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Biomek 96 well plating

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William J Buchser[1], Jason Waligorski[1]

[1]Washington University in St. Louis

WashU FIVE @ MGI

J Jace Park

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Protocol status: Working
We use this protocol and it's working

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Abstract

Randomized plating of cells using Biomek.

Troubleshooting

Creating Plate Layouts in FIVTools

1 Open FIVTools and click "Layouts."

2 Edit "Exp Name" to the correct FIV Number (make sure to keep "FIV" in the name)
1. Click once on the “Export Folder” bar so it updates the location

3 Choose the desired number of 96-well plates by typing 1, 2, 3, or 4 in "Count."
1. Select “96 Mote” to exclude plating from the outermost wells
2. Select “384 Full” for a 384 well plate
3. If the layout is not standard, adjust the “First Row”, “First Column”, “Last Row”, and “Last Column” boxes appropriately
4. If doing an antibody plate, check the “Ab Plate” box and:
   - Make “Count” = 0 for one antibody plate
   - Make “Count” = 2 for two 96-well plates and an antibody plate

4 Change “Cells per Well” to desired plating density.

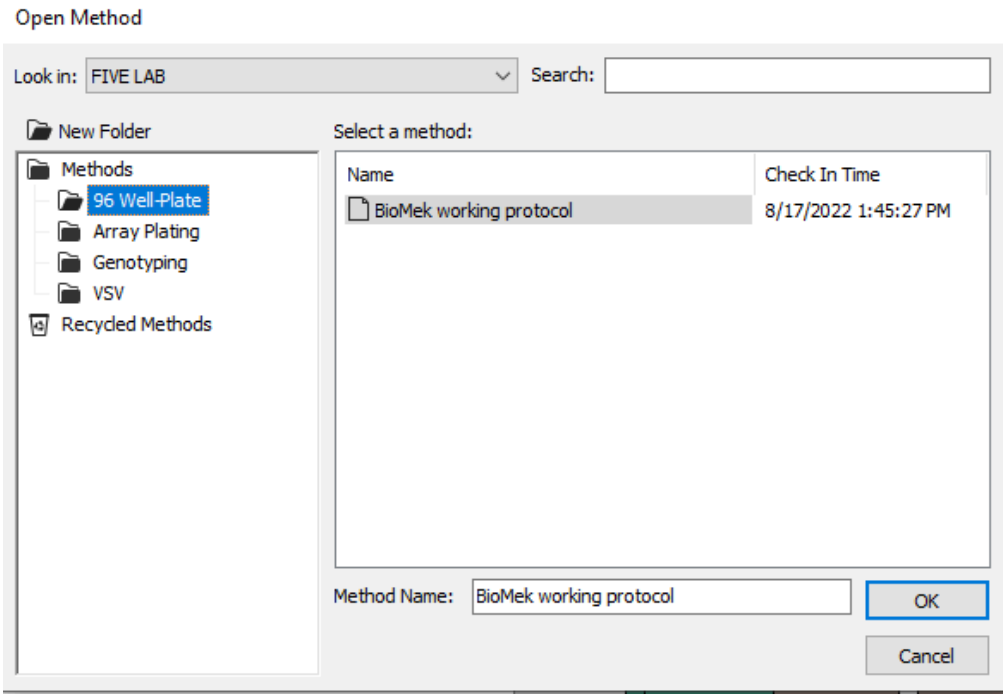
5 Copy experimental condition names (often genotypes, dosages, G1/G2, etc.) and click “PASTE Names” to auto-fill the 12-well source plate.
1. “Name” can also be manually filled
2. The “Well” column can only have row/column combinations of a 12-well plate (A-C, 1-4)
3. “Relative Representation” allows you to assign different weights for representation in the 96-well plates
4. Use the underscore symbol to have a cell type only show up in the antibody plate layout (for example: “_cell type”)

6 Click "Fill Plates"
1. Try to make sure the empty wells are not on any edges and if doing multiple plates that they cannot be flipped or rotated to the same location as another plate.

7 Go into the “4 Mapping” folder of the respective experiment and make sure there is a PlateMap, PlatingSetup, and script.

