

高感度DNAチップ3D-Gene[®]とDNAチップ実験の QAQCの取り組みについて

High-sensitive DNA chip 3D-Gene[®] and an approach of Quality assurance/Quality control in the DNA chip experiment



東レ株式会社 新事業開発部門
近藤哲司
Toray Industries, Inc.
New Projects Development Div.

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3. DNAチップ解析フローと品質保証・コントロール
Analysis flow and QAQC
4. 事例紹介とまとめ
Example of Clinical Study and summary

Outline of Toray Group

Toray is a global material company

- Foundation: January 1926
- President: Akihiro Nikkaku
- Head Office: Tokyo in Japan
- Employees: 45,789
- Net sales: JPN 2,011 B (USD 16.7 B)
- Op. Income: JPN 123 B (USD 1.03 B)

All figures herein are as of March 31st, 2015
Exchange rate: USD1.00=JPY120.02

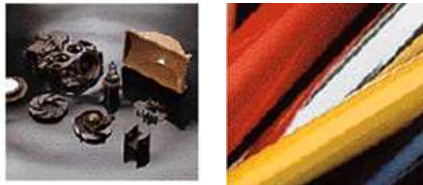
Business Fields

**Core Growth
Driving Business**

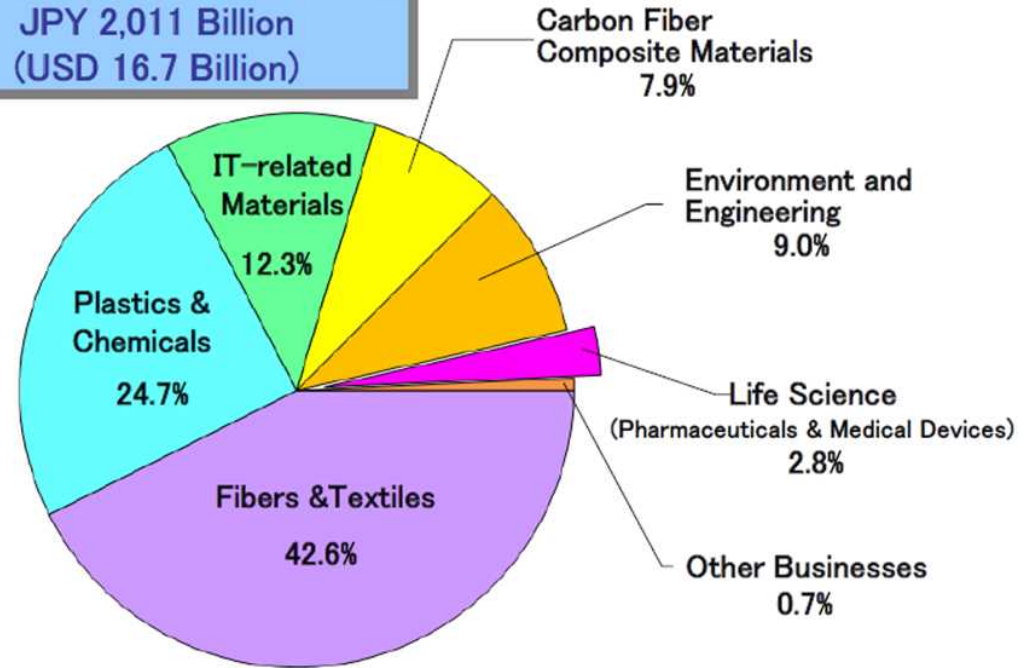
Fibers & Textiles



Plastics & Chemicals



Net Sales (March 31st, 2015)
JPY 2,011 Billion
(USD 16.7 Billion)



