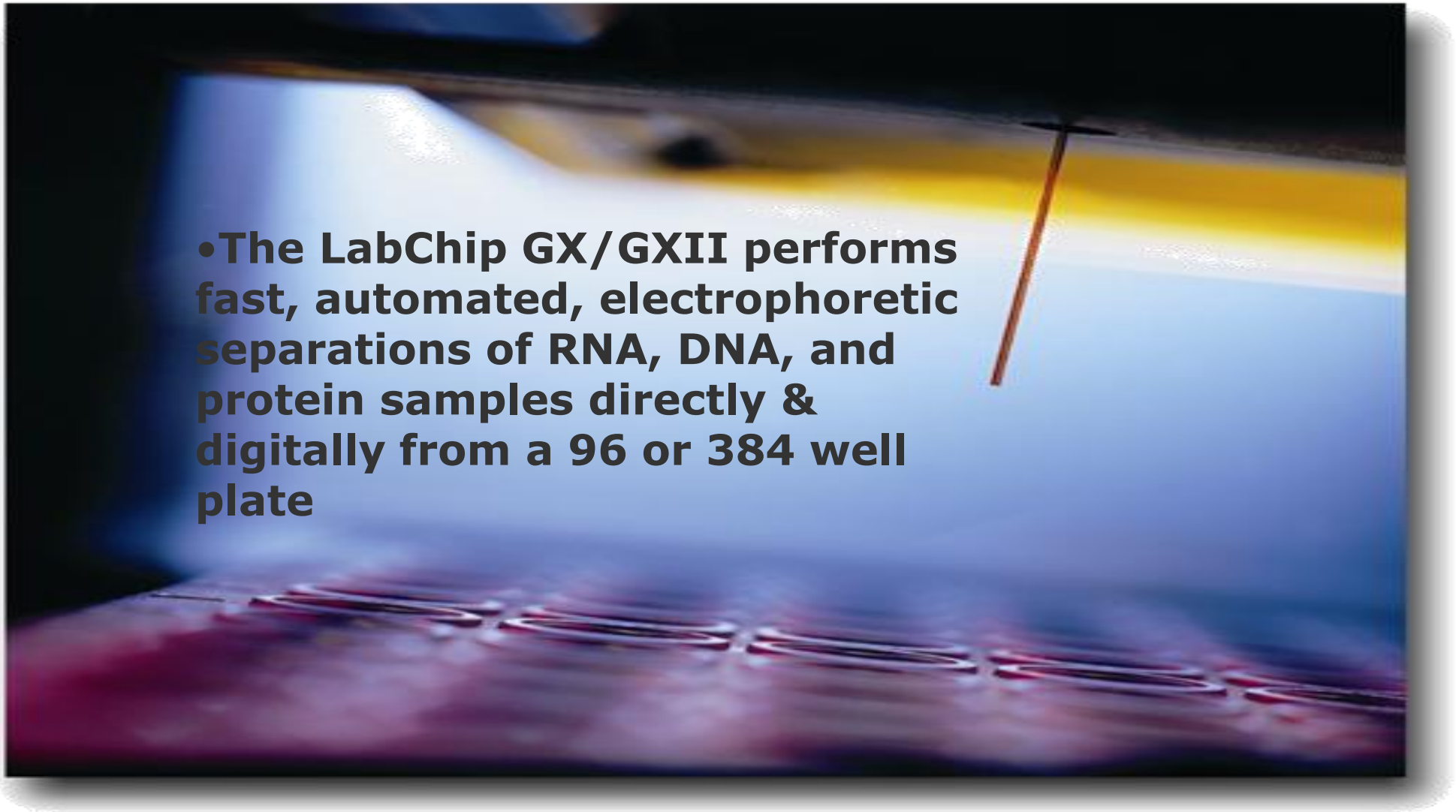
Caliper LabChip GX/GXII Microfluidic System

進階生物科技
產品專員　廖哲鋒

Caliper LifeSciences

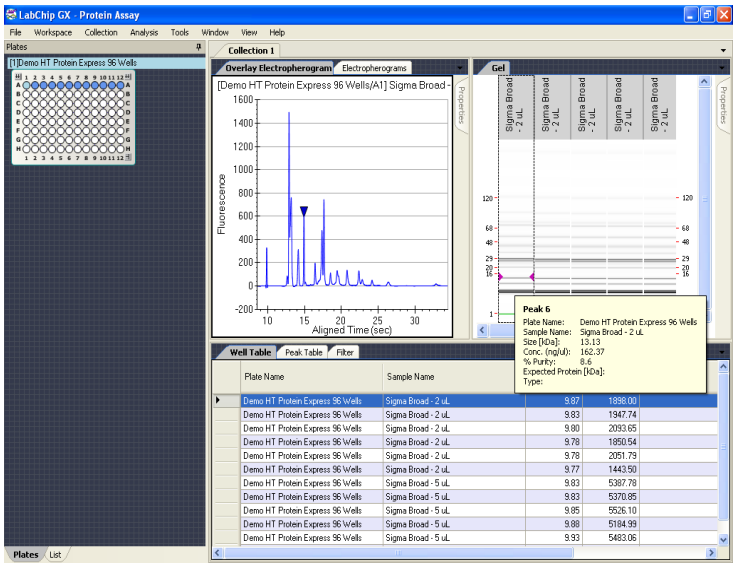

•The LabChip GX/GXII performs fast, automated, electrophoretic separations of RNA, DNA, and protein samples directly & digitally from a 96 or 384 well plate

LabChip GX & GXII System Components

LabChip GX/GXII Instrument

- PC Controller
- Integrated Barcode Scanner

LabChip GX/GXII Software

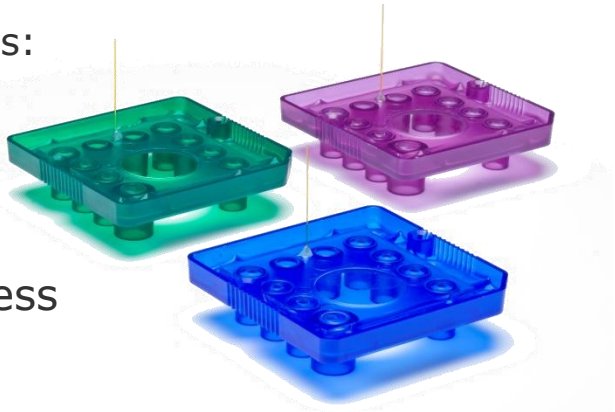


- Electropherogram, Virtual Gel, and Tabular Views
- Powerful Data Filters

LabChip Kits:

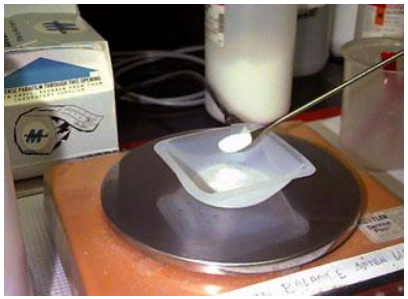
Wide Variety of Assays:

- HTRNA
- HTDNA 5K
- HTDNA 12K
- HTDNA 1K
- HT Protein Express



Replacement technology for agarose slab gel

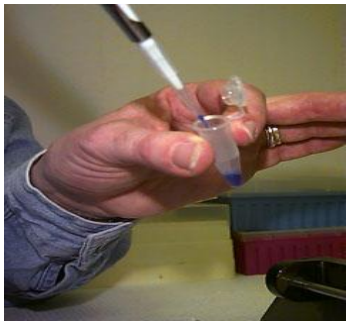
Traditional gel electrophoresis -- Timeline to Result



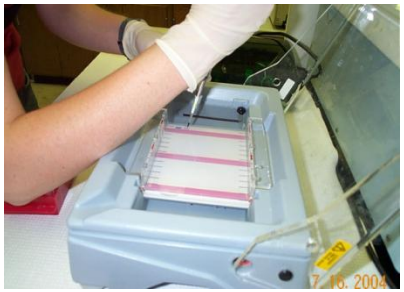
Weigh powder

Mix gel

Cast gel



Mix Dye

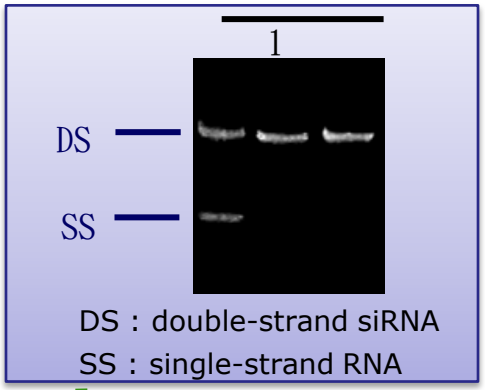


Running Time

30~40 min

Load & Run samples

Scan gel

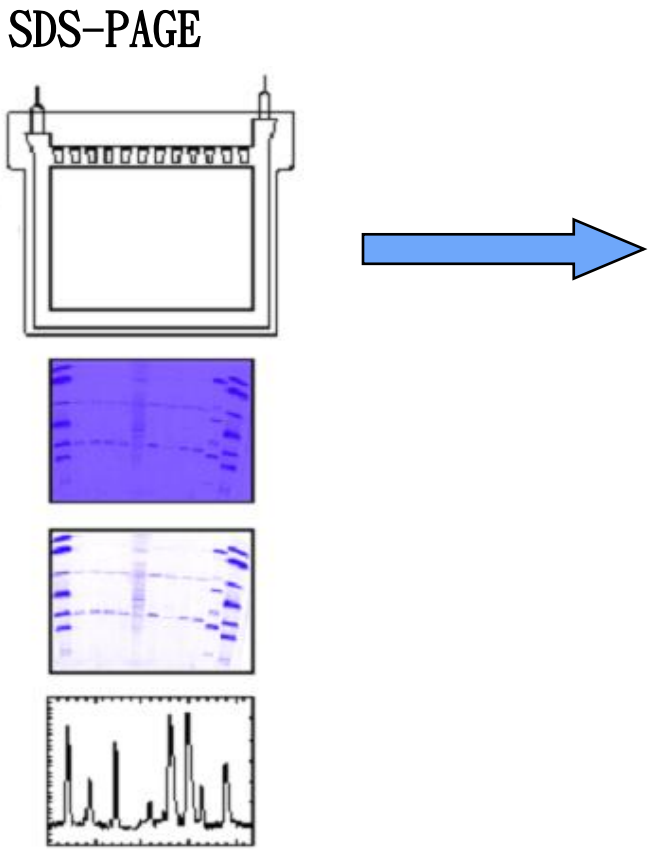


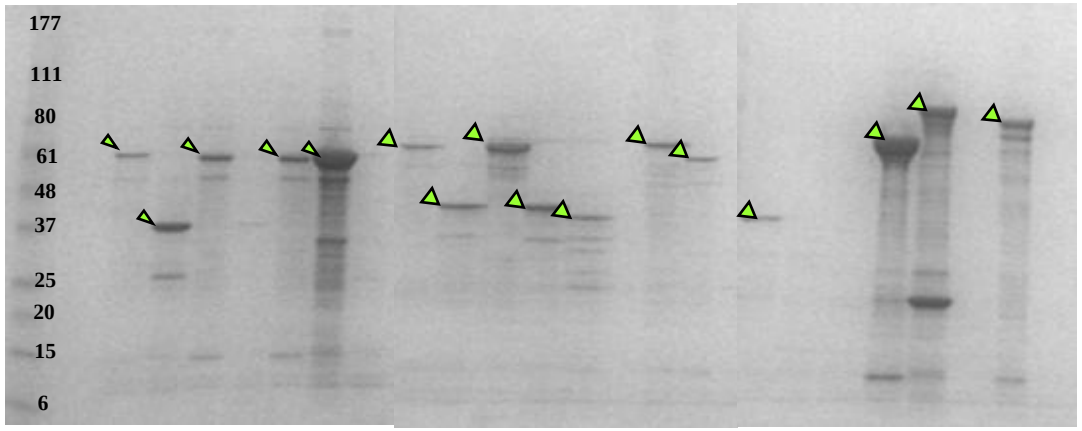
DS : double-strand siRNA

SS : single-strand RNA

2 - 4 hours !

SDS-PAGE Protein Gel Analysis



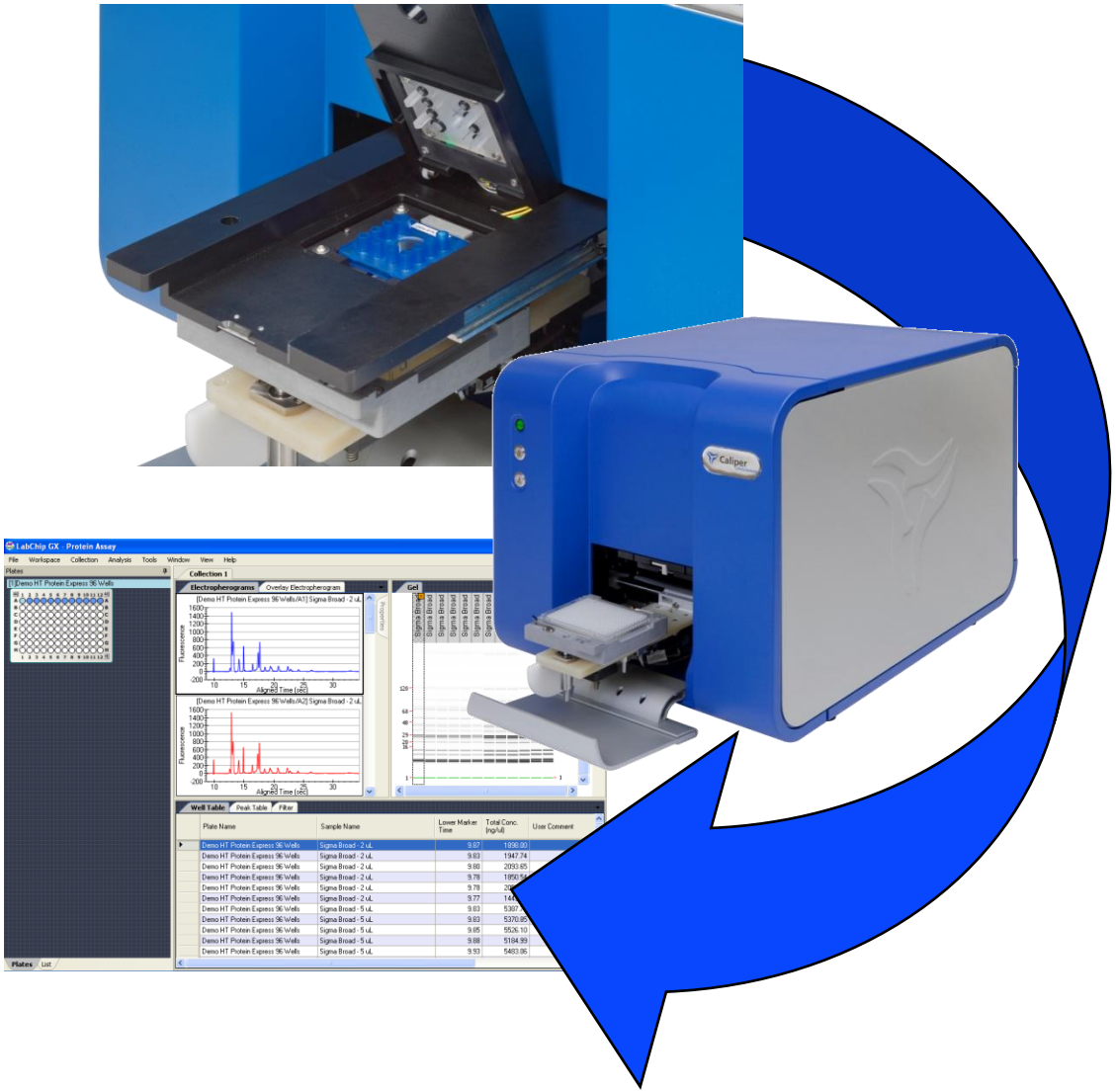


Limitations:

