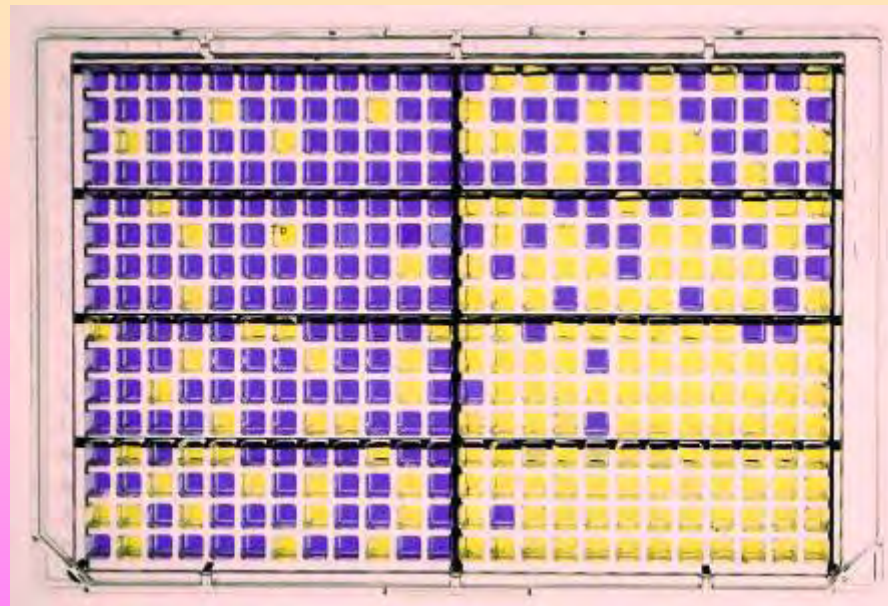




The Ames MPF procedure



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CH-4123 Allschwil
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www.aniara.com



Transport / Shipment

Ames MPF comes with semi-solid strains which are shipped at ambient temperature. Please note that the bacteria **MUST** be stored at -80°C upon arrival!

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Take a semi-solid bacterial stock vial from the freezer.



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Let stand for 5 minutes at room temperature, then add 200 μ l of Growth Medium



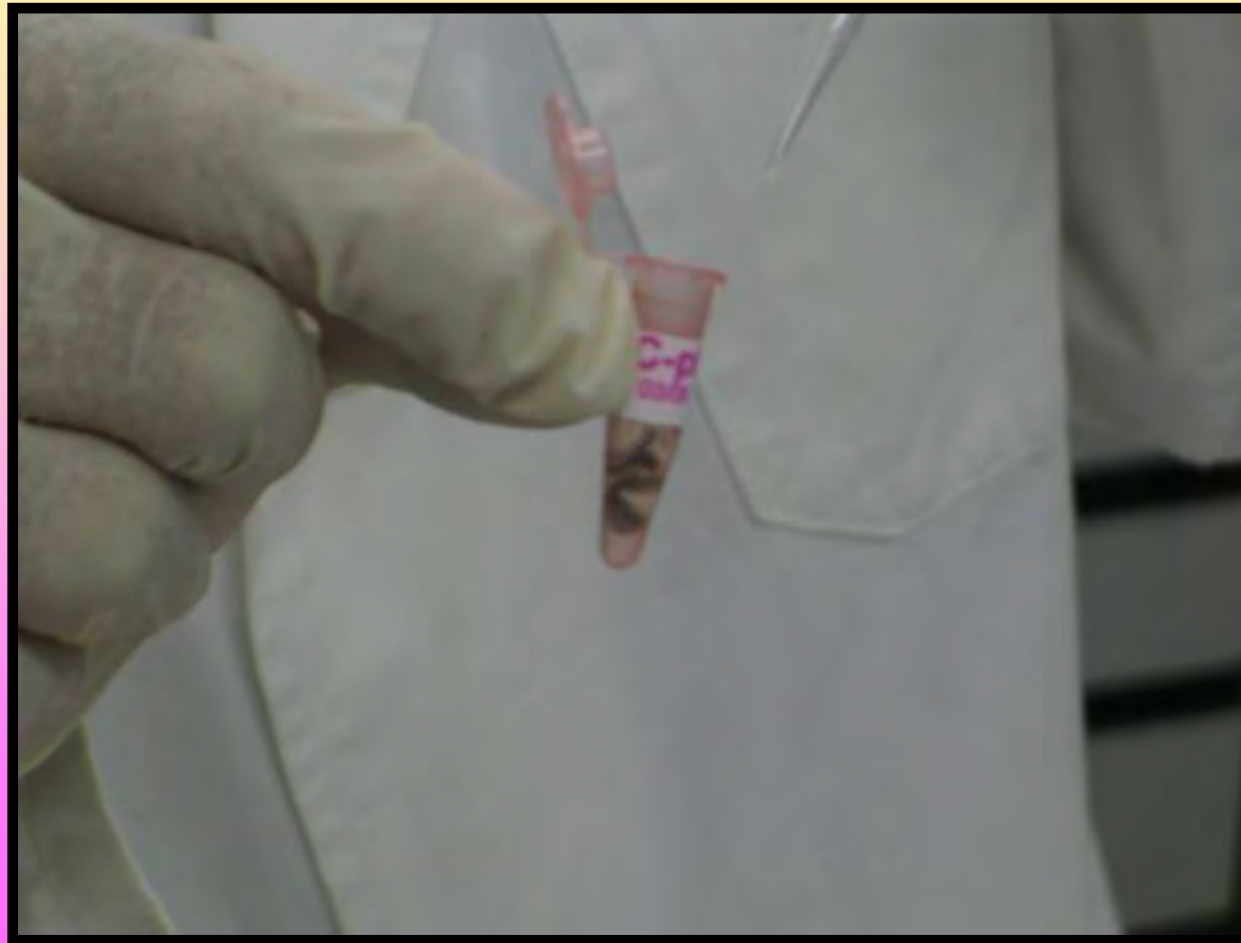
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Disrupt the pellet mechanically with the tip of the pipette...



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...until you have small enough fragments that can be further disrupted by pipetting.



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Disrupt the large clumps by pipetting up and down...



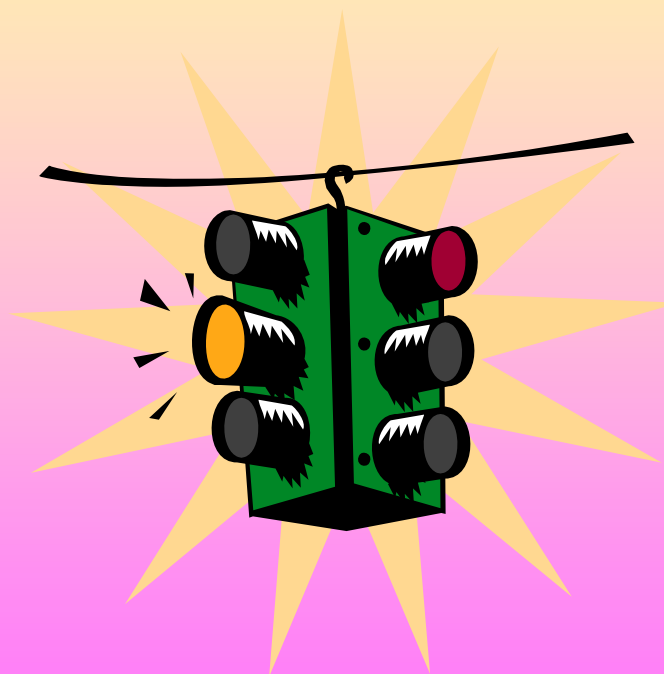
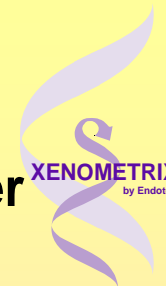
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... until the fragments are homogeneously small.



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Avoid the formation of foam and make sure the liquid never touches the orifice of the pipettor!



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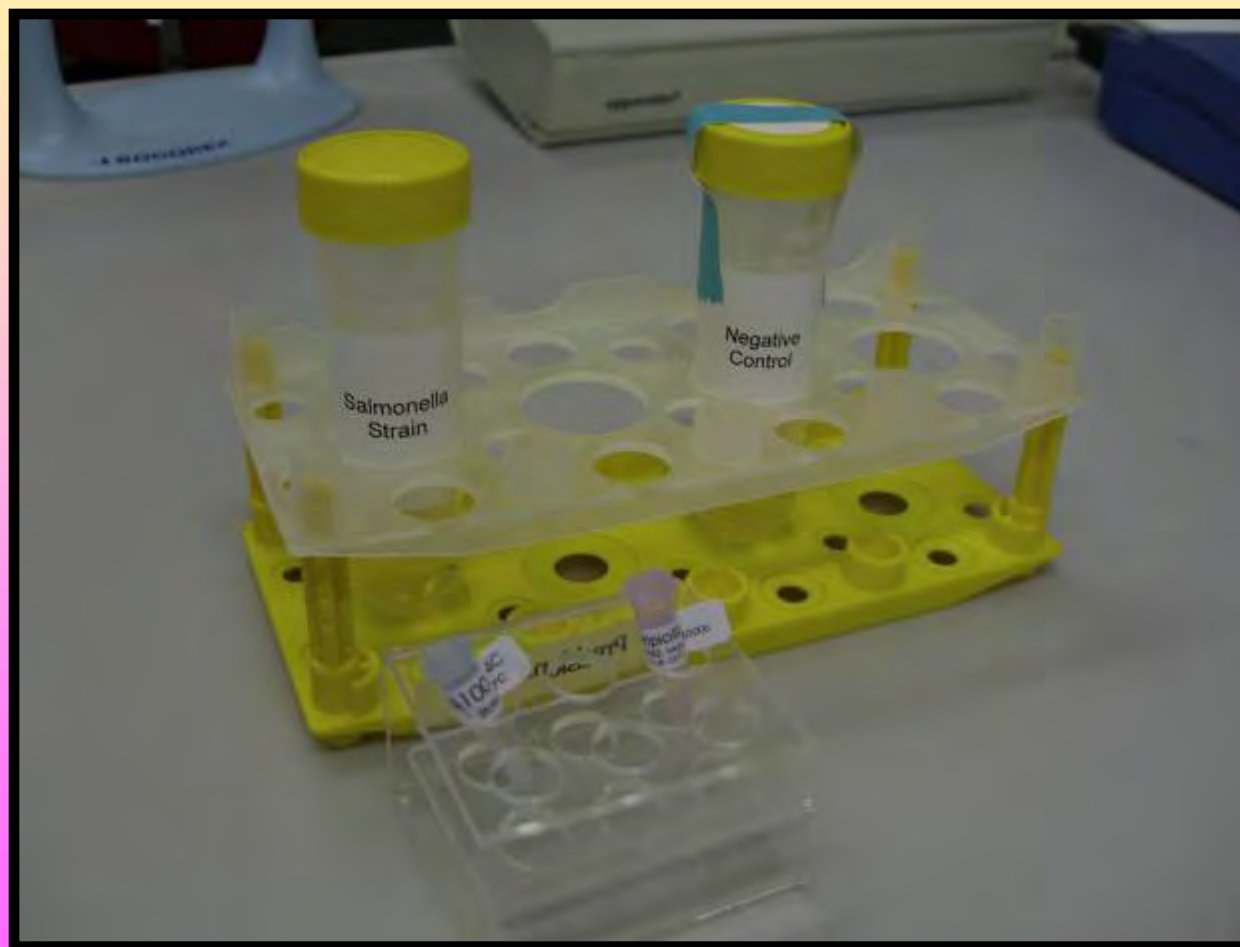


Transfer 50 μ l of the fine bacterial suspension to a 50 ml tube with Growth Medium for overnight incubation.



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Prepare the bacteria and a sterility control for overnight incubation.



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Make sure the caps are loosely fixed or are permeable for air!



