Molecular Diagnostics Kits and Microfluidics System for Detection of Infectious Pathogens and Cancer Cell Mutations

Prof. Michael Yang
Department of Biomedical Sciences
City University of Hong Kong

bhmyang@cityu.edu.hk

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Hong Kong Council for Testing and Certification
Hong Kong Accreditation Service

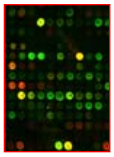
Research Interests

Development of biotechnology and nanomedicine for molecular diagnostics and therapeutic applications.

DNA Chips

Gene Expression Profiling & Genotyping

Established the 1st DNA Microarray Core Facility in Hong Kong supported by Innovation and Technology Fund (1996-2000).



AoE in Molecular Technology & Drug Discovery (2001-2011)
AoE in Marine Environmental Research & Technology (2003-13)
AoE in Chinese Medicine Development (2007-12)

Co-founded two biotech companies with CityU and successfully transferred the DNA chip technology for cervical cancer screening and molecular diagnostic services.

J. Inv. Dermatology, **2003**, *120*, 849-857.
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Exp. Cell Res. **2007**, *313*, 1722-1734.
Clin. Cancer Res. **2008**, *14*, 5061-5068.
Proteomics, **2008**, *8*, 3105-3117.
J. Clinical Neuroscience, **2009**, *16*, 285-294.
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J. Medical Virology, **2010**, *82*, 1724-1729.
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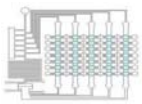
J. Cellular Biochem. **2013**, *114*, 1105-1114.
Biosens. Bioelec. **2014**, *53*, 406-413.

Microfluidics

Cellular Communication
Drug Screening Assays



Lab Chip, **2002**, *2*, 158-163.
Lab Chip, **2004**, *4*, 53–59.
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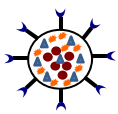
Nanomedicine

Stem Cell Differentiation
Targeted Cancer Therapy



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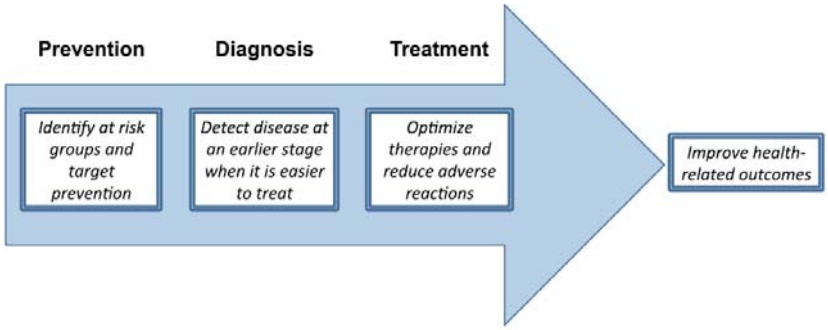
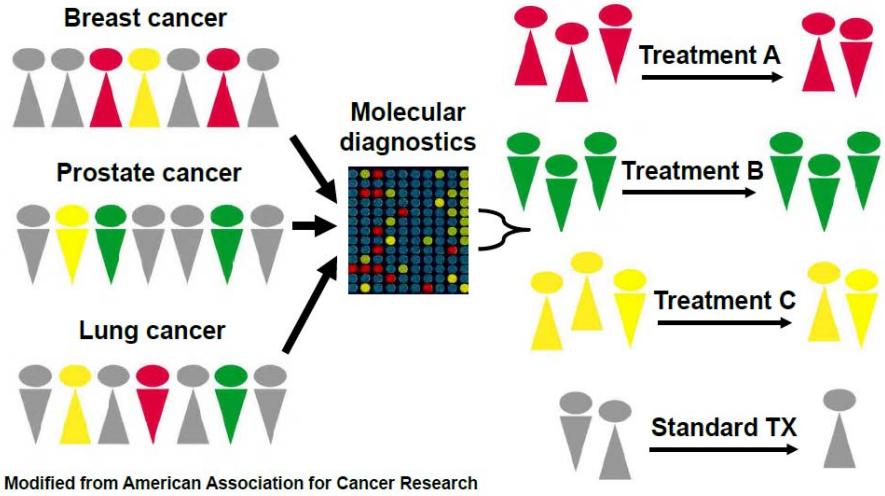
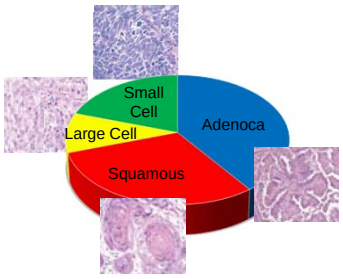
Precision Medicine



