APLICACIONES

Revolucione el laboratorio con todas las posibilidades de su BioAnalizador ADN, ARN y proteínas

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Agilent Technologies

Agenda

Introducción al BioAnalizador

Aplicaciones para DNA

- Analisis de productos de PCR
- Aplicaciones en alimentación
- Aplicaciones en estudios clínicos
- NGS

Aplicaciones para ARN

+Algoritmo RIN

+Small RNA

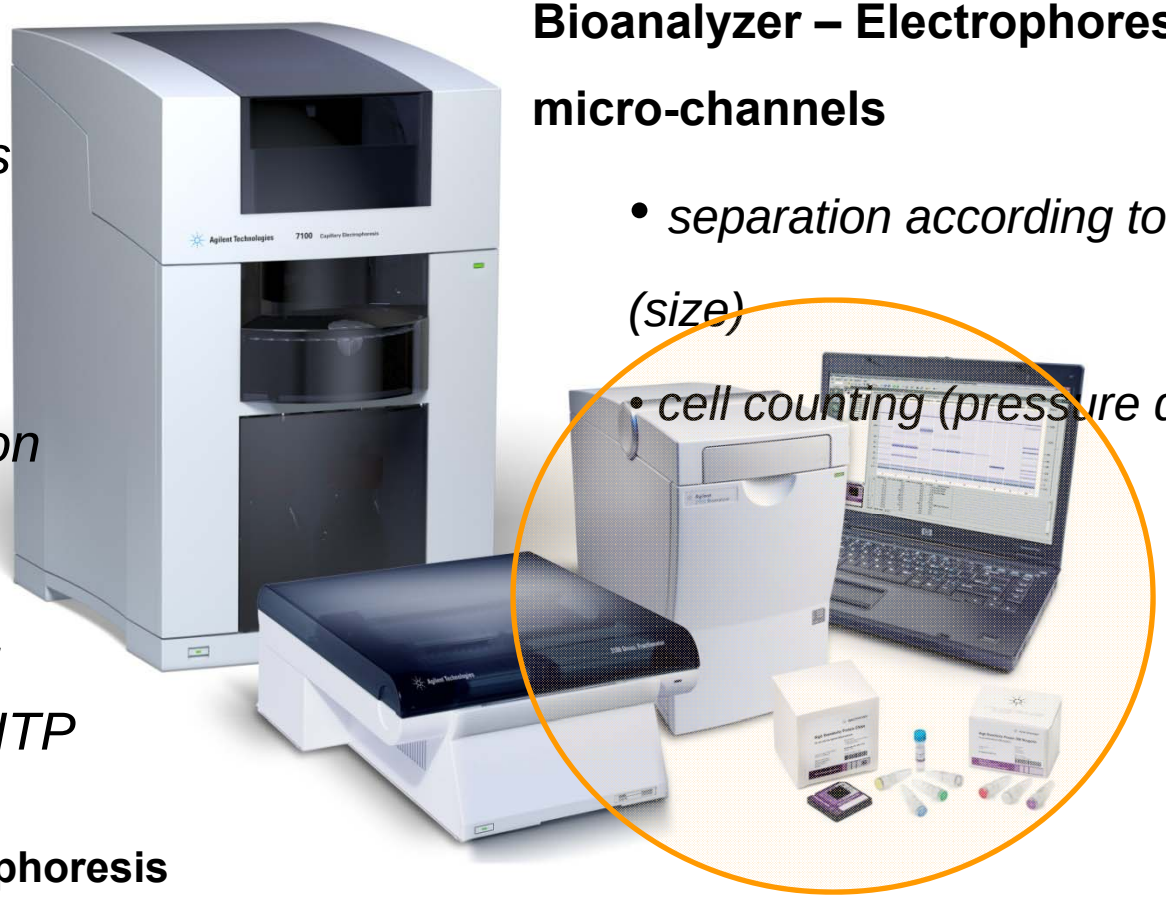
Aplicaciones para proteínas


WE MAKE IT. You make it happen.
OpenGenomics
Bioanalizador 2100
Una plataforma de posibilidades infinitas para el análisis de ADN, ARN, proteínas y células
Disfrute ya de nuestras promociones especiales*:
25% por la compra de un BioAnalizador 2100
25% en cualquier tipo de chips o reactivos para el análisis de ADN, ARN y proteínas
El Bioanalizador 2100 permite realizar automáticamente separaciones electroforéticas y análisis por fluorescencia de células mediante citometría de flujo.
Sus ventajas principales son:
• Kits de chips y reactivos listos para su utilización
• Consumo mínimo de muestra (1 a 4 µL) y resultados en 30 minutos
• Mejora de en la exactitud y fidelidad en las mediciones.
• Resultados comparables entre experimentos y laboratorios
• Datos digitales para la facilidad del análisis, presentación y archivo de datos
Para beneficiarse de estas ofertas, por favor, contacte con su representante local de Agilent y proporcione el código promocional S_1103_BIOA.
*hasta el 31 de marzo de 2011en Agilent Technologies
Agilent Technologies

Electrophoresis Product Portfolio

CE – Capillary Electrophoresis

- *various detectors*
  - *UV, LIF, MS, CCD*
- *various separation modi*
  - *CZE, MEKC, CGE, CiEF, CITP*



Bioanalyzer – Electrophoresis in micro-channels

- *separation according to mobility (size)*
- *cell counting (pressure driven)*

OGE – Offgel Electrophoresis

- *separation according to iso-electric point (charge)*

Overview Agilent Lab on a ChipTechnology

2100 Bioanalyzer Solutions

Agilent Technologies

El bioanalizador adaptable a cualquier tipo de ambiente de estudio

Field deployment for on-site detection of *B. pseudomallei* in environmental samples; analysis in a mobile lab

Bioanalyzer deployed at sea for fish species identification (NOAA)

Bioanalyzer used to test 250 samples in grain silos for GMOs (UK)

The Agilent 2100 bioanalyzer offers

Improved quality of *slab-gel-type analysis* through on-Chip electrophoresis…

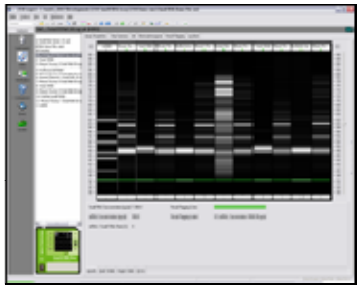
Easy to use analytical device that performs separation and quantitation of RNA, DNA and Proteins based on electrophoresis within a disposable glass chip.

…reducing the experiment to:

1. Load sample

2. Run analysis

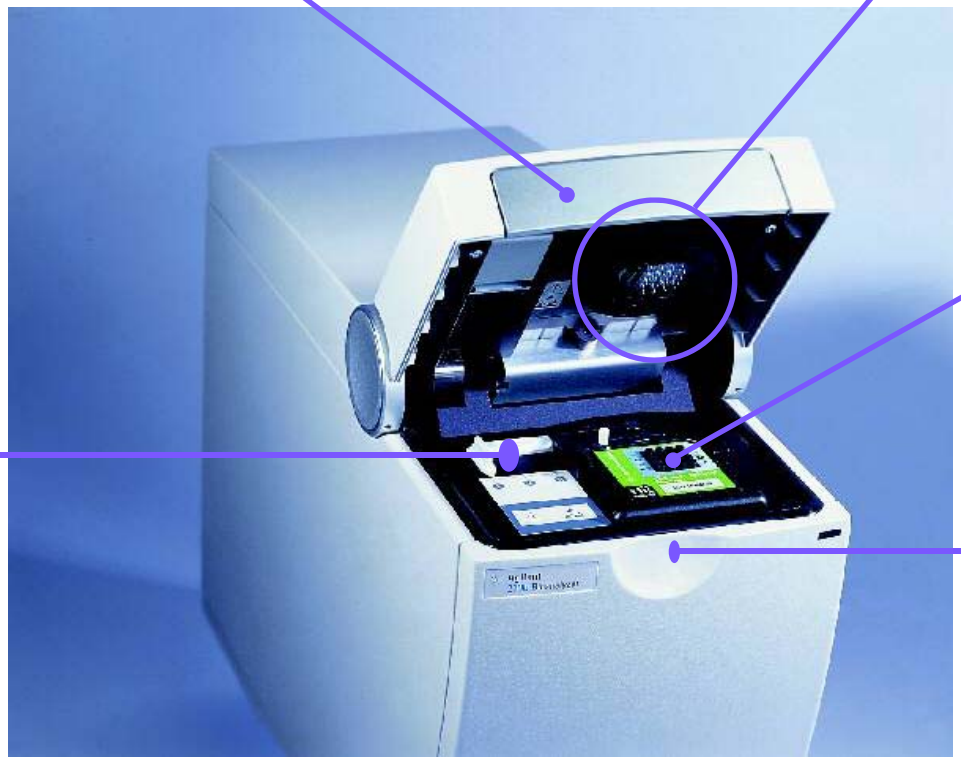
3. Analyze data



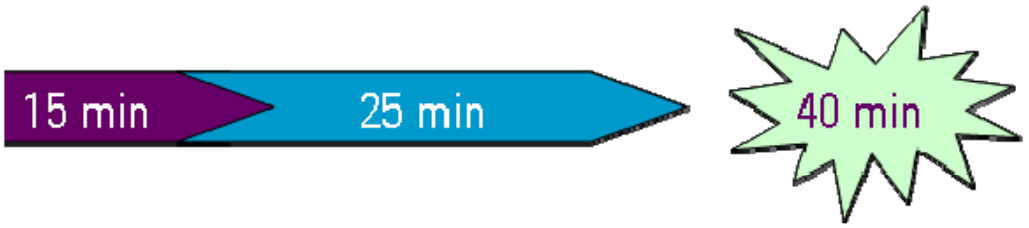
On a single Platform perform:

- **Sizing, Quantitation and Purity of Proteins (5 -230 kDa)**
- **Sizing, Quantitation and Purity of DNA fragments (25 – 12000 bp)**
- **Integrity check, Separation and Quantitation of RNAs**
- **Cell Fluorescence Assays with stained cells (Apoptosis, Transfection, Expression,)**

2100 Bioanalyzer Hardware

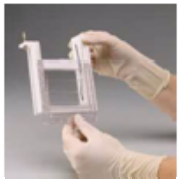


Lab-on-a-Chip or Gel - A Faster Answer !

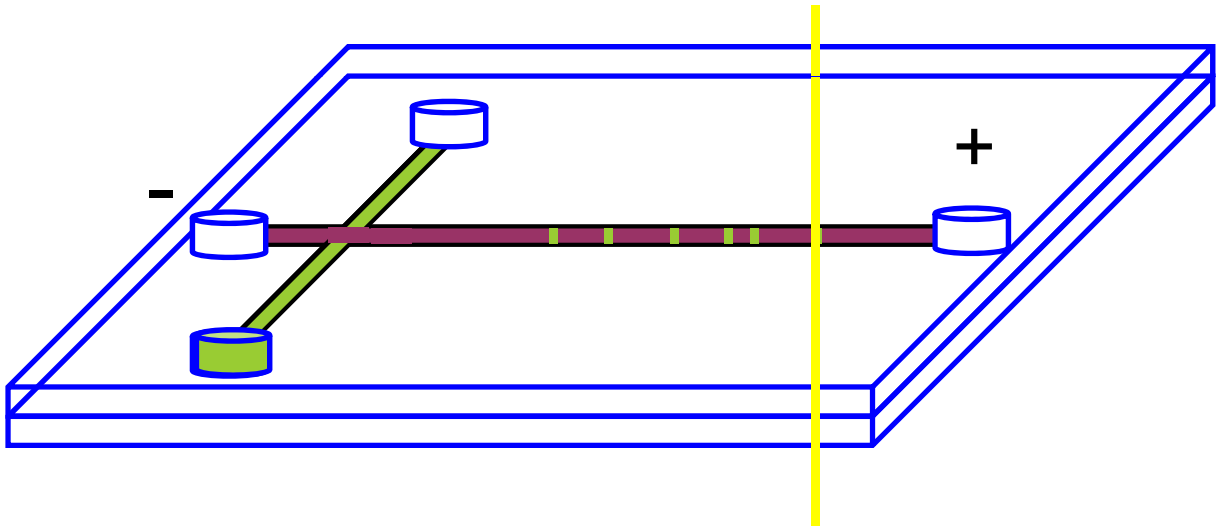


Prep Chip **Run Chip** **Analyzed - Archived data**

Prep Gel **Run Gel** **Stain / Destain** **Scan / Analyze**

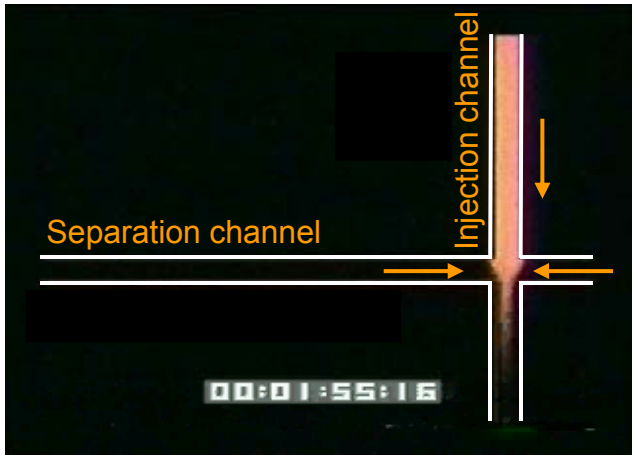


The LabChip® Approach - Simplified Model



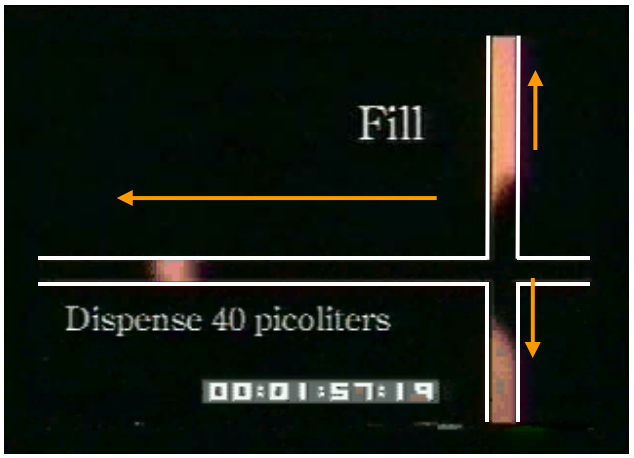
Lab-on-a-Chip - Principle of Injection & Separation

1

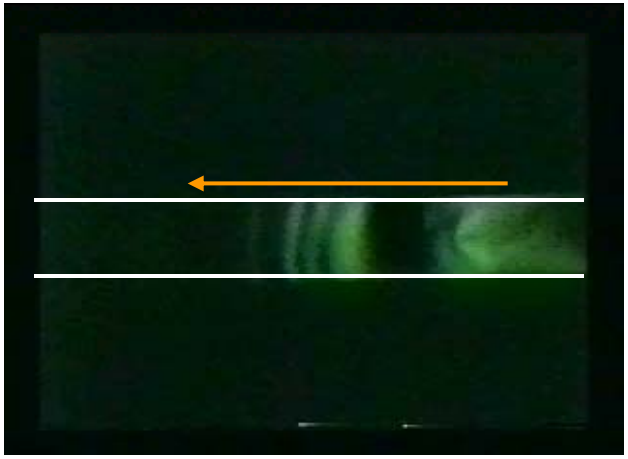


2

3

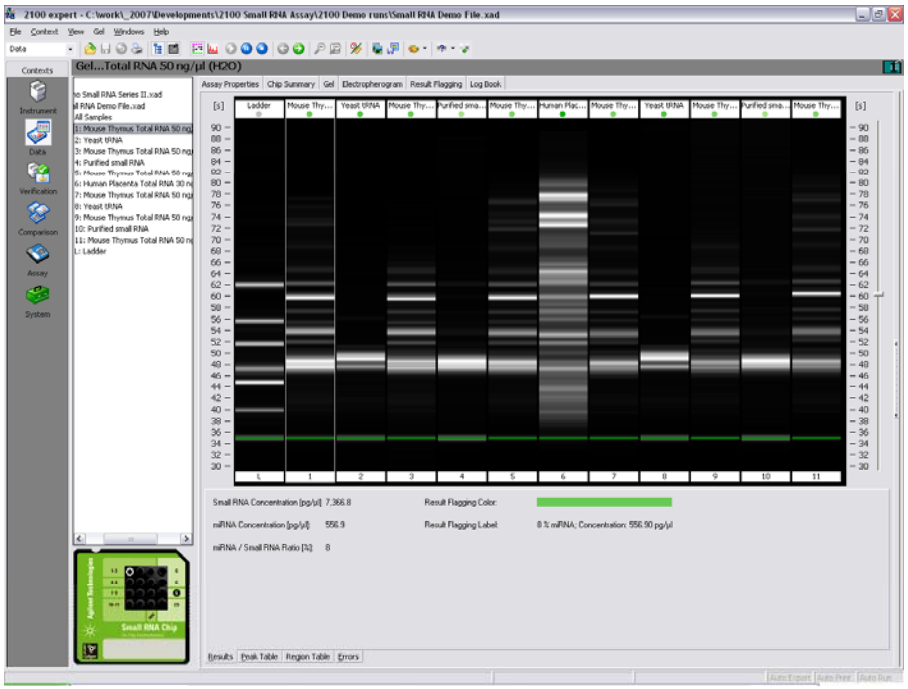


4



← Direction of electrodriven movement of liquids and molecules within liquids

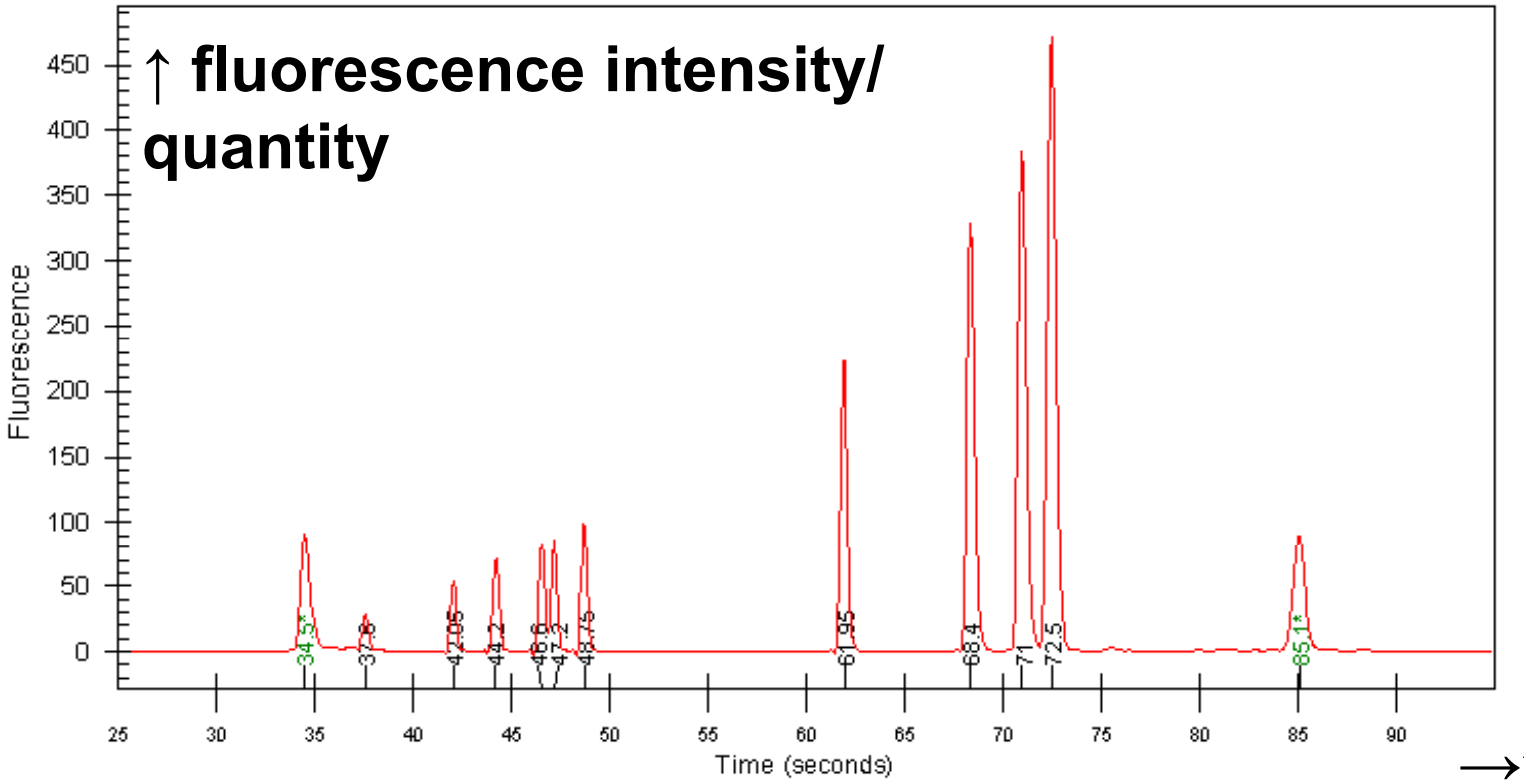
2100 expert SW
Version B.02.07



Third SW Generation

- Includes all the new Series II bioanalyzer kits
- Supporting new High Sens DNA and Protein
- Patended RIN (RNA Integrity Number)
- Color coded Result Flagging
- Allows compliance also on e-bioanalyzer (G2939AA)
- Improved comparison context (up to 48 samples per file)
- Improved manual integration
- Customizable result tables for printing and reports
- Not compatible with A-type Instruments

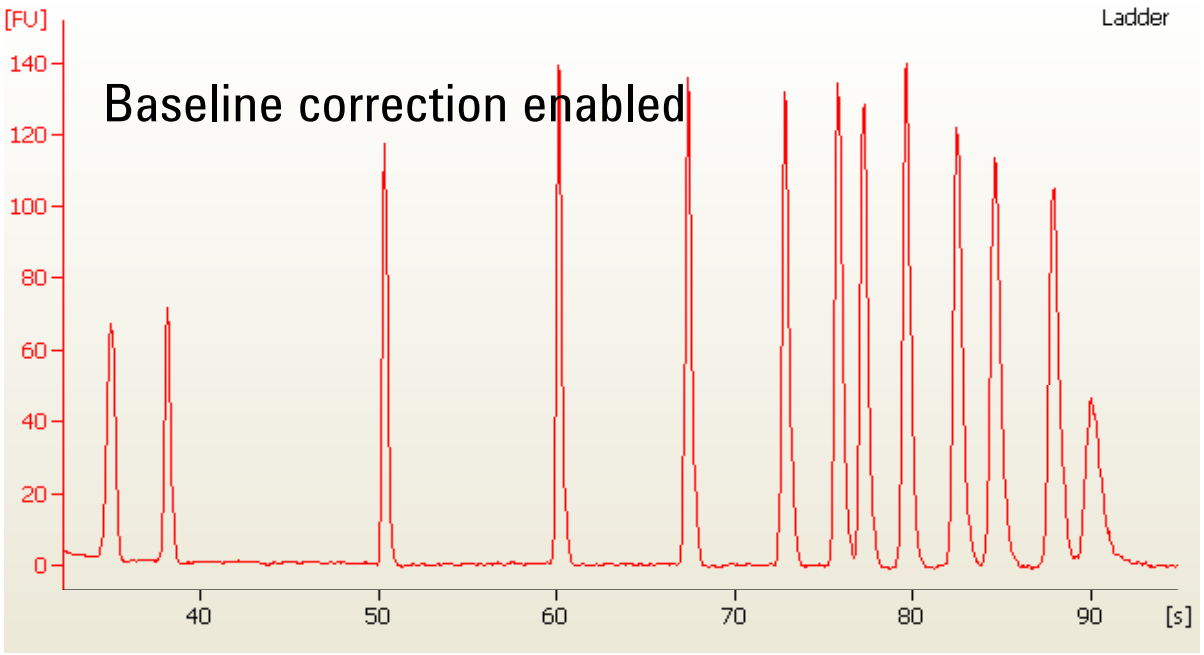
Gel view and electropherogram view



0. preparatory transformations

- **Noise reduction (Savitzky-Golay filtering**
- **Spike rejection**
- **Baseline correction**

Filter Width: (Secs)	1
Baseline Plateau: (Secs)	0.5
Polynomial Order:	6



1. Peak recognition

table of fluorescence intensities

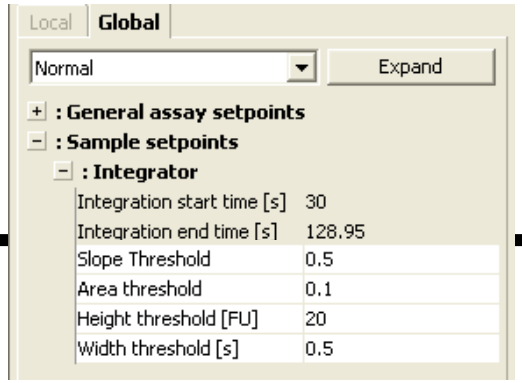
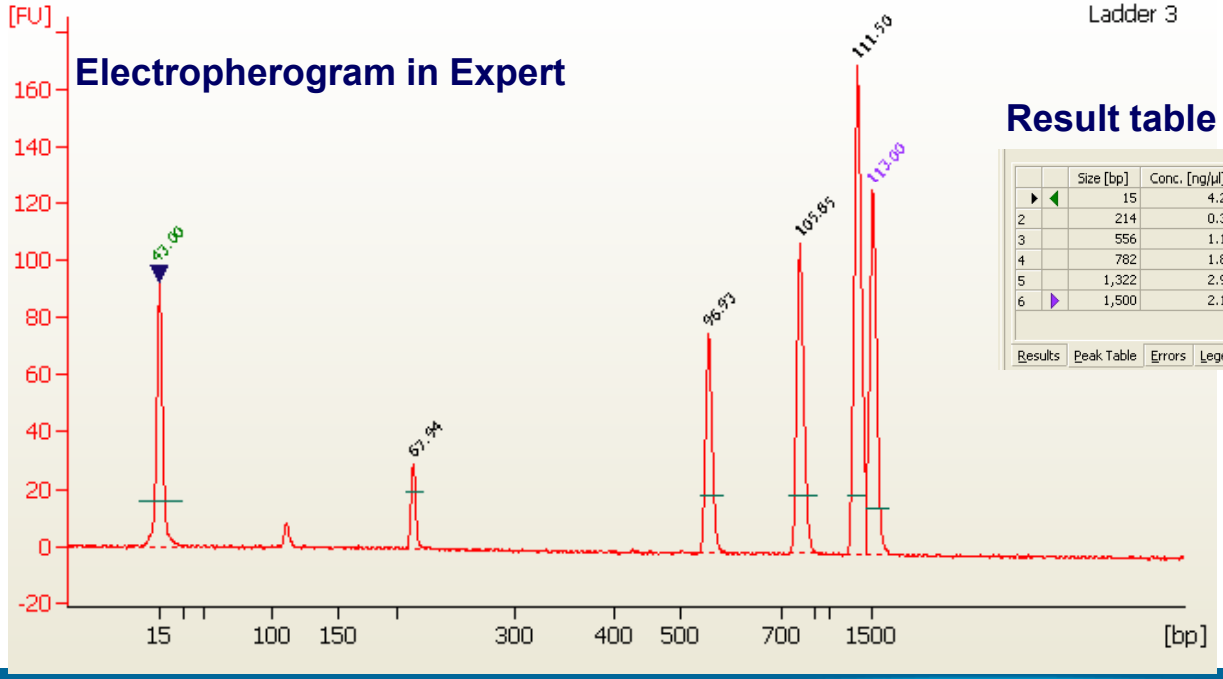


table of peaks

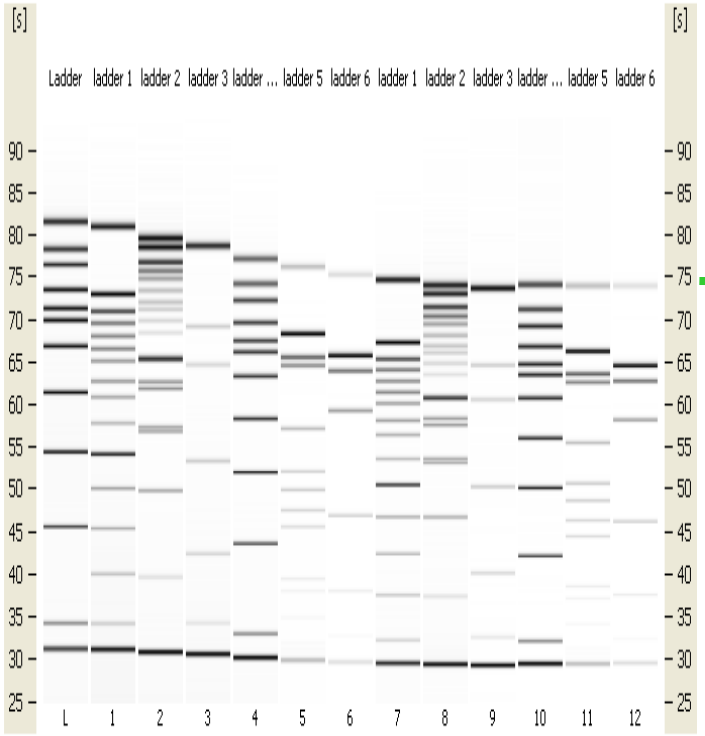


Result table in Expert

		Size [bp]	Conc. [ng/µl]	Molarity [nmol/l]	Observations
▶	◀	15	4.20	424.2	Lower Marker
2		214	0.34	2.4	
3		556	1.19	3.2	
4		782	1.84	3.6	
5		1,322	2.96	3.4	
6	▶	1,500	2.10	2.1	Upper Marker