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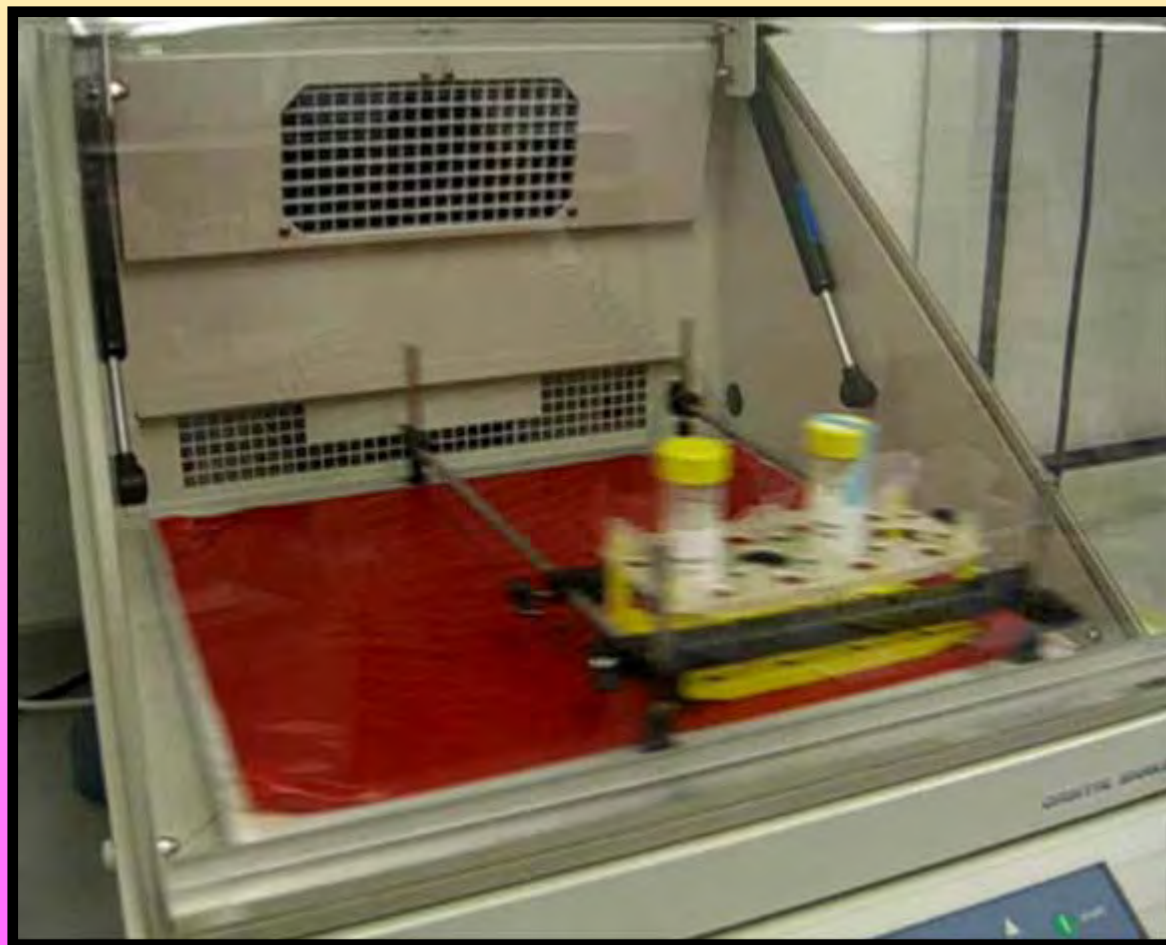
Place in a environmental shaker....



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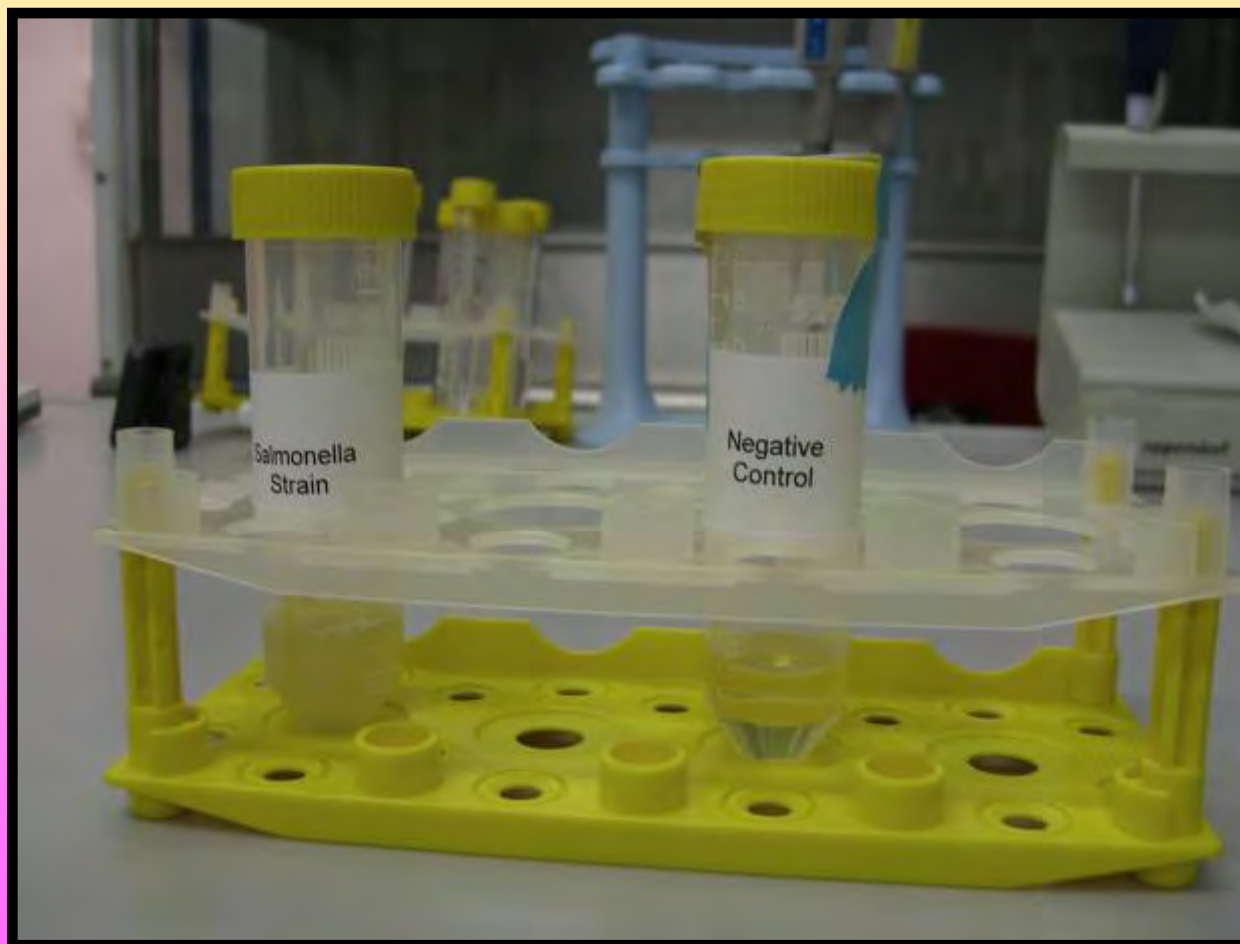


Then incubate for 12 – 16 hrs (overnight) at 37°C, 250 rpm.



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After the incubation the tube with bacteria should be turbid while the sterility control should be clear.



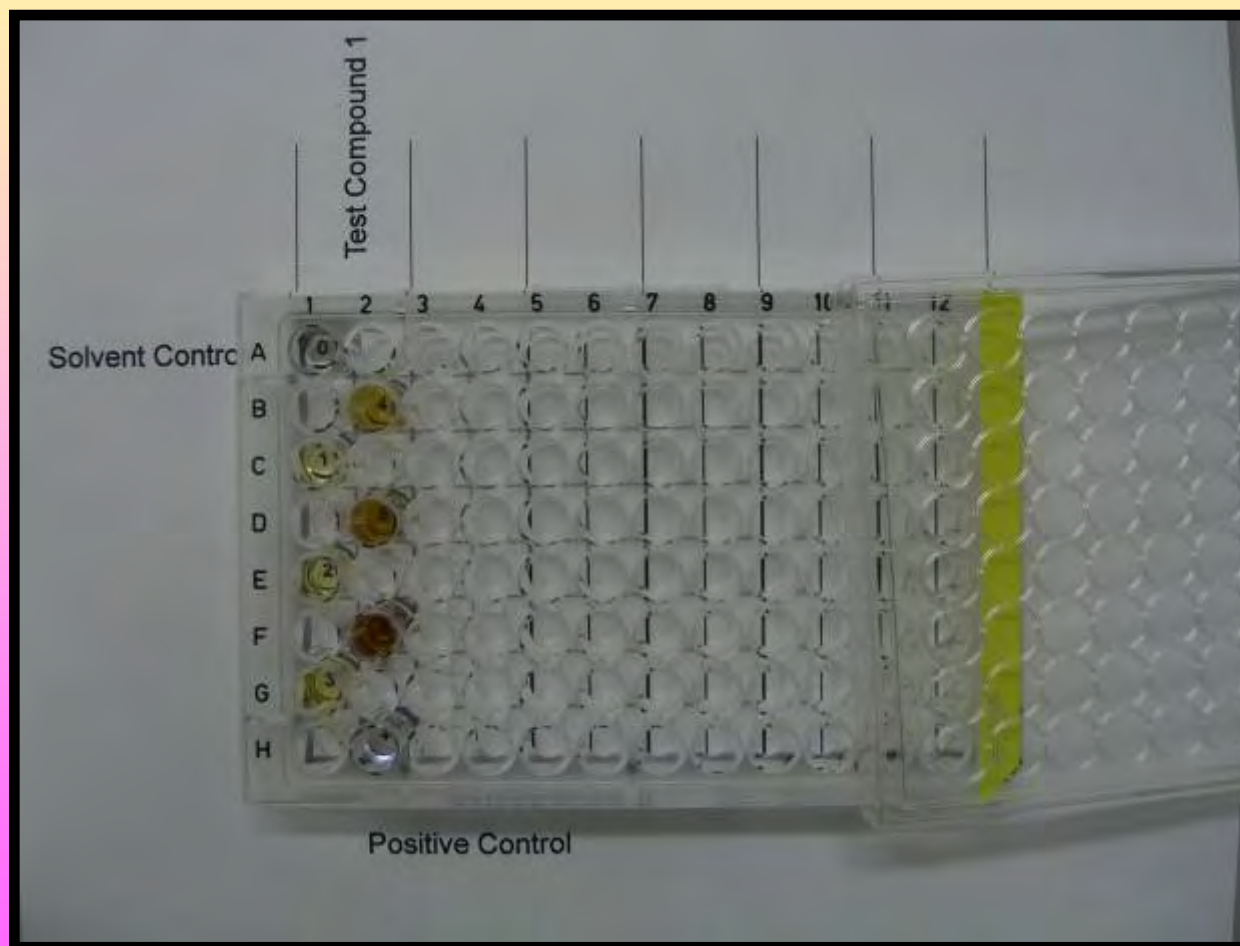
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The OD_{600} should be >2.0 . Measure a 1:10 diluted sample.



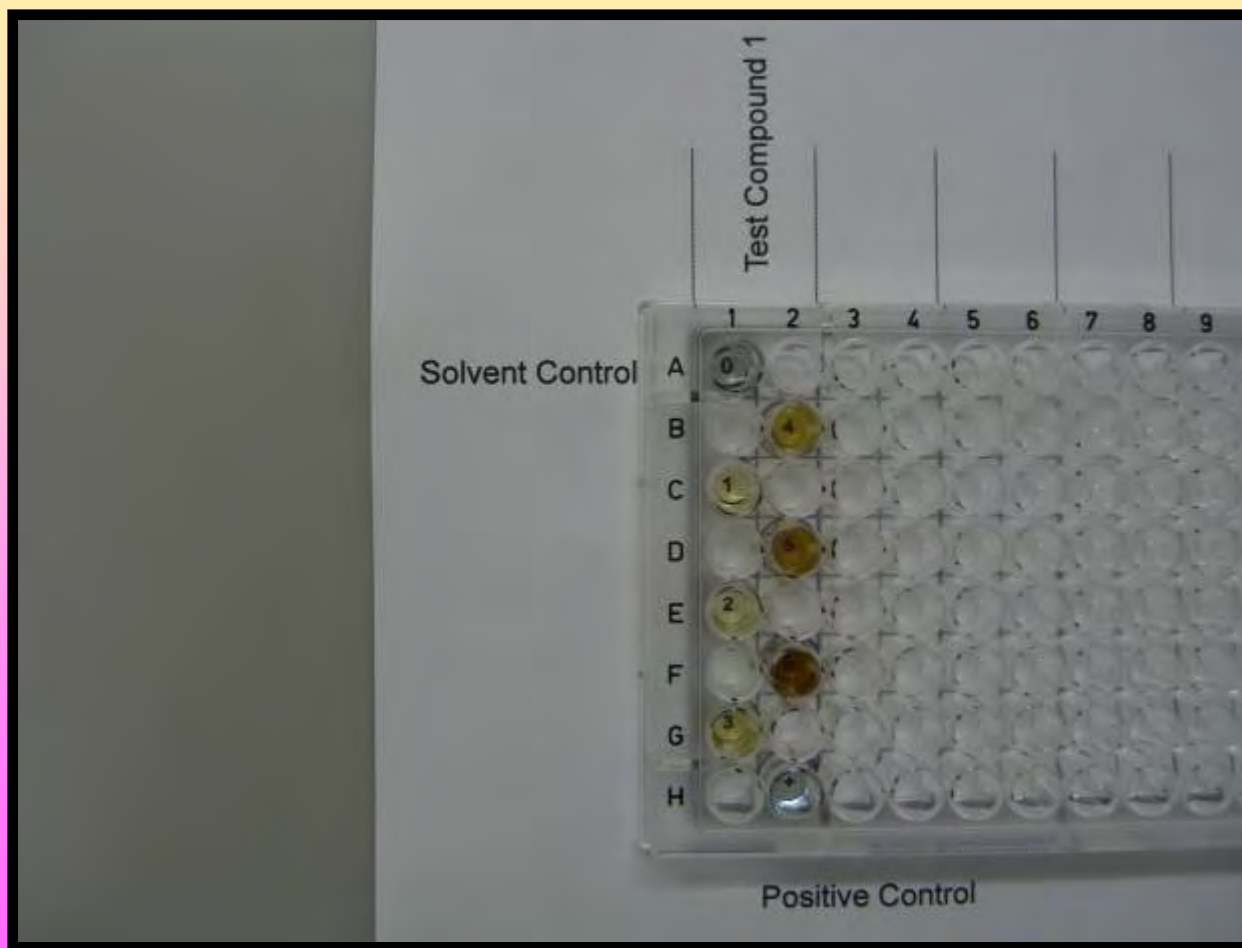
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Prepare the chemical dilution plate.



