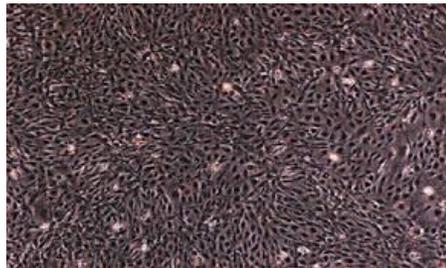


LINCS Proteomic Characterization Center for Signaling and Epigenetics

Jaffe Lab¹, Tsai Lab², MacCoss Lab³

¹Broad Institute of MIT and Harvard, ²MIT, ³University of Washington



Cell Lines

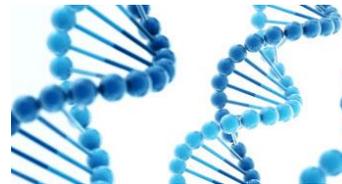


Neural Lineages

X

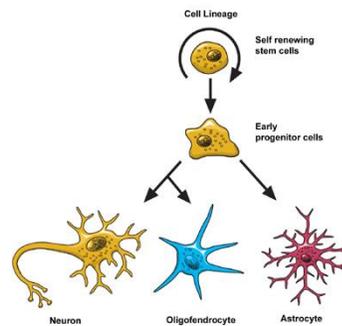


Drugs



Genes

X



Fates



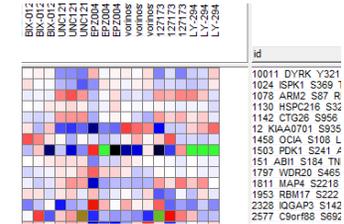
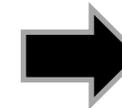
P100 / DIA
(phospho)



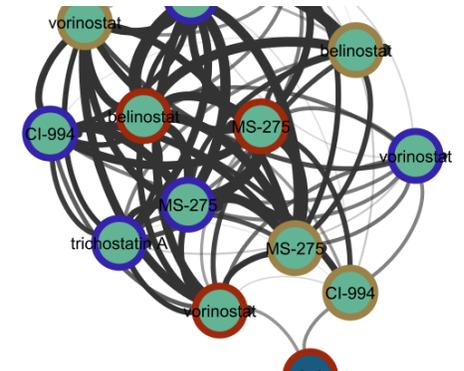
GCP
(histone marks)



L1000
(mRNA)



Signatures



Connectivity



Circuitry

Core Cell Lines



Cell Line	Provenance
MCF7	Breast Cancer
PC3	Prostate Cancer
A375	Skin Cancer
A549	Lung Cancer
NPC	H9 ESC-derived (normal)
<i>Coming soon...</i>	
YAPC	Pancreatic Cancer

Neural lineages – progress and strategies



Generation of human Cas9 NPCs

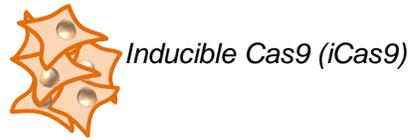
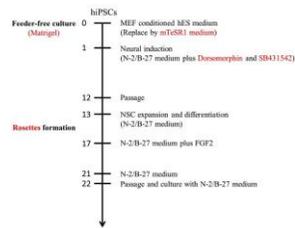
Lentivirus treatment of H9 ES cells
Selection with blasticidin

Neural progenitor cells (NPCs)

H9 ES cells



Clonal Expansion
NPC differentiation



Organoid creation in the Tsai lab:

