Product & Services

Based on an Innovative Platform Technology for Screening in Mammalian Cells

CytoPathfinder

Our Potential Customers

We are providing our solid–phase transfection(STF) plates in cellular research for

1.facilitating target discovery and alternative pathway analysis

2.RNAi-based compound profiling for designing rational combination of drugs

Our potential customers would be Researchers
Who have problem such as

- we have no suitable method to transfect into our target cell

also who have expectation such as
- we want to convert actual viral method to non viral

- we want to conduct large scale high-throughput screening more efficiently by small number of staff

- we want to conduct gene editing research work in house instead of outsourcing.

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Our Products and services

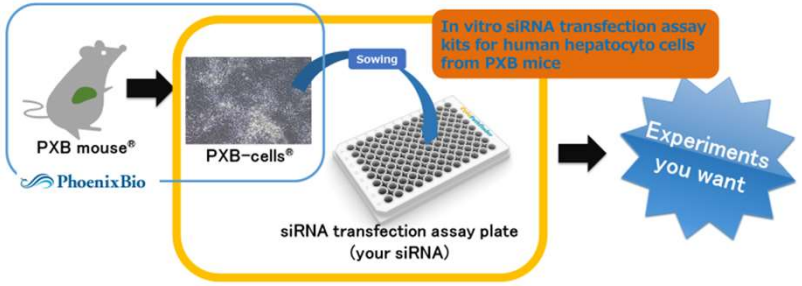
- siRNA / miRNA / Plasmid DNA (supplied from clients)
  - Plates for sale for use in customer's laboratory
    - Transfection Optimization plates
    - Customized plates
    - Large scale screening plates

- **Non viral method of Gene Editing using CRISPR−Cas9 System**
  (Cas9 and g-RNA are supplied from clients)

■ Types of solid-phase CRISPR / Cas9 plates (including under development)

Cas9	gRNA	
	Plasmid	RNA
Cas9-introduced cells	Kits for plasmid	Kits for RNA
plasmid	Kits for plasmid	under development
mRNA	under development	under development

- **In vitro siRNA transfection assay kits for human hepatocyto cells from PXB mice**



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How to use our products for screening processes?

Steps	Transfection condition screen	Assay Development	Screening
Objectives	•To find several Solid-phase transfection candidate conditions for target cells •To find appropriate culture condition for seeding (ie. Serum-free treatment, Seeding density, culture time, etc.)	•To find the best transfection condition under appropriate assay methods for screen •To find assay controls for screen •To find appropriate culture condition for screen	Cutdown
Products	Name : **Transfection Optimization Kit**  Transfection Optimization plate contents; •For Hs/Ms/Rn species •384 plate (standard : *Corning#3712*) •Freezer storage (at least 1 yr.) •ca.140 compositions/plate ✓Transfection reagents (types & conc.) ✓siRNA conc. ✓siRNA (Negative & CytoTox siRNA) ✓Accelerator conc.	Name : **Customized plate** Customized points; •Free layout •Can use various manufacture's siRNA/miRNA (Ambion, Dharmacon, Qiagen, etc.; negatively charged) •Can use various types of plate (6, 96, 384, 1536well, PDL/PLL-coated, etc.) •Variable number of replica plates •Freezer storage (at least 1 yr.)	
Necleic acid resources	**CytoPathfinder will provide**	**Client will provide (Basically)**	
Assays	Viability assays; •Luminescence (**recommended**) (Promega CellTiter-Glo, PE ATPLite, etc.) •Fluorescence (Promega CellTiter-Blue, etc.) •Absorbance (MTT, WST assay) •Image-based assay (GE INCell analyzer, PE Opera, etc.) *CytoPathfinder will provide the analysys software (Excel macro)	Various assays; •qPCR •Western blot •Reporter assay •ELISA •Image-based assay …etc.	

Innovative tools in vitro study compared to actual method

Materials

Processing

Products (Results)

Try & error by specialist

CytoPathfinder's TF plate

Easy and effective

Reducibility and Reliability

Data set for drug discovery research and medical research

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Automated system for STF plate manufacturing

① Improvement of reproducibility by automation of manufacturing

CytoPathfinder's system (Multi-well plate)

Manufacturing

Automatic dispenser
- Sample information management
- Automatic operation
- Calibration system integrated
- High reliability
- Dispense information management

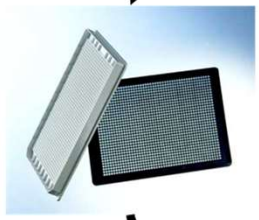
Resources (DNA, RNA…)
- Vector library
- Reporter system
- siRNA library
- Other molecules

information

Data storage
- Data integration of all information
- Mathematical analysis for all data

③ Experimental condition setting based on accumulated data

② Reliability due to strict quality control



Quality management

Cell
- Cell line
- Primary cell
- Normal cell

Time-lapse observation
- Cell culturing system integrated
- Auto-focus unit integrated
- Automatic data acquisition software
- High resolution image acquisition

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Advantage over conventional transfection method

