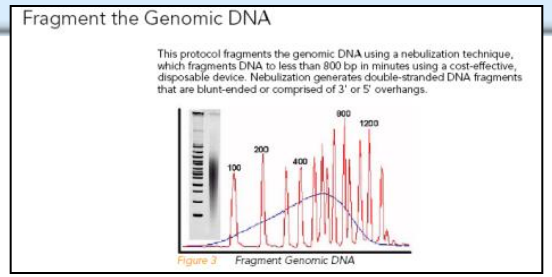
Cancer diagnostics

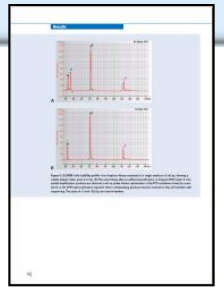
DNA QC in Next-Gen Sequencing - Situation

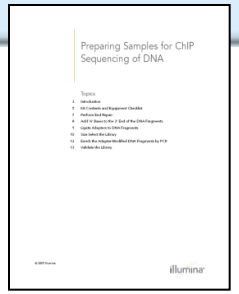
- Next Generation Sequencing is an emerging technology.
- Quality of sequences is one of the chief technical concerns about next gen sequencing platforms in a recent customer survey by Insight pharma reports
- Leading next generation sequencing vendors (*Illumina, Roche, Life Technologies*) recommend existing Bioanalyzer DNA assays in their workflows

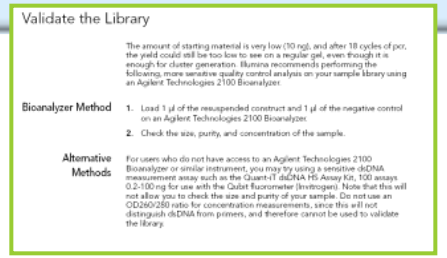
References

• Manuals of all Next Gen Sequencing Vendors: Illumina, Roche, Life Technologies

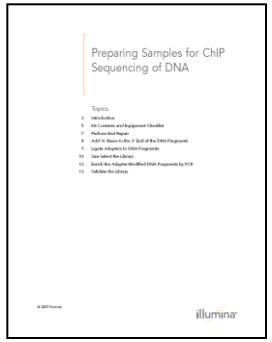








Bioanalyzer in Illumina/Solexa Workflow – Examples



Workflow

ChIP-Seq

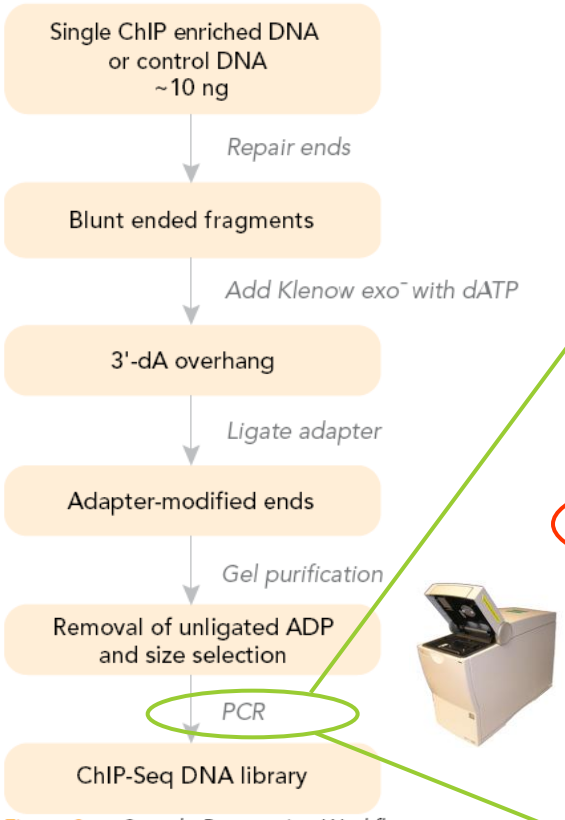


Figure 2 Sample Preparation Workflow

Validate the Library

The amount of starting material is very low (10 ng), and after 18 cycles of pcr, the yield could still be too low to see on a regular gel, even though it is enough for cluster generation. Illumina recommends performing the following, more sensitive quality control analysis on your sample library using an Agilent Technologies 2100 Bioanalyzer.

Bioanalyzer Method

1. Load 1 µl of the resuspended construct and 1 µl of the negative control on an Agilent Technologies 2100 Bioanalyzer.
2. Check the size, purity, and concentration of the sample.

Alternative Methods

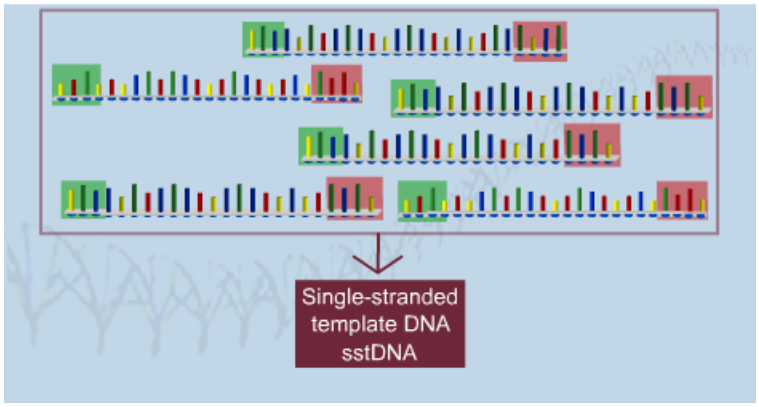
For users who do not have access to an Agilent Technologies 2100 Bioanalyzer or similar instrument, you may try using a sensitive dsDNA measurement assay such as the Quant-iT dsDNA HS Assay Kit, 100 assays 0.2-100 ng for use with the Qubit fluorometer (Invitrogen). Note that this will not allow you to check the size and purity of your sample. Do not use an OD260/280 ratio for concentration measurements, since this will not distinguish dsDNA from primers, and therefore cannot be used to validate the library.

Bioanalyzer in Roche/454 GS FLX Workflow

DNA Kit Family

Validate DNA fragmentation (< 800bp) with **DNA kit family**

DNA Library Preparation

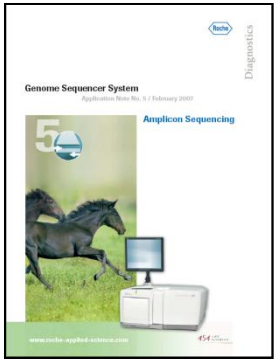


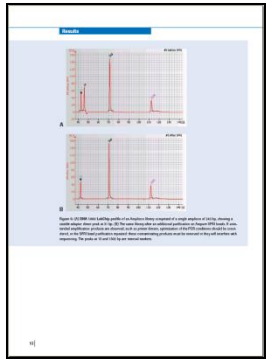
RNA Kit Family

Validate ssDNA library

RNA kit:

- estimate the size distribution
- to determine if fragments <300 bp successfully removed
- quantitation for correct dilution





454 recommends the use of Agilent Bioanalyzer in their manuals

http://www.454.com/downloads/protocols/Guide_To_Amplicon_Sequencing.pdf

http://cage.unl.edu/3.%20GS%20FLX%20Shotgun%20DNA%20Library%20Preparation%20QuickGuide.pdf

