Predictive Biology, Inc.

A Diversity Outbred ES Cell Platform for In Vitro Genetics

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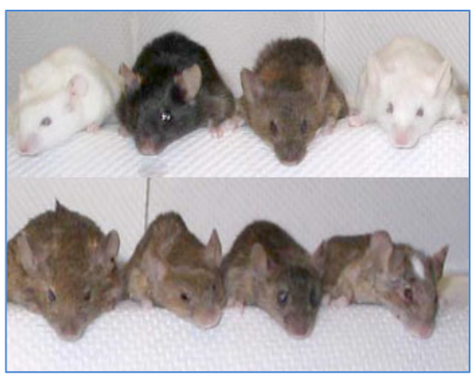
Predictive Biology, Inc.

Population-Based Rodent Resources for Environmental Health Sciences Meeting
March 18, 2015

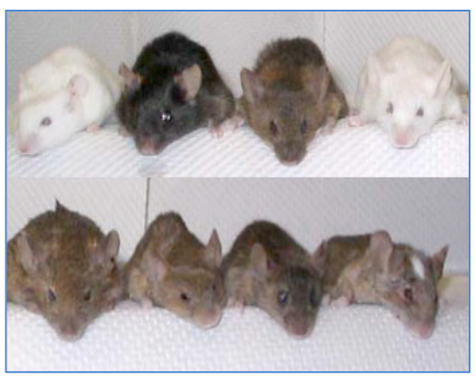
Predictive Toxicology Models



DO/CC/MDP

Human Diversity

Predictive Toxicology Models



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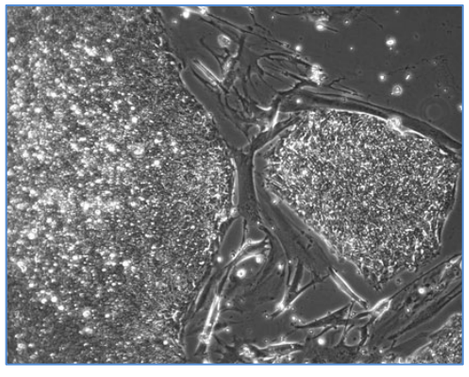
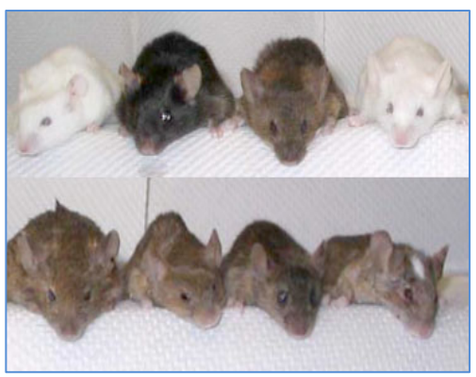
Human Diversity



Human Cell Lines

Predictive Toxicology Models

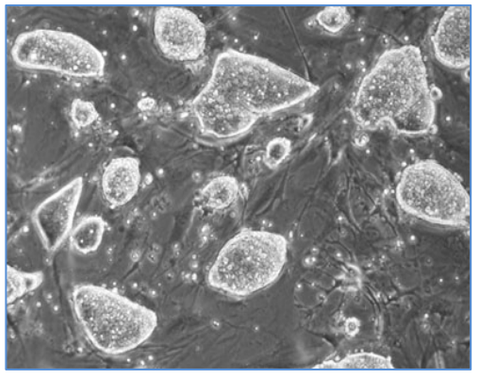


DO/CC/MDP

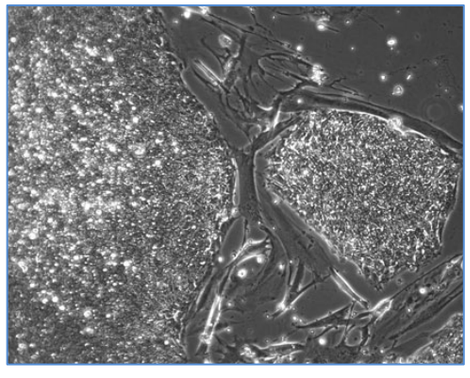
Human Diversity

PS

BM



MT



In Vitro Genetics (DO ESC)

Human Cell Lines

Diversity Outbred ES Cell Lines

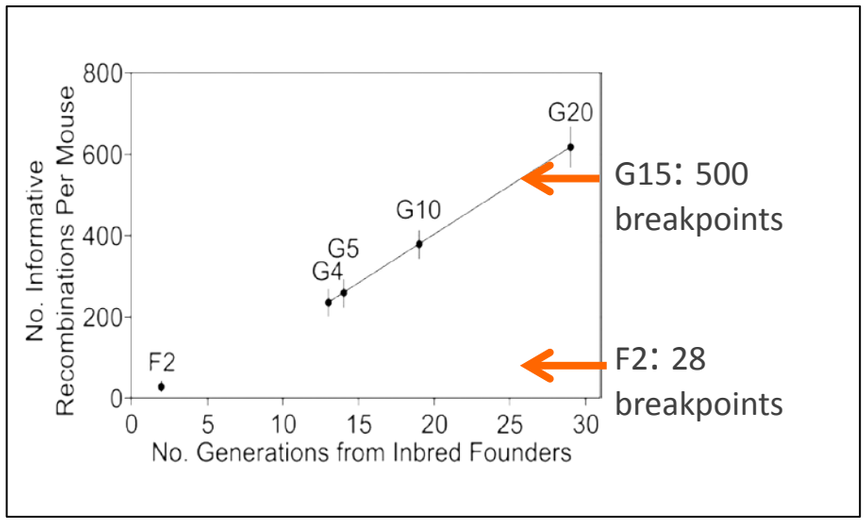
- Immortalizes heterozygous genome of DO
- Renewable, permanent screening resource
- ES cells provide access to many cell types
- Mature genome engineering tools
- Mixed cell models- 2D, 3D, organoid
- Gene x Environment studies
  - Timing and duration of exposure (windows of susceptibility)
  - Compound series and combinations

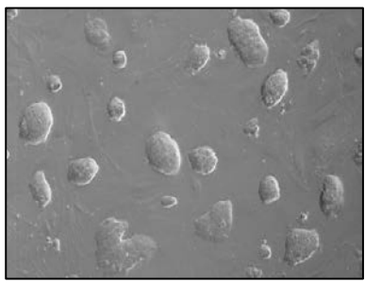
Challenges of In Vitro Genetics

- Cell type hard to define
- Cell model too simplistic
- Cell line drift
- Relevance of phenotype (assay measure)
- Robustness of assay (Z’ score)
- Percent variability due to genetics

