Generation of a Standard Curve to Convert CTG Values to Cell Number for PC9 Cells

- Protocol
  - Seed PC9 cells at different cell numbers per well in triplicate in 90ul RPMI+10%FBS growth medium
  - Incubate at 37C, 5% CO2
  - 24h after seeding, image wells by IncuCyte, then read wells by CTG
- Analysis
  - For all CTG values from wells containing cells, subtract CTG value from empty well (raw data minus background)
  - Calculate average value and standard deviation for each triplicate
  - Plot cell number seeded (X axis) vs. CTG reading (Y axis)

Plate Map – 1000 to 64000 cells/well by 2X increments, done in triplicate

	1000	**2000**	4000	8000	16000	32000	64000	cells /well				
A												
B												
C												
D												
E												
F												
G												
H												

Day 1 IncuCyte Image

Cell \ well

1000

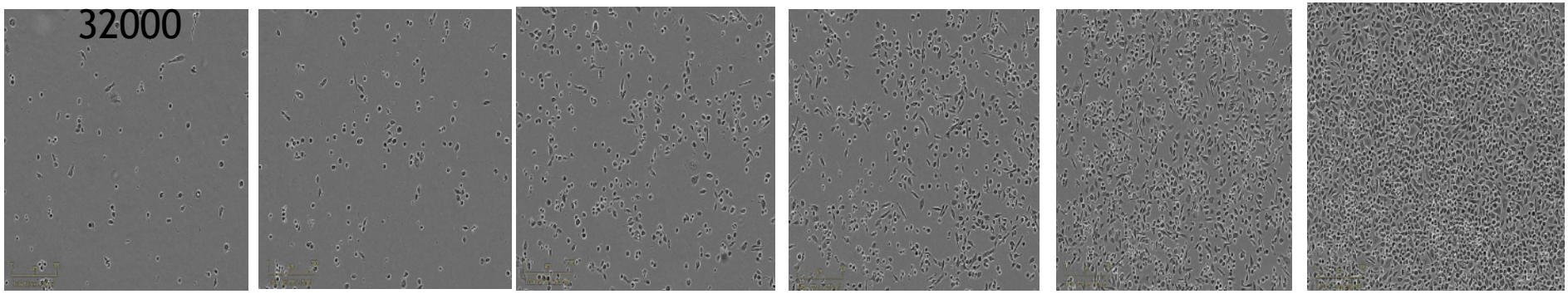
2000

4000

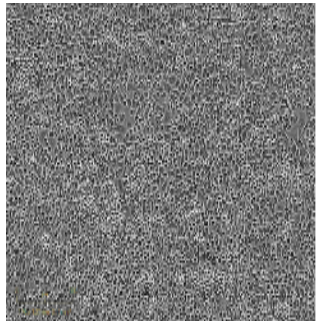
8000

16000

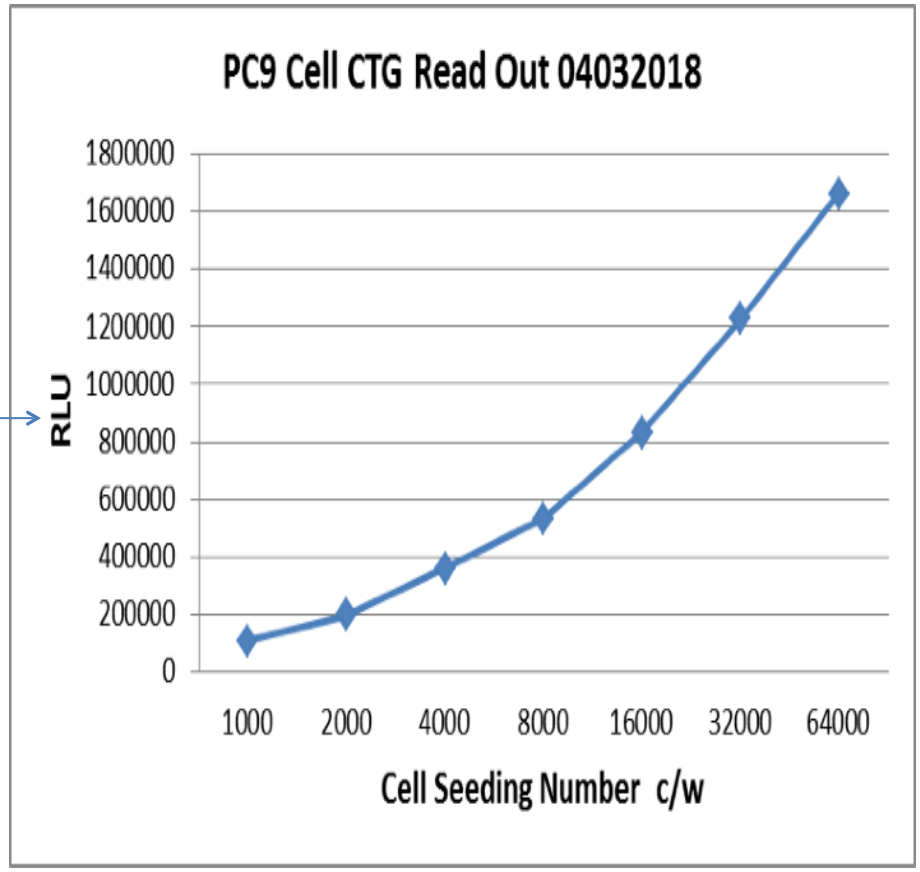
32000



64000

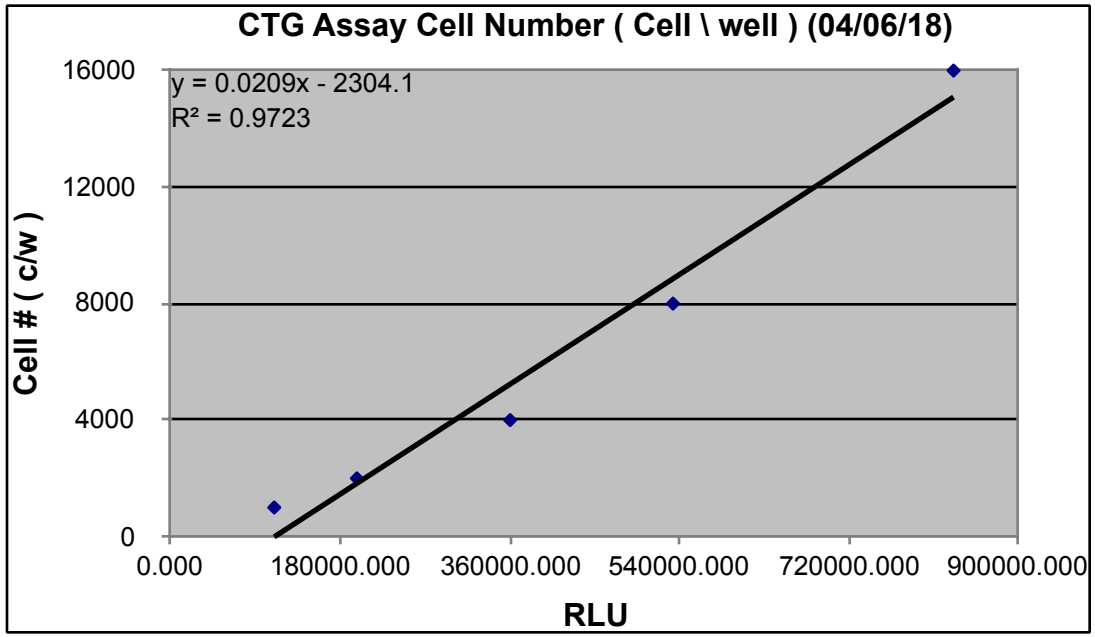


RLU =
- CTG reading from wells with cells
- Subtract empty well CTG value
- Average of triplicates



- Use standard curve to convert CTG values to cell numbers from all subsequent drug treatment experiments (formula at CTG cell# on excel)
- Use 2000 cells as the starting number of cells at the initiation of drug treatment (day 0 baseline)

Formula at CTG cell# on excel



Single Agent and Combo Effects of Dacomitinib and Osimertinib on PC9 Cell Viability in 3 Day Assays