Bioanalyzer in ABI SoliD Small RNA Sequencing Workflow

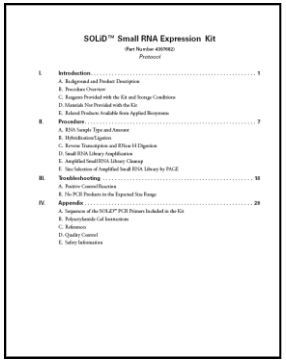
Small RNA Kit

Check small RNA content in total RNA samples

If small RNA content >0.5% use total RNA

If small RNA content <0.5% enrich small RNA first

Figure 1: Workflow for SOLiD™ Small RNA Library Preparation



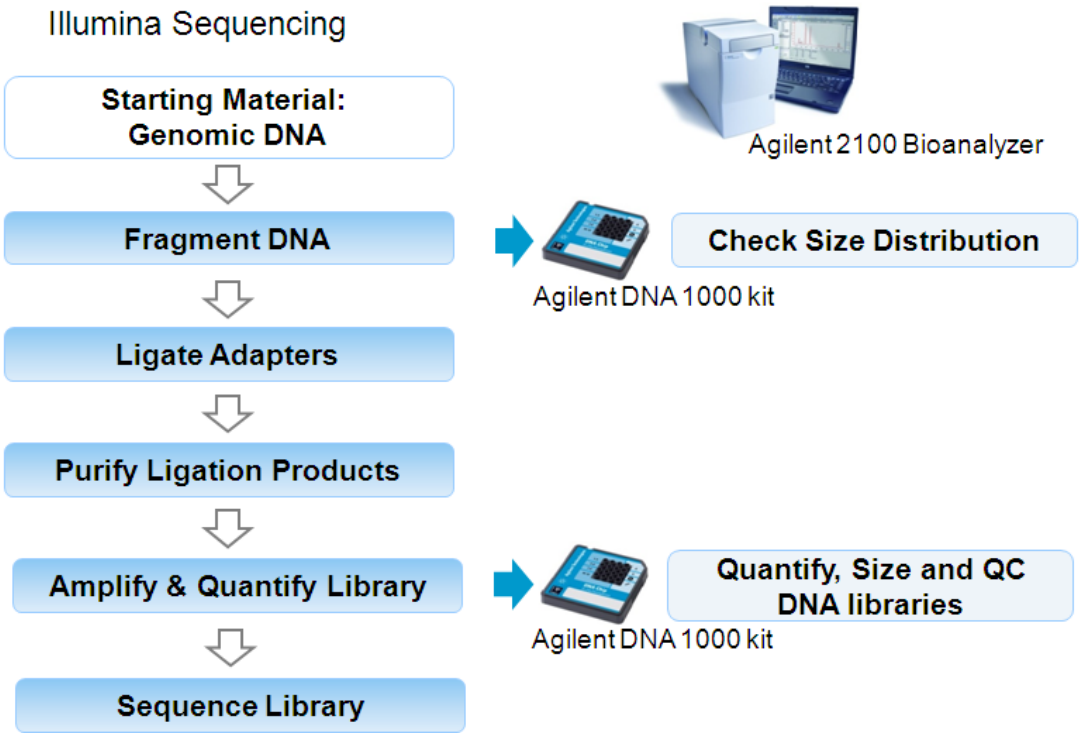
DNA Kit Family

verify the size and quality using an Agilent bioanalyzer or 6% native PAGE.

"…..we recommend evaluating the small RNA content of samples to determine whether to use total RNA or size-selected RNA in your reactions. This can be done using an Agilent bioanalyzer with the Small RNA Chip (#5067-1548)."

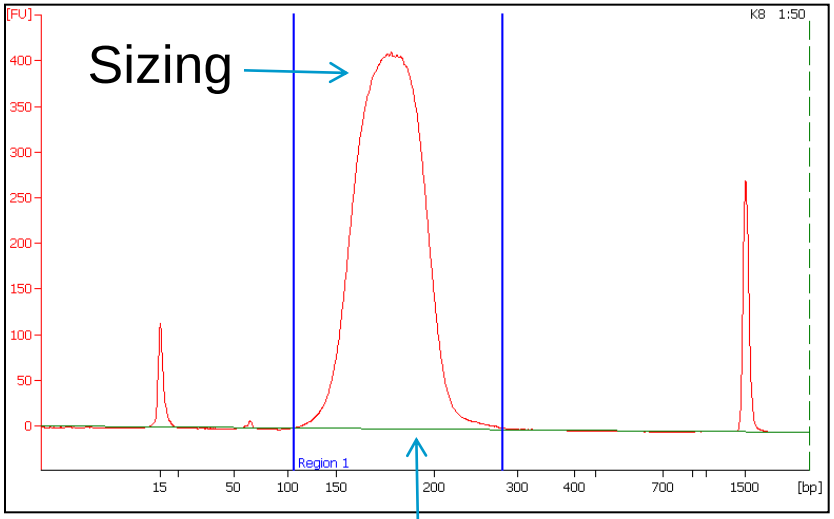
Sneak Preview – High Sensitivity DNA Kit

Simplified Illumina GAII Workflow



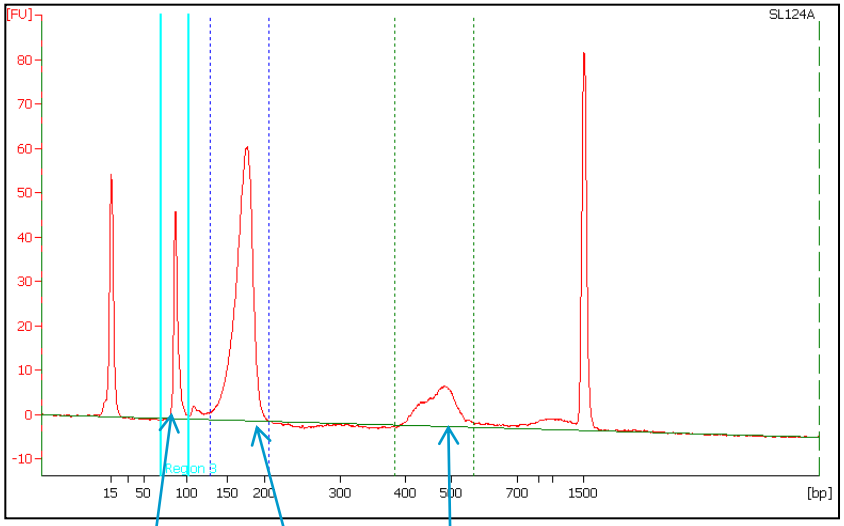
Quantification, Sizing and QC of NGS Libraries