DO ES Cell Lines

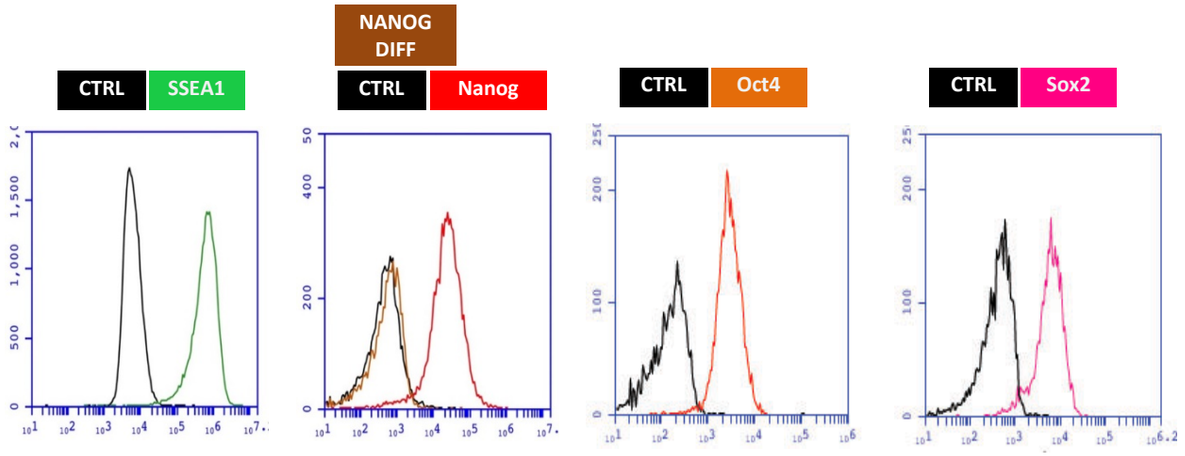
- 115 DO ES Lines (G9)
- 1,002 DO ES Lines (G15/16)
- Cryopreserved at Passages 1, 2 and 3
- Genotyped at 77K SNPs on MegaMUGA