- Concentration and purity often determined by eye
- Qualitative result
- Comparison across multiple runs is limited

Data provided courtesy of Dr. TVS Murthy, Harvard Institute of Proteomics

LabChip GX/GXII Process Flow

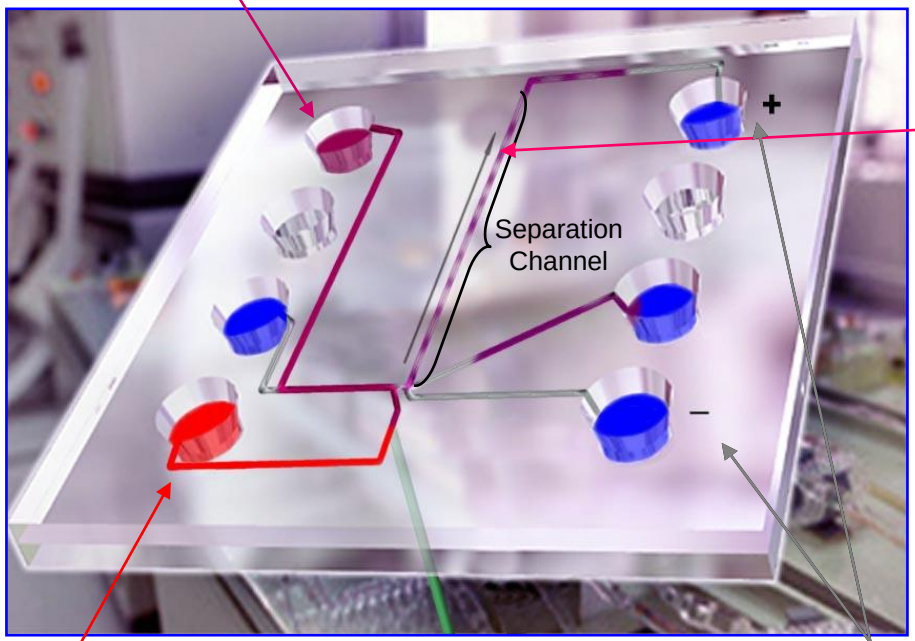


Steps:

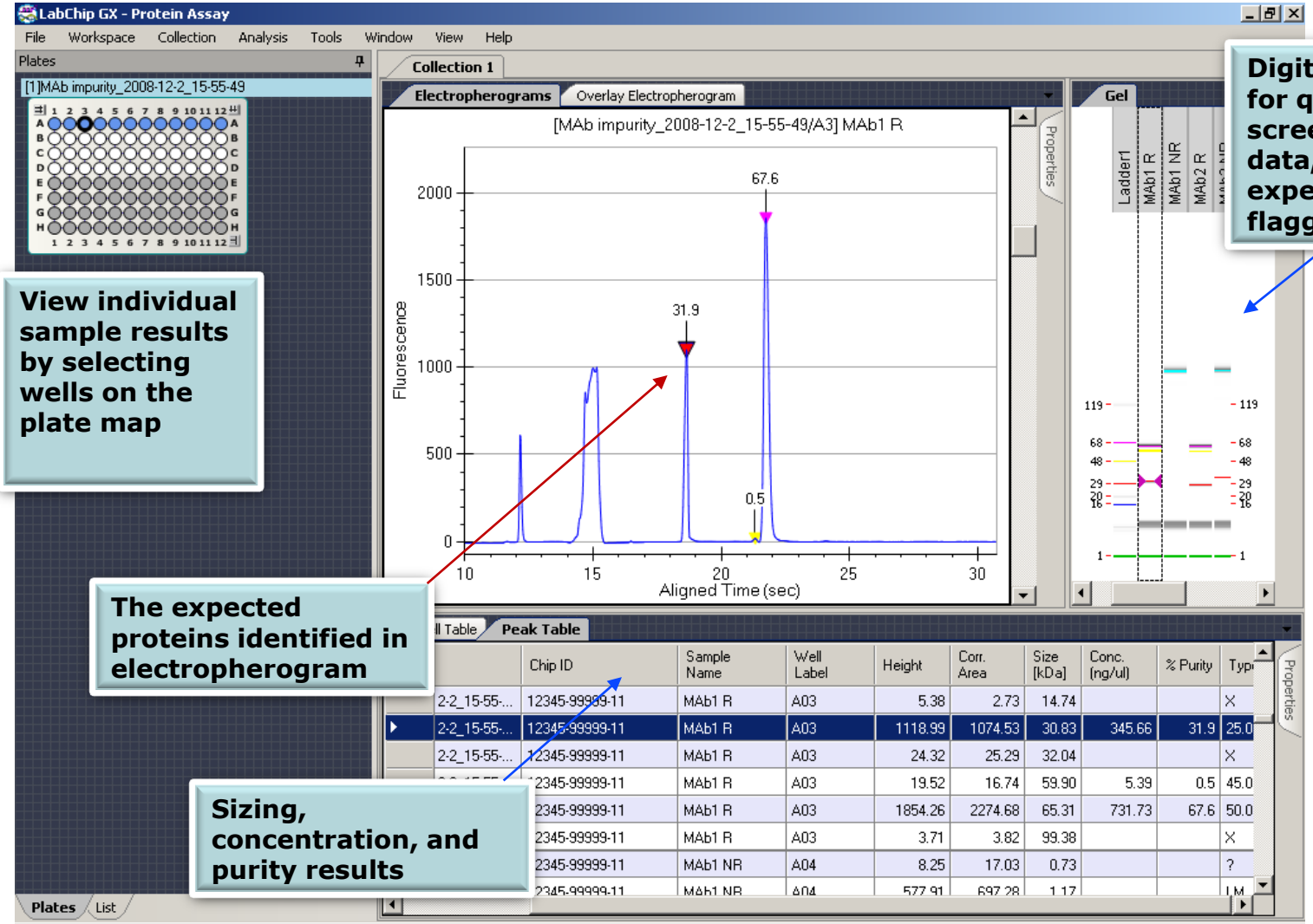
- Prepare the chip
- Insert sample plate
- Load the chip
- Run automated assay
- View results in minutes!

Microfluidic Technology

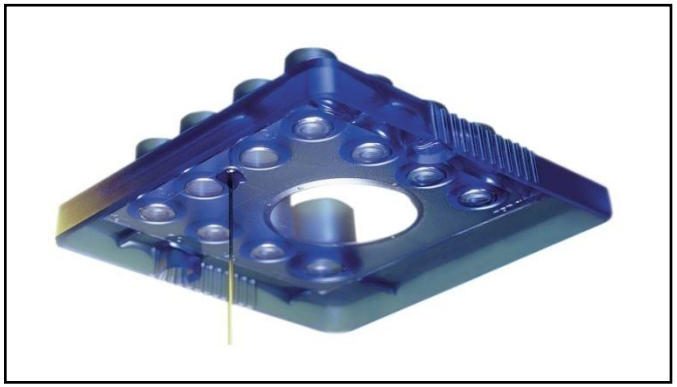
- Buffer and ladder placed on LCGX
- Dye-Gel Mix introduced
- Marker loaded
- Chip loaded into instrument
- START
- Chip pressurized
- DNA is separated and detected in the separation channel



Data Analysis Software



DNA Analysis on the LabChip GX Systems



DNA Chip

Integrates the entire agarose slab gel process onto a microfluidic chip

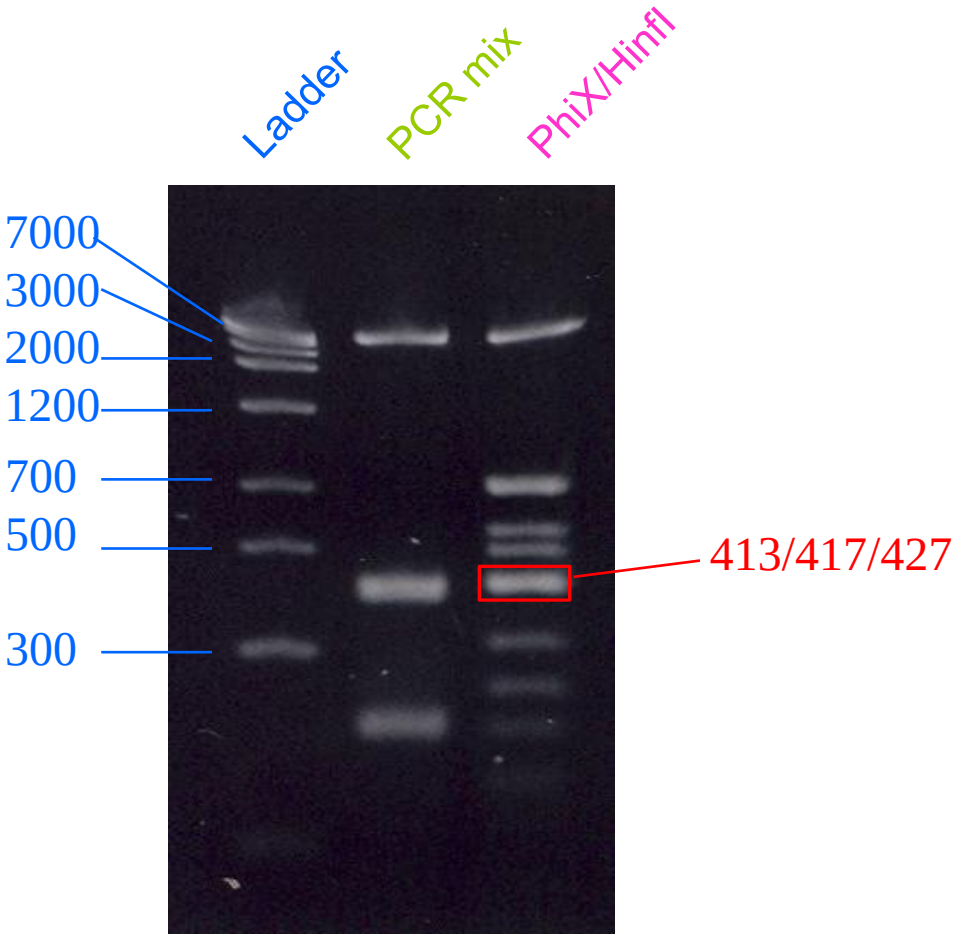
Automatic sampling, electrophoresis and reporting of dsDNA

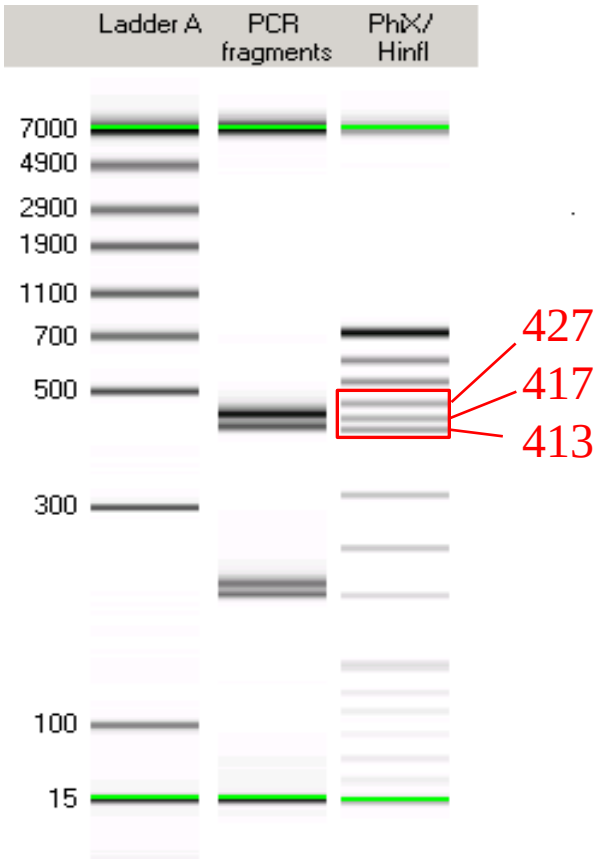
DNA sizing and quantitation in 30 seconds per sample

96-well plates analyzed in 45 minutes, 384 well plates in 4 hours

Ideal for the analysis of PCR products, restriction digests, RFLP

Resolution Comparison – 2% Agarose Gel and DNA5K Assay

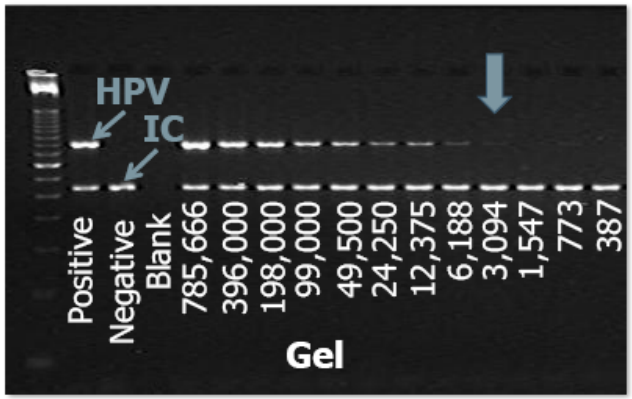


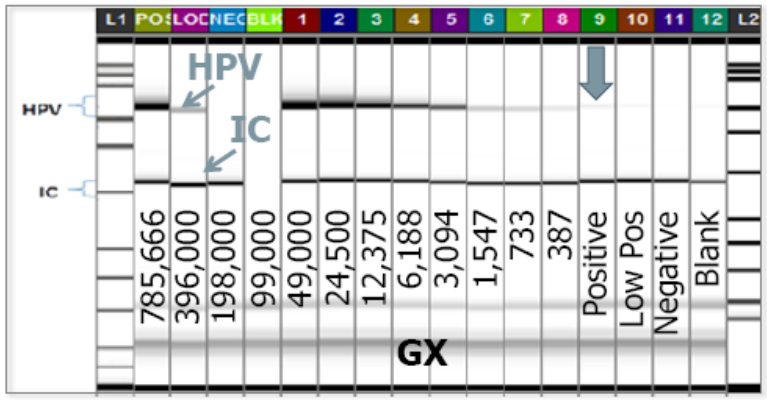


access genetics

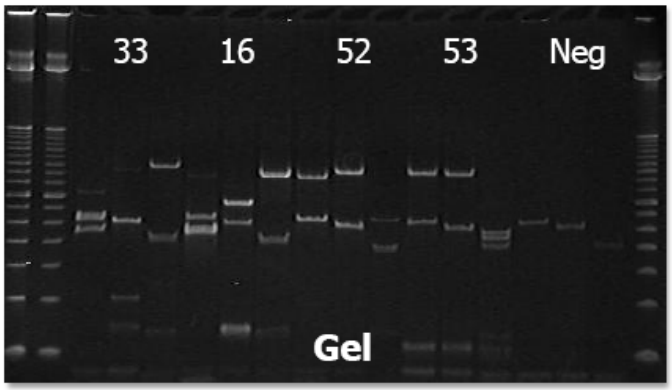
HPV Detection

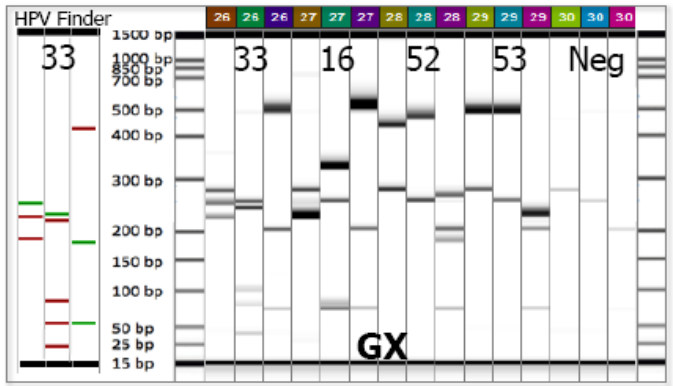
LabChip® GX data was compared to visual interpretation of gel electrophoresis.