High Quality DNA library



Quantification

Identification and quantification
of primer dimers and PCR artifacts



Primer
Dimers/
Adaptors

DNA library

PCR artifact

SureSelect Overall Sequencing Sample Prep Workflow

Genomic DNA

Shear Genomic DNA

DNA fragments with a base pair peak of 200

Repair ends

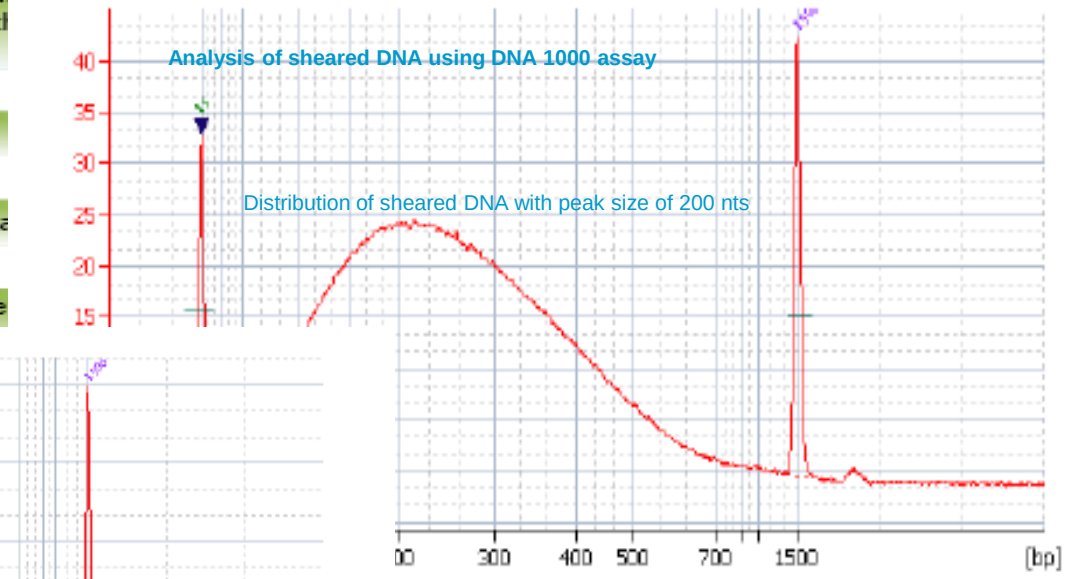
Blu… wit…

Ada…

Re…

Check size distribution

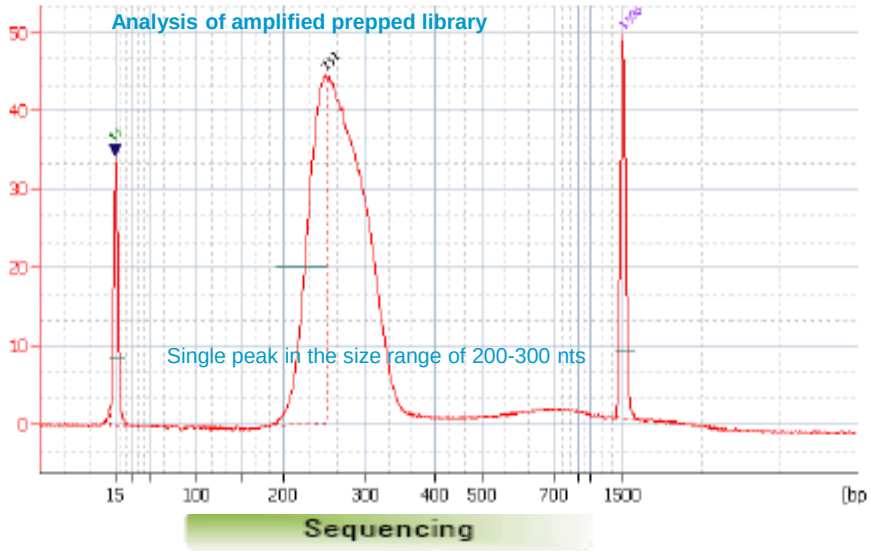
Agilent DNA 1000 kit



Genomic Locations

Probe Design in eArray

SureSelect Oligo Capture Library



Sequencing

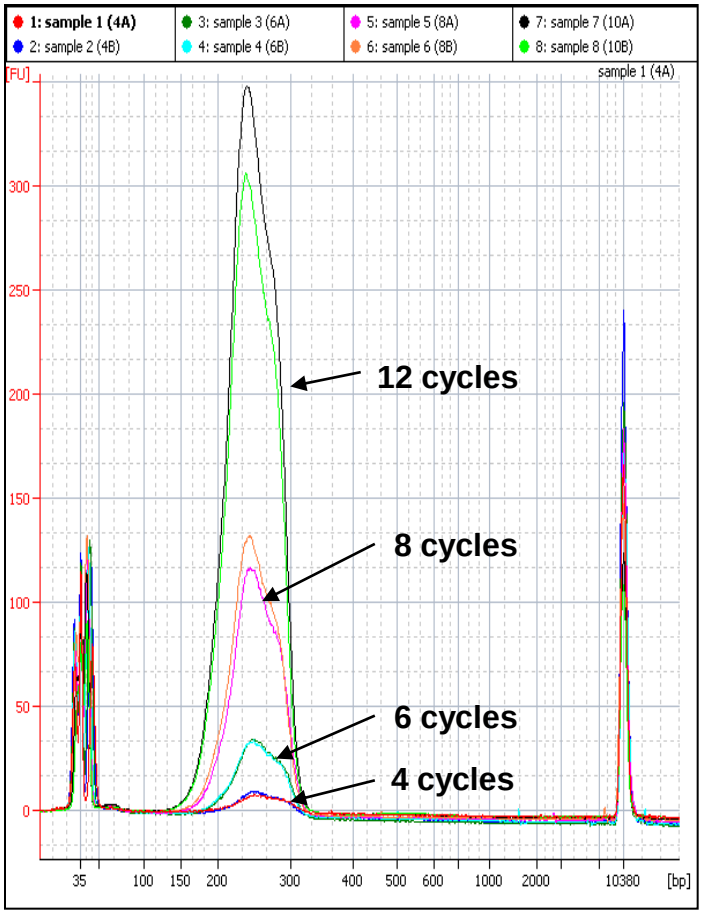
Quantify , size and QC DNA libraries

Agilent DNA 1000 kit

Improving the next-gen sequencing Sample Prep Workflow

Sizing and quantification of a Illumina GAII library. The library was enriched with Agilent's SureSelect Target Enrichment platform and amplified with varying PCR cycles.

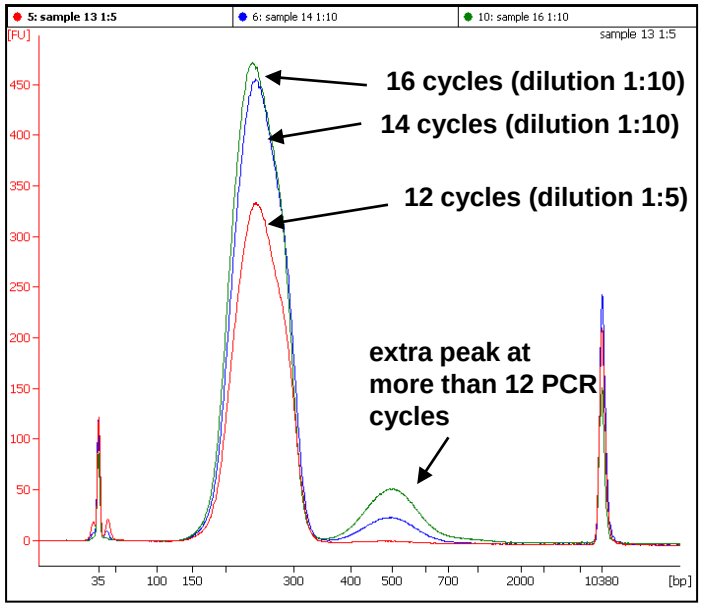
Sample ID	No. Cycles	Qubit BR ng/ul	Bioanalyzer ng/µl
4A	4	Too Low	0.090 0.015
4B	4	Too Low	0.083 0.007
6A	6	Too Low	0.304 0.017
6B	6	Too Low	0.301 0.010
8A	8	Too Low	1.13 0.07
8B	8	Too Low	1.34 0.07
10A	10	3.4	4.49 0.16
10B	10	3.4	4.54 0.17
A12	12	13.4	14.5 0.5
C12	12	12.5	13.9 0.6

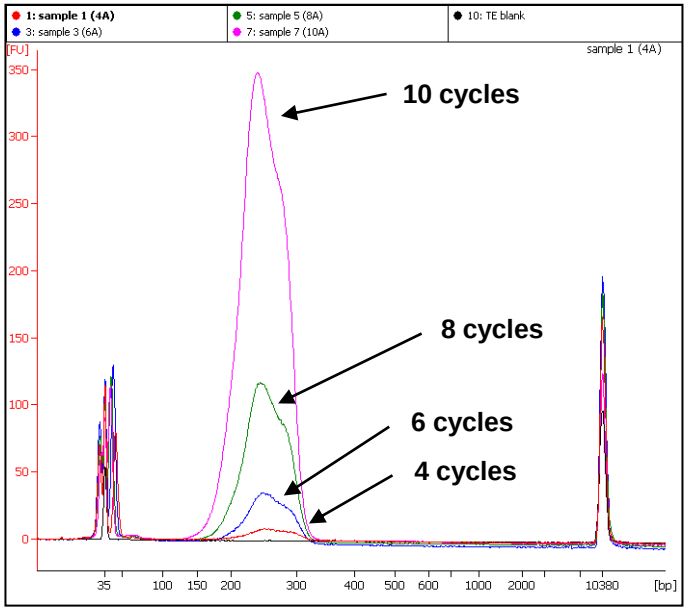


High Sensitivity DNA kit Applications – Reduction of Library Amplification Cycles

Sizing and quantification of a Illumina GAII library. The library was enriched with Agilent's SureSelect Target Enrichment platform and amplified with varying PCR cycles.