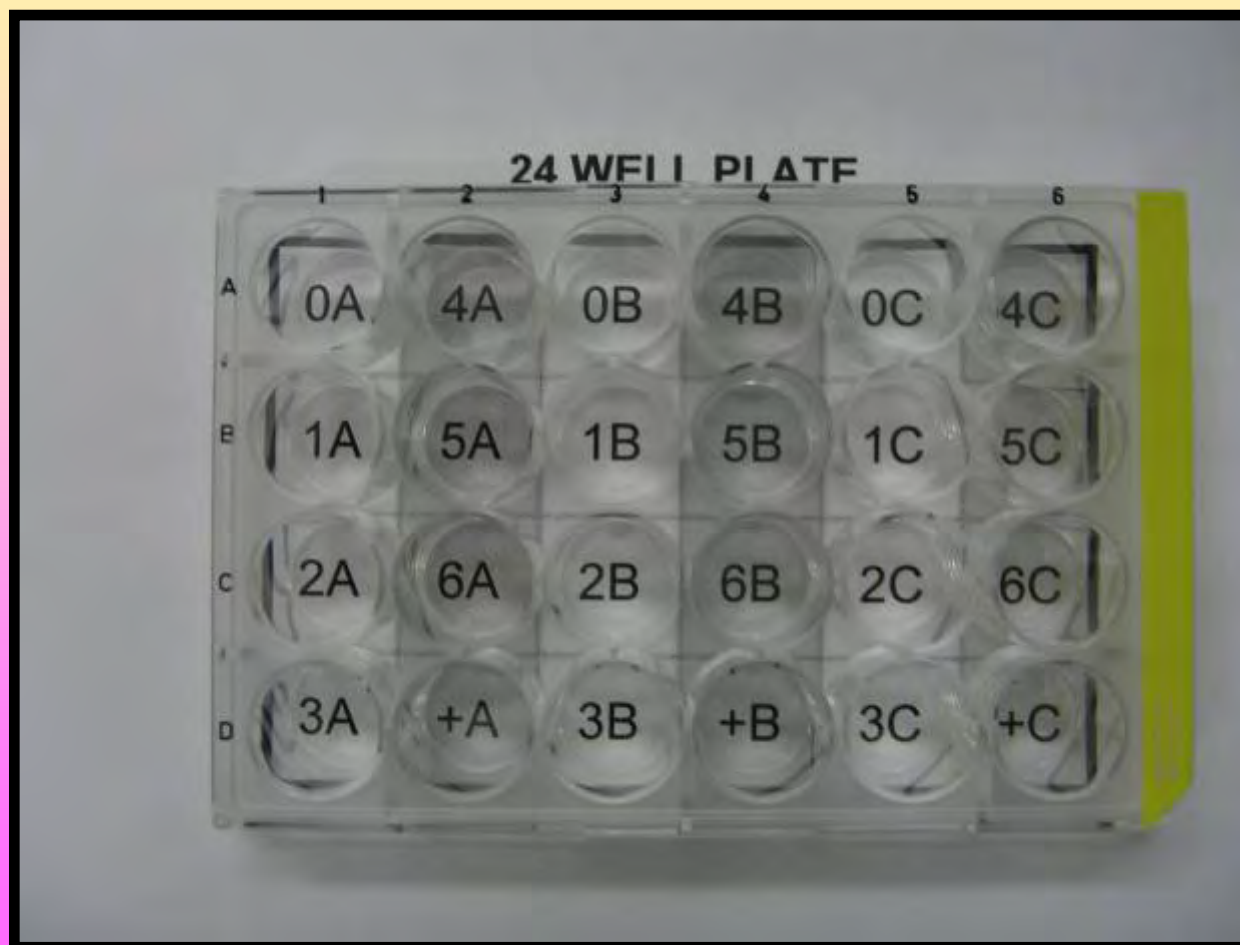
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Note the well arrangement: only every other well is used.
The solvent (negative) control is at pos. A1; the positive control
at pos. H2. In between, the test sample in 6 dilutions.



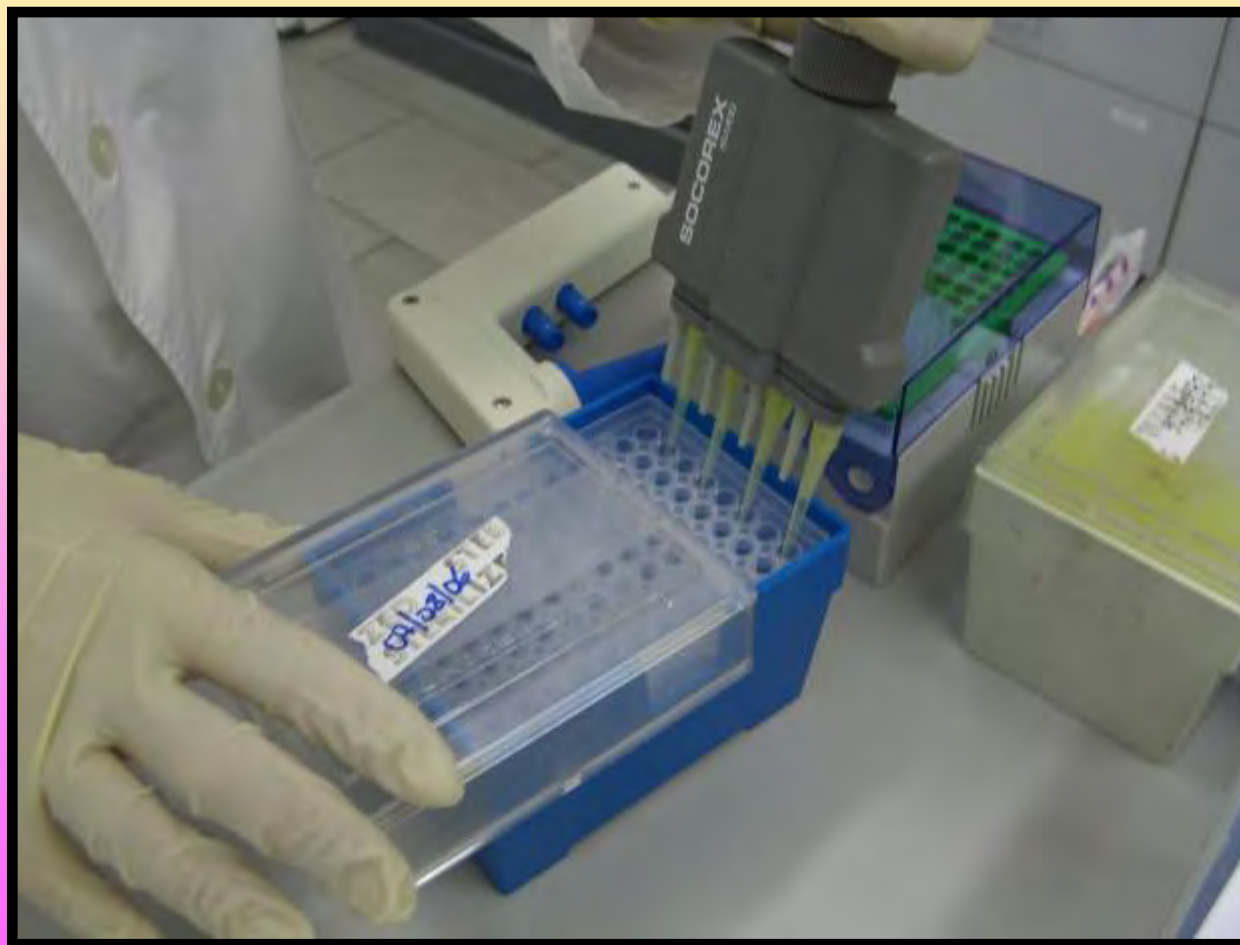
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This is the plate used for exposure: 3 x 8 wells to produce triplicates (A, B, C) of the samples prepared before in the chemical plate.



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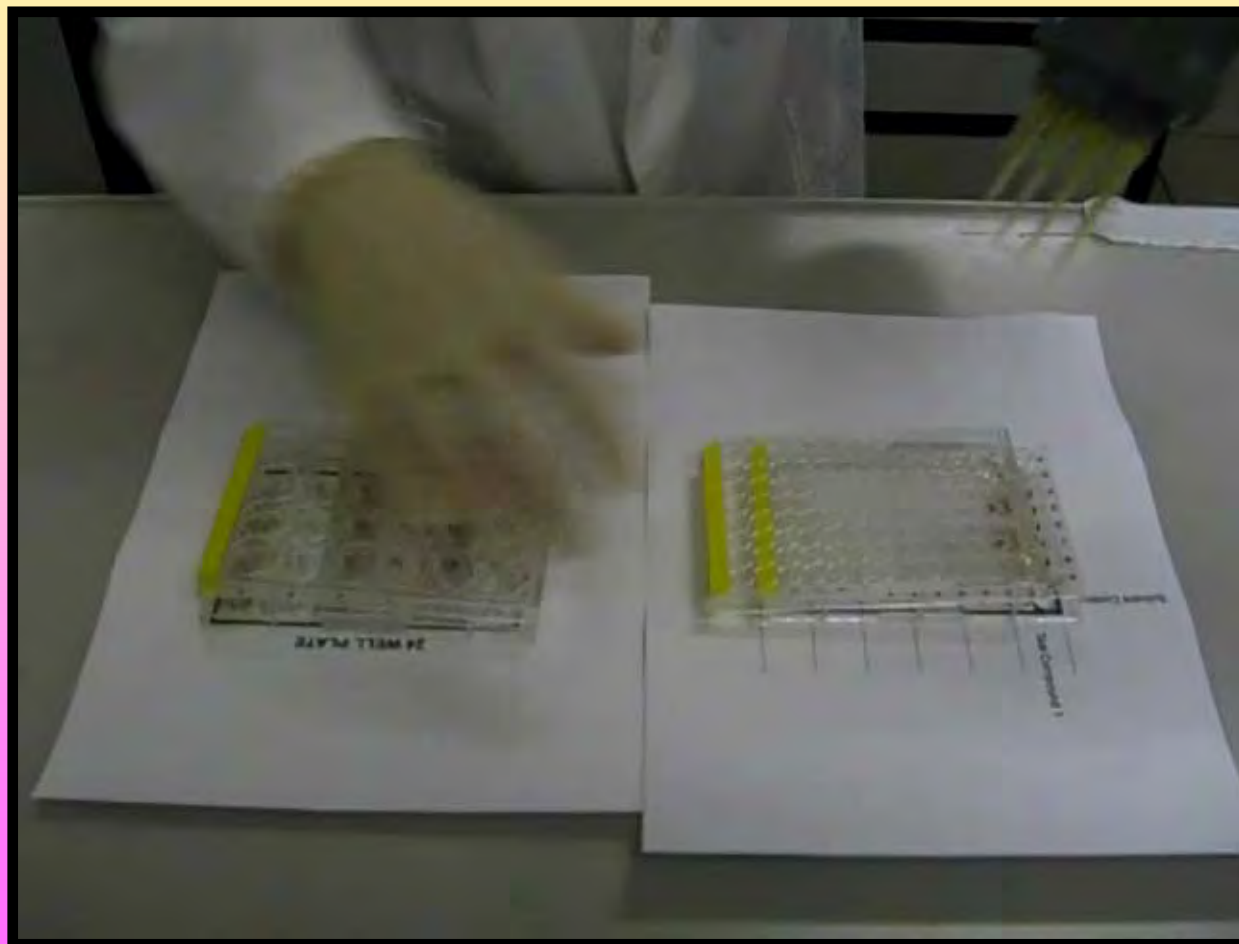
Using 4 tips on a 8-channel pipette....



