High-Throughput CellSensor® Reporter Assays For Multi-Pathway Analysis

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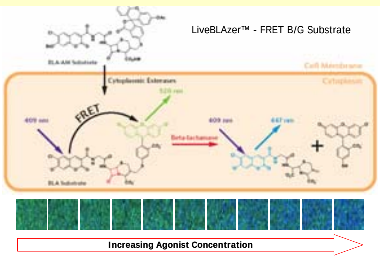
Abstract

Recent studies of cell signaling pathways revealed many genetic changes that contribute to various diseases. We have developed a portfolio of cell-based reporter assays that can be used to screen for perturbagens of over 20 different endogenous signaling pathways as well as specific protein targets (e.g. kinases) that are involved in these pathways. To build these pathway reporter assays, we stably engineered the beta-lactamase reporter under the control of pathway-specific response elements into various cell backgrounds that natively express either wild-type or mutated signaling pathways. Activation of a pathway either through ligand binding or a clinically relevant constitutively active mutation in the pathway leads to the expression of beta-lactamase, which can be easily detected and quantified by a live cell, ratiometric beta-lactamase substrate. These cell lines were functionally validated with small molecule inhibitors and/or RNAi against known players of each pathway and were shown to be robust enough for high through-put screening. We have profiled 45 commercially available small molecule inhibitors against 11 signaling pathways. The results highlight the utility of multi-pathway cell-based profiling to advance drug discovery by providing robust data for both on and off pathway activities.

Figure 1 – GeneBLAzer® Beta-Lactamase Reporter Assay Technology

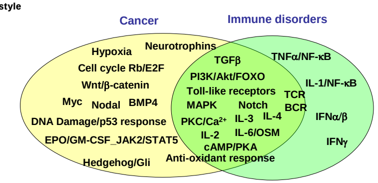


GeneBLAzer® Technology uses a FRET-based substrate to provide reliable and sensitive detection of beta-lactamase in cells. The advantages of this technology include:

1. Ratiometric readout reduces experimental noises
2. Fluorescent readout allows for live cell sorting by flow cytometry & imaging by microscopy
3. Sensitive detection due to enzyme turnover of substrate

Figure 2 – Invitrogen CellSensor® Cell Lines Offer Broad Pathway Coverage

Cell Backgrounds
HEK293T, 293Freestyle
CHO-K1
NIH3T3
ME-180
HCT-116
Jurkat
Ramos-1
THP-1
Ba/F3
CTLL-2
TF-1
HEL
HeLa
HepG2
ECV304
RAW264.7
SW480
A375


Cancer
Immune disorders
Hypoxia
Neurotrophins
Cell cycle Rb/E2F
Wnt/β-catenin
Myc
Nodal
BMP4
DNA Damage/p53 response
EPO/GM-CSF_JAK2/STAT5
Hedgehog/Gli
TGFβ
PI3K/Akt/FOXO
Toll-like receptors
MAPK
Notch
PKC/Ca2+
IL-3
IL-4
IL-2
IL-6/OSM
cAMP/PKA
Anti-oxidant response
TCR
BCR
TNFα/NF-κB
IL-1/NF-κB
IFNα/β
IFNγ

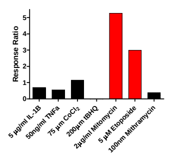
24 CellSensor® vectors
>50 CellSensor® lines

>20 pathways represented

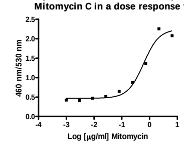
Figure 3 – CellSensor® Cell Lines for Toxicity Pathway Analysis

Pathways	Cell lines	Stimuli Tested
MAP Kinase	AP1-*bla* ME-180	EGF, HGF, MSP, IL-6, OSM, TNFα, IL-1β, PMA/Thaps
Hypoxia	HRE-*bla* ME-180	DFO, COCl2, Hypoxia Chamber
	HRE-*bla* HCT116	DFO, COCl2
DNA Damage/p53	p53RE-*bla* HCT116	Mitomycin, Etoposide, Nutlin
Nrf2/ARE	ARE-*bla* Hep G2	t-BHQ, β–Naphthoflaone, Sulforaphane

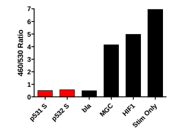
p53RE-*bla* HCT116 cells respond to DNA damaging agents



