Managing and Evaluating an HCS siRNA Screen of the p53 Pathway with AcuityXpress Software

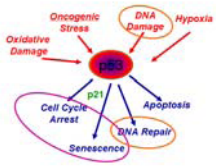
Laura Corson[1], Pierre Turpin[2], Thomas Hunsaker[1], Jeff Eastham-Anderson[1], James Lee[1], Emmy Verschuren[1], Rami Hannoush[1], David Davis[1], Yun Li[1], Grischa Chandy[2], Jignesh Joshi[1], James Fitzgerald[1], & Peter Jackson[1]
[1]Genentech Inc., [2]MDS Analytical Technologies

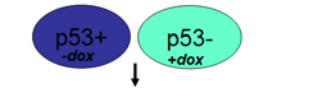
Abstract

An advantage of conducting High Content Screening (HCS) is the vast amount of biologically contextual data generated. However, in practice, the logistics of implementing image acquisition, image quantification, data management, and multi-parametric analysis can be complex and cumbersome. Here, we present a study demonstrating the power of using commercially-available software from MDS Analytical Technologies to facilitate the management and evaluation of an HCS siRNA screen, designed to yield insight into genes of the druggable genome that impinge upon the p53 tumor suppressor pathway. RPE cells +/- p53 were reverse transfected with 7657 siRNA pools and stained for p53, p21, Ki67, and pH2AX under basal conditions and upon treatment with a DNA damaging agent. Over 1.3 million images (~1 terabyte) were acquired and analyzed with the MetaXpress software. The AcuityXpress software was then used for a number of key downstream processes including (1) annotation, (2) data quality review, (3) data normalization, (4) image review, and (5) multi-parametric profiling. Data plotting, data analysis, data visualization, and group selection tools streamlined the identification of siRNAs with phenotypic effects of interest. Links between the AcuityXpress software and web-based databases further facilitated review and prioritization of screening hits. The HCA management and analysis software, AcuityXpress, makes full scale, multi-parametric siRNA screens of this complexity feasible in a small lab setting, and thus permits investigators to take more comprehensive, multipronged approaches to probe deep into biological pathways of interest.

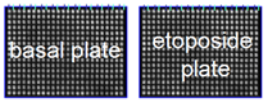
Screening Strategy

➢ One screen of RPE cells +/- p53 knockdown configured to yield significant insight into multiple components of the p53 pathway





Reverse Transfect Dharmacon siRNA Pools



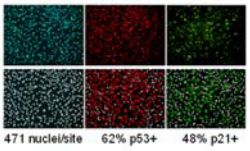
No etoposide treatment

3d

5hr treatment with low level of etoposide just prior to fixation

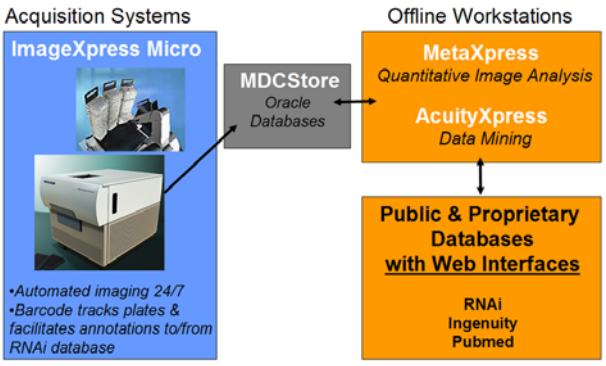
Stain for DNA, p53, p21, Ki67, H2AX

Image, Quantitate, & Analyze



- Total Nuclei Count
- % Positive for p53
- Average Intensity of p53
- % Positive for p21
- Average Intensity of p21
- % H2AX (not shown) – DNA Damage
- % Ki67 (not shown) – Cell Cycle Exit
- %2N or Less, %S, %4N or Greater

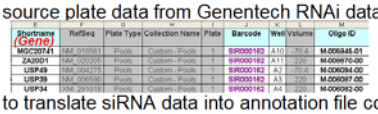
➢ 1.3 million images acquired (212 384-well plates, 4 sites/well) & analyzed with MDS COMPLETE SOLUTION FOR HCS



Benefits of Using AcuityXpress 2.0 for siRNA Screen

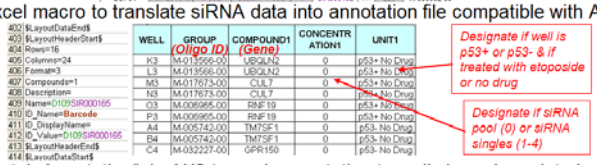
STREAMLINE DATA ANNOTATION

➢ Download siRNA source plate data from Genentech RNAi database as Excel file



Shortname (Gene)	RefSeq	Plate Type	Collection Name	Plate	Barcode	Well	Volume	Oligo ID
MDC20741	NM_018561	Pools	Custom - Pools	1	SIR000162	A10	70.4	M-006946-01
ZA20D1	NM_020205	Pools	Custom - Pools	1	SIR000162	A11	220	M-009670-00
USP49	NM_004275	Pools	Custom - Pools	1	SIR000162	A2	70.4	M-006094-00
USP39	NM_006590	Pools	Custom - Pools	1	SIR000162	A3	220	M-006097-00
USP34	NM_014709	Pools	Custom - Pools	1	SIR000162	A4	220	M-006082-00