High Sensitivity
HPV Detection

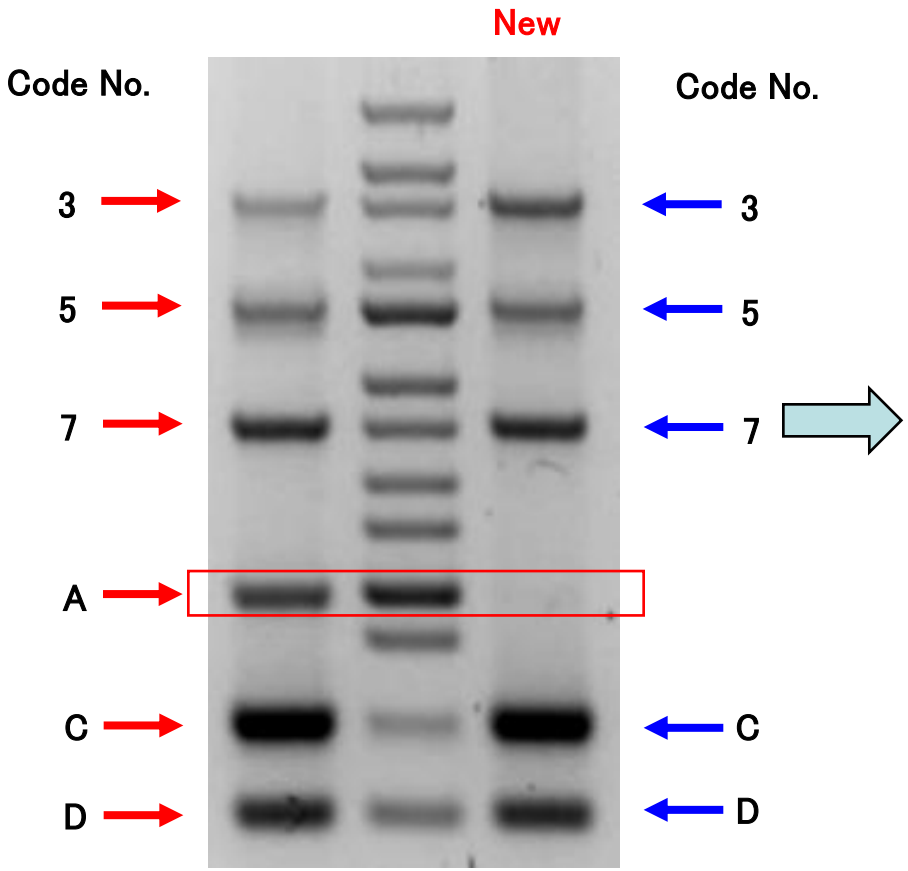




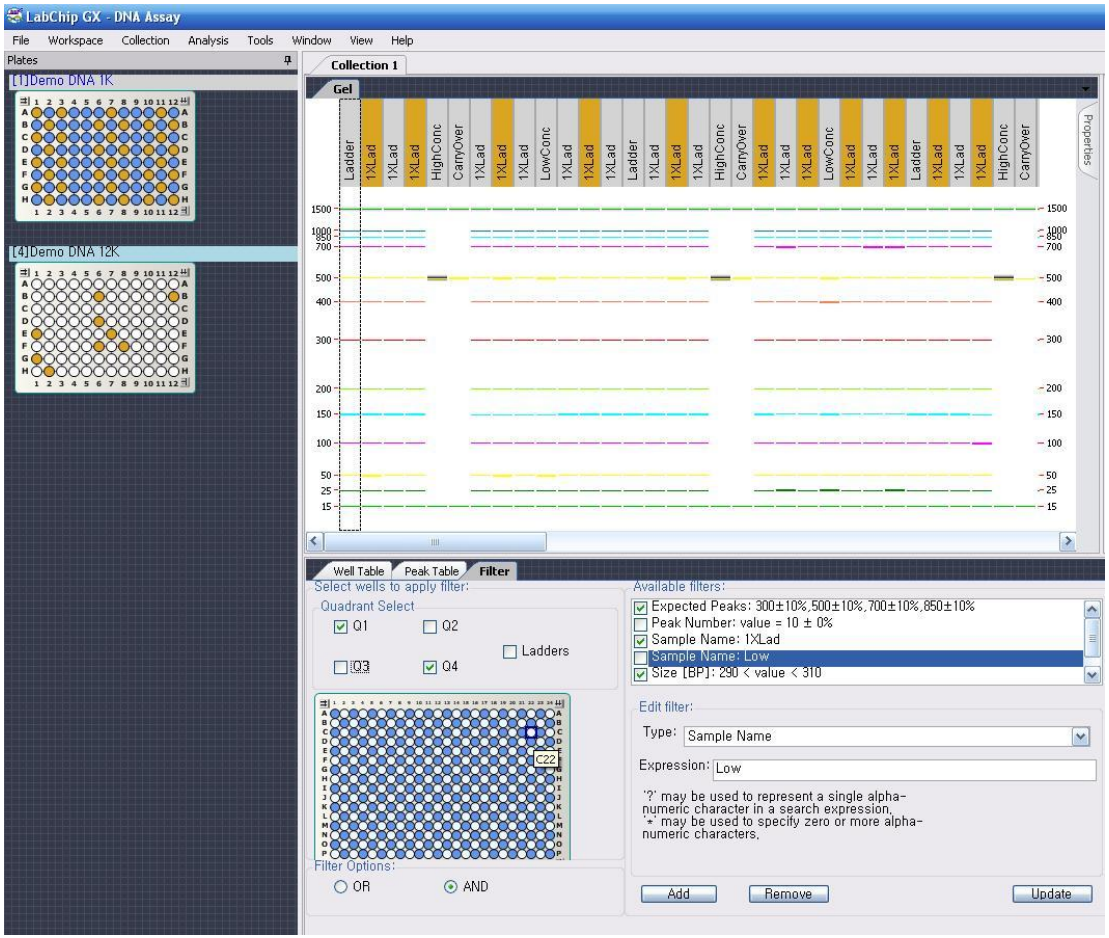
Auto Typing of strain
(HPV type 33 is shown here)

Five HPV ID types are shown for both methods. Each sample contains the IC and uses 3 lanes. TeleGene™ HPV Finder Tool displays the RFLP pattern for HPV type 33 (red) and the IC (green).

Allele-specific PCR Result Analysis



Verification and classification with LC-GX software



Influenza Viral Strain Typing

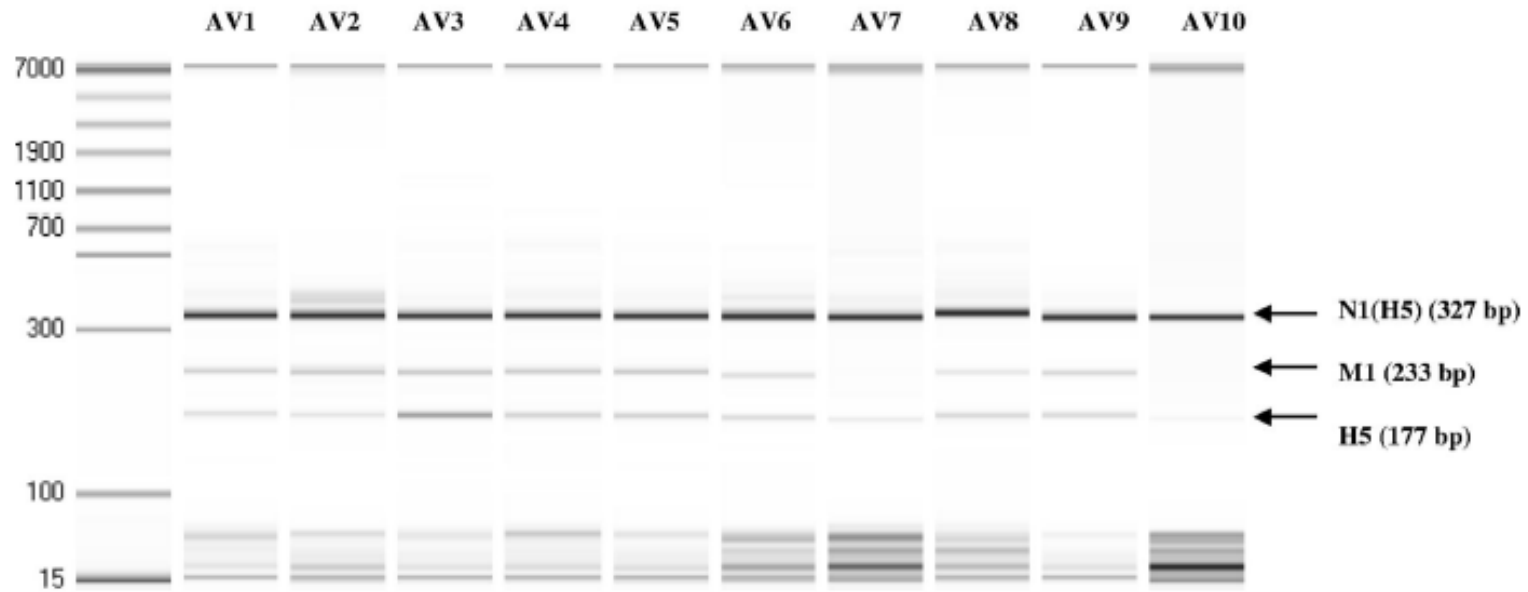


FIG. 2. NC400 data from the eMA influenza virus genotyping assay and microcapillary electrophoresis gel, showing the detection of influenza virus in 10 avian specimens at $1/10^2$ dilutions of RNA. Red fluorescent signals are represented by shading. Green fluorescent signals are represented by unshaded numbers. The SNR is the ratio of the RFU on a test site to the RFU on a background site (Bkgd). Typing and subtyping are called when the SNR is ≥3.0.

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0095-1137/09/$08.00+0 doi:10.1128/JCM.01807-08

Multiplex Assay for Simultaneously Typing and Subtyping Influenza Viruses by Use of an Electronic Microarray▽

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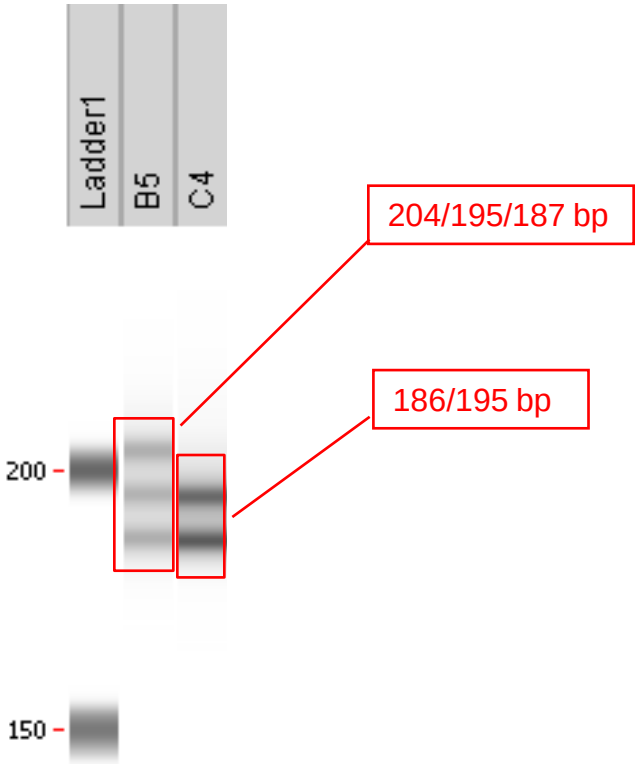
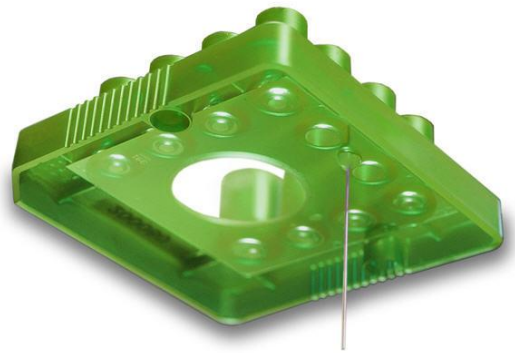
Nanogen, Inc., San Diego, California 92121,[1] and Department of Pediatrics[2] and Midwest Respiratory Virus Program,[3] Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Actual Performance –
親子鑑定-馬偕醫院醫研部



Sample Name	Size [BP]	Conc. (ng/ul)	Molarity (nmol/l)
B5	187	0.8322	6.75
B5	195	0.8169	6.34
B5	204	0.9356	6.96
C4	186	1.7749	14.43
C4	195	2.1572	16.78

RNA Analysis on the LabChip GX Systems



RNA Chip

Integrates the entire sample analysis process onto a microfluidic chip

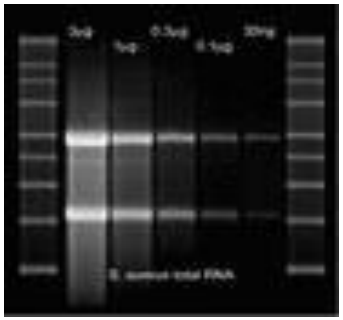
Automatic sampling, electrophoresis and reporting of RNA integrity

