Supplemental Figures for Thomas-Fowlkes et al.

Cell-Based *In Vitro* Assay Automation: Balancing Technology & Data Reproducibility/Predictability

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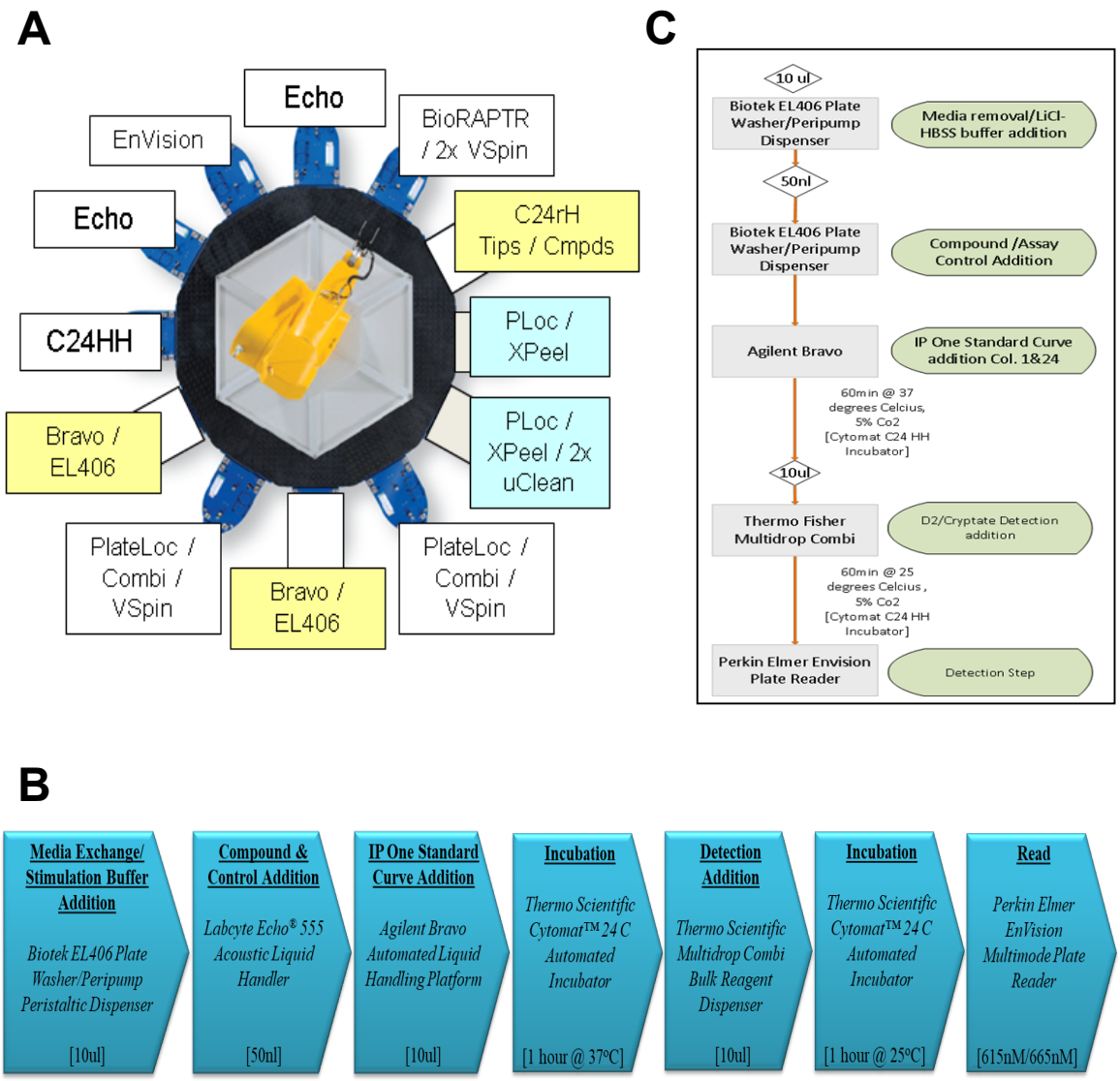
Supplemental Figure SF1: Fully Integrated Robotic Assay on a HighRes Biosolutions Microstar™ System

A) HighRes Biosolutions Microstar™ system layout equipped with a Staubli robotic arm operated with the Cellario scheduling software which offers dynamic scheduling with full error recovery and multiple protocol and plate stream execution. B) HighRes Biosolutions Microstar™ system components to support the assay workflow. C) IP-One assay workflow.