- Protocol
  - Seed PC9 cells at 2000 cells per well in 80 or 90ul RPMI+10%FBS growth medium
  - Incubate at 37C, 5% CO2
  - 24h after seeding (day 0 baseline), add single agent or combo drug treatment as indicated in plate map, with duplicate wells for each condition
  - After 3 days of drug treatment (day 3), read wells by CTG
- Bar Graph Analysis
  - For all CTG values from wells containing cells, subtract CTG value from empty well (raw data minus background)
  - Convert all CTG values to cell numbers using standard curve (formula at CTG cell# on excel)
  - Bar plot of cell numbers per selected drug treatment conditions in duplicate
  - Use 2000 cells seeded CTG read at day 0 converted through formula to cell number as untreated starting cell number (day 0)
- Concentration – Response Curve Analysis
  - All data is from CTG day 3 read, plates 3 and 4, drug treatment and control DMSO vehicle
  - For all CTG values, subtract empty well background
  - % control = 100% X (treatment CTG read / DMSO CTG read)
  - Concentration-response curve plotted log drug concentration (X-axis) versus % control (Y-axis) using GraphPad PRISM

Plate map

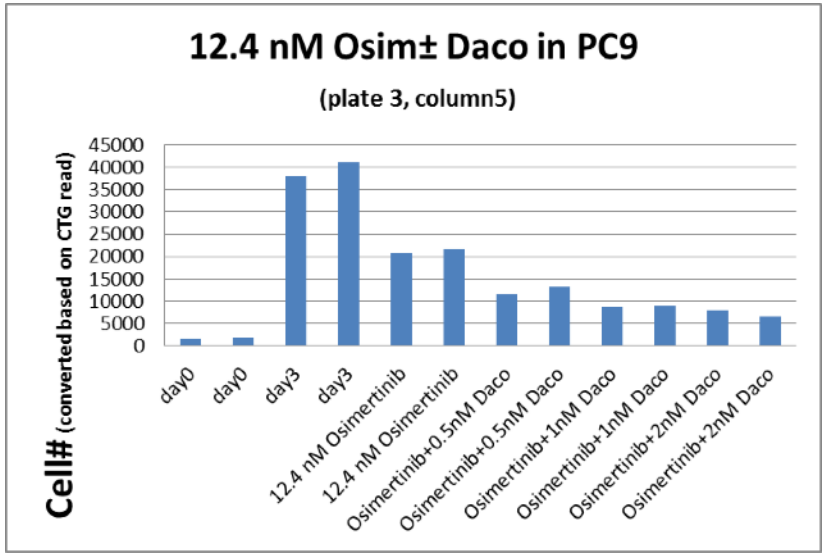
Plate 3: seed 2K cells/well; start drug treatment next day; CTG read after 3 days drug treatment

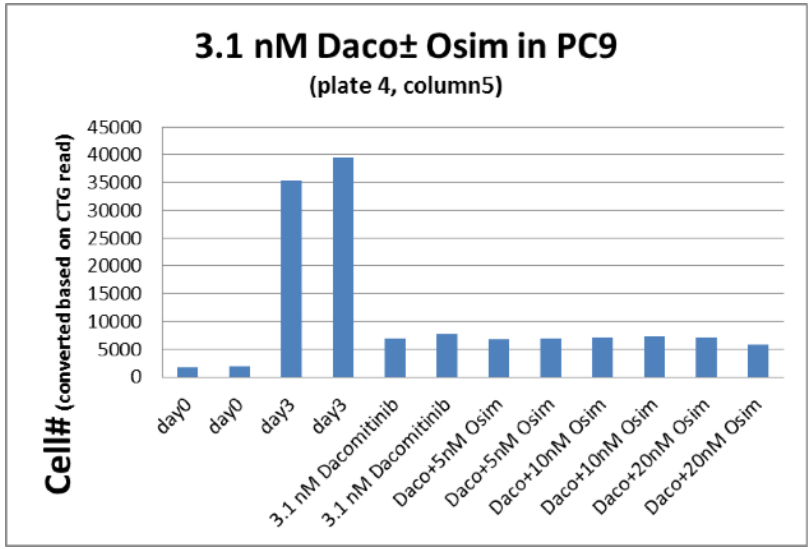
Osi nM	**1000**	333.3	111.1	37.04	12.35	4.115	1.372	0.457	0.152	0.051	0.017	DMSO	
A													no Daco
B													no Daco
C													with 0.5 nM Daco
D													with 0.5 nM Daco
E													with 1 nM Daco
F													with 1 nM Daco
G													with 2 nM Daco
H													with 2 nM Daco