- Enhanced solid-phase transfection efficiency with proprietary accelerators and accumulated know-how
- Simple to use methodology/simpler transfection process
- Varied formats possible
- Simple optimisation process
- Excellent reproducibility and reliability
- Reliable results with hard-to-transfect cells
- Fewer cells needed per assay
- Dual knockdown easily achievable

Time, cost saving for getting reliable data

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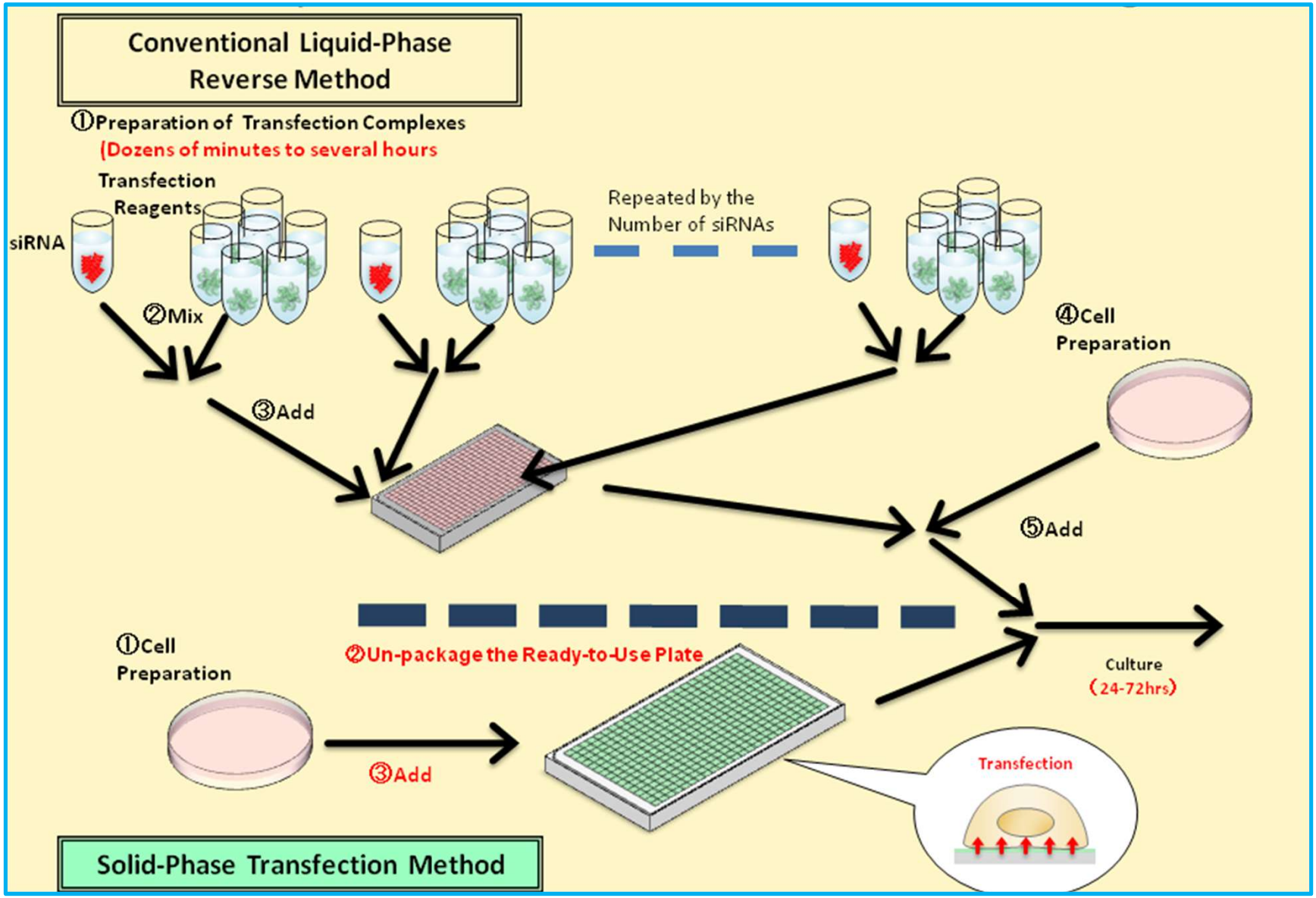
Advantage over conventional method
Simple Process- Time, Labor, Cost saving



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Time saving

Conventional methods: (multiple try & error experiments)

Low efficiency
High toxicity

High efficiency
Low toxicity

~6 months

STF:

Low efficiency
High toxicity

High efficiency
Low toxicity

~1 month

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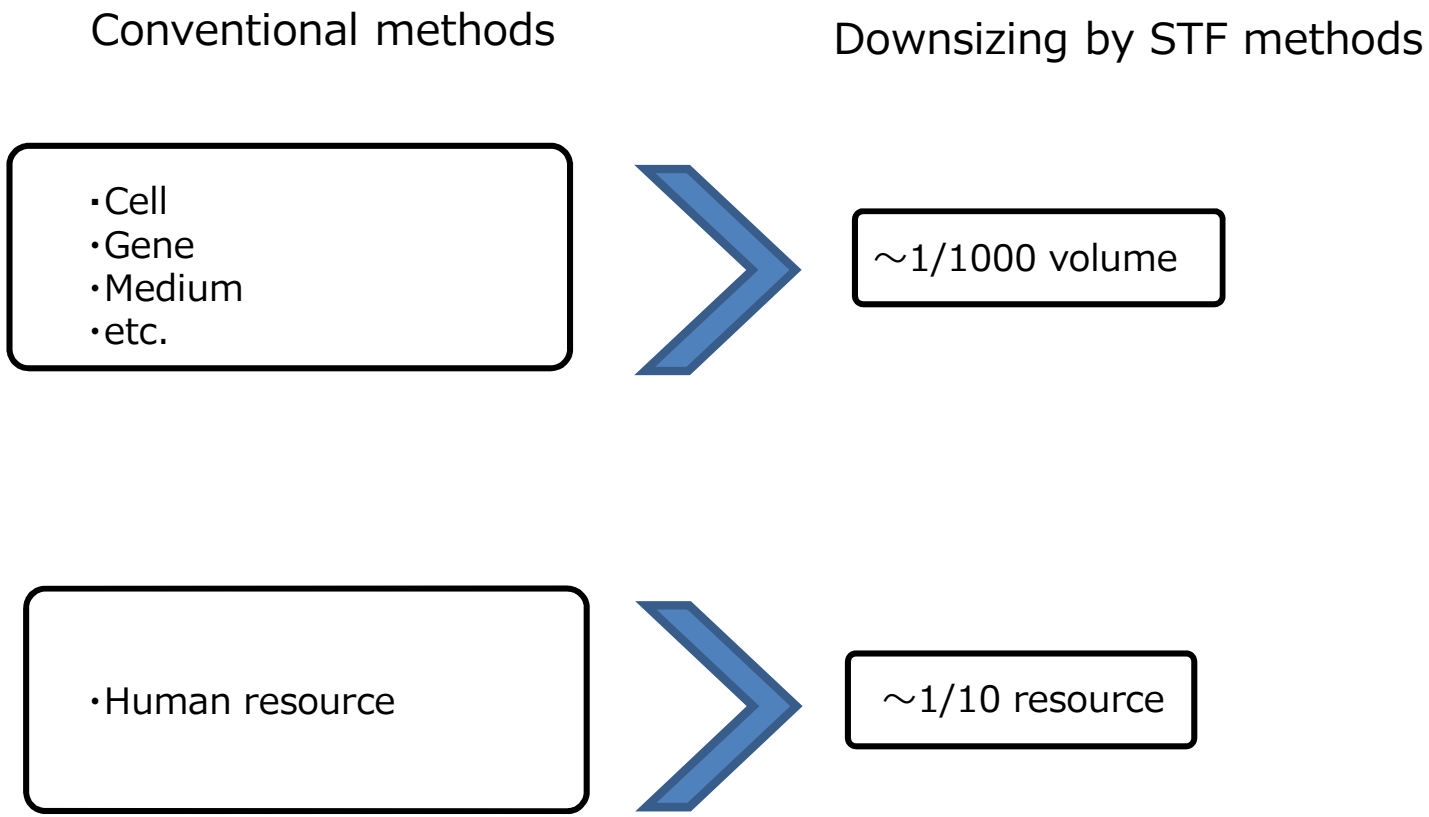
Cost saving



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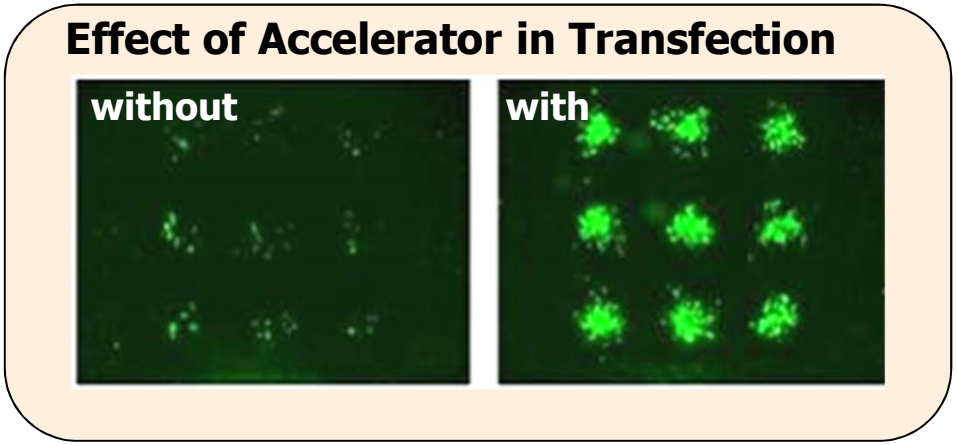
Advantage over conventional method
Enhanced Transfection Efficiency

- Patented accelerator increases rate and extent of transfection
  - Stabilises transfection complexes
  - Enhances uptake of complexes by cells
  - More cells transfected, less variability
- Facilitates well-to-well reproducibility