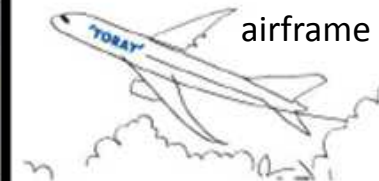
**Strategically Expanding
Businesses**

**Intensively Developing and
Expanding Businesses**

IT-related Products



*Carbon Fiber
Composite Materials*



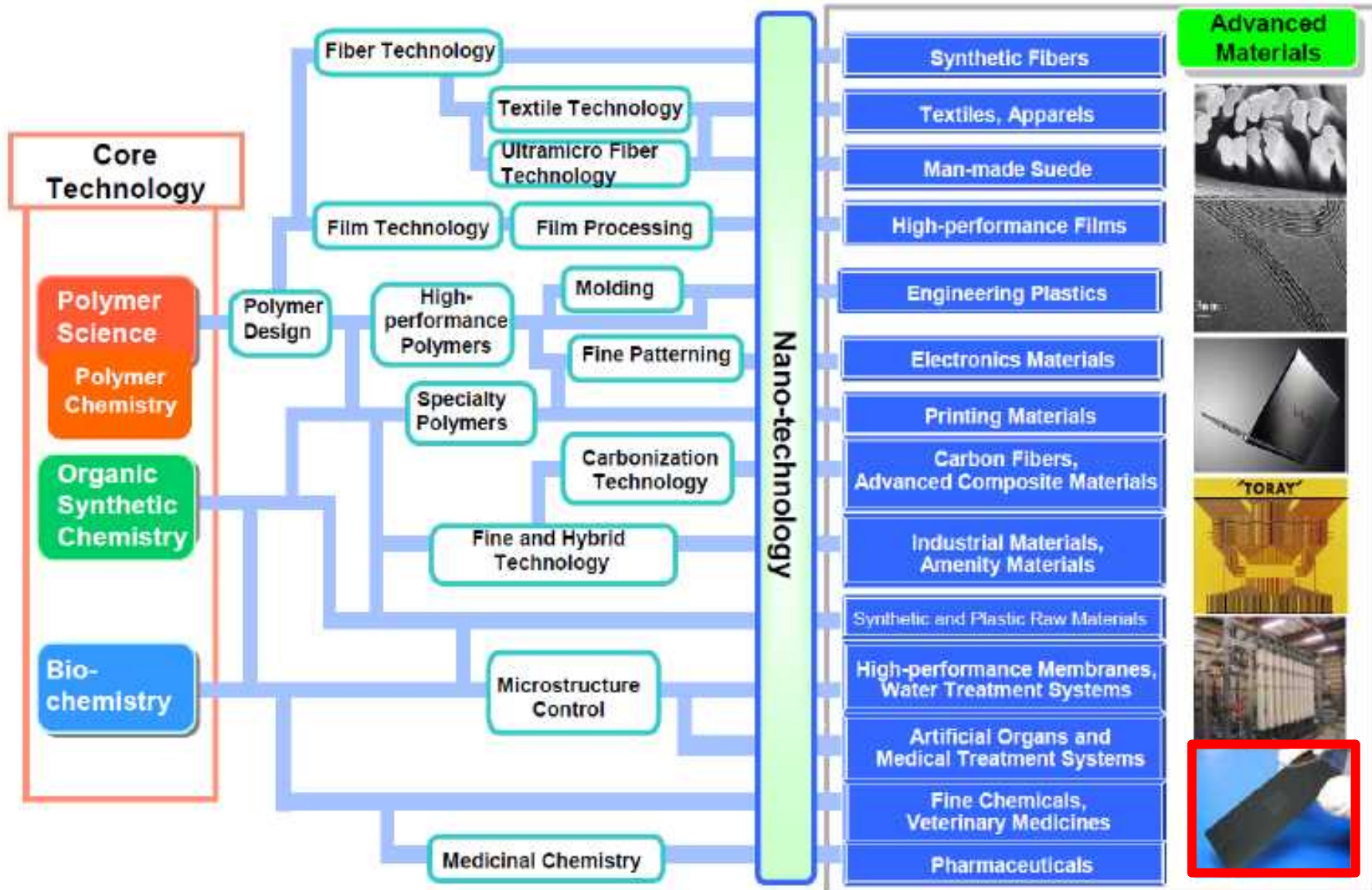
*Environment &
Engineering*



*Life Science
(Pharma. & Med. Devices)*



Technologies and Major Products



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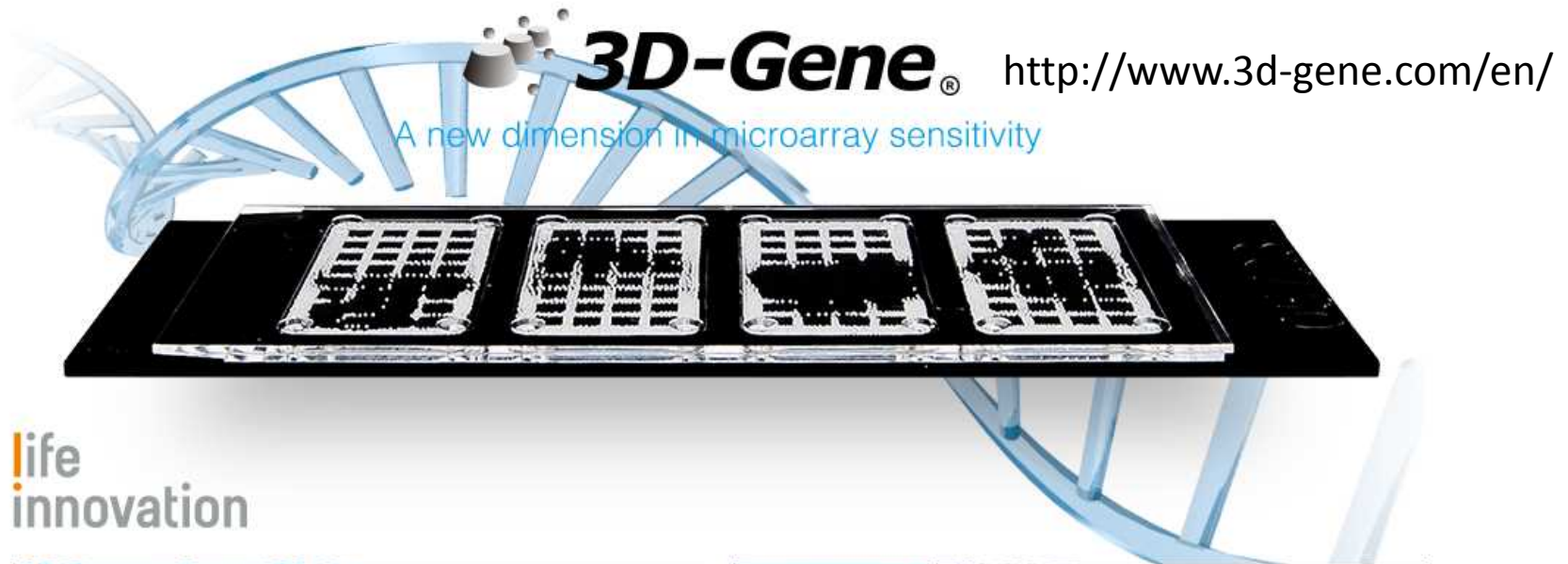
3. DNAチップ解析フローと品質保証・コントロール

Analysis flow and QAQC

4. 事例紹介とまとめ

Example of Clinical Study and summary

Highly Sensitive DNA microarray **3D-Gene®**



What is 3D-Gene®
Introduction of the features of 3D-Gene®



Products
Introduction of our product lineup



Services
Introduction of contract analysis service by 3D-Gene®

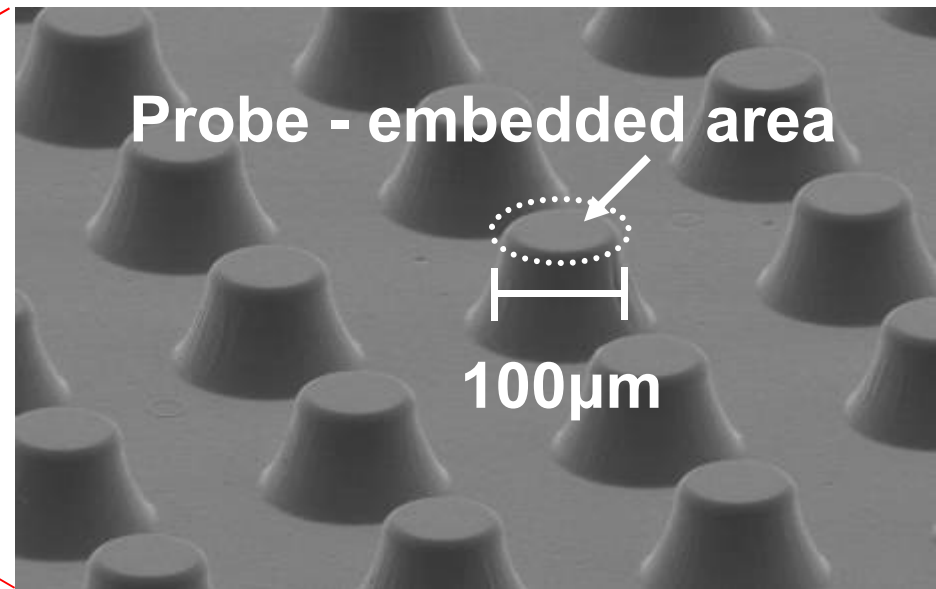
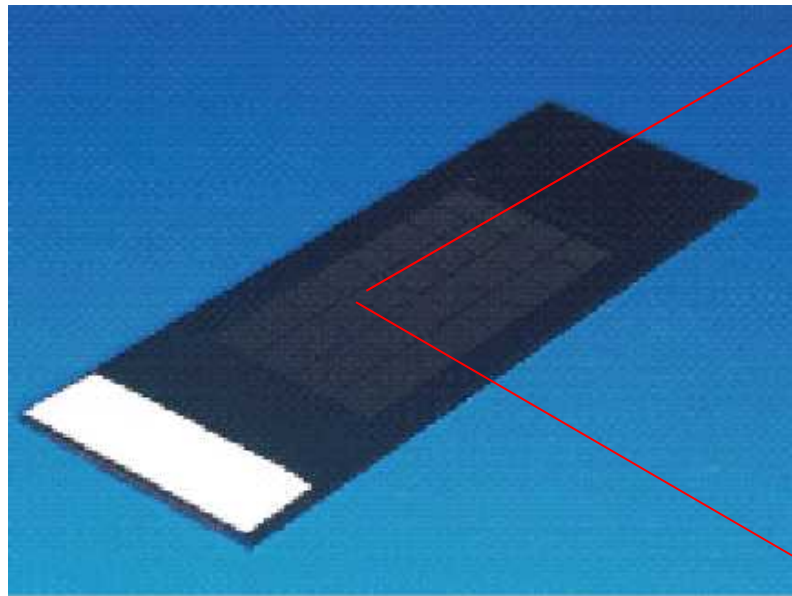


Case studies
Analysis case studies of 3D-Gene®

Technical Background (1): Highly Sensitive DNA microarray **3D-Gene**[®]