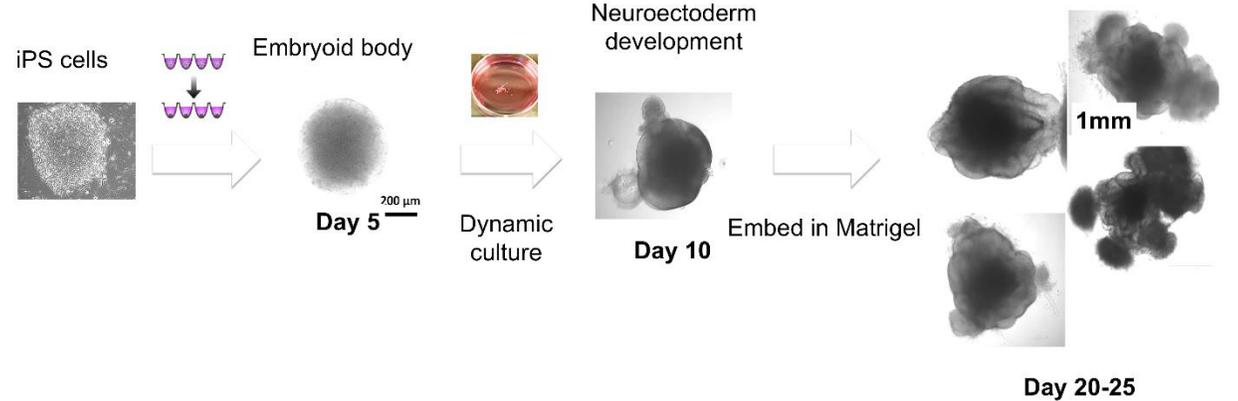
Two main approaches are being used in the Tsai lab to create three-dimensional cytosystems or “organoids”. These differ mainly in the medium components and the timing of Matrigel embedding.

Approach 1:

from Lancaster et al, *Nature* 501(7467):373-9, 2013.

Approach 2:

from Sasai et al, *Cell Stem Cell* 3(5):519-32, 2008.



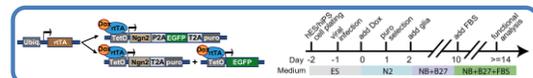
Future Directions: Proteomics using human Cas9 neurons.

CHALLENGE:

To create large cultures of homogenous, mature human neurons

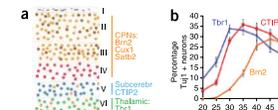
CANDIDATE APPROACHES:

Inducible Ngn2 Overexpression



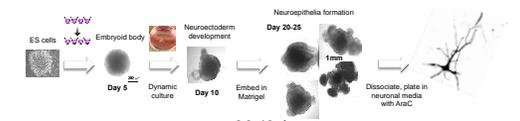
Zhang et al, 2013, *Neuron* 78, 785-798

Passive Differentiation



Shi et al, 2012, *Nat Neurosci* 15, 477-486

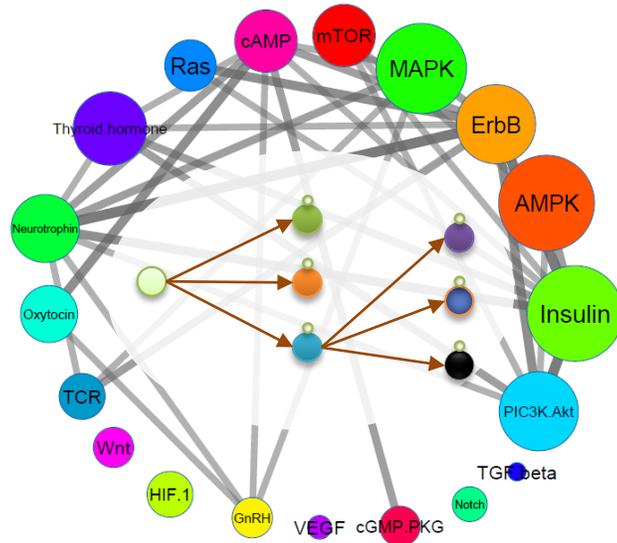
Organoid-derived neurons



-----30-40 days-----

Our assays

P100 is a targeted proteomic assay against 96 phosphopeptide probes that are commonly observed and modulated in diverse cell types