The accelerator provides visibly higher efficiency

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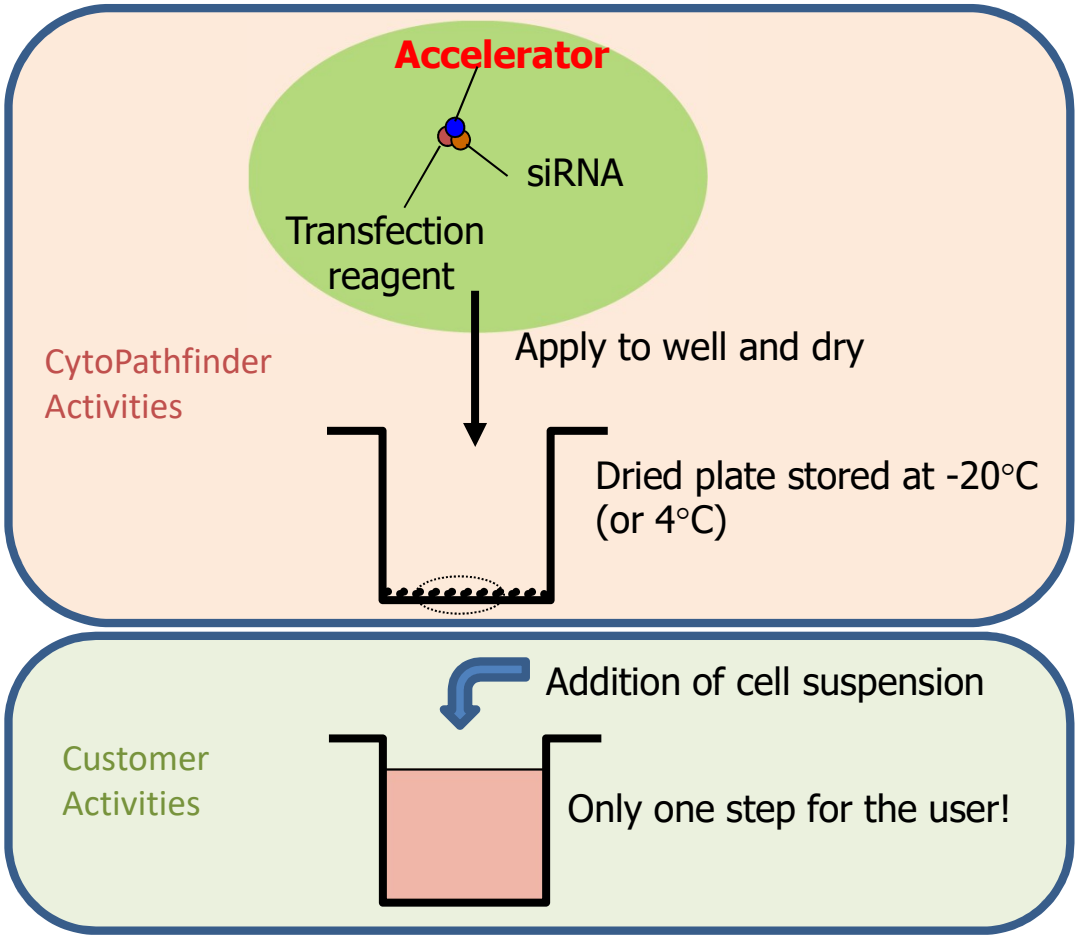
Advantage over conventional method
Simple to Use Methodology



CytoPathfinder supply Plates with the siRNA, Plasmid DNA transfection reagent and accelerator dried on the bottom of the wells.

Plates are stable for at least 12 months.

Customers simply add cells, incubate and read their preferred endpoint.

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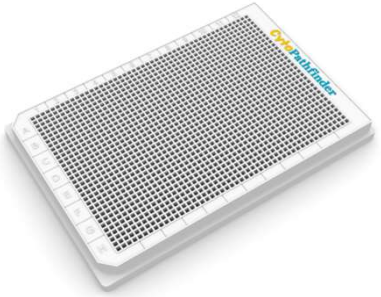
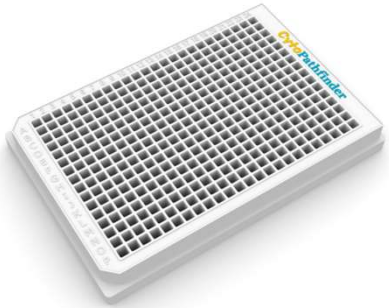
Advantage over conventional method
Varied Formats Possible

Wells facilitate high-throughput screening

- 96,384 ,1536 well plates as standard



1536 well plate



384 well plate

96 well plate

- **6 well plate**

  (under development)

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Advantage over conventional method
Simple Optimisation Process

- Optimisation plate with wide range of permutations used to select conditions prior to full screening
  - Major bottleneck in other siRNA transfection methods

- Efficiency and toxicity assessed using lethal and non-coding siRNAs

Variables: (materials)

Over one hundred and forty conditions can be tested on a single plate.

