The 7th International Conference on Systems Biology

Technological breakthrough for cell-based target discovery

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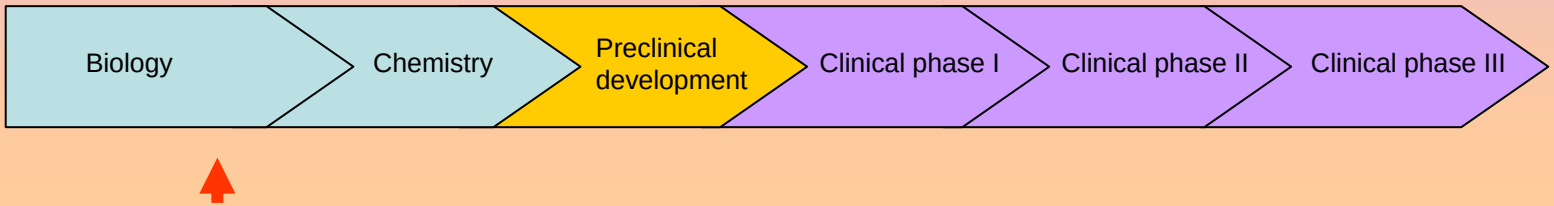
Oct. 9, 2006 at Pacifico Yokohama

The aims of cell-based technologies for target discovery



- **New & right targets**
- **Proof of concepts**

RNA interference expands the druggable targets: genome-wide target discovery

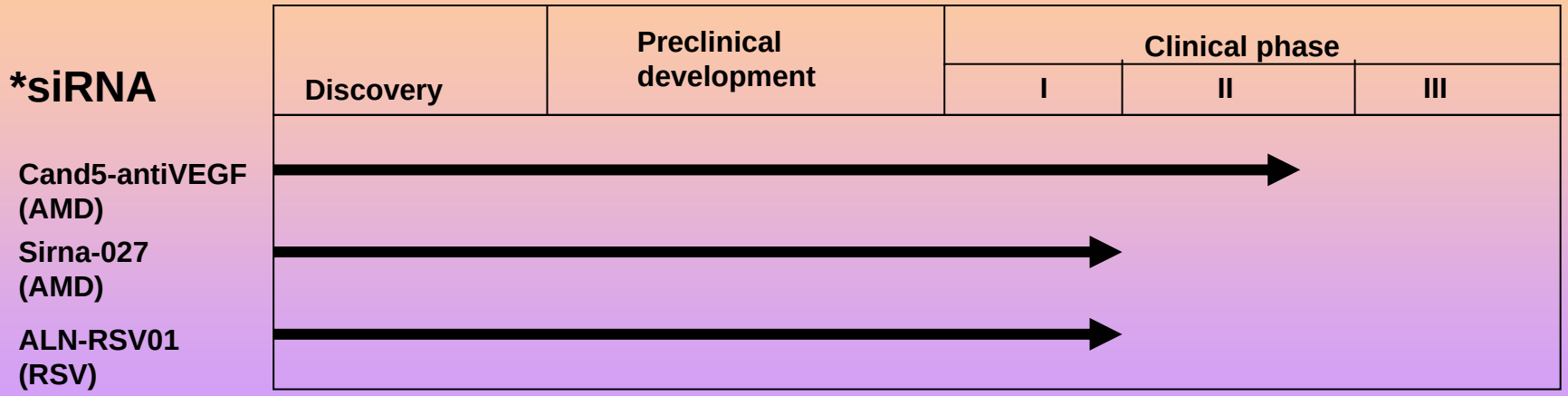
Low risk

Target gene → **Design of interfering RNA** → **Drug R&D**

High risk → **Screening of small compounds** → **Drug R&D**

Discovery or Preclinical Dev.

- Hepatitis C
- Hepatitis B
- Flu
- HIV
- Hairless
- Diabetes
- Respiratory disease
- Parkinson's disease
- Huntington's disease
- Neuropathic pain
- Hypercholesterolemia
- Oncology
- etc.



*siRNA:short interfering RNA

Unpredictable combinations of molecularly targeted agents

Table: Phase III trials of targeted agents combined with standard cancer treatments

Agent	Cancer	Chemotherapy		Outcome
		Concurrent administration	Preclinical support for combination?	
Gefitinib	Lung (two trials)	Y	Y	Failed (no difference in RR and OS)
Erlotinib	Lung (two trials)	Y	Y	Failed (no difference in RR and OS)
	Pancreas	Y	Y	Improved survival
Trastuzumab	Breast/HER2+	Y	Y	Improved survival
Bevacizumab	Colon (2 trials)	Y	Y	Improved survival
	Breast: first line	Y	Y	Improved survival with paclitaxel
	Breast: scond/third line	Y	Y	Failed (improved RR but no difference in PFS))
	Lung	Y	Y	Improved survival
Oblimersen	Melanoma	Y	?	Failed to improve OS
Cetuximab	Head and neck	Y	Y	Failed (improved RR but no statistically significant difference in PFS))
	Head and neck	Radiation	Y	Improved survival

OS, overall survival; PFS, progression-free survival; RR, response rate.

J. E. Dancey & H. X. Chen, Nature Rev. Drug Discovery 5, 649 (2006)

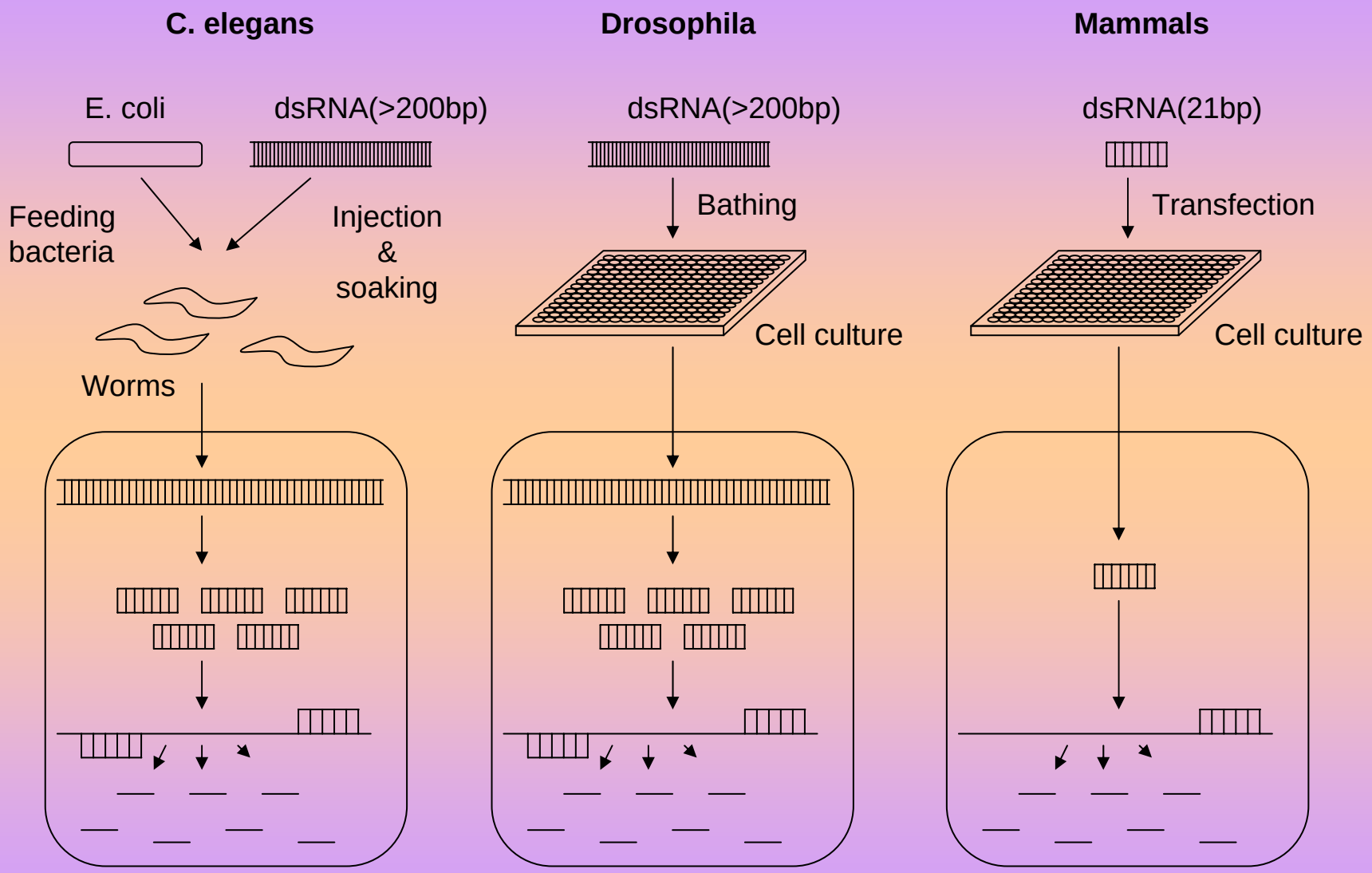
CP

Low risk in toxicology, High risk in Efficacy

Industry Median	Pre-Clinical	Phase I	Phase II	Phase III	Registration
Clinical Safety	0.5%	27.9%	13.4%	9.8%	30%
Efficacy	5.6%	17.5%	52.0%	72.5%	20%
Formulation	5.1%	5.8%	1.6%	0.0%	0%
Market Potential	6.2%	3.9%	7.9%	3.9%	30%
PK/Bioavailability	11.8%	14.9%	2.4%	0.0%	0%
Strategic	14.4%	12.3%	13.4%	5.9%	20%
Resources	1.5%	1.3%	0.8%	3.9%	0%
Toxicology	44.1%	10.4%	2.4%	3.9%	0%
Cost of Good	1.5%	1.3%	0.0%	0.0%	0%
Unknown	7.2%	1.3%	4.7%	0.0%	0%
Others	2.1%	3.2%	1.6%	0.0%	0%
Number of Projects	194	154	127	51	10

L. Suter, L. Babiss, E. B. Wheeldon, Chemistry & Biology 11, 161 (2004)

Cell-based large-scale functional screening (multi-well format)



Modified the fig in D. Kuttenkeuler & M. Boutros, Briefings in Functional genome and Proteomics 3, 168 (2004)