RNA sizing and quantitation in 80 seconds per sample

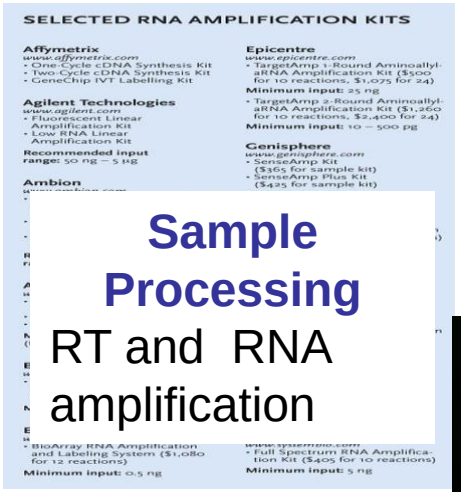
96-well plates analyzed in 150 minutes

Ideal for confirming RNA integrity in advance of RT-PCR or microarray study

Gene Expression WorkFlow

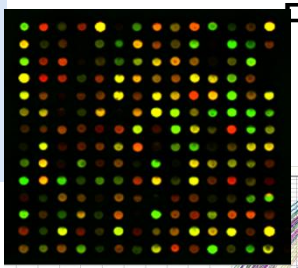


Sample Preparation:
RNA isolation and QC

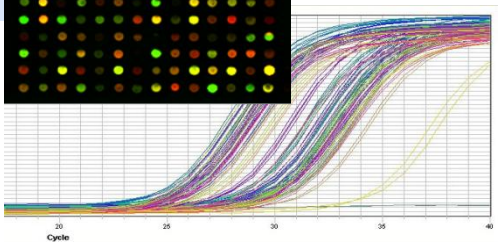


Sample Processing
RT and RNA amplification

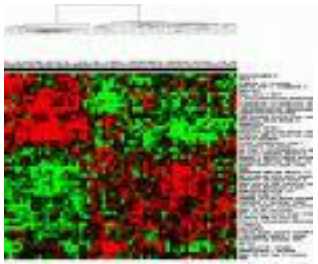
Gene Expression Tools:
Microarrays,

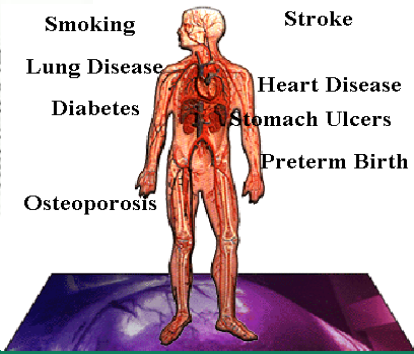


Real-Time PCR



Data Analysis:
Bioinformatics



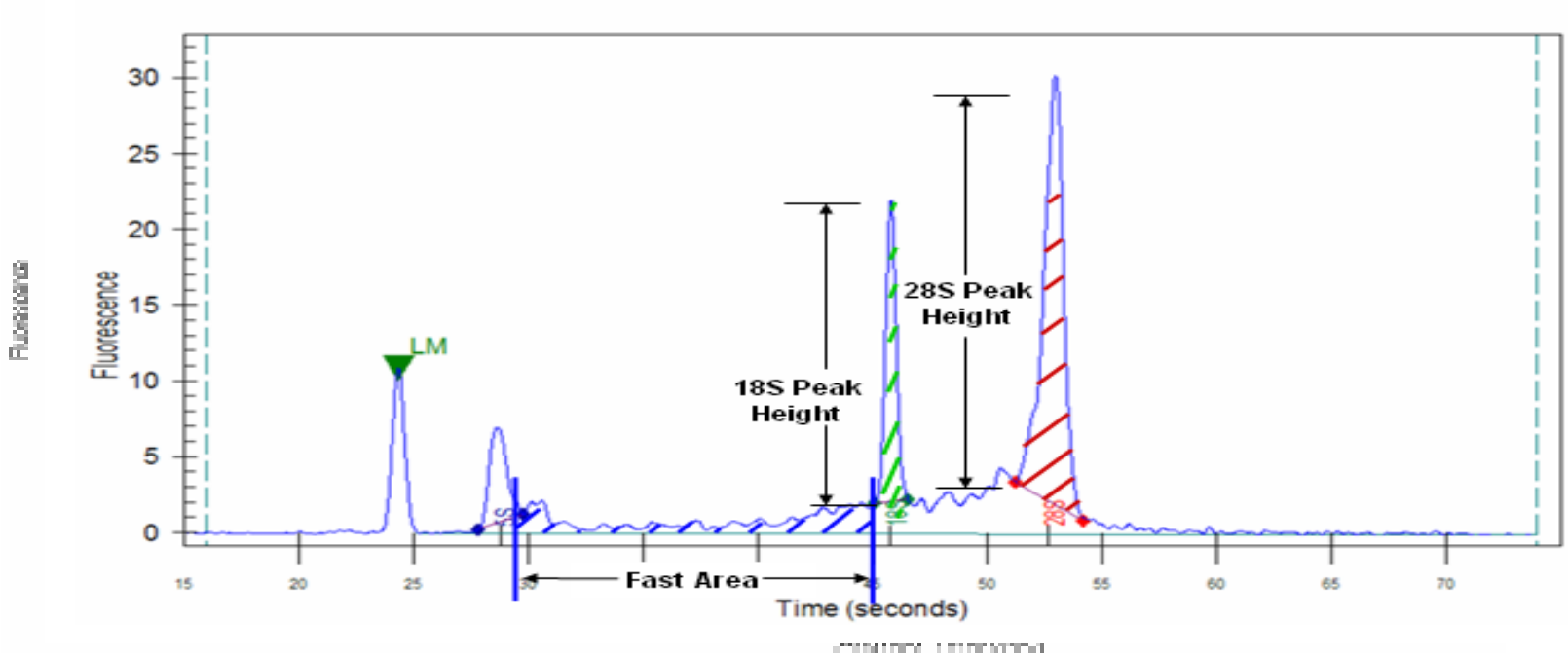


Downstream results are dependent upon high quality RNA samples

RNA Quality Score, definition

$$QS = A + \left(1 - \frac{FastRegionArea}{TotalArea}\right) * X_1 + \left(\frac{18SArea + 28SArea}{TotalArea}\right) * X_2 + \left(\frac{28SHeight}{18SHeight}\right) * X_3$$

- Only one number for assessing RNA Integrity
- Integrity score values FastRegionArea, 18SArea and 28SArea
- 18SArea and 28SArea will decrease whereas the FastRegionArea will increase over the degradation process

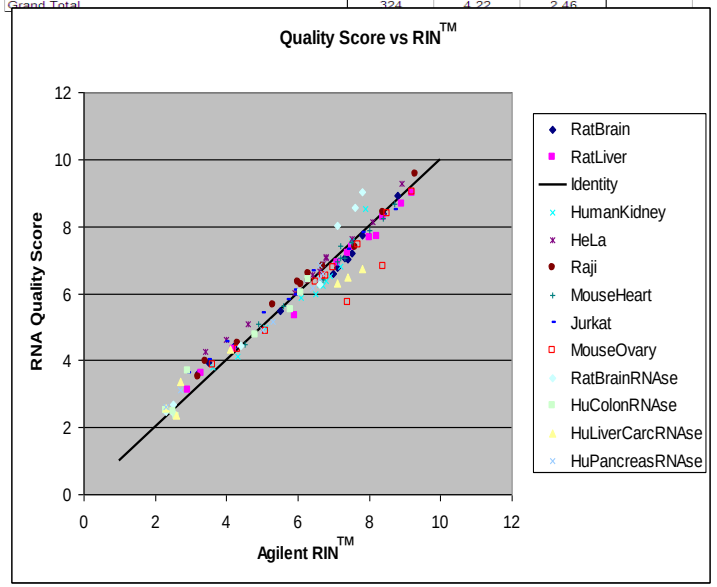


RNA Quality Score is reproducible (typically CV < 10%) and correlates well to other commercially available indices

- CVs shown of RNA samples from 4 different tissue types.
- Degraded by RNAse, in 8 levels of degradation.
- Samples prepared at 3 different concentrations (200, 100, 50 ng/uL)
- 3 repeats at each concentration, pooled for a total of 9 replicates for each degradation level.
- Table shows mean, standard deviation, and CV as a percentage of full RIN scale.
  - 10% = 1 RIN unit.
- Correlation shown is for 156 samples of 12 different tissues degraded by both RNAse and heating

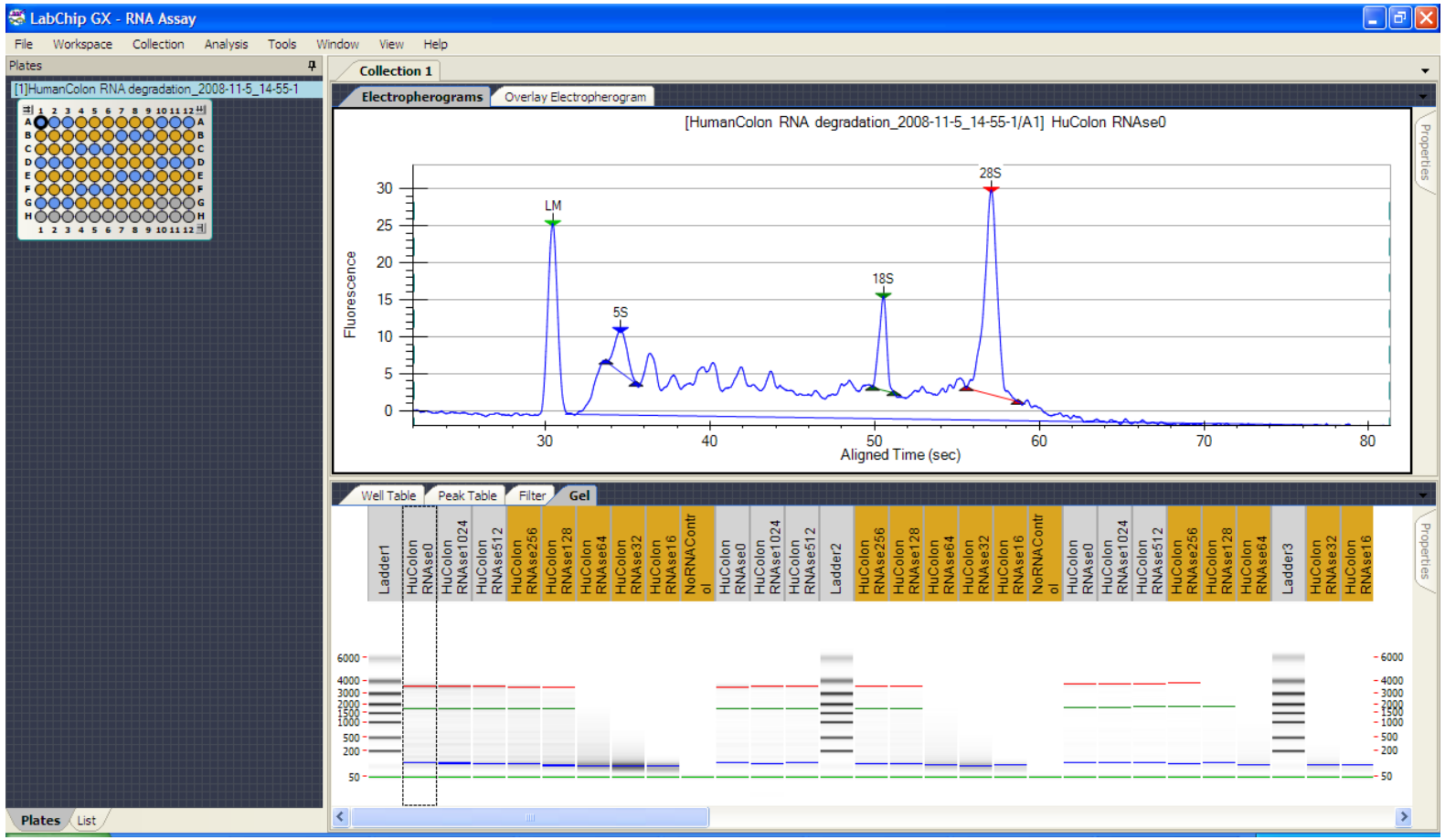
Degradation	Tissue	Sample Name	Count of RNA Quality Score	Average of RNA Quality Score	StdDev of RNA Quality Score	CV
RNAse	HuColon	HuColon RNAse0	9	6.12	0.18	1.8%
		HuColon RNAse1024	9	5.75	0.16	1.6%
		HuColon RNAse128	9	3.63	0.20	2.0%
		HuColon RNAse16	9	1.83	0.59	5.9%
		HuColon RNAse256	9	4.49	0.14	1.4%
		HuColon RNAse32	9	2.35	0.15	1.5%
		HuColon RNAse512	9	5.37	0.10	1.0%
		HuColon RNAse64	9			
		NoRNAControl	9			
	HuLiverCarc	HuLiverCar RNAse0	9			
		HuLiverCar RNAse1024	9			
		HuLiverCar RNAse128	9			
		HuLiverCar RNAse16	9			
		HuLiverCar RNAse256	9			
		HuLiverCar RNAse32	9	2.22	0.32	3.2%
		HuLiverCar RNAse512	9	6.36	0.17	1.7%
		HuLiverCar RNAse64	9	2.89	0.33	3.3%
		NoRNAControl	9	0.00	0.00	0.0%
	HuPancreas	HuPanc RNAse0	9	6.67	0.15	1.5%
		HuPanc RNAse1024	9	6.5	0.10	1.0%
		HuPanc RNAse128	9		0.15	1.5%
		HuPanc RNAse16	9		0.06	0.6%
		HuPanc RNAse256	9		0.17	1.7%
		HuPanc RNAse32	9		0.13	1.3%
		HuPanc RNAse512	9	6.35	0.12	1.2%
		HuPanc RNAse64	9	4.81	0.16	1.6%
		NoRNAControl	9	0.00	0.00	0.0%
	RatBrain	NoRNAControl	9	0.00	0.00	0.0%
		RB RNAse0	9	9.00	0.15	1.5%
		RB RNAse1024	9	8.47	0.22	2.2%
		RB RNAse128	9	4.34	0.10	1.0%
		RB RNAse16	9	1.89	1.05	10.5%
		RB RNAse256	9	6.00	0.19	1.9%
		RB RNAse32	9	2.42	0.13	1.3%
		RB RNAse512	9	7.89	0.19	1.9%
		RB RNAse64	9	2.58	0.13	1.3%
Grand Total			324	4.22	2.46	