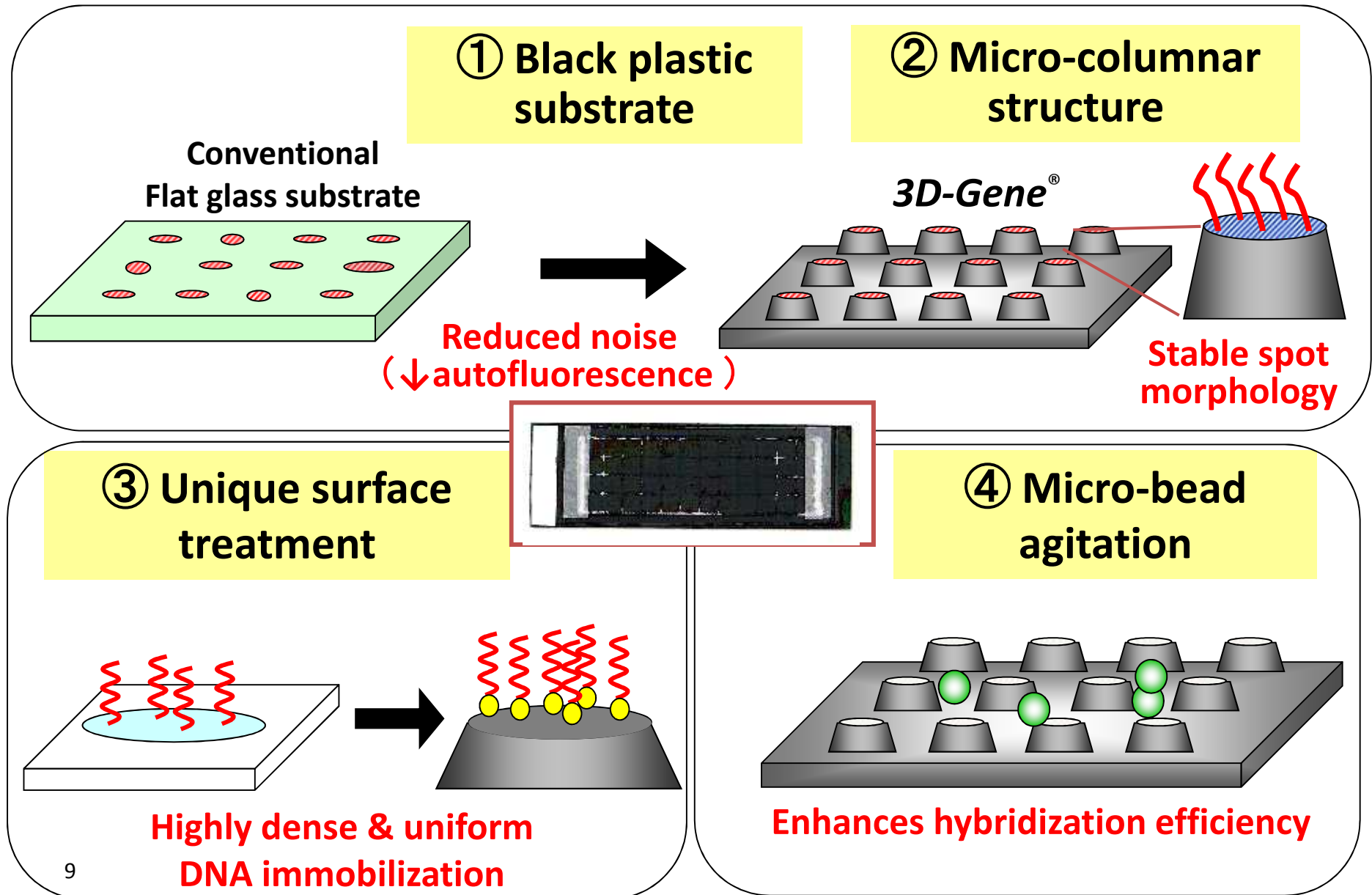


Unique micro-columnar structure
of probe - embedded area

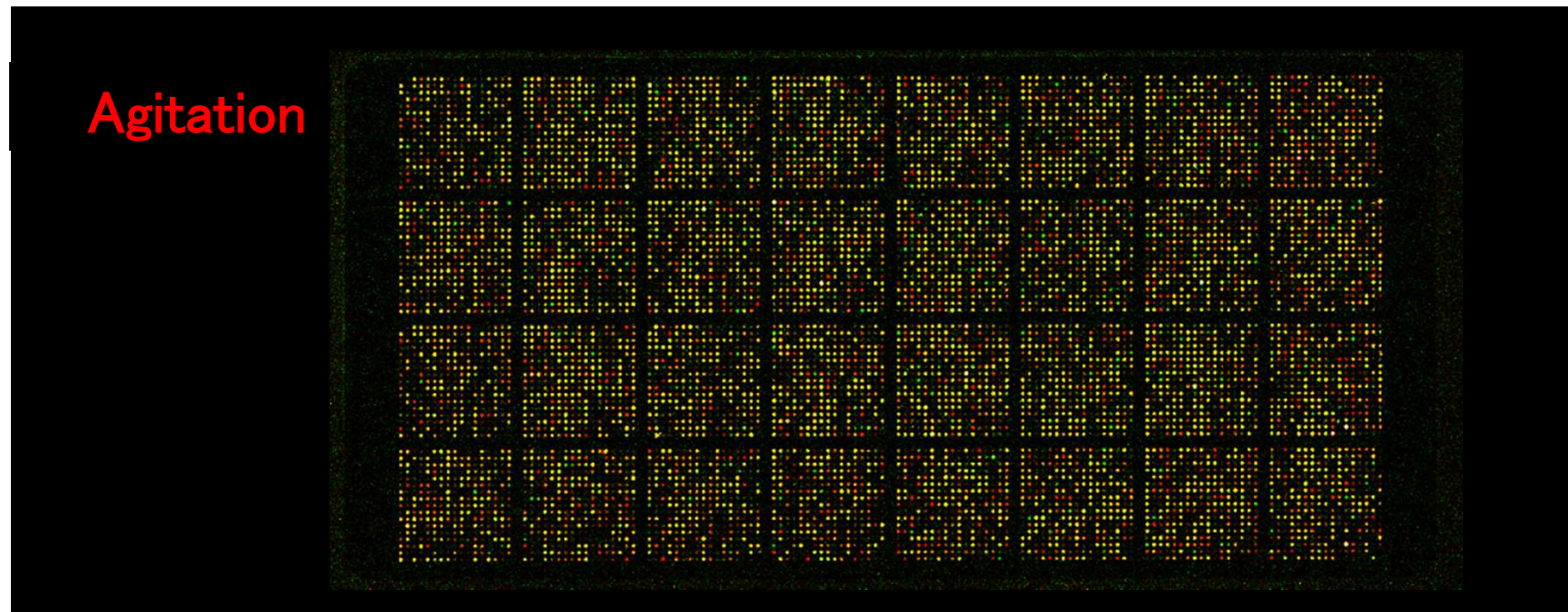


Micro-columnar structure (**3D**) detects genes (**Gene**)

3D-Gene[®] has four technologies features

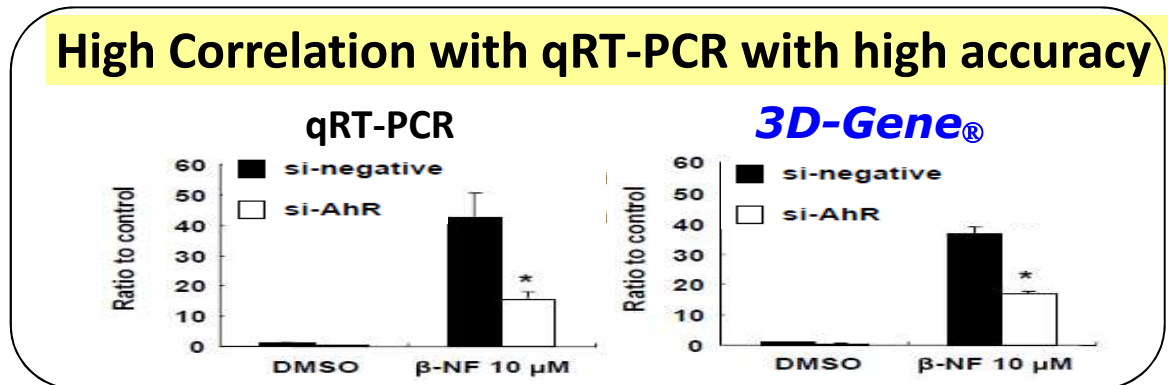
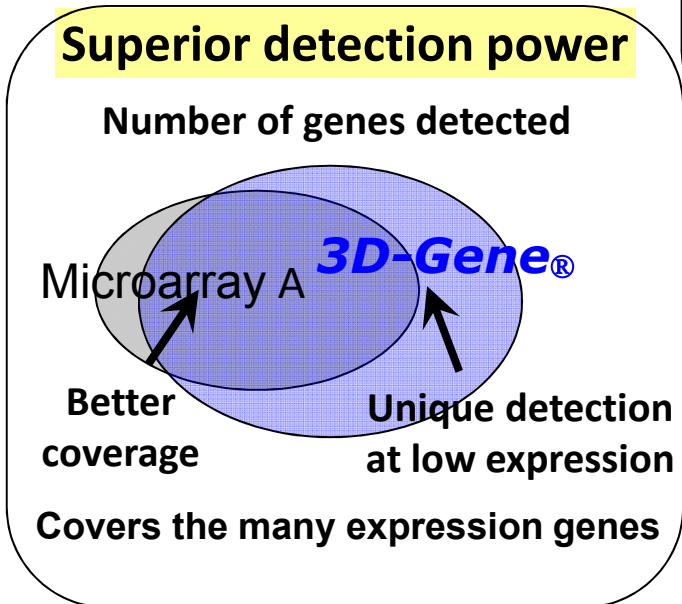
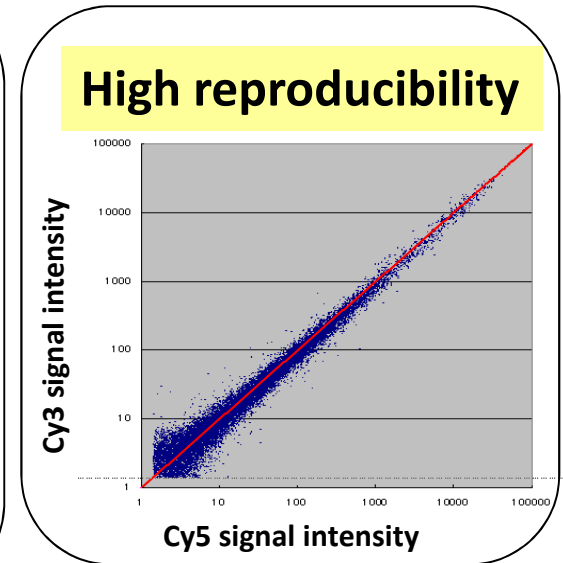
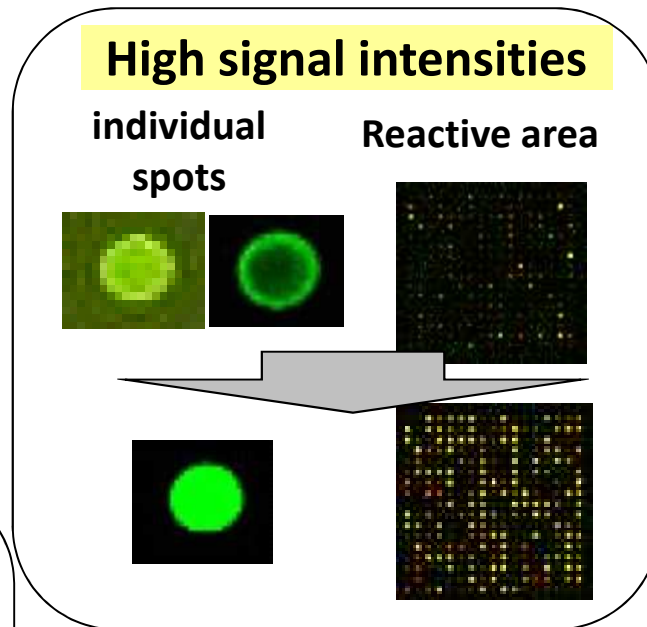
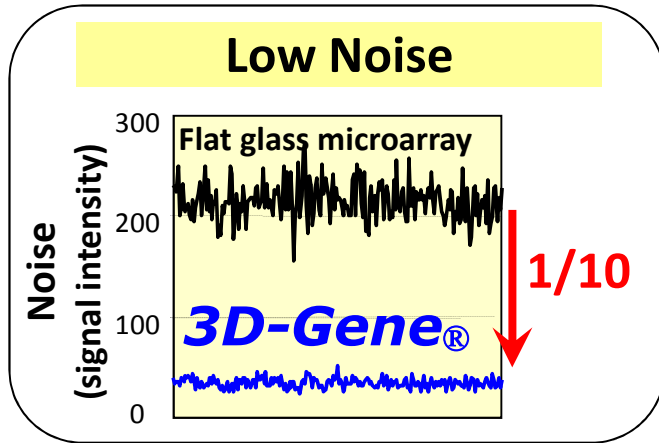