Genome-wide functional screening works as identification of certain pathway components

Model	Screening scale	Phenotype assay	Results	Ref.
C. elegance	RNAi bacterial library (>80% ORFs)	Longevity (whole body)	Daf-2/insulin-like signaling pathway, Energy generation metabolism, Protein turnover, New pathway components	Genes&Dev.19, 1544 (2005)
	dsRNA (98% ORFs)	Time-lapes image(Embryonic cell) 46 phenotype categories	661 genes responsible for 23 embryogenic phenotypes	Nature 434, 462 (2005)
	RNAi bacterial library (>94% ORFs)	RNAi monitoring GFP	90 components responsible for RNAi involved 11known genes	Science 308, 1164 (2005)
Drosophila (cultured cells)	dsRNA library (43% ORFs)	Hedgehog-responsive luciferase	4 known Hh pathway components, 4 unknown Hh pathway components, 11 multi-functional proteins	Science 299, 2003 (2003)
	dsRNA library (whole genome)	RNA monitoring luciferase	5 known RNAi pathway components, 2 unknown components in the pathway	PNAS 103, 11880 (2006)
	dsRNA library (>95% ORFs)	Wnt reporter (TCF-binding site+luciferase)	238 Wnt signaling pathway components (50% human orthologs, 18% associate with human disease, corelation to in vivo model animals (zebra fish).	Science 308, 826 (2005)
Mammalian cell lines				
HeLa (human)	dsRNA library (590 kinase)	Virus infection (endocytosis)	208 endocytic pathway components	Nature 436, 78 (2005)
SHSY5Y(human)	dsRNA library (85 tyrosine kinases)	Neurite extention	Cholinergic and dopaminergic neurite extention specific tyrosine kinases. Correlation to in vivo functions.	In submission

Multi-well cell-based functional analysis for target discovery

Merits

>Rapid genome-wide functional analysis
>New compornents in the known pathway
>Components suggesting new pathways

Demerits

>In vitro results may be artificial
>Insufficient injection or transfection
>Requiring robust statistical analysis
(multi-well is too large to manage multiple experimental repeats)

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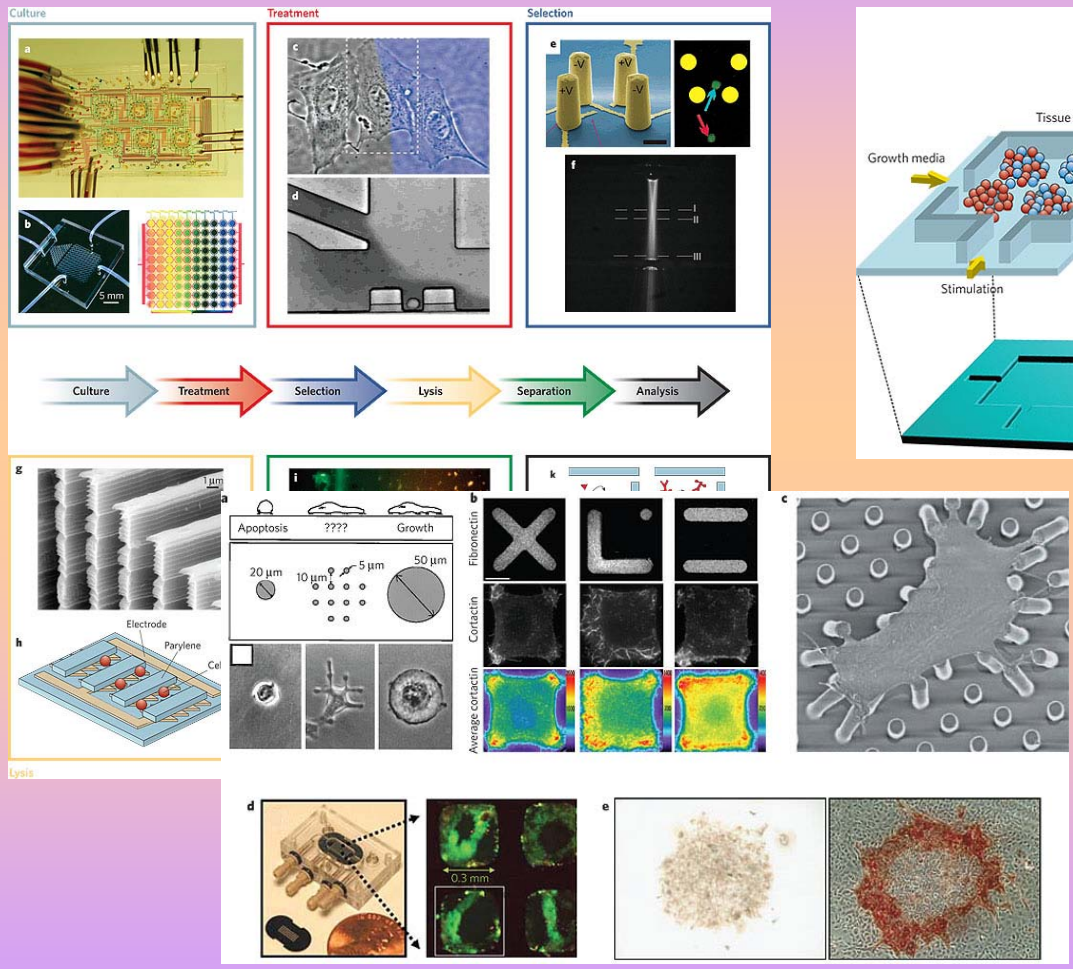
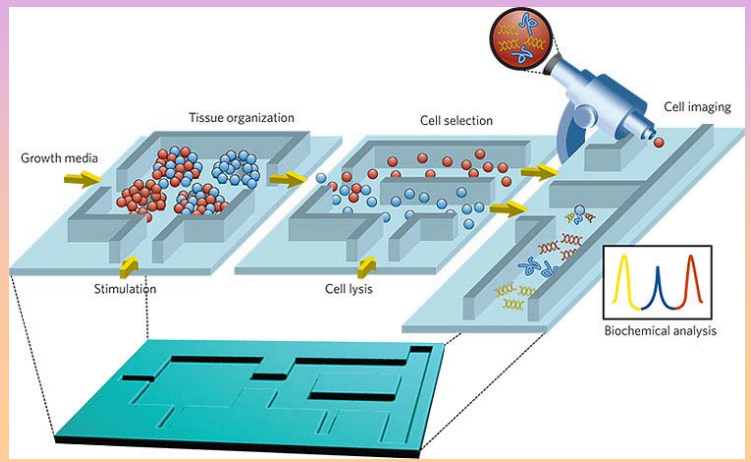
Challenge to right analysis of cells





Clinical samples

↓

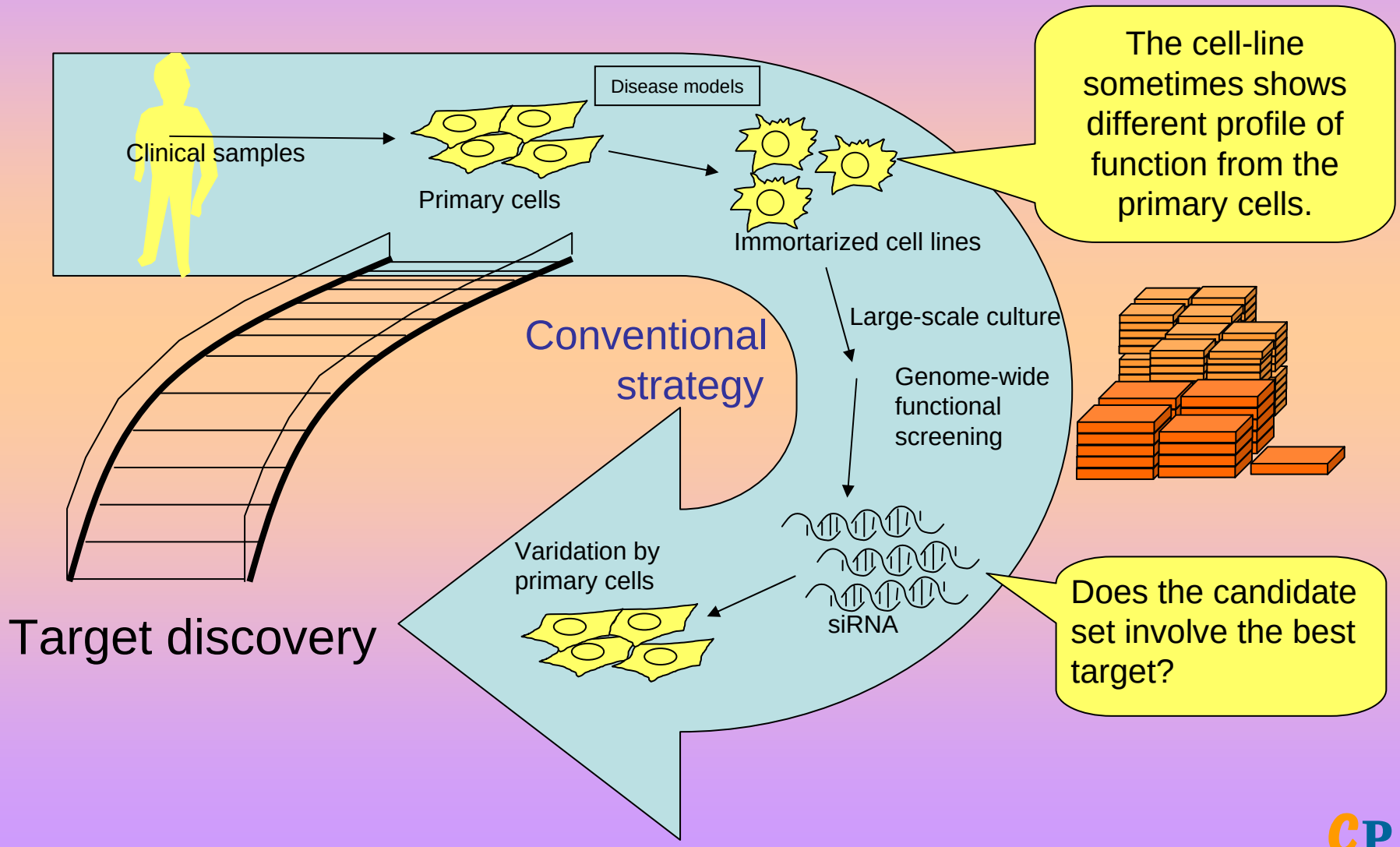
Separation

↓ (culture)