GCP is a targeted proteomic assay against ~ 60 probes that monitor combinations of post-translational modifications on histones



Our Compounds

Set 1: Epigenetically Active

Common Name	MOA
DMSO	negative control
GSK1210151A	Bromodomain inhibitor; BRD2,3,4
GSK525762A	Bromodomain inhibitor; BRD2,3,4
jq1	Bromodomain inhibitor; BRD4
zebularine	DNMT1 inhibitor
decitabine	DNMT1 inhibitor
EPZ-5676	DOT1L inhibitor
EPZ004777	DOT1L inhibitor
gsk126	EHZ2 inhibitor
EPZ-5687	EHZ2 inhibitor
CPI-169	EHZ2 inhibitor
UNC0646	G9a inhibitor
UNC0321	G9a inhibitor
BIX 01338	G9a inhibitor
BIX 01294	G9a inhibitor; ? also kinase inhibitor (mTOR)
vorinostat	HDAC inhibitor; general
entinostat (MS275)	HDAC inhibitor; general
tacedinaline (Ci-994)	HDAC inhibitor; general
trichostatin-a	HDAC inhibitor; general
belinostat	HDAC inhibitor; general
methylstat	JHDM inhibitor (Jumonji C)
GSK-J4	JMJD3 inhib
UNC1215	L3MBTL3 probe
1271738-62-5 (MI-2)	MLL inhibitor
OSI-027	mTOR inhibitor
resveratrol	Sirt1 activator (?)
EX527	Sirt1 inhib
salermide	Sirt1 inhib; Sirt2 inhib
geldanamycin	HSP90 inhibitor
rapamycin	mTOR inhibitor
staurosporine	Kinase inhibitor; general
LY-294002	Kinase inhibitor; pim-1, PI3k

Set 2: Neuroactive

Common Name	MOA
DMSO	negative control
Etoposide	Top2B inhibitor, DNA DSB inducer
KU-55933	ATM inhibitor
SMER-3	E3 ligase inhibitor
ruxolitinib	JAK 1 & 2 inhibitor
niclosamide	STAT3 signaling inhibitor
VU0155056	inhibitor of phospholipase D1/D2
tacrolimus	inhibitor of calcineurin
tretinoin	Retinoic Acid Receptor agonist
curcumin	NFkB inhibitor
semagacestat	gamma secretase inhibitor
dexamethasone	Glucocorticoid receptor agonist
Olaparib	PARP inhibitor
bafilomycin A1	inhibitor of the vacuolar-type H ⁺ -ATPase
Exifone	Antioxidant
vorinostat	HDAC inhibitor; general
KN-62	CaMKIIalpha inhibitor
calpain inhibitor II	calpain inhibitor
C646	CBP/p300 inhibitor
Compound E	gamma secretase inhibitor
KN-93	CaMKII/CaMKIV inhibitor
Gossypetin	HDAC1 activator
Ginkgetin	HDAC1 activator
Okadaic Acid	PP1 and PP2a inhibitor
momelotinib	JAK1/2 inhibitor
TBB	Casein kinase II inhibitor
Roscovitine	Cdk5 inhibitor
Rolipram	PDE4 inhibitor
RGFP966	HDAC3 inhibitor
sotrastaurin	inhibitor of protein kinase C beta
staurosporine	Kinase inhibitor; general
CHIR99021	GSK3 inhibitor