- ✓Nucleic acid concentration
- ✓TF reagents; type & concentration
- ✓Buffers

- Once optimal conditions selected, experimental plates produced by CytoPathfinder using siRNA library chosen by the customer

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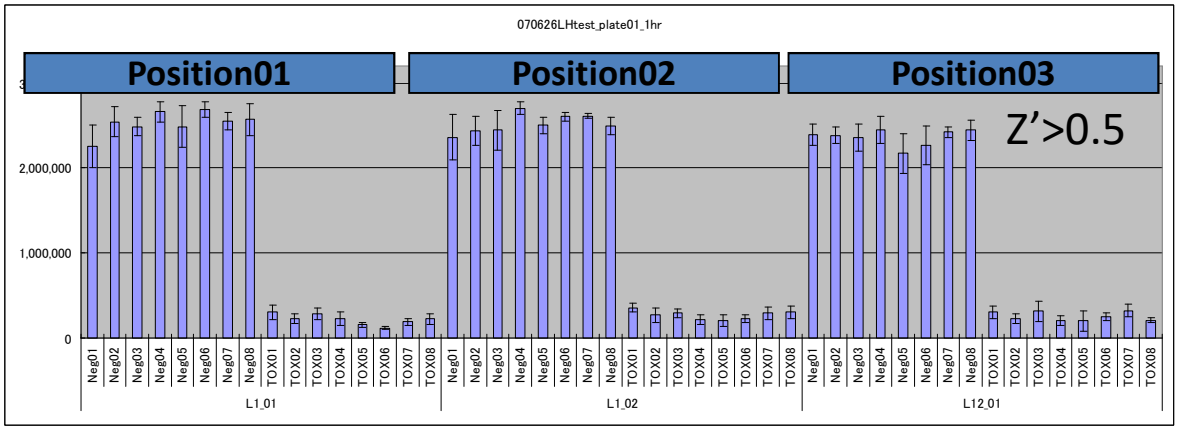
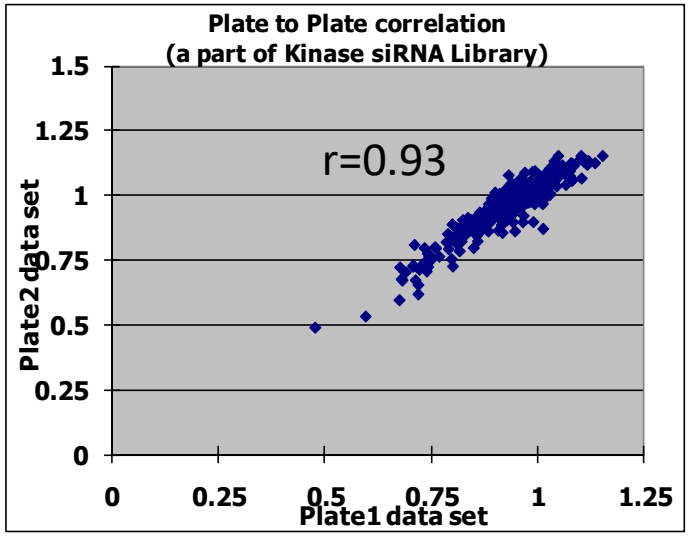
Advantage over conventional method
Excellent reproducibility and reliability

Plates





- Plate to plate correlation > 0.9
- Z' values > 0.8 readily achievable

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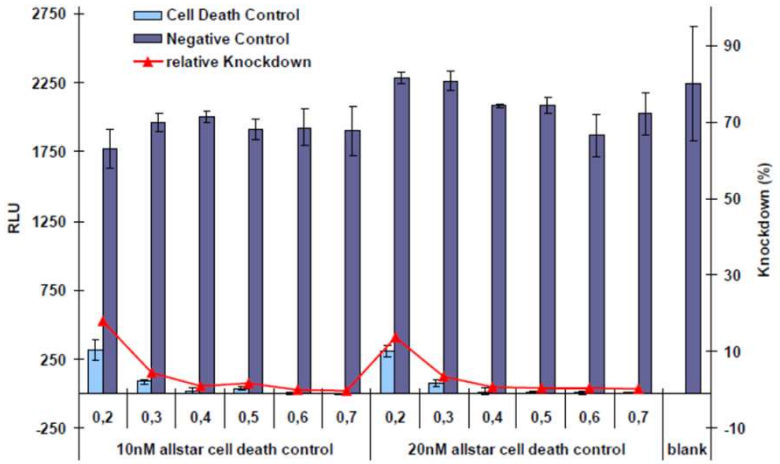
Advantage over conventional method
Reliable results with hard-to-transfect Cells

- Use of the accelerator also increases transfection in hard-to-transfect cells
- Allows excellent transfection with maintained cell viability
  - For example, human bronchial/tracheal epithelial cells