Results | Peak Table | Errors | Legend

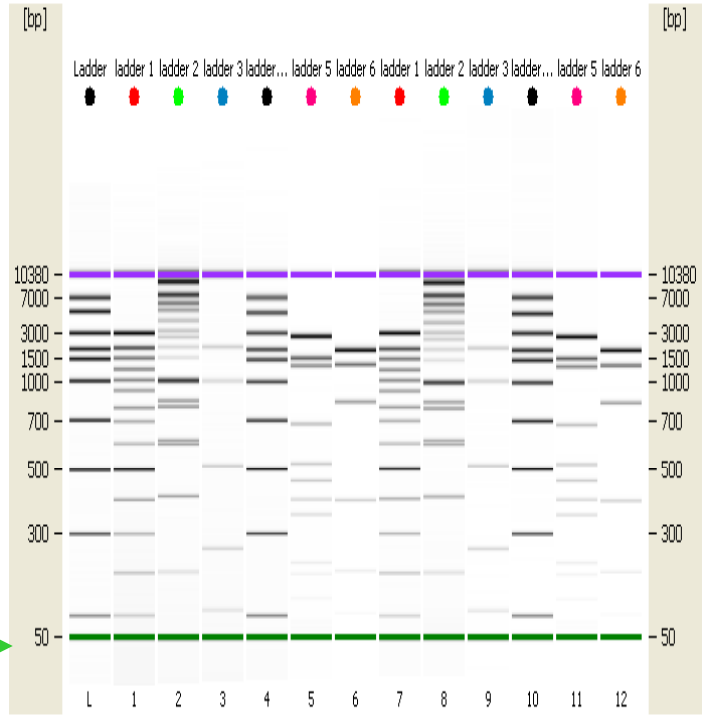
2. Alignment to markers



Analysis turned off –
Unaligned data

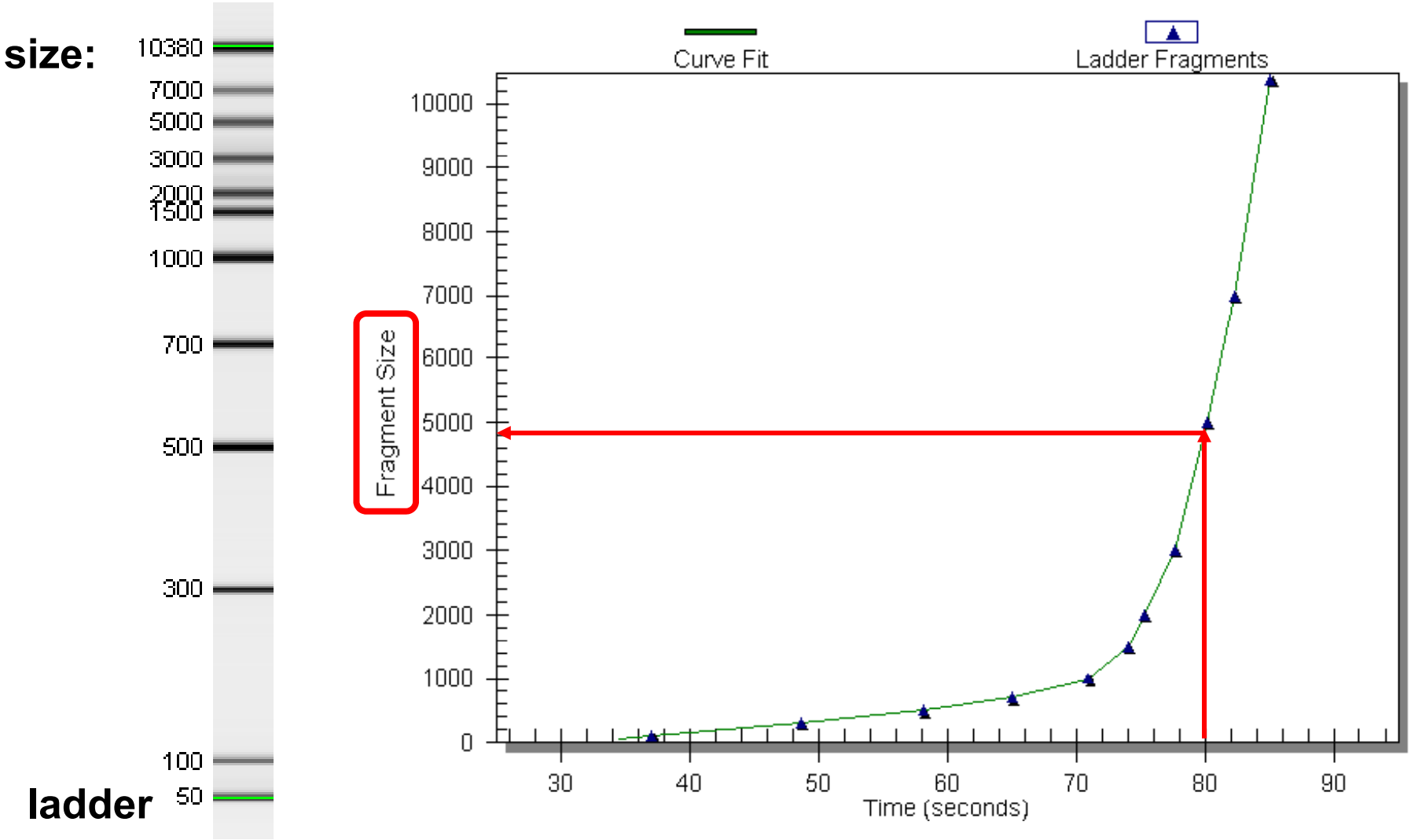
Upper marker

Lower marker



Analysis turned on –
Aligned data

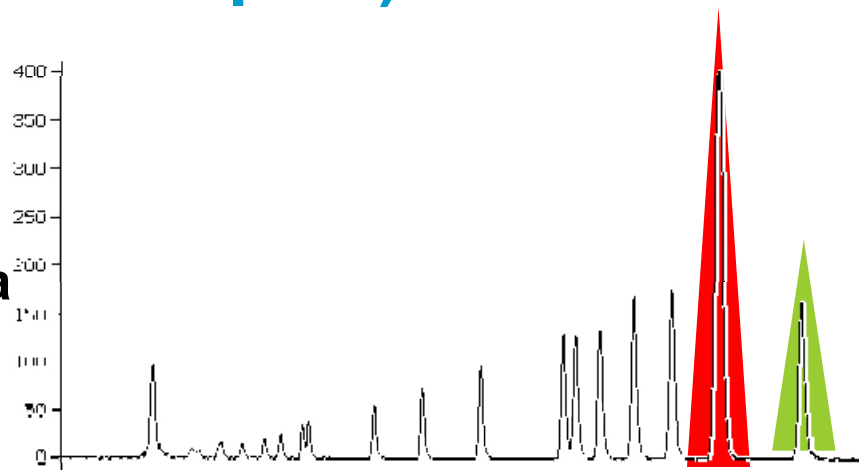
3. Sizing


size:
10380
7000
5000
3000
2000
1500
1000
700
500
300
100
50
ladder
Curve Fit
Ladder Fragments
Fragment Size
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
30
40
50
60
70
80
90
Time (seconds)

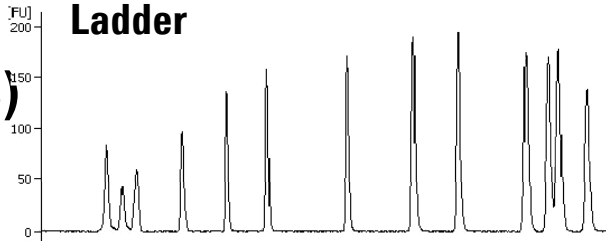
4. Quantification (DNA/Protein samples)

peak area

upper marker peak area (known concentration)



assay-specific calibration curve (standard areas, see assay properties)



concentration

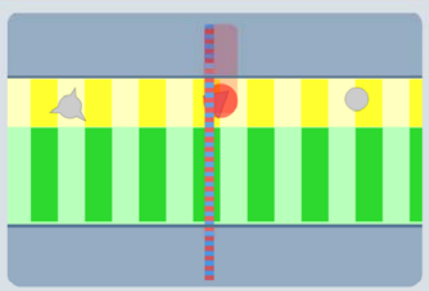
		Size [bp]	Aligned Migration Time [s]	Time corrected area	Conc. [ng/µl]	Molarity [nmol/l]	% of Total
1	◀	15	43.00	179.7	4.20	424.2	0.0
2		403	87.85	83.6	1.55	5.8	9.5
3		426	89.34	86.0	1.59	5.7	9.9
4		466	91.93	92.2	1.70	5.5	11.0
5		524	95.29	108.0	1.99	5.8	13.3
6		692	99.21	116.2	2.18	5.5	14.9
7		718	104.45	306.1	5.90	12.4	41.4
8	▶	1,500	113.00	109.7	2.10	2.1	0.0

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)

Flow Cytometry

Standard Flow Cytometers have 3-4 fluorescence detection channels:

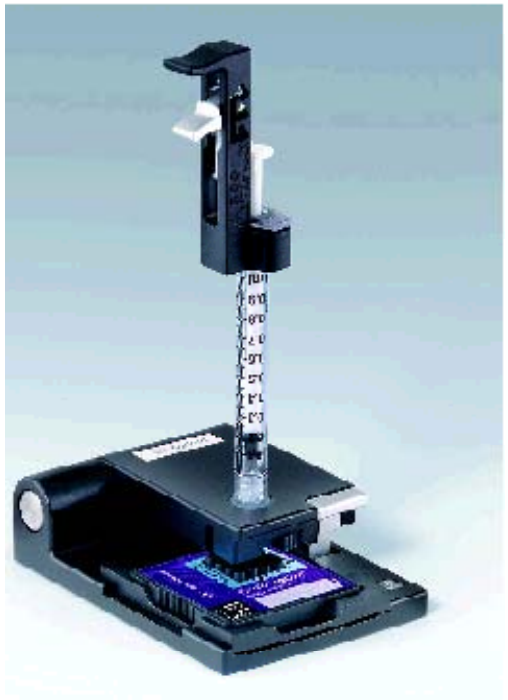
FL1: excitation 488 nm, detection 530 nm
FL2: excitation 488 nm, detection 585 nm
FL3: excitation 488 nm, detection 661 nm
FL4: excitation 635 nm, detection 670 nm

2100 Bioanalyzer LabChip Kits

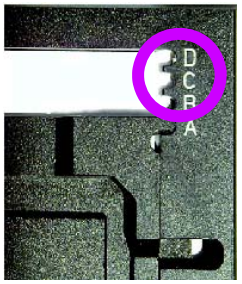
What you need to know

- Chips are disposable, they are used once and then thrown away
- Even if all wells on a chip are not been used the remaining wells cannot be used at a later time
- Some of the reagents in the LabChip kits are thermally unstable at room temperature so the kits have to be shipped cooled (on ice) and stored in a refrigerator
- The LabChip kits have a limited shelf life
  - Shelf life is 12 months from manufacture date
  - We guarantee a minimum of 4 months shelf-life when received by the customer
- Reagent kits (cooled) are available for customers who lose/waste their reagents or have reagents that exceeded their shelf-life

Chip Priming

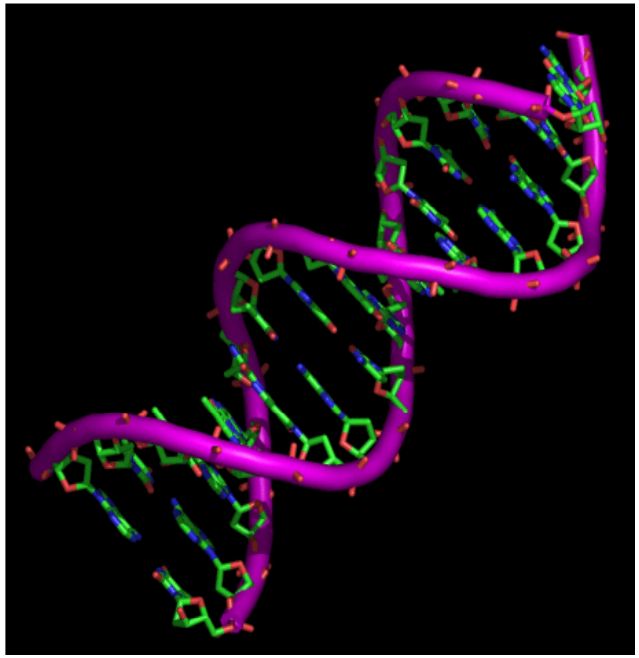


Set chip priming station base plate to C



Set metal clip to top (DNA 7500/12000)
OR
lowest (DNA 1000) position

30 sec pressurization (DNA 7500/12000)
OR
60 sec pressurization (DNA 1000)



APLICACIONES EN ADN

Aplicaciones plataforma DNA

Pureza de los productos de PCR

Amplificaciones multiples por PCR

Gene expression análisis via RT-PCR (validación target)

GMO testing

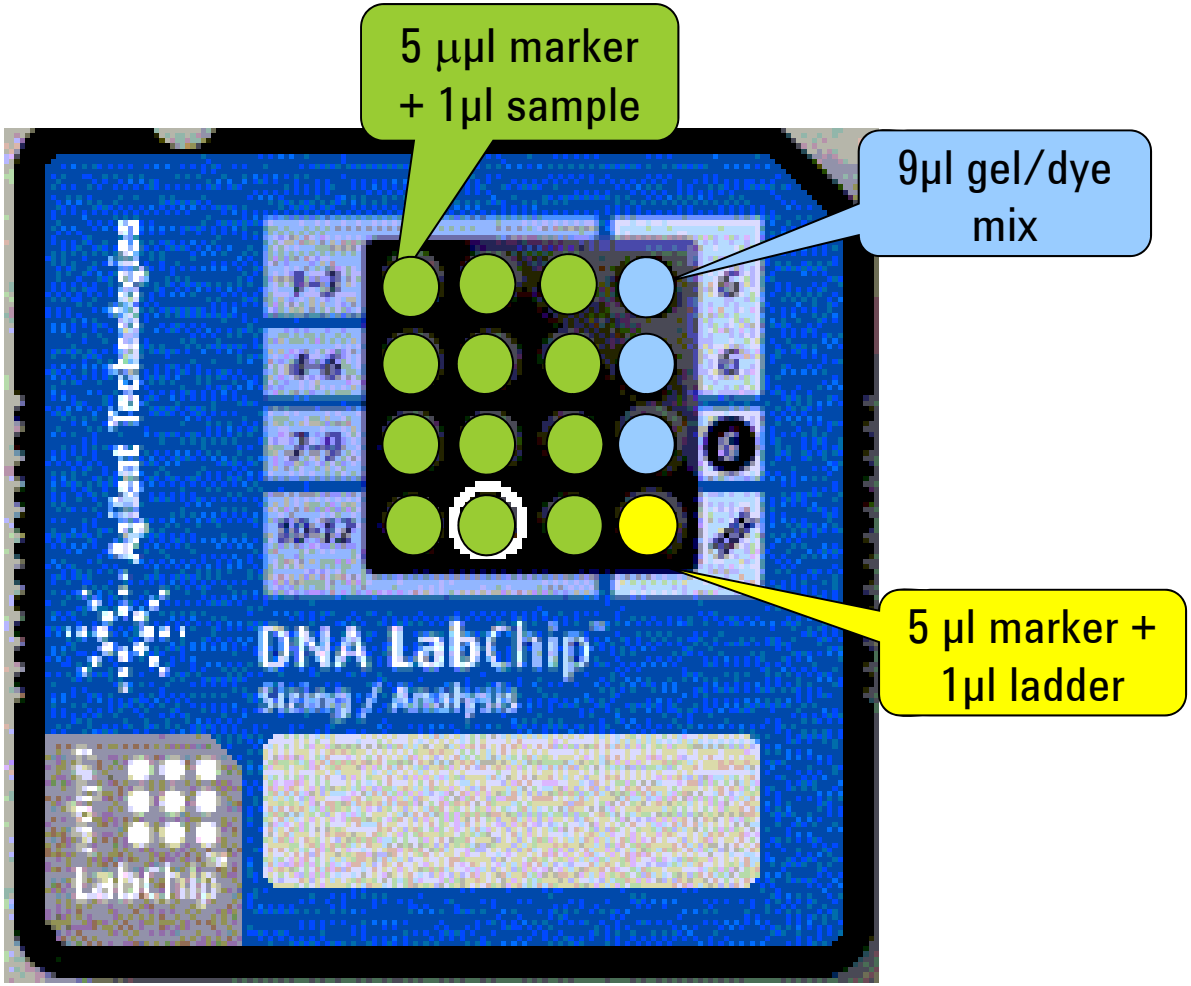
Detección de Patogenos (Veterinaria, hospitales, medio ambiente)

Aplicaciones en Genotipado

- **Duplicaciones/ delecciones**
- **Frequencia alélica**
- **Sub-tipado de bacterias**
- **Forense**

Cancer diagnóstico

Chip Preparation



DNA Analysis Kits and Reagents

Order No.	Description
5067-1504	DNA 1000 Kit
5067-1505	DNA 1000 Reagents

Order No.	Description
5067-1506	DNA 7500 Kit
5067-1507	DNA 7500 Reagents

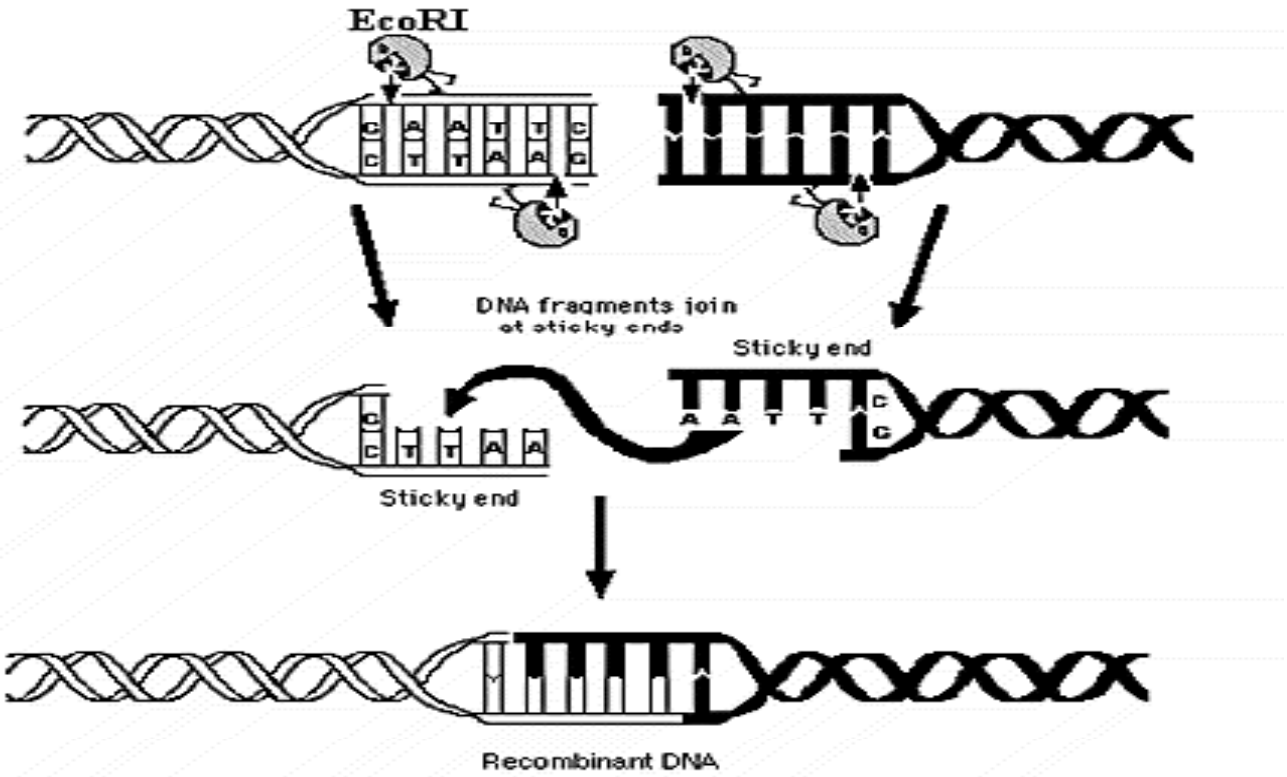
Order No.	Description
5067-1508	DNA 12000 Kit
5067-1509	DNA 12000 Reagents

Order No.	Description
5067-4626	High Sensitivity DNA Kit
5067-4627	High Sensitivity DNA Reagents

Analytical Specifications	DNA 1000 Assay	DNA 7500 Assay	DNA 12000 Assay	High Sensitivity DNA Assay
Sizing range	25–1000 bp	100–7500 bp	100–12000 bp	50-7000 bp
Sizing resolution	25–100 bp: 5 bp 100–500 bp: 5 % 500–1000 bp: 10 %	100–1000 bp: 5 % 1000–7500 bp: 15 %	100–1000 bp: 5 % 1000–12000 bp: 10 %	50-600 bp: ±10 % 600-7000 bp: ±20 %
Sizing accuracy	± 10 % CV*	± 10 % CV*	± 15 % CV*	± 10 % CV*
Sizing reproducibility	5 % CV*	5 % CV*	5 % CV*	5 % CV*
Quantitation accuracy	20 % CV*	20 % CV*	25 % CV*	20 % 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*	50-2000 bp: 15 % CV* 2000-7000 bp: 10 % CV*
Qualitative range	0.1–50 ng/µL*	0.1–50 ng/µL*	0.1–50 ng/µL*	5-500 pg/µL*
Maximum salt concentration in sample	250 mM for KCl 250 mM for NaCl 15 mM for $MgCl_2$	250 mM for KCl 250 mM for NaCl 15 mM for $MgCl_2$	250 mM for KCl 250 mM for NaCl 15 mM for $MgCl_2$	10 mM Tris and 1 mM EDTA
Physical Specifications				
Analysis time	35 minutes	30 minutes	30 minutes	45 minutes
Samples per chip	12	12	12	11
Sample volume	1 µL	1 µL	1 µL	1 µL
Kit stability	4 months	4 months	4 months	4 months
Kit size	300 samples/kit	300 samples/kit	300 samples/kit	110 samples/kit

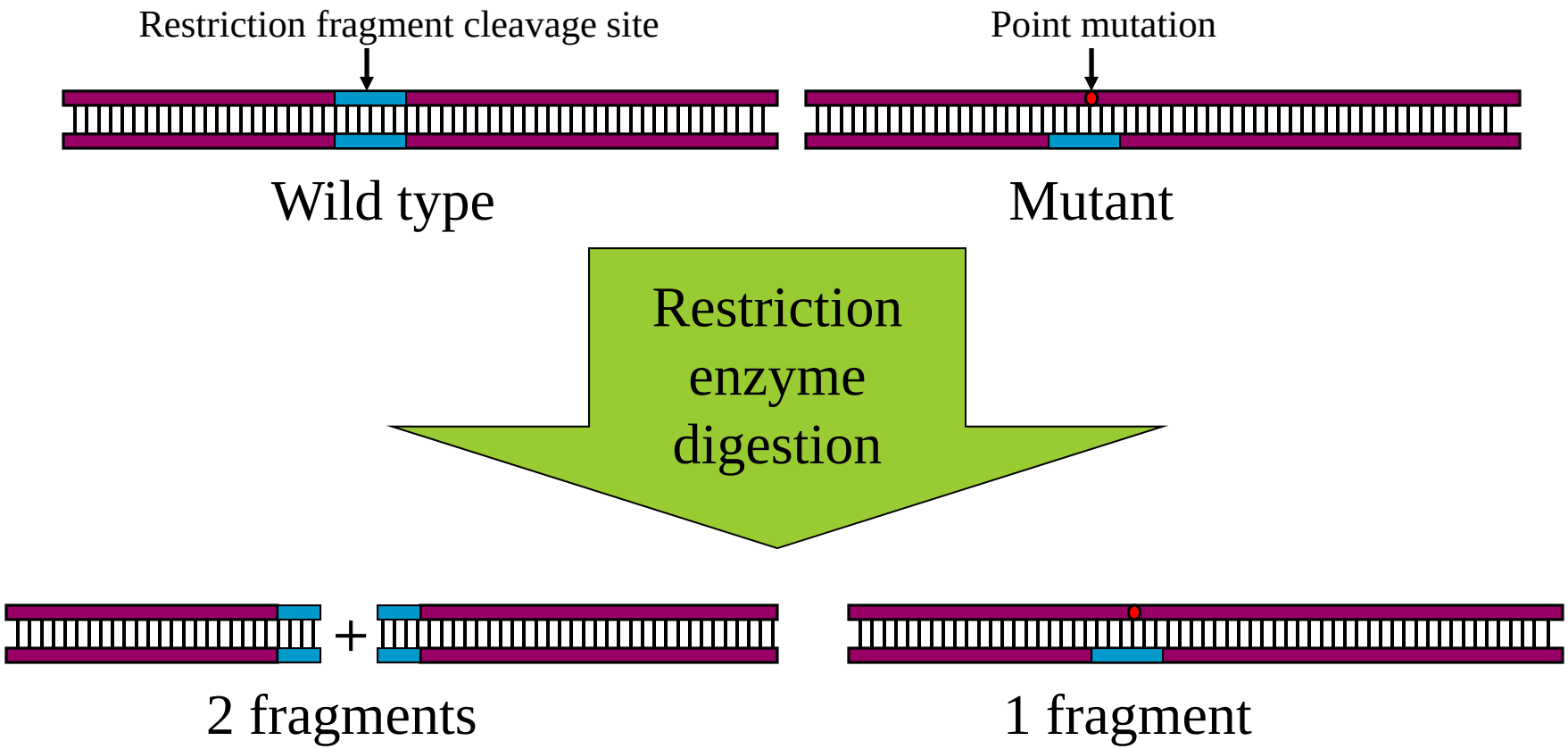
* Determined analyzing the DNA ladder as sample

Restriction Digests



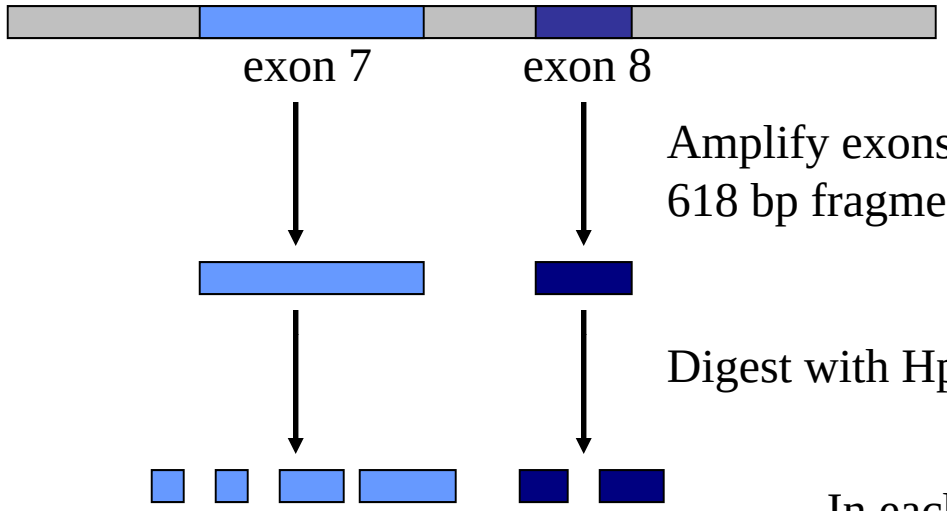
Restriction Enzyme
Action of EcoRI

Mutation Detection by RFLP



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)

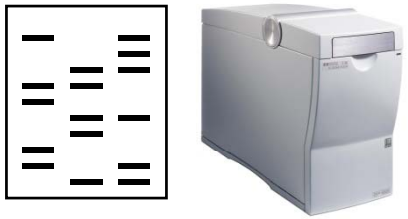
Digest with Hpa II

83 bp, 91 bp, 168 bp, 276 bp

91 bp, 109 bp

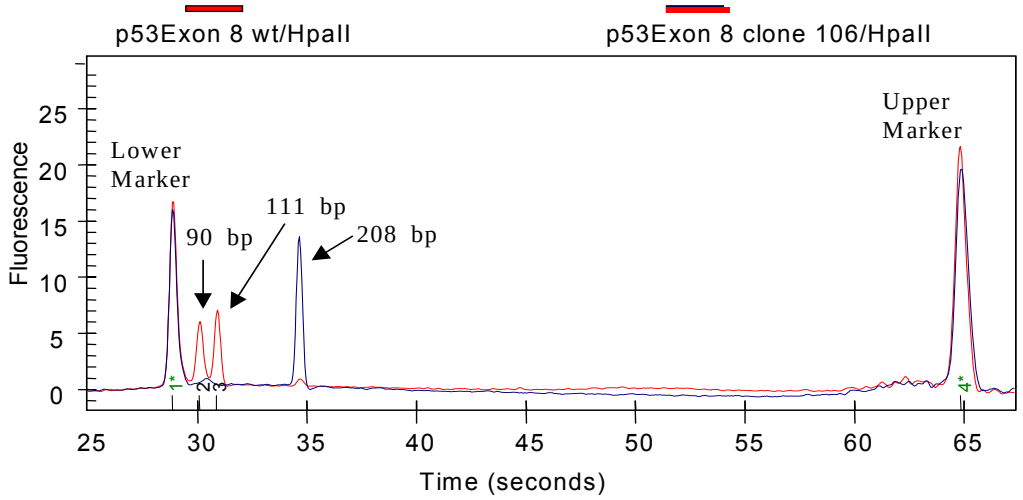
In each example one of the restriction sites can be deleted by a point mutation

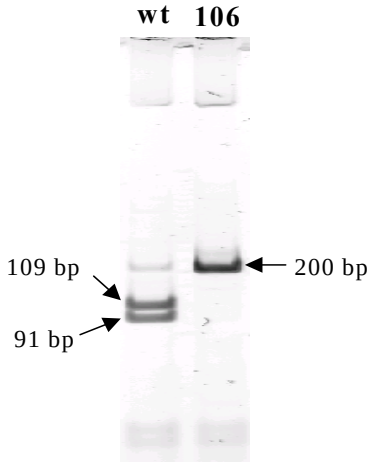
Analyze using Agilent 2100 bioanalyzer and 4-20 % acrylamide gel