- **Amplification cycles above a certain threshold can result in amplification artifacts.**
- **High Sensitivity DNA kit enables to reduce library amplification cycles**





Protein Kit Portfolio

P80

Range 5 - 80 kDa
Sensitivity: Coomassie
Samples: 10

Samples
-Antibodies (reduced)
-Small Proteins

P230

Range 14 - 230 kDa
Sensitivity: Coomassie
Samples: 10

Samples
-Antibodies (all types)
-Standard Proteins

Coomassie Range (5 ng/µl BSA)

Silver Stain Sensitivity

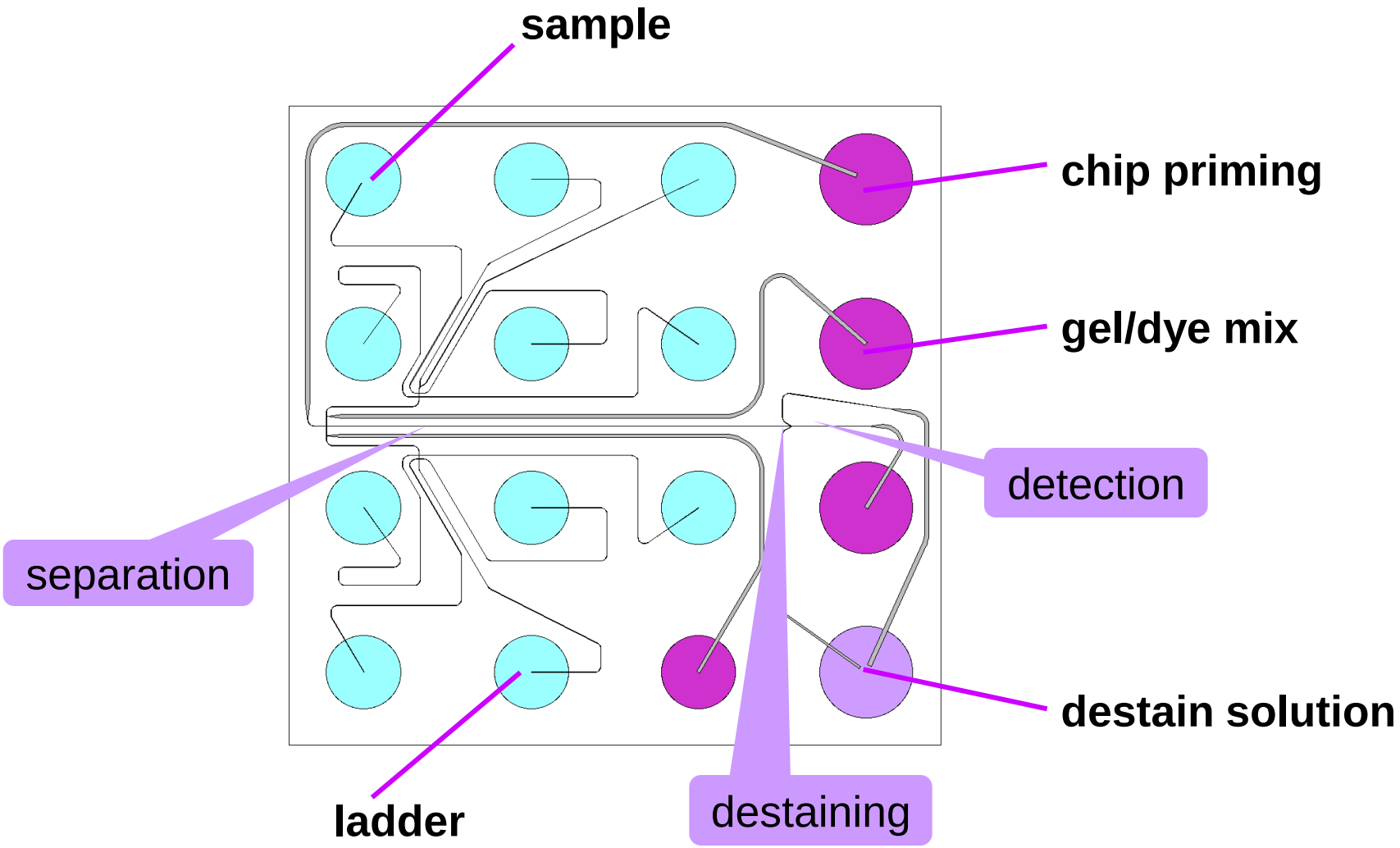
HSP-250

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

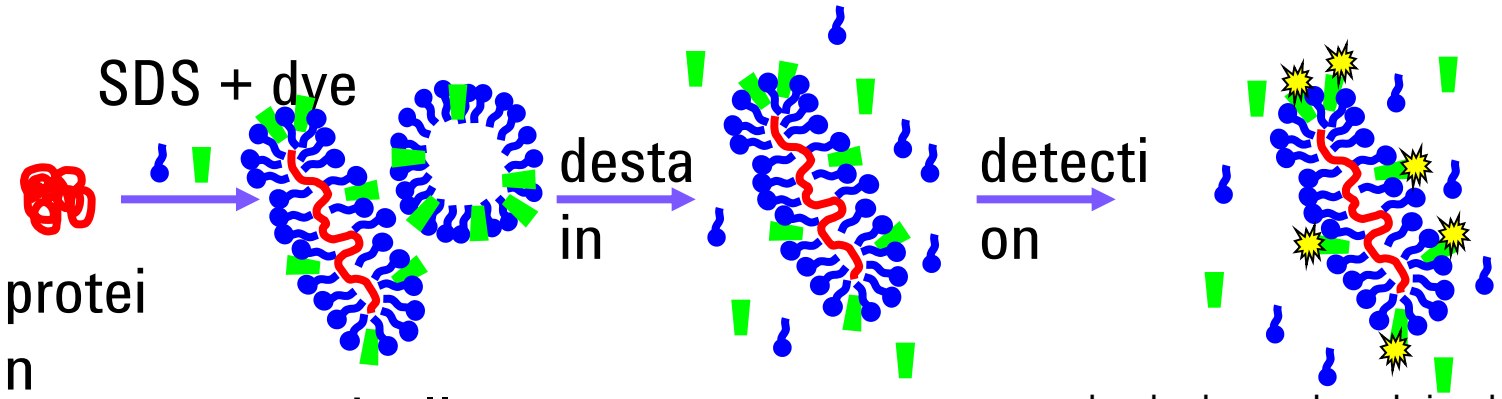
Requirements:
Software: Expert B.02.06 (+)
Instrument: all except G2938A