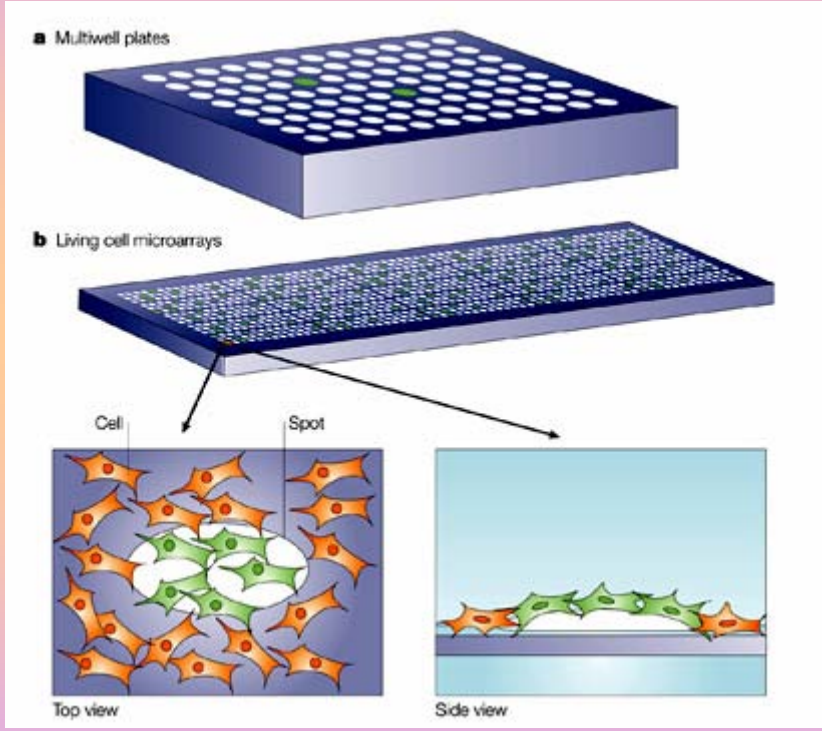
Analysis

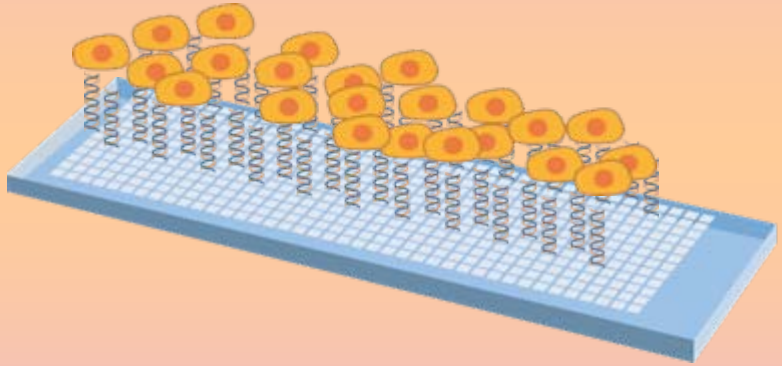
Data source: J. El-Ali, P.K.. Sorger and K. F. Jensen, Nature 442, 403 (2006)

Challenge to primary-cell-based screening



Cell array systems as miniaturized format for genome-wide functional assay

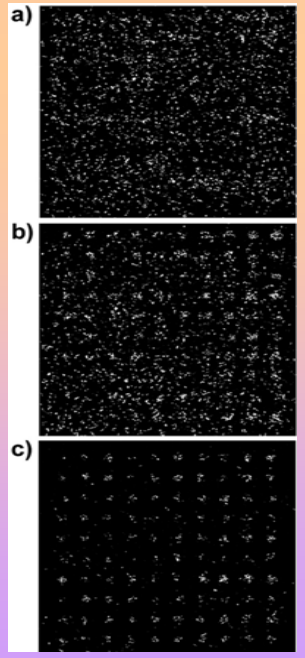




Data source: Nature Review Genetics

Development of practical format

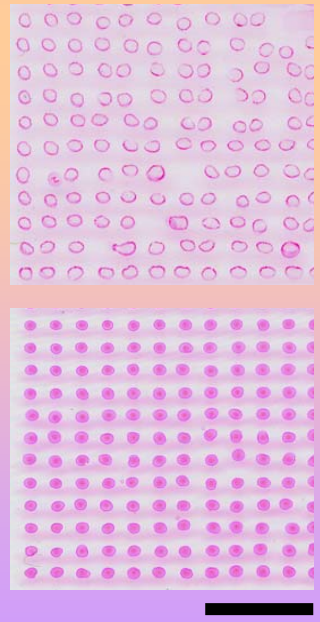
- **High transfection efficiency for primary cells**
- **High-content image-based assay**
- **Robust statistical analysis**



Multiple print (5 times)

Multiple print (3 times)