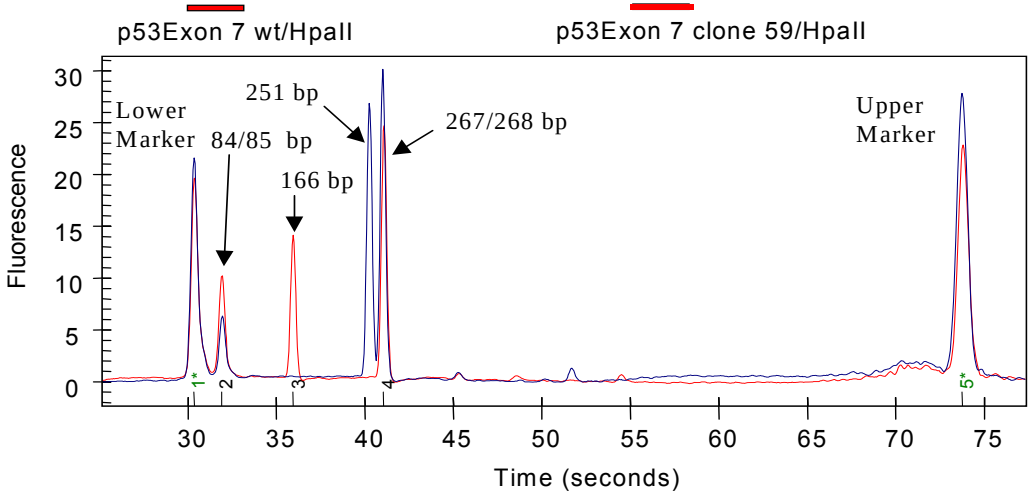


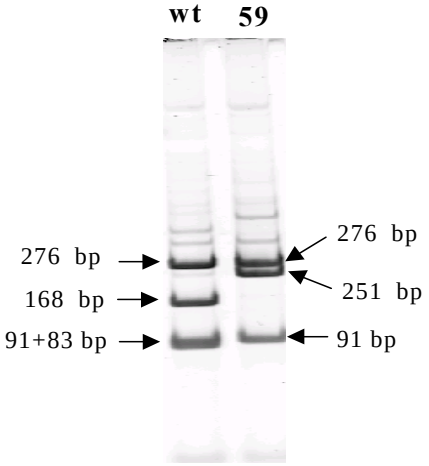
DNA 7500

Detection of Single Base Mutations (2)





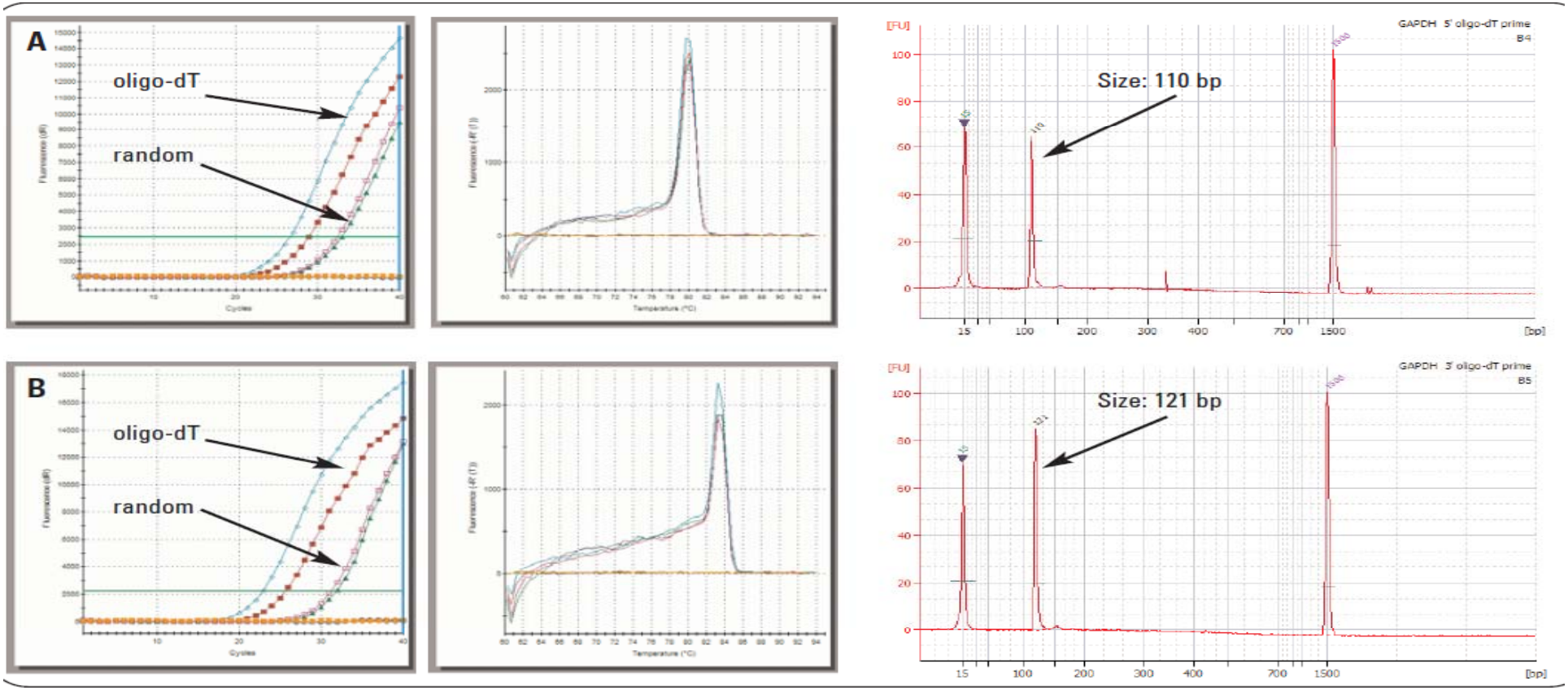




DNA 7500

Agilent Technologies

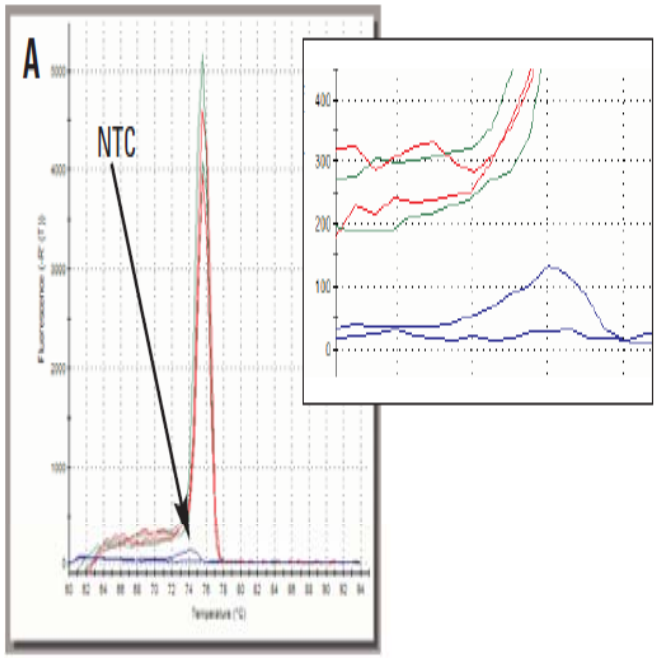
Optimize QPCR assay design

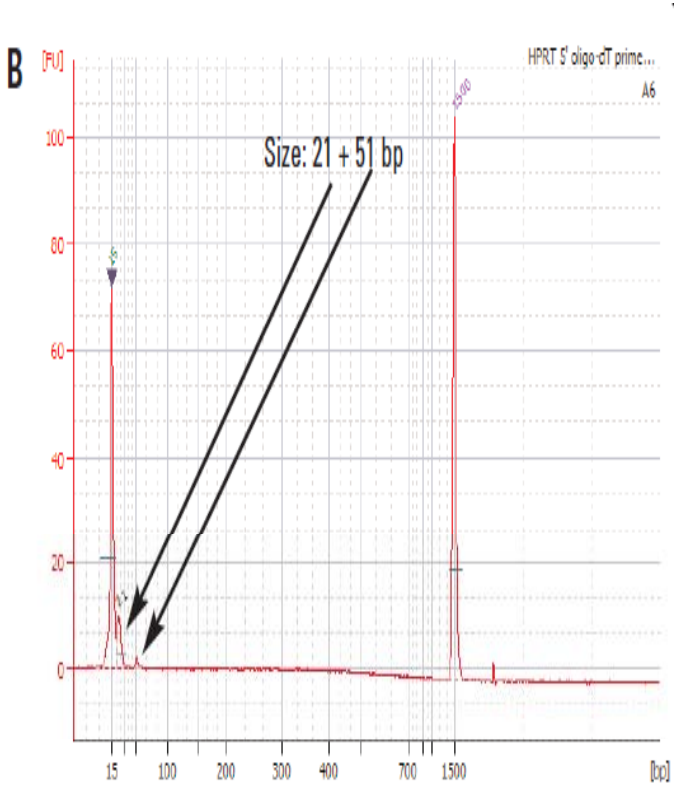


Assay Validation: Amplification plots, melt curve and bioanalyzer analysis of amplicons for GAPDH. A) 5'-assay, B) 3'-assay.

Optimize QPCR assay design

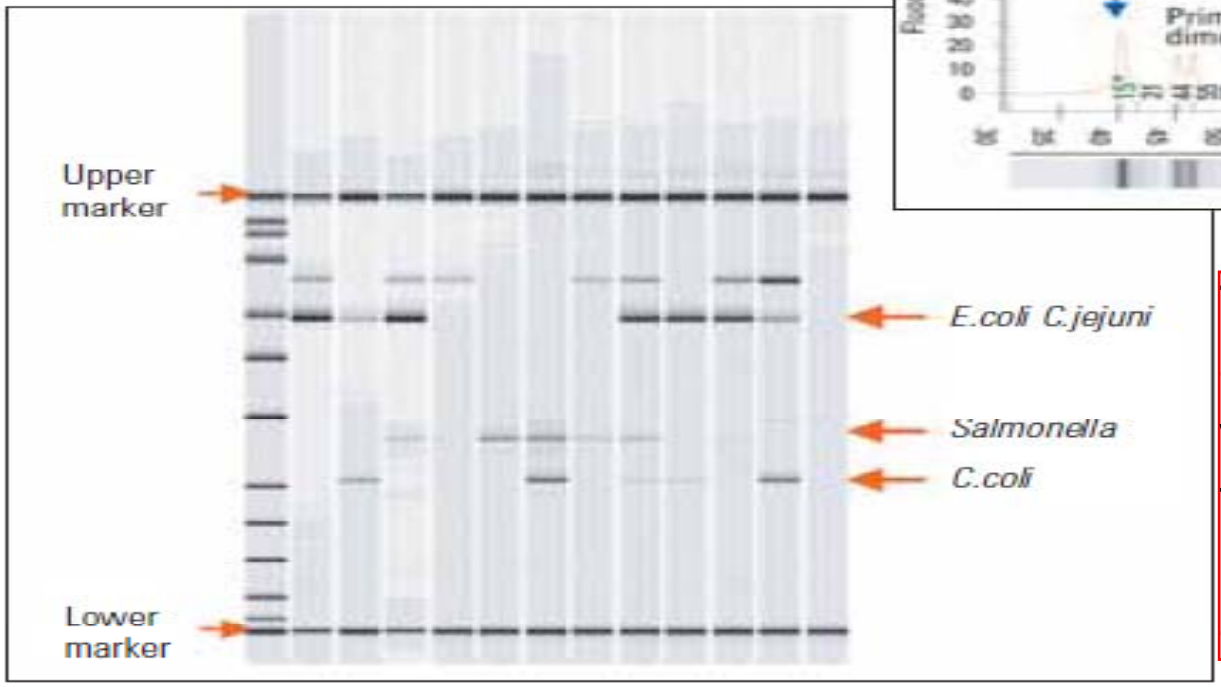
This highlights the high information content obtained by the 2100 Bioanalyzer and the poor discrimination capabilities of a SYBR Green based melt curve.

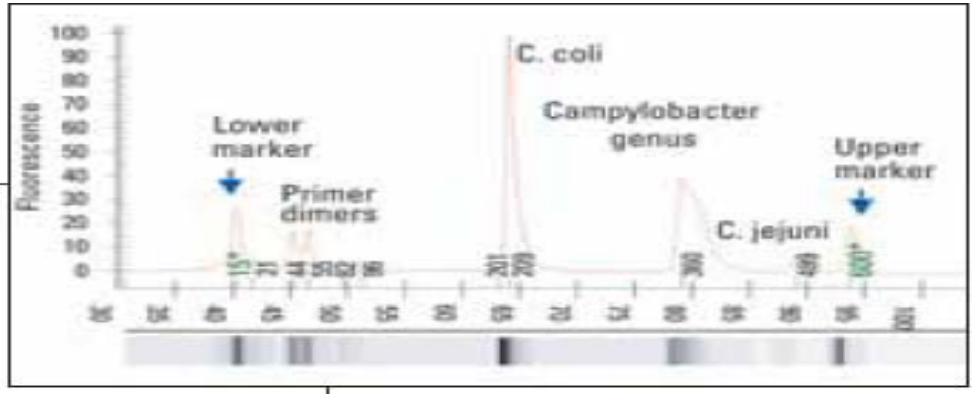




Validation of positive NTC results. Melt curve and bioanalyzer analysis of HPRT1 5'-amplicons. Enlargement of melt curve clearly shows a product in the NTC control with similar Tm to the positive control. Bioanalyzer analysis shows two minor peaks of 21 and 51 bp pointing to primer dimer formation.

Multiplex PCR analysis of bacteria in chicken

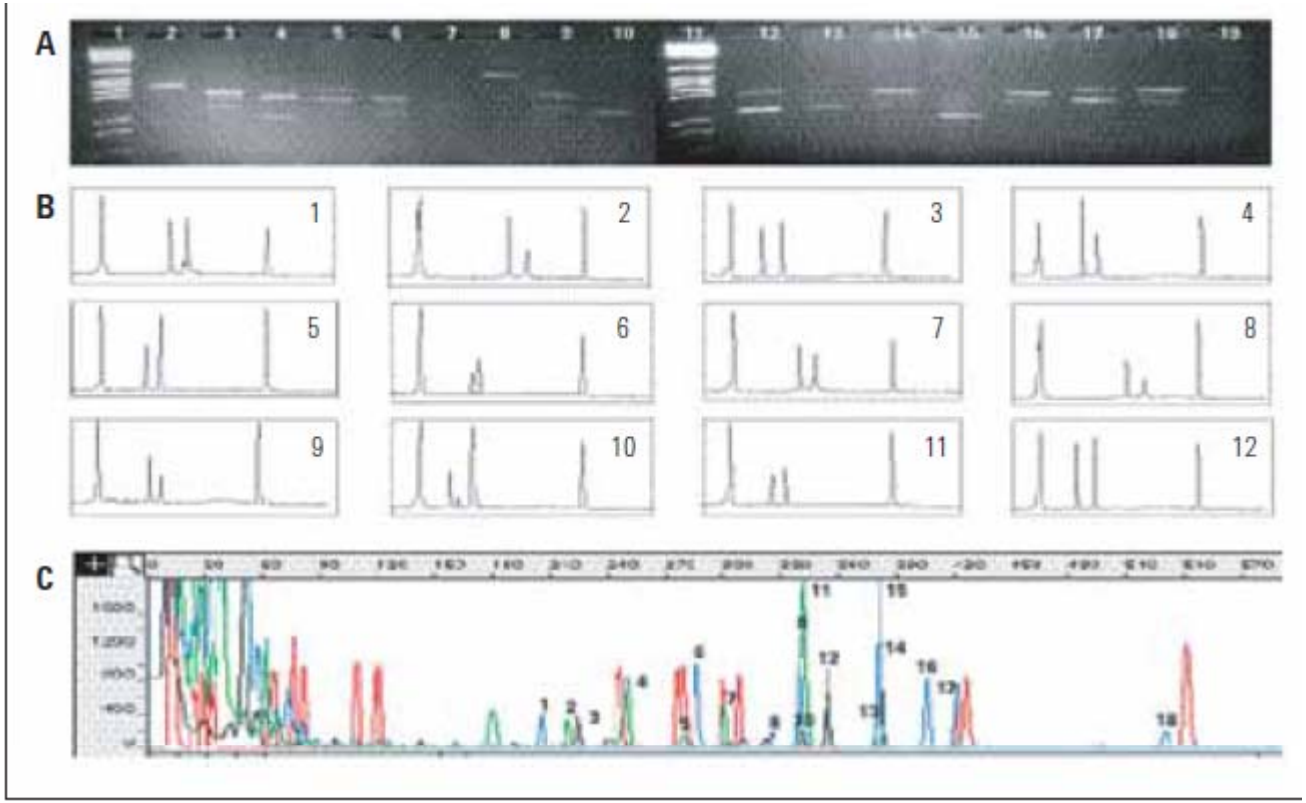




The electropherogram is one example where bacterial DNA from two species of the *Campylobacter genus could be detected.*

Standardized end-point RT-PCR

The direct comparison of the reproducibility for agarose gel analysis (A, CV = 0.50) and the ABI Prism310 Genetic Analyzer (C, CV = 0.39) with the 2100 Bioanalyzer (B, CV = 0.29) reveals that the 2100 Bioanalyzer is superior.



Complementary DNA from bronchial epithelial cells (BEC) was analyzed by a Standardized RT-PCR (StaRT) for the expression of 15 different genes.

Agilent Technologies
DNA Fish ID Solution

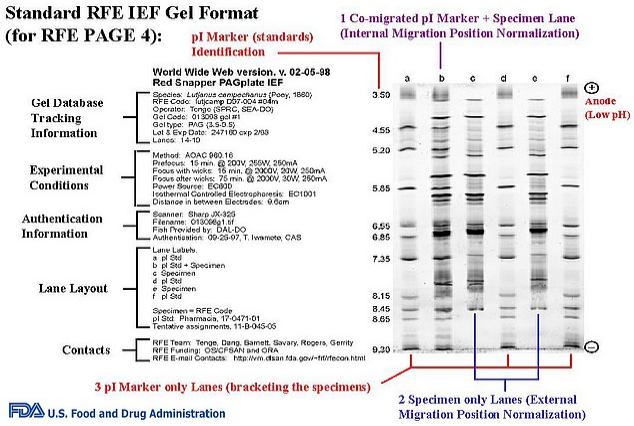
Métodos de identificación de pescados - Tradicional

- Requiere expertos para la identificación
- Solamente aplicable a pescado total

Morfologías

- El Procesado puede destruir proteinas.
- Polimorfismo limitado entre especies
- Dificultad de interpretación
- Influenciado por el medio ambiente
- Normalmente requiere correr muestrar autentificadas en paralelo

Métodos basados en proteínas

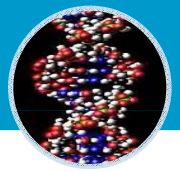


Regulatory Fish Encyclopedia:
U.S. Food and Drug Administration, 1993-2009

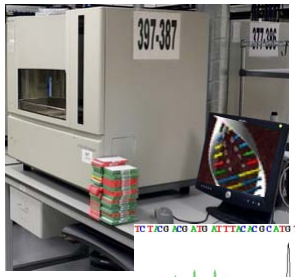
Métodos de identificación de pescados – DNA

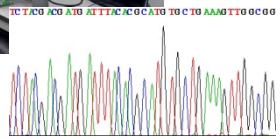
- Resultados objetivos
- Más resistentes al procesado de muestras
- Fácil de utilizar
- Alta especificidad/sensibilidad

DNA métodos



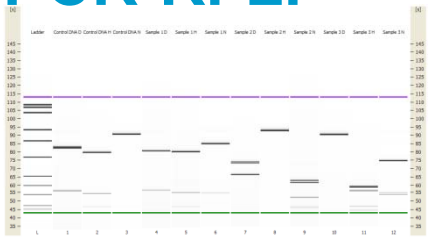
Secuenciación





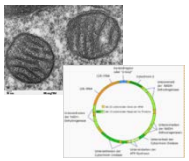
⇨ Alta inversión incial (> 100K $)
⇨ Alto coste de mantenimiento
⇨ No aplicable con muestras mezcladas
⇨ Métodos de elección para nuevas especies.

PCR-RFLP



⇨ Discriminación entre especies sumamente relacionadas
⇨ Trabaja con mezclas
⇨ Bajo coste inicial

Método de identificación de pescados– PCR RFLP



- ⇨ Mitocondrias: Alto número de copias
- ⇨ Comunmente empleado el gen Cytochrome B (CytB) o Cytochrome Oxidase I (COX1)
- ⇨ La secuencia del CytB aporta información para especies de pescados.

Extraer el ADN

Amplificar la región de interés en ADN

Fragmentar el ADN para generar los modelos especificos

PCR RFLP

⇨ La PCR RFLP es un metódo acreditado/aprobado en muchos países.

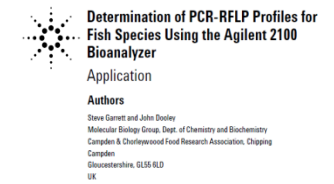
⇨ Transferencia del método a 2100 Bioanalyzer para mejorar el análisis edición 2004 y 2005 por Stephen Garrett de Campden BRI (Reino Unido)

Food Control **16** *(7),* 601-607, (2004)

Improved fish species identification by use of lab-on-a-chip technology

John J. Dooley, Helen D. Sage, Helen M. Brown and Stephen D. Garrett

Biochemistry Section, Department of Chemistry and Biochemistry, Campden and Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK



Determination of PCR-RFLP Profiles for Fish Species Using the Agilent 2100 Bioanalyzer

Application

Authors

Steve Garrett and John Dooley

Molecular Biology Group, Dept. of Chemistry and Biochemistry

Campden & Chorleywood Food Research Association, Chipping Campden

Gloucestershire, GL55 6LD

UK

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fish Species Identification Using PCR–RFLP Analysis and Lab-on-a-Chip Capillary Electrophoresis: Application to Detect White Fish Species in Food Products and an Interlaboratory Study

John J. Dooley, Helen D. Sage, Marie-Anne L. Clarke, Helen M. Brown, and Stephen D. Garrett*

Biochemistry Section, Department of Chemistry and Biochemistry, Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire, GL55 6LD, UK

J Agric Food Chem **53** *(9),* 3388-3357 (2005)

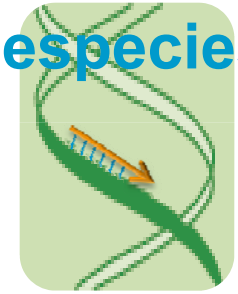
http://www.chem.agilent.com/Library/applications/5989-2982EN.pdf

Agilent DNA Fish ID Solution -

Purificación de la muestra

< 1 h
(dependiendo # muestras)

Amplificación de secuencias de identificación de especies



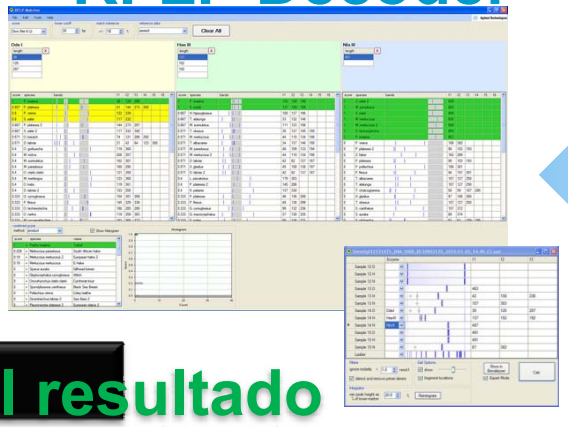
~ 1.5 h

Generación de modelos de especies específicos

~2.5 h

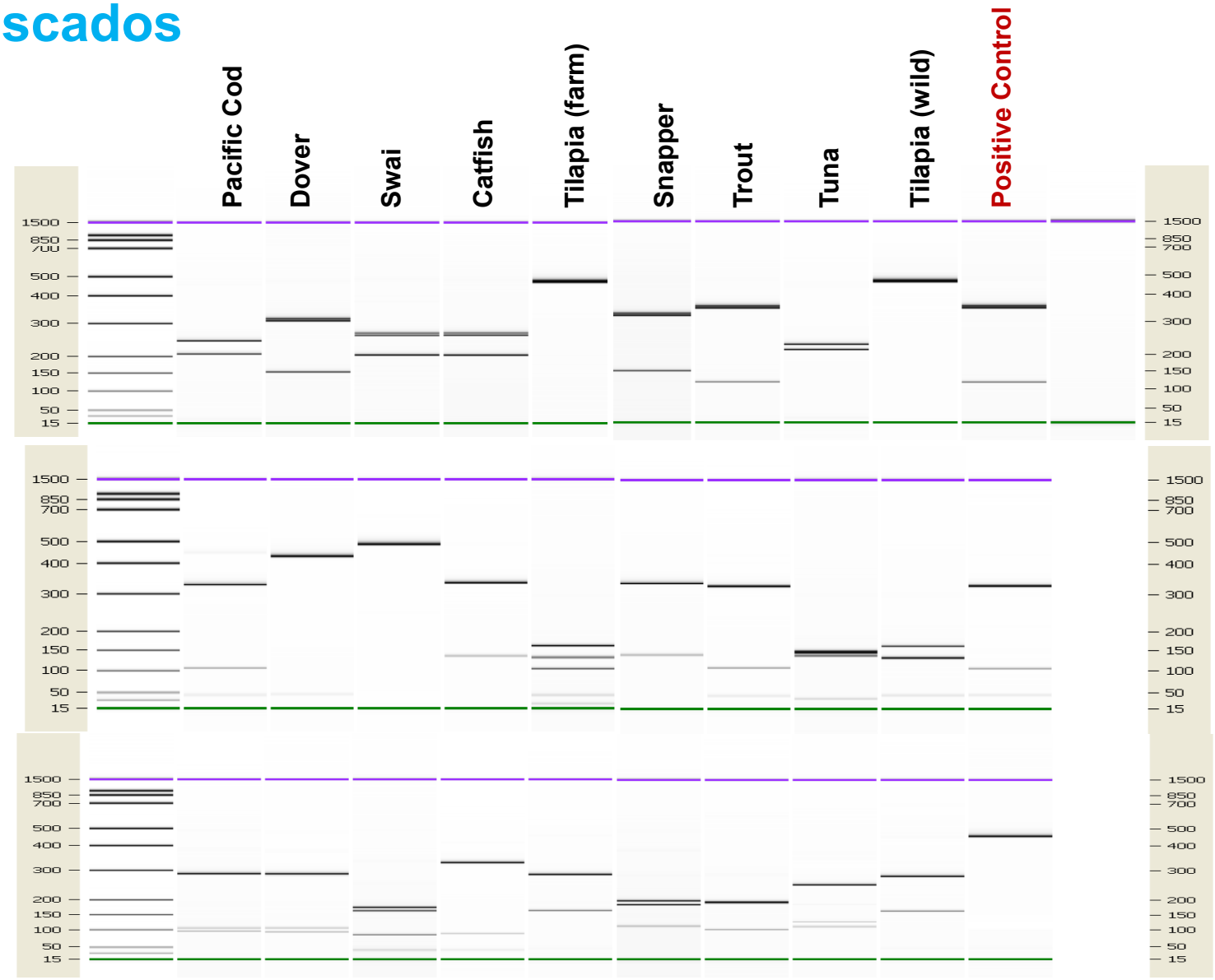
Análisis de modelos usando el RFLP Decoder

< 1 h
(dependiendo de # muestras)

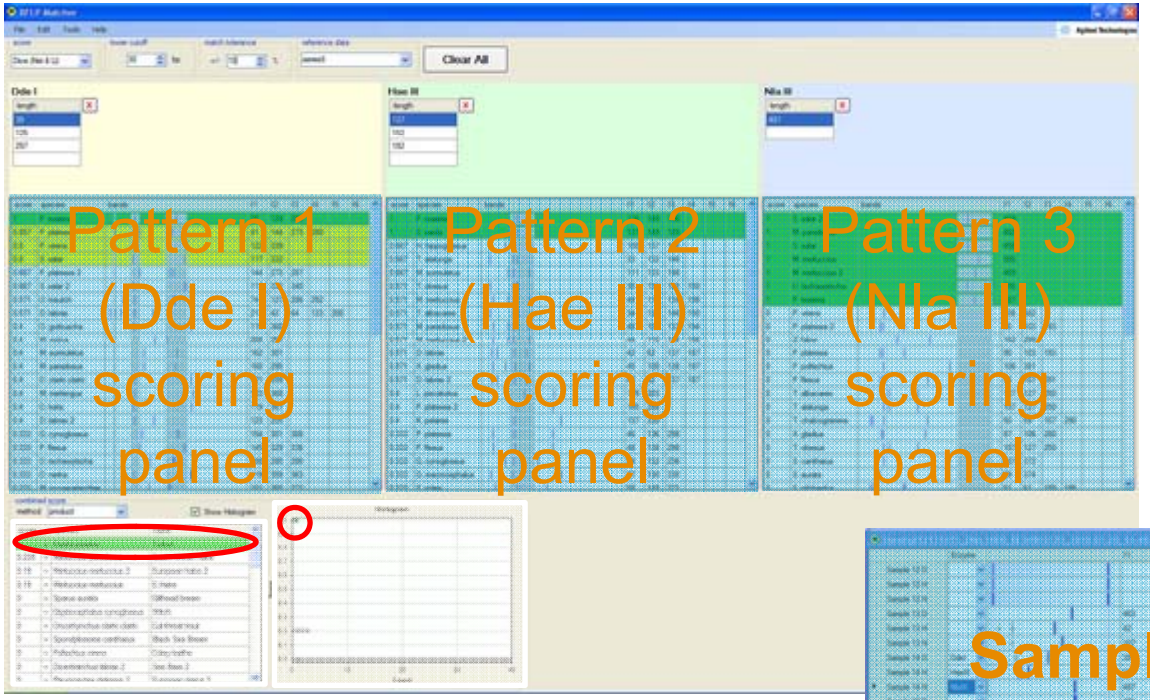


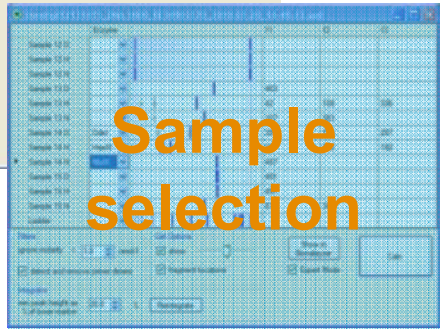
~ 6 h de muestra al resultado

Digestión restrictiva de los amplicons de diferentes muestras de pescados



Interpretación de Resultados – RFLP Decoder Software

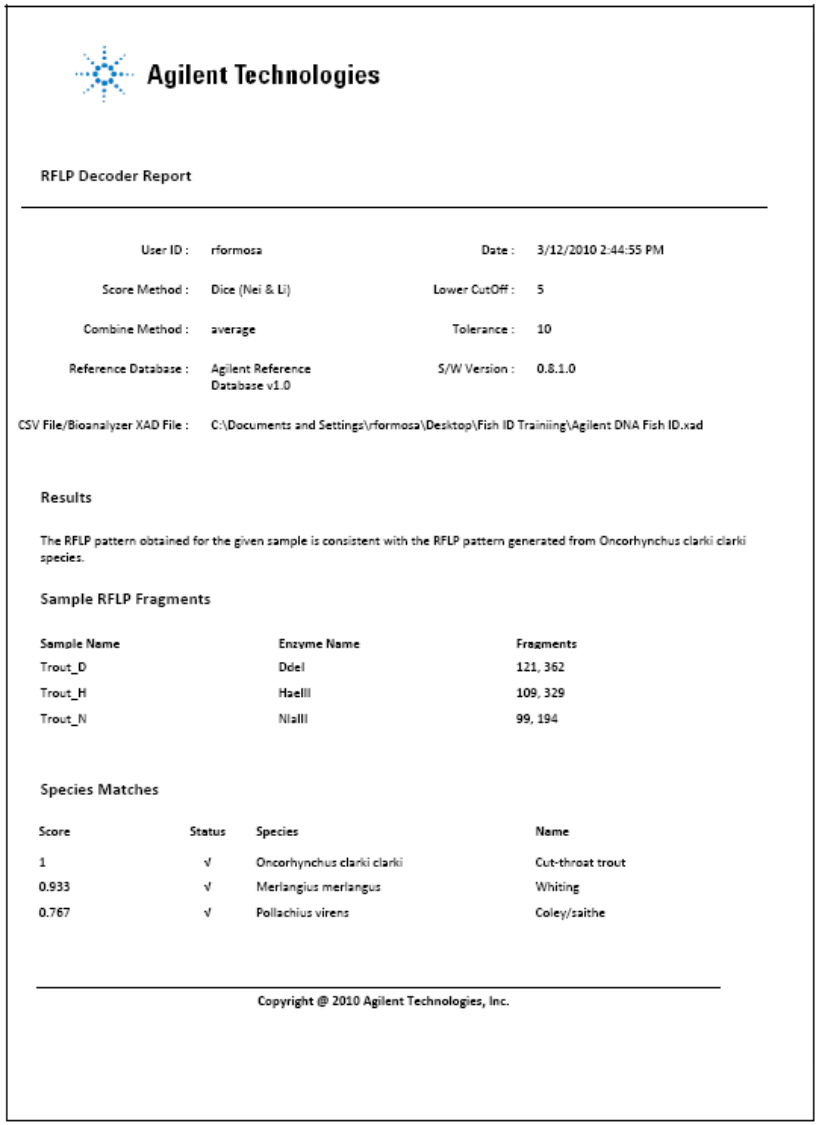




Puntuación combinada:
Indica la mayoría de las especies
El valor de la Puntuación indica la calidad del emparejamiento.