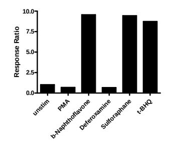
p53RE-*bla* HCT116 cells respond to Mitomycin C in a dose response format



Mitomycin C response can be knocked down by p53-specific RNAi



ARE-*bla* HepG cells respond to oxidative stress signal



ARE-*bla* HepG cells respond to tBHQ in a dose response format

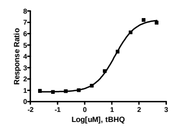
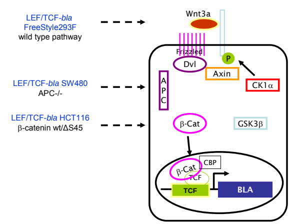


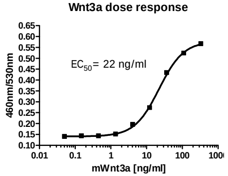
Figure 4 – CellSensor® Cell Lines for Step-Wise Interrogation of Wnt/β-catenin Pathway

Overview of the Wnt/β-catenin pathway



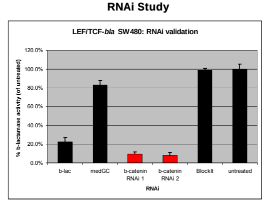
Wild-type Wnt Pathway: LEF/TCF-*bla* FreeStyle™ 293F

Wnt3a dose response



Constitutively Active Wnt Pathway (loss of APC mutant): LEF/TCF-*bla* SW480

RNAi Study



Inhibitor dose response

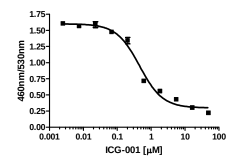
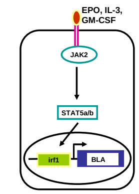
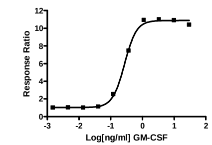


Figure 5 – CellSensor® Cell Lines for Various Cytokine-mediated JAK/STAT Pathways

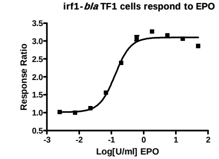
Cytokines	Cell lines	Pathways
IL-2	irf1--*bla* CTLL-2	JAK2/STAT5 (mouse)
IL-3	irf1-*bla* Ba/F3	JAK2/STAT5 (mouse)
	irf1-*bla* TF-1	JAK2/STAT5
	irf1-*bla* HEL	JAK2V617F/STAT5
IL-4	STAT6-*bla* RA1	JAK3/STAT6
IL-6 or OSM	SIE-*bla* HEK293T	JAK1_JAK2/STAT3
	SIE-*bla* ME-180	JAK1_JAK2/STAT3
GM-CSF or EPO	irf1-*bla* TF-1	JAK2/STAT5
IFNα or IFNβ	ISRE-*bla* HEK293T	JAK1_TYK2/STAT1/STAT2
	ISRE-*bla* Jurkat	JAK1_TYK2/STAT1/STAT2
IFNγ	GAS-*bla* ME-180	JAK1_JAK2/STAT1
	SIE-*bla* ME-180	JAK1_JAK2/STAT1
	SIE-*bla* THP1	JAK1_JAK2/STAT1



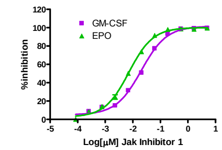
irf1-*bla* TF1 cells respond to GM-CSF



irf1-*bla* TF1 cells respond to EPO



JAK Inhibitor Treatment



JAK RNAi Knockdown

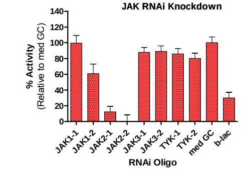


Figure 6 – CellSensor® Cell Lines for Compound Selectivity Profiling

IC50 (nM)