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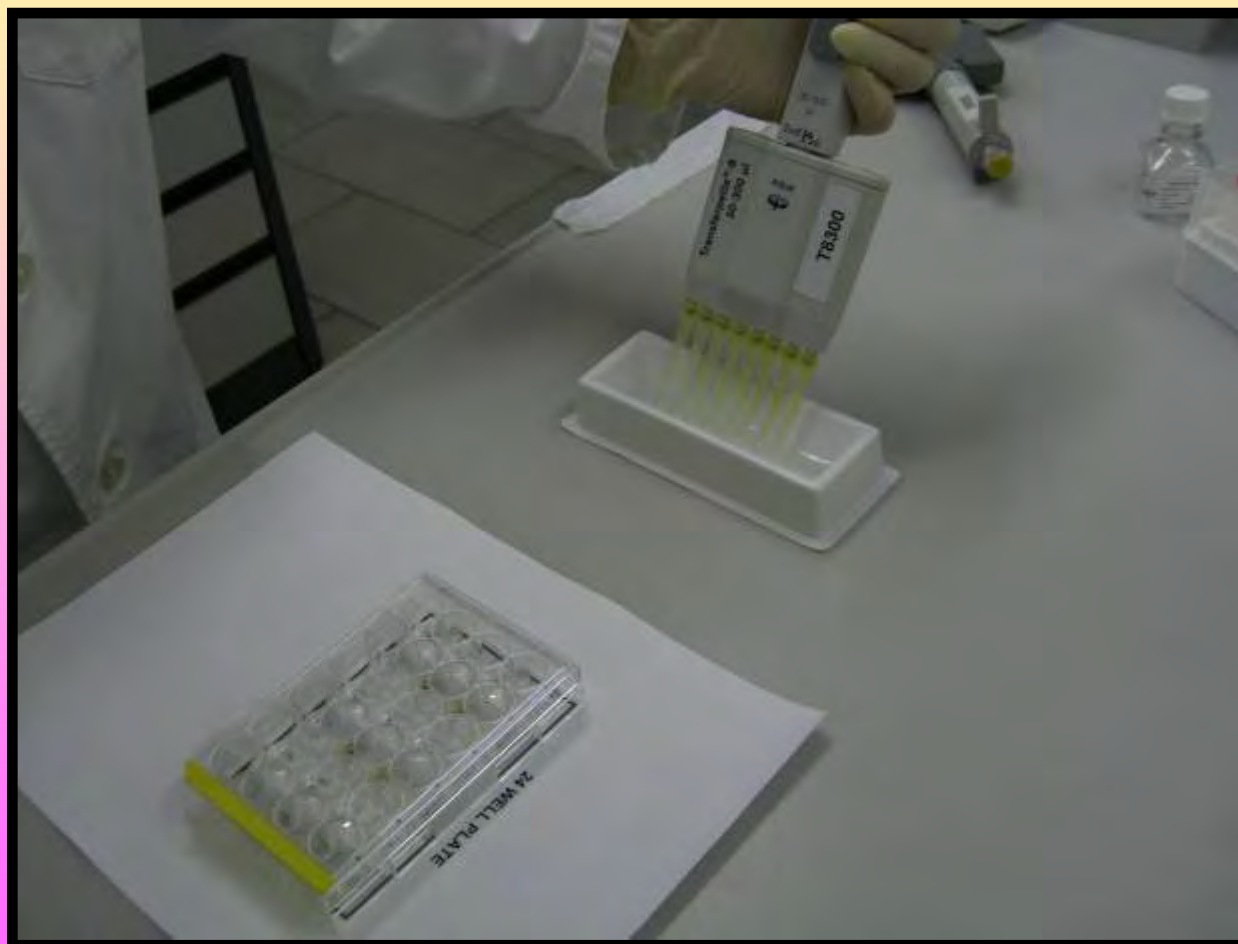


....and chemical from the second column is transferred to the 2nd, 4th, and 6th column of the exposure plate.



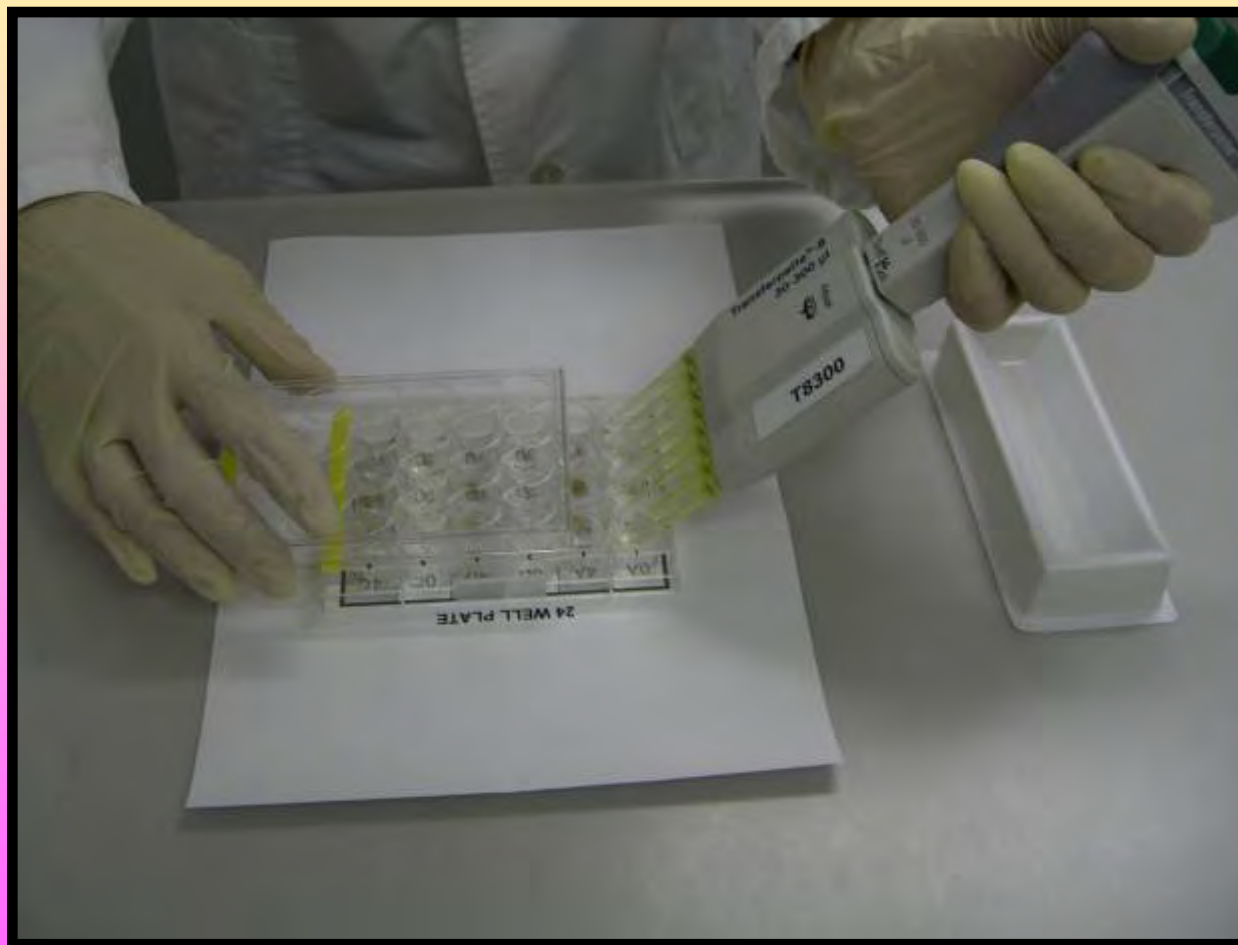
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Then you add 240 μ l of the bacterial suspension diluted in Exposure Medium to the chemical in the Exposure Plate.



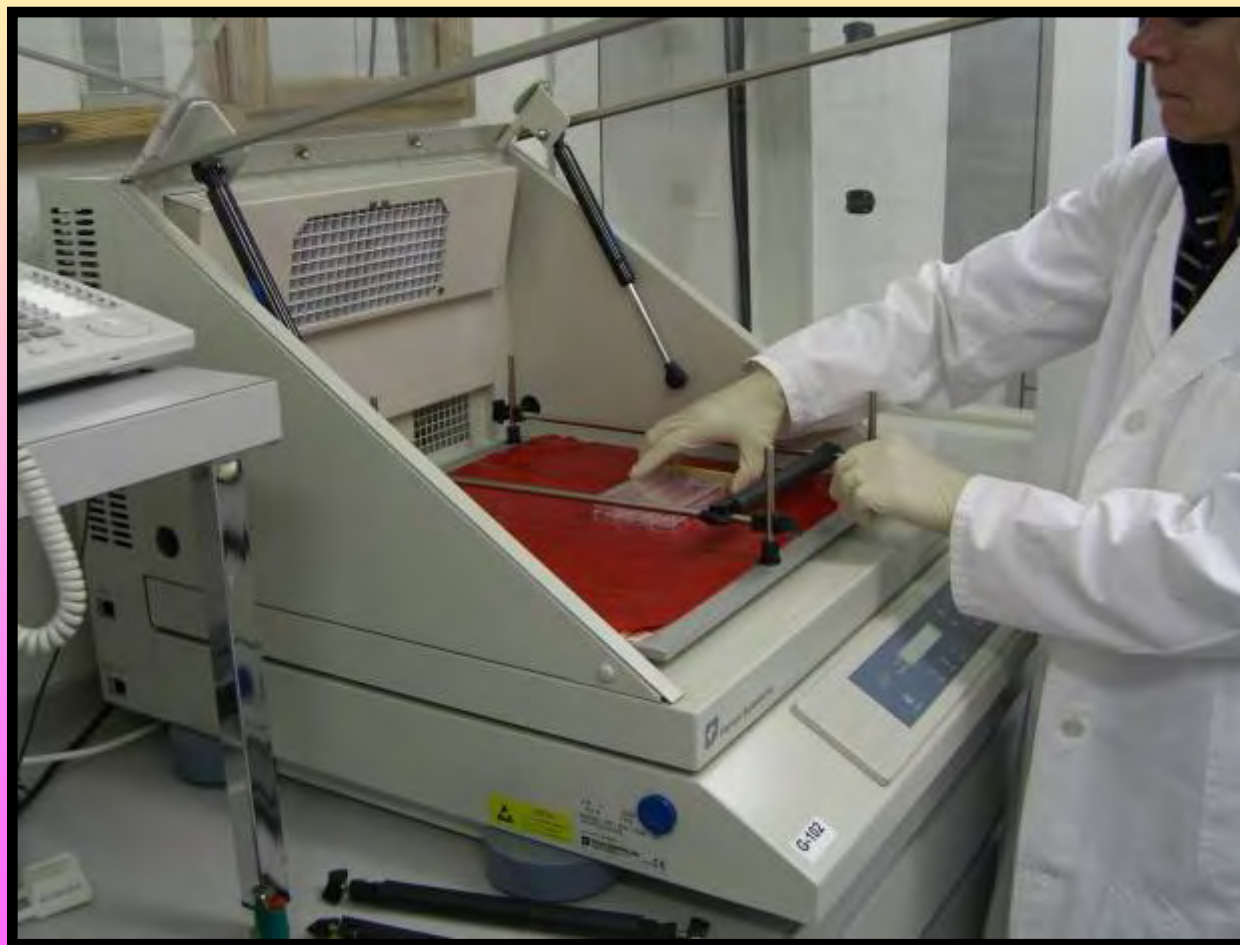
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Two tips with 120 μ l each fit into 1 well on the exposure plate.