Setting up Biomek

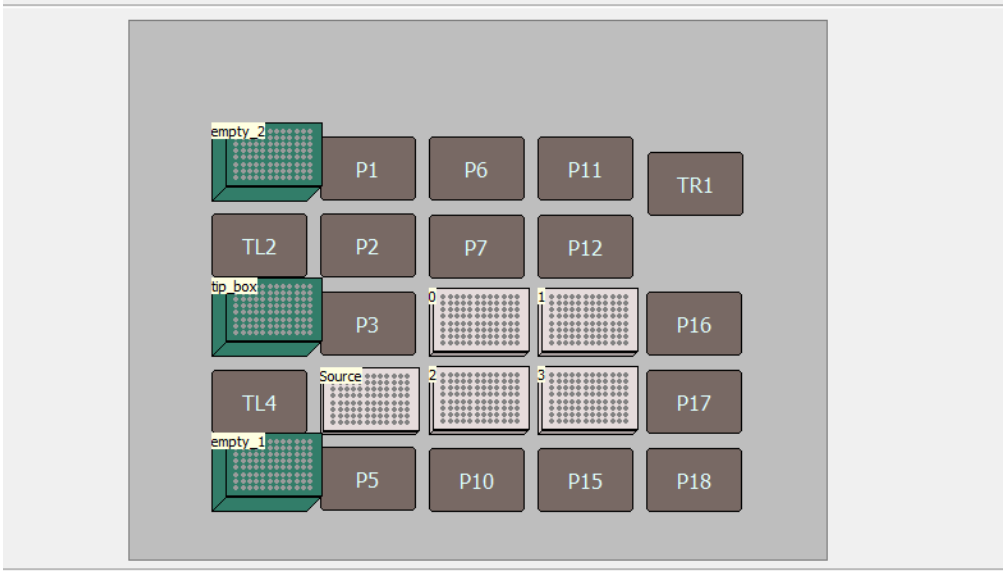
8 Find the correct method.



8.1 The algorithm will look like this:


Start
Instrument Setup
Set endCol to =0
Set endRow to =0
Set breaker to =False
Define Transfer
Begin Using Select Tips on Pod1
Set numDispense to =-1
For loopv = 1 to 9999 step 1
Scripted Let
If TipUnload = True
Then
Advanced Unload Tips
End
Else
End
If TipLoad = True
Then
Advanced Load Tips
End
Else
End
If breakOut = True
Then
Break
End
Else
If numDispense < 1 or TipLoad = True or TipUnload = True
Then
Set numDispense
Mix At Source With Select Tips
Aspirate From Source With Select Tips
End
Else
End
End
Dispense To =Plate With Select Tips
Set numDispense to =numDispense-1
Set endRow to =tipEndRow
Set endCol to =tipEndCol
End Scripted Let
End Loop
Advanced Unload Tips
End Using Select Tips
End Procedure
Run Transfer
Finish

9 Set up the deck in the configuration shown



9.1 Make sure the two tip rows closest to the front are full in the “tip_box” and the “empty_1” and “empty_2” are completely empty.

9.2 Only use the number of 96-well plates needed for your experiment and place them in order starting with “0.”

- Keep the lids on the plate until you are ready to begin the Biomek program.

10 Open the appropriate experiment folder, go to “4 Mapping” and open the “FIVXXX_script” file.

11 Copy the entire script.

12 Go to the “Scripted Let” step in the algorithm and paste your entire script.

- Scroll up to the top to make sure the correct experiment number and an appropriate time stamp are there

13 Go back to the “4 Mapping” folder and drag and drop the “FIVXXX_PlatingSetup” file onto and **EXCEL** sheet (DO NOT OPEN AS TEXT FILE).

14 After creating cell suspensions for each source condition and obtaining counts, input their counts into the appropriate positions of the “Cells/uL” column:

Date:	########		uL/Well:	100				
Exp:	FIV726		FF:	1.8		(12 well has 6 ml max)		
Plates:	2							
I35347			Change Me			Enter Count Below		
Condition	Source We	Dest Well	Cells/Wel	Cells Tota	uL Total	Cells/uL	uL Media	uL Cells
D I2S	A1	22	4000	158400	3960	100	2376	1584
A I2S	A2	24	4000	172800	4320	100	2592	1728
G I2S	A3	22	4000	158400	3960	100	2376	1584
E I2S	A4	24	4000	172800	4320	100	2592	1728
D Veh	B1	24	4000	172800	4320	100	2592	1728
A Veh	B2	24	4000	172800	4320	100	2592	1728
G Veh	B3	24	4000	172800	4320	100	2592	1728
E Veh	B4	24	4000	172800	4320	100	2592	1728

15 With a new 12-well plate, set up the Source Plate by adding the volumes in the “uL Media” and “uL Cells” columns into the appropriate wells:

	A	B	C	D	E	F	G	H	I	J
1	Date:	8/11/2022		uL/Well:	100					
2	Exp:	FIV726		FF:	1.8		(12 well has 6 ml max)			
3	Plates:	2								
4	I35347			Change Me			Enter Count Below			
5	Condition	Source Well	Dest Well	Cells/Wel	Cells Tota	uL Total	Cells/uL	uL Media	uL Cells	
6	D I2S	A1	22	4000	158400	3960	100	2376	1584	
7	A I2S	A2	24	4000	172800	4320	100	2592	1728	
8	G I2S	A3	22	4000	158400	3960	100	2376	1584	
9	E I2S	A4	24	4000	172800	4320	100	2592	1728	
10	D Veh	B1	24	4000	172800	4320	100	2592	1728	
11	A Veh	B2	24	4000	172800	4320	100	2592	1728	
12	G Veh	B3	24	4000	172800	4320	100	2592	1728	
13	E Veh	B4	24	4000	172800	4320	100	2592	1728	
14										
15										
16										

16 Place the Source Well in the correct position on the deck,open the lids to all plates, and close the front door.

17 Press “Run” (the green arrow) and confirm the configuration in the pop-up box.