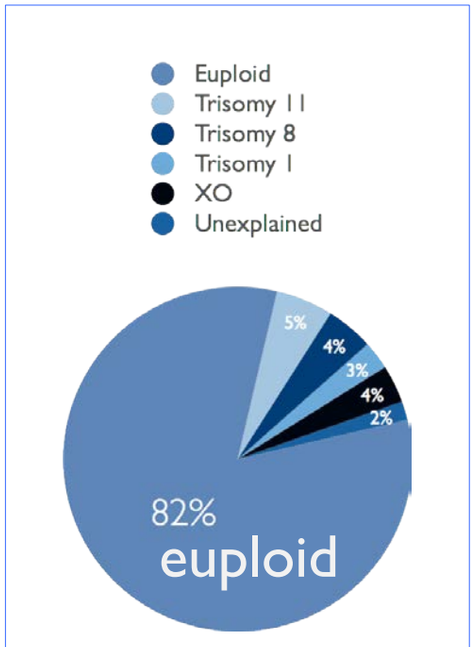


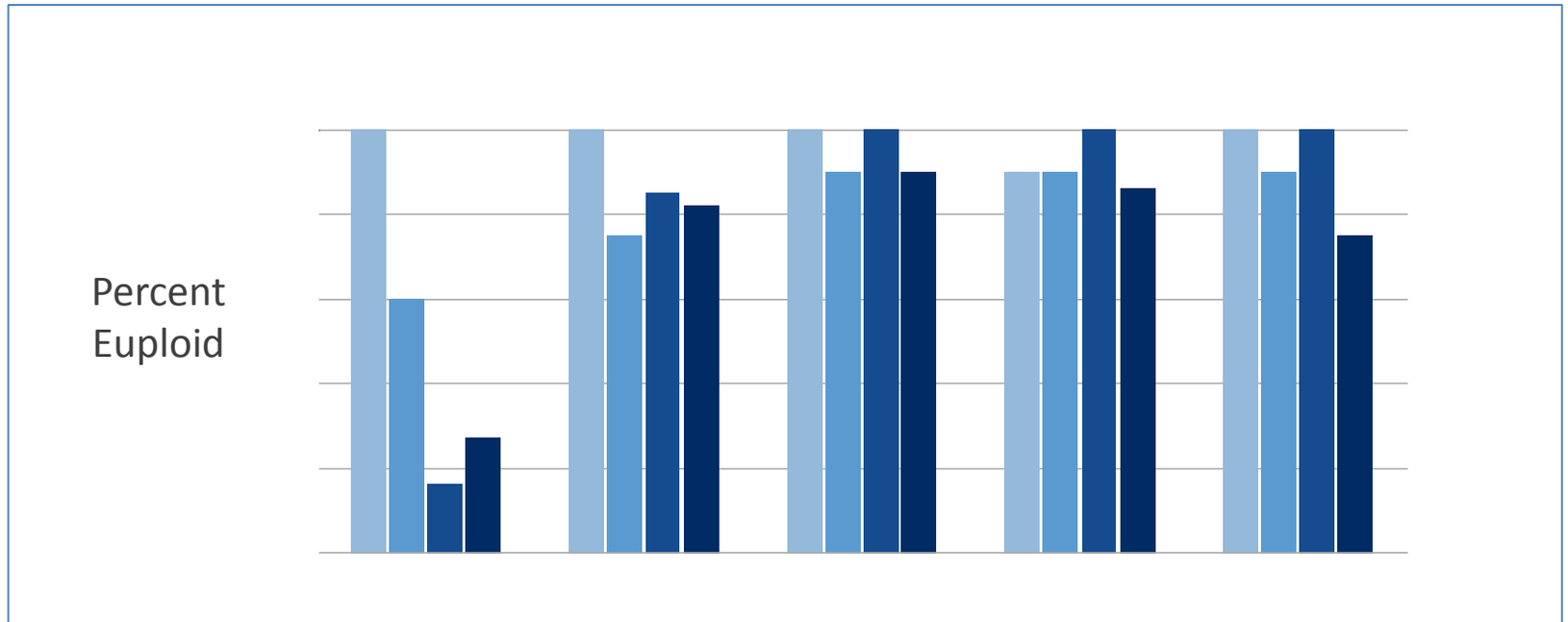
Growth

Differentiation



DO ES Lines are Mostly Euploid and Stable



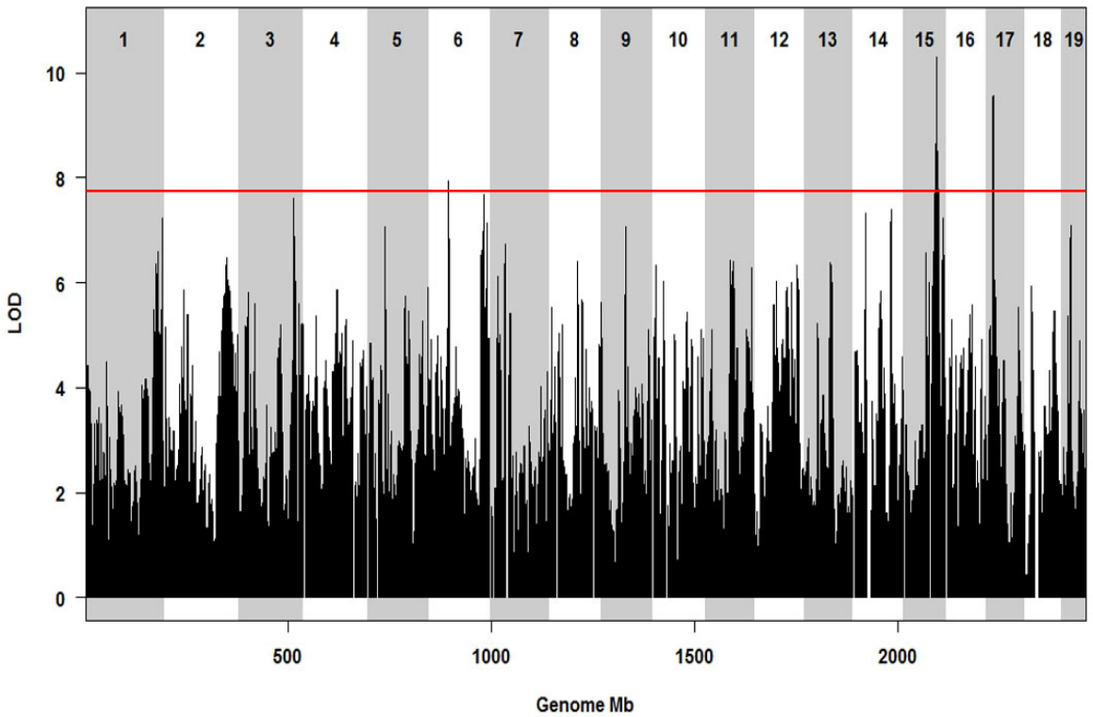


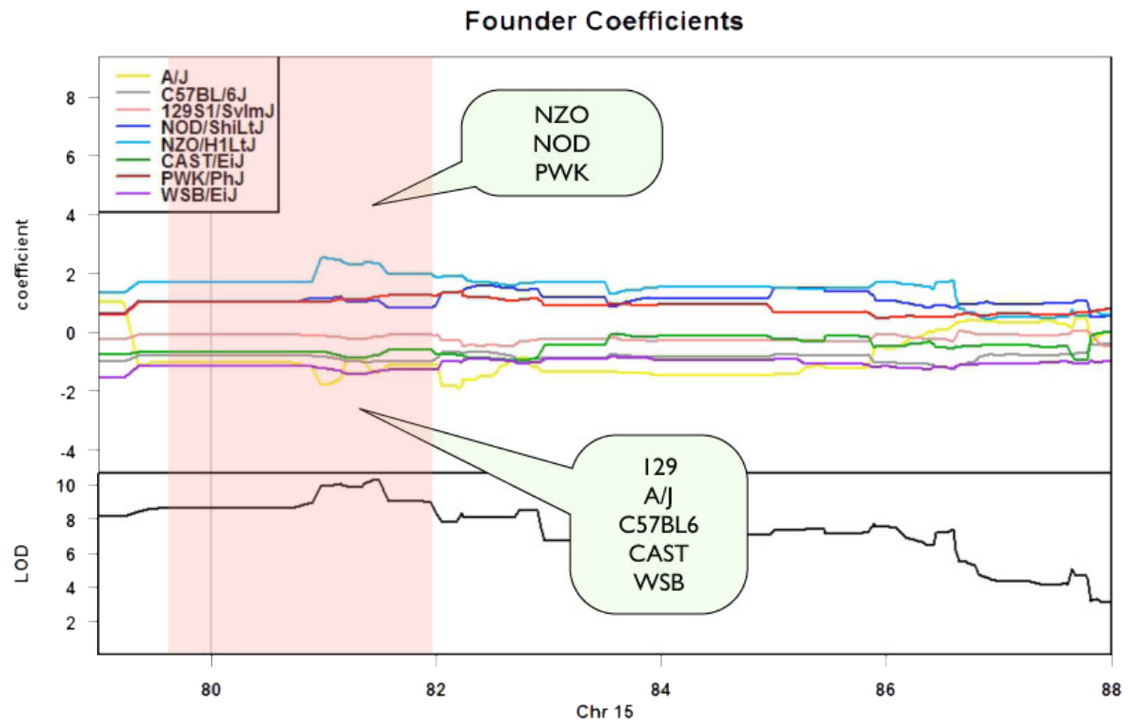
Pilot Expt: Cytotoxicity

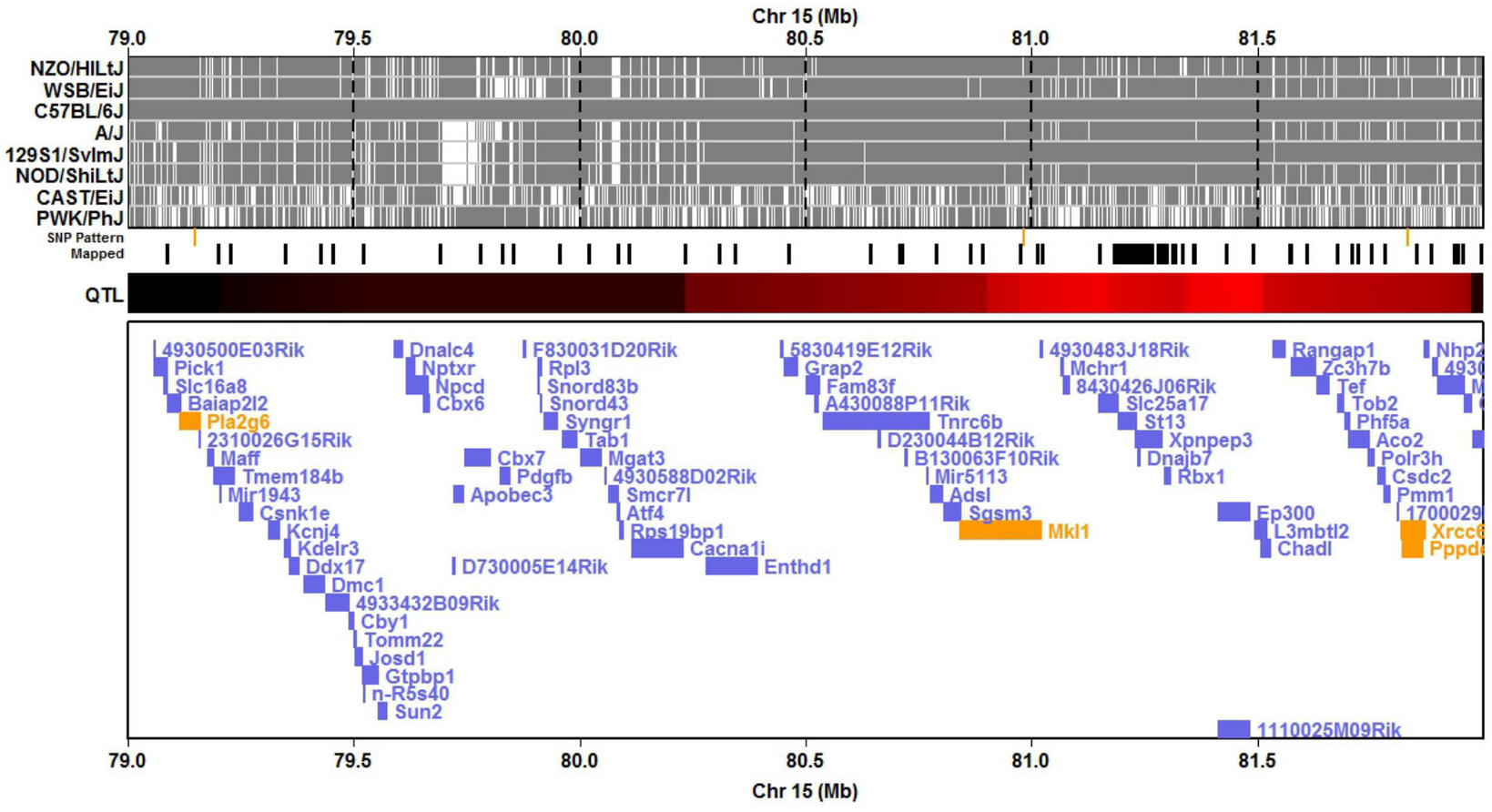
- 93 DO ES cell lines x 16 compounds
- 12 pt dose response
- Alamar Blue assay
- IC50 as phenotype score
- 4 technical replicates
- 2 biological replicates,10 passages apart
- DOQTL2 for mapping and analysis