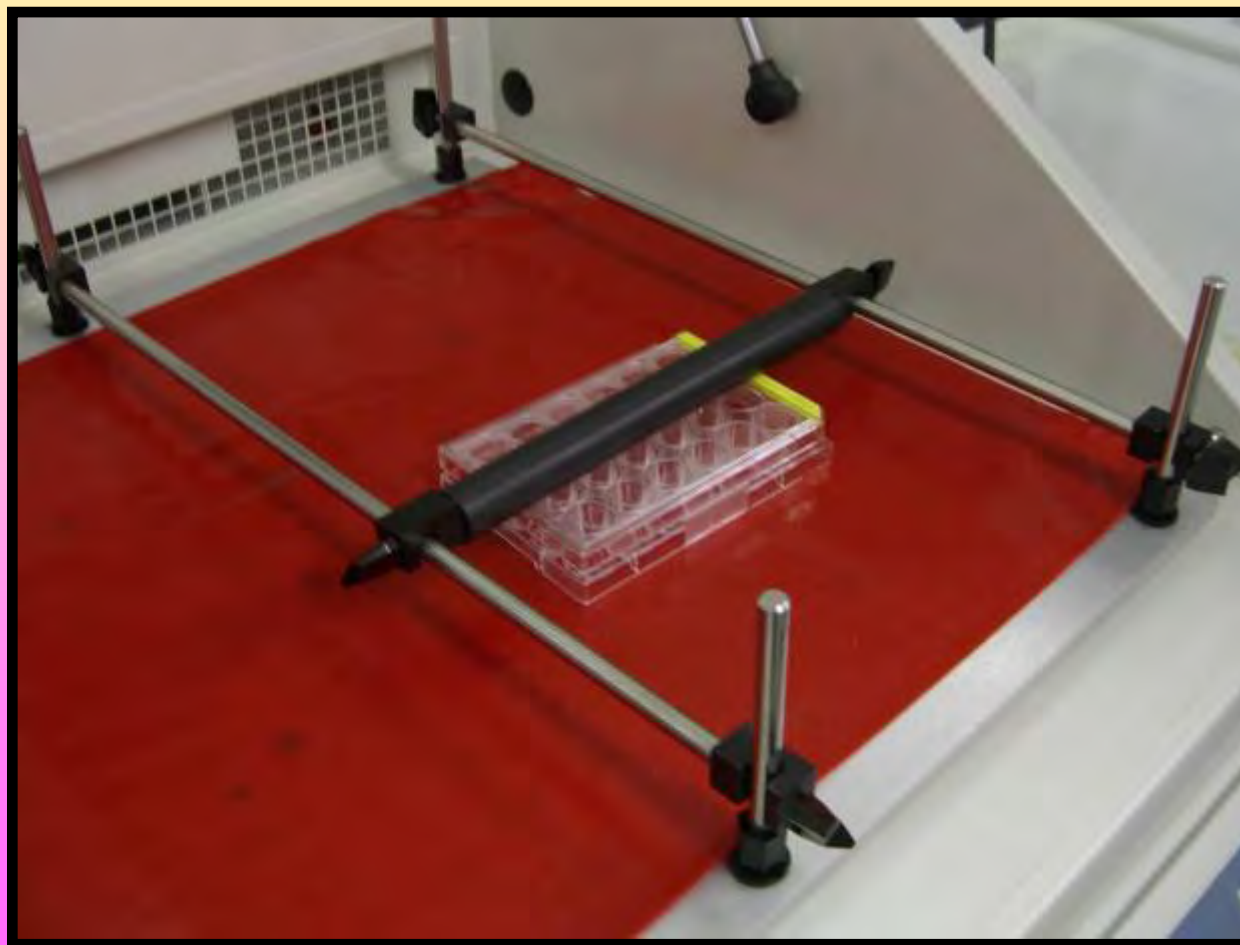
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Place the Exposure Plate in the incubator and incubate it for 90 min.



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The plate should be firmly attached.



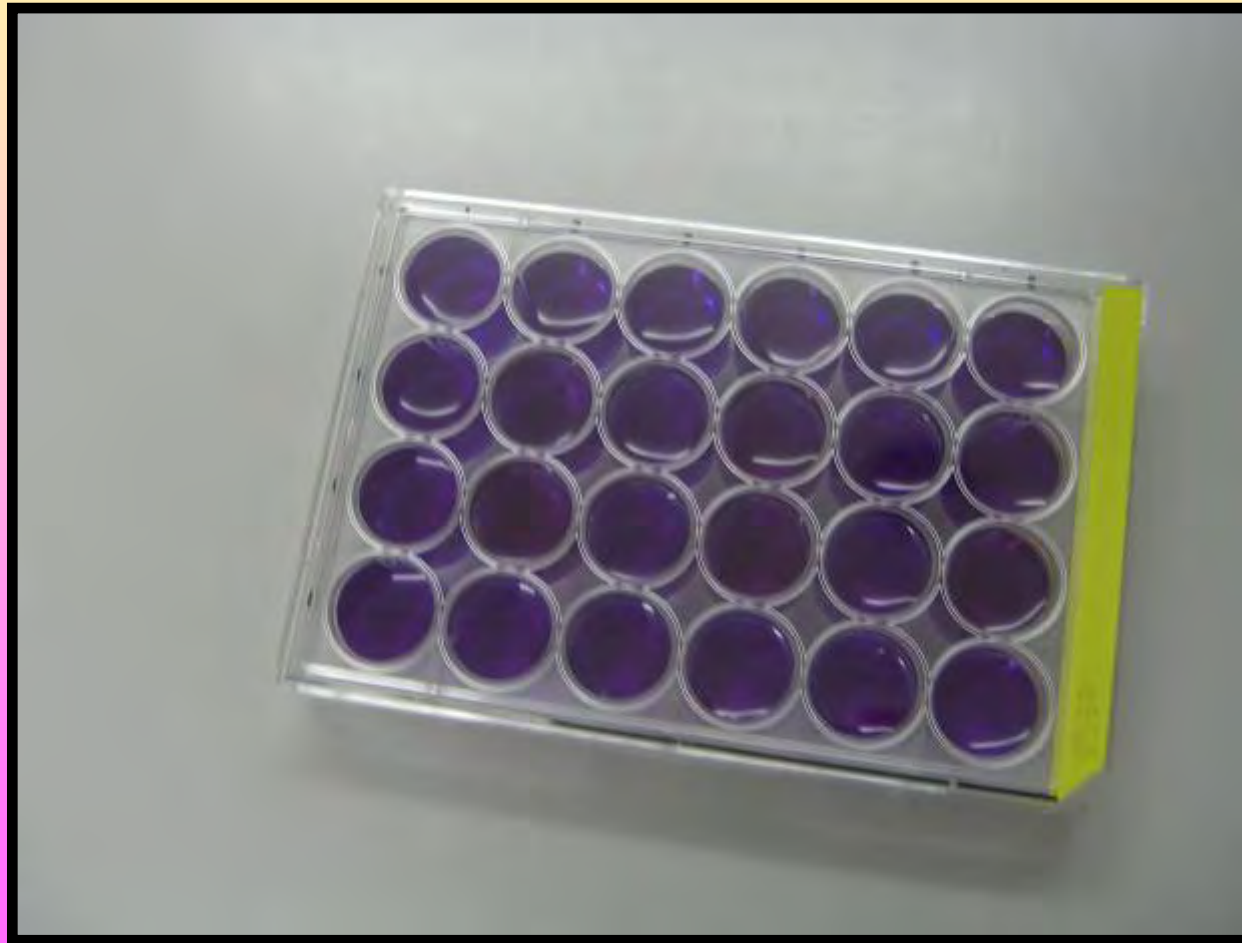
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After 90 min: add 2.8 ml of the purple Indicator Medium to each well of the exposure plate.



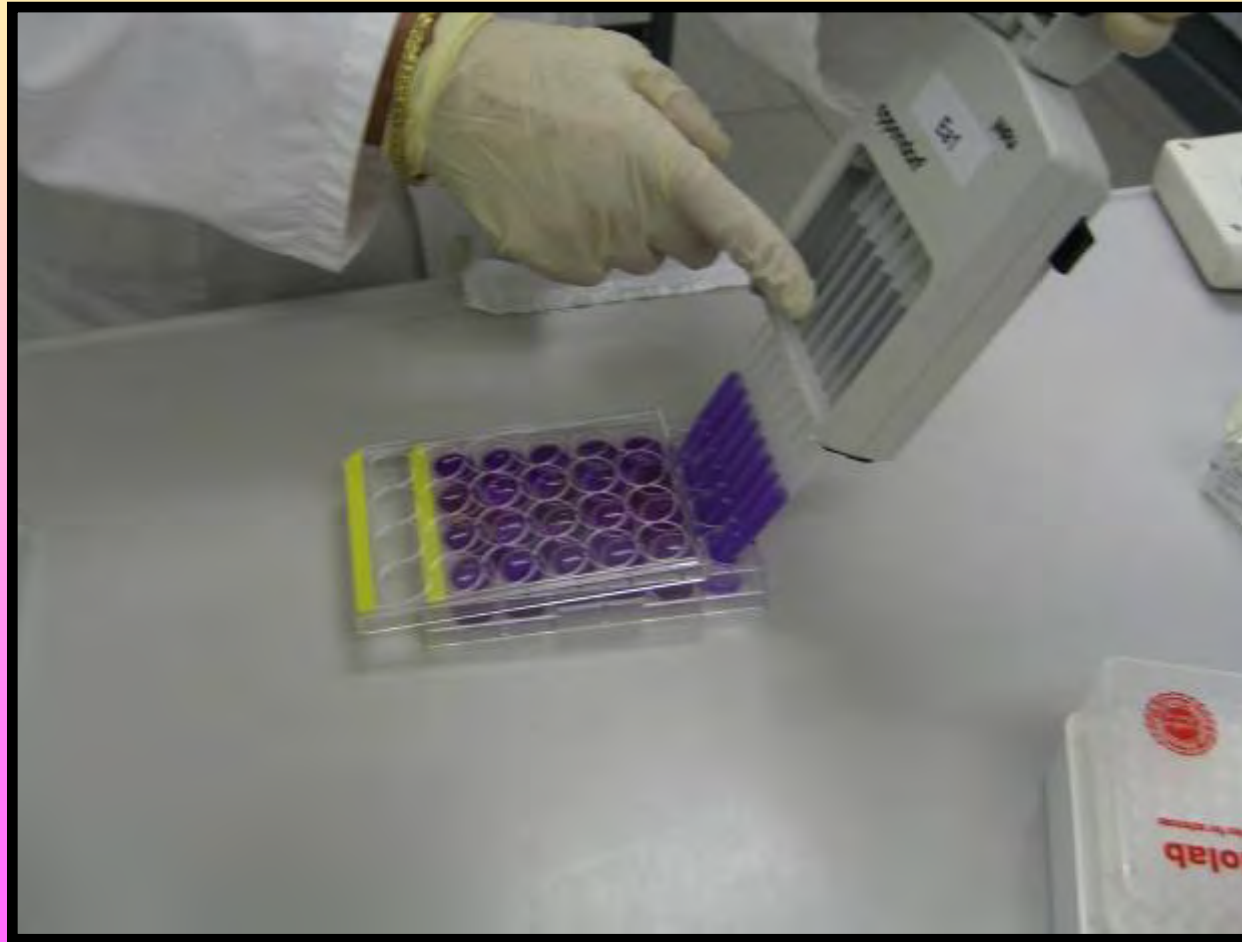
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The exposed cultures are now ready to be distributed into 384-well plates.



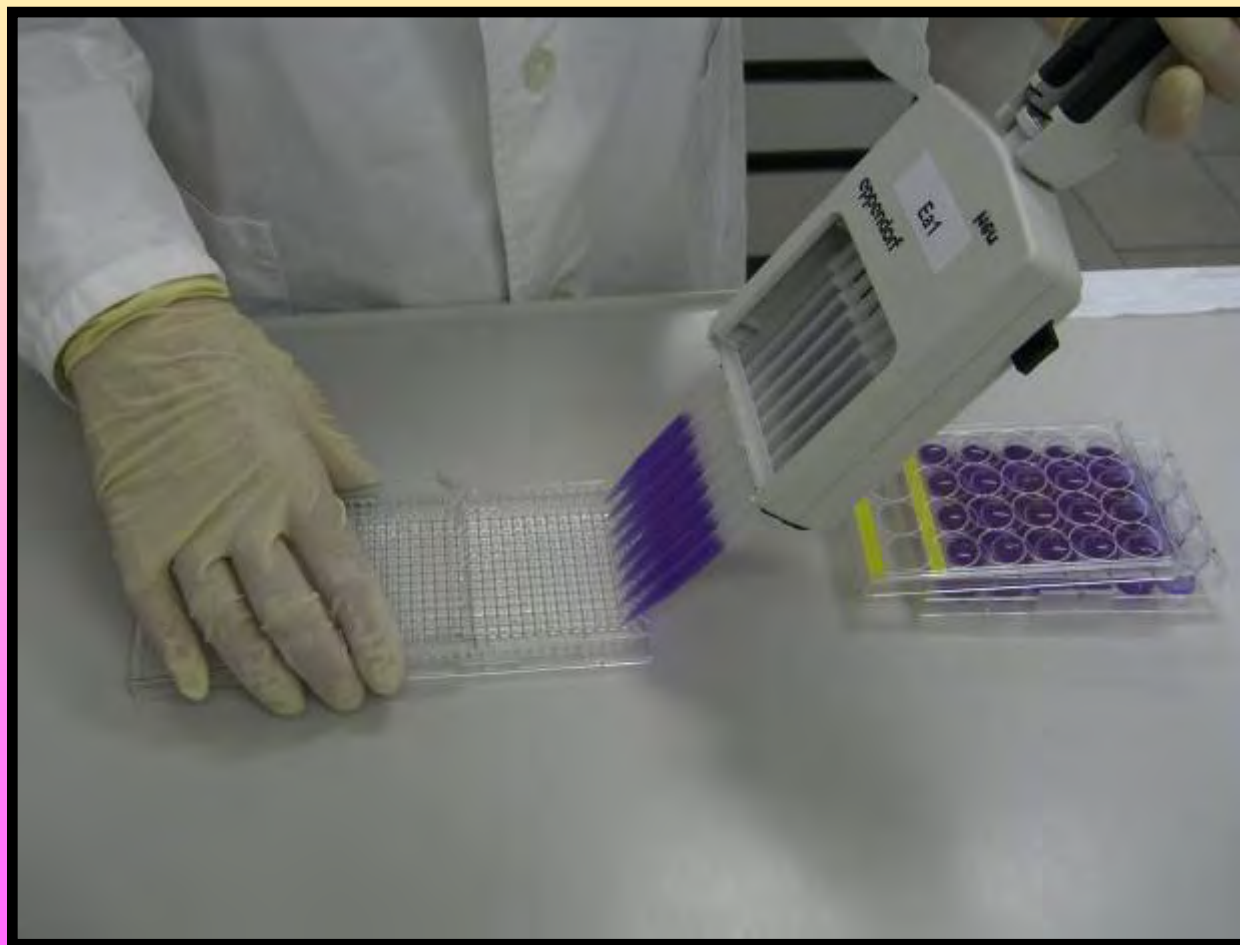
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Ideally, use a 8-channel dispenser. Again, 2 tips fit into each well, such that....