CV < 10%

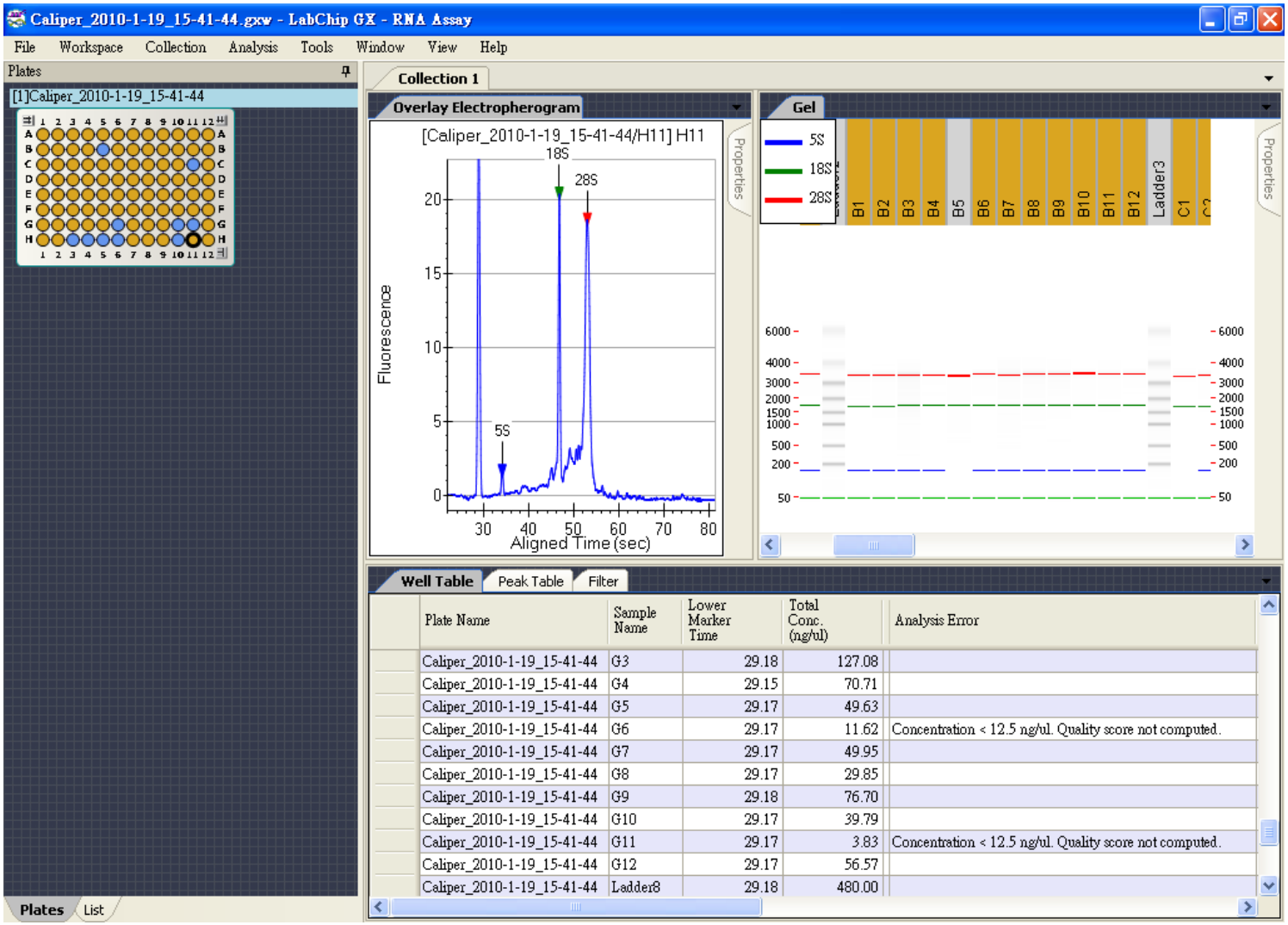


RNA Quality Score is automatically reported as a Well Result, and is available for use by the selection filter

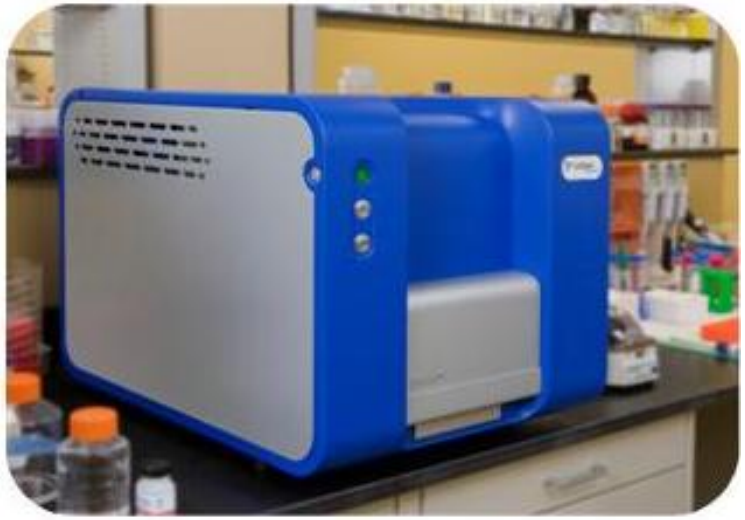
- Example of filter: RNA Quality Score < 5

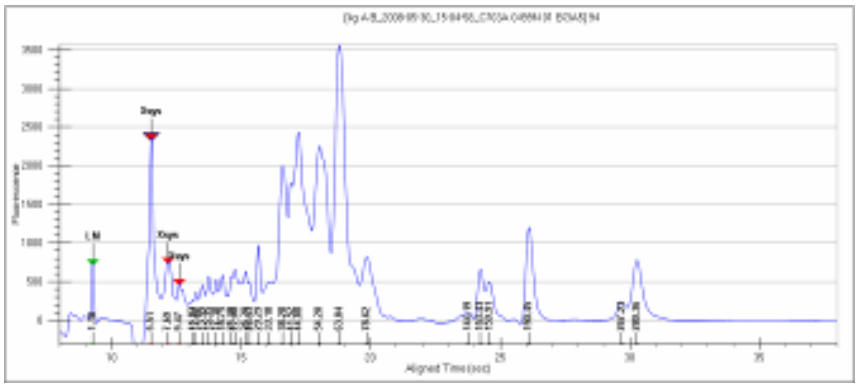


Actual Performance – 國家基因型鑑定中心



Protein Analysis on the LabChip GX II System

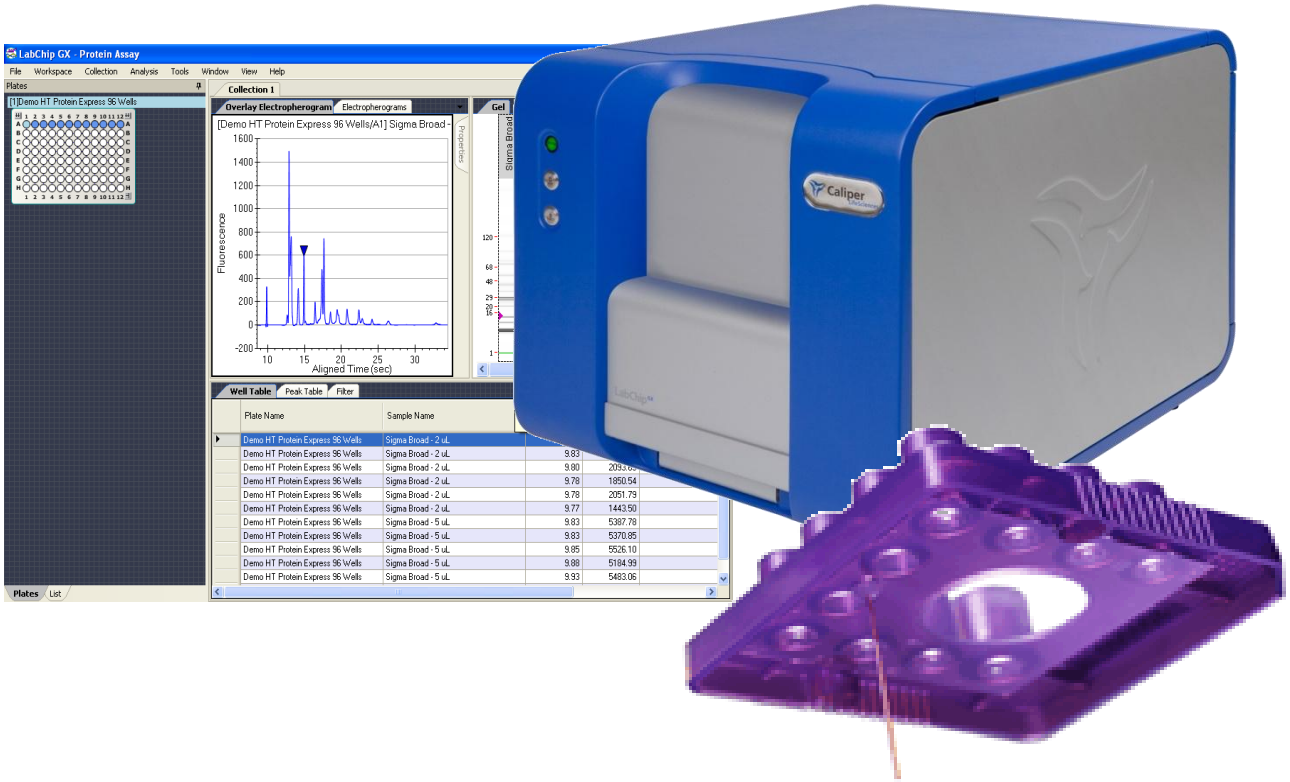




LCGXII Protein Assay Features

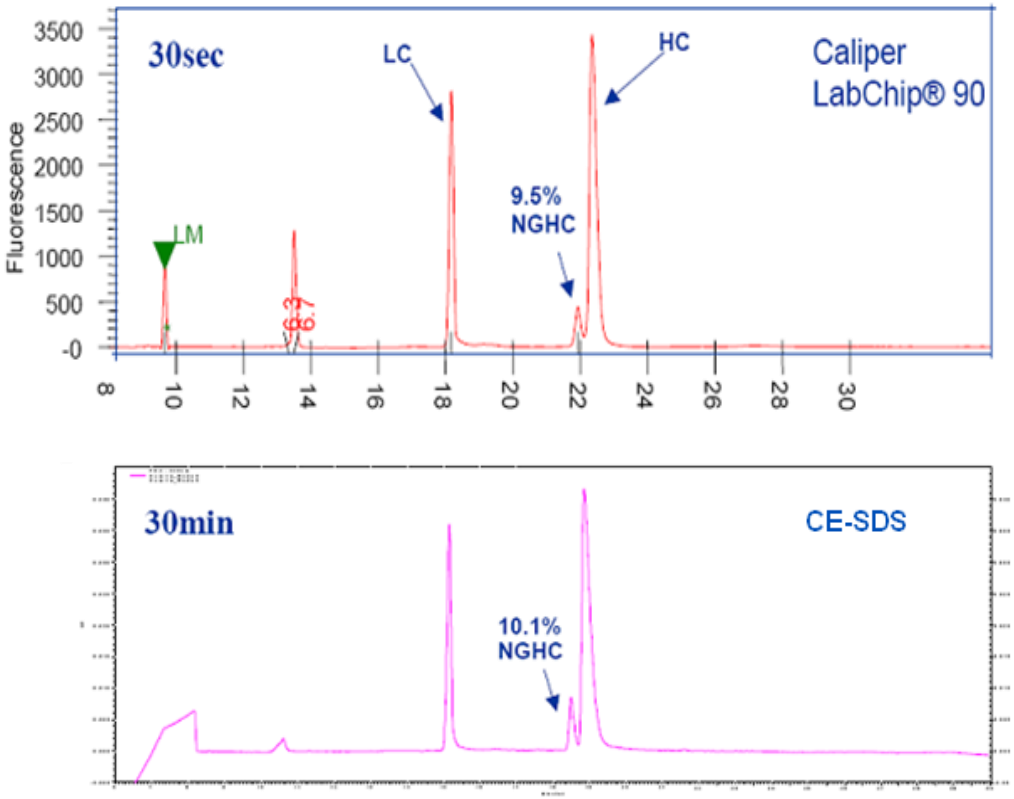
- Protein sizing from 14-200 kDa
- Process samples directly from 96-and 384-well plates
- Assays are complete in just 40 seconds
- Chip can be left in instrument to run up to 400 samples within 8 hours on a single preparation
- 70 times faster than traditional CE
- Ideal for the analysis of cell lysates, column fractions, purified and partially purified proteins, antibodies
- 21 CFR Part 11 compliant software

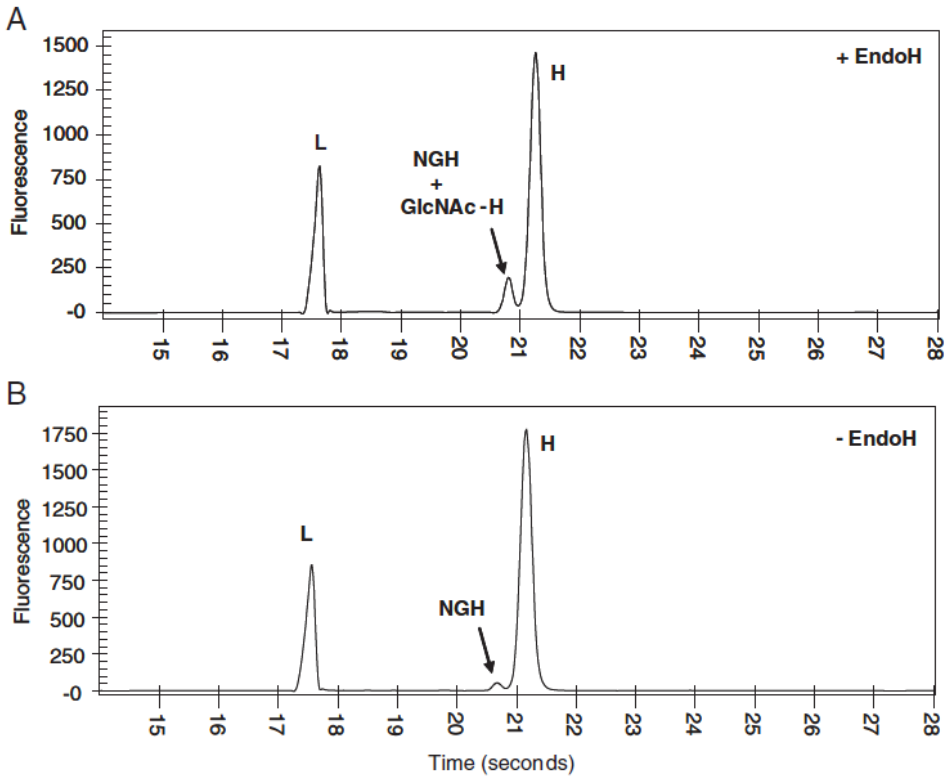
Summary of Protein Assay



Antibody Analysis (IgG)