Compounds	Known targets/mode of actions	Hypoxia HRE-bla ME180 (CoCl2)	IFNg/JAK/STAT SIE-bla ME-180 (IFNg)	IL-6/JAK/STAT SIE-bla ME-180 (IL-6)	IL-1/NFkB NFkB-bla ME180 (IL-1)	TNFa/NFkB NFkB-bla ME180 (TNFa)	TNFa/JNK AP1-bla ME180 (TNFa)	EGFR/MAPK AP1-bla ME180 (EGF)	IFNa/JAK/STAT ISRE-bla HEK293T (IFNa)	TGF-beta SBE-bla HEK293T (TGF-beta)	PKC/Ca2+ NFAT-bla Jurkat (Thapsigargin)	cAMP/PKA CRE-bla Jurkat (Forskolin)
Staurosporine	Pan-kinase inhibitor	<1	12	13	101	84	2	2	29	90	40	18
K-252a	Pan-kinase inhibitor	22	94	41	305	695	418	176	101	1200	807	261
Ro-31-8220	Pan-AGC kinase inhibitor (e.g. PKA, PKC)	1030	614	420	668	1321	465	470	1740	2280	1430	2770
CDK1/2 Inhibitor II	CDK1/2, others (e.g. ROCK2, PDK1)	7700	784	987	>10000	5206	345*	191*	2790	373*	>10000	>10000
IKK Inhibitor III	IKK2>IKK1	3900	2772	3700	8530	5938	3761	3354	1070*	>10000	5500	3760
PD153035	EGFR	3670	1397	4840	4960	6330	1310	26	8990	6590	>10000	>10000
SU11652	RTKs (e.g. PDGFRb, VEGFR2, FGFR1, Kit family) [not EGFR]	565*	526*	6570	>10000	4690	51*	>10000	5560	1080*	4880	>10000
Dequalinium analog, C-14	PKC	617	3020	3190	>10000	4714	8220	9730	937*	>10000	2240	1480
Go 6983	PKCs	6960	3620	4920	3470	4155	>10000	340*	6190	>10000	2030	>10000
Roscovitine	CDKs	>10000	4872	4570	2840	3893	3874	3120	>10000	7700	7140	>10000
AKT Inhibitor IV	AKT activation	232	>10000	>10000	>10000	4346	>10000	>10000	614	>10000	1360	974
JAK Inhibitor I	JAKs	602	31	17	>10000	>10000	>10000	>10000	109	>10000	>10000	>10000
IGF1R Inhibitor	IGF1R	470	705*	>10000	>10000	>10000	>10000	>10000	591*	981*	>10000	>10000
H-1152	ROCK1	5610	3513	4313	7820	5250	>10000	5030	>10000	6600	>10000	>10000
SU5416	VEGFR [not EGFR]	1400	>10000	>10000	>10000	>10000	>10000	>10000	>10000	992*	6110	>10000
Tyrphostin	EGFR	>10000	8874	>10000	>10000	>10000	2500	85	>10000	>10000	>10000	>10000
PP2	Src family kinases (e.g. Lck, Fyn, Hck)	372*	>10000	>10000	>10000	>10000	326*	>10000	>10000	>10000	>10000	>10000
Hypericin	Pan-kinase inhibitor [poor aqueous solubility]	3620	4341	>10000	>10000	>10000	>10000	>10000	>10000	6390	>10000	>10000
SU6656	Src family kinases	>10000	580*	2840	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
U0126	MEK1/2 activation	>10000	>10000	>10000	>10000	>10000	>10000	955	>10000	>10000	3460	>10000
TGFbR1 Inhibitor	TGFbR	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	90	>10000	>10000
SB 202190	p38 MAPK [not ERK or JNK]	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	252*	>10000	>10000
Rapamycin	mTOR, p70S6k	384*	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
VEGF R2 Kinase Inhibitor III	VEGFR [not EGFR]	447*	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
JNK Inhibitor II	JNKs	851*	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
Y-27632	ROCK1/2	>10000	>10000	>10000	>10000	>10000	1831	9220	>10000	>10000	>10000	>10000
KN-93	CaMK II	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	2590
SB203580	p38 MAPK [not JNK]	>10000	>10000	>10000	>10000	>10000	>10000	>10000	7410	>10000	>10000	>10000
LY294002	PI3K	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000

low nM	submicromolar	low micromolar	above-range
<100 nM	100-1000 nM	1000-10000 nM	>10000 nM

Summary

- We have developed a panel of validation CellSensor® cell lines that can be used for endogenous signaling pathway analysis.
- Cell lines were further validated with specific Stealth™ RNAis or small molecular inhibitors against an essential player of the pathway.
- Use of CellSensor® cell lines to examine mutant and wild-type signaling pathways or to step-wise interrogate a pathway can facilitate a deeper understanding of disease biology.
- Many of our CellSensor® cell lines can be used for studying pathways initiated by various cytokines.
- CellSensor® cell lines can be used to generate compound potency and selectivity profiles.

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