Supplemental Table ST1: Liquid Handling Parameters for the HighRes Biosolutions Microstar™ Robotic Platform Automation Protocol for GPCR IP-One assay workflow

		Step 1: Pre-Dispense of Wash Buffer	Step 2: Initial Dispense	Step 3: Media Removal	Step 4: Plate Washing	Step 5: Pre-Dispense Assay Buffer	Step 6: Dispense Assay Buffer
	# Cycles				1	2	
Dispense Parameters	Buffer	A	A		A		
	Volume	50 ul /tube	50 ul/well		50 ul/well	10 ul /tube	10 ul/well
	Flow Rate	4	2		2	Medium	Medium
	Offset		**Z**: 120 steps (15.24 mm above carrier) **X**: -7 steps (0.32 mm left of center) **Y**: -4 steps (0.30 mm back of center)	**Z**: 48 steps (6.1 mm above carrier) **X**: 15 steps (0.69 mm right of center) **Y**: 10 steps (0.74 mm front of center)	**Z**: 120 steps (15.24 mm above carrier) **X**: 0 steps (center of well) **Y**: 0 steps (center of well)		**Z**: 338 steps (15.45 mm above carrier) **X**: 0 steps (center of well) **Y**: 0 steps (center of well)
	Delay Vacuum		Delay start until volume dispensed: 25 ul/well		Delay start until volume dispensed: 50 ul/well		
Aspiration Parameters	Travel Rate			6 CW, 14.7 mm/sec	6 CW, 14.7 mm/sec		
	Delay			0 msec	0 msec		
	Offset				**Z**: 30 steps (3.81 mm above carrier) **X**: 15 steps (0.69 mm right of center) **Y**: 10 steps (0.74 mm front of center)		
	Secondary Offset				**Z**: 29 steps (3.68 mm above carrier) **X**: -15 steps (0.69 mm left of center) **Y**: -10 steps (0.74 mm back of center)		

Supplemental Table ST2: Optional Methods For Removing Growth Medium from Assay Plates

The most commonly used methods for removing buffer from microplates were evaluated in an effort to simplify and standardize the protocol to be user independent.

	Manual Removal (Dump-Pat)	BioTek Plate Washer / Dispenser	BlueWasher Centrifugal Washer	Suspension Cell
Use	• Used for adherent cell based assays only • Invert and quickly shake plate to remove buffer and blot dry • Commonly used in low and medium throughput processes	• Used for adherent cell based assays only • Automated process to aspirate from and dispense into assay plates • Can be used in low, medium or high throughput processes	• Used for adherent cell based assays only • Uses centrifugation to remove buffer from assay plates • Can be used in low, medium or high throughput processes	• Eliminate the overnight cell adherent process • Can be used in low, medium or high throughput processes • Aligns with other HTRF assay protocols
Pros	• No specialized instrumentation required	• Standardize wash and dispense protocols across users • Variety of programming options which can be used with or without a dispensing protocol • Can be fully integrated into uHTS processes	• Standardize wash and dispense protocols across users • No contact with cell monolayer • Pre-programmed options which can be used with or without a dispensing protocol • No residual buffer remains in the wells • Can be fully integrated into uHTS processes	• No specialized instrumentation required • Assay can be set up and run on the same day • Complete removal of growth media during wash step
Cons	• Highly inconsistent results across operators, or by the same operator within a day or across days • Not automation friendly	• Specialized instrumentation required • Complicated programming • Risk of disturbing cell monolayer and losing cells • Known residual volume remaining in the wells • Highly inconsistent between cell types • Not routinely used in low or medium throughput processes	• Specialized instrumentation required	• Highly inconsistent results across different cell types • Increased variability and reduction in overall assay window compare to adherent cell assays