The bead agitation improved hybridization effectively



Signal intensity is drastically increased by the effect of beads agitation

Four technological advantages lead to supreme data quality

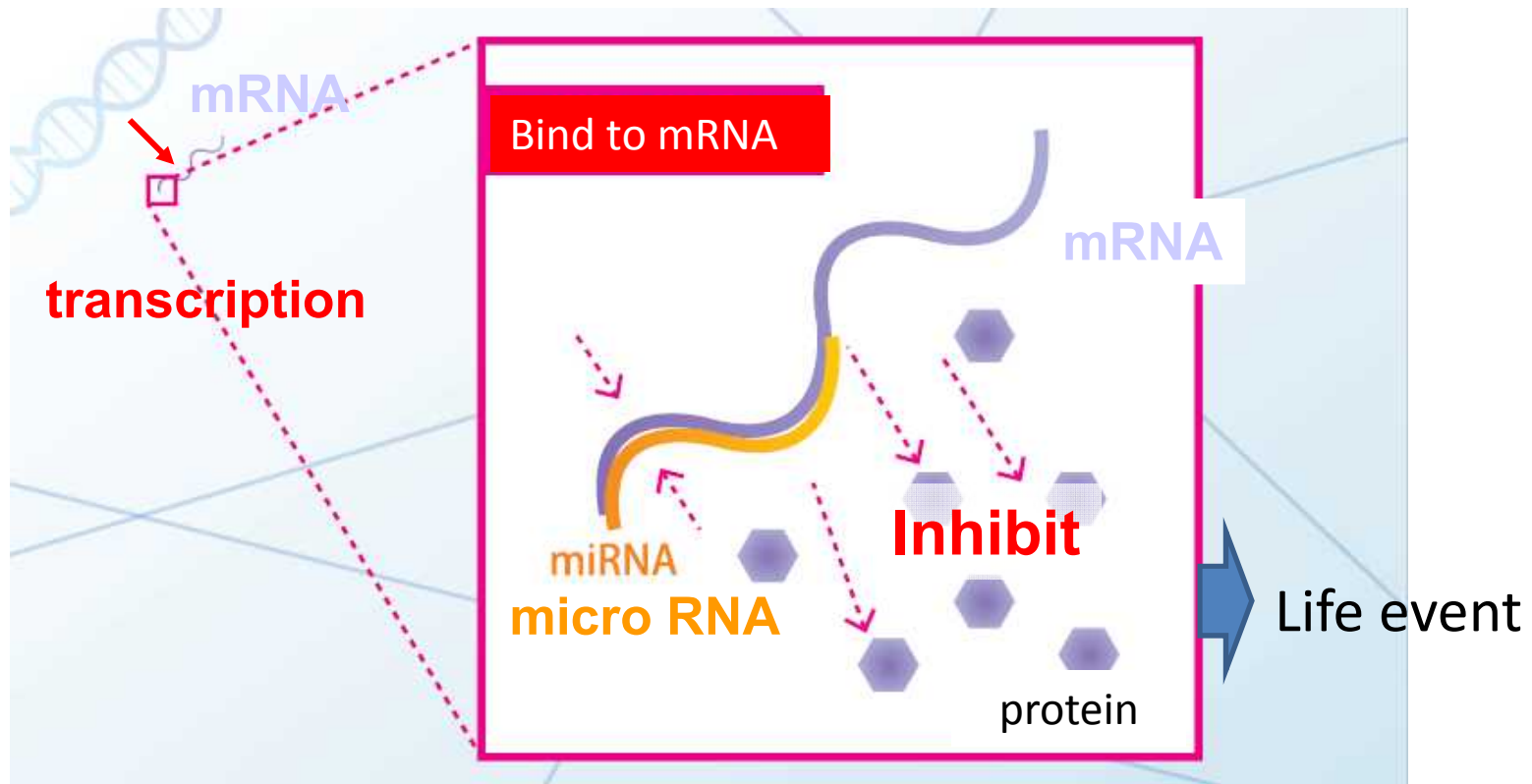


These features result in highly sensitive microarray

microRNA (miRNA)

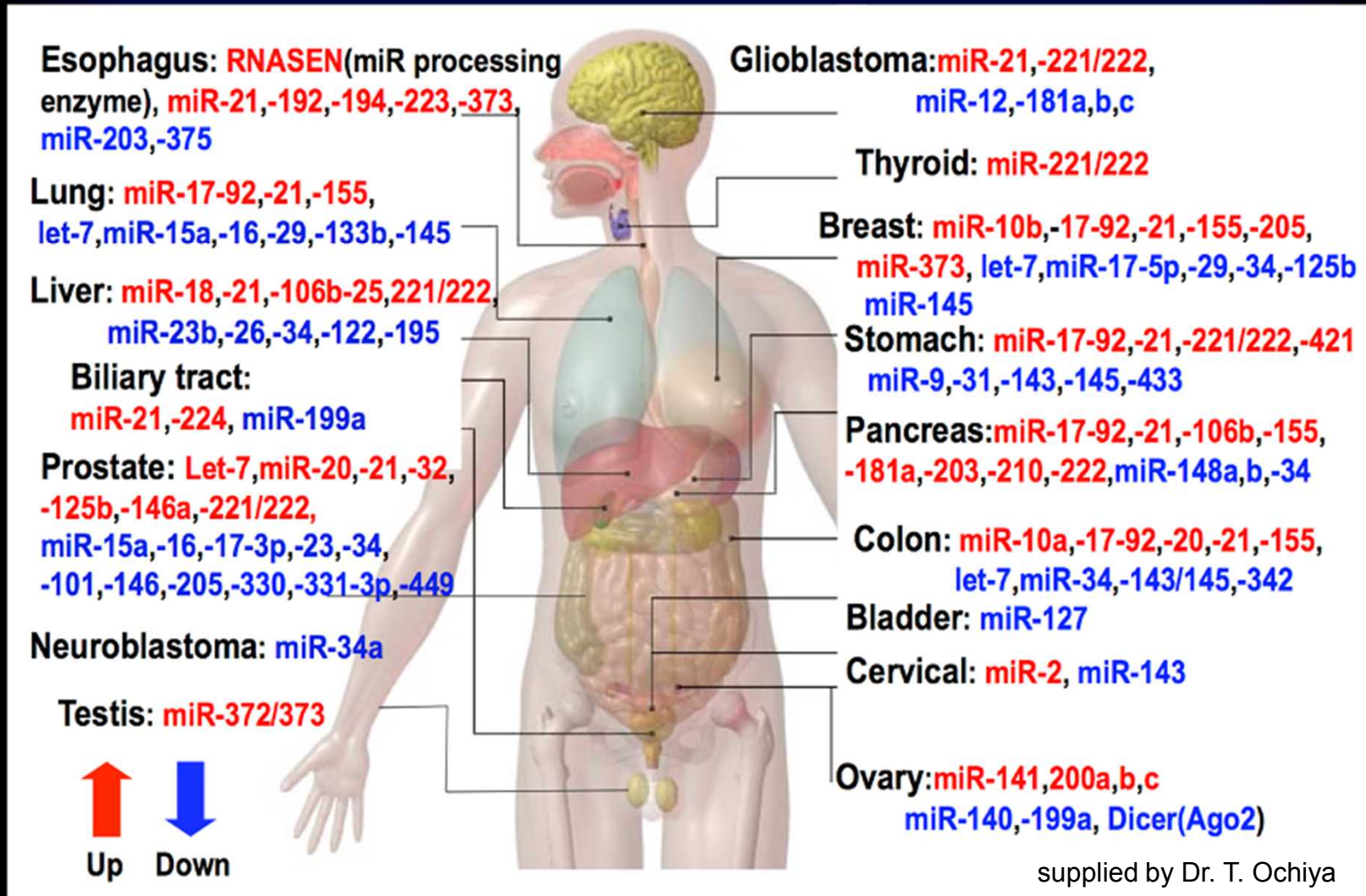
Single-stranded RNA molecules of 21-23 nucleotides in length,
which regulate gene expression

gene(DNA)