Compound	Rank Order Correlation of 93 Cell Lines	QTL Detected	QTL LOD	Threshold LOD
6-Thioguanine	0.410	1	8.16	7.63
Malachite Green	0.394	1	7.91	7.82
Benzethonium Cl	0.368	3	10.29, 9.57, 7.95	7.70
Ethidium Bromide	0.348	1	8.25	7.54
Tamoxifen Citrate	0.324	0	-	7.62
Curcumin	0.263	1	8.45	7.65
Doxorubicin	0.260	0	-	7.75
5-Fluorouracil	0.174	0	-	7.61
6-Mercaptopurine	0.100	0	-	7.64
Verapamil	0.079	0	-	7.61

- QTL detected for compounds with higher correlation
- Replicable phenotype over 10 passages


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
LOD
Genome Mb


Founder Coefficients
A/J
C57BL/6J
129S1/SvImJ
NOD/ShiLtJ
NZO/H1LtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ
NZO
NOD
PWK
coefficient
129
A/J
C57BL6
CAST
WSB
LOD
Chr 15


Chr 15 (Mb)
NZO/HlLtJ
WSB/EiJ
C57BL/6J
A/J
129S1/SvImJ
NOD/ShiLtJ
CAST/EiJ
PWK/PhJ
SNP Pattern
Mapped
QTL
4930500E03Rik
Pick1
Slc16a8
Baiap2l2
Pla2g6
2310026G15Rik
Maff
Tmem184b
Mir1943
Csnk1e
Kcnj4
Kdelr3
Ddx17
Dmc1
4933432B09Rik
Cby1
Tomm22
Josd1
Gtpbp1
n-R5s40
Sun2
Dnalc4
Nptxr
Npcd
Cbx6
Cbx7
Pdgfb
Apobec3
F830031D20Rik
Rpl3
Snord83b
Snord43
Syngr1
Tab1
Mgat3
4930588D02Rik
Smcr7l
Atf4
Rps19bp1
Cacna1i
D730005E14Rik
Enthd1
5830419E12Rik
Grap2
Fam83f
A430088P11Rik
Tnrc6b
D230044B12Rik
B130063F10Rik
Mir5113
Adsl
Sgsm3
Mkl1
4930483J18Rik
Mchr1
8430426J06Rik
Slc25a17
St13
Xpnpep3
Dnajb7
Rbx1
Ep300
L3mbtl2
Chadl
1110025M09Rik
Rangap1
Zc3h7b
Tef
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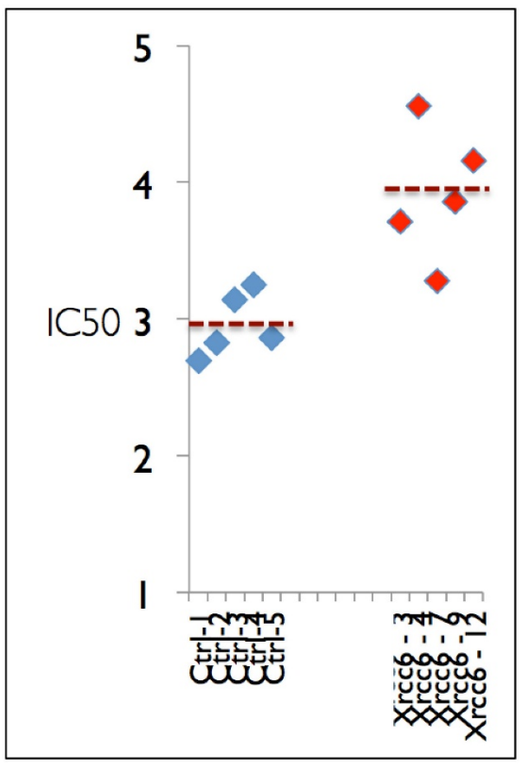
80.0
80.5
81.0
81.5


IC50
Ctrl-1
Ctrl-2
Ctrl-3
Ctrl-4
Ctrl-5
Xrcc6 - 3
Xrcc6 - 4
Xrcc6 - 7
Xrcc6 - 9
Xrcc6 - 12

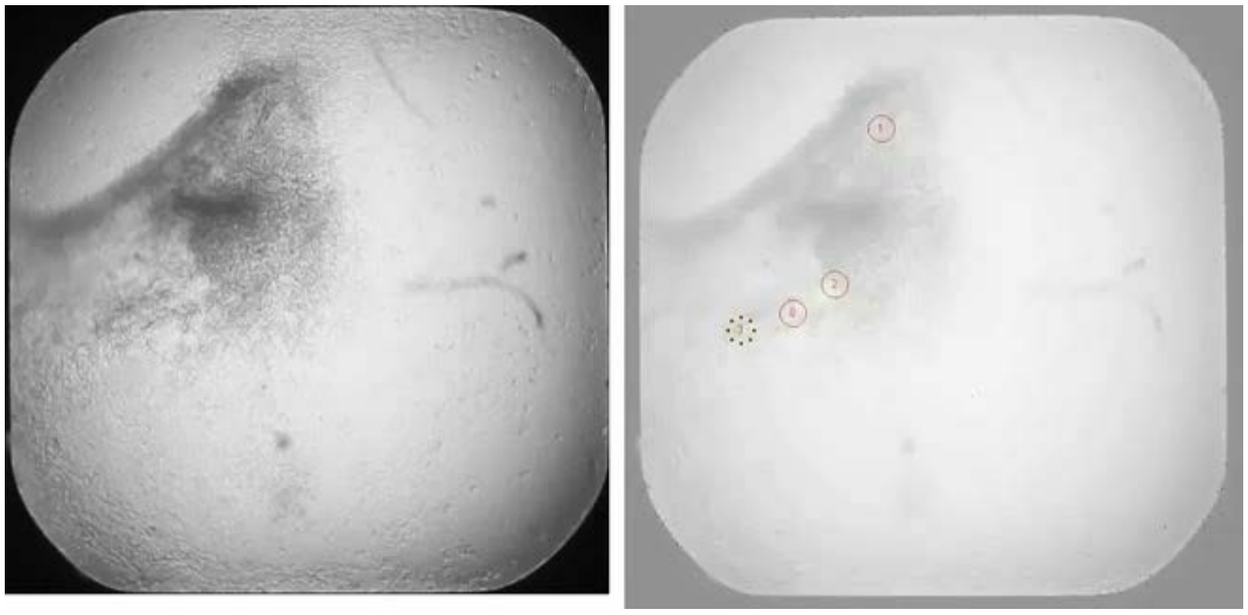
ES Cell Cytotoxicity Screen

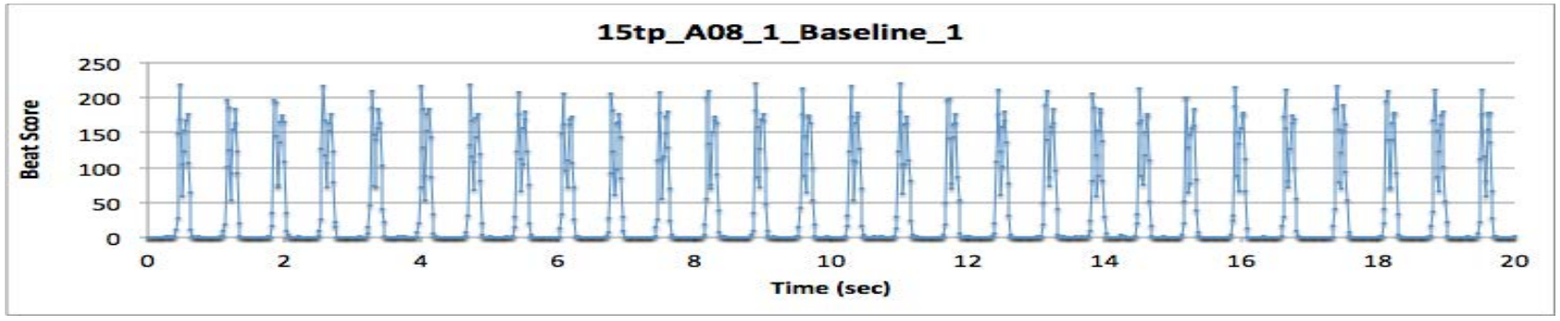
- 320 cell line screening panel
- 80 compounds from 1000 Genomes HTS study
- How many QTL genes and variants found?
- How many will translate to hIPSC?
- Will similar compounds share QTL?