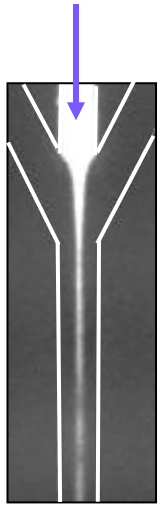
Silver stain Range (200 pg/µL BSA)

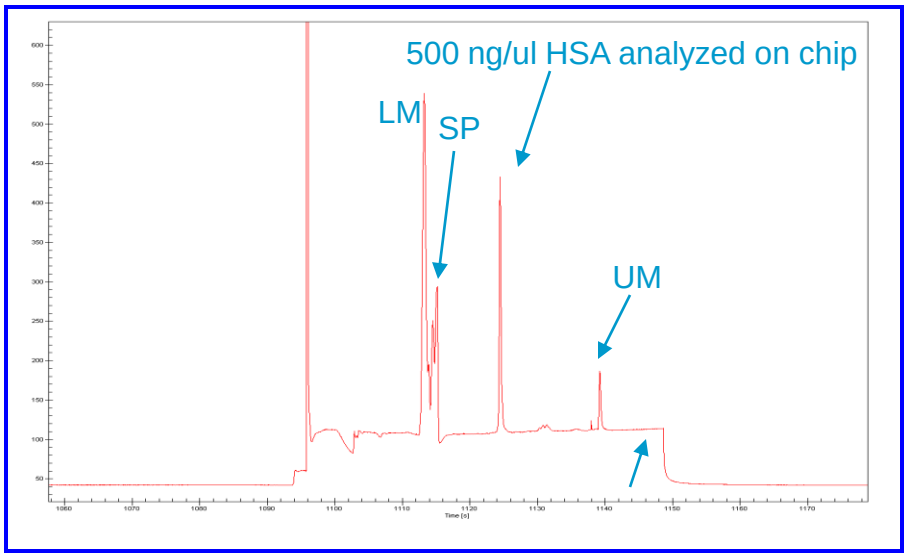
Protein Chip Layout

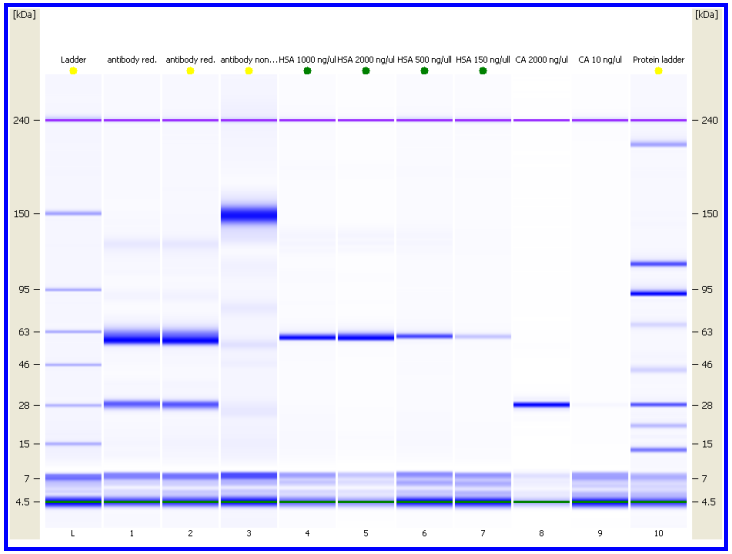


Principles (I) -- P80/P230 Assay

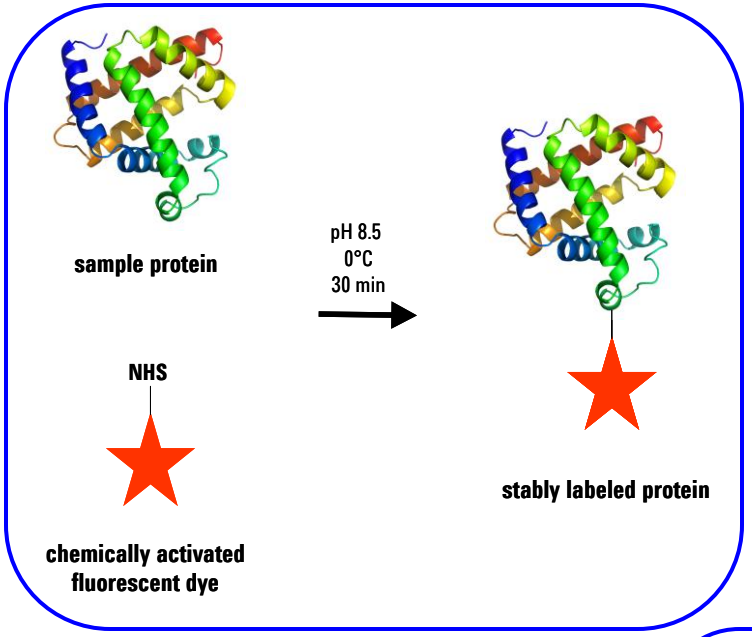


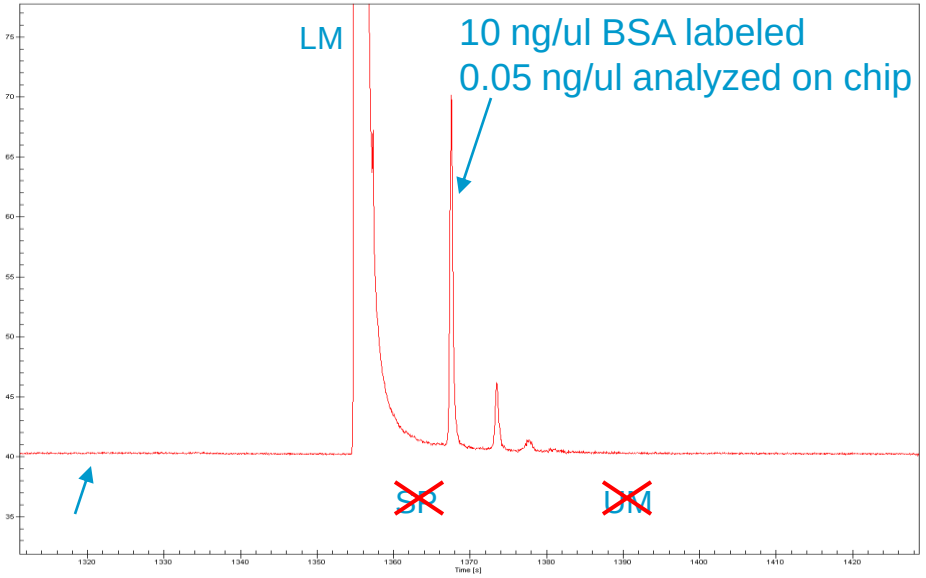




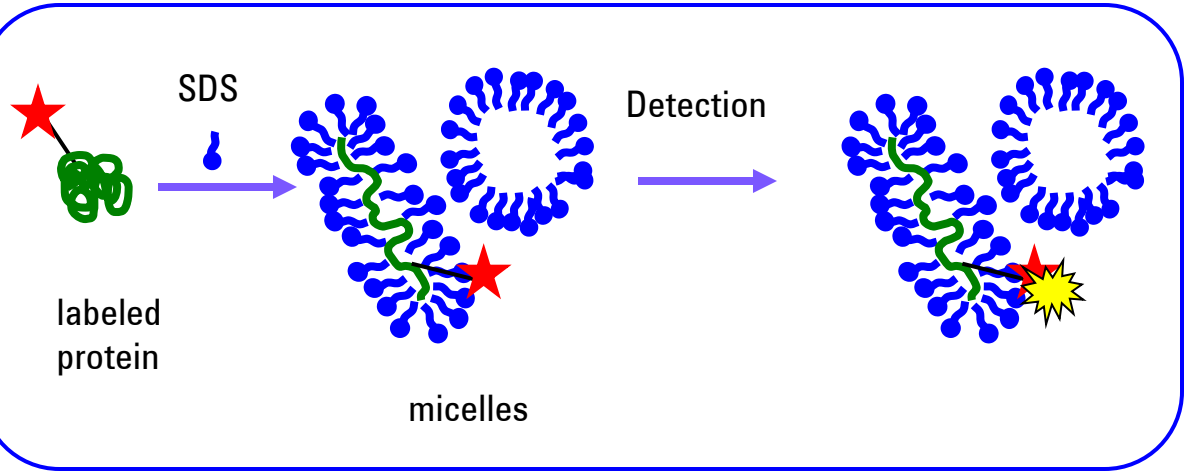


Principles (II) -- High Sensitivity Protein Assay

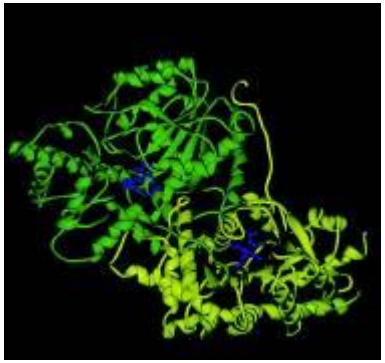




Fractionation,
Purification,
Dilution, etc.



Protein LabChip Applications



Cell Lysates

- identification of over-expressed proteins
- comparison of different expression patterns

Column Fractions

- monitoring of protein isolation and purification process
- check fractions for impurities

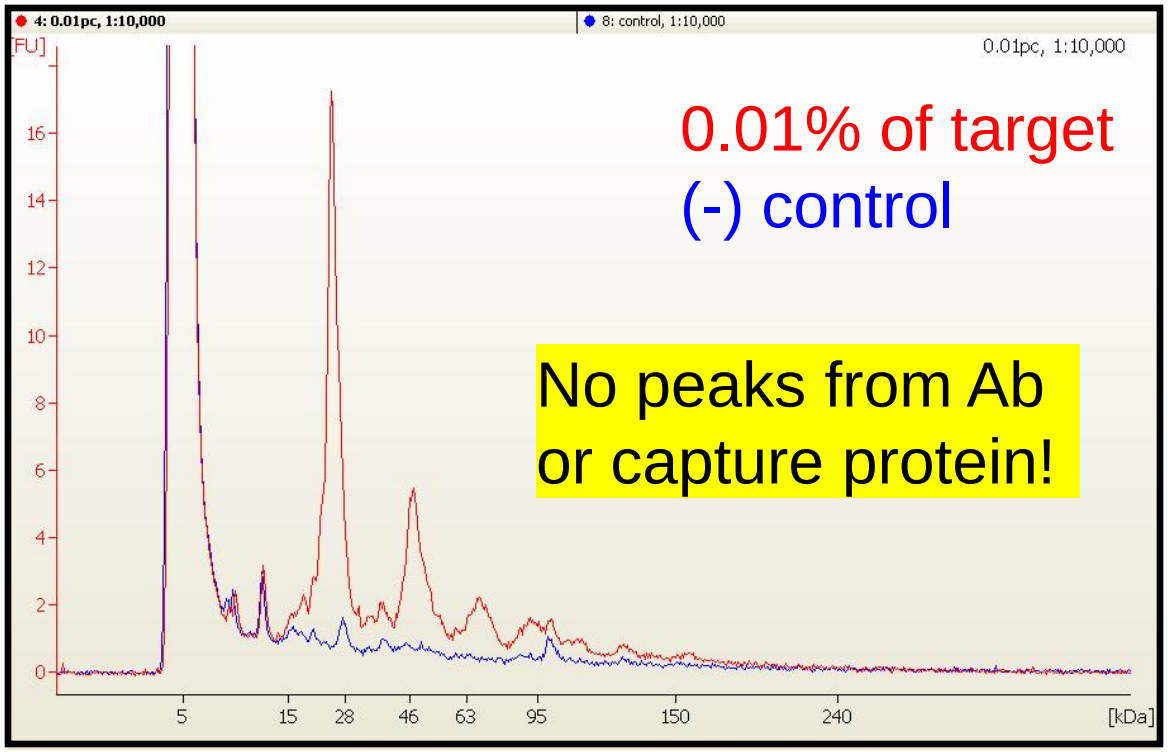