miRNA can be new Biomarker as potential

Cancer-related miRNA and Their Aberrant Expression



Serum miRNA analysis is quite hot research area for clinical researchers

3D-Gene™ miRNA data comparison

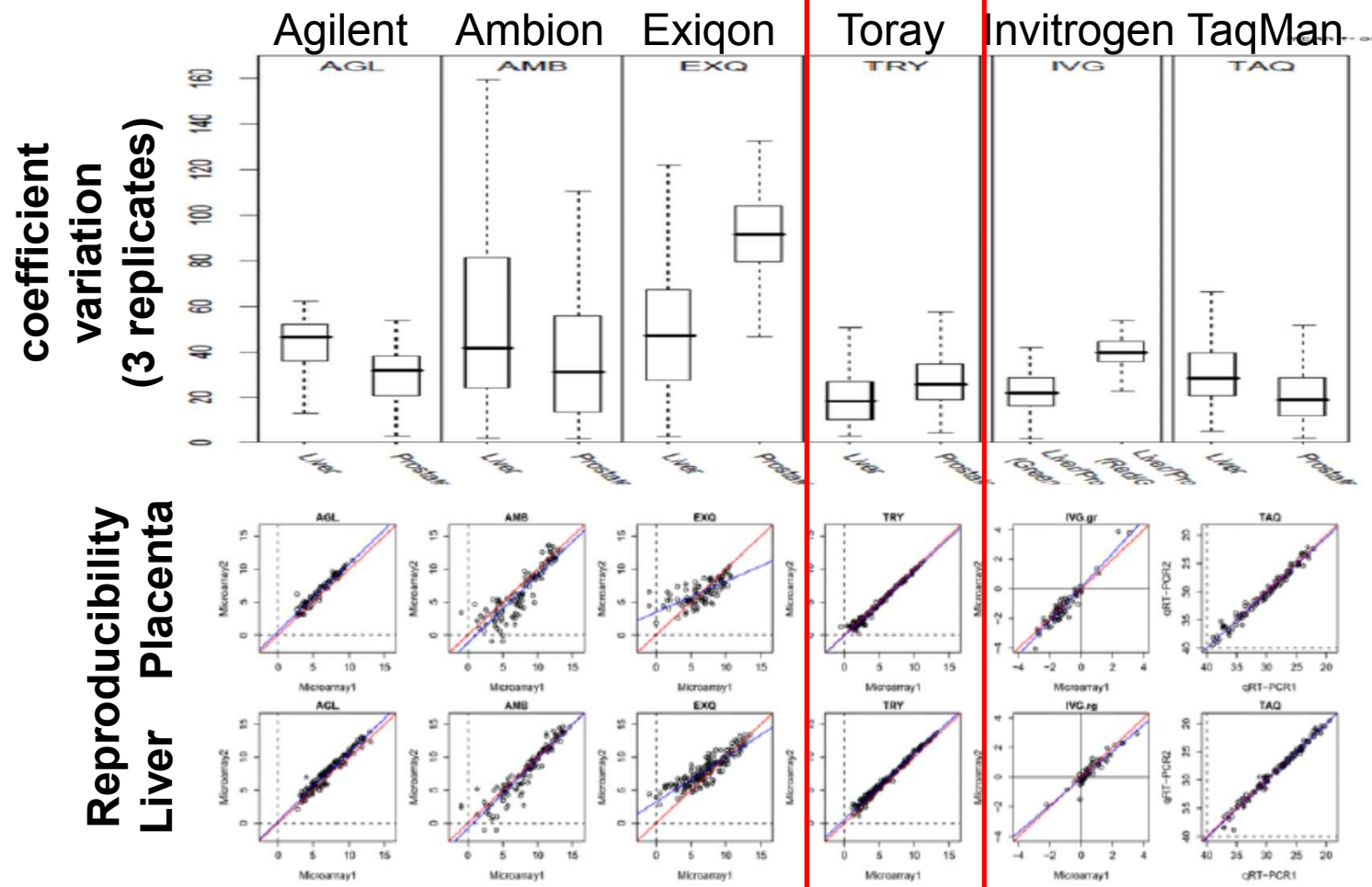
OPEN ACCESS Freely available online

PLOS one

Intra-Platform Repeatability and Inter-Platform Comparability of MicroRNA Microarray Technology

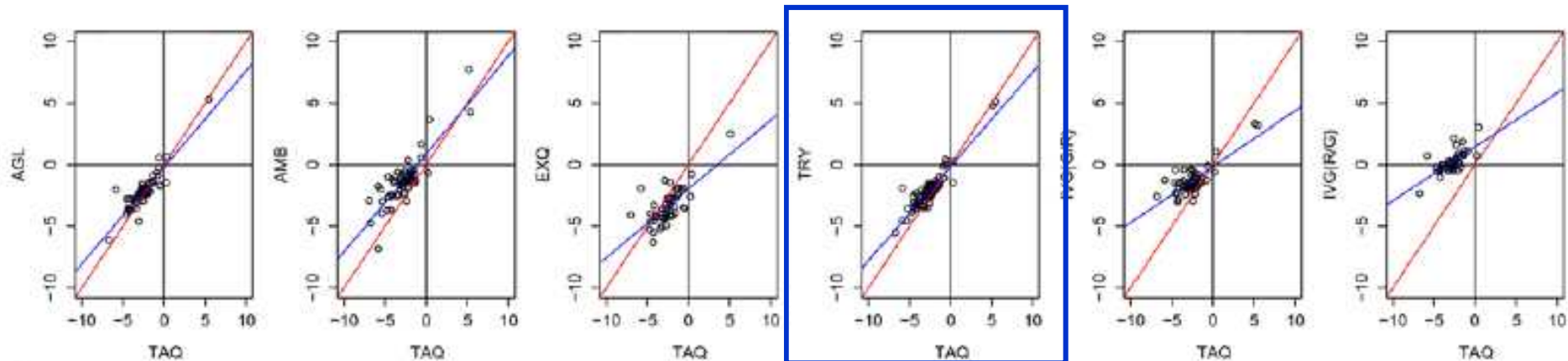
MAQC for comprehensive miRNA expression profiles

May 2009 | Volume 4 | Issue 5 | e5540



3D-Gene[®] : evaluation by 3rd party

Correlation with TaqMan qRT-PCR



	AGL	AMB	EXQ	TRY	IVG(G/R)	IVG(R/G)
# of probes	59/142	62/142	50/142	82/142	56/142	43/142
Rs	0.85	0.72	0.67	0.86	0.49	0.69
slope	0.78	0.80	0.56	0.77	0.46	0.44
Int	-0.21	0.88	-1.97	-0.13	-0.16	1.45

PLoS ONE 2009;4(5):e5540

3D-Gene[®] has high correlation with TaqMan[®]

Specificity of **3D-Gene**® on let-7 family

Sequences of human let-7 family

let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUGUGGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUACAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU

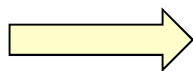
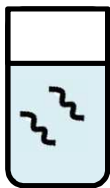
The red letters are mismatch nucleotides against let-7a.

high similarly sequence

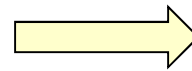
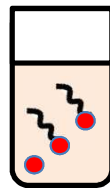
		miRNAs							
		let-7a	let-7b	let-7c	let-7d	let-7e	let-7f	let-7g	let-7i
probe	let-7a	100%	3%	20%	1%	2%	3%	0%	0%
	let-7b	6%	100%	8%	1%	0%	3%	0%	1%
	let-7c	22%	46%	100%	1%	1%	4%	0%	0%
	let-7d	21%	6%	9%	100%	0%	1%	0%	1%
	let-7e	1%	0%	0%	0%	100%	3%	0%	0%
	let-7f	2%	1%	0%	1%	0%	100%	0%	0%
	let-7g	0%	0%	0%	0%	0%	0%	100%	11%
	let-7i	0%	0%	0%	0%	0%	0%	11%	100%

Intensity of the signals obtained with each probe sequence was normalized with a value of a completely matched sequence, and the ratios (%) were obtained.

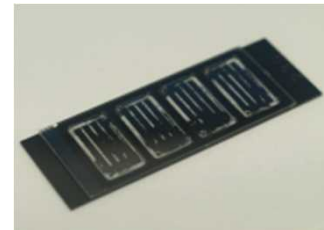
Let-7 family
chemically
synthesized



Individually
labeling



Detected with **3D-Gene**®



3D-Gene[®] miRNA microarray

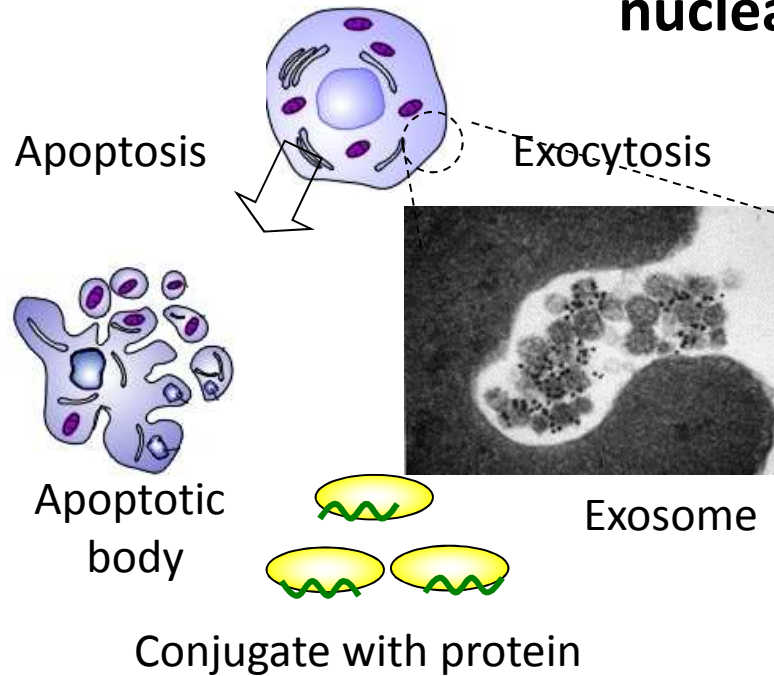
- **High Sensitivity**
- **High Reproducibility**
- **High correlation with RT-PCR**
- **High Specificity**

Useful for many application

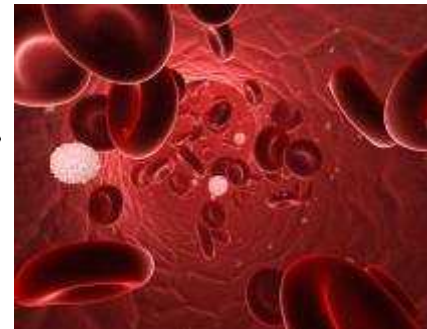
Circulating microRNA in blood(Serum/Plasma)

Tissue / Cells

nuclease-resistant(stable)