Single print



5 mm

S. Baghdoyan et al. Nuc Acid. Res. 32, e77(2004)

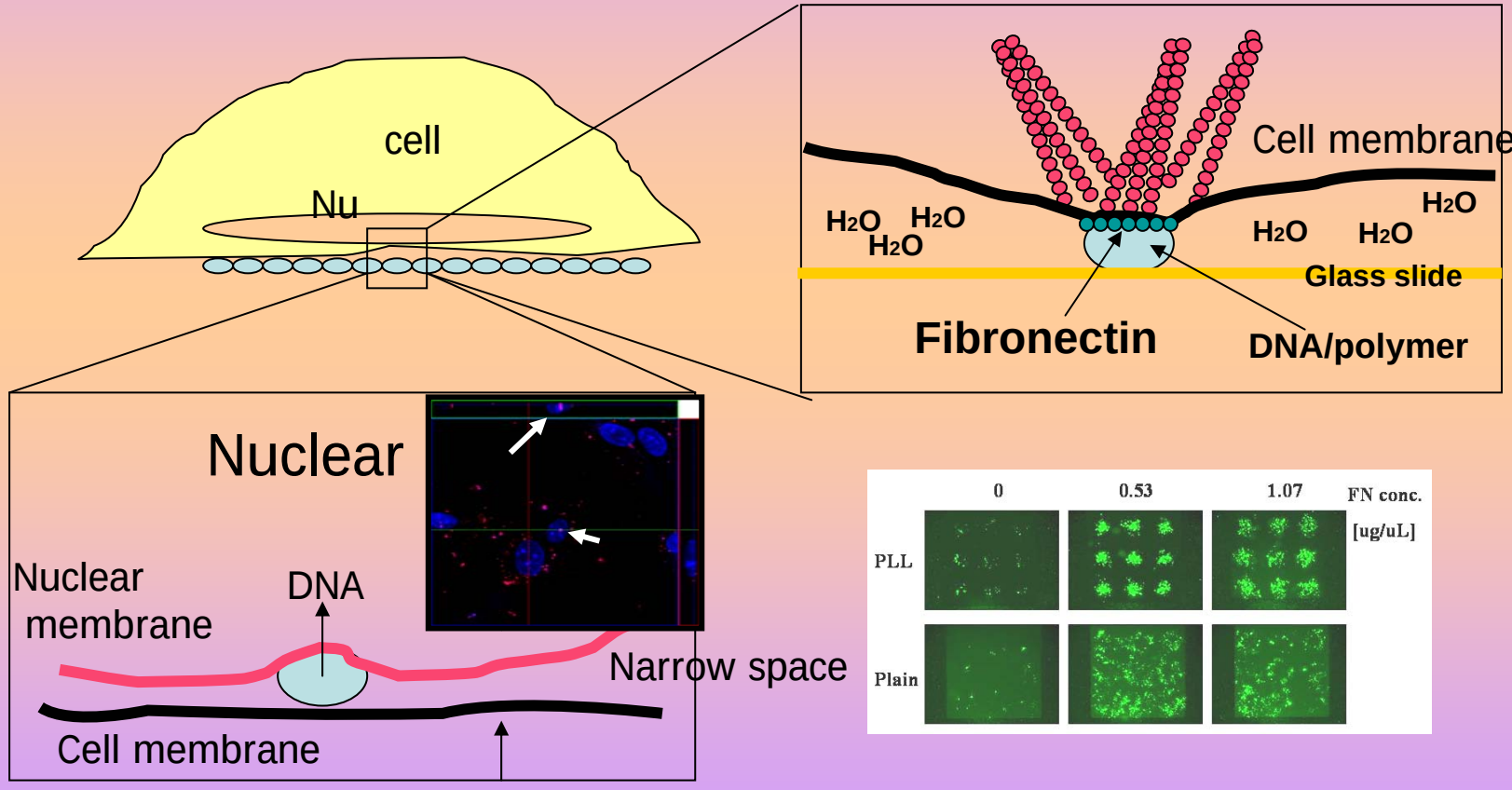
Issues on manufacturing

Transfection efficiency

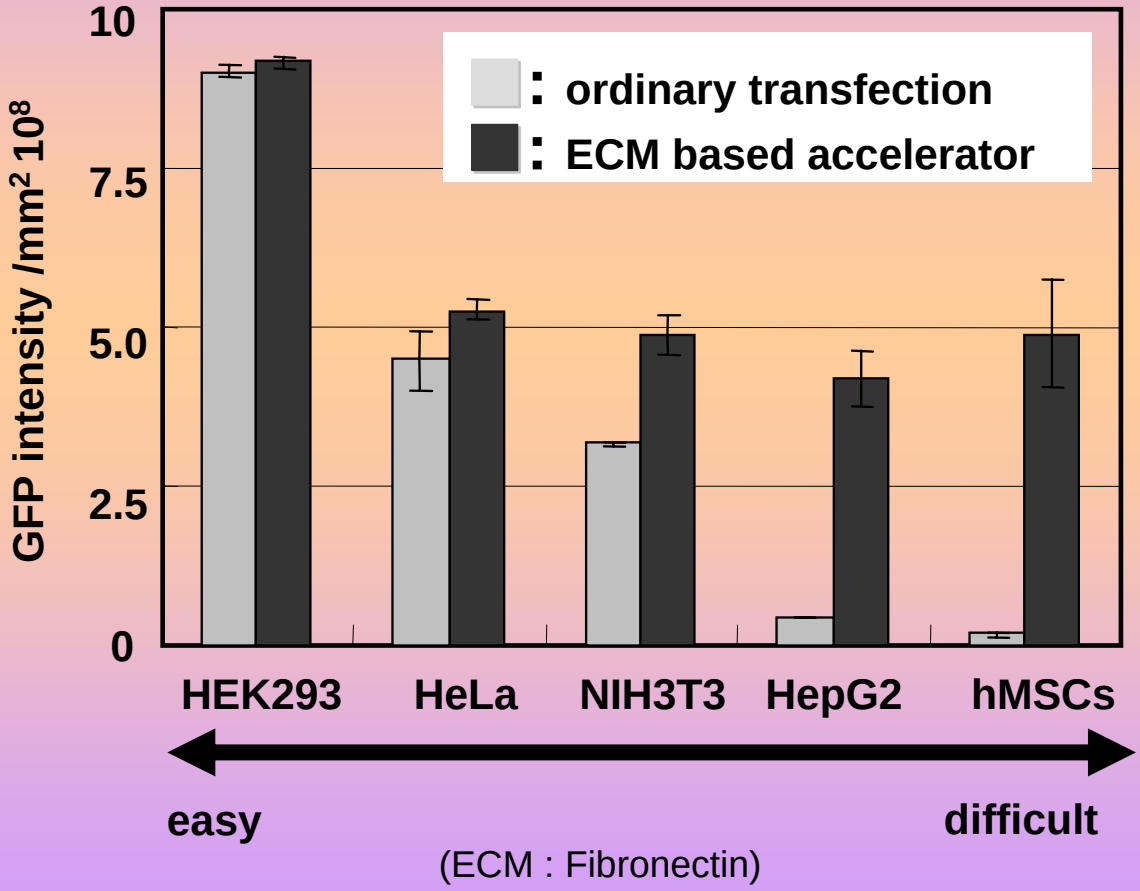
Localization

Uniformity of spots

Solid-phase non-viral transfection accelerators



Optimal accelerators increase solid-phase transfection efficiency



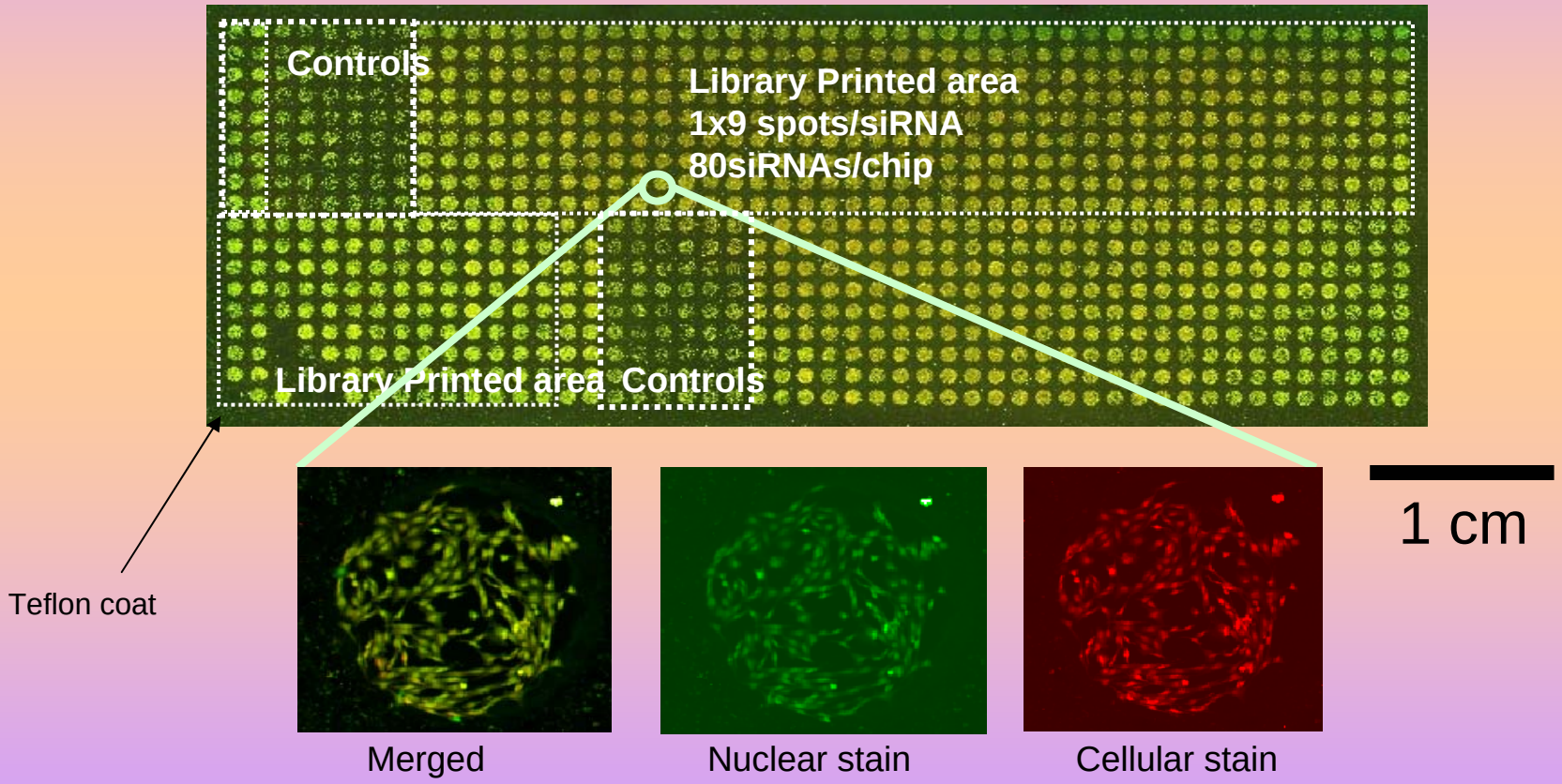
(ECM : Fibronectin)

Cells	derive	Efficiency%
hMSC	**bone marrow**	**40**
HepG2	**liver**	**80**
MCF7	Breast	80
U251	brain	80
SHSY5Y	Brain	60
NTERA-2	embryo	80

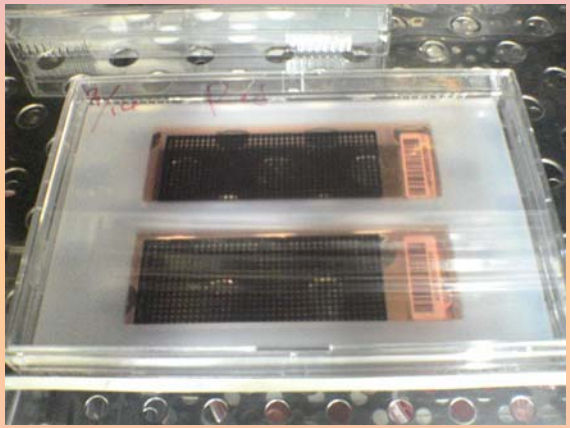
（over 10 times higher efficiency)

Masato Miyake：Bio Venture 7/8, 22 (2004) (Domestic journal)