Molecular Classification of Diseases



Molecular Subsets and Target-based Therapeutics for Lung Cancer



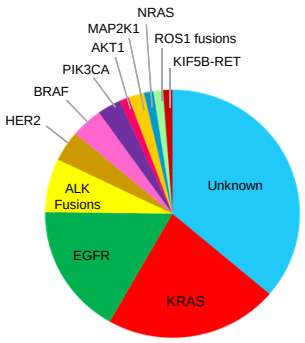
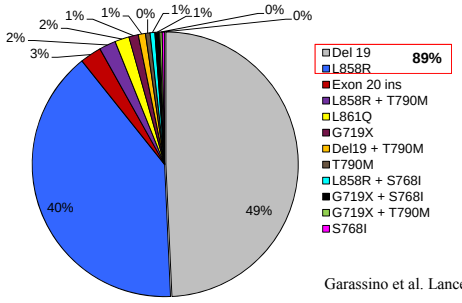


Table 1. Frequency of Mutations and Availability of Targeted Therapies

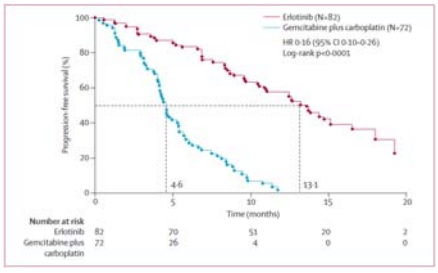
Gene	Alteration	Frequency in NSCLC
AKT1	Mutation	1%
ALK	Rearrangement	3–7%
BRAF	Mutation	1–3%
DDR2	Mutation	~4%
EGFR	Mutation	10–35%
FGFR1	Amplification	20%
HER2	Mutation	2–4%
KRAS	Mutation	15–25%
MEK1	Mutation	1%
MET[a]	Amplification	2–4%
NRAS	Mutation	1%
PIK3CA	Mutation	1–3%
PTEN	Mutation	4–8%
RET	Rearrangement	1%
ROS1[a]	Rearrangement	1%

Key:
Drugs approved in NSCLC.
Drugs approved in NSCLC but for other molecular subtype.
Drugs approved in other cancer.
Drugs in clinical development.

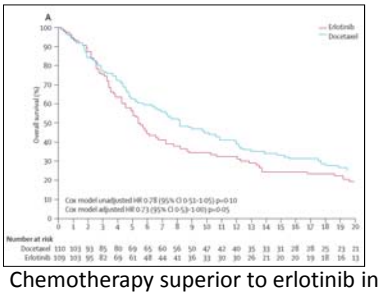
Targeting the Mutant EGFR with Erlotinib