Histograma:
La Separación de las principales especies de aquellas con menor puntuación ofrece una mayor confianza en el resultado.

Coincidencia de patrones: RFLP Report



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RFLP Decoder Report

User ID :	rformosa	Date :	3/12/2010 2:44:55 PM
Score Method :	Dice (Nei & Li)	Lower CutOff :	5
Combine Method :	average	Tolerance :	10
Reference Database :	Agilent Reference Database v1.0	S/W Version :	0.8.1.0

CSV File/Bioanalyzer XAD File : C:\Documents and Settings\rformosa\Desktop\Fish ID Trainiing\Agilent DNA Fish ID.xad

Results

The RFLP pattern obtained for the given sample is consistent with the RFLP pattern generated from Oncorhynchus clarki clarki species.

Sample RFLP Fragments

Sample Name	Enzyme Name	Fragments
Trout_D	DdeI	121, 362
Trout_H	HaeIII	109, 329
Trout_N	NlaIII	99, 194

Species Matches

Score	Status	Species	Name
1	√	Oncorhynchus clarki clarki	Cut-throat trout
0.933	√	Merlangius merlangus	Whiting
0.767	√	Pollachius virens	Coley/saithe

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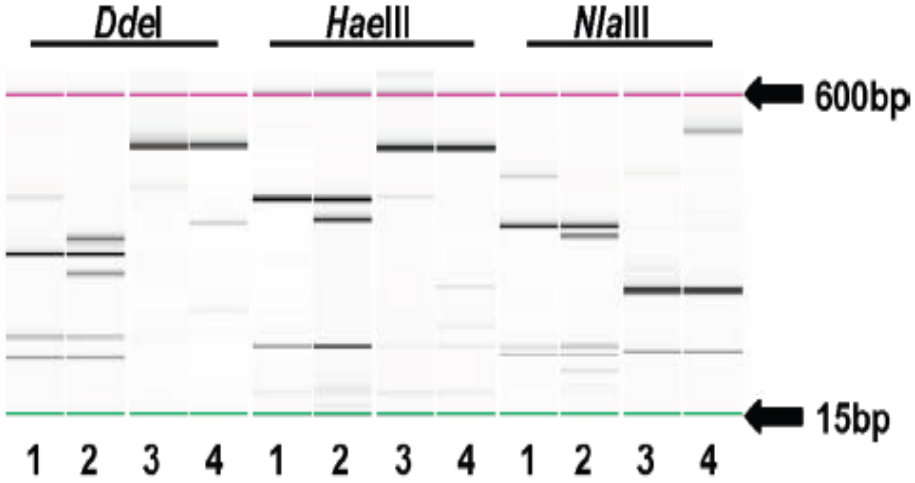
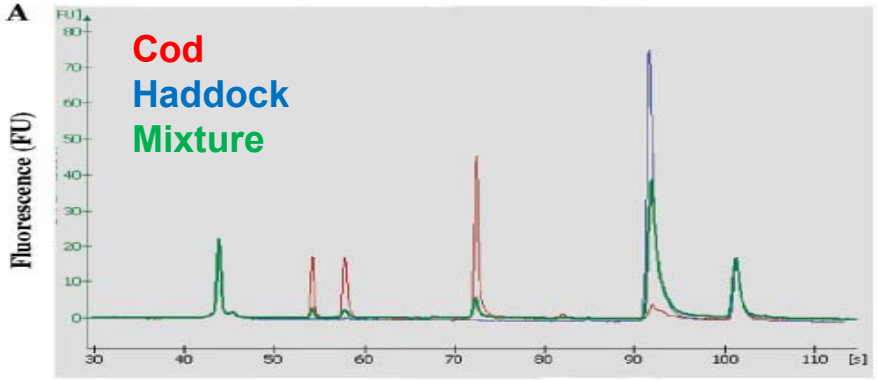
Modelos de RFLP con mezclas de pescados

(d) Details of DNA Admixtures Used in this Study

species 1	species 2
5% coley	95% Atlantic cod
5% hoki	95% Atlantic cod
5% whiting	95% haddock
5% SA hake	95% haddock
5% coley	95% Pacific cod
5% hoki	95% haddock
5% SA hake	95% Atlantic cod
5% whiting	95% Pacific cod
5% hoki	95% coley
5% whiting	95% Atlantic cod
5% A. pollock	95% Atlantic cod
5% hoki	95% Pacific cod
5% coley[b]	95% Pacific cod
5% whiting[b]	95% haddock
5% SA hake[b]	95% Atlantic cod
10% coley[b]	90% Pacific cod

[a] Admixtures prepared on a w/w basis. [b] Admixture contains 59% (v/v) total fish DNA and 41% (v/v) soya DNA.

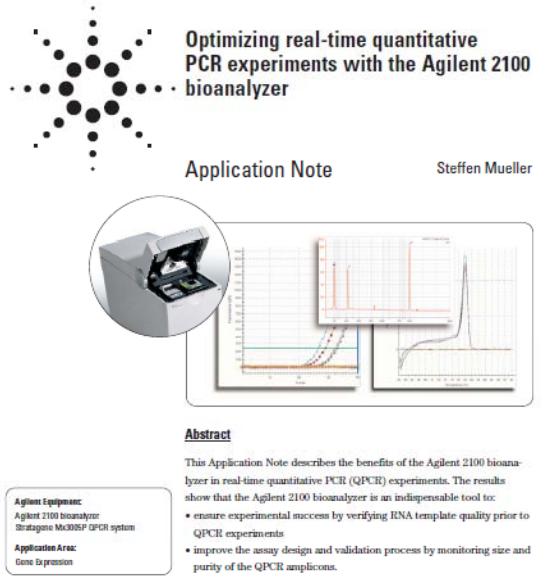




Limites de detección en mezclas: 5%

J. Agric. Food Chem. **2005**, *53*, 3348–3357

FOOD MARKET
SINERGIAS BIOANALIZADOR Y QPCR



Optimizing real-time quantitative PCR experiments with the Agilent 2100 bioanalyzer

Application Note

Steffen Mueller

Abstract

This Application Note describes the benefits of the Agilent 2100 bioanalyzer in real-time quantitative PCR (QPCR) experiments. The results show that the Agilent 2100 bioanalyzer is an indispensable tool to:

- ensure experimental success by verifying RNA template quality prior to QPCR experiments
- improve the assay design and validation process by monitoring size and purity of the QPCR amplicons.

Agilent Equipment:
Agilent 2100 bioanalyzer
Stratagene Mx3005P QPCR system

Application Area:
Gene Expression

El Bioanalizador puede sustituir

El sistema de Fotodocumentación de geles

El QPCR complementa

Ensayos ELISA tanto en DNA como en Proteinas

Ensayos microbiológicos con patógenos

(MENOS tiempo y MAS especificidad)

ELIMINAMOS LOS NEGATIVOS EN MENOS TIEMPO

Agilent Technologies

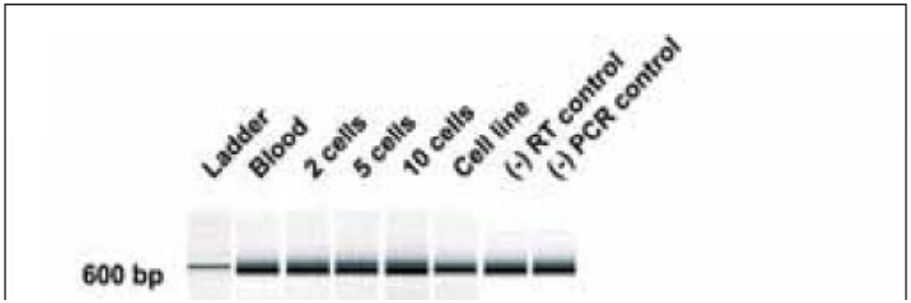
LOS POSITIVOS TENEMOS QUE CONTRASTARLOS CON MICROBIOLOGÍA

Quien ya tiene QPCR **Y** utiliza la tecnología **SYBR Green**, debe hacer un gel o bien un Bioanalizador para definir si lo que a amplificado es 1 sola Diana o varias. De lo contrario deberían utilizar sondas para ser selectivos

Focus en la sensibilidad:
Detección de células cancerígenas en sangre total

Sangre total
5ml-EDTA

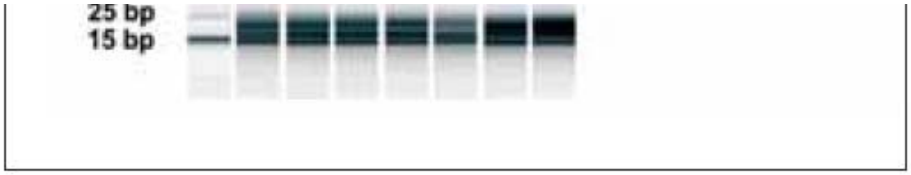
↓



		Prior surgery	1,5 months	3 months	4,5 months	6 months
Patient 1	ELISA	+	-	-	-	-
	PCR	+	-	-	-	+
Patient 2	ELISA	-	-*	-*	-*	-
	PCR	-	-*	+*	+*	+
Patient 3	ELISA	+	-	-	-	-
	PCR	-	-	-	-	+

de marcadores tumorales

↓



BioAnalizador

Data kindly provided by AdnaGen

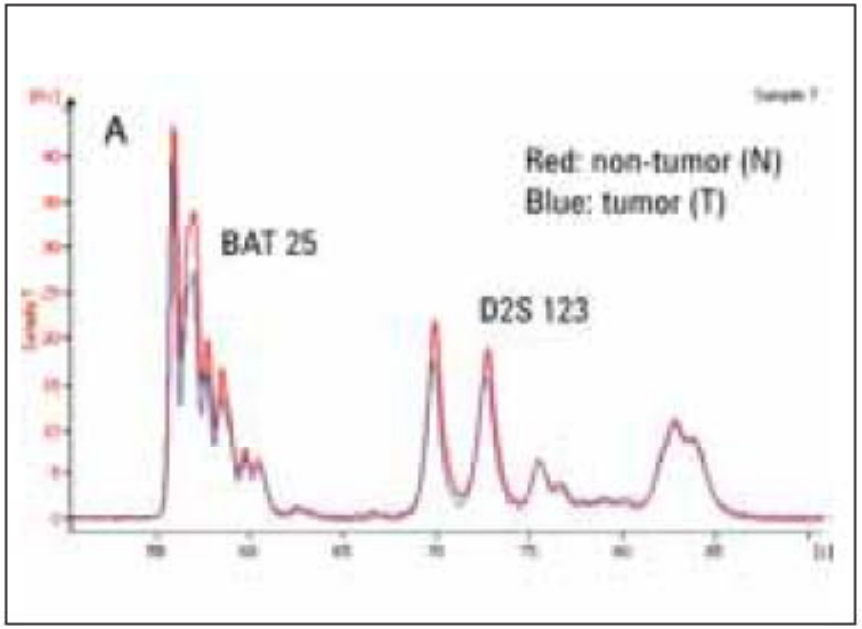
Detección de SNPs en los genes p16 y K-ras

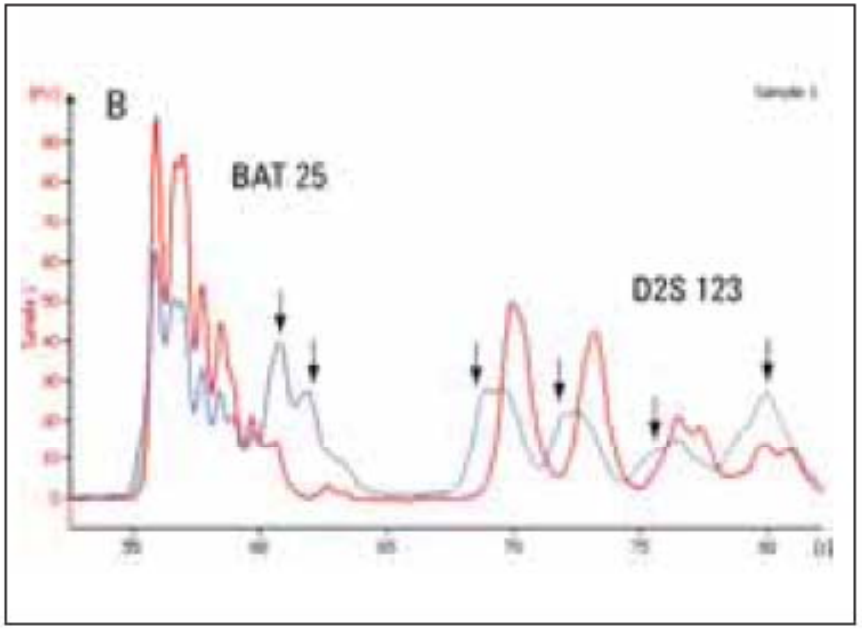
Sample	1	2	3	4	5	6	7	8	9	10	11
Genotype316	C	C	CG	CG	CG	CG	CG	C	C	C	G
Genotype356	C	C	CT	CT	CT	C	C	CT	CT	T	C
Agilent Gel											
Main Band	198	198	197	198	198	198	198	198	199	198	195
Extra Band 1			204	204	204						
Extra Band 2			216	215	215	214	214				

	1	2	3	4	5
ForSeq					
RevSeq					
Genotype	CG	CG	CG	CC	CC (CG)

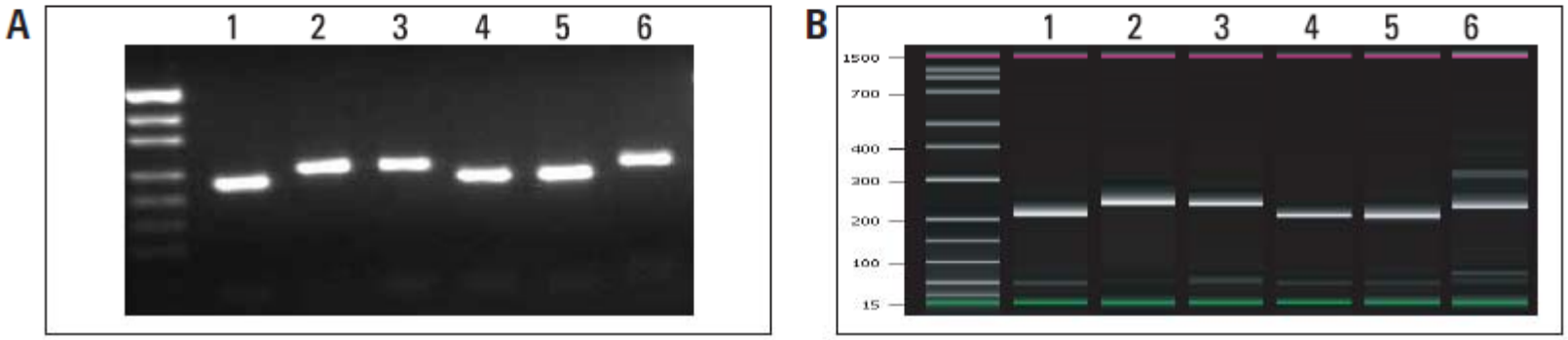
Inestabilidad de microsatélites en carcinoma sin marcaje adicional

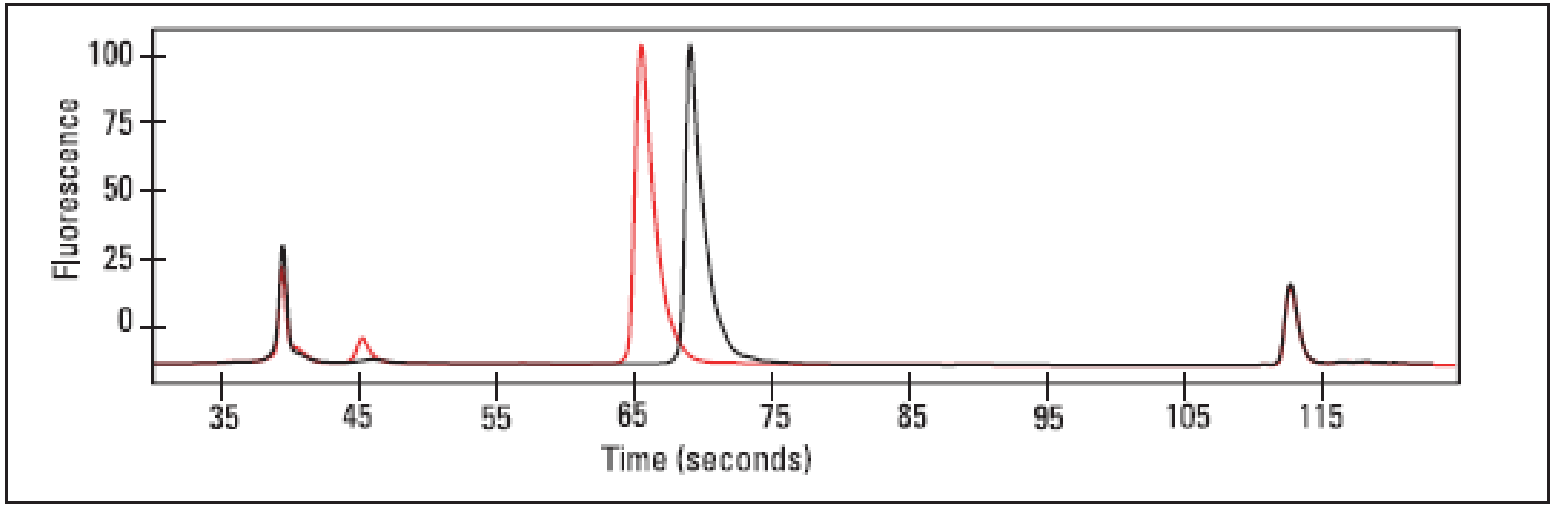
Comparación entre tejido normal y tumoral para el análisis del patrón de los microsatélites tras amplificación por PCR.





Genotipado de *H. pylory*





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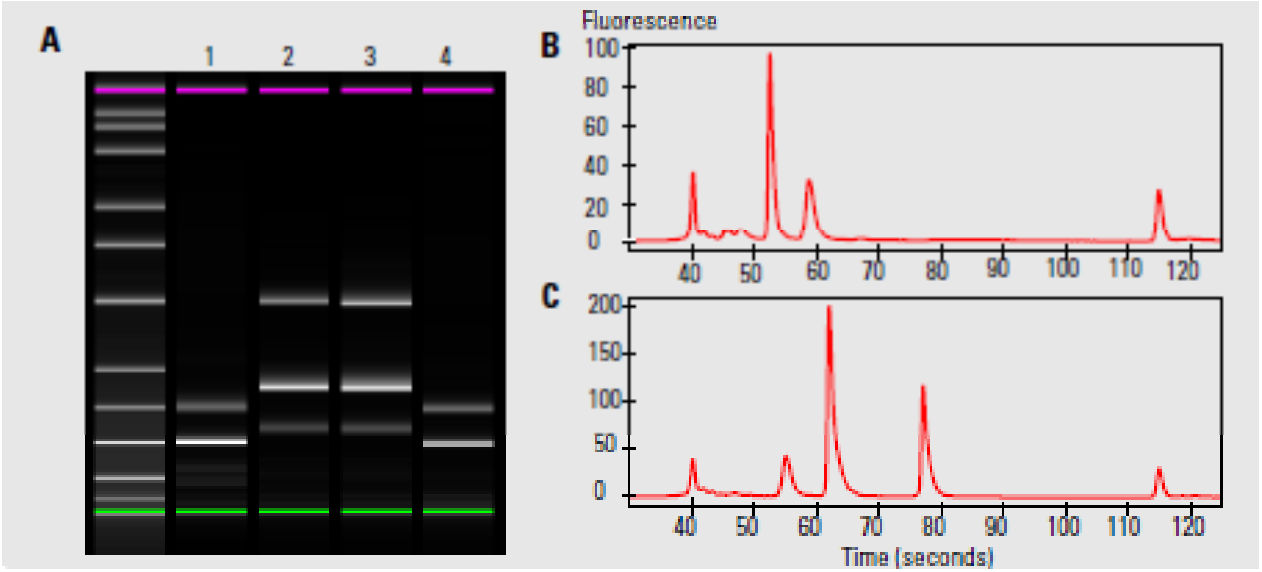
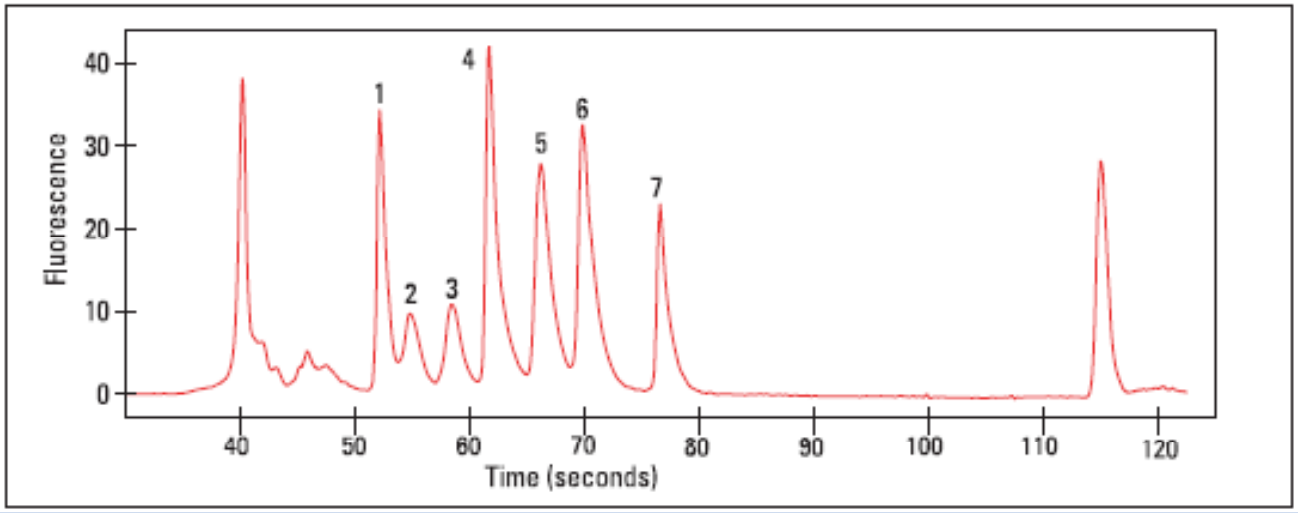
Genotipado de *H. pylory*





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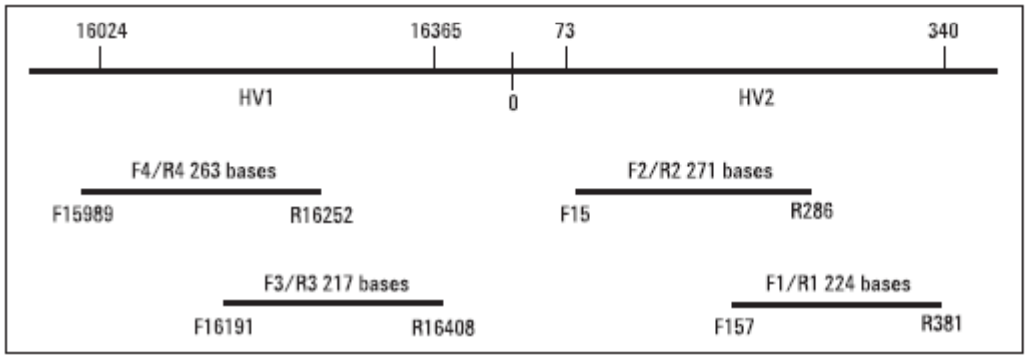
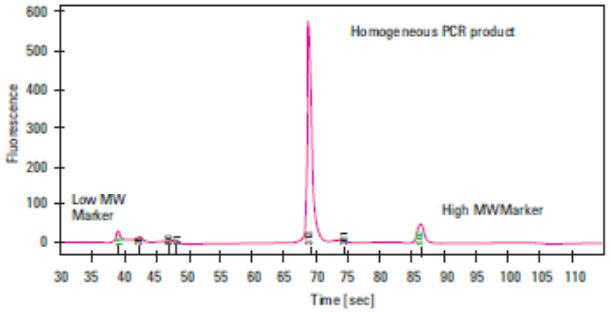
Análisis de ADN mitocondrial



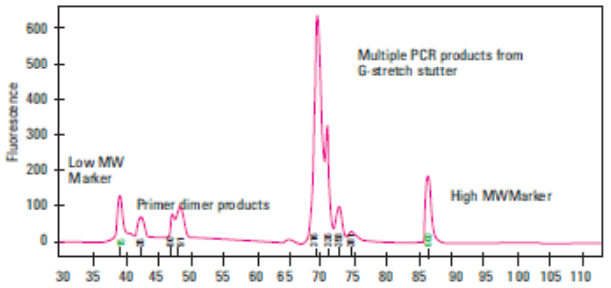
Amplified areas in human mtDNA

mtDNA PCR product analysis



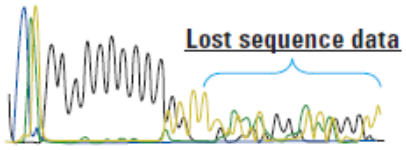
Sequence data from pure PCR product

CATGGGGAGGGGGTTTTGATGTGGATTGGGTT



Sequence data from multiple PCR product

CATGGGGGGGGGGTTTTANNTGNATGATNNGGTT



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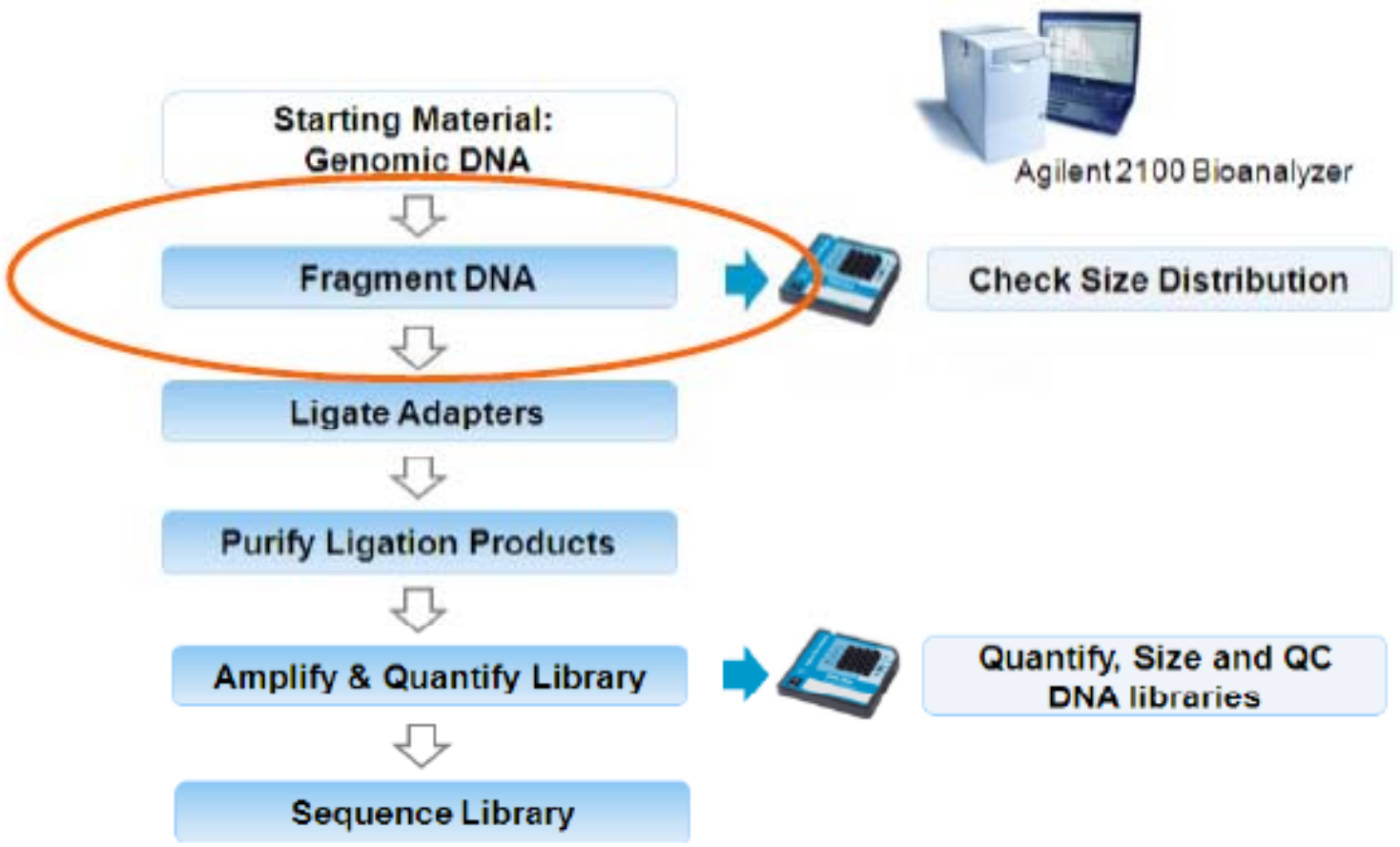
Control de calidad para Next-generation sequencing



DNA Fragmentation - Monitoring the fragment size distribution

Michael A Quail, Iwanka Kozarewa, Frances Smith, Aylwyn Scally, Philip J Stephens, Richard Durbin, Harold Swerdlow & Daniel J Turner

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SA, UK. Correspondence should be addressed to D.J.T. (djt@sanger.ac.uk).