Transfection microarray allows image-based analysis



On-chip image-based assay of tyrosine kinase siRNAs inhibited neurite extension



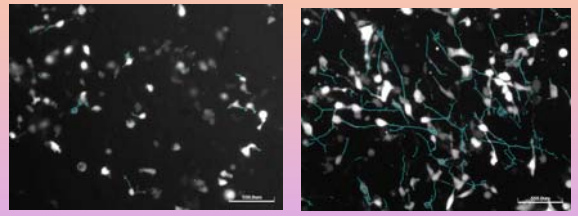
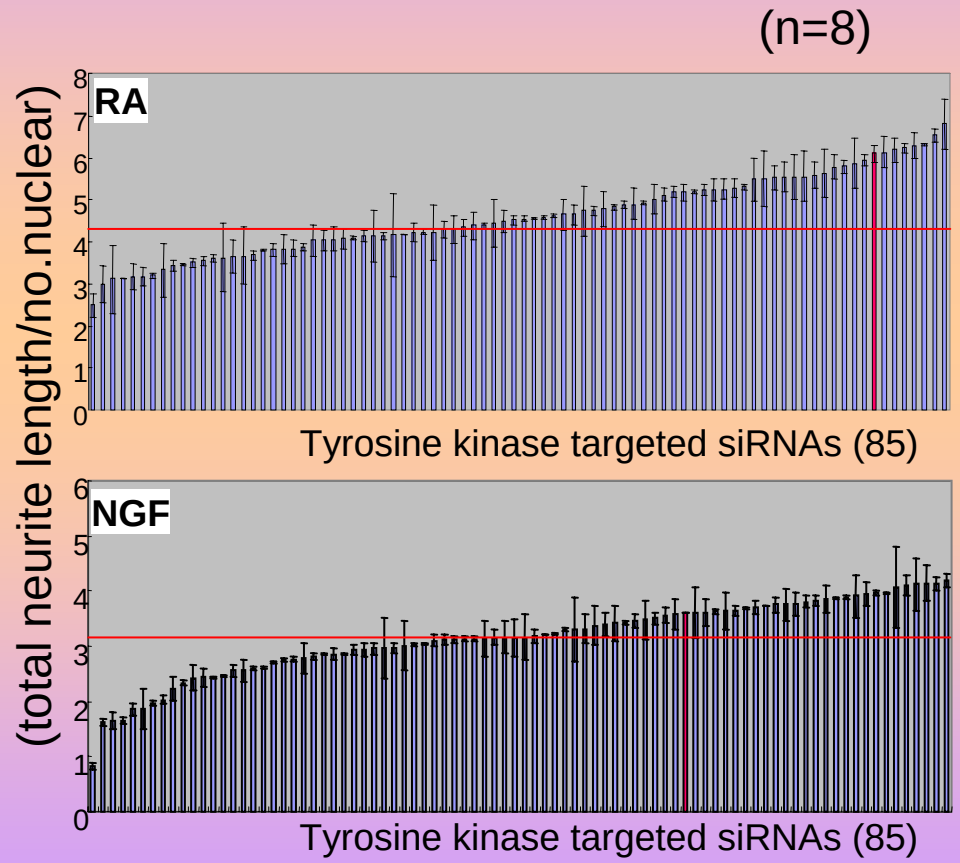
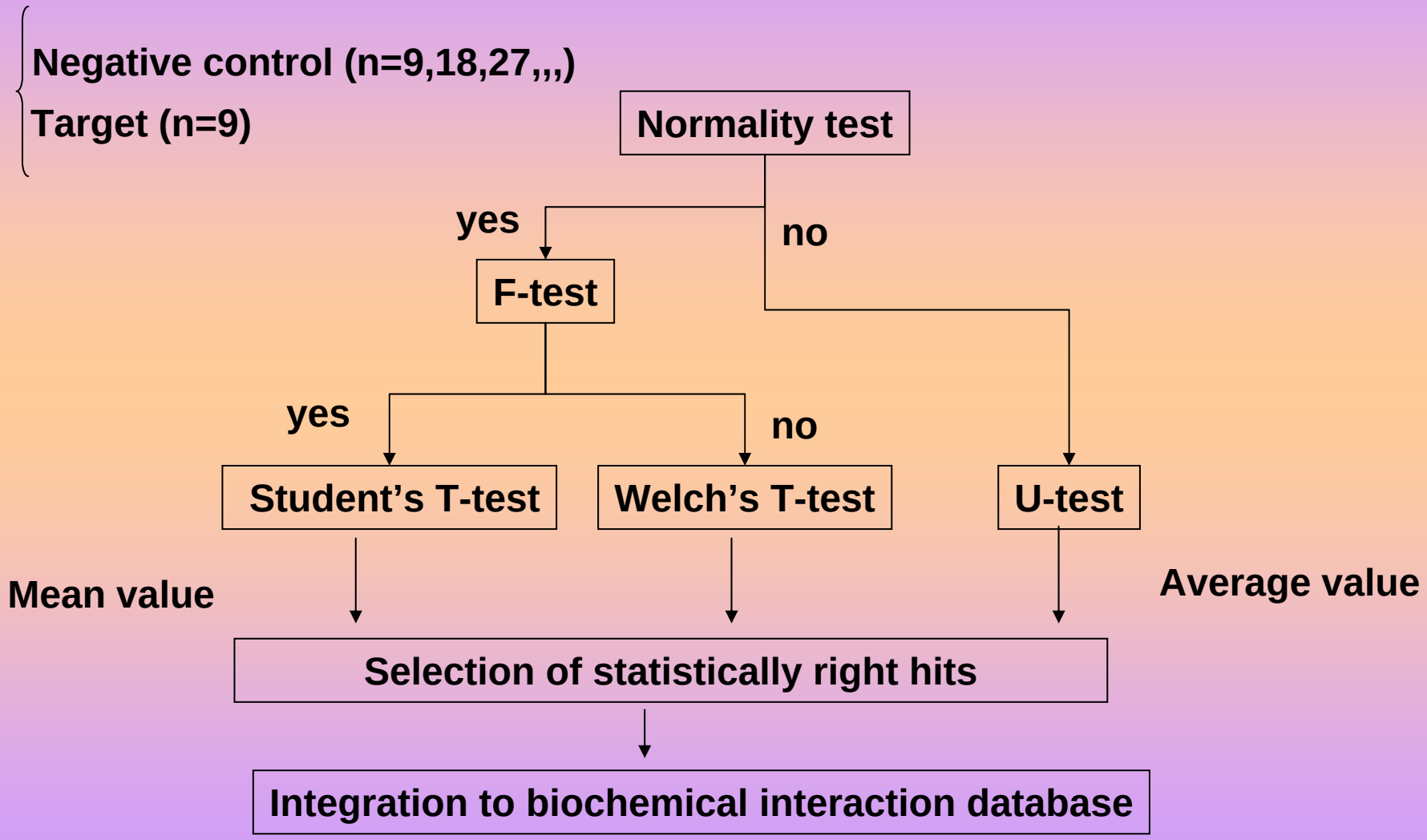


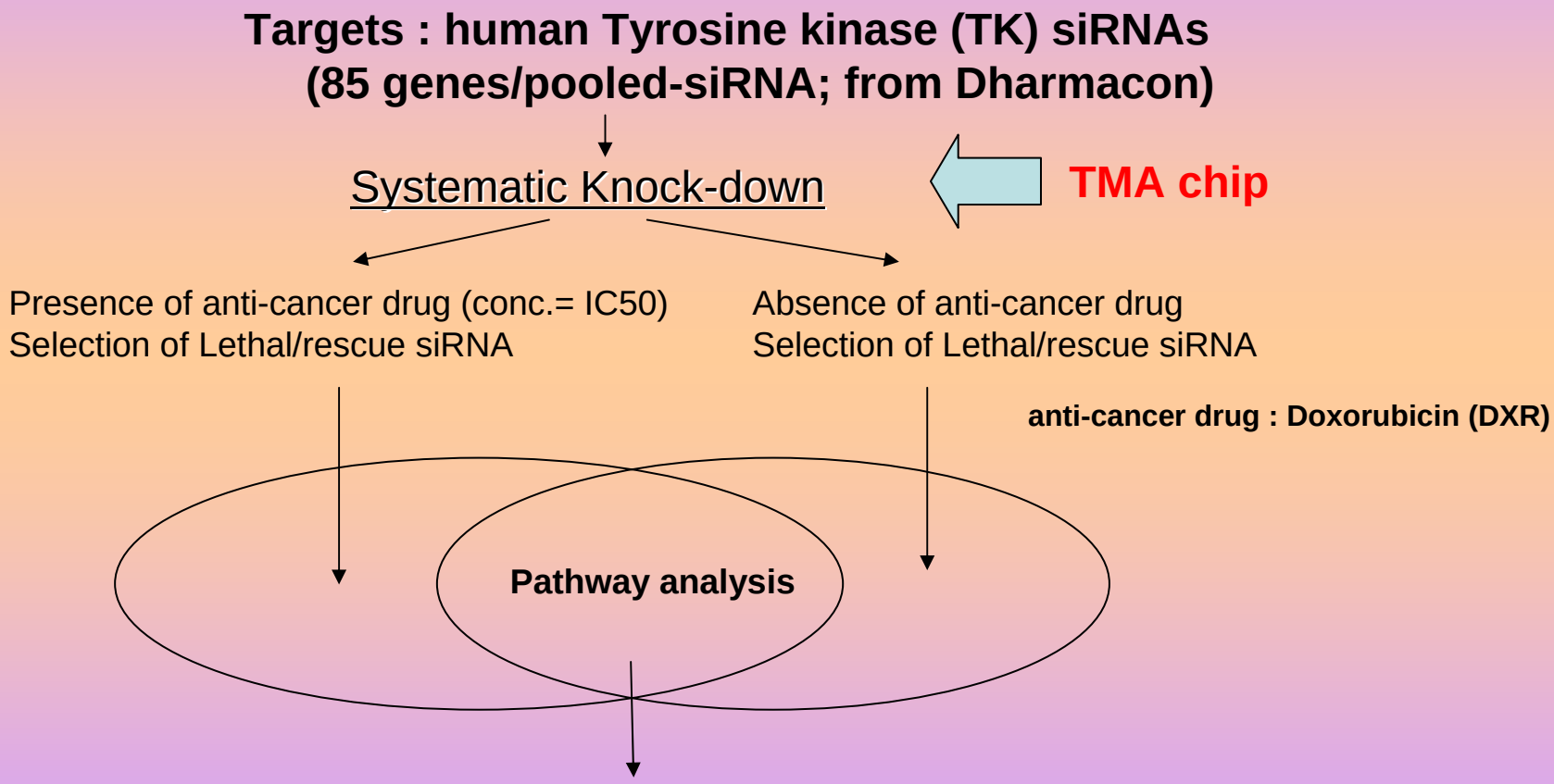
Image-based quantification of frequency of neurite outgrowth



TMA format allows robust statistical analysis



Pathway components analysis responsible for DXR sensitivity



Exploratory new targets which independent to DXR

Experimental condition

1.Cell line

	ER α	HER2	DXR IC_{50}(nM)
T-47D（ATCC HTB-133)	＋	－	156
SK-BR-3（ATCC HTB-30)	－	＋	163

2.Culture condition
DMEM+10%FBS (5% CO2)

3.anti-cancer drug
Doxorubicin (DXR)

4.siRNA library
human Tyrosine Kinase siRNA library (85 genes, pooled)

Data collection

（Four days after siRNA transfection, viability was measured by using WST and Live/Dead assay）