Circulating body fluid
e.g., **blood**

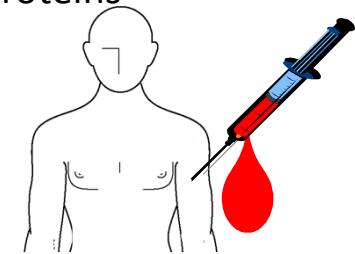


Microvesicle

Diameter 30-100nm
Phospholipid bilayer

Enclose RNA like miRNA
and proteins

Collect



Diagnostic marker



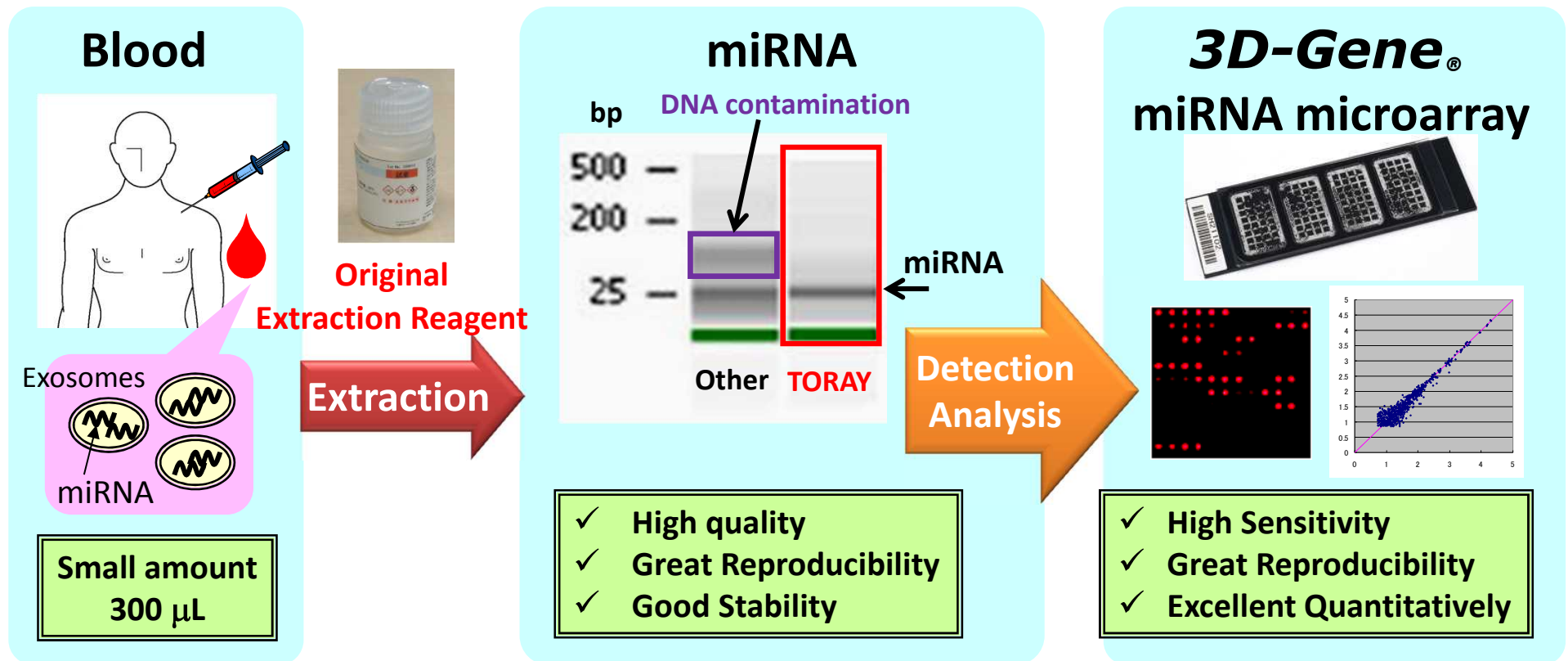
3D-Gene® detection

Features

- :Reflect the condition of organ/tissue
- :Collection is easy
- :Stable (freeze-thaw)
- ⇒ Considered as Easy and Useful Diagnostic marker

Advantage of the 3D-Gene system for studying circulating miRNA

- RNA extraction technology especially focusing on serum/plasma analysis.
 - Extraction tech. : NO DNA contamination



Starting material is recommended only 300μL plasma / serum

Cancer diagnosis with small amount of blood



Comprehensive analysis
of miRNAs in serum

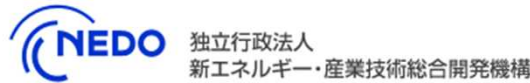


NCC



NCGG

体液中マイクロRNA測定技術
基盤開発プロジェクト



バイオチップコンソーシアム

Early stage detection of
13 cancers and dementias



1. Specific diagnostic markers will **improve mortality rate** and contribute **the reduction of medical cost**.
2. Samples and clinical information stored in **bio-bank** of NCC/NCGG make the **rapid research and development**.
3. Hope for the **prediction of the therapeutic effects or new drug development** are also promising.

データベースならびに臨床検査システムの開発



JMACの主導によりデータベースを設計・構築・運用

国際的な視点からデータベースのベンチマーク評価⇒ **国際評価の向上**
データベースの活用 ⇒ **診断システムの創出**

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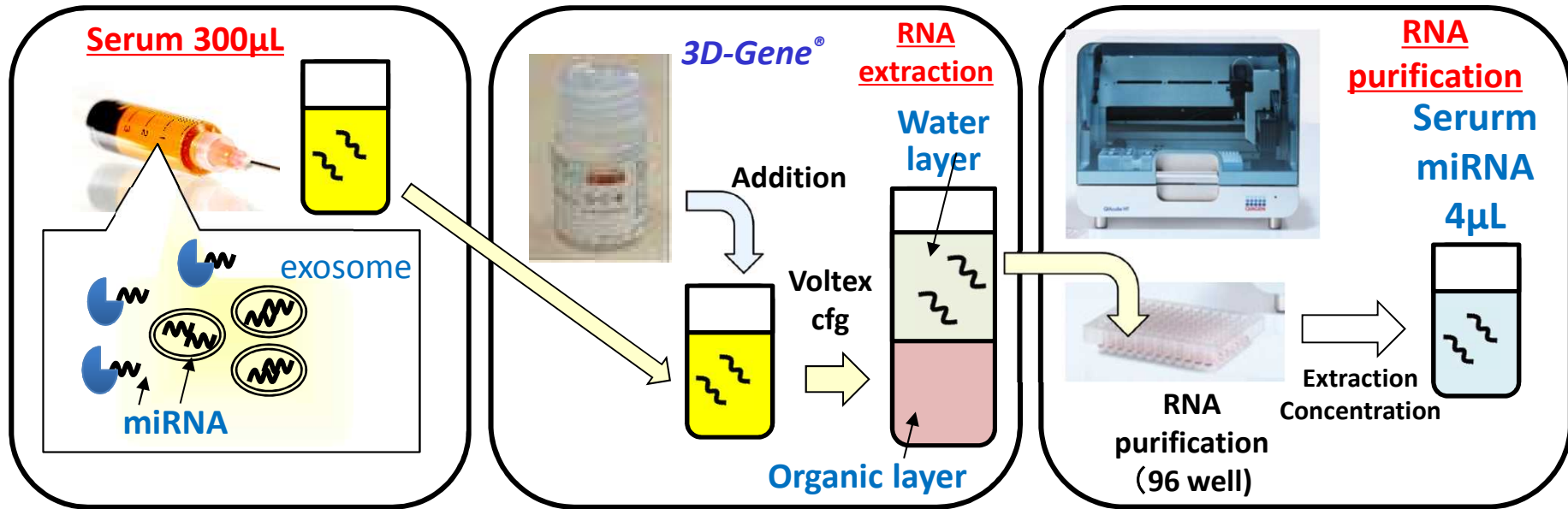
Analysis flow and QAQC