Garassino et al. Lancet 2013; Zhou et al. Lancet 2011

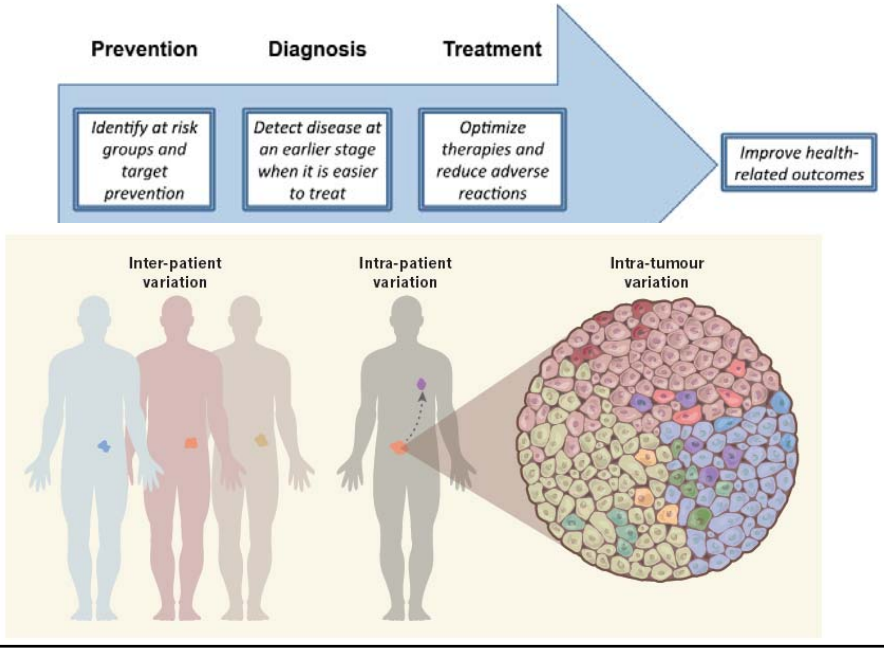


Erlotinib superior to chemotherapy in EGFR mutated lung cancer

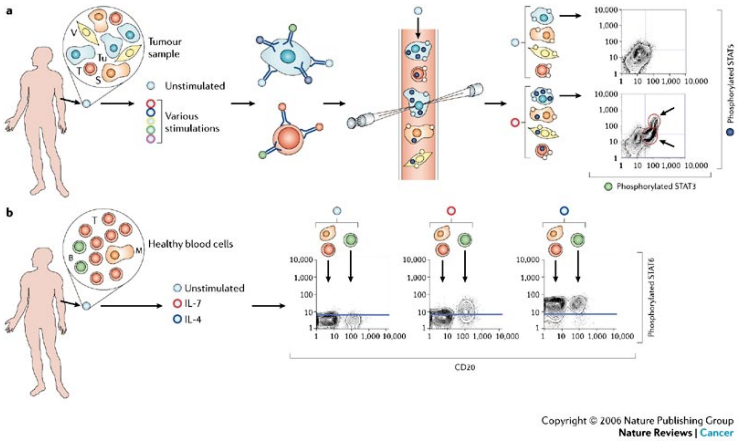


Chemotherapy superior to erlotinib in EGFR non-mutated (wild-type) cancer.

Precision Medicine



Single Cell Analysis



- Biopsy/primary tumor
- Circulating tumor cells
- Mutation analysis
- Gene expression analysis