PUBLISHED ONLINE 25 NOVEMBER 2008; DOI:10.1038/NMETH.1270

NATURE METHODS | VOL.5 NO.12 | DECEMBER 2008 | 1005

- **Comparison of fragmentation by nebulization with AFA technology.**
- **Monitor performance on an Agilent Bioanalyzer 2100 DNA 1000 chip.**
- *For a 200-bp (20 bp) library, the yield produced by AFA was four- to fivefold greater than that produced by nebulization.*

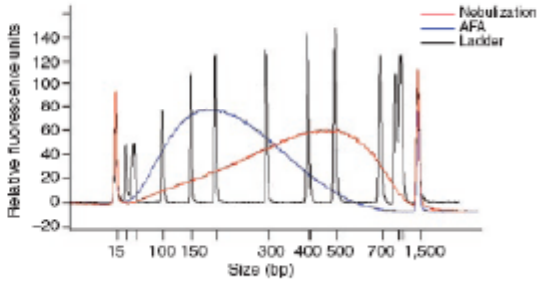


Figure 2 | Sample fragmentation. Comparison of fragmentation by nebulization with AFA technology. We fragmented 4.5 µg of human genomic DNA by nebulization or AFA, purified the samples using a spin column, eluted the DNA in 30 µl of 10 mM Tris pH 8.5, and ran 1 µl of each eluate on an Agilent Bioanalyzer 2100 DNA 100 chip. For a 200-bp (±20 bp) library, the yield produced by AFA was four- to fivefold greater than that produced by nebulization.

High sensitivity DNA kit

Para la cuantificación y discriminación por tamaño de fragmentos de dsDNA y smear de dsDNA de entre 50 – 7000 bp hasta sensibilidades de pg/µl.

Incrementar la sensibilidad el ADN significa:

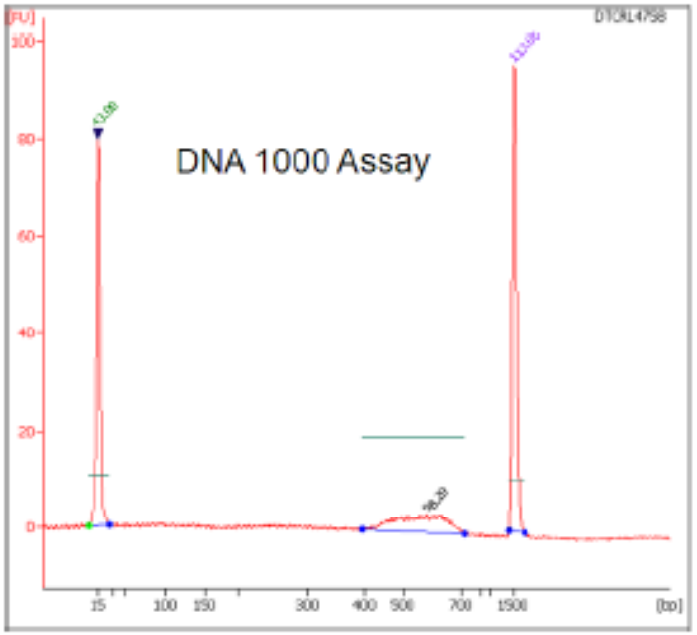
- ❑ Muestras poco concentradas se pueden cuantificar
- ❑ La cantidad de ciclos de PCR para la amplificación de la librería se puede reducir considerablemente

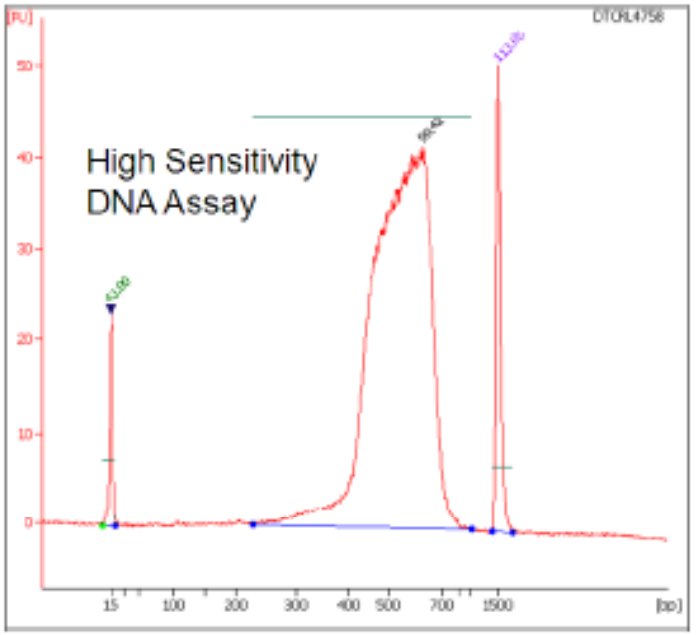
Agilent High Sensitivity DNA Kit
(5067-4626)

High Sensitivity DNA Reagents
(5067-4627)

High Sensitivity DNA Kit – Sensibilidad mejorada

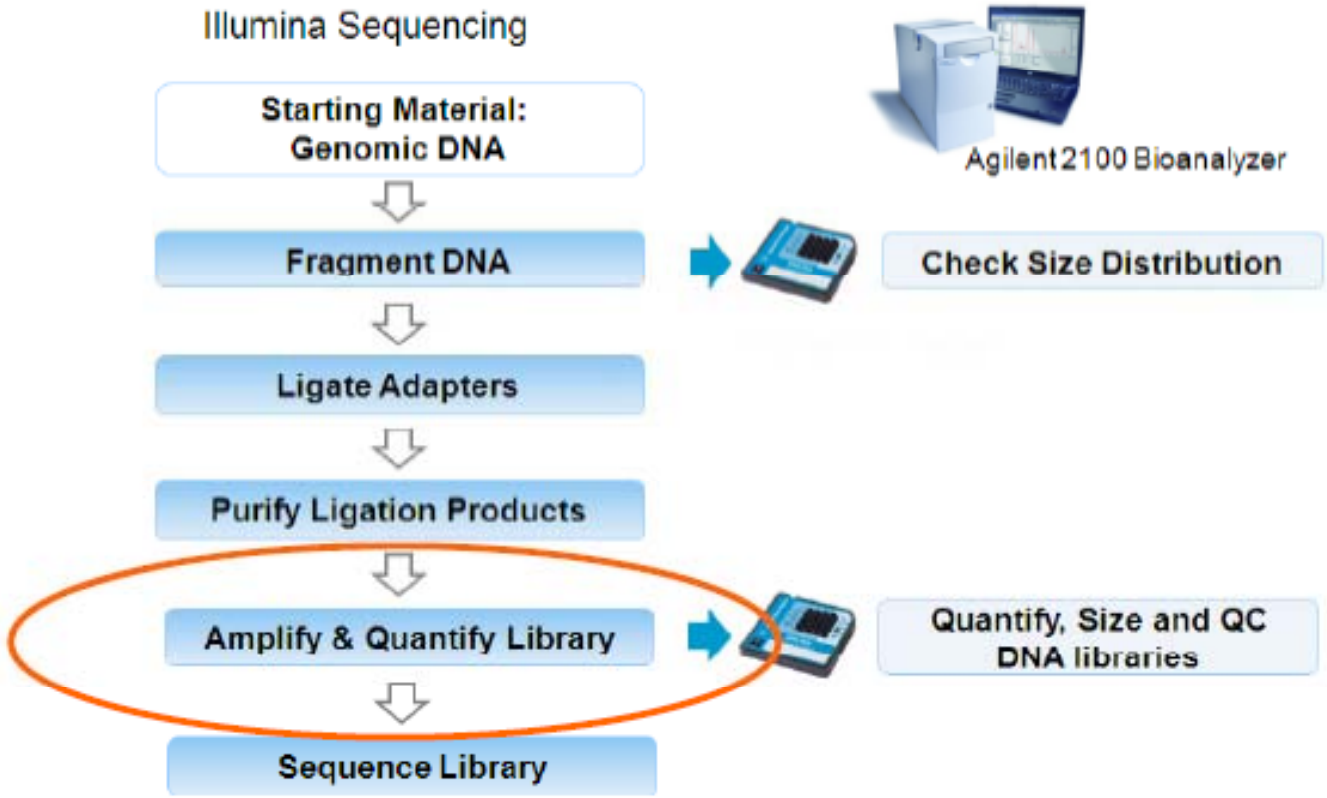
- Aumenta la sensibilidad en un factor de 20-30.
- El rango cuantitativo del High Sensitivity DNA kit se ha determinado entre 5-500 pg/µL por fragmento de DNA y entre 100-10000 pg/µl en AND fragmentado de tamaños entre 50-7000 bp.





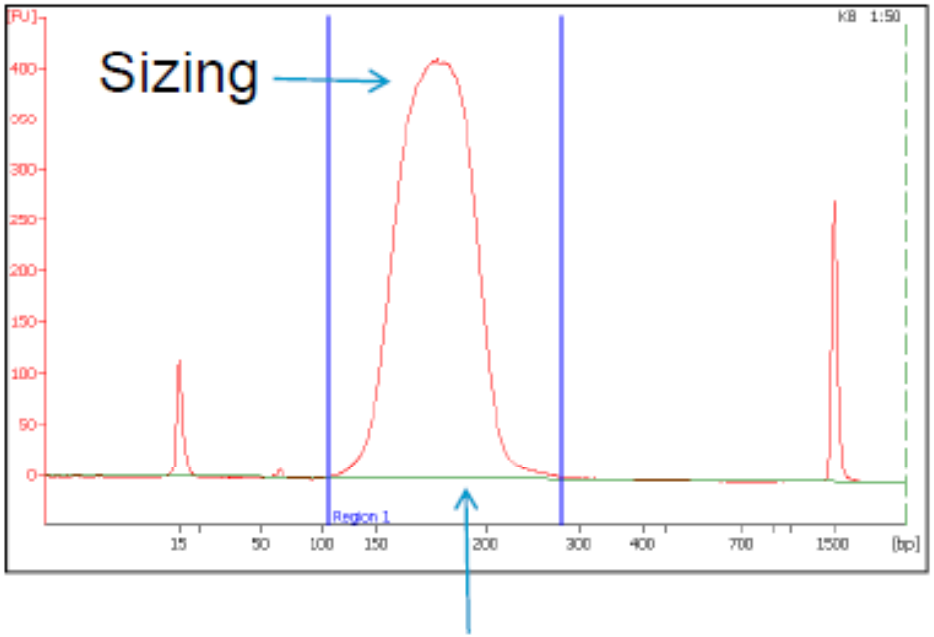
La misma librería de ADN analizada mediante DNA 1000 kit y el High Sensitivivity DNA kit

Control de calidad para Next-generation sequencing

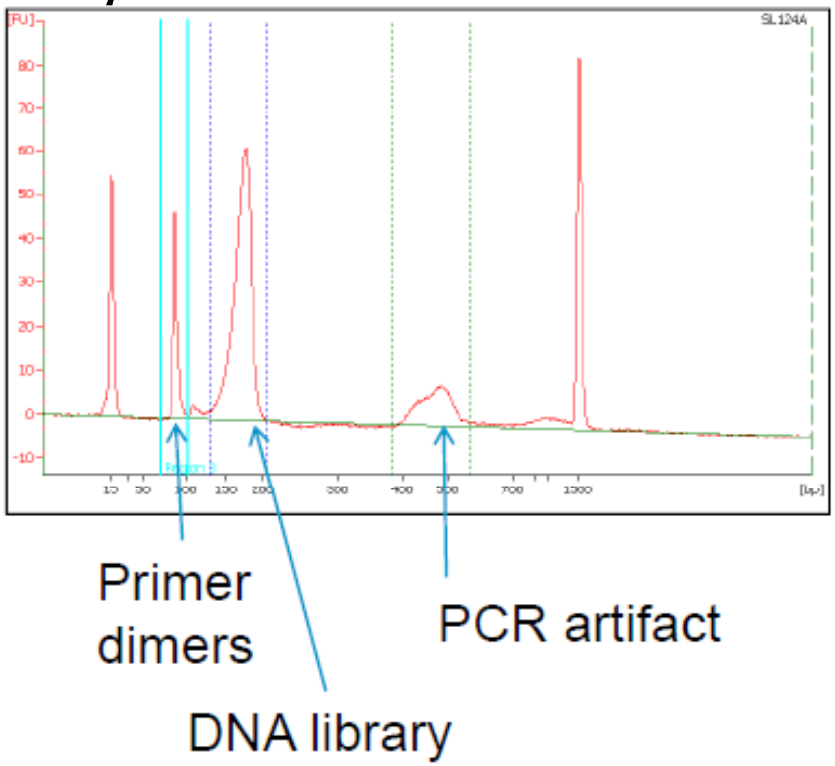


Cuantificación, tamaño y control de calidad de librerías para NGS

Librería de ADN
de alta calidad



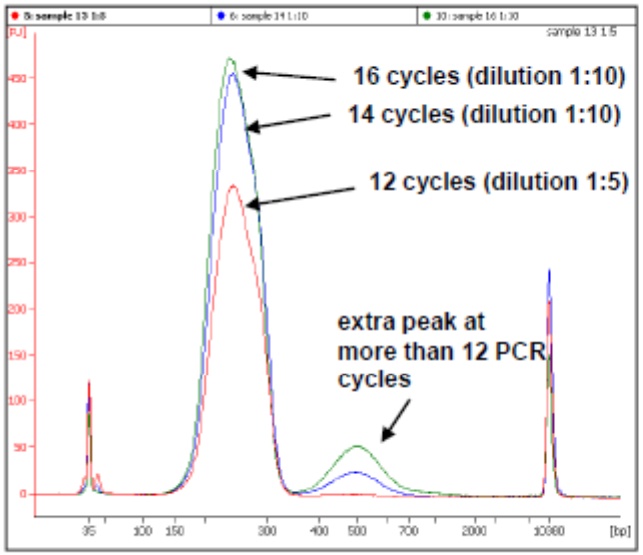
Identificación y cuantificación
de primer dimers
y artefactos en la PCR

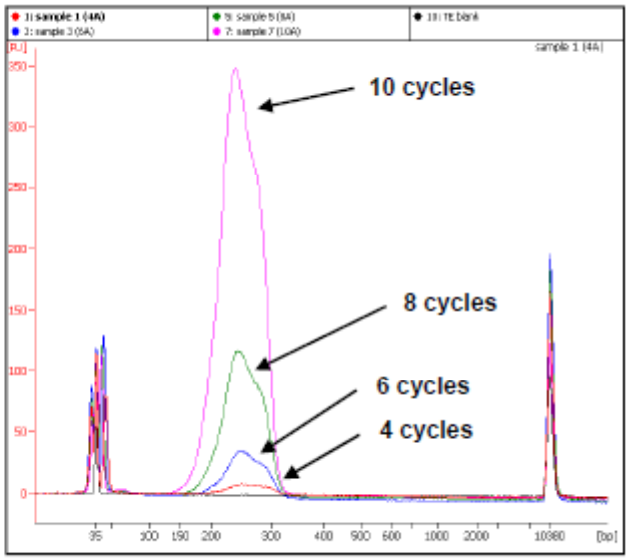


High Sensitivity DNA Kit – Reducción del número de ciclos de amplificación

Tamaño y cuantificación para una librería de Illumina GAII. La librería se enriqueción conSureSelect Target Enrichment de Agilent y se amplificó variando el número de ciclos de PCR

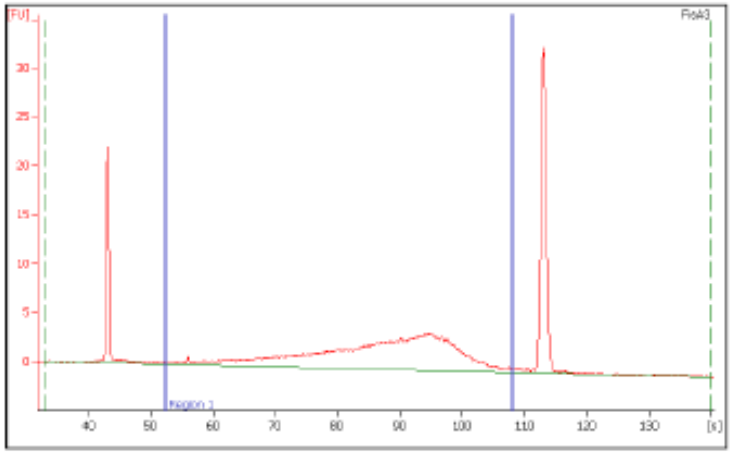
- **Los ciclos de amplificación a partir de cierto nivel pueden resultar en artefactos por amplificación**
- **Mediante el análisis con el High Sensitivity DNA se permite reducir los cliclos innecesarios de amplificación**





High Sensitivity DNA Kit– Análisis de muestras con poca cantidad incial para NGS

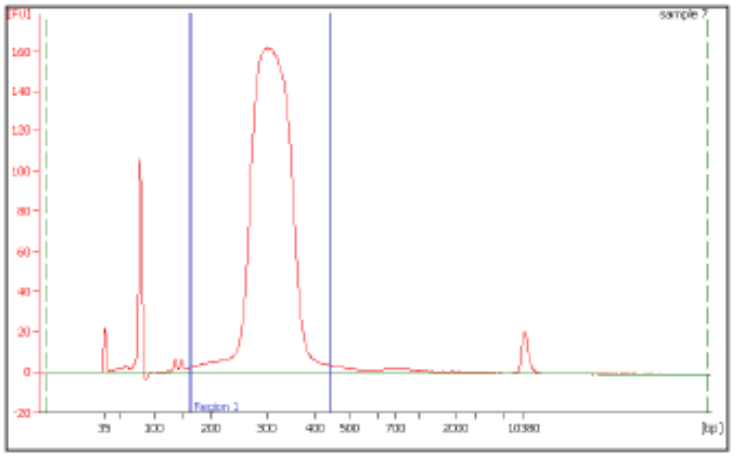
Enables the anaylsis of low-concentrated starting material as derived from microdissected tissues or post IP material for ***ChIP-Seq***



ChIP Pre-library samples

Average Size: 827 bp

Conc: 335 pg/µl



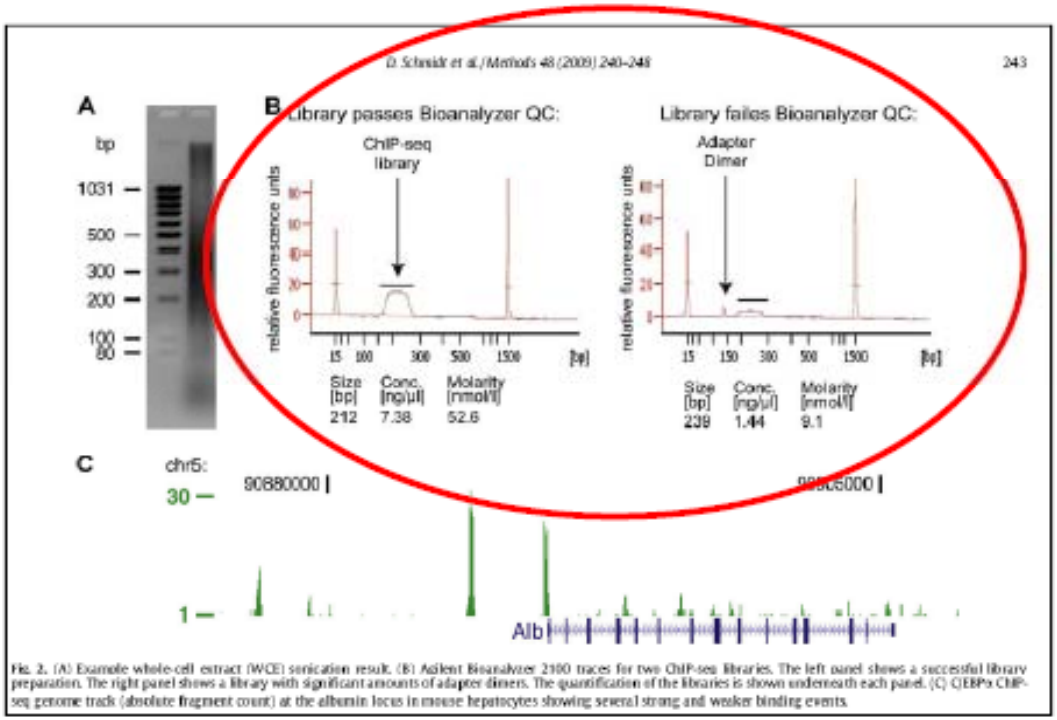
ChIP-Seq library

Average Size: 310 bp

Conc: 8.6 ng/µl

Control de calidad de librerías para ChIP-Seq

Schmidt et al. ***ChIP-seq: Using high-throughput sequencing to discover protein–DNA interactions*** Methods 48 (2009) 240–248



APLICACIONES EN ARN

Agilent RNA Analysis Kits and Reagents

Order No.	Description
5067-1511	RNA 6000 Nano Kit
5067-1512	RNA 6000 Nano Reagents
5067-1529	RNA Nano Ladder

Order No.	Description
5067-1513	RNA 6000 Pico Kit
5067-1514	RNA 6000 Pico Reagents
5067-1535	RNA Pico Ladder

Order No.	Description
5067-1548	Small RNA Kit
5067-1549	Small RNA Reagents
5067-1550	Small RNA Ladder

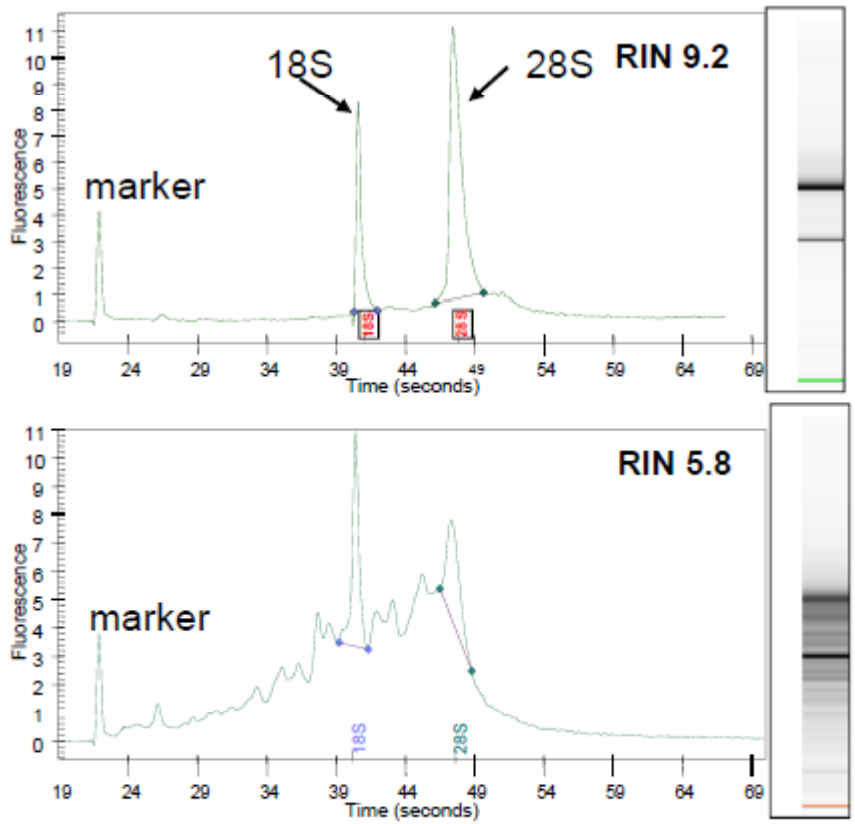
Analytical Specifications	RNA 6000 Nano Total RNA Assay	RNA 6000 Nano mRNA Assay	RNA 6000 Pico Total RNA Assay	RNA 6000 Pico mRNA Assay	Small RNA
Quantitative range	25–500 ng/µL	25–250 ng/µL	–	–	50–2000 pg/µL of purified miRNA in water
Qualitative range	5–500 ng/µL	25–250 ng/µL	50–5000 pg/µL in water	250–5000 pg/µL in water	50–2000 pg/µL of purified miRNA in water
Sizing range	–	–	–	–	6–150 nt
Sensitivity (signal/noise > 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 Nano 12/chip = 300 samples/kit	RNA Pico 11/chip = 275 samples/kit	RNA Pico 11/chip = 275 samples/kit	–

* Determined analyzing the RNA ladder as sample

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

Integridad del ARN – Calidad del ARN total de la muestra

Paso clave en trabajo tras la preparación de la muestra de ARN anterior a realtime-PCR o microarrays

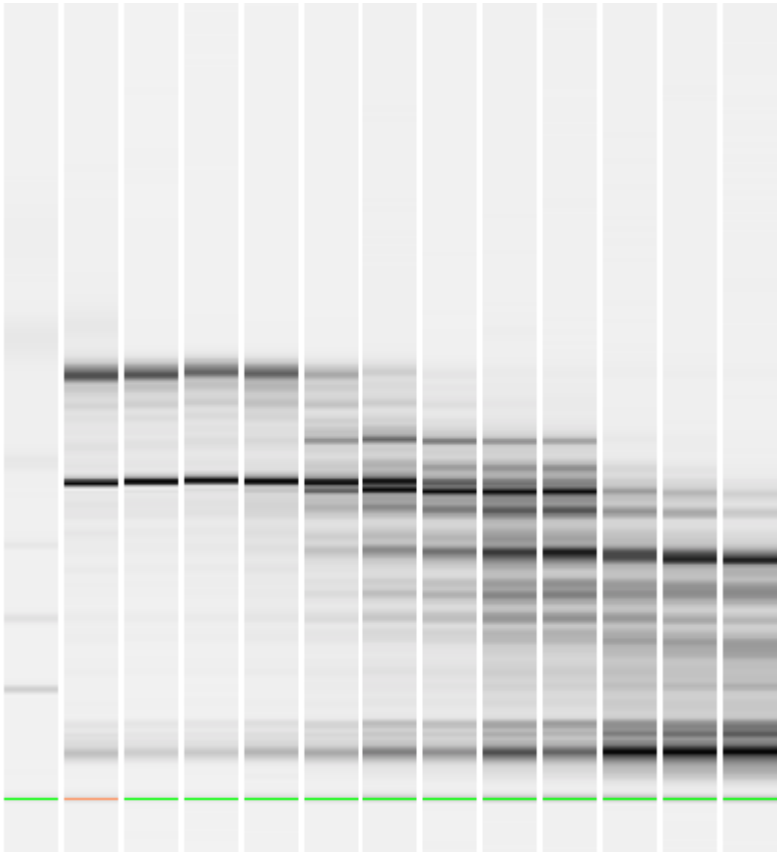


ARN de alta calidad

ARN de baja calidad

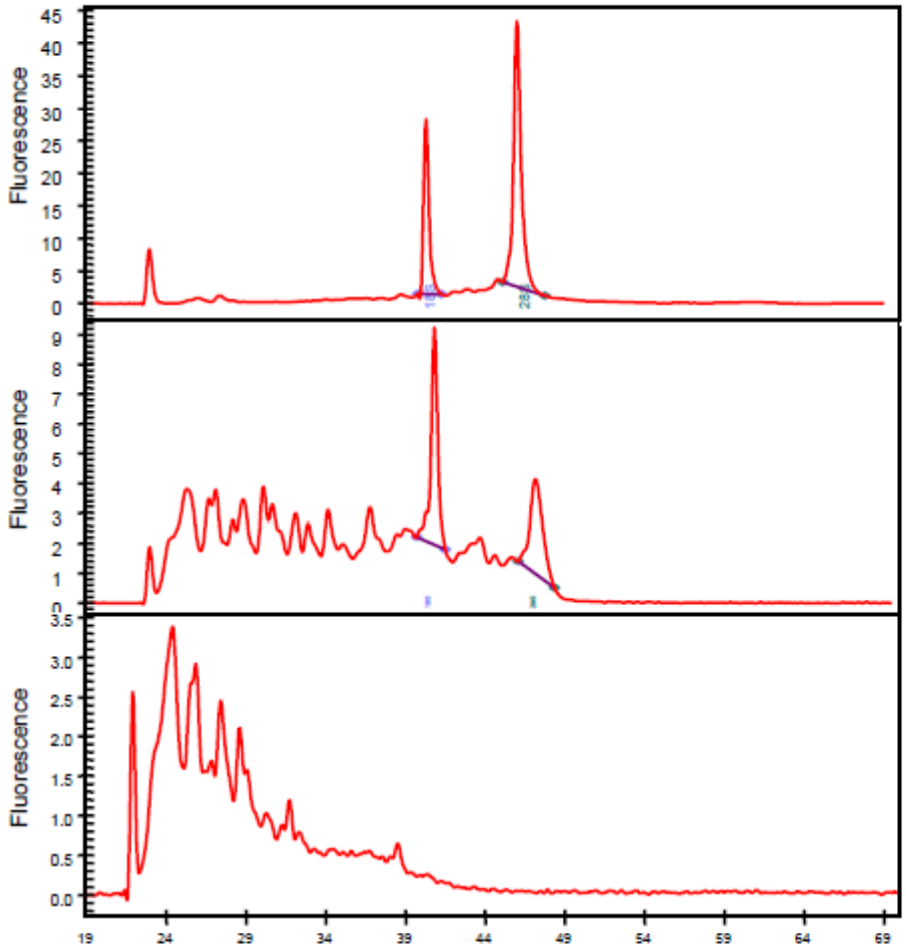
Schroeder et al.: The RIN: an RNA integrity number for assigning integrity values to RNA measurements, BMC Mol Biol. 2006 Jan 31;7:3.