1,3-Dicyclohexylcarbodiimide	Cycloheximide	PF-5274857
1,3,5-Triglycidyl isocyanurate	Daunomycin HCL	PFI-1
1,6-Hexamethylene diacrylate	Dequalinium Chloride	pimozide
1,8-Dihydroxy-4,5-dinitroanthraquinone	Dexamethazone	Potassium dichromate
17beta-Estradiol	Dieldrin	Purmorphamine
2-Amino-4-chlorophenol	Diethylene glycol diacrylate	Pyrimethamine
2-Amino-4-methylphenol	Diglycidyl resorcinol ether (DGRE)	quinidine
2,2',4'-Trichloroacetophenone	Digoxin	ranolazine
2,3,4,5-Tetrachloronitrobenzene	diltiazem	Rapamycin (Sirolimus)
2,4-Difluoronitrobenzene	Docetaxel	RGFP966
4-Chloro-o-phenylenediamine	Domiphen bromide	risperidone
5-Fluorouracil	domperidone	Romidepsin
6-Mercaptopurine monohydrate	doxorubicin	Sirtinol
6-Thioguanine (6-TG)	droperidol	Sitaxentan
9-Aminoacridine Chloride	Ethidium bromide	Sodium dichromate dihydrate (VI)
acrylamide	EVP4593	Sotalol
Aldrin	EX 527	Sotrastaurin
Alisertib	Exemestane	t-Butylhydroquinone
Azathioprine	flecainide	Tamoxifen citrate
Azimilide	Fumaronitrile	Temozolomide
Batimastat	GF109203X	tetra-N-Octylammonium bromide
Bazedoxifene HCl	Hexachlorophene	Tetraethylene glycol diacrylate
Benzethonium chloride	Hexamethyl-p-rosaniline chloride	Tetramethylthiouram disulfide
bepridil	ibutilide	TGX-221
beta-Nitrostyrene	Iodochlorohydroxyquinoline	Thalidomide
Bortezomib	Malachite green oxalate	Tofacitinib Citrate
Bosutinib	metoprolol	Topotecan HCl
Brefeldin A	mexiletine	Turmeric (>98% curcumin)
Carfilzomib	N-Methyl-p-aminophenol sulfate	UK 383367
Catechol	N,N-Dimethyl-p-nitrosoaniline	valproic acid
Cetylpyridinium bromide	N,N'-Di-sec-butyl-p-phenyldiamine	Veliparib
Chlorambucil	nitrendipine	Verapamil HCl
Chloranil	Nocodazole	Vismodegib
Chlorhexidine	o-Phenanthroline	Vorinostat
Chlorpromazine	ondansetron	VX-765
cisapride	Palbociclib Isethionate	WZ4003
clarithromycin	Pentaerythritol triacrylate	Y-27632
clozapine	PF-04691502	Zinc pyrithione
Colchicine	PF-3845	Ziram
CP-91149	PF-4708671	

Visual Beating Assay

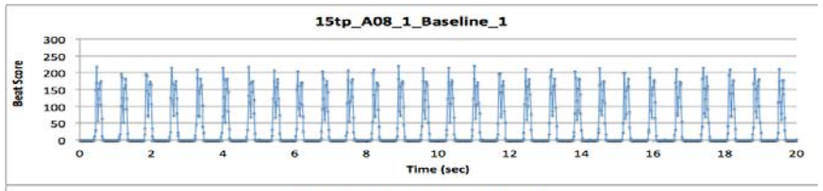
- Non-invasive, label free visual assay in 384w plates
- Automated, highly scalable, low cost
- Uses cardiomyocytes from EB, monolayer, or purified
- Mouse ESC or hIPSC derived CMs