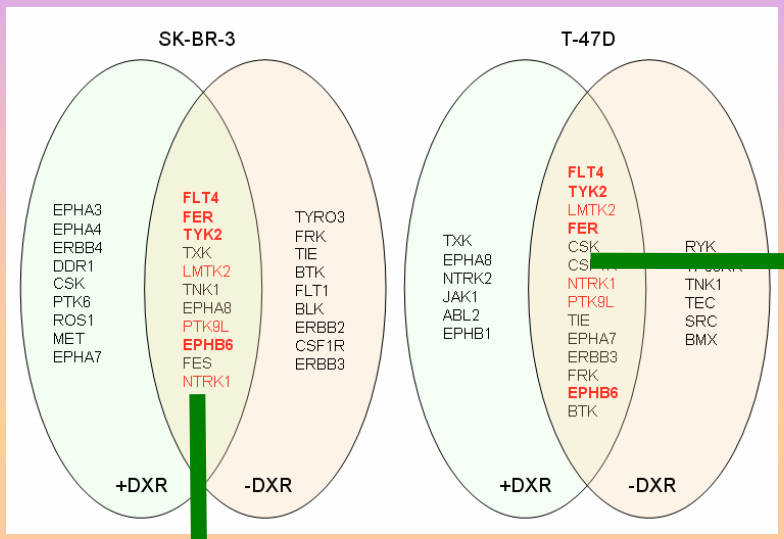
P-test value cut off（<0.01）n=4

SK-BR-3＋DXR　　SK-BR-3　　T-47D＋DXR　　T-47D

RNAi　　RNAi　　RNAi　　RNAi

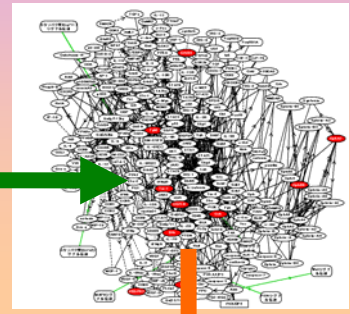
AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ
SK-BR3	+DXR						SK-BR3	-DXR						T-47D							T-47D					
Comparison	Difference	q			P value		Comparison	Difference	q			P value		Comparison	Difference	q			P value		Comparison	Difference	q			P value
Ds+Sc vs FLT4	-0.8185	11.5380	**	Down	P<0.01		Ds+Sc vs FLT4	-1.1790	5.7090	**	Down	P<0.01		Ds+Sc vs FLT4	-0.9451	9.1390	**	Down	P<0.01		Ds+Sc vs CSF1R	-0.9418	4.3440	**	Down	P<0.01
Ds+Sc vs EPHA3	-0.4290	4.4240	**	Down	P<0.01		Ds+Sc vs FER	-0.8285	3.7460	**	Down	P<0.01		Ds+Sc vs TYK2	-0.3367	3.4760	**	Down	P<0.01		Ds+Sc vs TIE	-0.8763	4.3110	**	Down	P<0.01
Ds+Sc vs EPHA4	-0.4285	4.4190	**	Down	P<0.01		Ds+Sc vs TYRO3	-0.9243	3.5600	**	Down	P<0.01		Ds+Sc vs TXK	-0.2061	2.2820	ns	Down	P>0.05		Ds+Sc vs RYK	-0.8636	3.9870	**	Down	P<0.01
Ds+Sc vs FER	-0.2597	3.6610	**	Down	P<0.01		Ds+Sc vs LMTK2	-0.6369	3.0850	*	Down	P<0.05		Ds+Sc vs EPHA8	-0.2546	2.1800	ns	Down	P>0.05		Ds+Sc vs FLT4	-0.8801	3.7870	**	Down	P<0.01
Ds+Sc vs TYK2	-0.2404	3.5440	**	Down	P<0.01		Ds+Sc vs FRK	-0.6028	2.9200	*	Down	P<0.05		Ds+Sc vs NTRK2	0.1646	2.0900	ns	Up	P>0.05		Ds+Sc vs EPHA7	-0.7986	3.6830	**	Down	P<0.01
Ds+Sc vs ERBB4	-0.2437	3.4350	*	Down	P<0.05		Ds+Sc vs TYK2	-0.7344	2.8290	*	Down	P<0.05		Ds+Sc vs JAK1	-0.1951	1.8960	ns	Down	P>0.05		Ds+Sc vs FER	-0.8270	3.5590	**	Down	P<0.01
Ds+Sc vs TXK	-0.2285	3.3700	*	Down	P<0.05		Ds+Sc vs NTRK1	-0.6569	2.7700	ns	Down	P>0.05		Ds+Sc vs ABL2	-0.2213	1.8950	ns	Down	P>0.05		Ds+Sc vs TP53RK	-0.7116	3.2820	*	Down	P<0.05
Ds+Sc vs DDR1	-0.3152	3.2500	*	Down	P<0.05		Ds+Sc vs PTK2	-0.5603	2.3630	ns	Down	P>0.05		Ds+Sc vs LMTK2	-0.1951	1.8870	ns	Down	P>0.05		Ds+Sc vs TNK1	-0.6520	3.2080	*	Down	P<0.05
Ds+Sc vs LMTK2	-0.2290	3.2290	*	Down	P<0.05		Ds+Sc vs TIE	-0.5509	2.1600	ns	Down	P>0.05		Ds+Sc vs FER	-0.1908	1.8450	ns	Down	P>0.05		Ds+Sc vs EPHB6	-0.7336	3.1570	*	Down	P<0.05
Ds+Sc vs TNK1	-0.2177	3.2100	*	Down	P<0.05		Ds+Sc vs BTK	-0.6493	2.1530	ns	Down	P>0.05		Ds+Sc vs CSK	-0.2041	1.7480	ns	Down	P>0.05		Ds+Sc vs BTK	-0.6411	2.9570	*	Down	P<0.05
Ds+Sc vs CSK	-0.3010	3.1040	*	Down	P<0.05		Ds+Sc vs FES	-0.4428	2.1450	ns	Down	P>0.05		Ds+Sc vs CSF1R	-0.1914	1.6390	ns	Down	P>0.05		Ds+Sc vs TEC	-0.5878	2.8920	*	Down	P<0.05
Ds+Sc vs EPHA8	-0.2992	3.0850	*	Down	P<0.05		Ds+Sc vs TNK1	-0.5416	2.0860	ns	Down	P>0.05		Ds+Sc vs NTRK1	-0.1063	1.5820	ns	Down	P>0.05		Ds+Sc vs TYK2	-0.5265	2.5900	ns	Down	P>0.05
Ds+Sc vs PTK6	-0.1659	3.0540	*	Down	P<0.05		Ds+Sc vs FLT1	-0.4158	2.0140	ns	Down	P>0.05		Ds+Sc vs PTK9L	-0.1063	1.5820	ns	Down	P>0.05		Ds+Sc vs CSK	-0.5485	2.5300	ns	Down	P>0.05
Ds+Sc vs PTK9L	-0.1632	3.0050	*	Down	P<0.05		Ds+Sc vs BLK	-0.6023	1.9970	ns	Down	P>0.05		Ds+Sc vs TIE	-0.1424	1.5770	ns	Down	P>0.05		Ds+Sc vs PTK9L	-0.5185	2.3940	ns	Down	P>0.05
Ds+Sc vs ROS1	-0.1788	2.8260	ns	Down	P>0.05		Ds+Sc vs EPHB6	-0.3848	1.8640	ns	Down	P>0.05		Ds+Sc vs EPHA7	-0.1724	1.4770	ns	Down	P>0.05		Ds+Sc vs NTRK1	-0.5128	2.3680	ns	Down	P>0.05
Ds+Sc vs EPHB6	-0.1970	2.7770	ns	Down	P>0.05		Ds+Sc vs ERBB2	-0.3774	1.8280	ns	Down	P>0.05		Ds+Sc vs EPHB1	-0.1723	1.4750	ns	Down	P>0.05		Ds+Sc vs ERBB3	-0.5108	2.1980	ns	Down	P>0.05
Ds+Sc vs MET	-0.1497	2.7570	ns	Down	P>0.05		Ds+Sc vs CSF1R	-0.5499	1.8240	ns	Down	P>0.05		Ds+Sc vs ERBB3	-0.1503	1.4530	ns	Down	P>0.05		Ds+Sc vs SRC	-0.4595	2.1170	ns	Down	P>0.05
Ds+Sc vs FES	-0.1934	2.7260	ns	Down	P>0.05		Ds+Sc vs TXK	-0.4511	1.7370	ns	Down	P>0.05		Ds+Sc vs FRK	-0.1471	1.4230	ns	Down	P>0.05		Ds+Sc vs FRK	-0.4758	2.0480	ns	Down	P>0.05
Ds+Sc vs NTRK1	-0.1460	2.6890	ns	Down	P>0.05		Ds+Sc vs ERBB3	-0.3566	1.7270	ns	Down	P>0.05		Ds+Sc vs EPHB6	-0.1376	1.3310	ns	Down	P>0.05		Ds+Sc vs LMTK2	-0.4706	2.0250	ns	Down	P>0.05
Ds+Sc vs EPHA7	-0.2522	2.6010	ns	Down	P>0.05		Ds+Sc vs EPHA8	-0.4903	1.6260	ns	Down	P>0.05		Ds+Sc vs BTK	-0.1424	1.2200	ns	Down	P>0.05		Ds+Sc vs BMX	-0.4318	1.9910	ns	Down	P>0.05
Ds+Sc vs FRK	-0.1705	2.5430	ns	Down	P>0.05		Ds+Sc vs ERBB4	-0.3176	1.6150	ns	Down	P>0.05		Ds+Sc vs TP53RK	-0.1705	1.1540	ns	Down	P>0.05		Ds+Sc vs STYK1	-0.4063	1.9720	ns	Down	P>0.05
Ds+Sc vs FGFR2	-0.1785	2.5170	ns	Down	P>0.05		Ds+Sc vs PTK6	-0.3724	1.5710	ns	Down	P>0.05		Ds+Sc vs PTK2B	0.0829	1.1510	ns	Up	P>0.05		Ds+Sc vs ABL1	-0.4155	1.9160	ns	Down	P>0.05
Ds+Sc vs EGFR	-0.2439	2.5150	ns	Down	P>0.05		Ds+Sc vs MUSK	-0.3618	1.5260	ns	Down	P>0.05		Ds+Sc vs FES	-0.1126	1.0890	ns	Down	P>0.05		Ds+Sc vs TYRO3	-0.3885	1.9110	ns	Down	P>0.05
Ds+Sc vs TIE	-0.1699	2.5050	ns	Down	P>0.05		Ds+Sc vs EPHA7	-0.4588	1.5210	ns	Down	P>0.05		Ds+Sc vs RET	0.0729	1.0850	ns	Up	P>0.05		Ds+Sc vs JAK1	-0.4391	1.8900	ns	Down	P>0.05
Ds+Sc vs JAK1	-0.1742	2.4560	ns	Down	P>0.05		Ds+Sc vs FYN	-0.3111	1.5070	ns	Down	P>0.05		Ds+Sc vs RYK	0.0726	1.0800	ns	Up	P>0.05		Ds+Sc vs FLT3	-0.4298	1.8500	ns	Down	P>0.05
Ds+Sc vs YES1	-0.1665	2.4550	ns	Down	P>0.05		Ds+Sc vs FGR	-0.3079	1.4920	ns	Down	P>0.05		Ds+Sc vs ACK1	-0.1206	1.0330	ns	Down	P>0.05		Ds+Sc vs ROR1	-0.3945	1.8210	ns	Down	P>0.05
Ds+Sc vs ERBB2	-0.1719	2.4230	ns	Down	P>0.05		Ds+Sc vs FLT3	-0.3026	1.4660	ns	Down	P>0.05		Ds+Sc vs YES1	-0.0923	1.0220	ns	Down	P>0.05		Ds+Sc vs EPHA3	-0.3915	1.8050	ns	Down	P>0.05
Ds+Sc vs STYK1	-0.2312	2.3840	ns	Down	P>0.05		Ds+Sc vs RYK	-0.3436	1.4490	ns	Down	P>0.05		Ds+Sc vs ERBB4	-0.1051	1.0160	ns	Down	P>0.05		Ds+Sc vs RET	-0.3875	1.7890	ns	Down	P>0.05
Ds+Sc vs FGFR1	-0.1684	2.3730	ns	Down	P>0.05		Ds+Sc vs FGFR4	-0.2974	1.4410	ns	Down	P>0.05		Ds+Sc vs PDGFRB	0.0731	1.0150	ns	Up	P>0.05		Ds+Sc vs FGFR2	-0.3811	1.6400	ns	Down	P>0.05
Ds+Sc vs PTK2	-0.1285	2.3670	ns	Down	P>0.05		Ds+Sc vs KIT	-0.3386	1.4280	ns	Down	P>0.05		Ds+Sc vs NTRK3	0.0664	0.9884	ns	Up	P>0.05		Ds+Sc vs TXK	-0.3305	1.6260	ns	Down	P>0.05
Ds+Sc vs JAK2	-0.1677	2.3640	ns	Down	P>0.05		Ds+Sc vs CSK	-0.4161	1.3800	ns	Down	P>0.05		Ds+Sc vs MATK	0.0651	0.9686	ns	Up	P>0.05		Ds+Sc vs ROS1	-0.3418	1.5780	ns	Down	P>0.05
Ds+Sc vs CSF1R	-0.2277	2.3480	ns	Down	P>0.05		Ds+Sc vs PTK2	-0.3477	1.3690	ns	Down	P>0.05		Ds+Sc vs ERBB2	-0.0979	0.9471	ns	Down	P>0.05		Ds+Sc vs EPHA4	-0.3263	1.5050	ns	Down	P>0.05
Ds+Sc vs FYN	-0.1659	2.3380	ns	Down	P>0.05		Ds+Sc vs TP53RK	-0.4083	1.3540	ns	Down	P>0.05		Ds+Sc vs EPHA4	-0.1076	0.9215	ns	Down	P>0.05		Ds+Sc vs EPHB3	-0.3115	1.4370	ns	Down	P>0.05
Ds+Sc vs NTRK2	-0.1345	2.3170	ns	Down	P>0.05		Ds+Sc vs RET	-0.3206	1.3520	ns	Down	P>0.05		Ds+Sc vs LYN	-0.0589	0.8776	ns	Down	P>0.05		Ds+Sc vs BLK	-0.2983	1.3760	ns	Down	P>0.05
Ds+Sc vs INSR	-0.1620	2.2840	ns	Down	P>0.05		Ds+Sc vs SRC	-0.3498	1.3470	ns	Down	P>0.05		Ds+Sc vs FLT3	-0.0869	0.8407	ns	Down	P>0.05		Ds+Sc vs ERBB4	-0.3043	1.3100	ns	Down	P>0.05
Ds+Sc vs EPHA1	-0.2209	2.2780	ns	Down	P>0.05		Ds+Sc vs LTK	-0.3123	1.3170	ns	Down	P>0.05		Ds+Sc vs ALK	-0.0979	0.8387	ns	Down	P>0.05		Ds+Sc vs LYN	-0.2758	1.2730	ns	Down	P>0.05
Ds+Sc vs ERBB3	-0.1582	2.2300	ns	Down	P>0.05		Ds+Sc vs IGF1R	-0.2673	1.2950	ns	Down	P>0.05		Ds+Sc vs INSR	-0.0831	0.8037	ns	Down	P>0.05		Ds+Sc vs PDGFRB	-0.2903	1.2560	ns	Down	P>0.05
Ds+Sc vs DDR2	-0.2152	2.2190	ns	Down	P>0.05		Ds+Sc vs JAK3	-0.2549	1.2350	ns	Down	P>0.05		Ds+Sc vs EPHB3	-0.0929	0.7959	ns	Down	P>0.05		Ds+Sc vs PTK6	-0.2661	1.2290	ns	Down	P>0.05
Ds+Sc vs FLT1	-0.1542	2.1740	ns	Down	P>0.05		Ds+Sc vs ACK1	-0.3704	1.2280	ns	Down	P>0.05		Ds+Sc vs STYK1	-0.0908	0.7773	ns	Down	P>0.05		Ds+Sc vs EPHA8	-0.2615	1.2060	ns	Down	P>0.05
Ds+Sc vs MUSK	-0.1180	2.1730	ns	Down	P>0.05		Ds+Sc vs NTRK2	-0.3377	1.2170	ns	Down	P>0.05		Ds+Sc vs MET	0.0499	0.7428	ns	Up	P>0.05		Ds+Sc vs HCK	0.3224	1.1940	ns	Up	P>0.05
Ds+Sc vs MST1R	-0.1169	2.1520	ns	Down	P>0.05		Ds+Sc vs ROR1	-0.2864	1.2080	ns	Down	P>0.05		Ds+Sc vs EGFR	-0.0806	0.6903	ns	Down	P>0.05		Ds+Sc vs EPHB1	-0.2568	1.1840	ns	Down	P>0.05
Ds+Sc vs FGR	-0.1502	2.1170	ns	Down	P>0.05		Ds+Sc vs PTK7	-0.2863	1.2070	ns	Down	P>0.05		Ds+Sc vs KDR	-0.0654	0.6328	ns	Down	P>0.05		Ds+Sc vs PTK2	-0.2735	1.1830	ns	Down	P>0.05
Ds+Sc vs FGFR4	-0.1455	2.0520	ns	Down	P>0.05		Ds+Sc vs LYN	-0.2774	1.1700	ns	Down	P>0.05		Ds+Sc vs ROS1	0.0496	0.6303	ns	Up	P>0.05		Ds+Sc vs ALK	-0.2538	1.1710	ns	Down	P>0.05
Ds+Sc vs KDR	-0.1440	2.0300	ns	Down	P>0.05		Ds+Sc vs MST1R	-0.2731	1.1520	ns	Down	P>0.05		Ds+Sc vs SYK	0.0569	0.6299	ns	Up	P>0.05		Ds+Sc vs ITK	0.2901	1.1690	ns	Up	P>0.05
Ds+Sc vs MERTK	-0.1089	2.0050	ns	Down	P>0.05		Ds+Sc vs ROS1	-0.2709	1.1430	ns	Down	P>0.05		Ds+Sc vs EPHA3	-0.0734	0.6289	ns	Down	P>0.05		Ds+Sc vs ERBB2	-0.2625	1.1290	ns	Down	P>0.05
Ds+Sc vs RET	-0.1089	2.0050	ns	Down	P>0.05		Ds+Sc vs FGFR3	-0.2344	1.1360	ns	Down	P>0.05		Ds+Sc vs BLK	-0.0726	0.6218	ns	Down	P>0.05		Ds+Sc vs AXL	-0.2415	1.1140	ns	Down	P>0.05
Ds+Sc vs RYK	-0.1137	1.9590	ns	Down	P>0.05		Ds+Sc vs INSR	-0.2239	1.0850	ns	Down	P>0.05		Ds+Sc vs BMX	-0.0721	0.6175	ns	Down	P>0.05		Ds+Sc vs PTK7	-0.2243	1.0360	ns	Down	P>0.05