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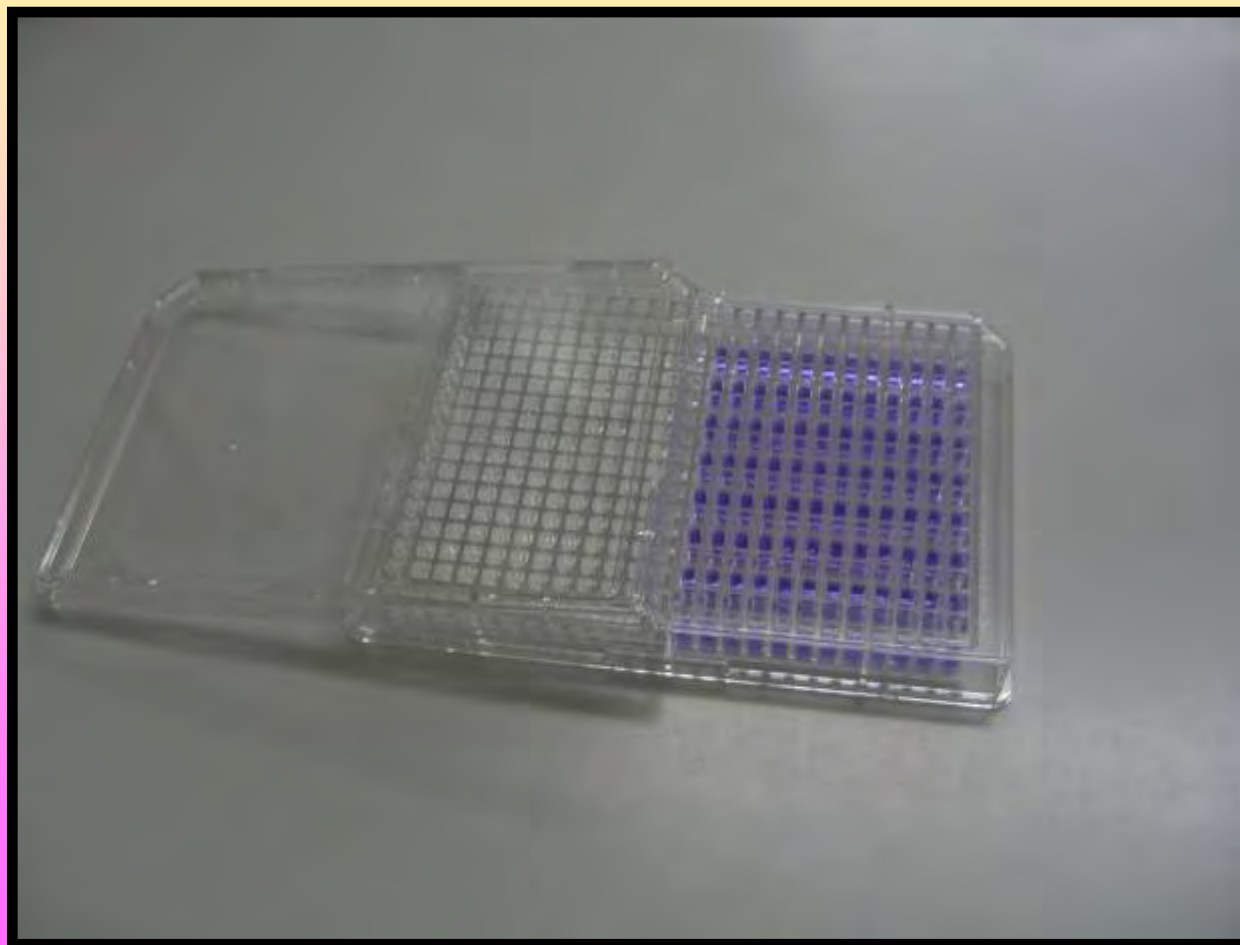
.... 50 μ l are distributed into the wells of every other row of the 384-microtiter plate.



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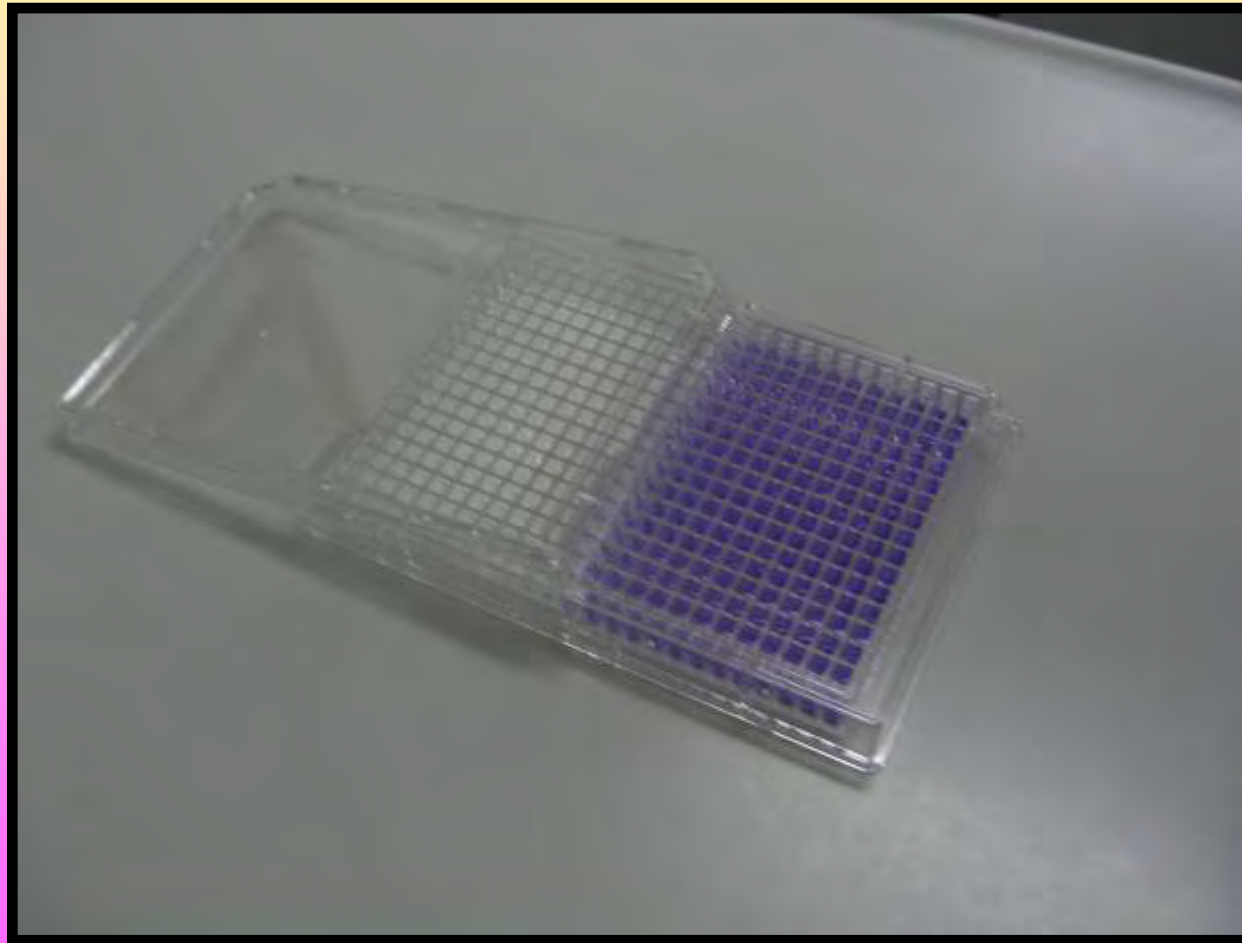
After 1 pass you therefore have filled 8 rows, 12 wells each.



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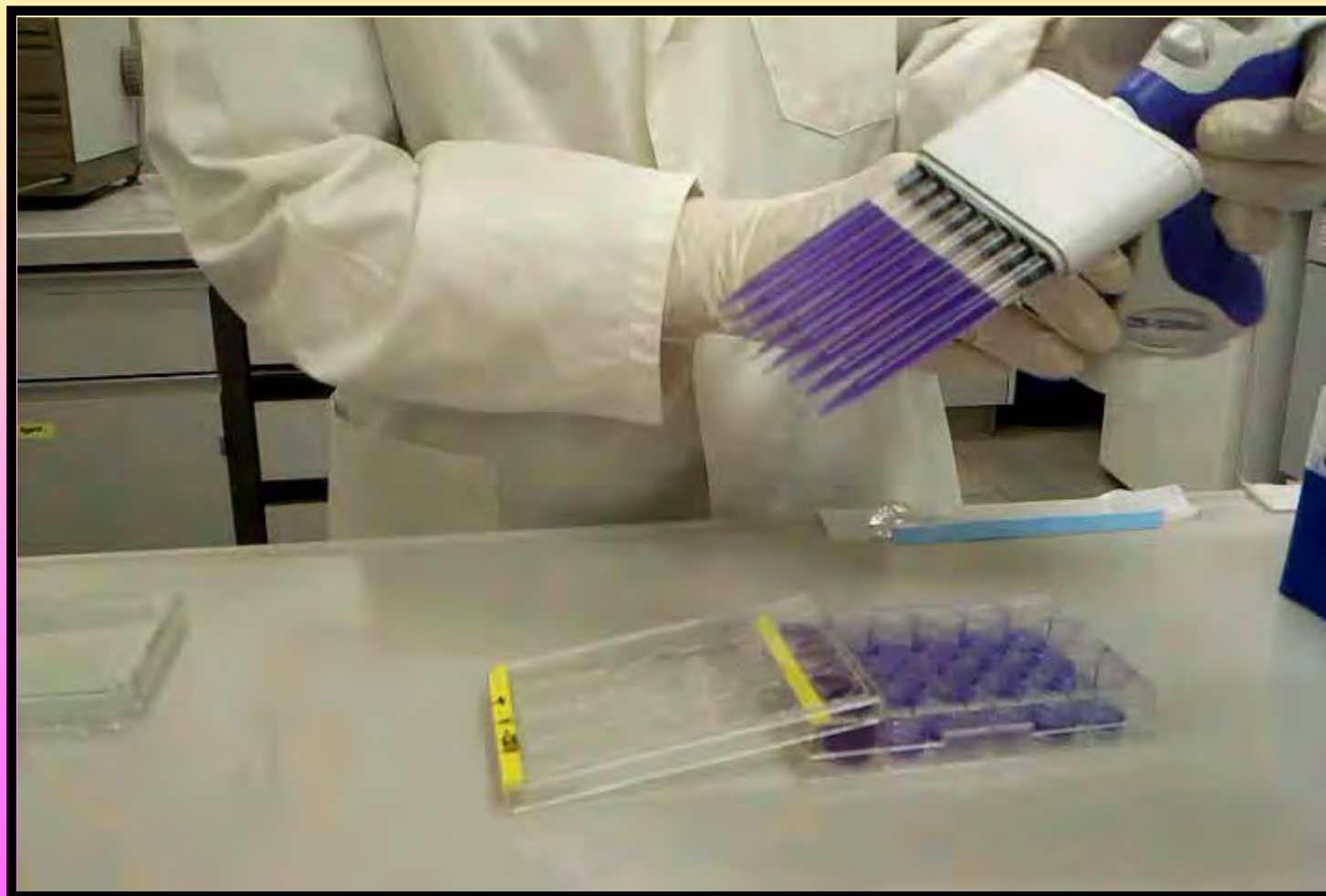
After a second pass you have now filled half of the 384-well plate.