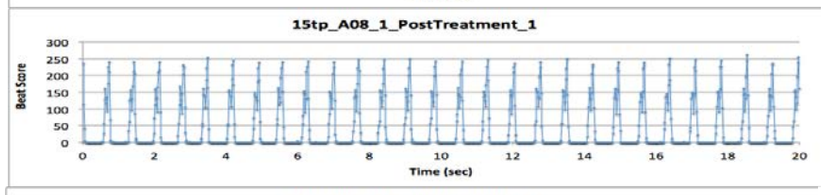


Cisapride Affects Rhythmicity, Not Contractility

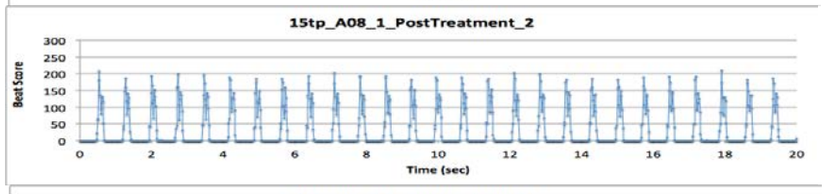
Baseline



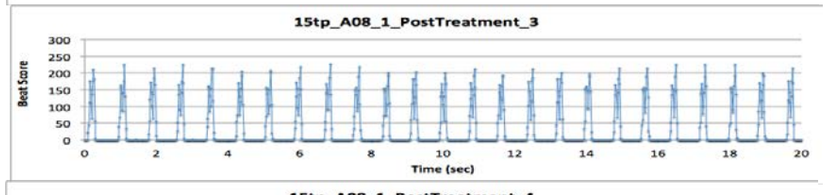
30 nM



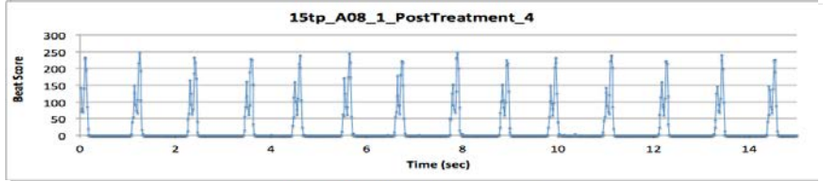
90nM



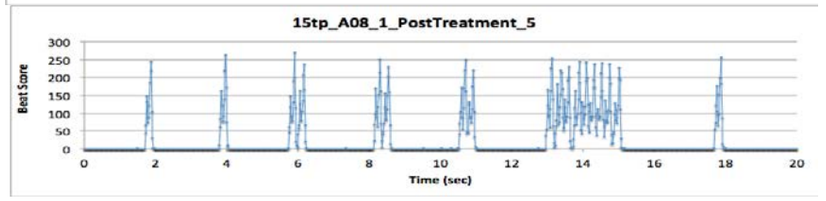
200 nM



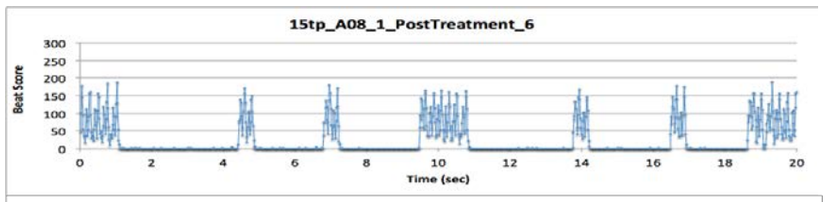
400 nM



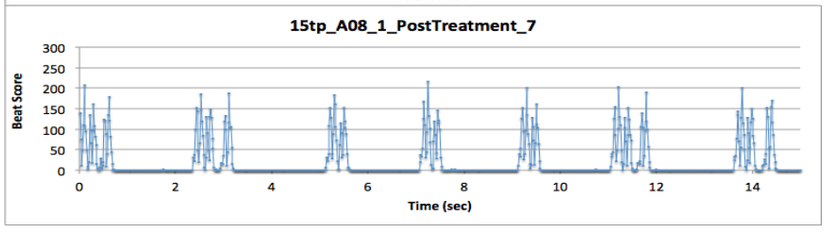
0.8 uM



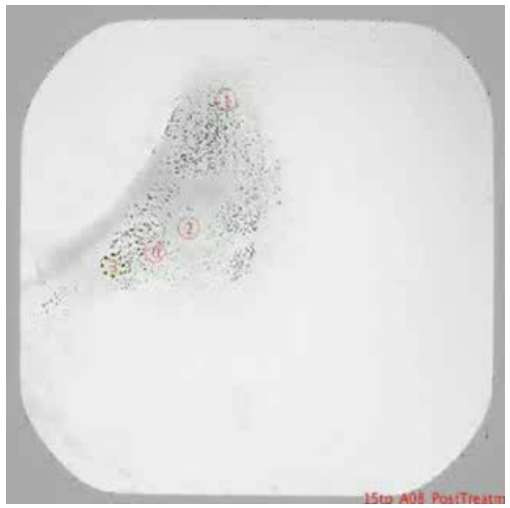
1.6 uM



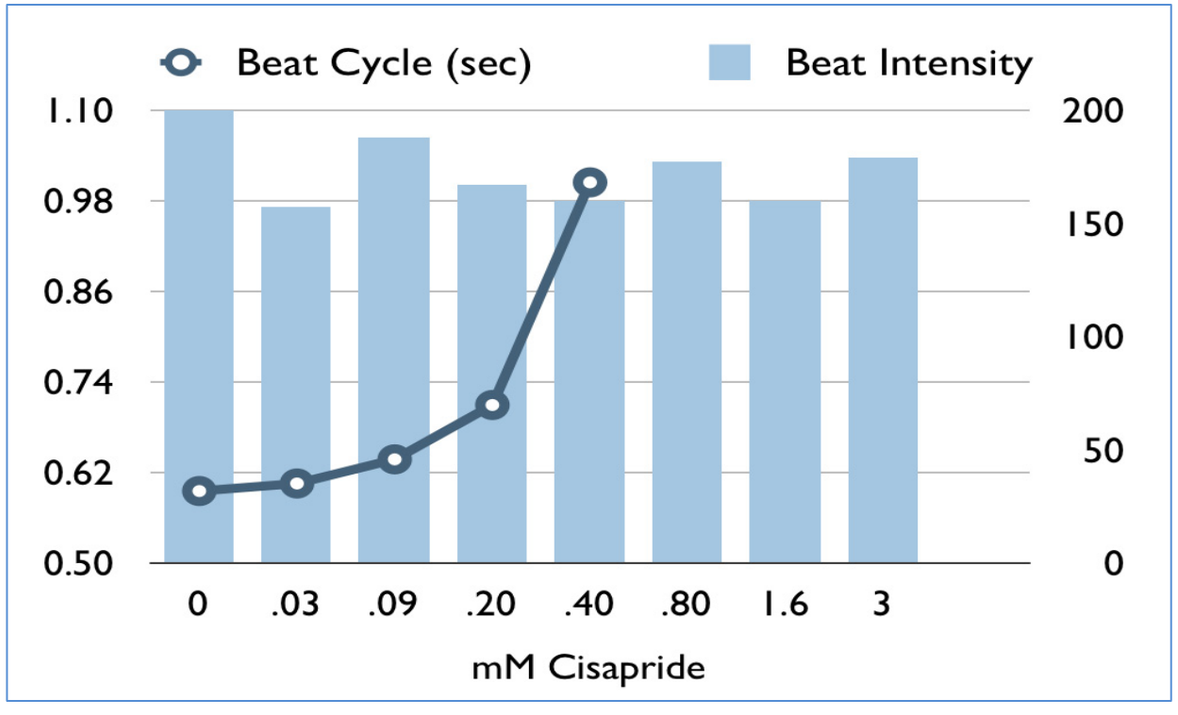
3.0 uM



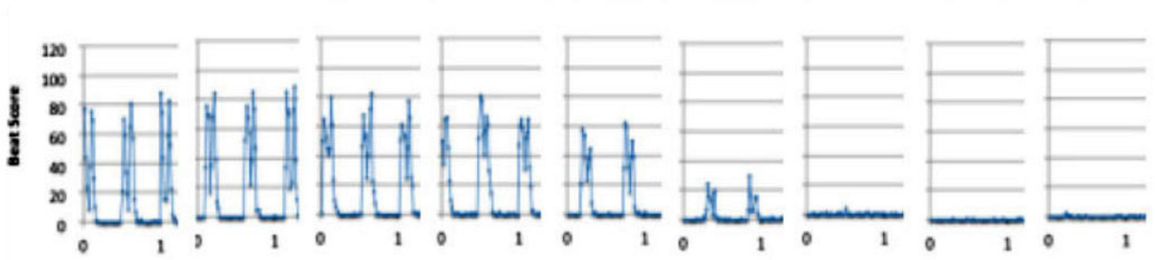
uM	beat cycle (sec)	cv
0	**0.595**	0.040
0.03	**0.605**	0.036
0.09	**0.637**	0.018
0.20	**0.709**	0.032
0.40	**1.004**	0.057
0.80	0.888	**1.110**
1.60	0.764	**1.109**
3.00	0.797	**1.063**