RIN – RNA Integrity Number



- 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

RIN –Integridad del ARN

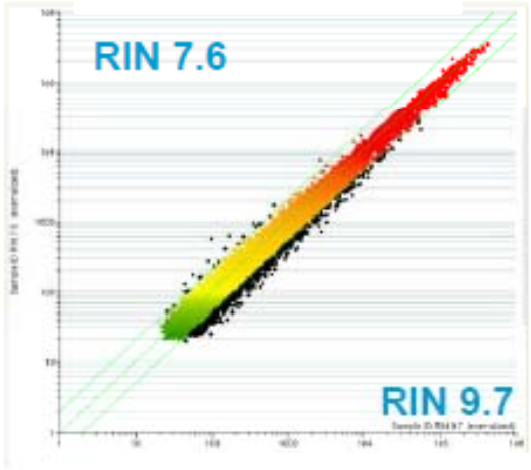


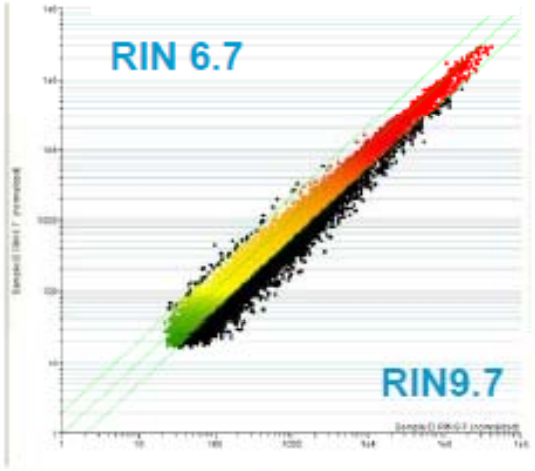
Intact RNA: RIN 10

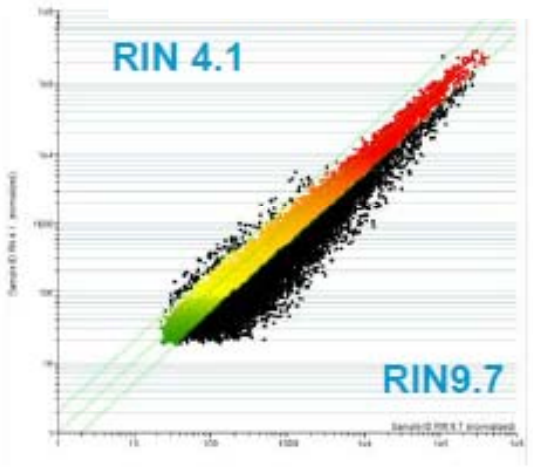
Partially degraded RNA: RIN 5

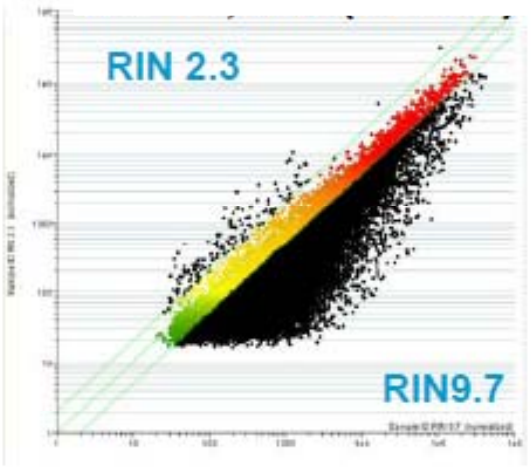
Strongly Degraded RNA: RIN 3

Porqué la calidad sí importa – Efectos en los resultados de expresión génica

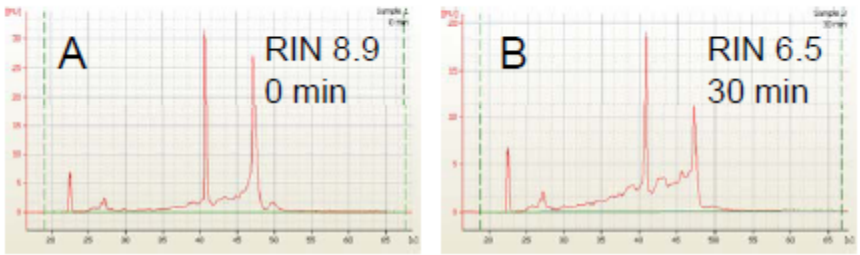


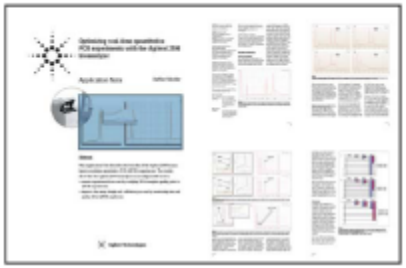


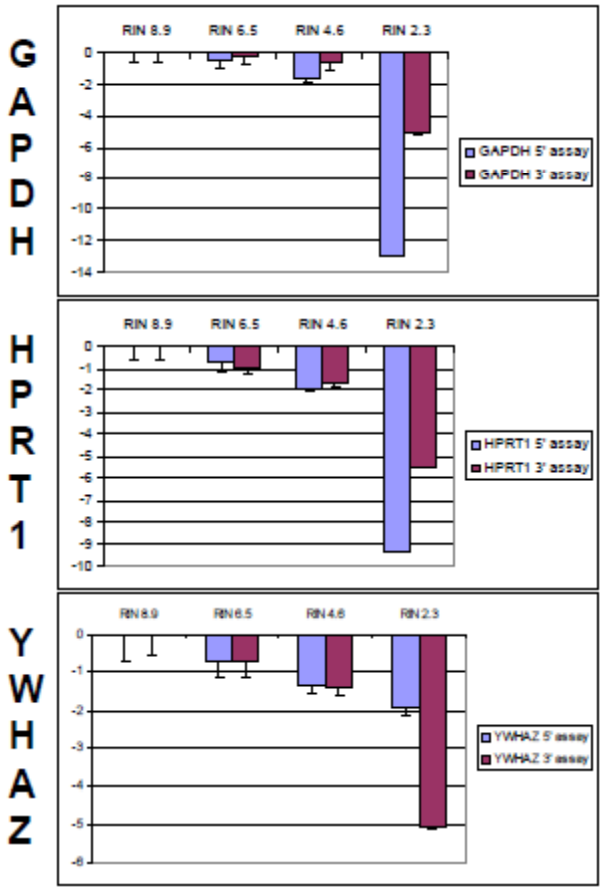




RNA QC/QPCR – Calidad del ARN y niveles de expresión génica

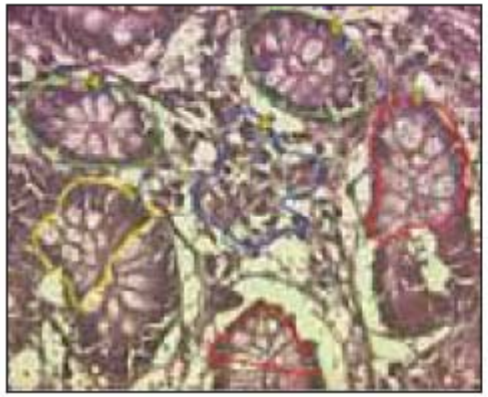


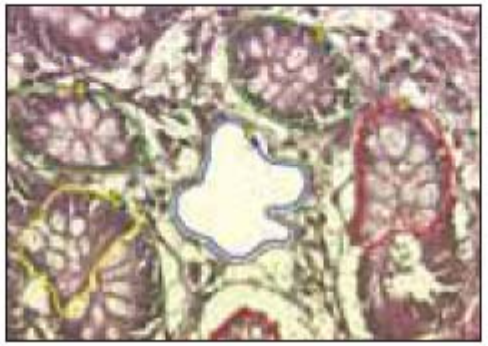


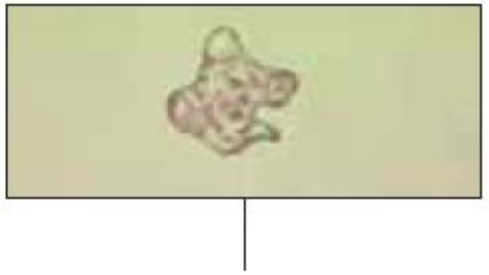


RNA QC/qPCR Application Note (5989-7730EN)

Microdisección y análisis de ARN

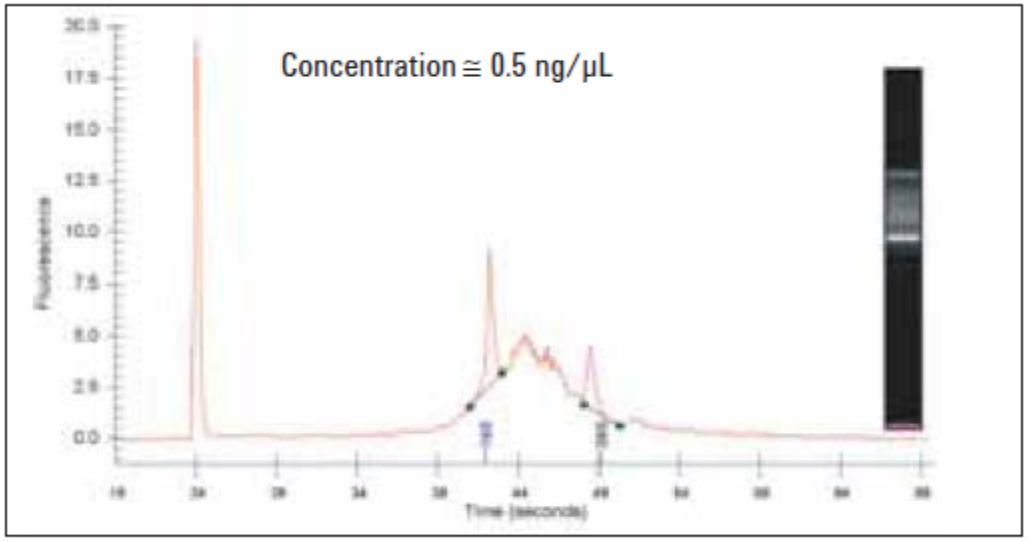




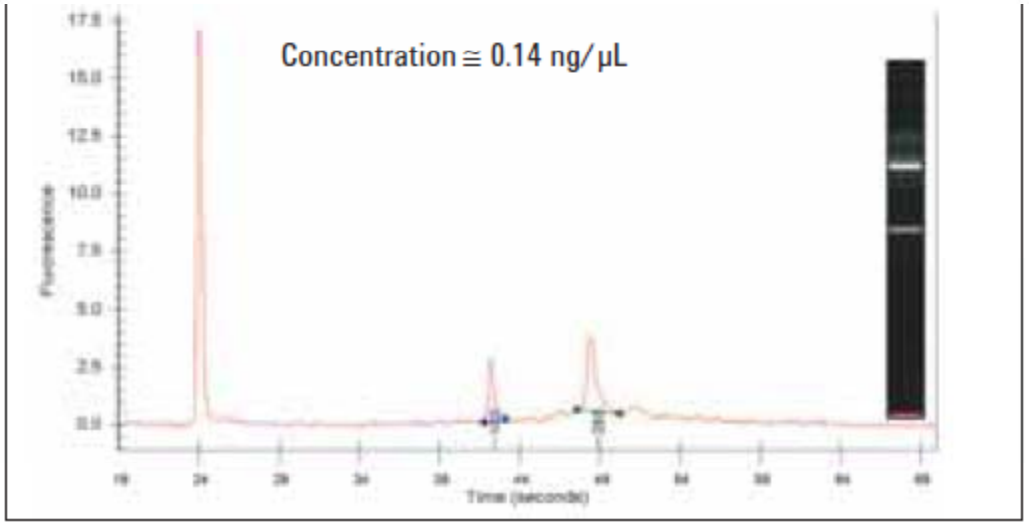


Laser micro dissection

Microdisección y análisis de ARN: contaminación por ADN de la muestra



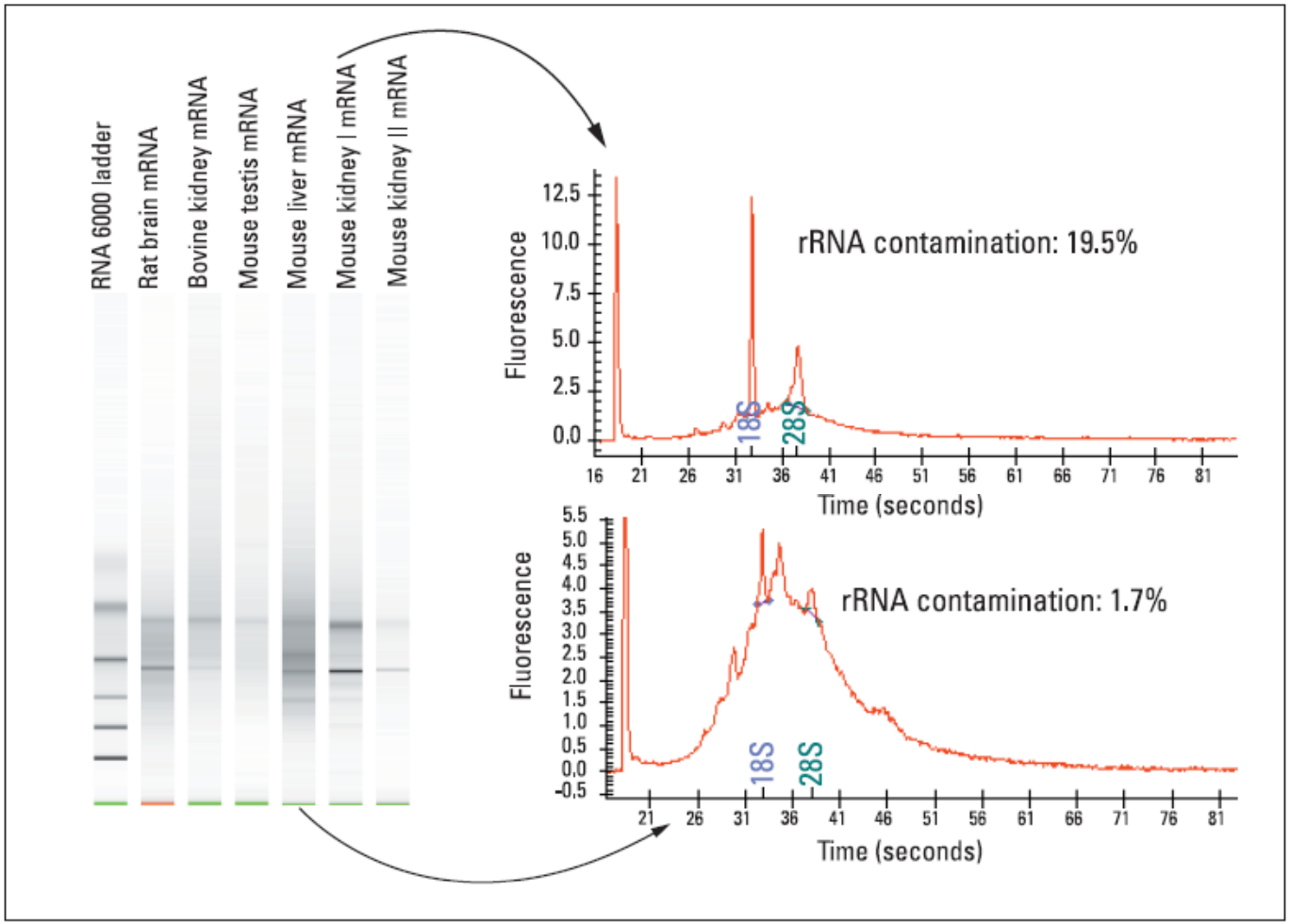
Efectos de la digestión de ADN en columna con DNasa



Sobreestimación de la concentración

Análisis del mRNA

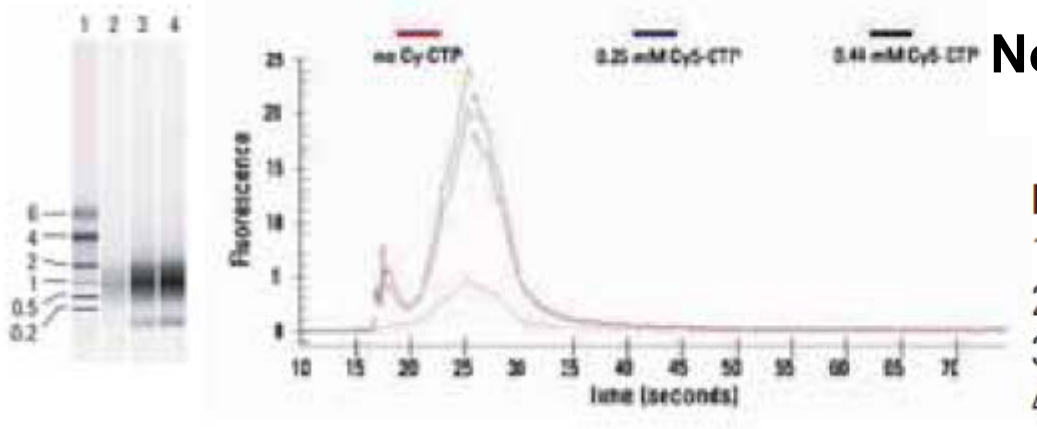
Contaminación con rRNA de muestras de mRNA



Agilent Technologies

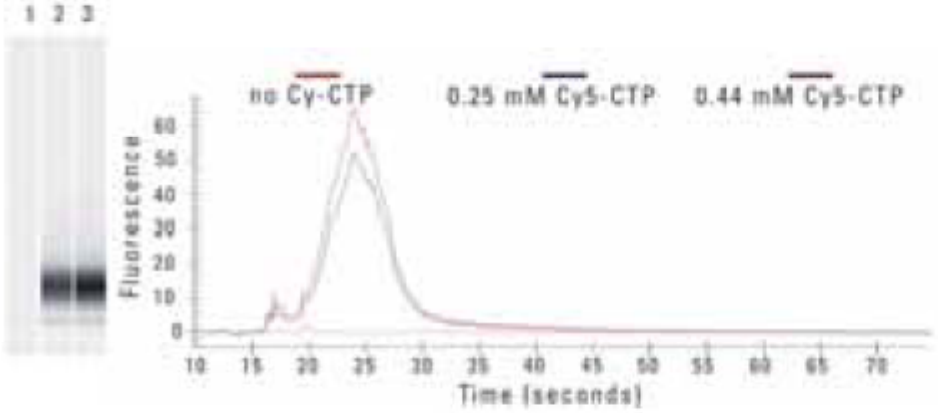
Analisis de muestras marcadas con Cy5

Muestras de cRNA con o sin tinción incoporado en la matriz de gel



No intercalating dye

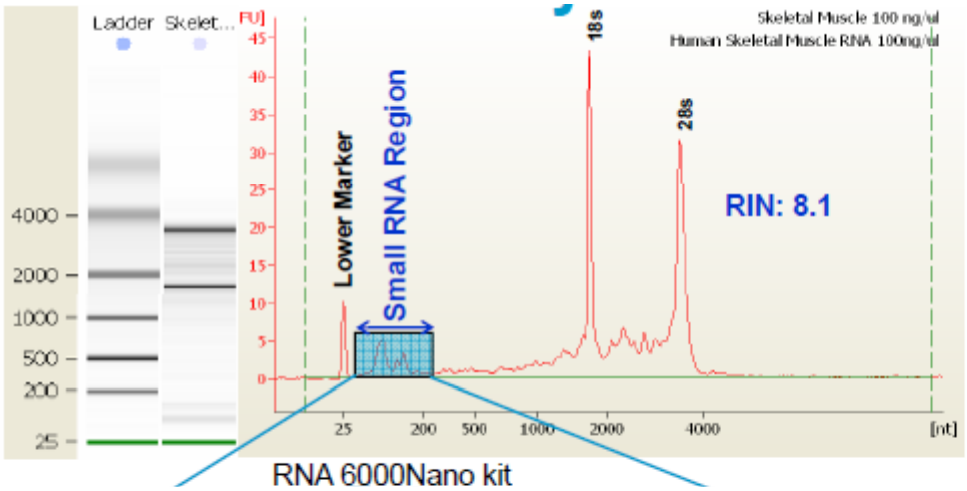
Lanes:
1. RNA transcript ladder
2. Unlabeled cRNA
3. Cy5 labeled cRNA
4. Cy5 labeled cRNA



Assay conditions:
- Cy5 labeled nucleic acids
- no intercalating dye used
- 5nM Cy5 dCTP added to gel matrix and sample buffer for focusing

Lanes:
1. Unlabeled cRNA
2. Cy5 labeled cRNA
3. Cy5 labeled cRNA

Small RNA kit

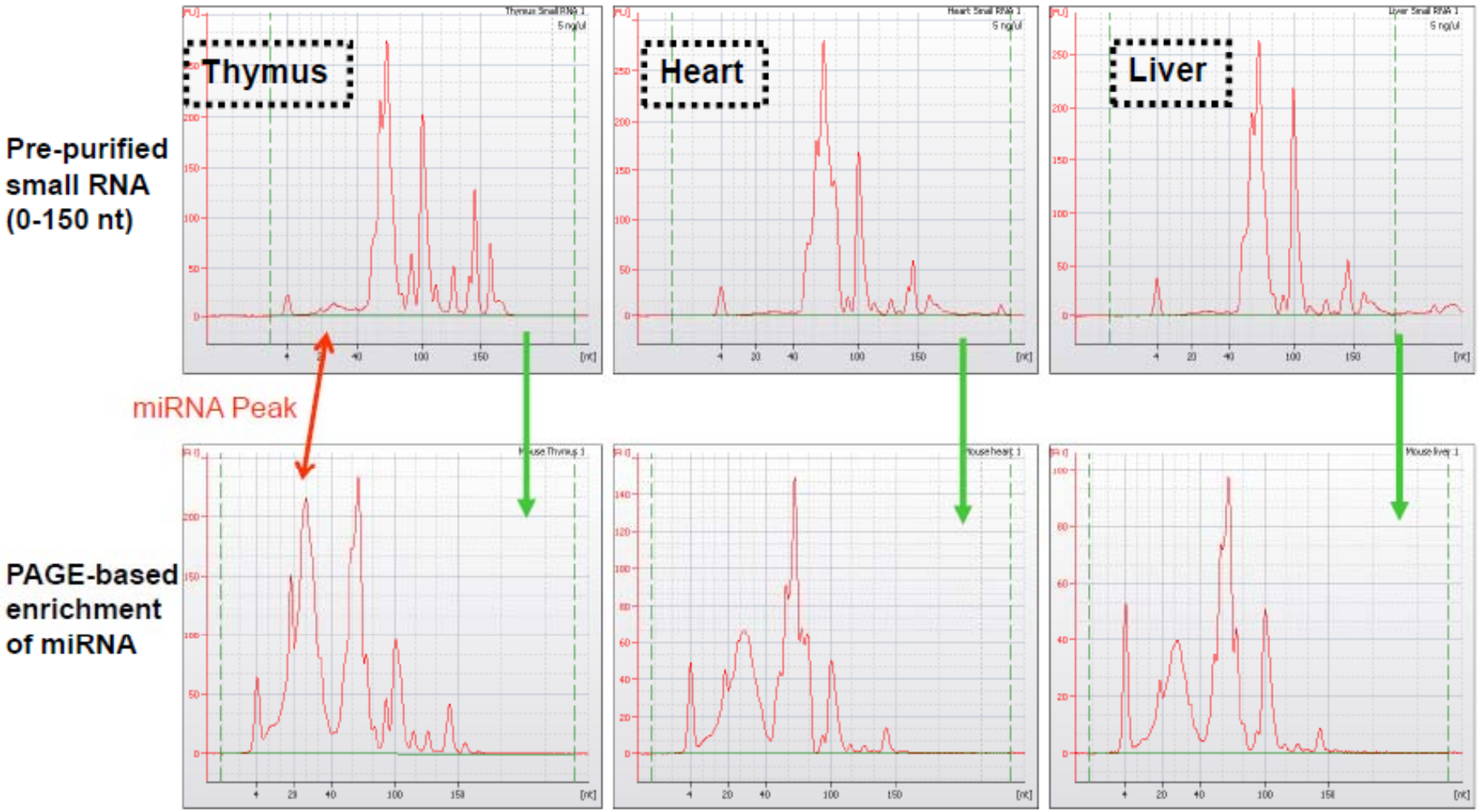


RNA 6000Nano

Size range: 25-6000nt

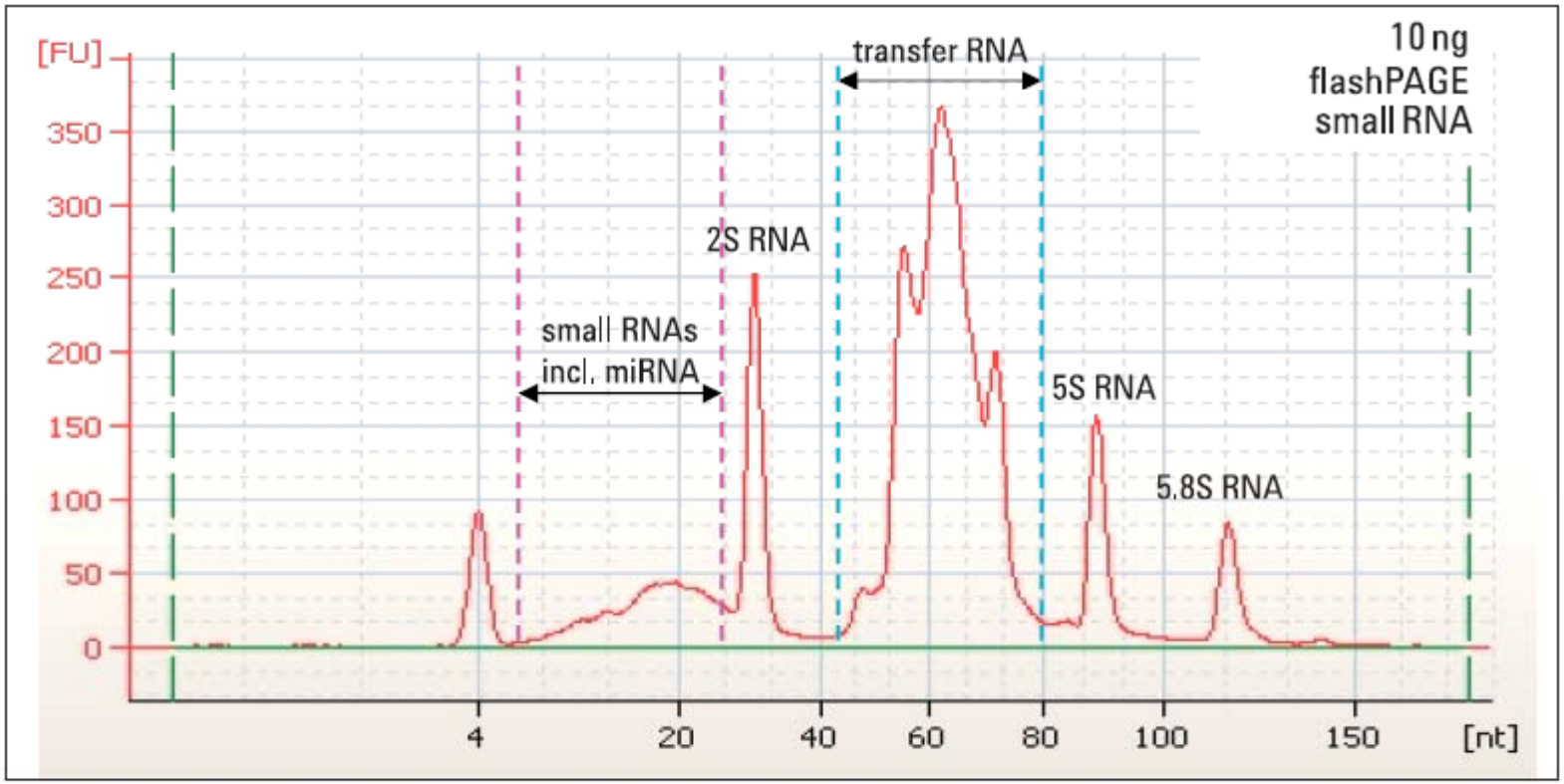
Results: Integrity, Total RNA amount, gDNA contamination

Small RNA Extraction - Monitoring the enrichment of miRNA



Agilent Technologies

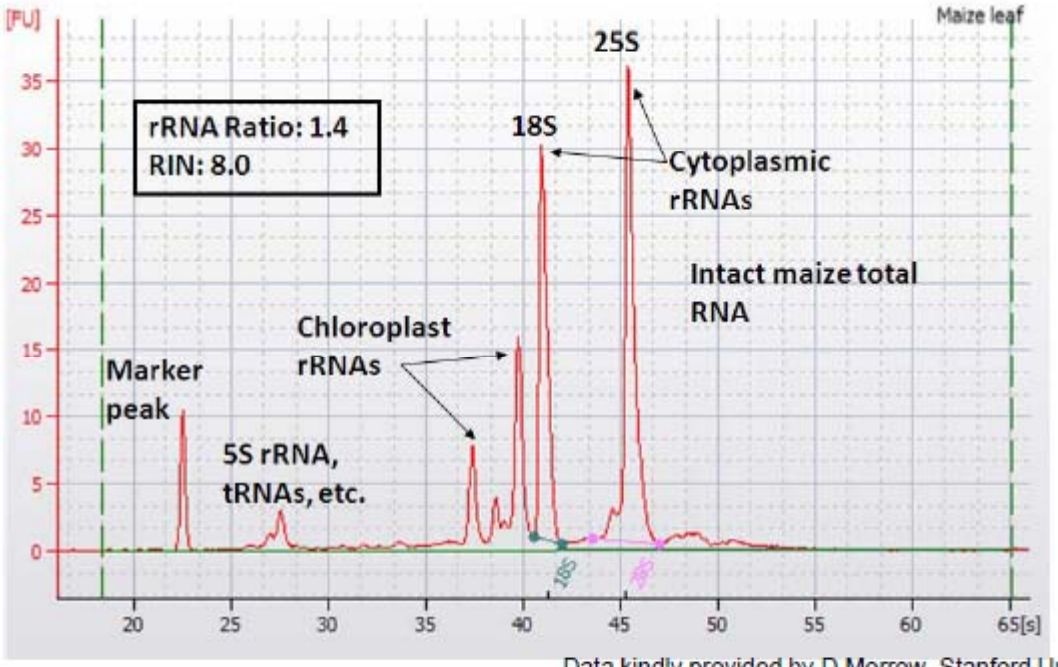
Small RNA – distinción de especies de small RNA