Analytical Platform for Precision Medicine

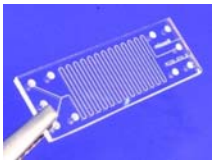
Sample Processing
Microfluidics

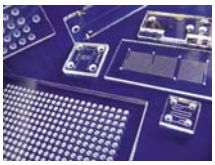
→

Data Generation
PCR/NGS
Microfluidics

→

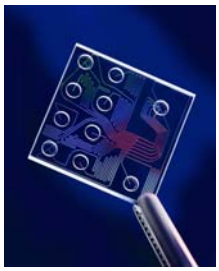
Data Processing
Bioinformatics



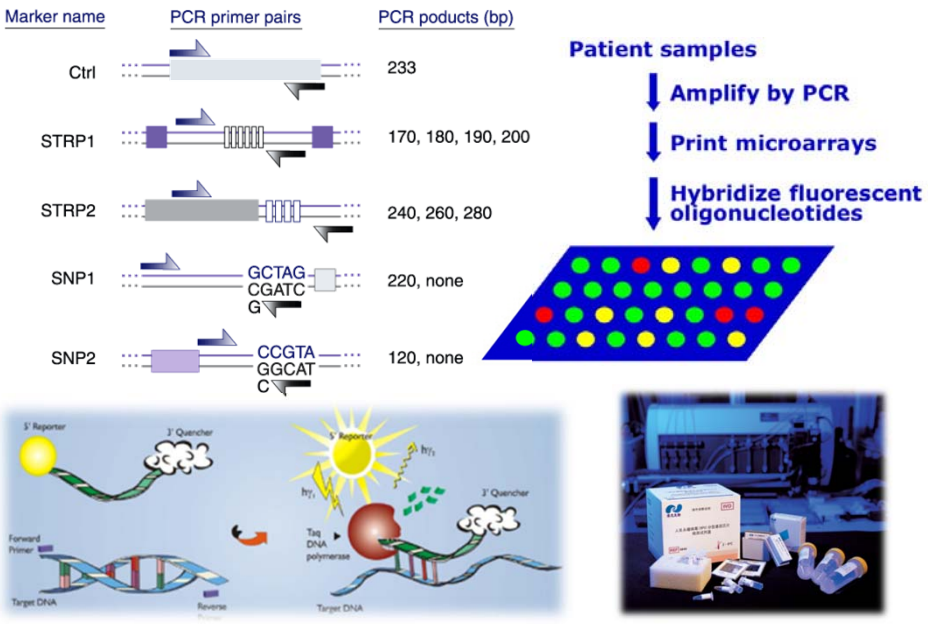


- **High-throughput Data Acquisition**
- **Massive, Parallel Data Analysis**
- **Miniaturization and Multiplexing**
- **Automation and Mass Production**

Faster…Smaller…Cheaper…



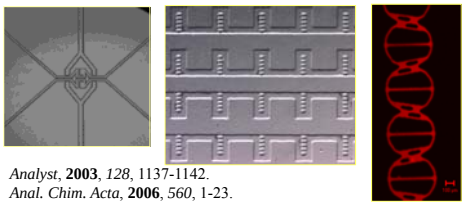
Multiplex PCR and DNA Chips for Molecular Diagnosis



Microfluidics Technology Platform

Fabrication of Microfluidics

- **Rapid prototyping of different microfluidic structures**
- **One-step to generate multi-level PDMS structures**



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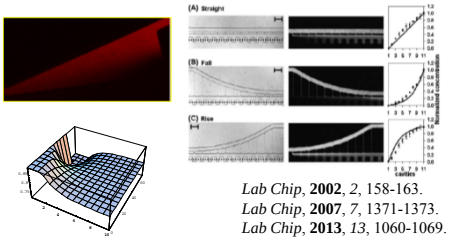
Biosens. Bioelec, **2013**, *41*, 675-683.

Generation of Concentration Gradient

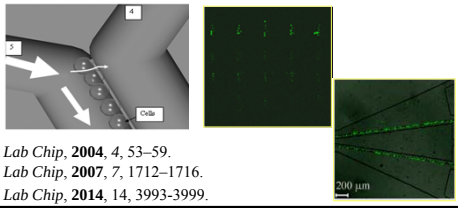
- **Discrete and continuous concentration gradients**
- **Parallel and perpendicular to flow over channel length**



Lab Chip, **2002**, *2*, 158-163.
Lab Chip, **2007**, *7*, 1371-1373.
Lab Chip, **2013**, *13*, 1060-1069.

Controlled Manipulation of Particles

- **Hydrodynamic cell docking parallel to liquid flow**
- **Linear and array formation of individual cells**

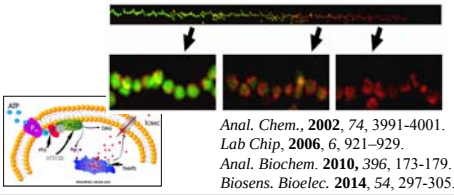


Lab Chip, **2004**, *4*, 53–59.
Lab Chip, **2007**, *7*, 1712–1716.
Lab Chip, **2014**, 14, 3993-3999.

On-chip Analysis of DNA/Proteins/Cells

US Patent No 7,560,267. PRC Patent . 03120720.0.

- **Multiple sets of dose-dependent bioassays**
- **Cell array for parallel individual cell data acquisition**



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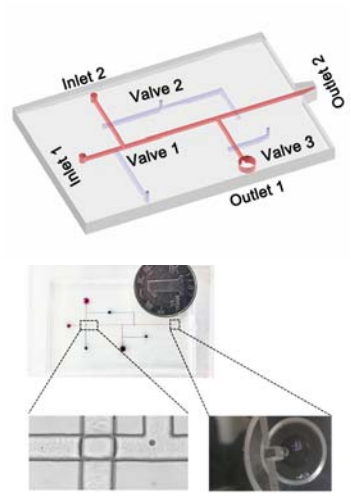
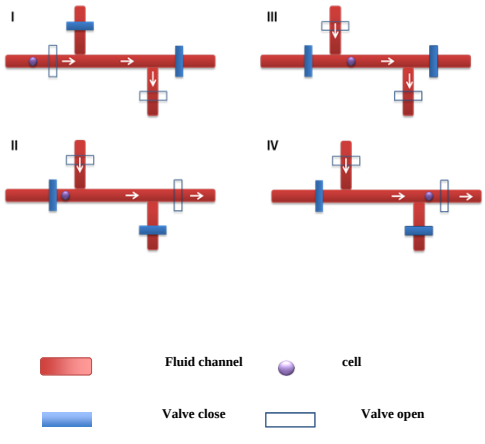
Microfluidic chip for single cell isolation