4. 事例紹介とまとめ

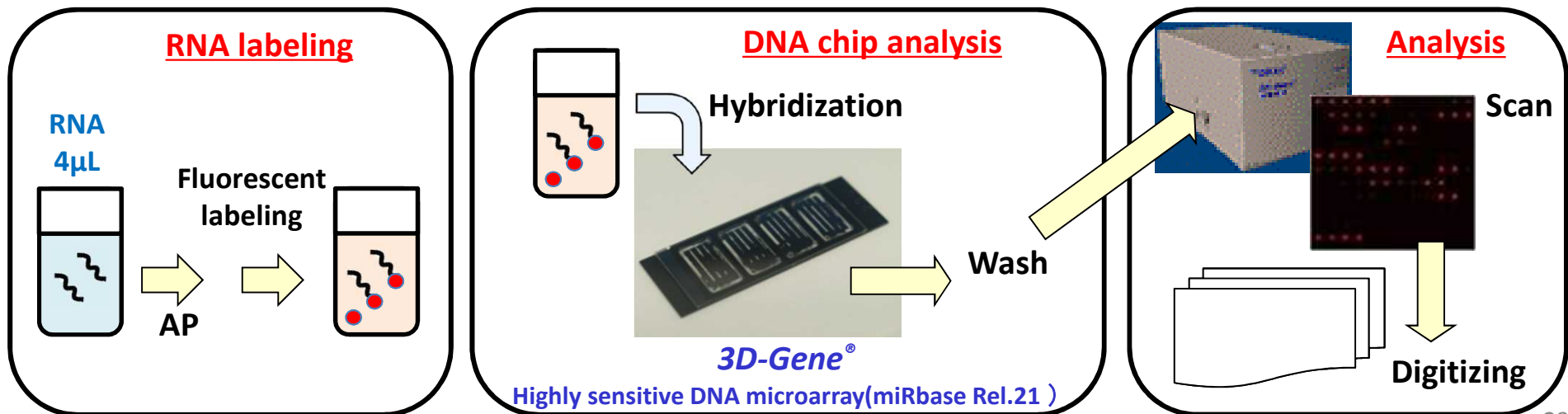
Example of Clinical Study and summary

Comprehensive analysis for serum miRNAs

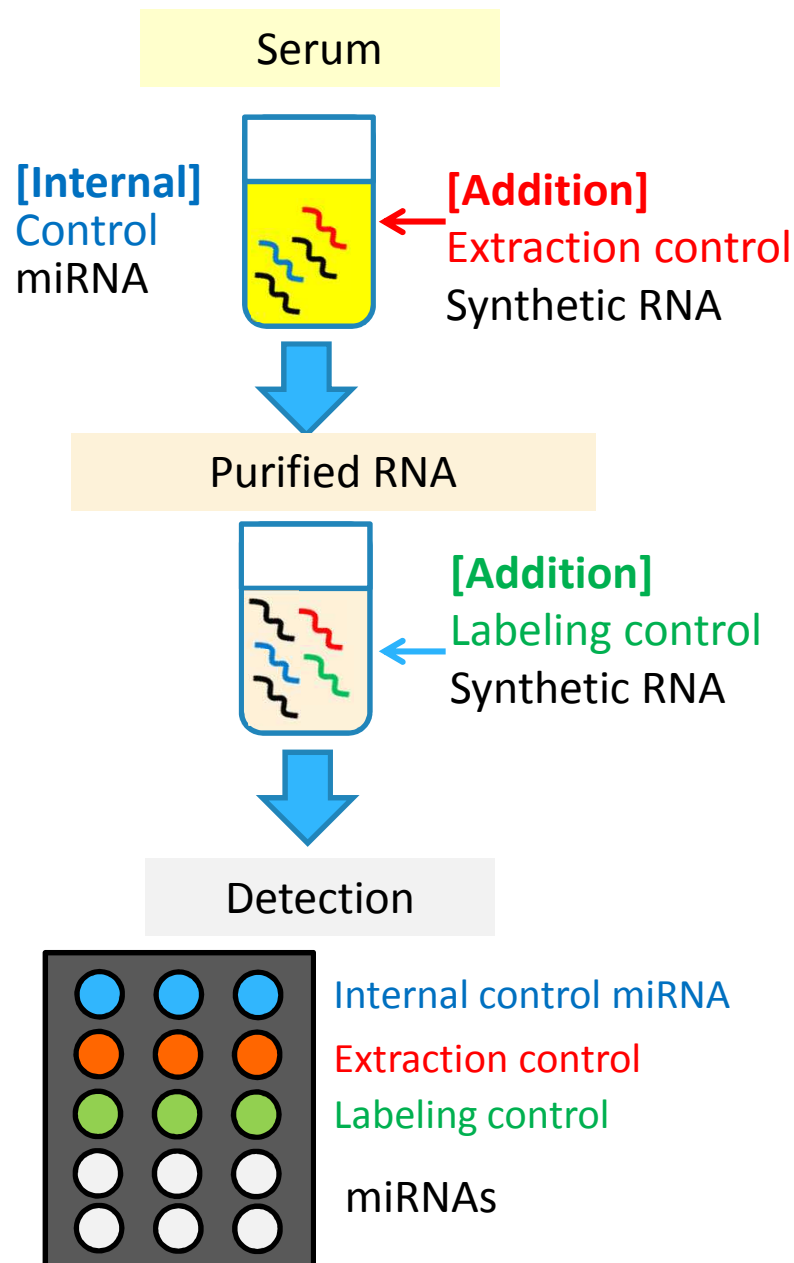
1. RNA extraction from serum



2. Comprehensive miRNA analysis with DNA microarray



Quality control methods in project



1 Extraction control :

Used for the extraction efficiency

- Three synthetic RNA were added before the purification of RNA solution.

2 Labeling control :

Used for labeling efficiency

- Three synthetic RNA were added before labeling of RNA.

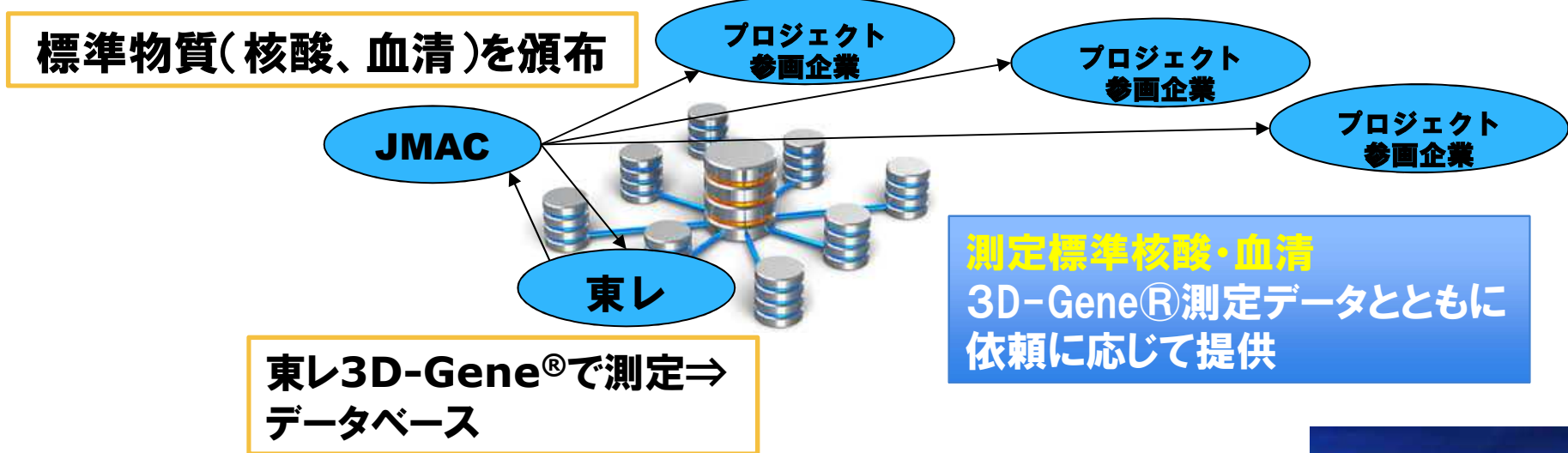
3 Internal miRNA :

Used for normalization

- Internal control miRNAs were selected from database of human serum miRNA of more than 700 samples analyzed by **3D-Gene**[®]
- Selection criteria was small CV (SD/average) by geNorm

実用化戦略

JMACの国際標準化戦略と連携し、
標準物質の頒布とデータベースの活用を行う



■標準物質開発

: 正確で簡便な値付け技術

■データベースの開発

: 真正性、信頼性、完全性、利便性が考慮されたデータベース

⇒ 信頼できるマーカーやシステムの開発と試験デザインの実施



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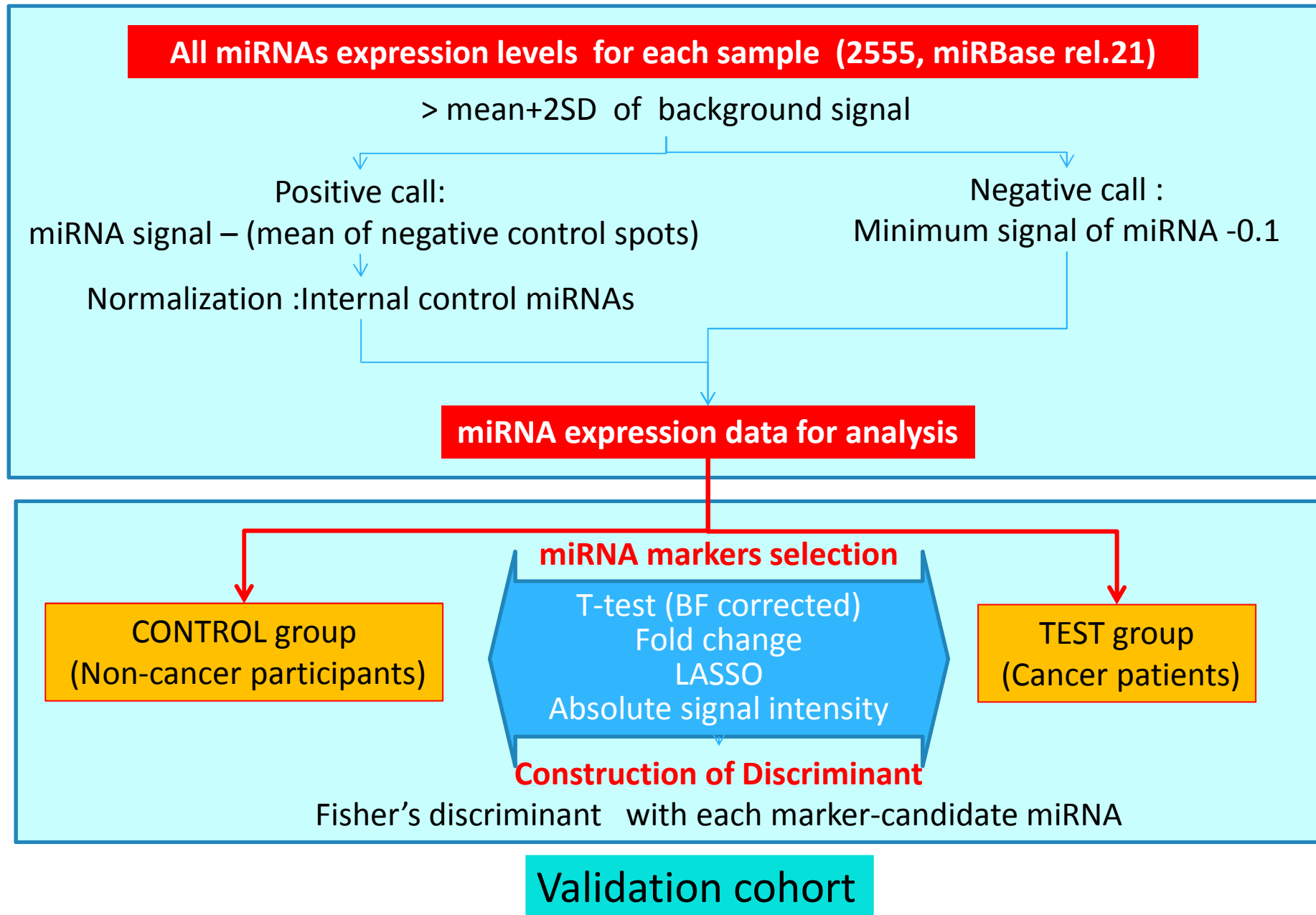
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Example of Clinical Study and summary