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① record of STF into Cell Line

type	name	tissue / disease	nuecleic asid
adherent	HeLa	cervix / adenocarcinoma	siRNA, Plasmid, RNA aptamer
adherent	T-47D	mammary gland, breast / ductal carcinoma	siRNA
adherent	SK-BR-3	mammary gland, breast / adenocarcinoma	siRNA
adherent	MCF7	mammary gland, breast / adenocarcinoma	siRNA, Plasmid, RNA aptamer
adherent	MDA-MB-231	mammary gland, breast / adenocarcinoma	siRNA
adherent	MDA-MB-435S	mammary gland, breast / ductal carcinoma	siRNA
adherent	HEK-293	embryonic kidney	siRNA, Plasmid
adherent	HEK-293T	embryonic kidney	siRNA, Plasmid
adherent	Caco-2	colon / colorectal adenocarcinoma	siRNA
adherent	HCT116	colon / colorectal adenocarcinoma	siRNA
adherent	SW480	colon / colorectal adenocarcinoma	siRNA
adherent	HepG2	liver / hepatocellular carcinoma	siRNA, Plasmid
adherent	NT2 （NTERA-2）	testis / malignant pluripotent embryonal carcinoma	siRNA, Plasmid
adherent	NIH/3T3	embryo	siRNA, Plasmid, RNA aptemer
adherent	3T3-L1	embryo	siRNA, RNA aptemer
adherent	MDCK	kidney / normal	siRNA
adherent	Malme-3M	lung / malignant melanoma	siRNA
adherent	SK-MEL-28	skin / malignant melanoma	siRNA
adherent	SK-MEL-5	skin / malignant melanoma	siRNA
adherent	A-375	skin / malignant melanoma	siRNA
adherent	NCI-H460	lung / carcinoma, large cell lung cancer	siRNA
adherent	A549	lung / carcinoma	siRNA, RNA aptemer
mixed	SH-SY5Y	bone marrow / neuroblastoma	siRNA
mixed	PC-12	adrenal gland / pheochromocytoma	siRNA, Plasmid
suspension	Jurkat	peripheral blood / acute T cell leukemia	siRNA
suspension	K-562	bone marrow / chronic myelogenous leukemia	siRNA, Plasmid
adherent	Neuro2a	Mouse neuroblastoma	Plasmid
suspension	THP-1	Human Monocytes/ acute monocytic leukemia	siRNA

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② record of STF into Normal / Primary Cell

Name	tissue / disease	nuecleic asid
Mice Bearing Human Tumor Xenografts		siRNA
Rat Brain Cortex Neuronal Cells		siRNA
Primary Rat Astrocytes		siRNA
Primary Rat Neuronal Cells		siRNA
Primary Mouse Neuronal Cells		siRNA
PMEF		siRNA
HUVEC	Normal Human Umbilical Vein Endothelial Cells	siRNA, Plasmid
Human Retinal Microvascular Endothelial Cells		siRNA
Rat Retinal Ganglion Cells		siRNA
HMEC	Normal Human Mammary Epithelial Cells	siRNA
NHBE	Normal Human Bronchial/Tracheal Epithelial Cells	siRNA
UASMC	Normal Human Umbilical Artery Smooth Muscle Cells	siRNA
NHDF-Neo	Normal Human Neonatal skin fibroblest	siRNA, Plasmid
Primary Human Hepatocytes from PXB-Mice®		siRNA
Chronic Lymphocytic Leukemia		siRNA
Normal Human B1 Cells		siRNA
Human Peripheral Blood CD14+ Monocytes		siRNA
hMSC	Human Mesenchymal Stem Cells	siRNA, Plasmid
mNSC	Mouse Neuronal Stem Cells	siRNA

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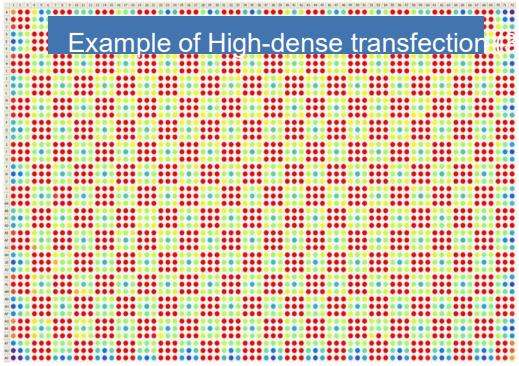
Advantage over conventional method
Fewer Cells per Assay

- High transfection efficiency permits dense formats:
  - ✓ 384 well plates 3,000 cells/well
  - ✓ 1536 well plates 500 cells/well
  - ✓ 3456 well plates 100-200 cells/well

- Allows broader gene analysis with scarce cells
  - ✓ Primary cells
  - ✓ Human tumour cells with low passage number