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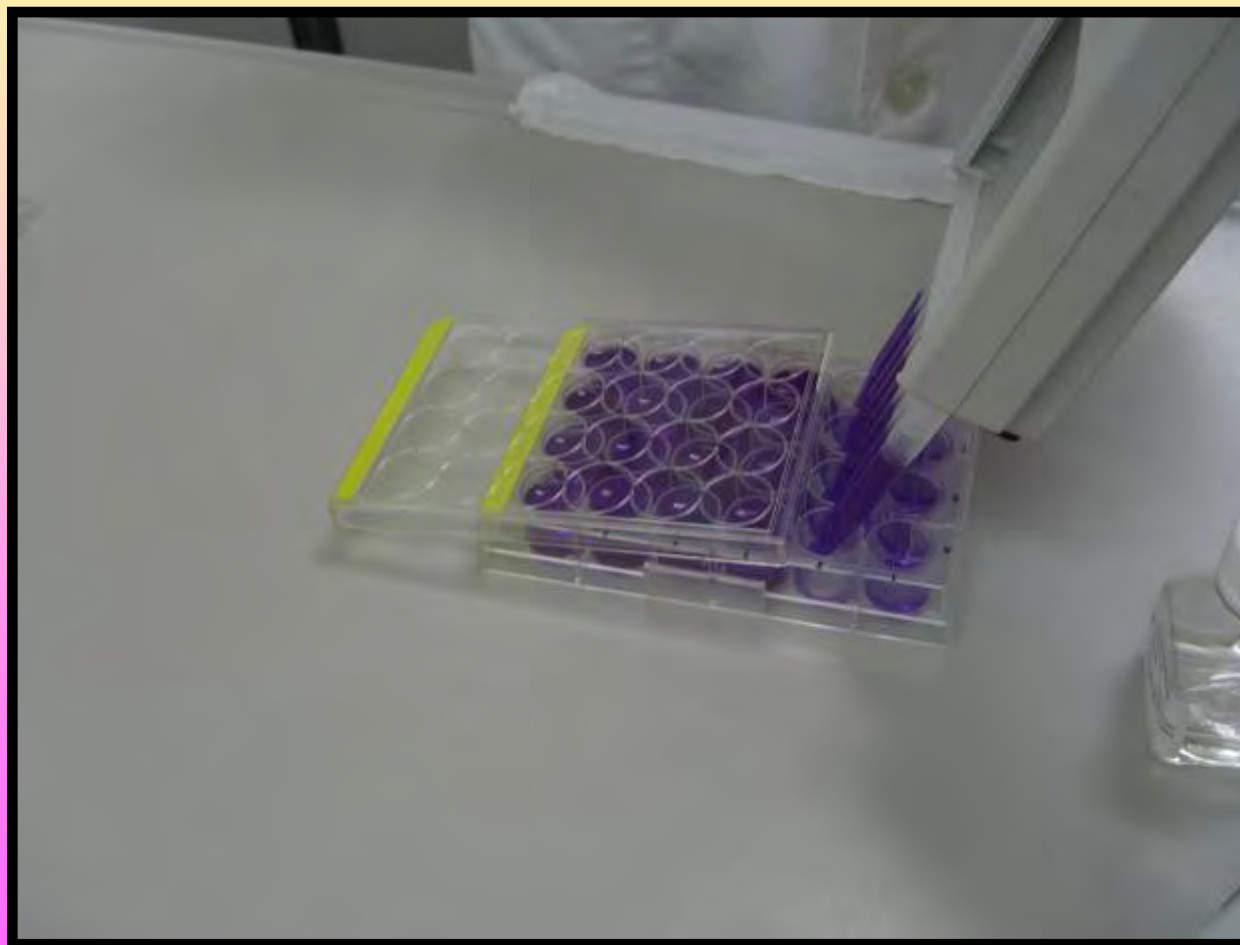


See how quickly you can fill the first half of a 384-well plate!



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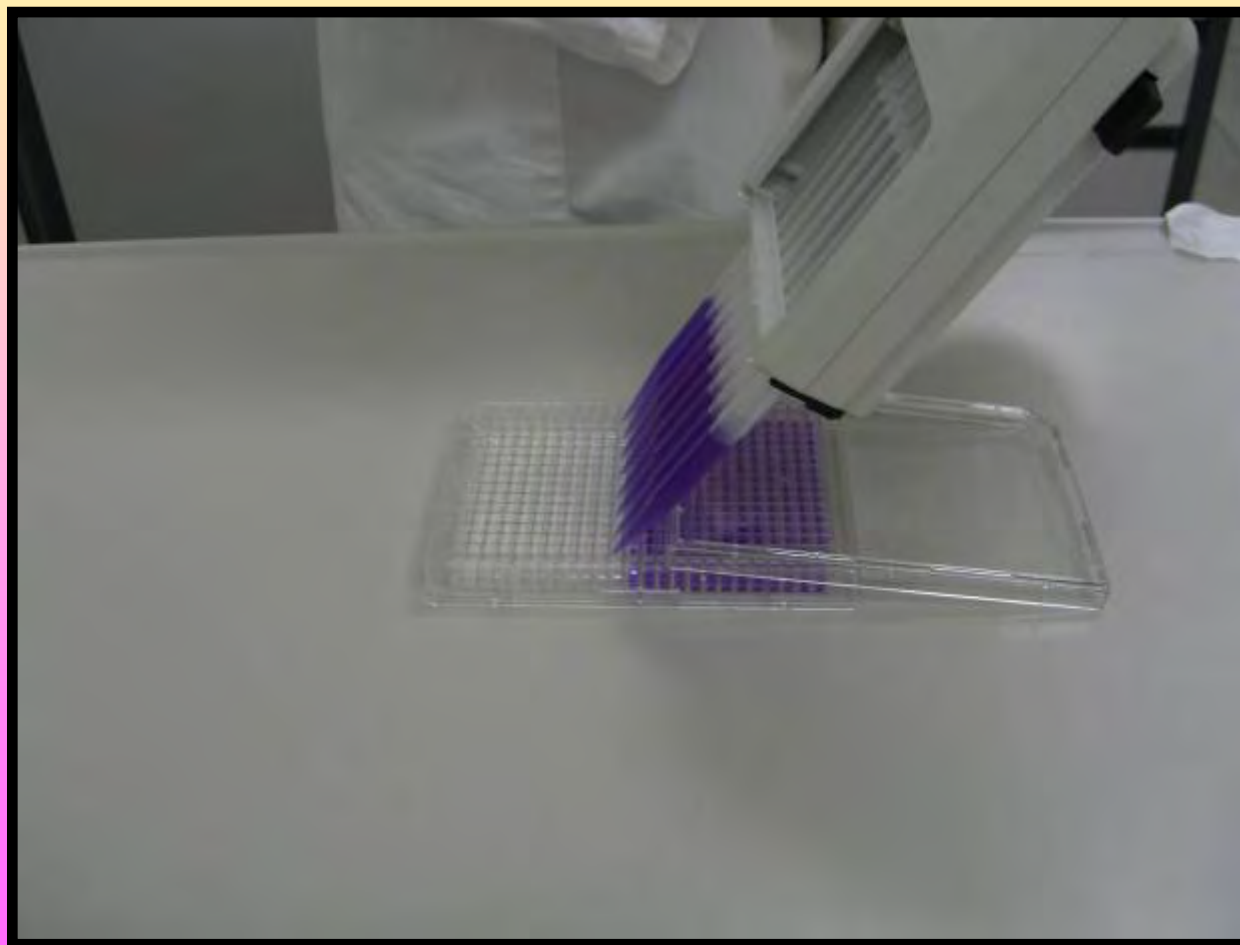
The second column of the Exposure plate....



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.... is similarly distributed to the right half of the 384 microplate.

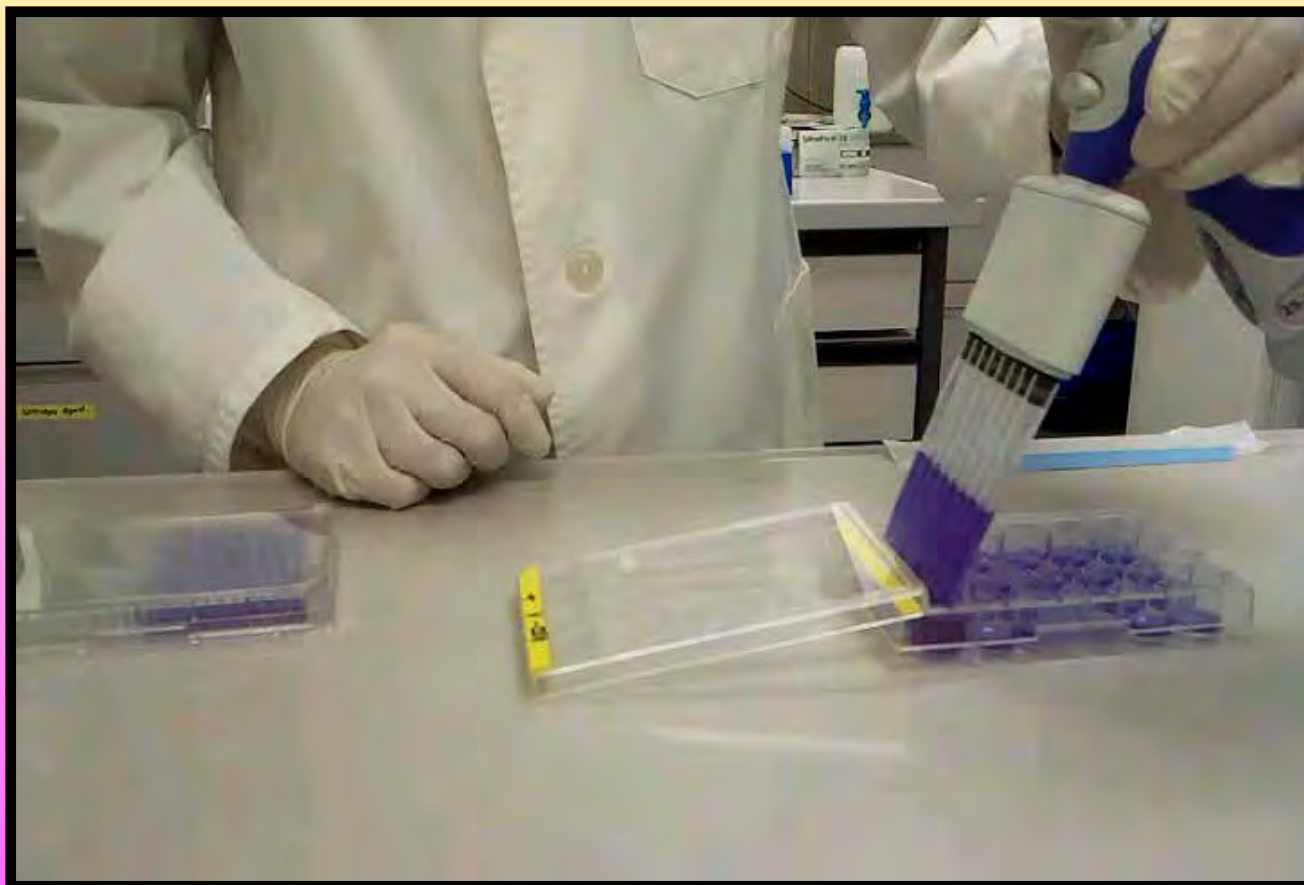


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The second half of the plate fills as fast!

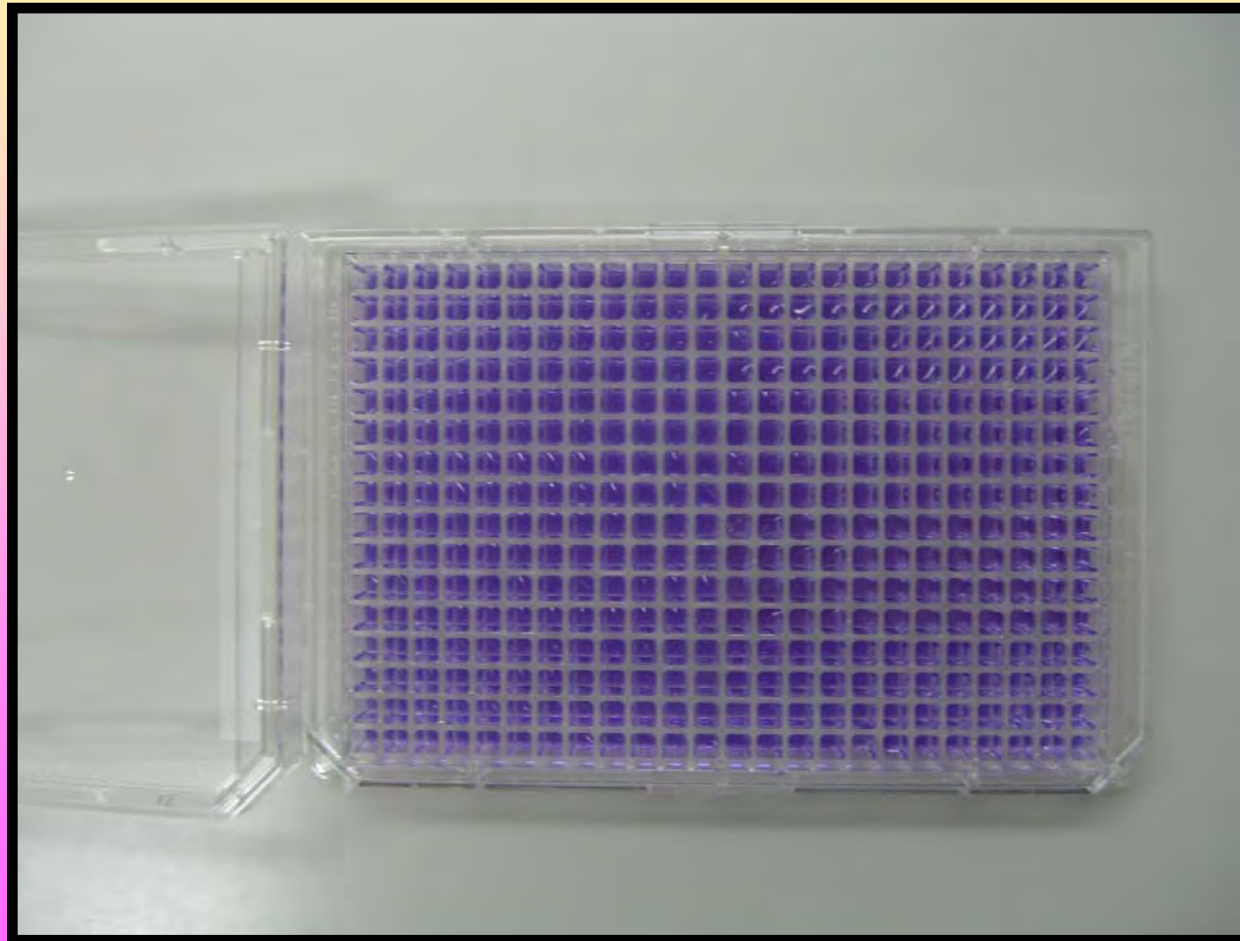
(Yes, Sini is left-handed!)