➢ Use Excel macro to translate siRNA data into annotation file compatible with AX2



	WELL	GROUP (Oligo ID)	COMPOUND1 (Gene)	CONCENTRATION1	UNIT1
402	$LayoutDataEnd$				
403	$LayoutHeaderStart$				
404	Rows=16				
405	Columns=24	K3	M-013566-00	UBQLN2	0 p53+ No Drug
406	Format=3	L3	M-013566-00	UBQLN2	0 p53+ No Drug
407	Compounds=1	M3	M-017673-00	CUL7	0 p53+ No Drug
408	Description=	N3	M-017673-00	CUL7	0 p53+ No Drug
409	Name=D109SIR000165	O3	M-006965-00	RNF19	0 p53+ No Drug
410	ID_Name=Barcode	P3	M-006965-00	RNF19	0 p53+ No Drug
411	ID_DisplayName=	A4	M-005742-00	TM7SF1	0 p53- No Drug
412	ID_Value=D109SIR000165	B4	M-005742-00	TM7SF1	0 p53- No Drug
413	$LayoutHeaderEnd$	C4	M-032227-00	GPR150	0 p53- No Drug
414	$LayoutDataStart$				

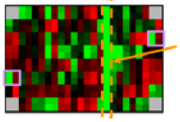
Designate if well is p53+ or p53- & if treated with etoposide or no drug

Designate if siRNA pool (0) or siRNA singles (1-4)

➢ Use "Batch Annotation" in AX2 to apply annotation to wells based on plate barcode

QC & NORMALIZE DATA

➢ Use heatmaps to identify/exclude problematic wells



Liquid handling missed antibody addition in this column

➢ Normalize to designated controls on every plate

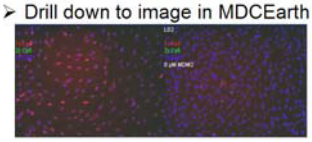
➢ Center & scale datasets (z scores)

FACILITATE DATA & IMAGE REVIEW

➢ Review phenotypic characteristics of interest

Compound [Well Annotation], Group [Well Annotation]	'p53+ No Drug' - [Total Cell...	'p53- No Drug' - [Total Cell...	'p53+ No Drug' - [p53 Avg Inten...	'p53- No Drug' - [p53 Avg Inten...	'p53+ No Drug' - [p21 Avg Inten...	'p53- No Drug' - [p21 Avg Inten...
MDM2 otp, [TEST]	11.253	67.236	108.210	18.550	105.699	27.579
TP53, [L-003329-00]	150.621	109.242	7.597	1.533	5.743	5.345
TP53, [M-003329-01]	125.865	109.166	-4.838	-4.362	-4.455	-0.674
USP7, [TEST]	47.473	82.216	120.118	13.768	83.946	2.335
p21, []	114.293	106.055	47.389	1.684	2.999	1.084
siCONTROL, []	100.956	112.406	28.350	1.613	30.289	6.629

➢ Drill down to image in MDCEarth

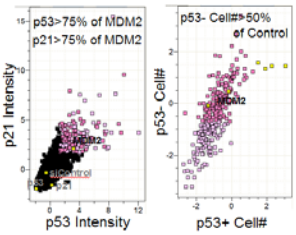


STREAMLINE MULTI-PARAMETRIC ANALYSES

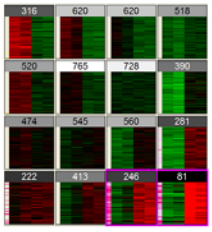
➢ Use variety of analytical methods to identify siRNAs yielding phenotype of interest

Example: MDM2-Like → High basal levels of p53 & p21, normal p53- cell#, reduced p53+ cell#

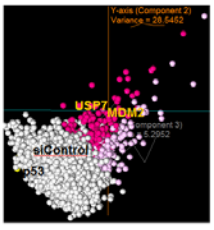
GRAPHS & QUICKLISTS



SOM

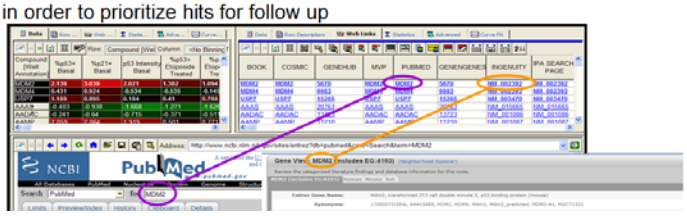


PCA



REVIEW BIOLOGICAL CONTEXT

➢ Hyperlink to external databases for published information on genes of interest in order to prioritize hits for follow up



End Product: A searchable database of genes of the druggable genome that impinge upon the p53 pathway

➢ Query dataset for genes with phenotype of interest

Examples: Which genes are required for inducing a p53-p21 response?
(% p53+, %p21+ in etoposide plate)

Which genes mediate a p53-dependent effect on proliferation/survival?
(Nuclei count p53- vs p53+ wells)

Which genes influence basal p53 levels?
(% p53+ in basal plate)

➢ Query YFG to understand potential interplay with p53 pathway

Genentech

Molecular Devices