LabChip vs. Conventional CE Methods



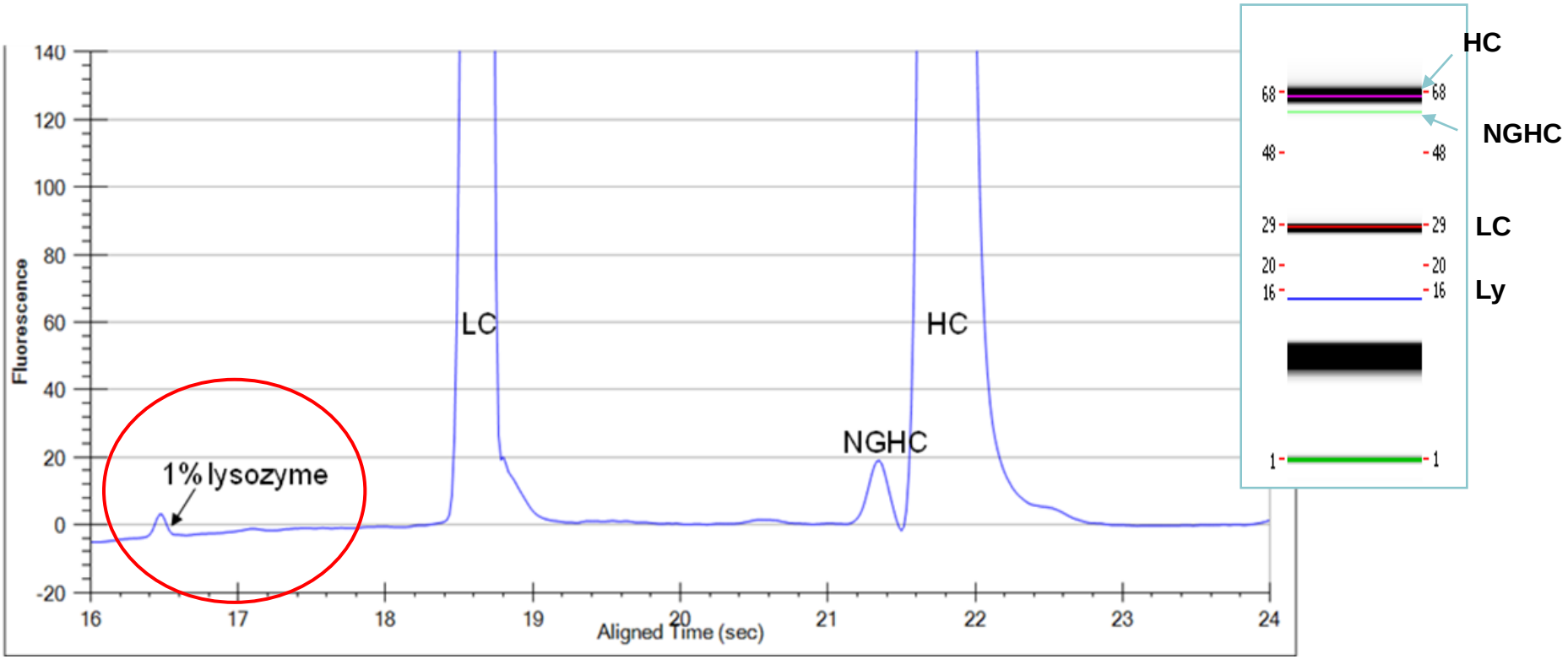


"Microchip CE-SDS… provide sufficient resolution and sensitivity for this purpose but on a time scale approximately **70 times faster** (41 s versus 50 min per sample) than conventional CE separation"

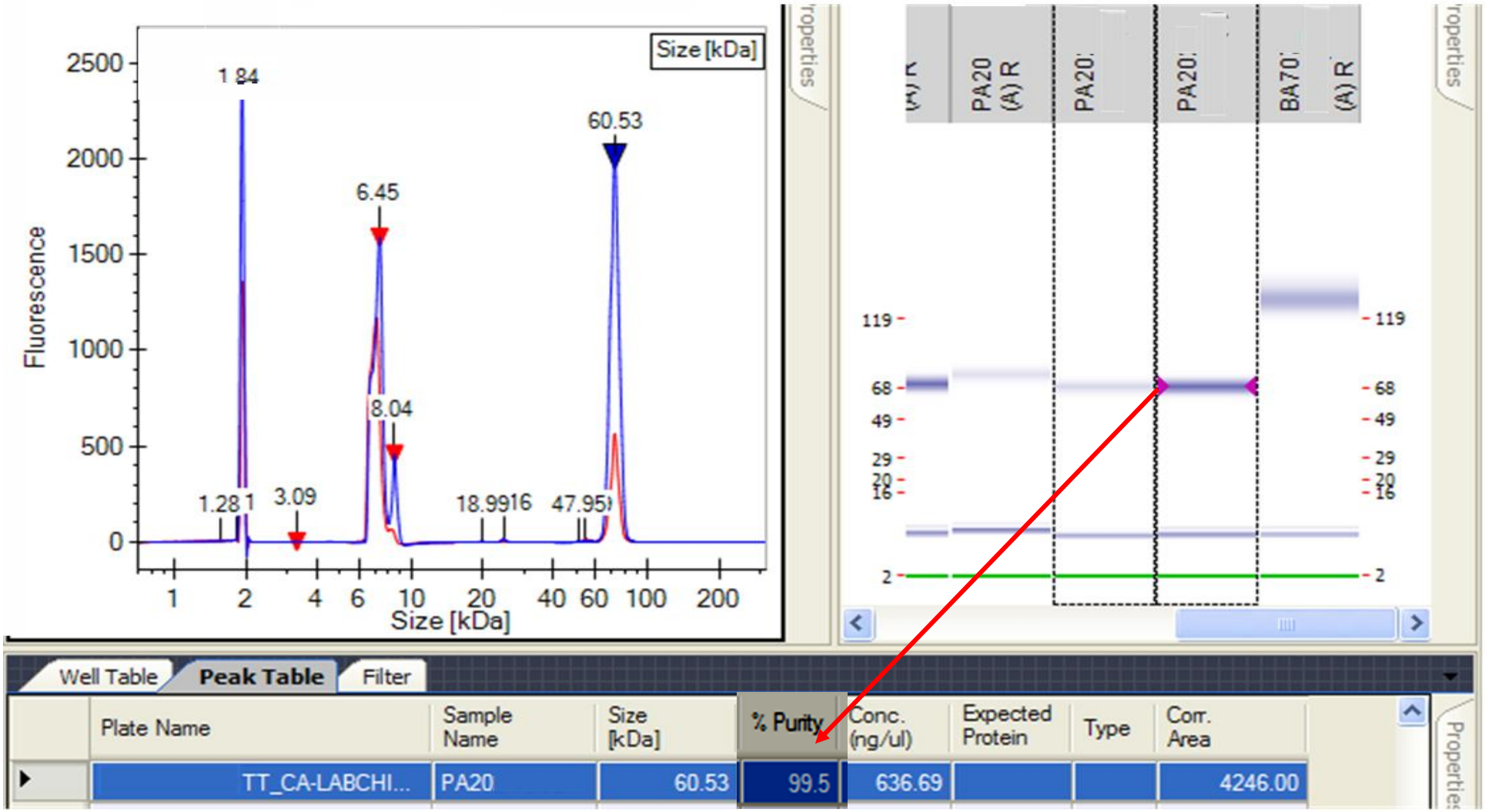
*Chen X. et al. Microchip assays for screening monoclonal antibody protein quality. Electrophoresis, **29** (2008) 4993-5002*

Low Level Impurity Detection

- Impurities as low as 0.5% to 1% are clearly identified
- Lysozyme was spiked into the sample at 1% of total protein and was readily identified

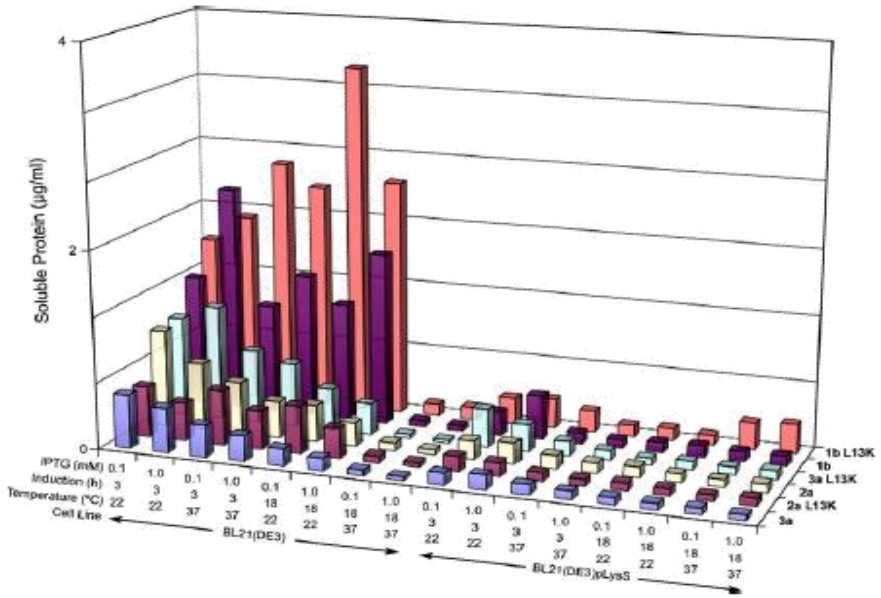


QC testing of Vaccine Purity



Protein Expression Optimization using DOE

VERTEX



Optimal expression condition is easily observed

*Swalley, S.E. et al., Quantitative Analysis of Protein Expression, Anal. Biochem, **351** (2006) 122-127*

Goal

Develop high throughput purification and expression system and test effectiveness of identifying factors influencing protein production.

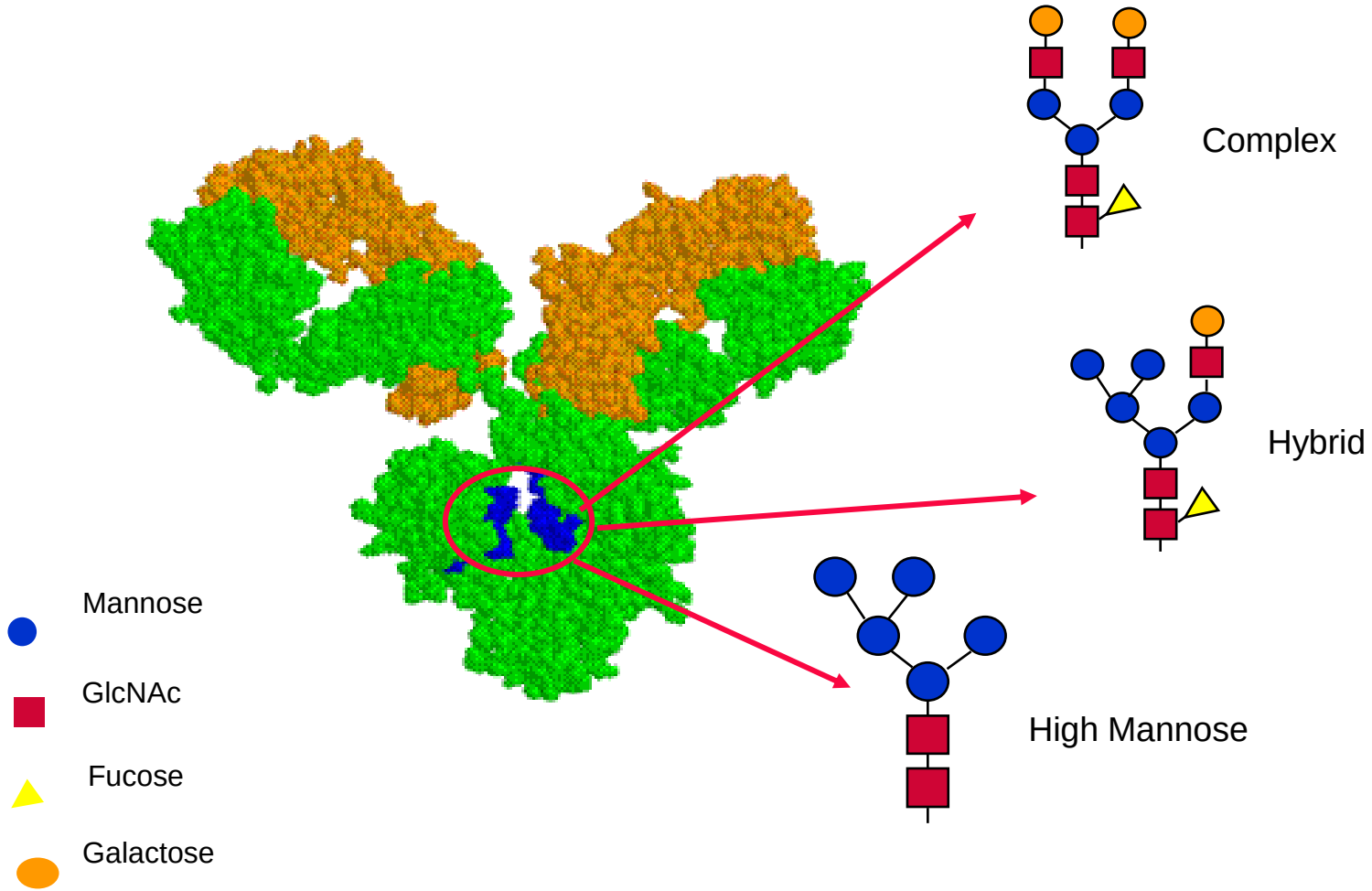
Methods

Utilize Caliper LabChip to run full factorial quantitative analyses of expression experiments of two level combinations on four factors.

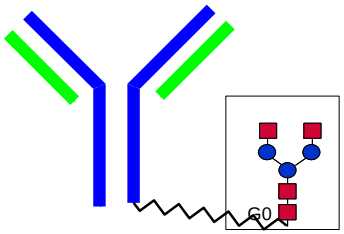
Authors' Conclusion:

"Finally, the process that we have described reduces both experimentation cost and time while increasing throughput, ***making possible what–if experiments that, up to now, had been either prohibitively expensive or too complex to perform***."

Antibody N-linked Glycan Analysis

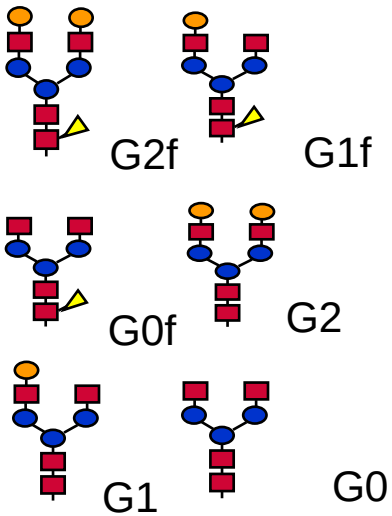


Glycan Analysis – Common Methods



Cleave
Purify

Glycans



Fluorophore Labelling

Exoglycosidase arrays

- **HPLC - Fluorescence**
- **CGE-LIF**
- **Gel electrophoresis (FACE)**
- **MALDI ToF/ToF-ToF**
- **ESI-MS/MS**
- **LC fractionation - NMR**

Glycan structure assignment including linkage

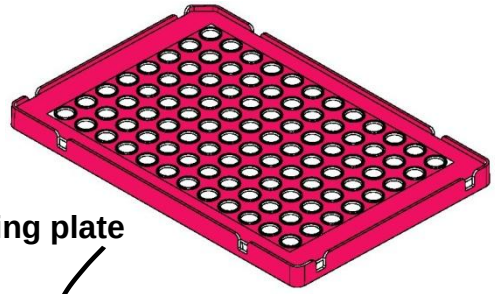
- **CZE-MS**
- **HPAEC- PAD**

Glycan structure assignment

Process takes 3-5 days

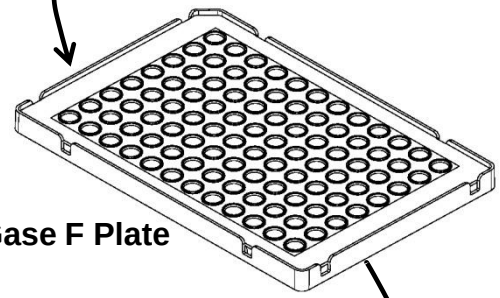
Glycan Profiling workflow

1
- Add Samples (10 to 60ug of monoclonal antibody) to Denaturing Plate
- Seal. Heat for 10 minutes at 70C.