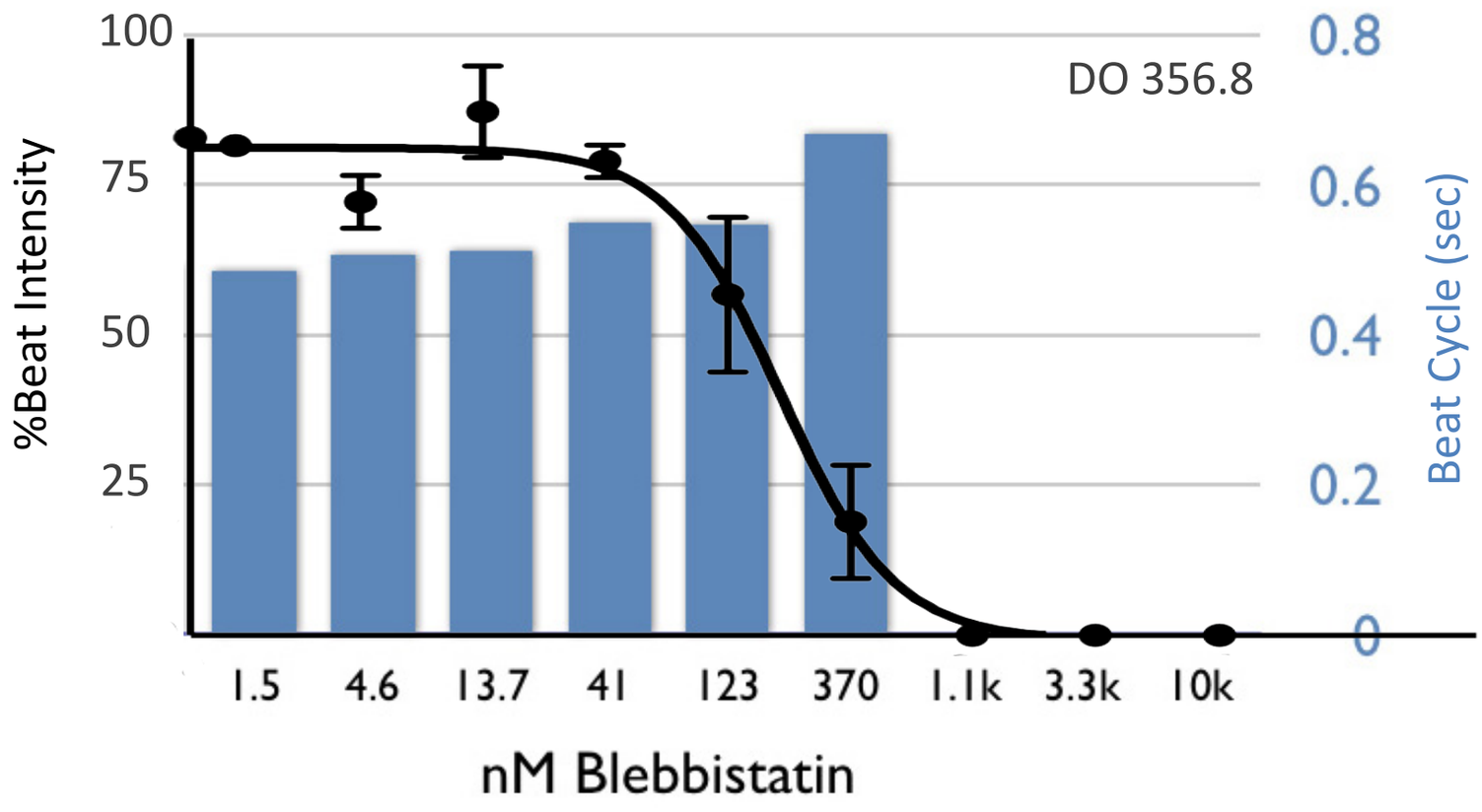


1.6 uM



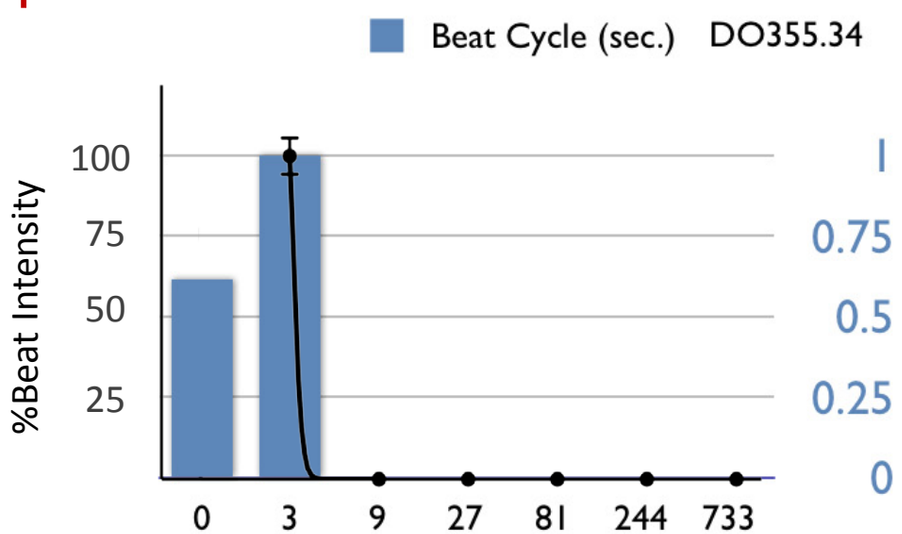
Blebbistatin Affects Contractility, Not Rhythmicity