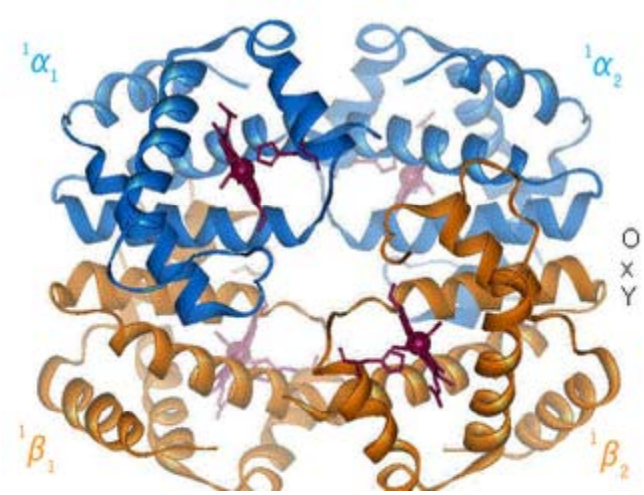
Analisis de RNAs de células Schneider de *Drosophila* (10ng RNA total)

Nuevo test de ARNpara plantas

- Nuevo test para ARN de plantas en el 2100 Expert software B.02.07
- Método de identificación reproducible de fragmentos de rRNA de diferentes muestras de plantas para determinar la integridad del ARN
- Algoritmo RIN estándar
- Compatible con los kits estándar de ARN



Data kindly provided by D Morrow, Stanford University



APLICACIONES EN PROTEINAS

Protein Analysis Kits and Reagents

Order No.	Description
5067-1515	Protein 80 Kit
5067-1516	Protein 80 Reagents

Order No.	Description
5067-1517	Protein 230 Kit
5067-1518	Protein 230 Reagents

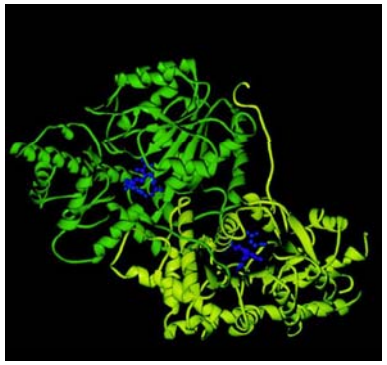
Order No.	Description
5067-1575	High Sensitivity Protein 250 Kit
5067-1576	High Sensitivity Protein 250 Reagents
5067-1577	High Sensitivity Protein 250 Labeling Reagents
5067-1578	High Sensitivity Protein 250 Ladder

Analytical Specifications	Protein 80 Assay	Protein 230 Assay	High Sensitivity Protein 250 Assay
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 time	30 minutes	25 minutes	30 minutes
Samples per chip	10	10	10
Sample volume	4 µL	4 µL	5 µL
Kit stability	4 months	4 months	6 months
Kit size	250 samples/kit	250 samples/kit	100 samples/kit

CAII = Carbonic Anhydrase, BSA = Bovine Serum Albumin, BLG = beta-Lactoglobulin

Aplicaciones del Protein LabChip



Células lisadas

- Identificación sobre proteinas expresadas
- Comparación de diferentes formas de expresión

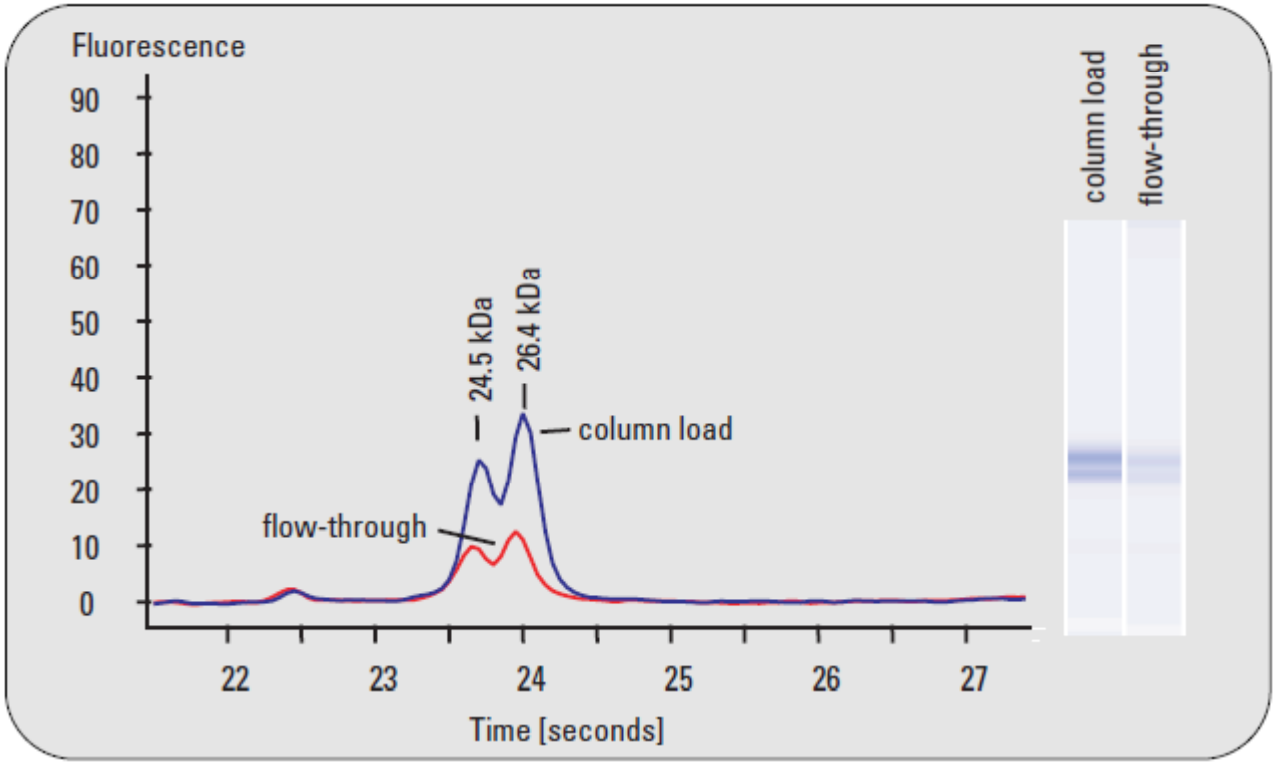
Fracciones de columna

- Monitorización de aislamiento de proteinas y purificación.
- Comprobación de fracciones(impurezas)

Purificar proteinas

- monitorizar impurezas
- QC de anticuerpos monoclonales y policlonales

Análisis de la capacidad de las columnas



Optimization of a Protein Purification Workflow

Application Note 5990-6153EN

The Bioanalyzer Protein 250 Kit is used to distinguish between His-tagged and cleaved protein species during a protein purification process. It helps with:
→Optimization of the sample purification process: cleavage of the His-tag
→Final sample QC.

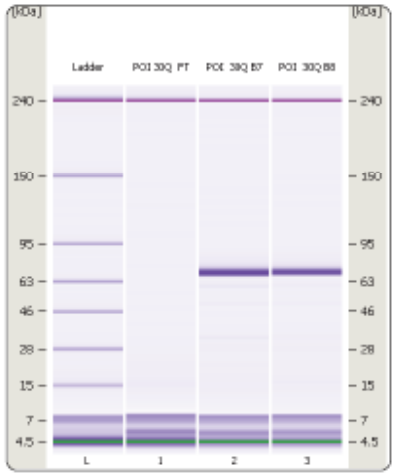
Optimization of on-column cleavage

	[kDa]	Rel. Conc. [ng/µL]	% Total
Unop	71.9	310.2	38.3
cleavag	81.9	414.7	51.3
Optimized	70.9	993.0	78.0
cleavage	83.2	11.4	0.9

➔ **Initial purity of the target protein: 38%**

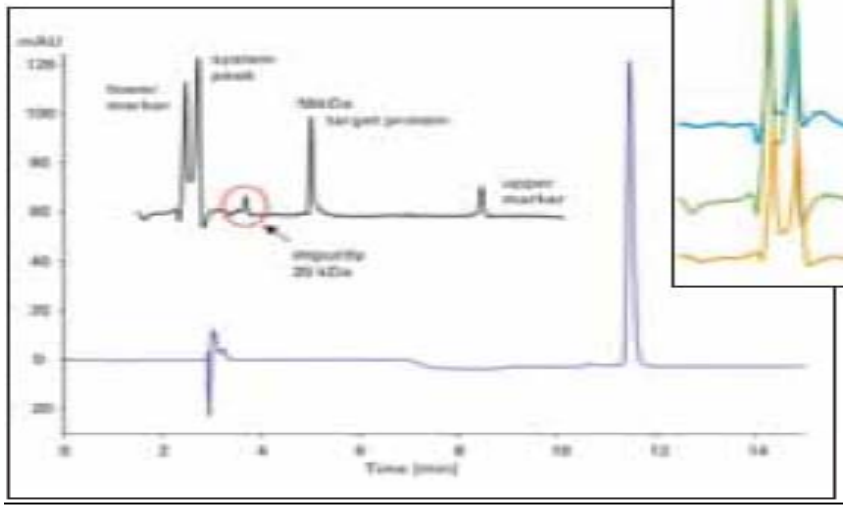
Final QC from optimized process

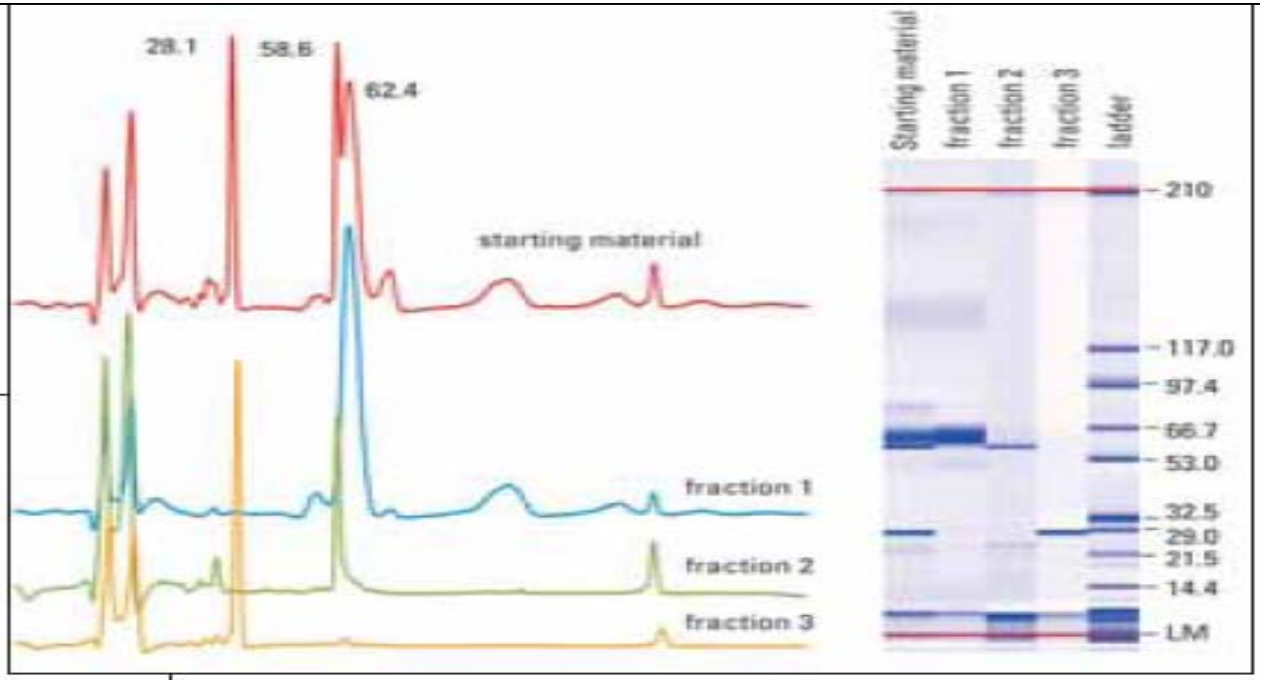
Size [kDa]	Rel. Conc. [ng/µL]	% Total
4.5	0.0	0.0
6	0.0	0.0
8.0	0.0	0.0
16.2	10.9	0.7
33.8	11.8	0.8
50.4	12.8	0.8
60.6	13.0	0.8
70.9	1,484.8	96.9
240.0 (marker)	60.0	0.0
324.8	0.0	0.0



➔ **Final purity of the target protein: 97%**

Complementing RP-HPLC protein purification

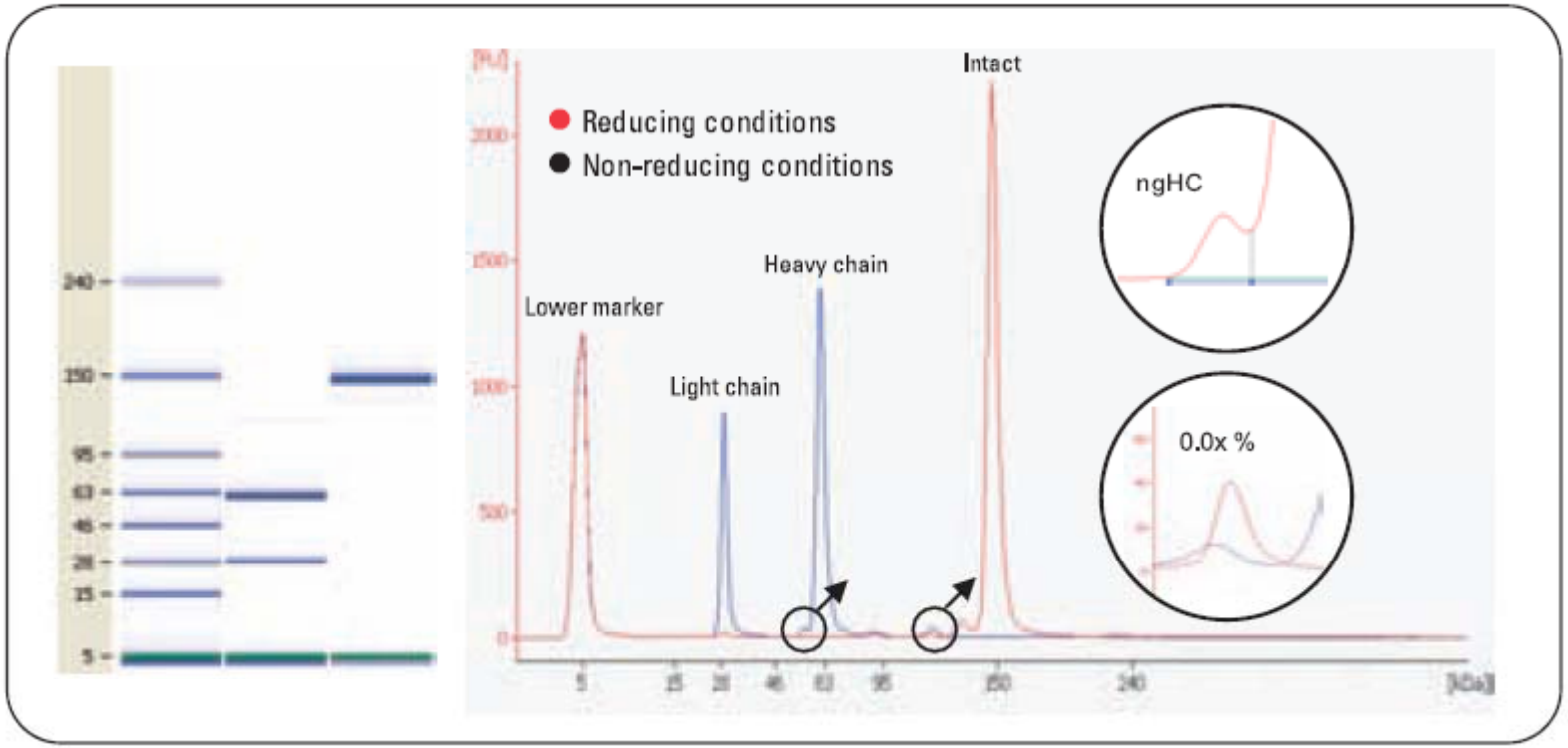




Protein purification and characterization was carried out facilitating an 1100 Series purification system for reverse phase HPLC assisted by the 2100 Bioanalyzer.

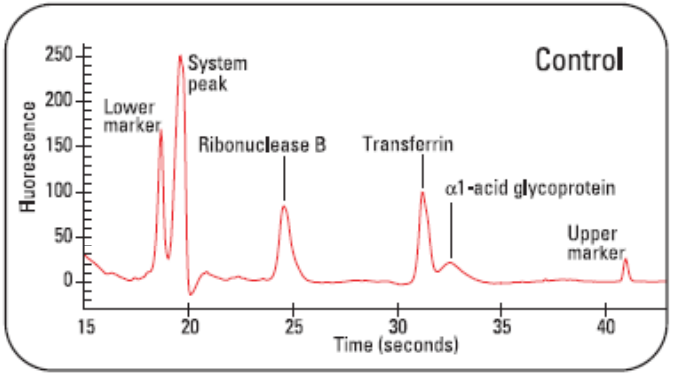
Análisis de anticuerpos

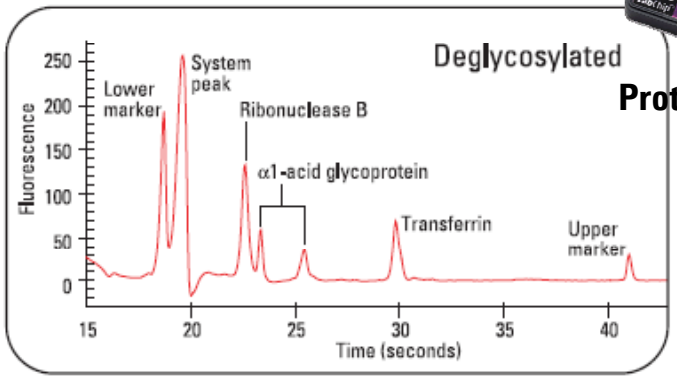
High sensitive Protein 250 kit

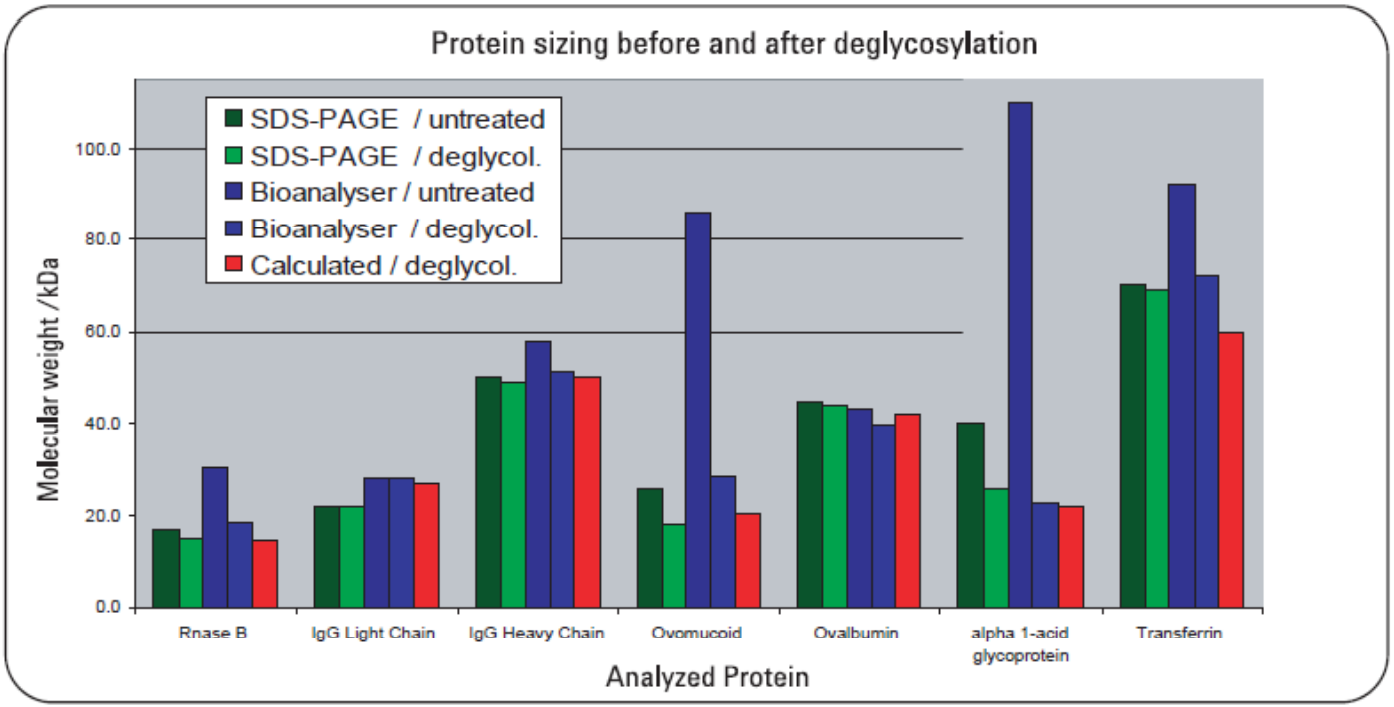


Glicosilación de proteínas

Protein 230 kit







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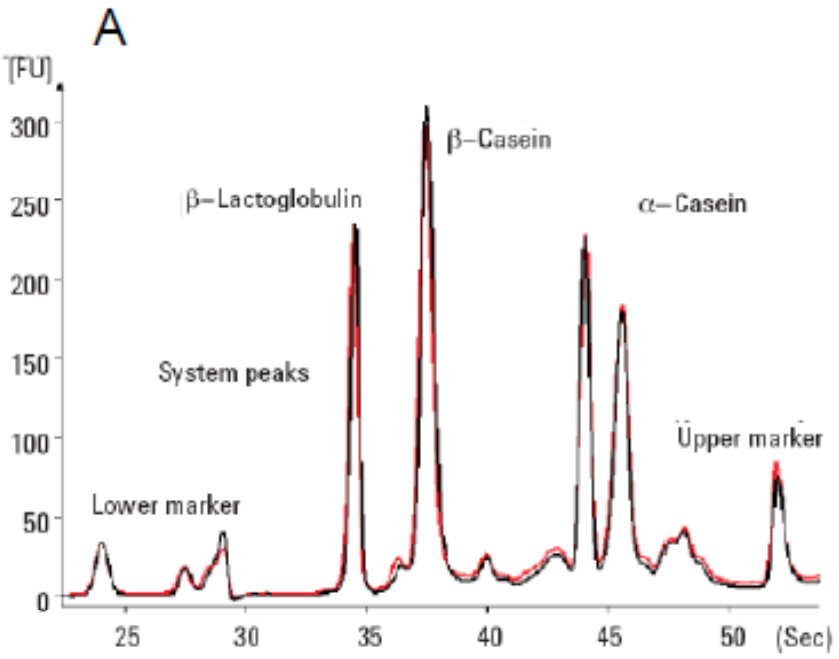
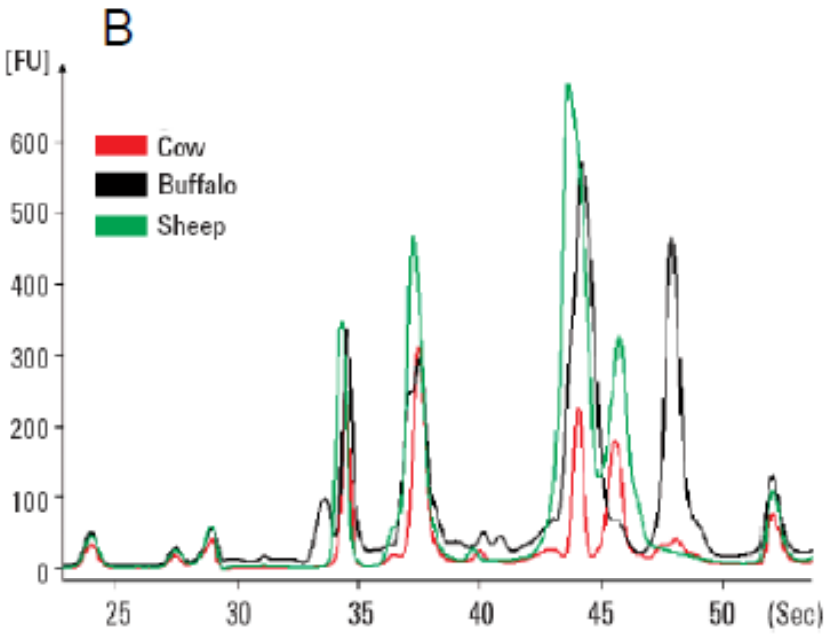
Análisis de proteínas en leche

Protein 80 kit





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Immunoprecipitation for quantification of β-galactosidase from crude *E. coli* cell lysate

Products:

2100 Bioanalyzer

Industry:

Academia

Author:

Ela Herwig, Technical University of Vienna

Application note publication number: 5990-7008EN

Publication date: January 1st, 2011

Highly sensitive and specific methods for the detection of target proteins in crude sample:

→ Traditional method: Western Blot of SDS-PAGE slab gels

→ New alternative method: Immunoprecipitation in combination with the High Sensitivity Protein 250 kit for the 2100 Bioanalyzer.

Advantages (see also 5989-4097EN):

→ Feature Combination of Western Blot and ELISA!

Sensitive: HSP-250 Kit for Bioanlayzer
Selective: Antibody-based in-solution pull-down
Quantitative: Electronic data from Bioanlayzer

mary

and secondary antibodies)

- Semi-quantitative & reproducible!
- → **Customer application example with β-Galacotsidase**

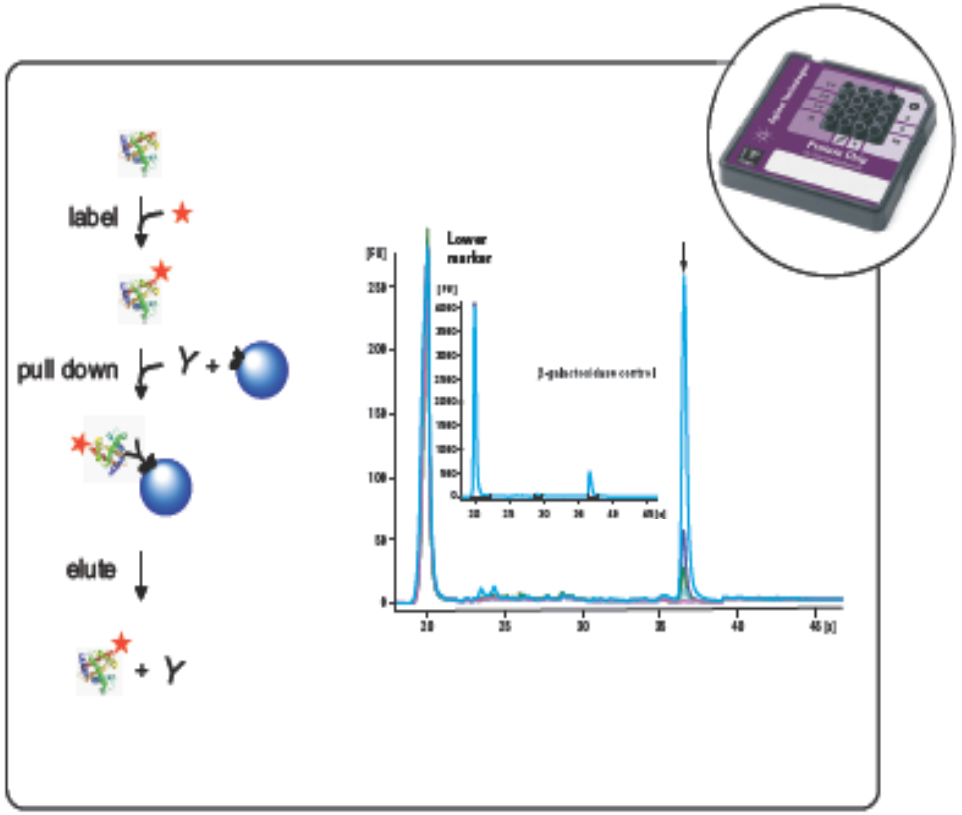
Immunoprecipitation for the specific detection of β-Galacotsidase (β-Gal)

This application example from the Technical University of Vienna shows:

- How to set up the **immunoprecipitation method**
- **Tuning** of the method:
  - Effects of Antibody selection
  - Effects of Antibody concentration
- How to establish a **calibration curve** for quantification
- **Reproducibility** of the method in determining the level of endogenously expressed target protein β-Gal

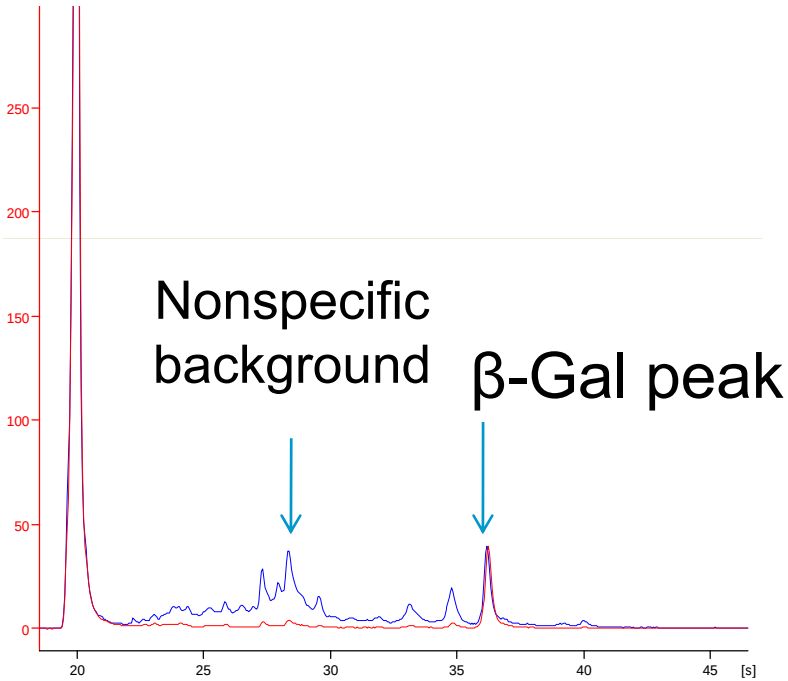
1. Immunoprecipitation workflow

1. Labeling of crude lysate expressing the target protein using reagents of the HSP-250 kit (5067-1575).
2. Immunoprecipitation of the target protein with specific antibody and Protein G coupled to magnetic beads.
3. Elution of all bead-bound proteins with SDS sample buffer of the HSP-250 kit.
4. Direct analysis on the 2100 Bioanalyzer using the HSP-250 Protein Chip and reagents.