Z' = 0.77

Neg. siRNA Cytotoxic siRNA



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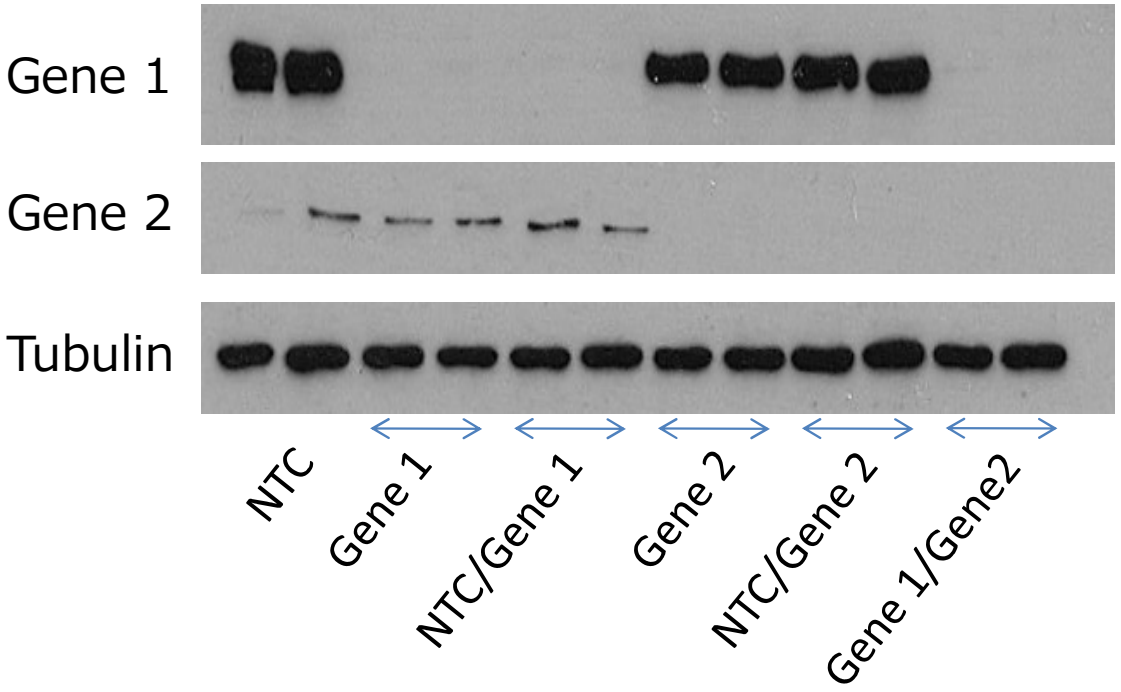
Advantage over conventional method
Dual Knockdown Easily Achievable

- Western blot showing knockdown of Gene 1 and Gene 2 separately and together (plus non-targeting control RNA – NTC)
- Effectiveness retained in dual knockdown



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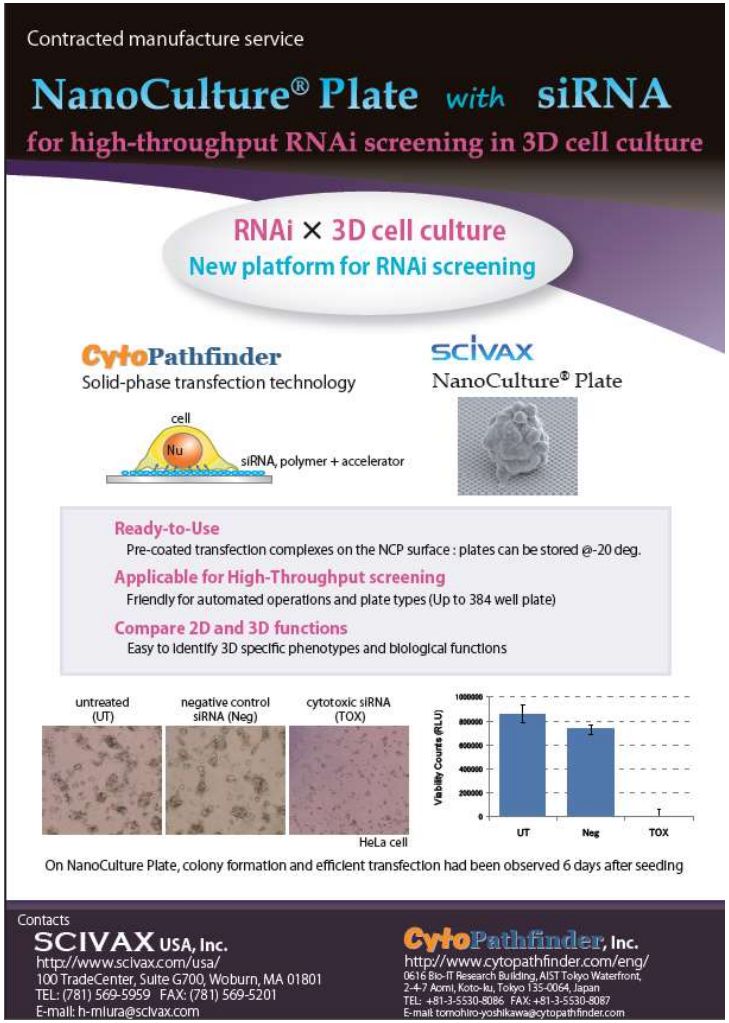
Advantage over conventional method
Solid-phase transfection combined with cutting-edge technologies