Set 3: Ser/Thr kinase pathways

Common Name	MOA
DMSO	negative control
Selumetinib	Mek1/2 inhibitor
PD0325901	Mek1/2 inhibitor
Everolimus	mTOR inhibitor
vemurafenib	Raf inhibitor
TG101348	Jak2 inhibitor
Tofacitinib	Jak3 inhibitor
Pravastatin	Stat1 inhibitor
SCH 900776	Rep. stress/CHK1 inhibitor
flavopiridol	CDK inhibitor
PD-0332991	CDK/4,6 inhibitor
Dinaciclib	CDK/1,2,5,9 inhibitor
RO4929097	Notch/gamma secretase inhibitor
BMS-906024	Notch/other inhibitor
Verteporfin	Hippo inhibitor
SP600125	Jnk inhibitor
vorinostat	HDAC inhibitor; general
CC-401	Jnk inhibitor
VX-970	Rep. stress/ATR inhibitor
losmapimod	p38 MAPK inhibitor
PRI-724	Notch/Wnt/Hedgehog inhibitor
dactolisib	Ras/PI3K inhibitor
afuresertib	Ras/AKT inhibitor
BYL719	Ras/PI3K-P110a inhibitor
Pazopanib	PDGFR and VEGFR; Also c-KIT, FGFR, inhibitor
Nilotinib	Multikinase inhibitor
lenalidomide	immunomodulator
AR A014418	GSK3 inhibitor
BMS-345541	IkkB inhibitor
IPI-145	Ras/PI3K-P110g,d inhibitor
staurosporine	Kinase inhibitor; general
PS-1145	Ikk inhibitor



Our Genes



Epigenetic Writers/Erasers/Readers						Autism Spectrum Disorder Genes			
CHD8	HDAC2	KDM2A	KMT2A	SIRT6	BRD9	SHANK3	CTNNB1	ASH1L	PRMT3
DNMT1	HDAC3	KDM2B	KMT2D	SIRT7	CBX1	GRIN2B	DYRK1a	ASH2L	PRMT5
DNMT3A	HDAC4	KDM3A	KMT2C	SUV39H1	CBX2	GRM5	ADNP	CHD1	PRMT6
DNMT3AP1	HDAC5	KDM3B	KMT2E	SUV39H2	CBX3	NLGN3	UBE3a	CHD1L	PRMT7
DNMT3B	HDAC6	KDM4A	SETD1A	SUV420H1	CBX4	NLGN4X	UBE3b	CHD2	PRMT8
DNMT3L	HDAC7	KDM4B	SETD1B	SUV420H2	CBX5	TSC1	CTCF	CHD3	
DOT1L	HDAC8	KDM4C	SETD2	WHSC1	CBX6	TSC2	EPC2	CHD4	
EHMT1	KAT2A	KDM4D	SETD7	WHSC1L1	CBX7	PTEN	TLK2	CHD5	
EHMT2	KAT2B	KDM4E	SETD8	NELFA	CBX8	NRXN1	EHMT1	CHD6	
EZH1	KAT5	KDM5A	SETDB1	ASXL1	EED	CACNA1C	ARID1B	CHD7	
EZH2	KAT6A	KDM5B	SETDB2	BRD1	EP300	CNTN4	CREBBP	CARM1	
HAT1	KAT6B	KDM5C	SIRT1	BRD2	NSD1	CNTNAP2	SYNGAP1	CHD9	
HDAC1	KAT7	KDM5D	SIRT2	BRD3	SMYD1	FMR1	SEMA5A	PRDM2	
HDAC10	KAT8	KDM6A	SIRT3	BRD4	SMYD2	MECP2	GRIP1	PRMT1	
HDAC11	KDM1A	KDM6B	SIRT4	BRD7	SMYD3	SCN1A	PTCHD1	PRMT9	
SUZ12	KDM1B	KDM8	SIRT5	BRD8	CLOCK	SCN2a	EIF4G1	PRMT2	