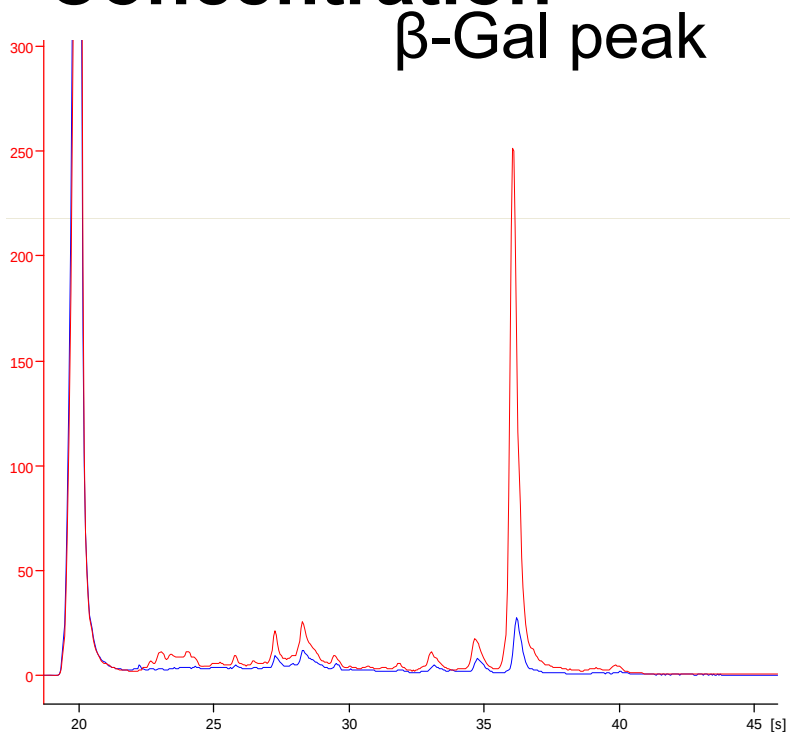
2. Set-up

A. Antibody Selection



One antibody shows significantly more non-specific background (blue) than the other (red)!

B. Antibody Concentration



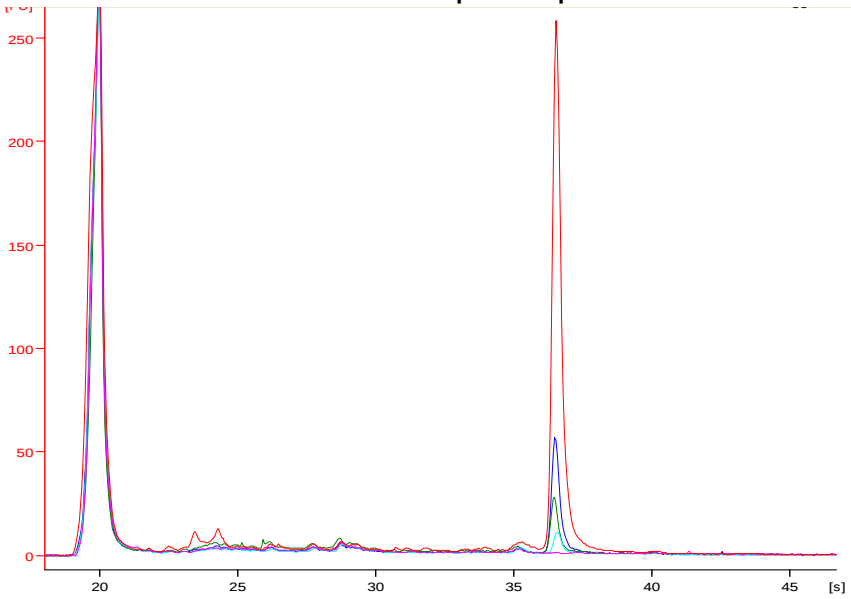
Antibody concentration influences target signal and non-sepcific background.

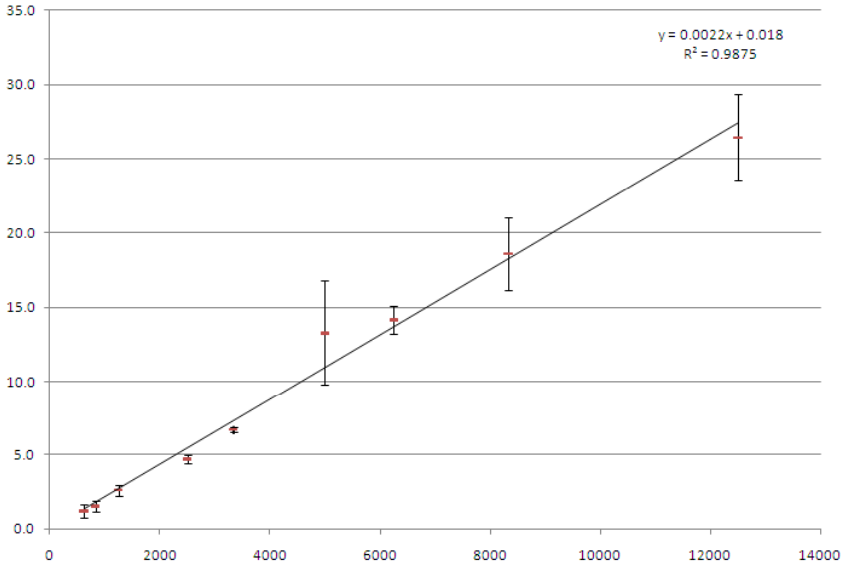
3. Calibration curve for quantification

Increasing concentration of spiked-in β-Gal into crude lysate returns increasing signal from IP.

➔ Calibration Curve based on the linear relationship between initial β-Gal concentration in the lysate and peak intensities.

β-Gal peak





4. Detection of endogenously expressed β-Gal

Detection and quantification of endogenously expressed β-Galactosidase in crude lysate:

Dilution factor of applied antibody	Migration time corrected area	Calculated amount of β-galactosidase (ng/µL)	Mean value (ng/µL) (n = 3)
1000	54,4	24,8	22,5 ± 1,9
2000	23,5	21,4	
5000	9,4	21,4	

Approach is proving its reliability as results are consistent and reproducible, even across experiments in which the antibody concentration was varied.

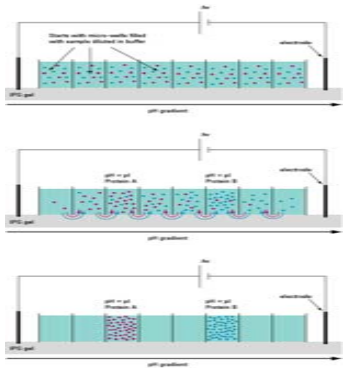
Geles en 2D: Combinación de Offgel y Bioanalizador

High sensitive Protein 250 kit

E. coli lysate (50 µg)

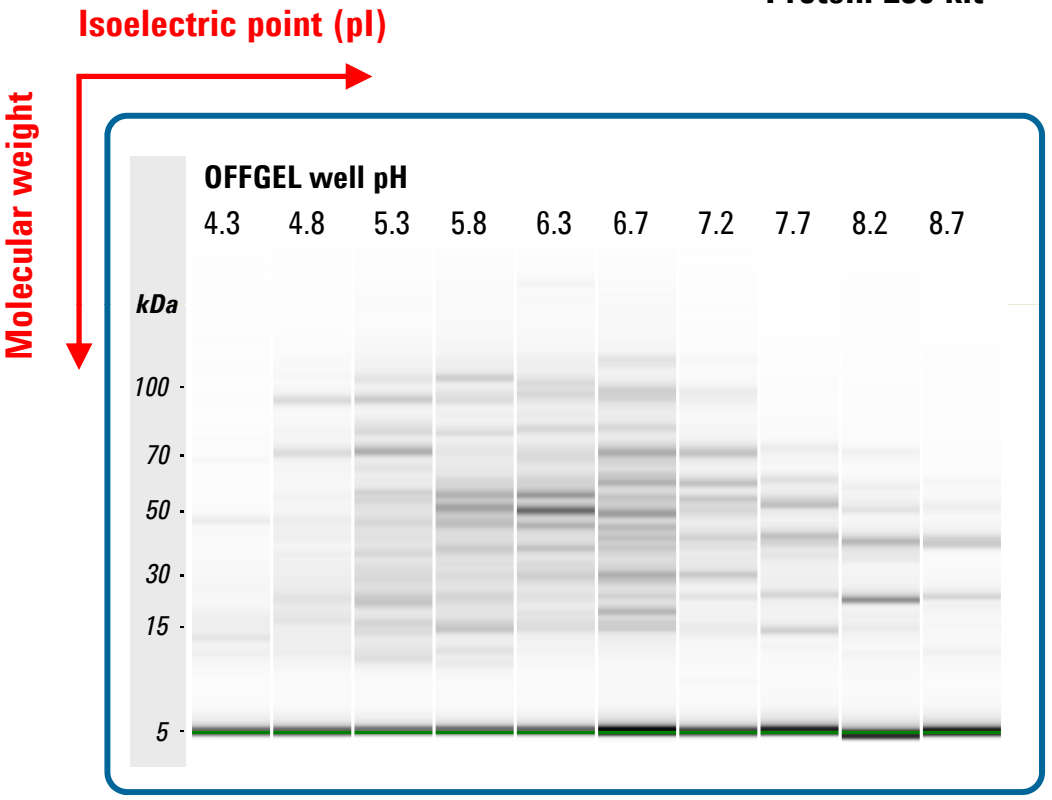
Protein clean up and labeling

OFFGEL Electrophoresis



2100 Bioanalyzer

HiSens Assay



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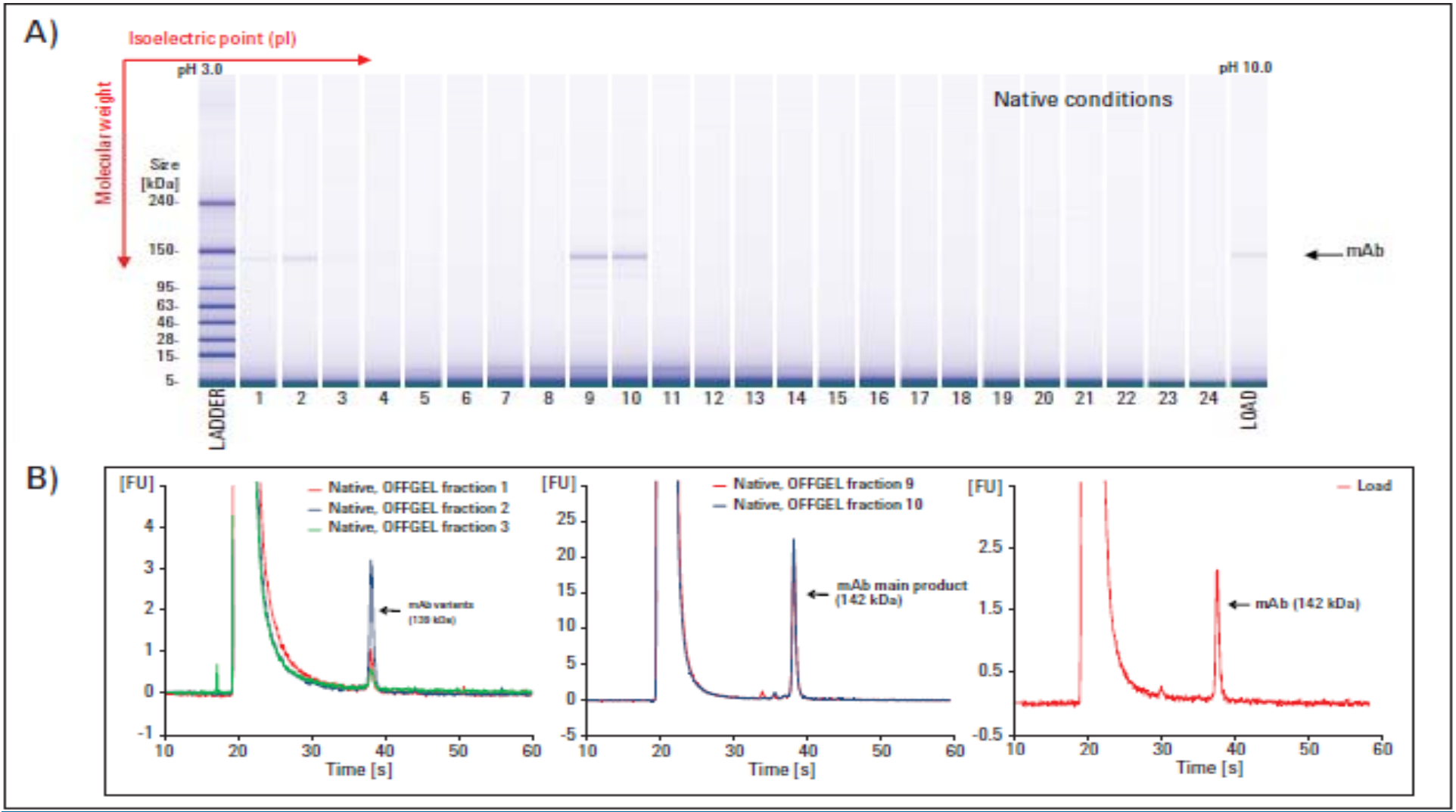
Monitoring antibody charge variants



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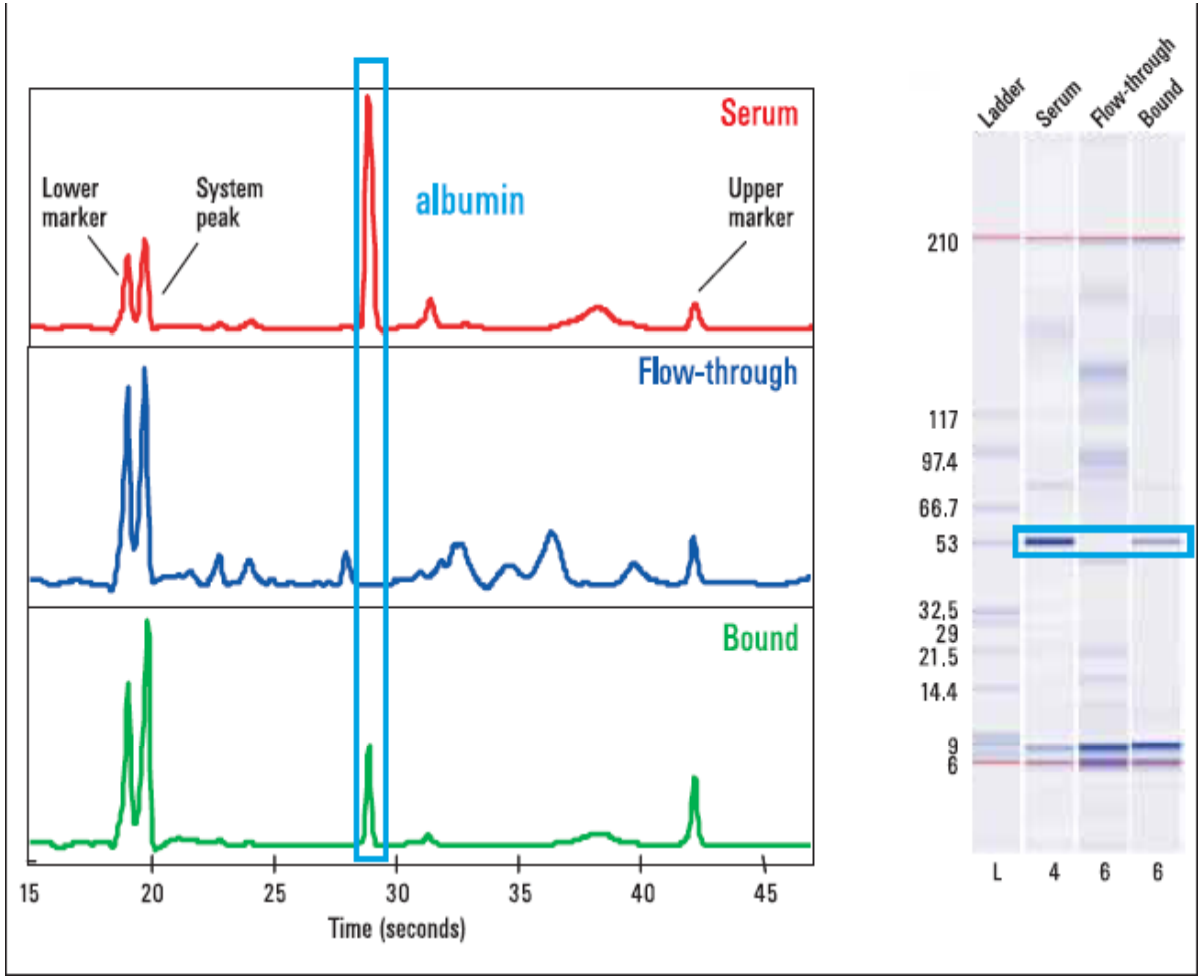
Depletion of high abundant proteins from blood samples

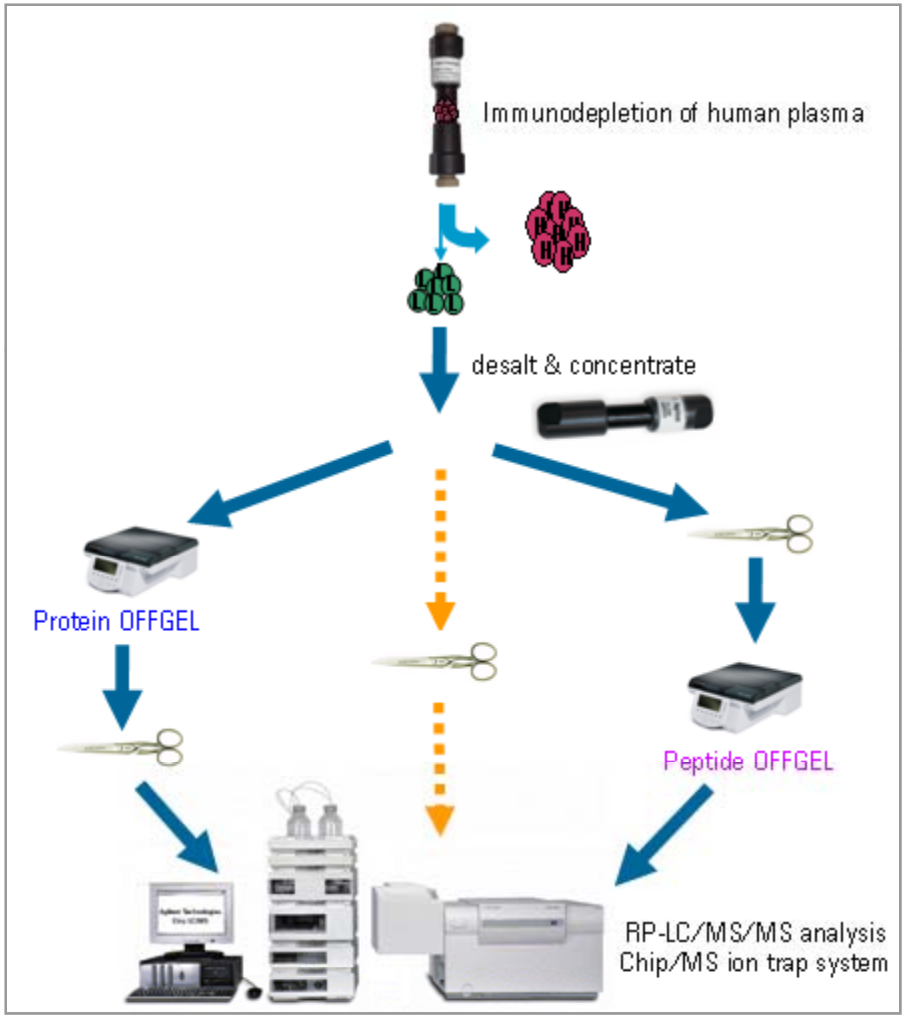


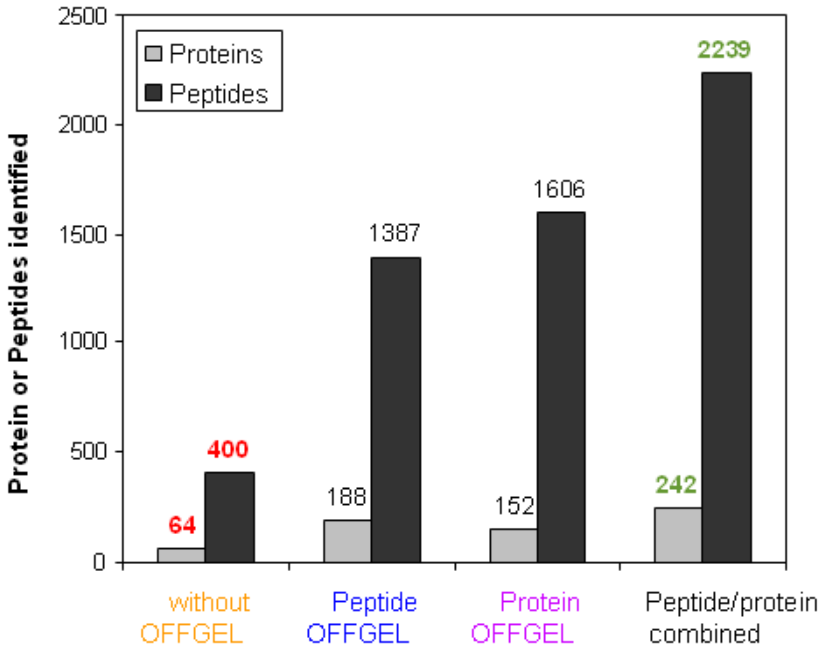
Depletion of high abundant proteins in human blood plasma was facilitated by the Agilent Multiple Affinity Removal System

OFFGEL

OFFGEL Incremento de la sensibilidad en MS

ejemplo de trabajo

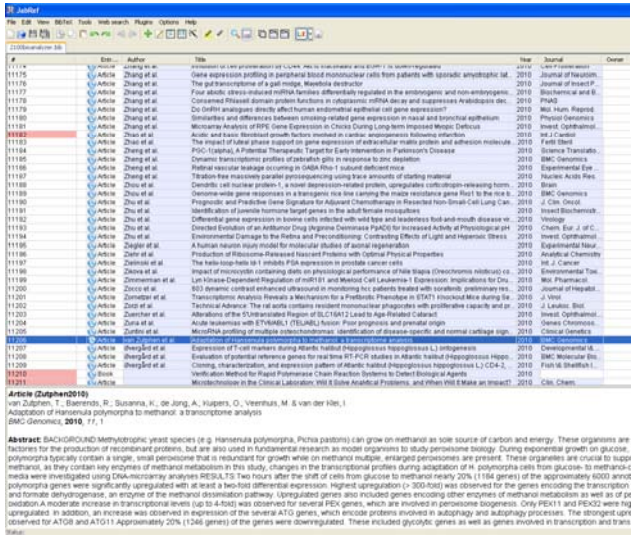




Incremento de 4x en la detección de proteínas tras el fraccionamiento con el OFFGEL

Bioanalyzer Literature

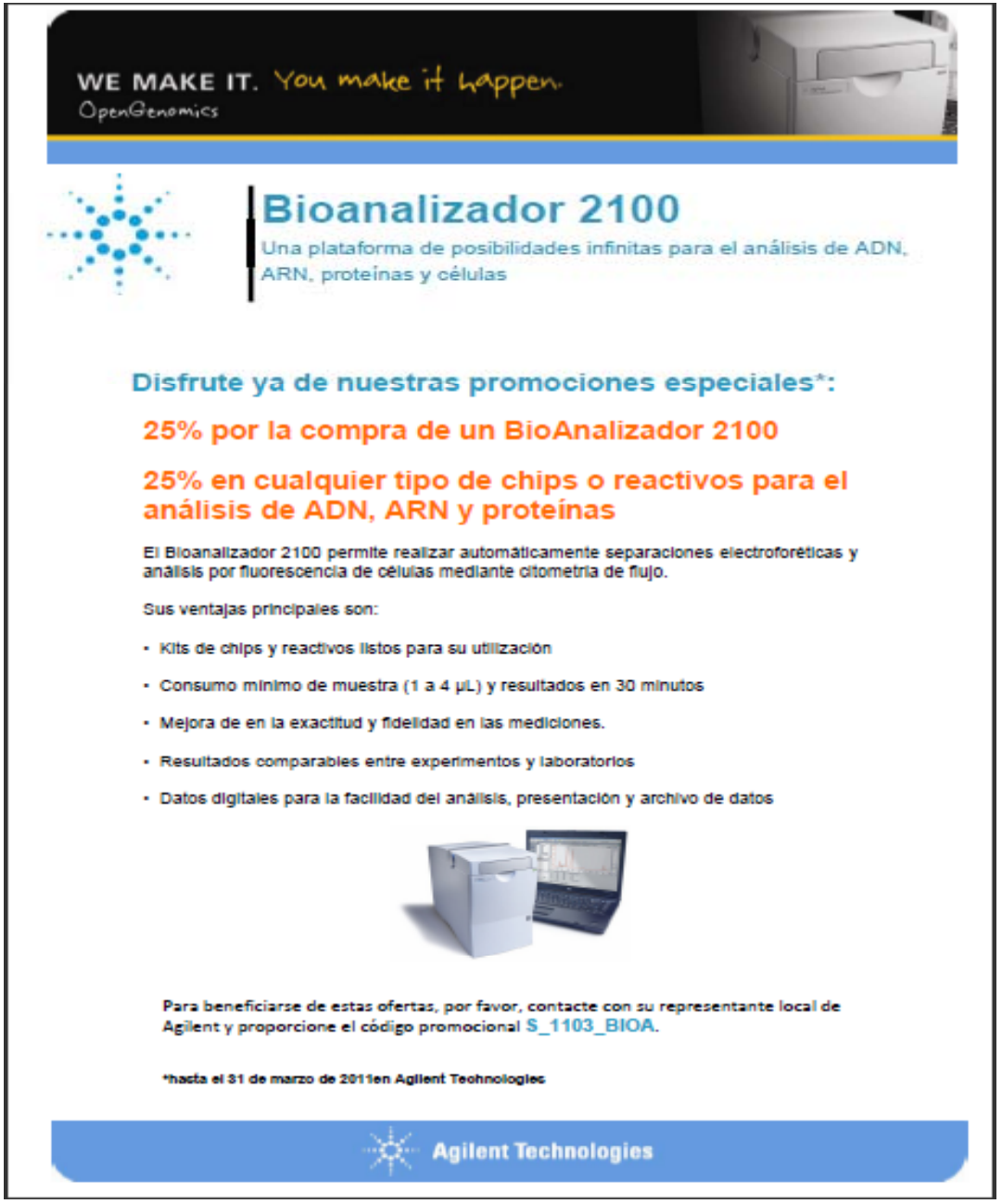
Bioanalyzer Application Compendium 5990-6987EN
Now with all the latest applications in NGS!



Bioanalyzer Reference Database

- includes publications in scientific journals and books
- 11211 references
- updated every 6 months
- available for download under Bioanlayzer Resources:

http://www.genomics.agilent.com/GenericA.aspx?PageType=Custom&SubPageType=Custom&PageID=2153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*hasta el 31 de marzo de 2011en Agilent Technologies
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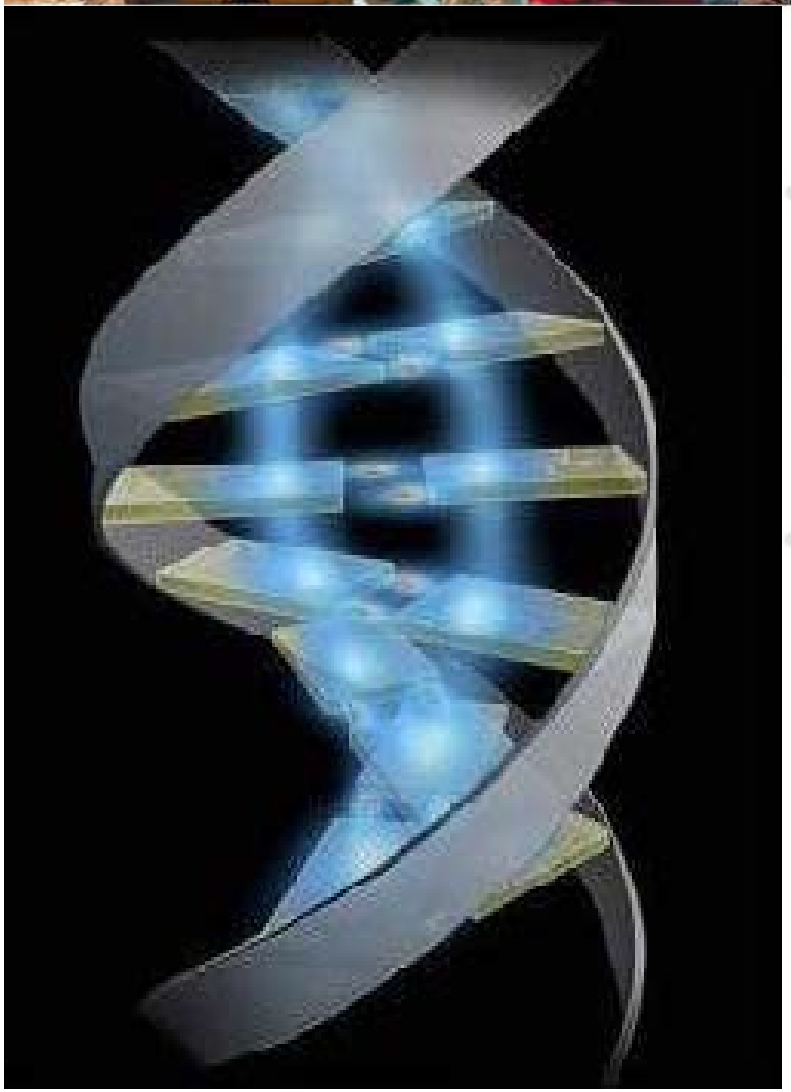

Muchas gracias!!

¿Preguntas?

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