Purified Proteins

- monitoring of impurities in protein preps
- integrity check for monoclonal or polyclonal antibodies (antibody QC)

Immunoprecipitation: IP/HSP-250

His-tagged protein in *E. coli* background

Anti-His + Protein A magnetic beads



C. Wenz & A. Rüfer, *Electrophoresis* 2009, 30, 4264–4269

1 mg/ml E. coli Lysate
+/- Target Protein(s)

↓

HSP 250 Protein Labeling

↓

Immunoprecipitation

Incubation with specific Antibody

Incubation with Protein A Beads

Wash with Buffer (3x)

Elution with 50% **HSP-250 Sample Buffer**

↓

Direct On-Chip Analysis

2100 Bioanalyzer
HSP-250 Assay

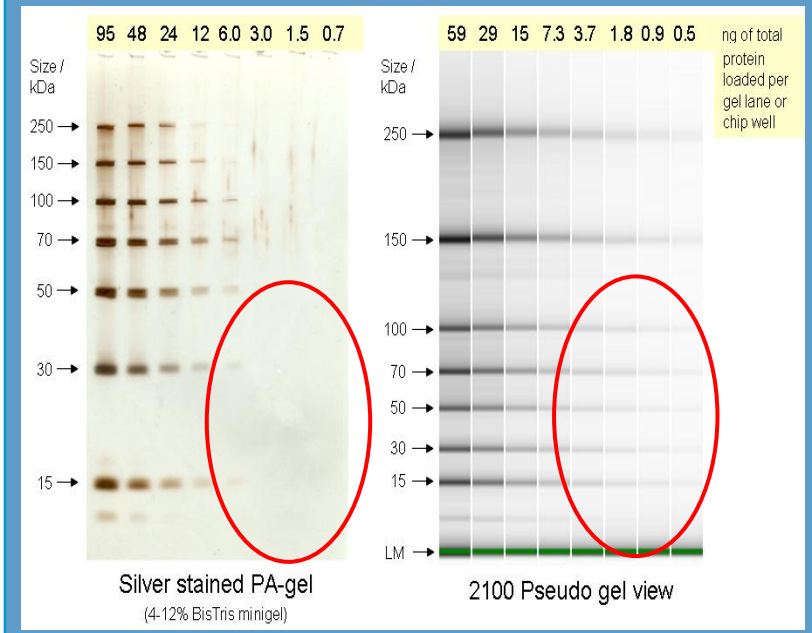
High Sensitivity Protein 250 Kit (HSP-250)

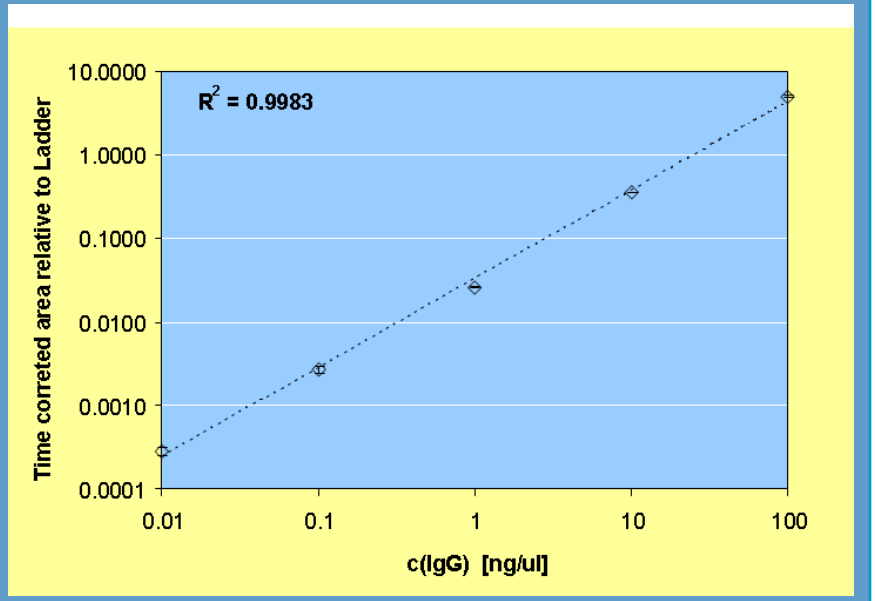
Highest sensitivity:

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

Extra wide linear dynamic range:

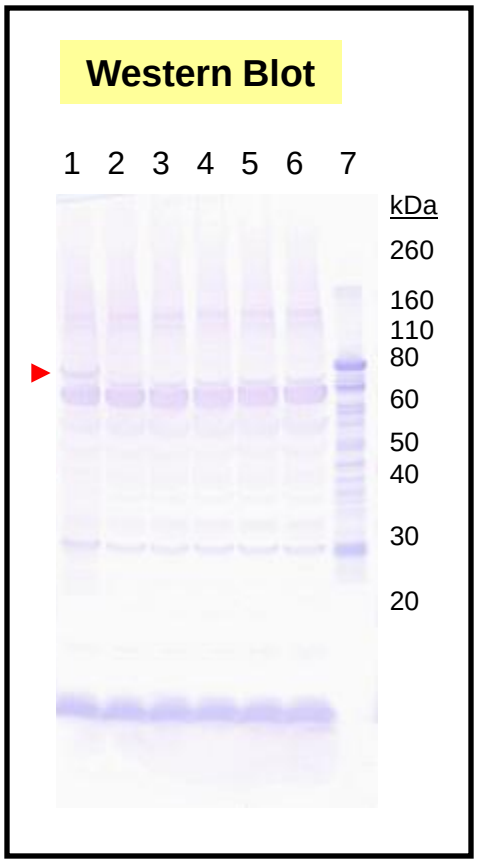
4 orders of magnitude linear dynamic range assuring excellent determination of impurities

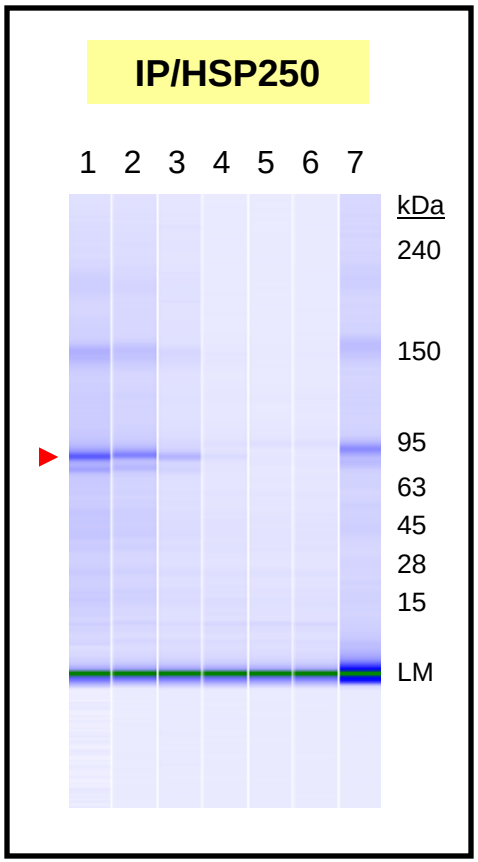




Direct comparison of samples run on SDS-PAGE with Silver staining and on 2100 Bioanalyzer.

Western Blot with PTEN-GST Fusion in *E. coli* Background





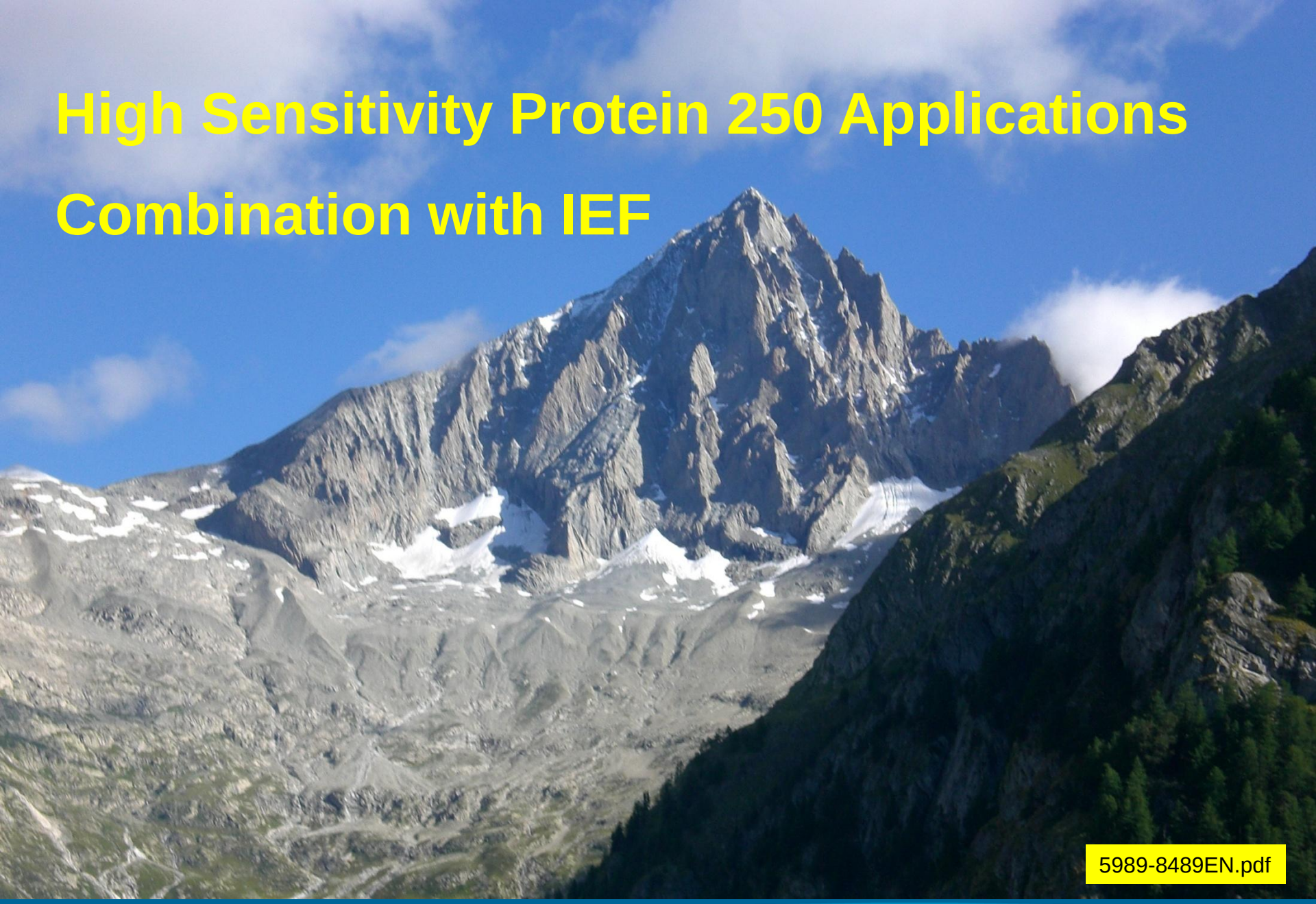
1: 1 % PTEN
2: 0.1 % PTEN
3: 0.01 % PTEN
4: 0.001 % PTEN
5: 0.0001 % PTEN
6: *E. coli* only
7: PTEN only