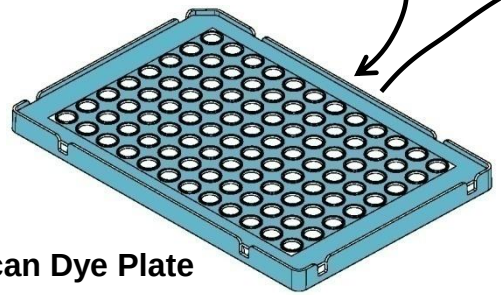
Denaturing plate

2
- Transfer denatured samples to PNGase F Plate.
- Seal. Incubate for 1 hour at 37C



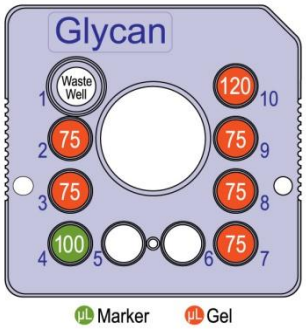
PNGase F Plate

3
- Transfer 8ul of digested sample to Dye Plate .
- Incubate for 2 hours at 55C, or until dry.



Glycan Dye Plate

4
- Load chip with Gel, and Marker.



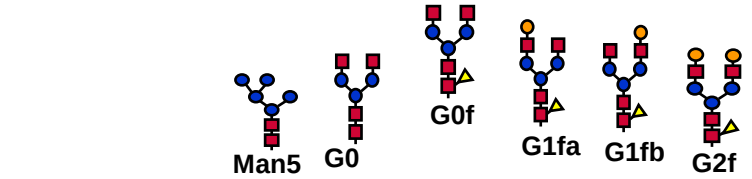
5
- Add water to reconstitute.
- Run on LabChip GXII.

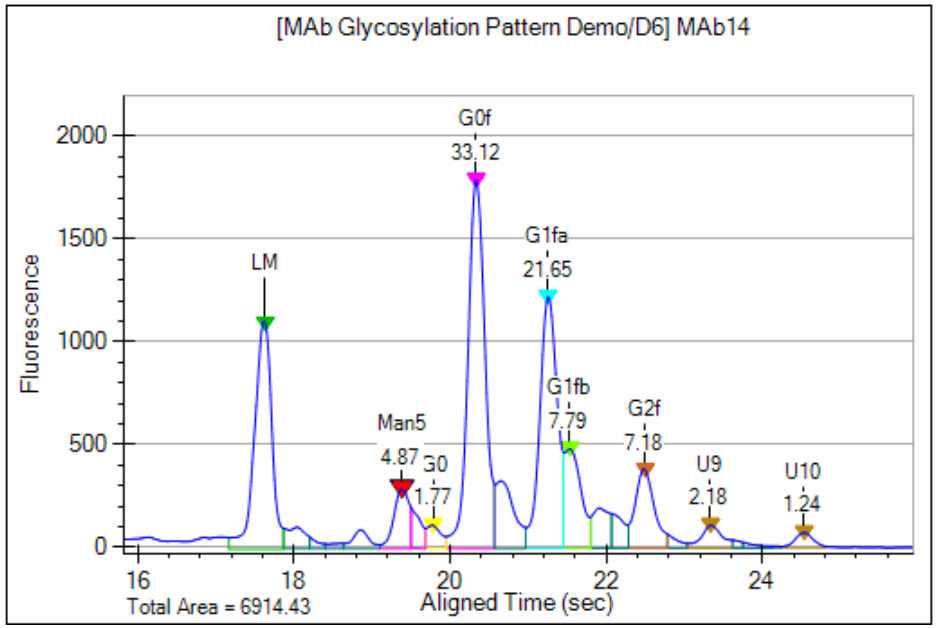
- **Notes:**
  - Plates can be cut to process as few as 24 samples at a time.
  - Requires LabChip GXII instrument with software version 2.2 or greater.
  - Requires LabChip GX Plate Adapter for Deep-Well PCR plates.

Process takes 1/2 day

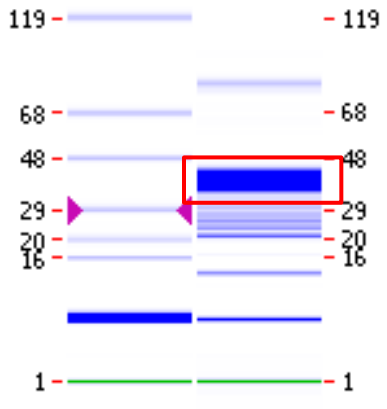
ProfilerPro Glycan Profiling

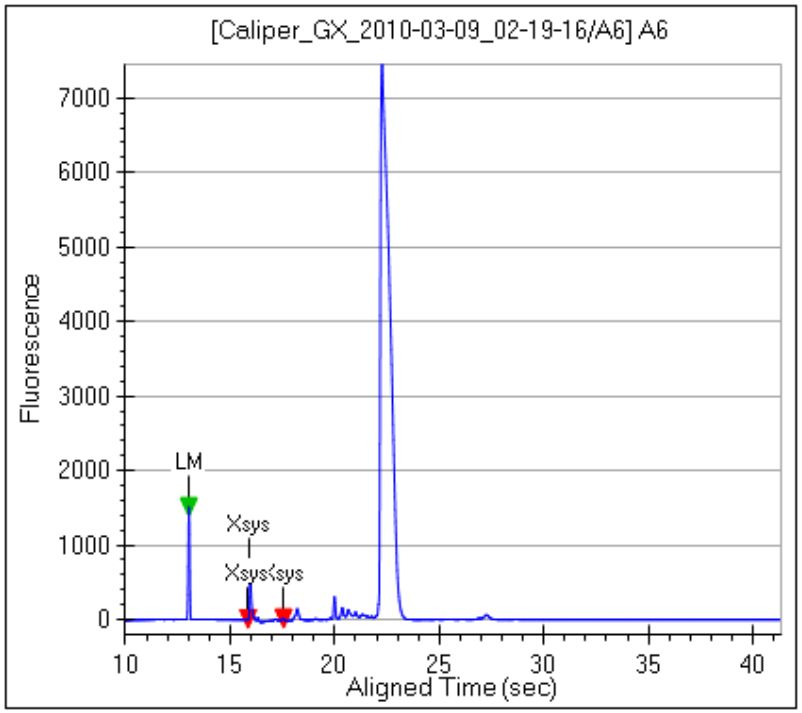
- Applications
  - Analysis of the glycosylation patters of recombinant/monoclonal antibodies (MAb).
  - Fast separation time–less than 50 seconds per sample—makes this system valuable for use in high throughput screening of antibody glycosylation patterns in early stage development.
  - Microchip based CE analysis gives resolution sufficient to quantify the relative abundance of the major glycan types found on antibodies.
    - Neutral N-linked glycans
      - Biantennary partially fucosylated complex type.
      - High-mannose type oligosaccharides





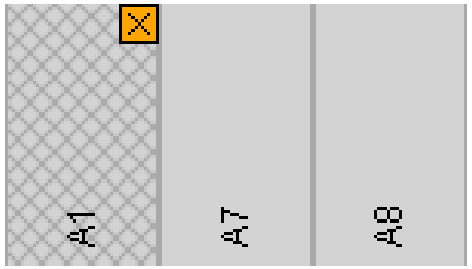
王惠鈞老師實驗室A6 sample蛋白質純化之結果

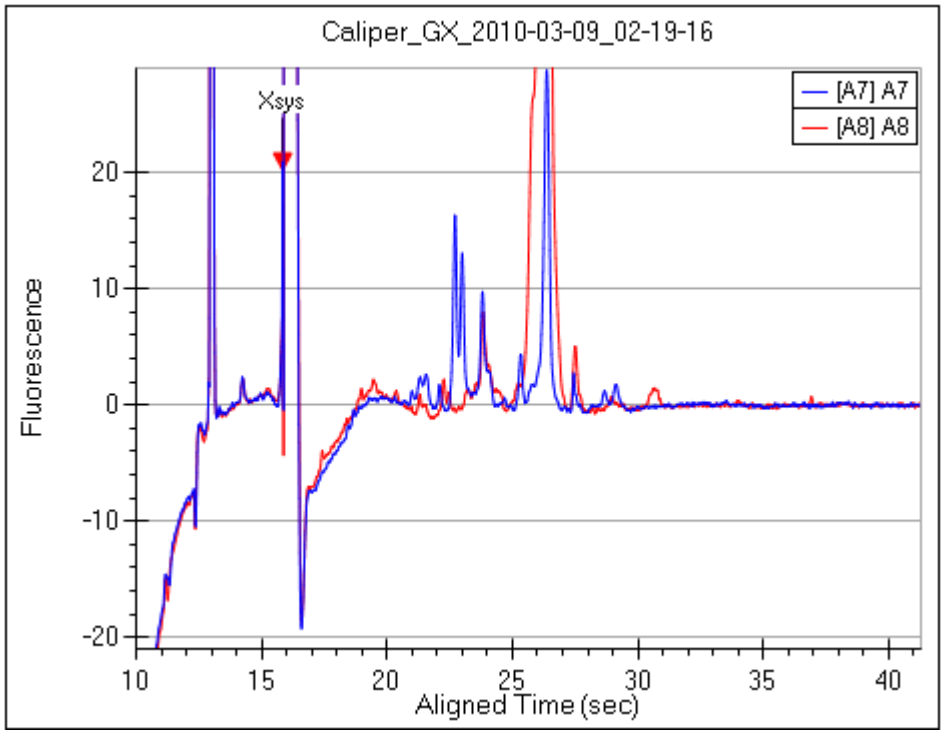




Sample Name	Size [kDa]	Conc. (ng/ul)	% Purity
A6	37	4785.8	95.76

蔡明道老師實驗室 (sample用不同buffer去elute之比較)





Sample Name	Size [kDa]	Conc. (ng/ul)	% Purity
A7	74.18	11.55	67.5
A8	73.89	192.65	100


Caliper
LifeSciences

Specifications and Reordering Information for LabChip GX Kits

	HT DNA 1K	HT DNA 5K	HT DNA 12K
Sizing Range	25 bp-1000 bp	100 bp-5000 bp	100 bp-12000 bp
Sizing Resolution[1]	± 15% from 25-100 bp ± 10% from 100-150 bp ± 5% from 150-700 bp ± 10% from 700-1000 bp	± 15% from 100-150 bp ± 10% from 150-500 bp ± 15% from 500-1500 bp ± 20% from 1500-5000 bp	± 10% from 150-1000 bp ± 15% from 1000-2000 bp ± 20% from 2000-8000 bp ± 25% from 100-150 bp, 8000-12000 bp
Sizing Accuracy	± 10 %	± 10 %	± 10%
Sizing Precision	± 5%	± 5%	5% CV
Linear Concentration Range	0.1 ng/µL-50 ng/µL per fragment	0.25 ng/µL-50 ng/µL per fragment	0.25 ng/µL-50 ng/µL per fragment
Sensitivity	0.1 ng/µL	0.25 ng/µL	0.25 ng/µL
Maximum Total DNA Concentration	80 ng/µL, 50 ng/µL per fragment	80 ng/µL, 50 ng/µL per fragment	60 ng/µL total, 50 ng/µL per fragment
Carry-Over	< 0.25%	< 0.25%	< 0.5%
Quantitation Accuracy	± 30% or ± 1 ng/µL, whichever is greater	± 30% or ± 1 ng/µL, whichever is greater	± 40% or ± 1 ng/µL, whichever is greater
Quantitation Precision	± 20% from 25-500 bp ± 10% from 500-1000 bp	± 20%	20% CV from 100-5000 bp 25% CV from 5000-12000 bp
Maximum Salt Concentration	125mM	125mM	125mM
Additives[2]	BSA/ detergents should not exceed 0.05mg/mL/ 0.01% (v/v)	BSA/ detergents should not exceed 0.05mg/mL/ 0.01% (v/v)	BSA/ detergents should not exceed 0.05mg/mL/ 0.01% (v/v)
Chip Lifetime[3]	2000 samples per chip	2000 samples per chip	2000 samples per chip
Reagent Kit Lifetime	up to 9 chip preps	up to 9 chip preps	up to 9 chip preps
Samples per Chip Prep	400 samples (four 96-well plates or one 384-well plate)	400 samples (four 96-well plates or one 384-well plate)	400 samples (four 96-well plates or one 384-well plate)
Standard Assay - Specifications are defined for this Assay	HT DNA 1K Standard: For sizing of DNA fragments in 25 to 1000 base pair range. (Analysis time per sample - 68 seconds)	HT DNA 5K Standard: For sizing of DNA fragments in 100 to 5000 base pair range. Fastest analysis time per sample compared with all available assays. (Analysis time per sample - 28 seconds)	HT DNA 12K Standard: For sizing of DNA fragments in 100 to 12000 base pair range. (Analysis time per sample - 65 seconds)
Extra Assays	HT DNA 1K High Resolution: For sizing of DNA fragments in 25 to 1000 base pair range. Greater resolution with longer analysis time per sample. (Analysis time per sample - 120 seconds)		HT DNA 12K High Resolution: For sizing of DNA fragments in 100 to 12000 base pair range. Greater resolution with longer analysis time per sample. (Analysis time per sample - 130 seconds) HT DNA 12K Extended Time: To be used only if peaks are cut off using the standard HT DNA 12K script (occurs in some high salt sample buffers). (Analysis time per sample - 80 seconds)

HT Protein Express

Sizing Range	**14 to 200 kDa**
Sizing Accuracy	± 20%
Sizing CV	± 10%*
Resolution [1]	± 10% difference in MW across the sizing range, 50% valley Resolution is comparable to a 10 cm 4-20% SDS-PAGE gel*
Linear Dynamic Range	5 - 2000 ng/µL*
Relative Concentration CV	30% up to 120 kDa relative to the ladder* Above 120 kDa, quantitation is not specified
Maximum total Protein Concentration	10 mg/ml
Sensitivity	~ 5 ng/µL per protein (CA)*
Maximum Salt Conc./pH range	1M NaCl/ pH 6.5 to 8.5
Samples per Chip Prep	384 samples (three 96-well plates)
Reagent Kit Lifetime	3 Chip preps
Chip lifetime [2]	384 samples (three 96-well plates)
Standard Assay - Specifications are defined for these Assays	**HT Protein Express 100 -** analysis of proteins from 14 to 100kDa (Analysis timer per sample - 34 seconds)
	HT Protein Express 200 - analysis of proteins from 14 to 200kDa (Analysis timer per sample - 41 seconds)
	HT Protein Express 100/200 - The protein 100 assay should be used for sizing proteins 100 kDa and smaller. The protein 200 assay should be used for sizing proteins between 100 and 200 kDa.
Extra Assays	**HT Protein Express 100/200 High Sensitivity -** The HT-Protein Express 100/200 High Sensitivity scripts provide 2X the sensitivity of the standard scripts but, the sizing is less robust to varying buffer conditions.
	High Molecular weight - for analyis of proteins up to 600kDa (Analysis time per sample- 69 seconds)

[1] Resolution is defined as half height or better separation of two peaks. Actual separation performance can depend on the sample and application. Peaks that are resolved less than half height can still be accurately identified by the system software.

[2] Expected chip lifetime is based on use under normal laboratory conditions and adherence to Caliper preparation protocols, sample guidelines and storage conditions. Individual laboratory results may vary.