Text-based path-finding



Common targets network

*



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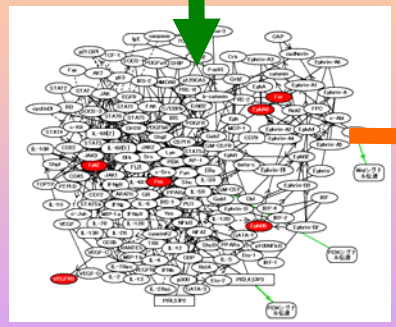


Common targets network

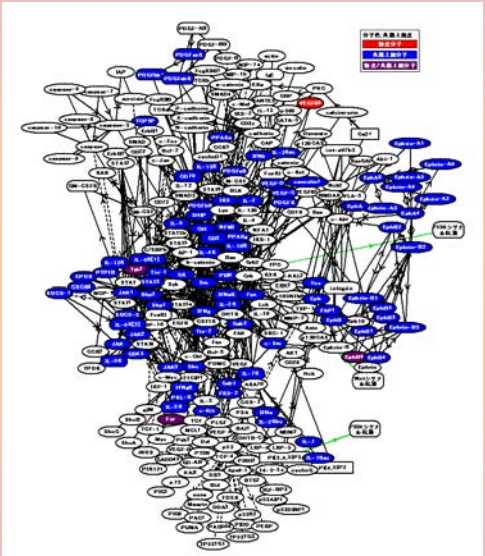
*

Common targets network between SK-BR-3 and T-47D

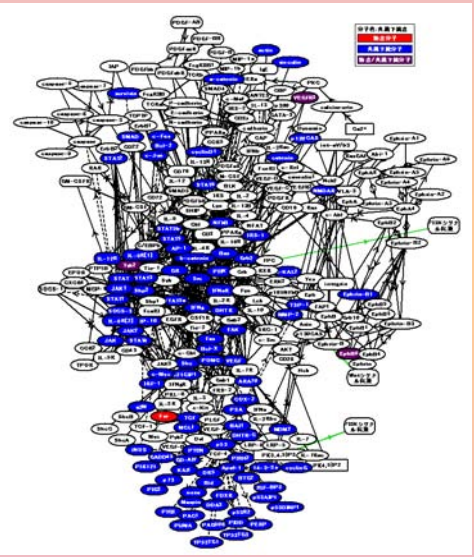
***using KeyMolnet database**

Identification of common pathways among the different cell-lines

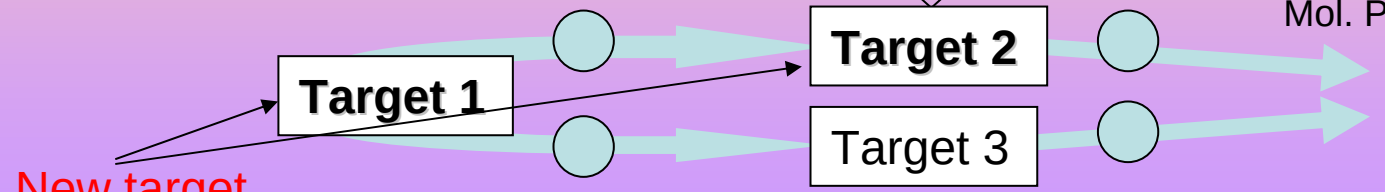
VEGF up-stream search



Target 1 down-stream search



SK-BR-3, T-47D common pathway



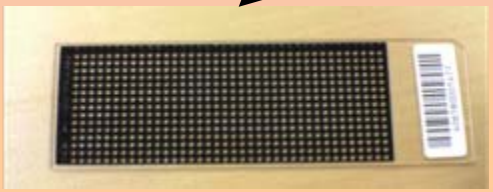
DXR enhancer target candidate
Mol. Pharmacol. (2004) 66:635