Advantages of the IP/HSP-250 Method:

- Sensitivity
- Specificity
- Time-to-result: 3 hours
- Cost: less primary & no secondary antibodies

High Sensitivity Protein 250 Applications

Combination with IEF



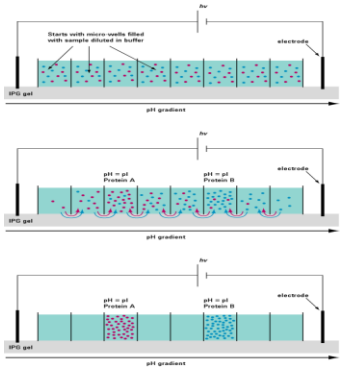
5989-8489EN.pdf

Combination with OFFGEL Fractionation

E. coli lysate
(50 µg)

Protein clean up and labeling

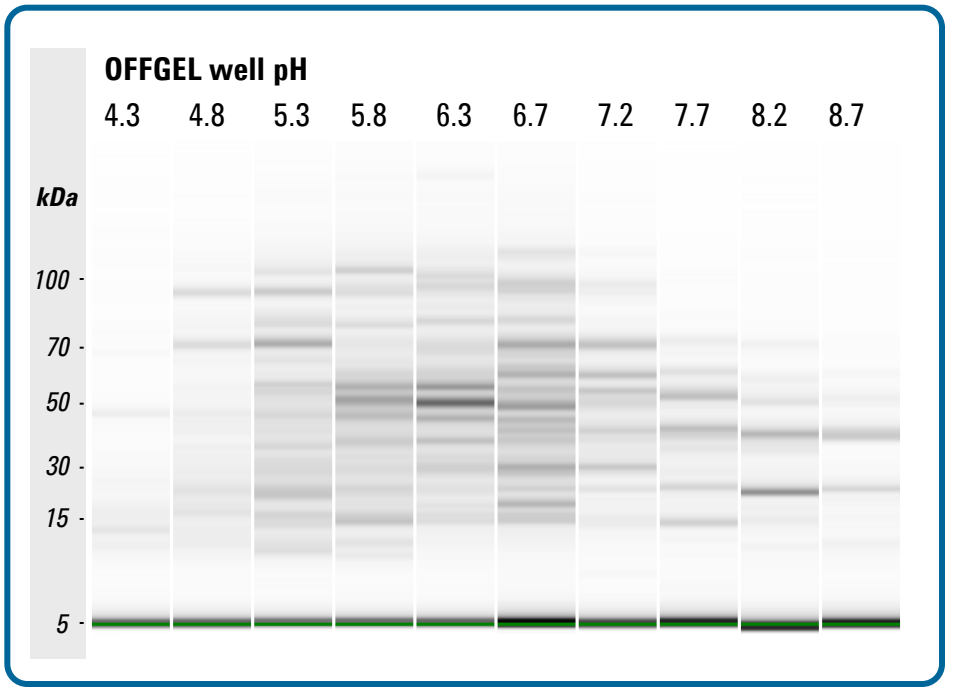
OFFGEL Electrophoresis



2100 Bioanalyzer
HiSens Assay

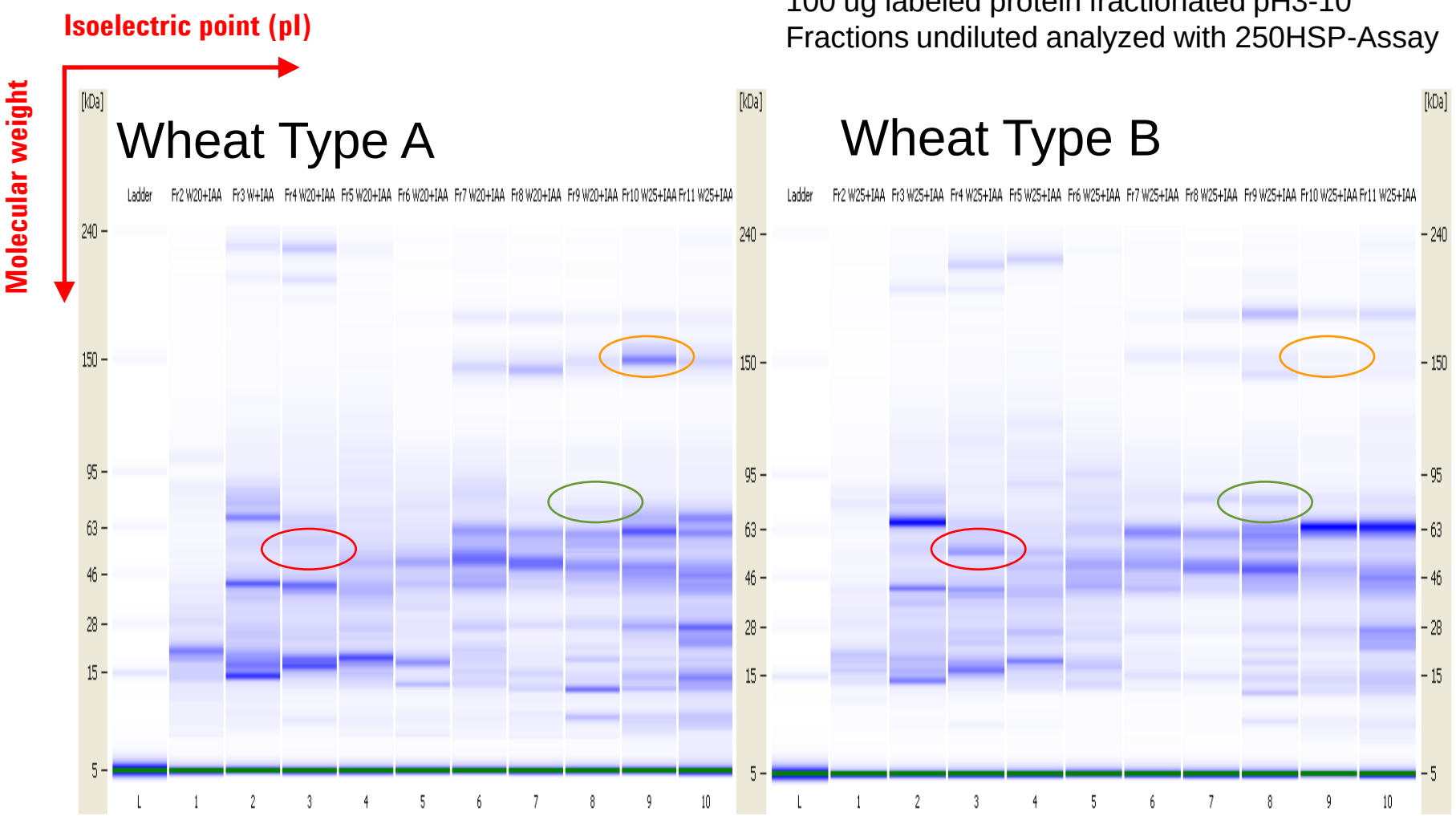
Isoelectric point (pI)

Molecular weight



Wheat Analysis

Protein extraction
Alkylation with IAA
Acetone precipitated
Labeling at 10 ug/ul total protein (Bradford)
100 ug labeled protein fractionated pH3-10
Fractions undiluted analyzed with 250HSP-Assay



Q & A

BioAnalyzer Support:

Bioanalyzer@agilent.com

Send .xad file not .pdf file