* in PBS

Compatible Buffers, Salts and Additives

Buffer and Salts	Concentration Limit	Additives	Concentration Limit
Tris Chloride	250 mM	Octyl Glucoside	2.5%
Tris Glycine	250 mM	Pluronic F68	0.1%
Hepes	500 mM	Sarcosyl	10%
PBS	8 X	CHAPS	0.5%
Sodium Citrate	150 mM	Tween 20	0.8%
Sodium Phosphate	250 mM	Triton X-100	0.6%
Sodium Acetate	600 mM	SDS	2%
Sodium Chloride	1000 mM	Zwittergent 3-14	0.4%
Sodium Azide	6%	PEG 3350	1%
Sodium Hydroxide	500 mM	Glycerol	30%
Potassium Chloride	900 mM	Urea	8 M
Ammonium Bicarbonate	1000 mM	Sucrose	1 M
Magnesium Chloride	300 mM	DMSO	25%
Imidazole	900 mM	EDTA	100 mM
PhosphoSafe		Ethanol	50%
BugBuster	2.5 X		
BPER			
POP Culture			
Insect POP Culture			

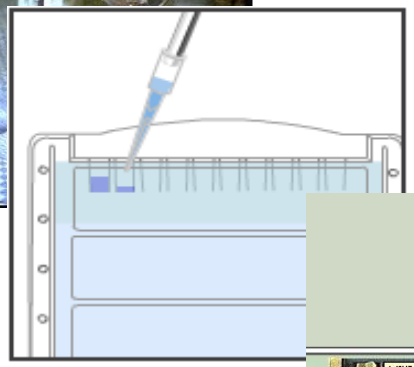
Incompatible Buffers, Salts and Additives

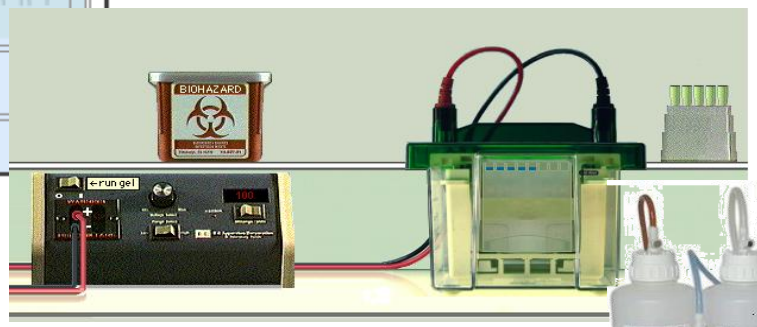
Buffer and Salts	Concentration Limit	Additives	Concentration Limit
RIPA	All	–	–

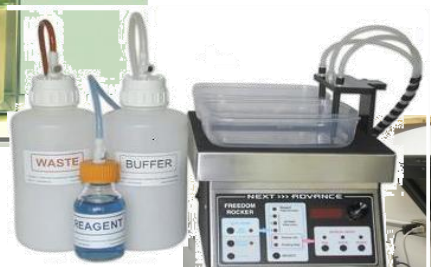
HT RNA Version 2 Assay Specifications

Linear Range:	25 to 250 ng/µL (high range) 5 to 50 ng/µL (low range)
Quantitation reproducibility:	< 20% CV (from chip to chip and instrument to instrument)
Quantitation Accuracy:	< +/- 30% error with ladder as sample
Size range:	100 to 6000 nucleotides (suitable for total RNA)
Carryover:	< 0.5% following 500 ng/µL sample.
RNA sample volume:	2 µL of user sample for high range assay 6 µL of user sample for low range assay
Run time:	80 sec per sample (about 2.5 hours for 96 well plate)
Setup time:	About 1/2 hour for chip and sample prep
No. of samples per chip prep:	200 samples max, then dye/gel must be replaced
Reagent kit lifetime:	Up to 5 chip primes (Typical modeled workflow is 96 samples per prime, thus 480 samples per reagent kit. Some high volume users may realize the full 200 sample capacity per prime --> 1000 samples per reagent kit)
Chip lifetime:	>2000 samples

Manual Gel -- Timeline to Result







Mix gel

Cast gel

Cure gel

Load samples

Run gel

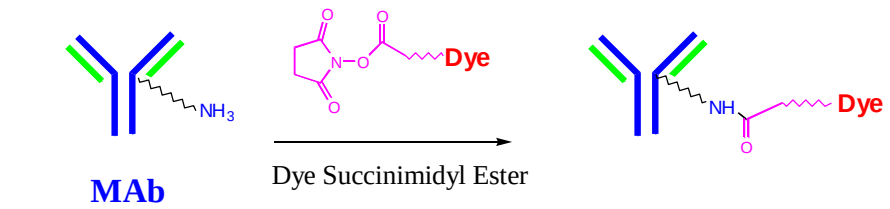
Stain/Destain the gel

Scan gel

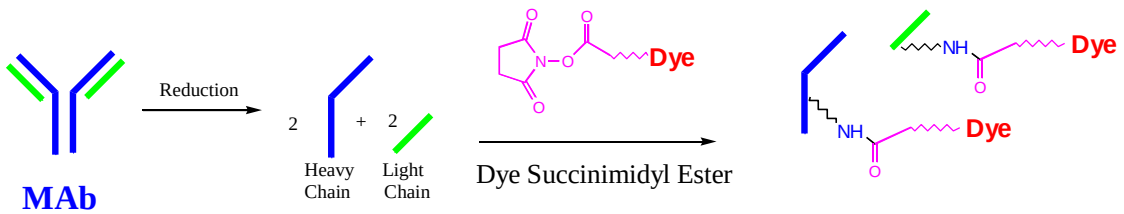
>4 hours !

High Sensitivity Pico Protein Assay

- Replaces on-chip staining with off-chip labeling
- NHS esters reaction with amine groups of lysine residues
- 100X Increase in sensitivity
- Sizing Range – 14 to 200KDa
- Linear dynamic range of over 4 logs have been achieved, both for reduced and intact Mab
- LOD – 50 pg/µl target antibodies
- LOD – 10 pg/µl target proteins

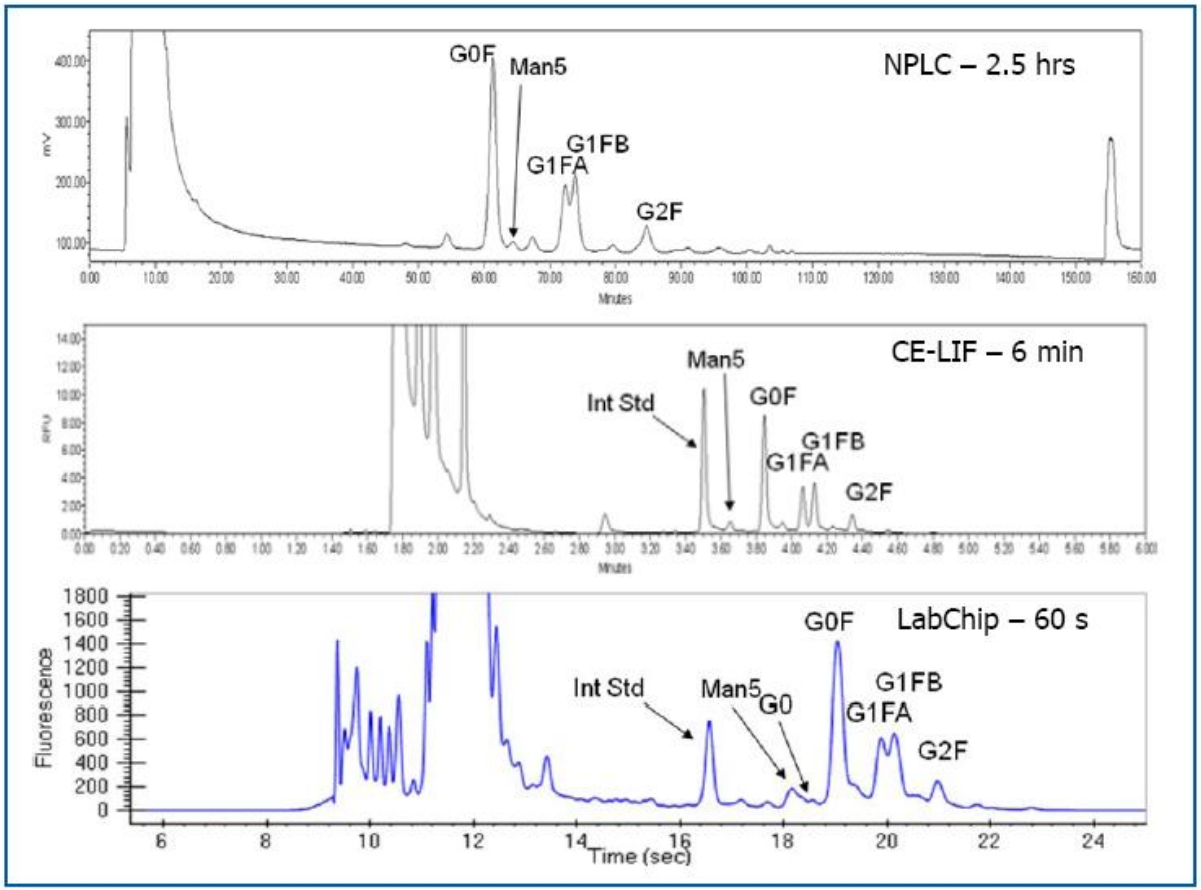


Intact Antibody



HC and LC

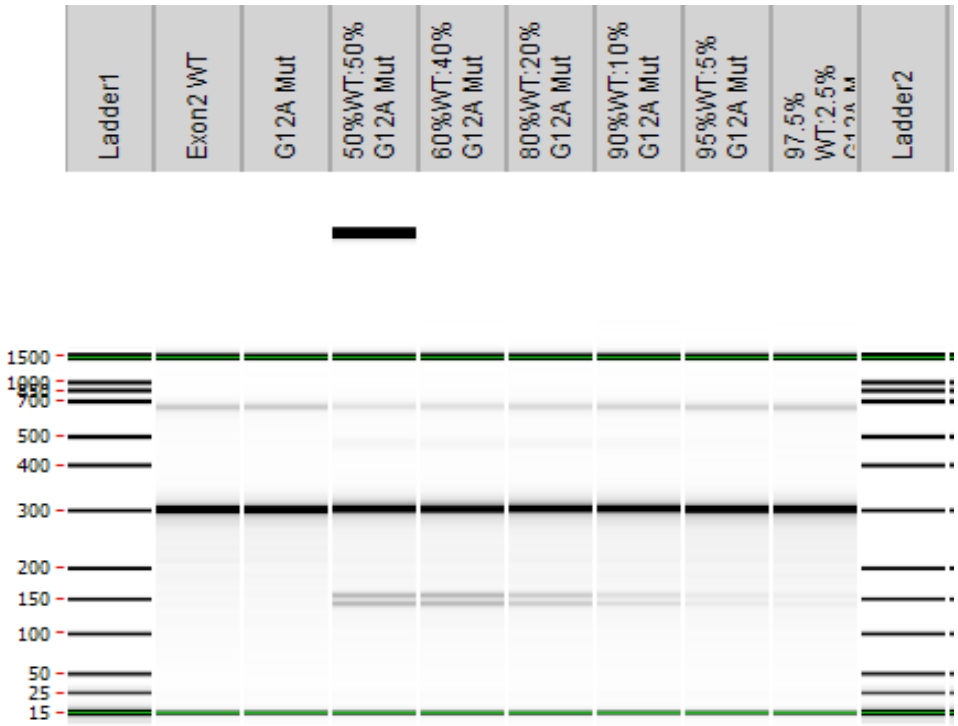
LabChip N-Glycan Assay Comparison to NPLC and CE-LIF

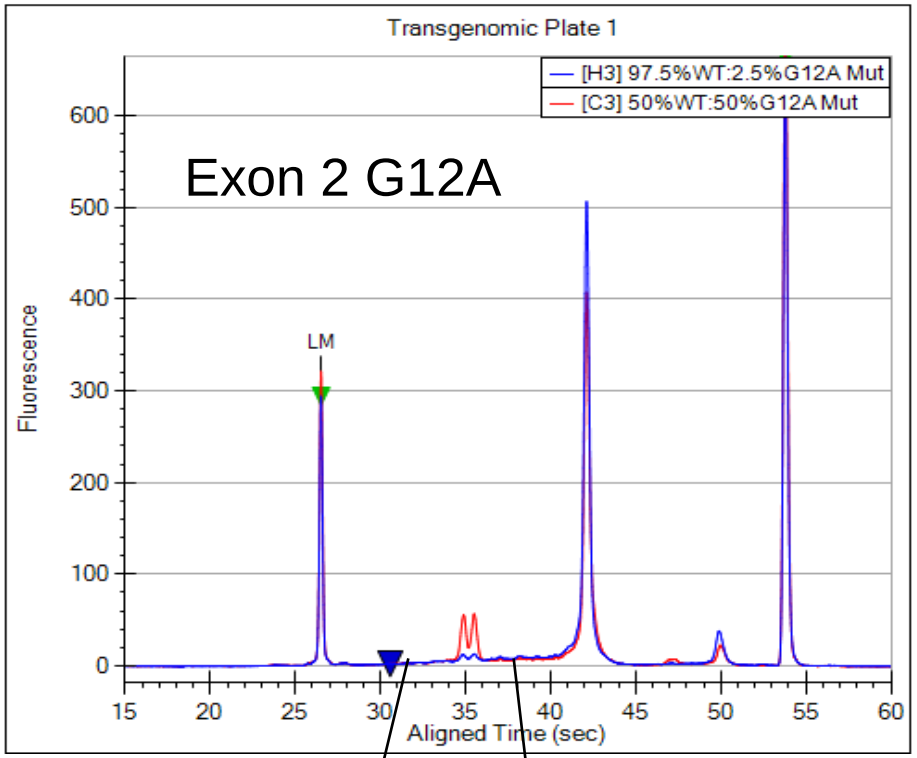


LabChip analysis is 160X faster than NPLC and 6X faster than CE-LIF analysis with comparable results

NPLC analysis with 2-AB label (top), CE-LIF analysis with APTS label (center), LabChip N-Glycan Profiling Assay with fluorescent label (bottom). Data courtesy of Pfizer Inc., Chesterfield, MO.

Transgenomics "Surveyor" Assay: K-ras Mutations

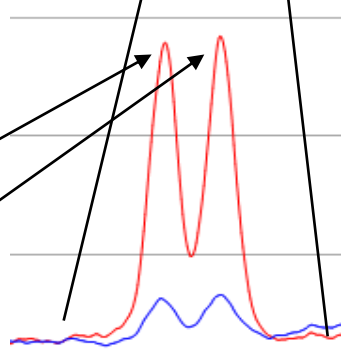




Frag Size (BP)

144

156



FDA recommendations
Cetuximab (Erbitux) and Panitumumab (Vectibix)
Class Labeling Changes to anti-EGFR monoclonal antibodies, cetuximab (Erbitux) and panitumumab (Vectibix): *KRAS* Mutations
On July 17, 2009, changes were made to the product labels of cetuximab (ErbituxImClone Systems, Branchburg, NJ) and panitumumab (Vectibix Amgen, Thousand Oaks, CA).