Single Cell Mutation Detection of Targeted Genes

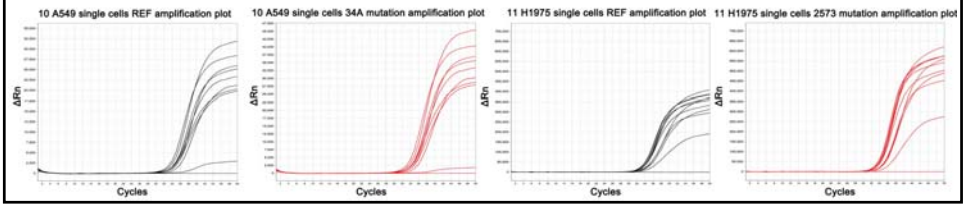
BRAF mutation		
Mutation Type	Amino Acid Change	Nucleotide Change
BRAF	V600E	1799T>A

KRAS mutation		
Mutation Type	Amino Acid Change	Nucleotide Change
34A	G12S	34G>A
34C	G12R	34G>C
34T	G12C	34G>T
35A	G12D	35G>A
35C	G12A	35G>C
35T	G12V	35G>T
38A	G13D	38G>A

EGFR mutation		
Mutation Type	Amino Acid Change	Nucleotide mutation
E18	G719S	2155G>A
	G719A	2156G>C
	G719C	2155G>T
E2303/2307	S768I	2303G>T
	V769-D770ins ASV	2307_2308ins9
E2310/2319	D770-N771insG	2310_2311insGGT
	H773-V774ins H	2319_2320insCAC
E2369	T790M	2369C>T
E2573	L858R	2573T>G
E2582	L861Q	2582T>A

EGFR mutation		
Mutation Type	Amino Acid Change	Nucleotide Change
E19	E746-T751>I	2235_2252>AAT
	E746-T751del	2236_2253del 18
	E746 - T751insA, E746-T751>A	2237_2251del 15
	E746-S752>A	2237_2254del 18
	E746-S752>V	2237_2255>T 2237_2250>T(complex)
	L747-A750>P	2238_2248>GC
	L747-T751>Q	2238_2252>GCA
	E746-S752>D	2238_2255del 18
	L747 - E749	2239_2247del9
	L747 - A750P	2239_2248g>C
	L747 - T751P	2239_2251a>C
	L747-T751	2239_2253del 15
	L747-S752	2239_2256del 18
	L747-T753	2239_2258>CA
	L747-P753Q, L747-T751>S	2240_2251del 12
	E746-A750(1)	2235_2249del 15
	E746-A750(2)	2236_2250del 15
	L747-T751	2240_2254del 15
	L747-P753insS	2240_2257del 18

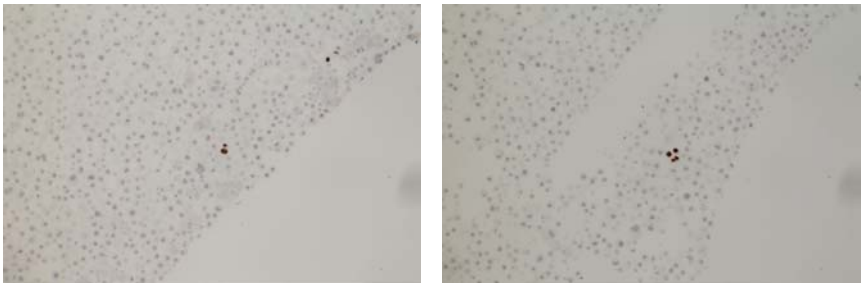
Taqman™



Profile of Pleural Fluid Sample from Lung Cancer Patient

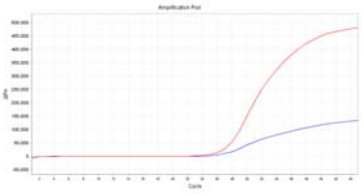
Thyroid Transcription Factor 1 (TTF-1) Staining

The no. of positive cells are very rare.