Explore ncRNA functional screening

Explore 2D/3D functional screening



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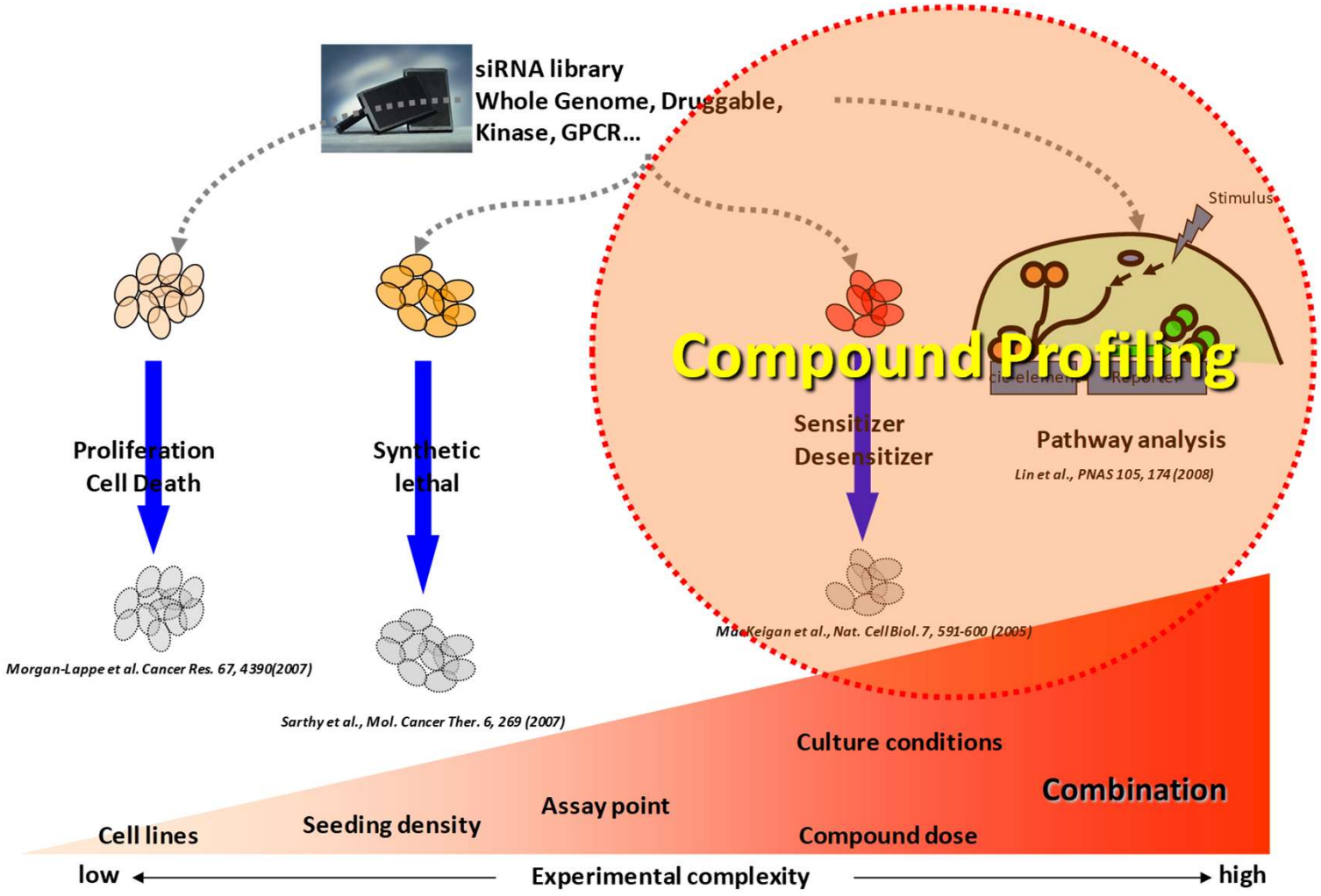
Advantage over conventional method
Compound profiling; understanding complex cellular system



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Advantage over conventional method -Summary-

method	chemicai			physical	biological
	Our STF	Liquid phase		Electroporation	Virus vector
		forward	reverse		
reproducibility	high	low～medium	high(in case of automatic)	low	low～medium
Availability for hard to transfect cells	high (induced pluripotent stem cell,embryonic stem cell,hepatocyte, stem cell isolated from body tissue, primary cell, floating cell)	low～medium	low～medium	high(primary stem cells etc)	high
Transfection efficiency	same level as reverse method or higher	medium	Medium～high	high	high
Preparation of sample	Only add cells	complex	automation	Device needed	complex
Selection of optimization condition	Simple selection using optimization plates	Try and error	Try and error	Try and error	Try and error
Varied formats	Available max1,536well		Max density384 well	impossible	
Minimize number of cells	1,536 well, 500cells/well	384well, 3,000cells/well	384 well, 3,000cells/well	impossible	impossible
Double knockdown	available	impossible	impossible	difficult	difficult
storage	possible (12 months at-20°C)	impossible	impossible		
Scale of library	small～large	small	small～large (in case of automation)	small～large	small
others				Low viability of cells Damage of nucleic acid	Contamination, insertion mutation,inactivity by immunity

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Standard price of STF plate

FOB Japan price (US$)

Nucleic acid	Kind of plate	Well format	Number of order	Unit price
siRNA/	Optimization	384	any number	290/1 plate
Plasmid DNA		96	any number	290/set 4plates
siRNA/	Customized*	1536/384/96	1	1,000/1plate
Plasmid DNA			2	670
			3～19	430
			20～49	380
			50～99	330
			100～	290

*siRNA/Plasmid DNA library is supplied from customer in case of customized plate production
Above price is standard price level (FOB),Protocol of custom plate will vary according to your target cell, so fixed price will be estimated and negotiated including export package and air freight .

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