Analytical steps



Study of Serum microRNA Diagnostic Marker researched by 3D-gene

Cancer Science

Japanese Cancer Association **JCA**

Open Access

Novel combination of serum microRNA for detecting breast cancer in the early stage

Akihiko Shimomura,^{1,2} Sho Shiino,³ Junpei Kawauchi,⁴ Satoko Takizawa,⁴ Hiromi Sakamoto,⁵ Juntaro Matsuzaki,⁶ Makiko Ono,^{1,6} Fumitaka Takeshita,⁷ Shumpei Niida,⁸ Chikako Shimizu,¹ Yasuhiro Fujiwara,¹ Takayuki Kinoshita,³ Kenji Tamura^{1,2} and Takahiro Ochiya⁸

Cancer Sci March 2016, vol. 107, no. 3, 326–334

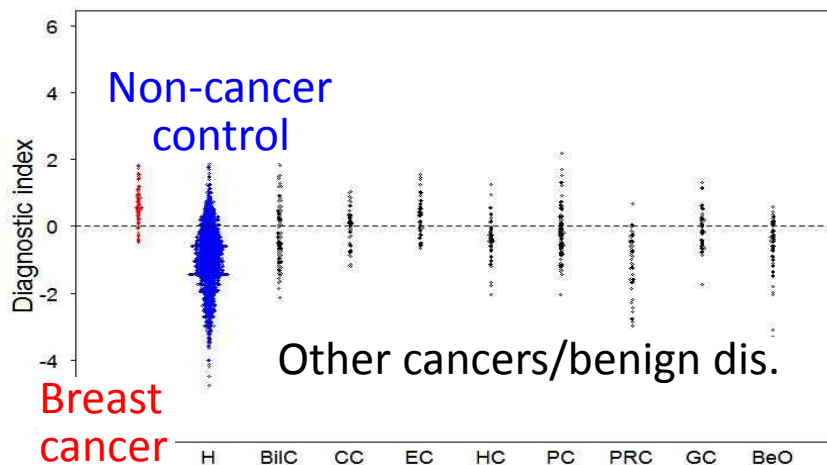
	Training cohort	Test cohort
Number of patients (1,280)	74	1,206
Tumor stage	Stage 0 (256)	0
	Stage 1 (483)	0
	Stage 2 (444)	52
	Stage 3 (44)	22
	Stage 4 (53)	0
		256
		483
		392
		22
		53

Discriminant performance of combined 5 miRNAs

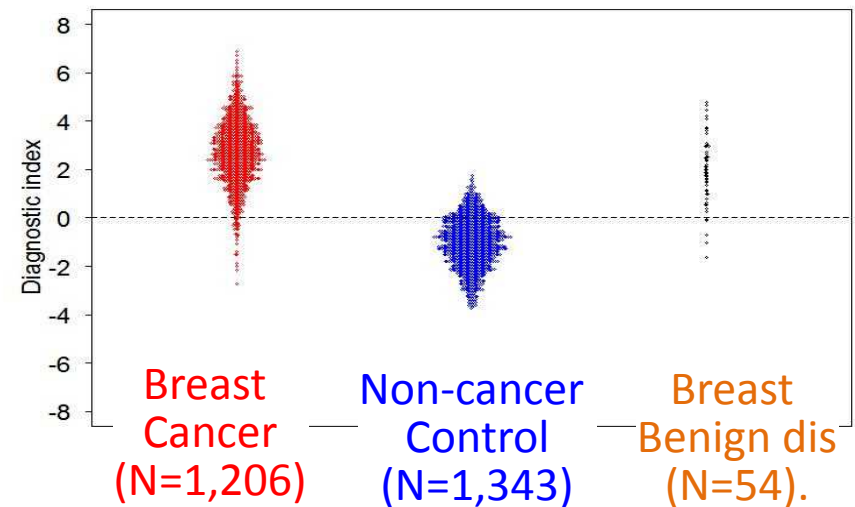
$$\text{Diagnostic index} = (0.25 \times \text{miR-1246}) + (0.49 \times \text{miR-1307-3p}) - (1.06 \times \text{miR-4634}) + (1.89 \times \text{miR-6875-5p}) + (0.31 \times \text{miR-6861-5p}) - 13.94$$

Dis-criminant	Training set			Test set		
	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)
Diagnostic index (5miRNAs)	78.7	87.8	78.5	89.7	97.3	82.9

A. Training set

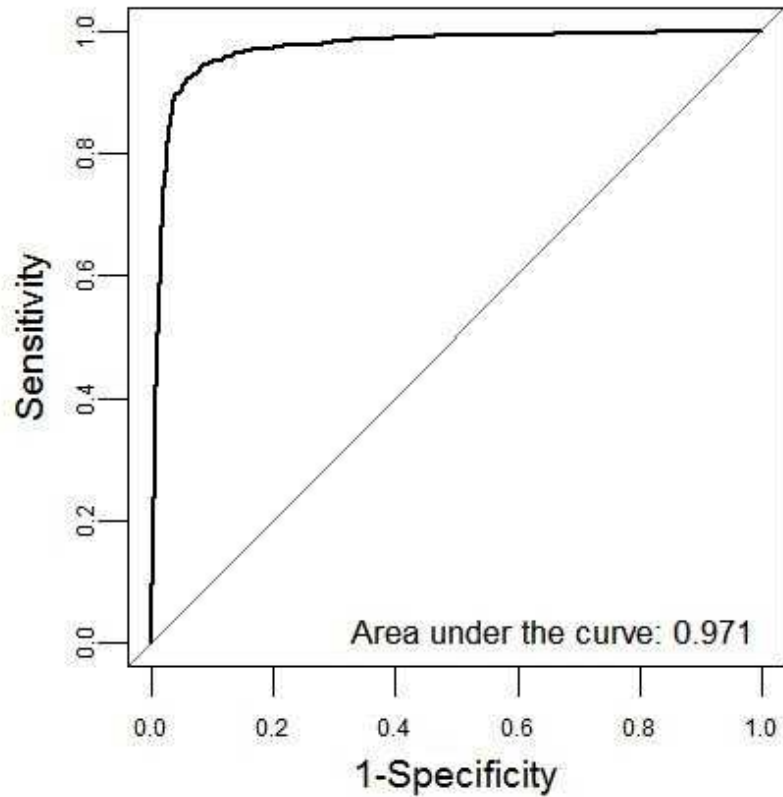


B. Test set



Discriminant performance combined 5 miRNAs results in **more than 85%**

Performance in combined 5 miRNAs



Patients with breast cancer (1206)				
	Stage or TNM	Positive	Negative	Sensitivity
Stage	Stage 0 (256)	251	5	0.980
	Stage 1 (483)	474	9	0.981
	Stage 2 (392)	375	17	0.957
	Stage 3 (22)	22	0	1.000
	Stage 4 (53)	51	2	0.962
T	T Tis (257)	252	5	0.981
	T1 (505)	496	9	0.982
	T2 (385)	369	16	0.958
	T3 (22)	20	2	0.909
	T4 (34)	33	1	0.971
N	Unknown (M1) (3)	3	0	1.000
	N N0 (1070)	1040	30	0.972
	N1 (109)	107	2	0.982
	N2 (14)	13	1	0.929
	N3 (4)	4	0	1.000
	Unknown (M1) (9)	9	0	1.000
M	M M0 (1153)	1133	31	0.973
	M1 (53)	51	2	0.962

Summary

- **3D-Gene**[®] system, which includes the RNA extraction kit, the highly sensitive DNA microarray, is suitable to detect the comprehensive miRNA expression in 300 μ L of serum /plasma.
- In the national project, we have been constructing miRNA profiling database of various cancer serum and we have been developing cancer diagnosis device.
- We control the experiments and analysis data quality by using developed standard substance in national project.