Plate 4: seed 2K cells/well; start drug treatment next day; CTG read after 3 days drug treatment

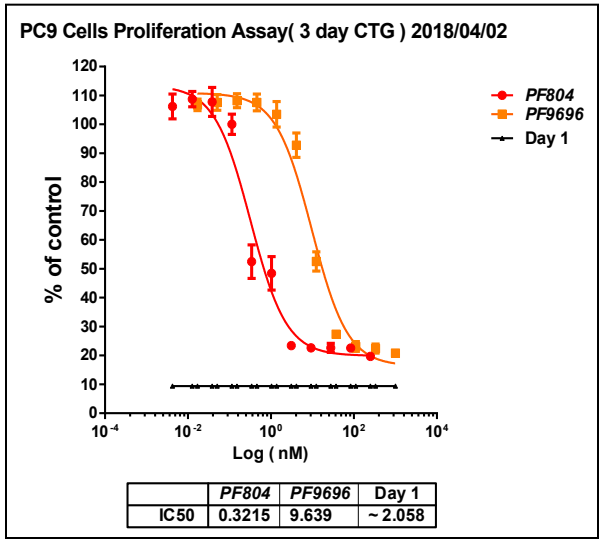
Daco nM	**250**	83.33	27.78	9.259	3.086	1.029	0.343	0.114	0.038	0.013	0.004	DMSO	
A													no Osi
B													no Osi
C													with 2.5 nM Osi
D													with 2.5 nM Osi
E													with 5 nM Osi
F													with 5 nM Osi
G													with 10 nM Osi
H													with 10 nM Osi

Bar Graph Plots

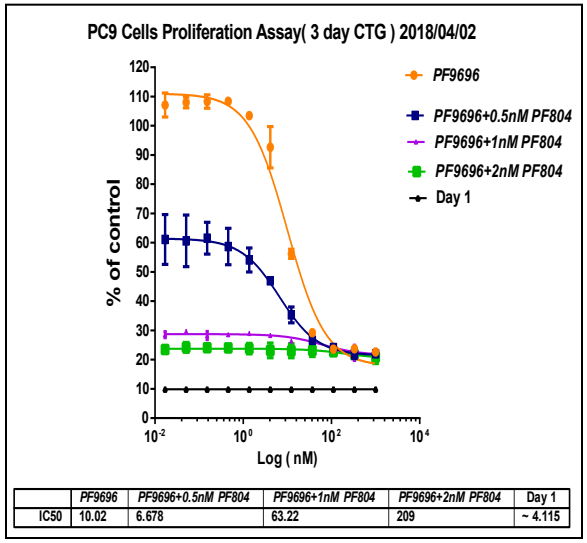




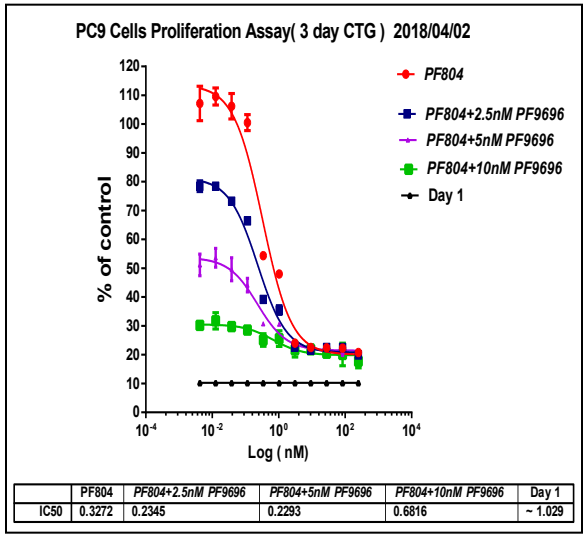
Concentration-Response Curves



	PF804	PF9696	Day 1
IC50	0.3215	9.639	~ 2.058



	PF9696	PF9696+0.5nM PF804	PF9696+1nM PF804	PF9696+2nM PF804	Day 1
IC50	10.02	6.678	63.22	209	~ 4.115



	PF804	PF804+2.5nM PF9696	PF804+5nM PF9696	PF804+10nM PF9696	Day 1
IC50	0.3272	0.2345	0.2293	0.6816	~ 1.029

PF804=Dacomitinib
PF9696=Osimertinib