Pleural Fluid Sample (MPE-1) from Lung Cancer Patient

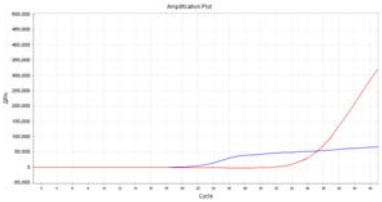
Sample	FAM CT	JOE CT	L858R
MPE-1	26.96	24.97	++



L858R total mutation rate: 7/21=0.33

SAMPLE	FAM	JOE	SAMPLE	FAM	JOE
LC9+		37.17	LC9-	36.14	36.28
LC9+	36.26	37.34	LC9-	35.20	36.35
LC9+		37.63	LC9-		36.59
LC9+		37.78	LC9-		36.77
LC9+	35.96	37.89	LC9-	37.11	36.98
LC9+		39.77	LC9-	36.38	37.01
LC9+	36.42	42.59	LC9-		37.02
			LC9-		37.27
			LC9-		37.41
			LC9-		37.57
			LC9-		37.77
			LC9-		38.51
			LC9-		38.81
			LC9-		39.88
Pos	28.855	27.503	Blk		

Sample	FAM	JOE	T790M
MPE-1	37.17	26.19	+



T790M total mutation rate: 8/17=0.47

SAMPLE	FAM	JOE	SAMPLE	FAM	JOE
LC9+	38.89	36.03	LC9-	42.64	36.05
LC9+	37.72	36.19	LC9-	37.51	36.35
LC9+	-	36.40	LC9-	-	37.23
LC9+	-	36.44	LC9-	42.36	37.31
LC9+	42.14	36.59	LC9-	-	37.97
LC9+	38.93	37.00	LC9-	-	38.24
LC9+	-	37.95	LC9-	-	38.25
			LC9-	-	39.31
			LC9-	40.46	39.32
			LC9-	-	40.05
Pos	33.87	29.04	Blk	-	-

Population Analysis vs Single Cell Analysis

Single Cell Gene Expression Analysis

Amplification Protocol

Single cell isolation by microfluidic chips

↓

Cell lysis

↓

Inverse transcription

↓

Exonuclease treatment

↓

cDNA preamplification

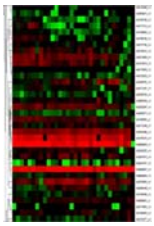
↓

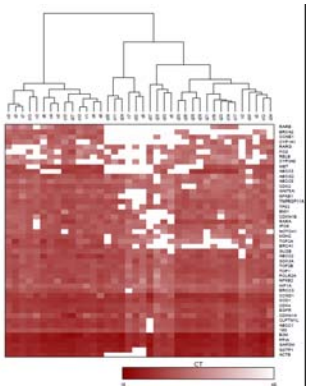
10-100 Dilution for qPCR

CLPTM1L	NFKB1	ERBB4
TP53	TOP2B	CDKN1B
RELB	ABCC2	EGFR
ERCC3	ABCC3	B2M
TNFRSF11A	GSK3A	GAPDH
GSTP1	CYP1A2	RARB
NFKB2	ABCC1	18S
CYP1A1	BRCA2	TOP2A
BMI1	CCND1	FOS
RARA	CYP3A5	POLR2A
CCNE1	RARG	NOTCH1
MSH2	TOP1	MET
SOD1	BRCA1	IPO8
CDKN2A	WNT5A	CYP3A4
WNT11	CDKN1A	GUSB
ERBB3	ABCG2	ABCB1
CDKN2D	PPIA	CDK4
HIF1A	ACTB	WNT5B
CDK2	ABCC5	

Drug metabolism/resistant genes

47 cells; 1 blank





Summary

- Molecular Diagnostic Kits based on PCR and DNA Chip Technology
  - Infectious pathogens
  - Viral genotyping
  - Tumor gene mutations

- Single Cell Analysis Systems based on Microfluidics Technology
  - Single cell selection and isolation
  - Single cell mutation analysis
  - Single cell gene expression analysis
  - Circulation tumor cell identification

Acknowledgements

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