VEGFR3

Breast cancer specific TK gene
BBRC(2004)318:882

Transfection microarray system

TMA array printer
- sample information management
- automatic operation
- calibration system integrated
- high reliability
- print information management



Optimized glass slide for TMA

Cell
- Cell line
- Primary cell
- Normal cell

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

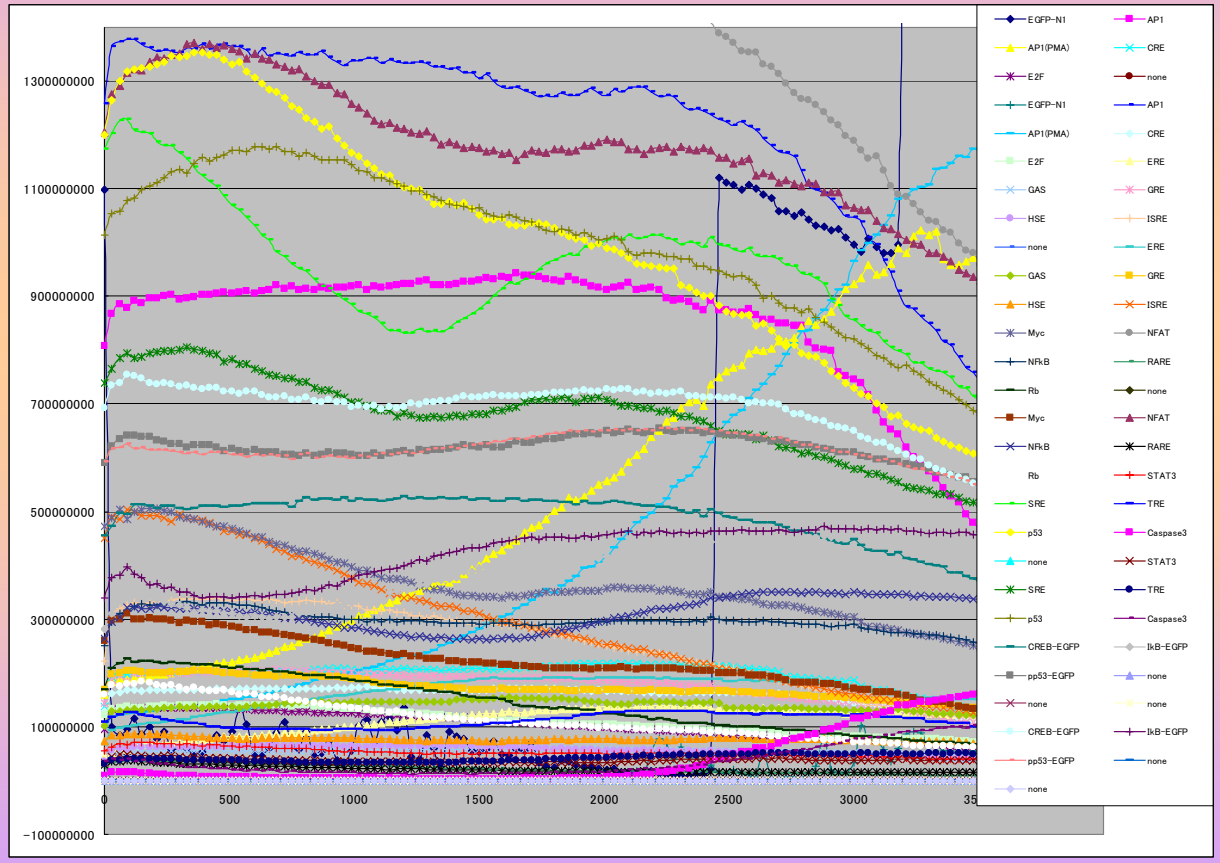
information

information

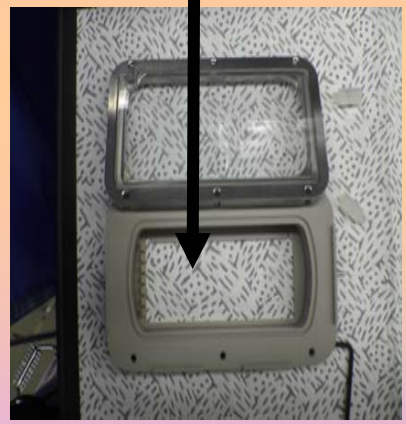
Time-lapse observation
- Cell culturing system integrated
- auto-focus unit integrated
- automatic data acquisition software
- adaptive for TMA format
- high resolution image acquisition

Data storage and image analysis
- Data integration all information
- Image analysis and quantification
- mathematical analysis for all data

Real-time & paralleled Pathway monitoring



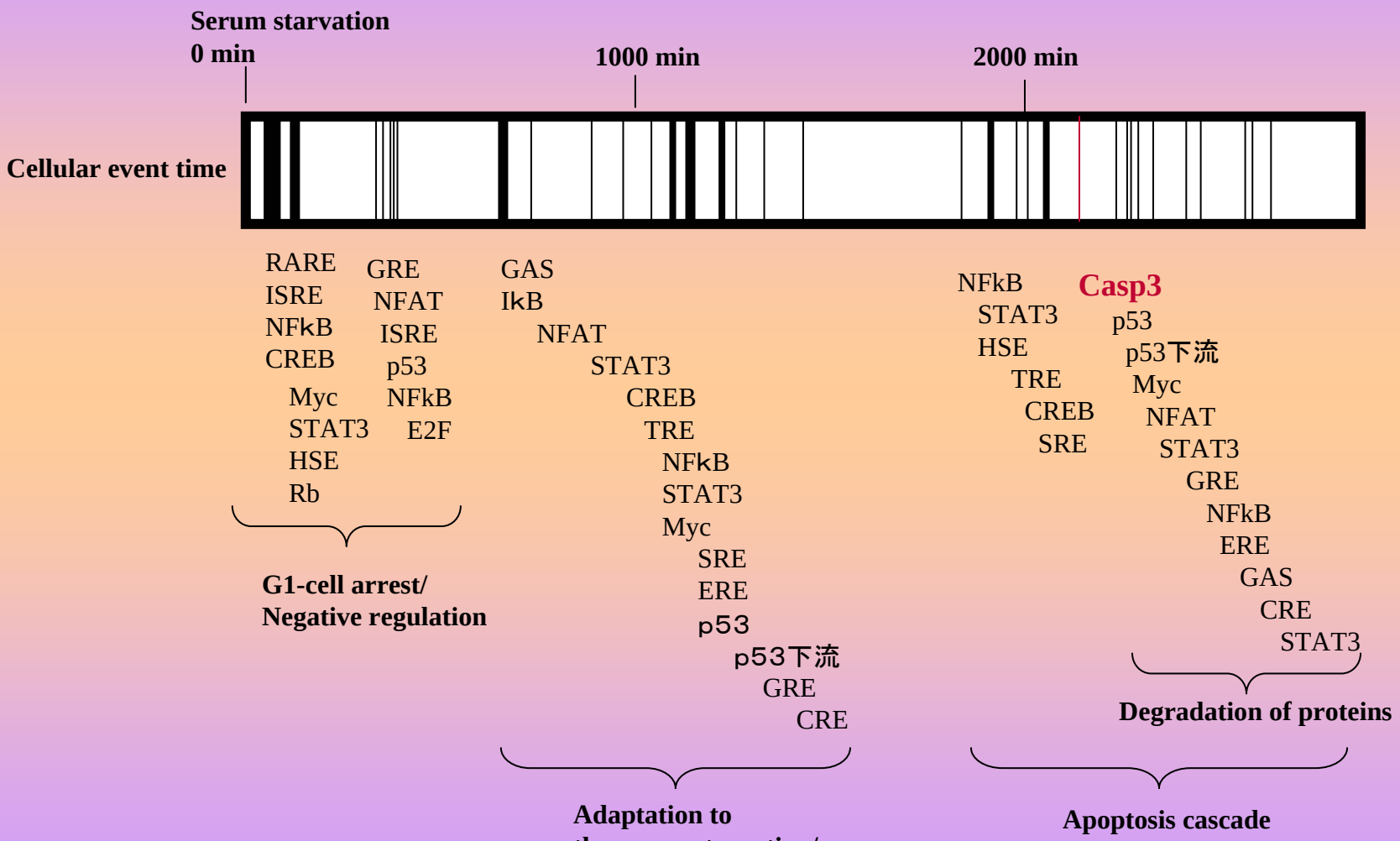
Transfection array



Incubation pod

Miyake et al. (in preparation)

Challenge to non-statistical analysis: Cellular event analysis



Next stage of cell-based functional analysis

- Molecular process for efficacy
- Proof of concept of combination therapy

Prospects of cell-based target discovery

- Genome-wide functional analysis would be powerful to find new druggable pathways and components.
- Transfection microarray, which allows primary cell, would be a robust miniaturized experimental tool for the genome-wide functional analysis.
- Next challenge would be understanding of process of efficacy and application to combination therapy.
- New bioinformatics methods may be needed to integrate the statistical data and the non-statistical data.

Contributions

CytoPathfinder

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Thank you !

CP