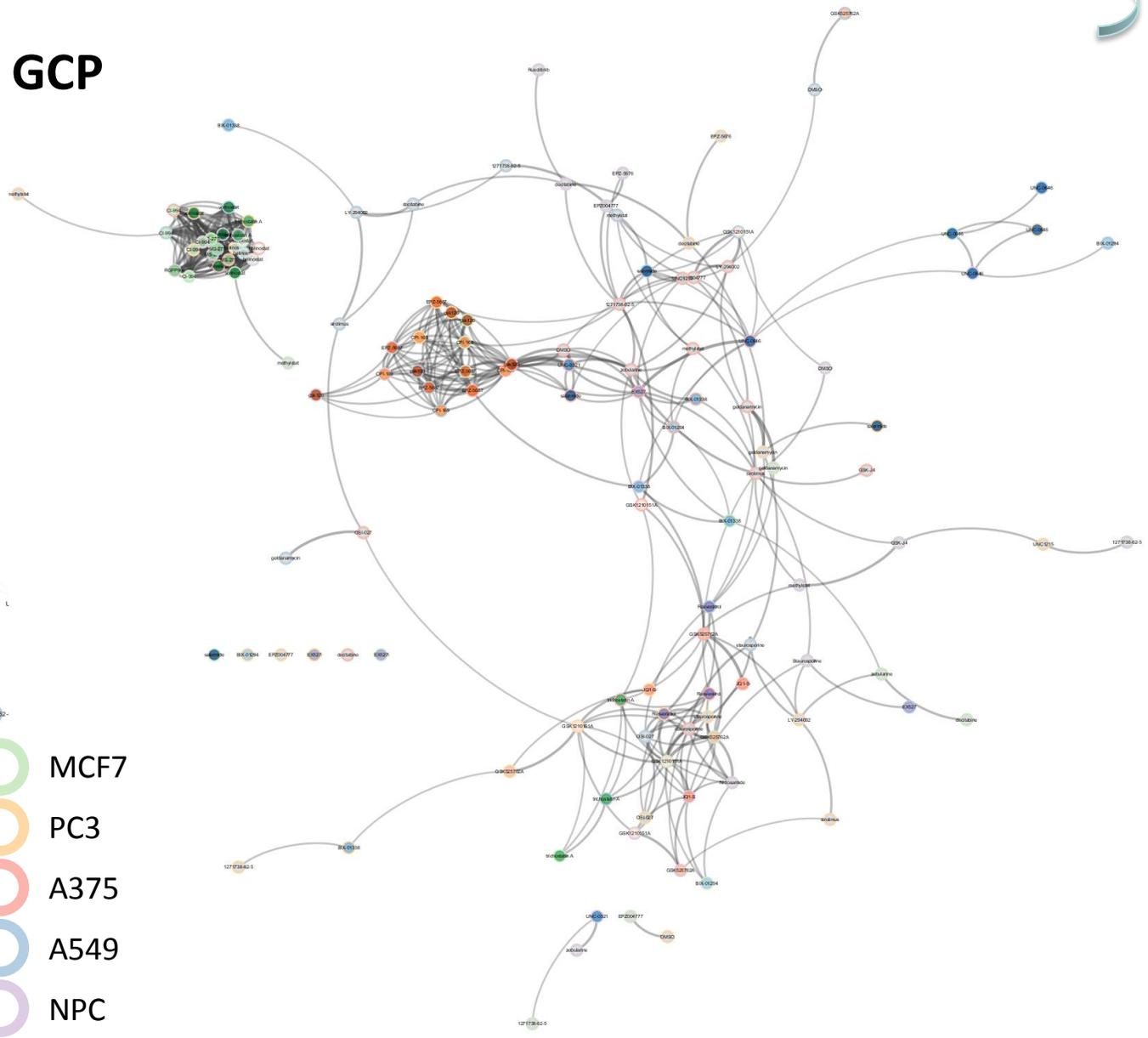
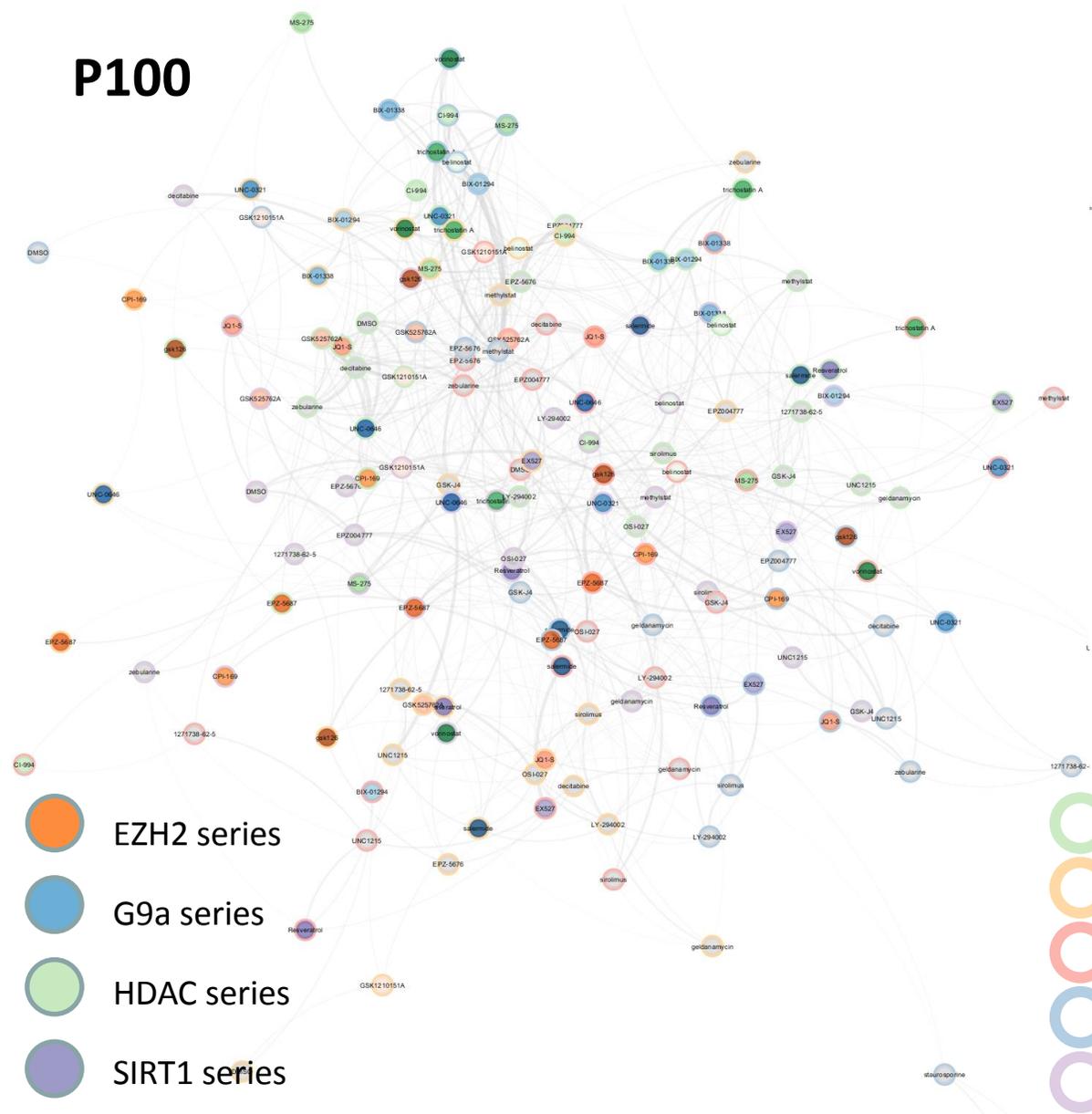
- To be disrupted in all core cell types by CRISPr/Cas9 (3 sgRNA's/gene)

Proteomic Connectivity Maps – Epigenetically Active Drugs



P100

GCP



-  EZH2 series
-  G9a series
-  HDAC series
-  SIRT1 series

-  MCF7
-  PC3
-  A375
-  A549
-  NPC