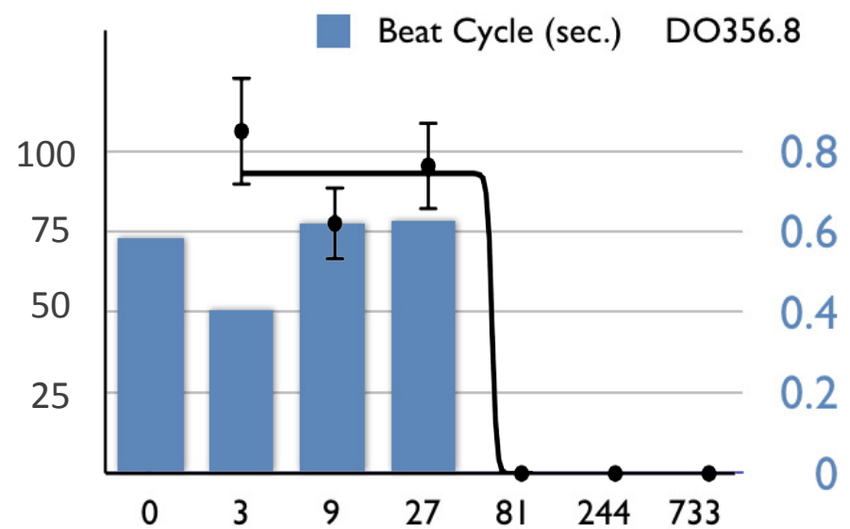




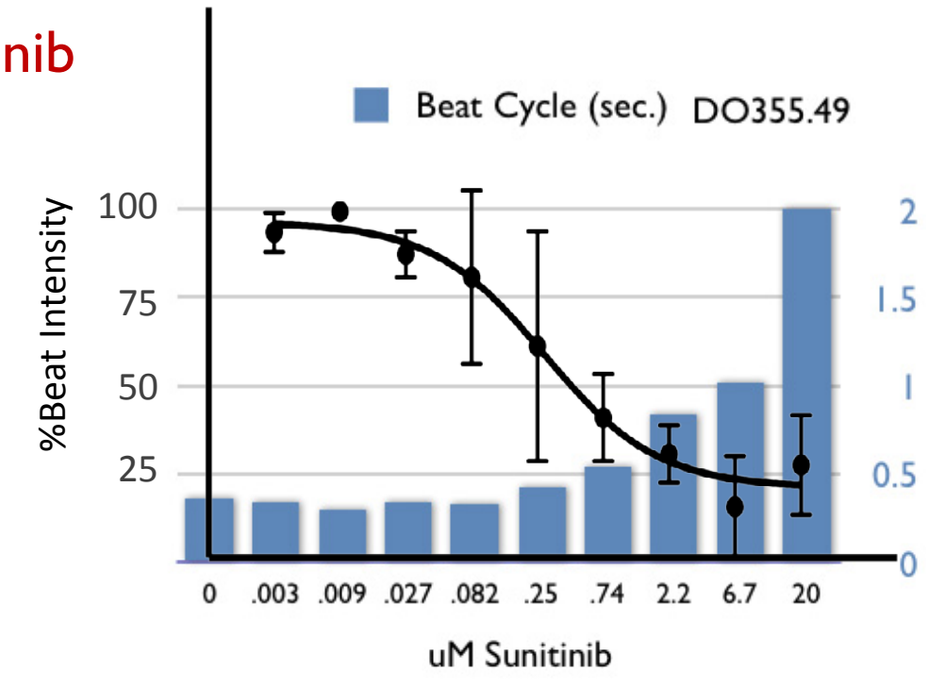
Variable Drug Response In DO Lines

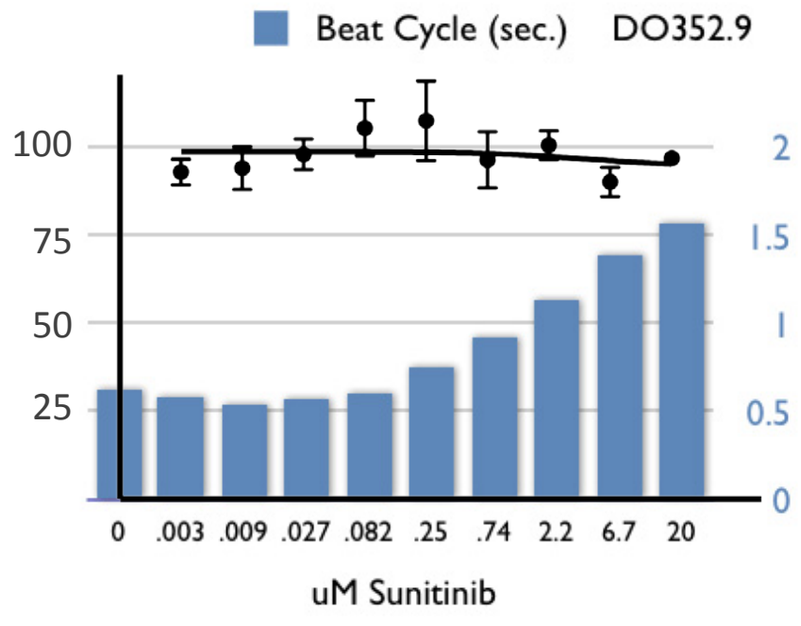
Isradipine



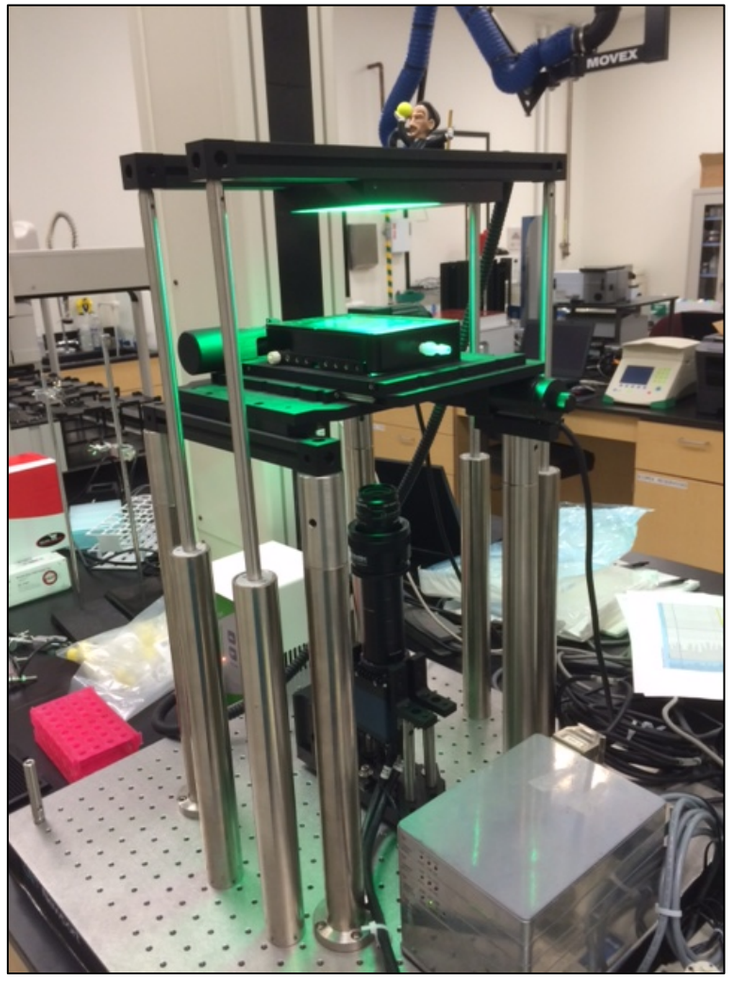


Sunitinib





High Speed Beating Imager



	Gen 1	Gen 2
Platform	Microscope with Camera	Machine Vision Camera
Scan Specs	60 fps, 20sec capture	58 fps, 20sec capture
Wells Per Capture	1	12
Analysis	Offline Software	Onboard FPGA
Time to Scan 384 Well Plate	150 minutes	16 minutes
Time to Analyze Videos	18 hours	4 minutes

Cardiotoxicity Screen

- 30 compound screen

- TKI anticancer drugs
- CIPA arrhythmia set

- Use IC10 or IC50 as phenotype

- Map, test candidate genes

- Test hIPSC derived CMs

Compound	Class	Compound	Class
Doxorubicin	Anthracycline	**Azimilide**	CIPA High Risk
Geldenamycin	Antibiotic	**Bepridil**	CIPA High Risk
Gefitinib	Tyr. Kin. Inhibitor	**Ibutilide**	CIPA High Risk
Alisertib	Tyr. Kin. Inhibitor	**Quinidine**	CIPA High Risk
Paclitaxel	Tyr. Kin. Inhibitor	**Sotalol**	CIPA High Risk
Sunitinib	Tyr. Kin. Inhibitor	**Pimozide**	CIPA Medium Risk
Sorafenib	Tyr. Kin. Inhibitor	**Chlorpromazine**	CIPA Medium Risk
Crizotinib	Tyr. Kin. Inhibitor	**Clarithromycin**	CIPA Medium Risk
Dasatinib	Tyr. Kin. Inhibitor	**Clozapine**	CIPA Medium Risk
Lapatinib	Tyr. Kin. Inhibitor	**Droperidol**	CIPA Medium Risk
Erlotinib	Tyr. Kin. Inhibitor	**Risperidone**	CIPA Medium Risk
1,2 bis (2-Chloroethoxy) Methane	NTP	**Diltiazem**	CIPA Low Risk
chloroacetaldehyde	NTP	**Flecainide**	CIPA Low Risk
Thioglycolic acid	NTP	**Cisapride**	Reference Arrythmic
2,2 Thiodiacetic Acid	NTP	**Isradipine**	Reference Contractile

Summary

- Developing an in vitro genetics platform with DO ES cell lines
- Scalable, automatable genetics of compound response
- Initial screening projects in cytotoxicity, cardiotoxicity and neurotoxicity, with results in late 2015 thru 2016.
- Goal to make mapping of toxicity modifier genes ‘routine’
- Identify mechanisms, genes and pathways underlying compound toxicity

Acknowledgements

Cytotoxicity : **Jacqueline Bergseid/Shikha Sharma**

Cardiotoxicity: **Shivanjali Joshi-Barr/Stephanie Brown**

Neurotoxicity: **Lesley Page/Amy Carlo**

DOQTL: **Dan Gatti, Jackson Laboratory**

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