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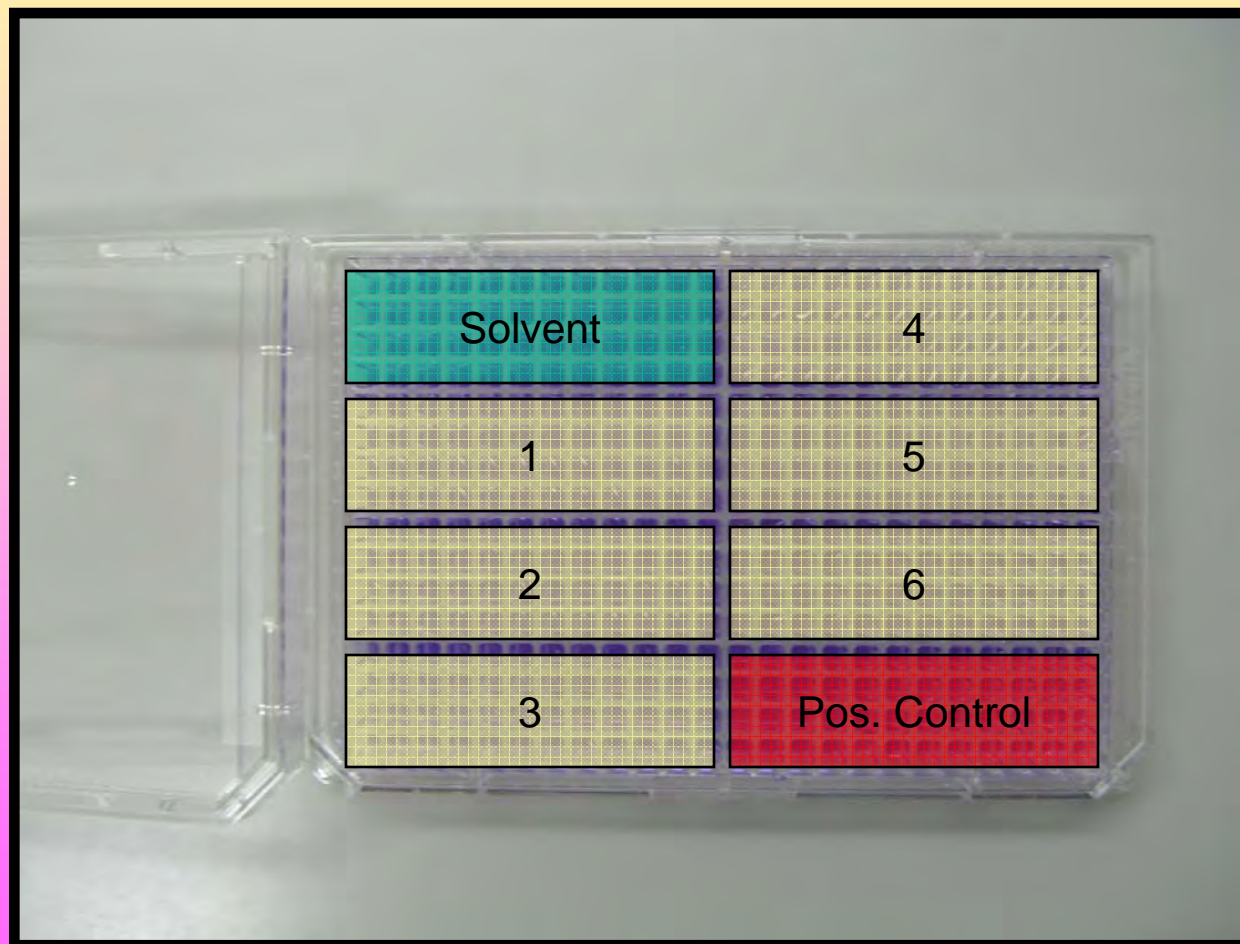


One complete plate corresponds to....



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....1 chemical in 6 dilutions with positive and negative controls.
There will be 3 identical plates for triplicate values.



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After filling all plates they are placed in a plastic bag....



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....and sealed.



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The sealed bag with the plates is placed in a dry incubator....



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....and incubated for 48 hrs at 37°C.



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2-days later.....

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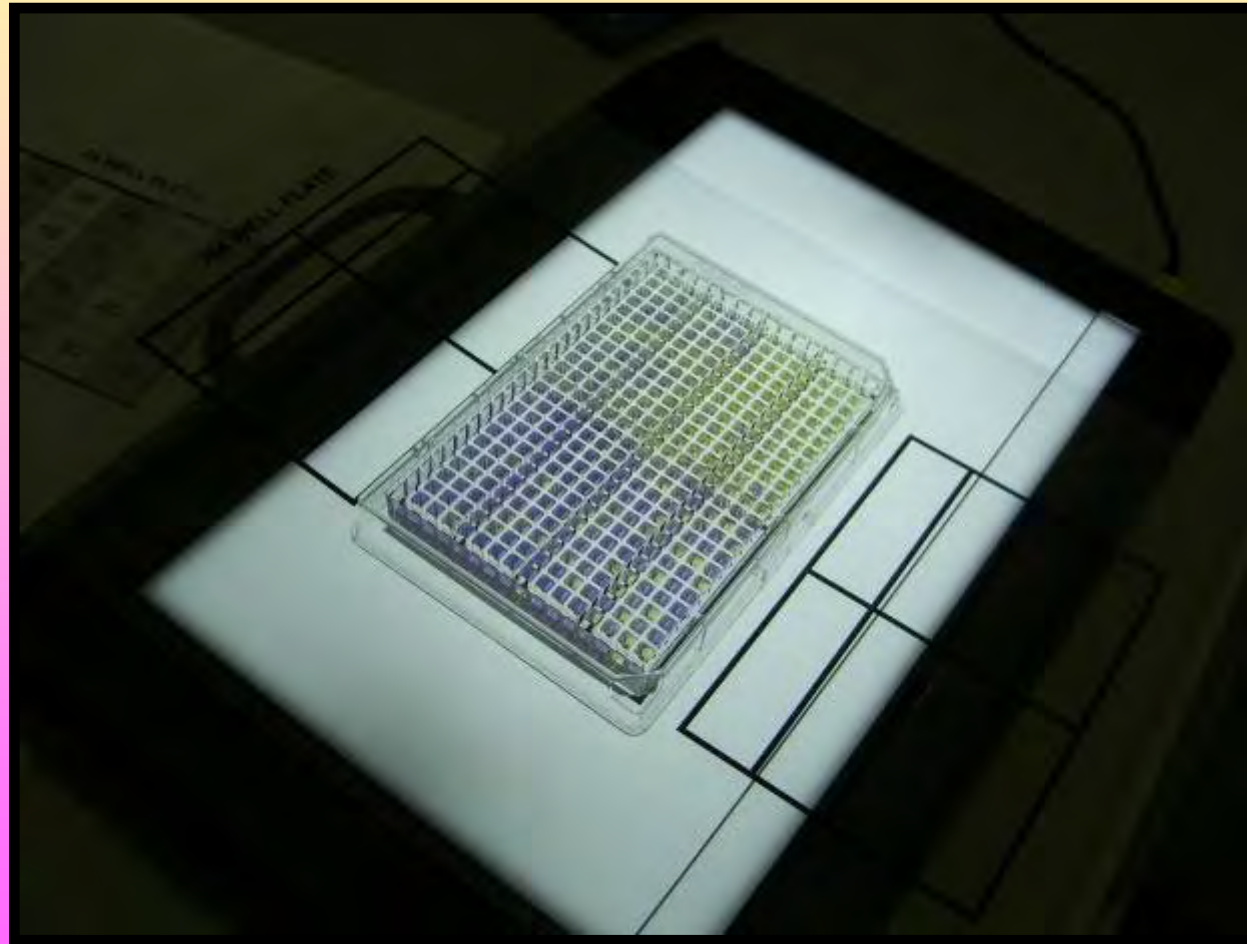
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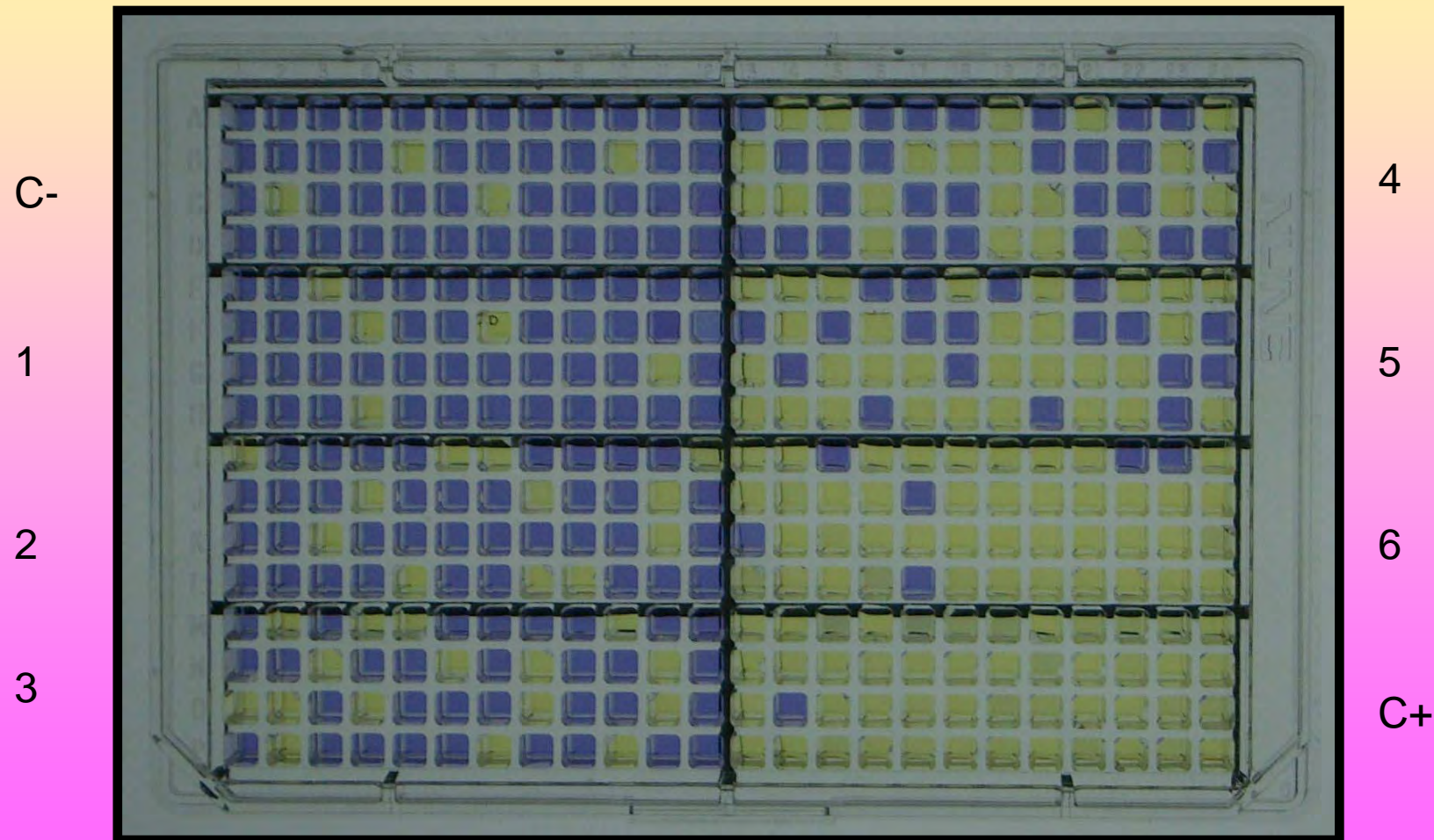
After 2 days the plates can be read. Ideally this is done on top of a light box. The use of the provided reading grid helps with the counting of positive wells.



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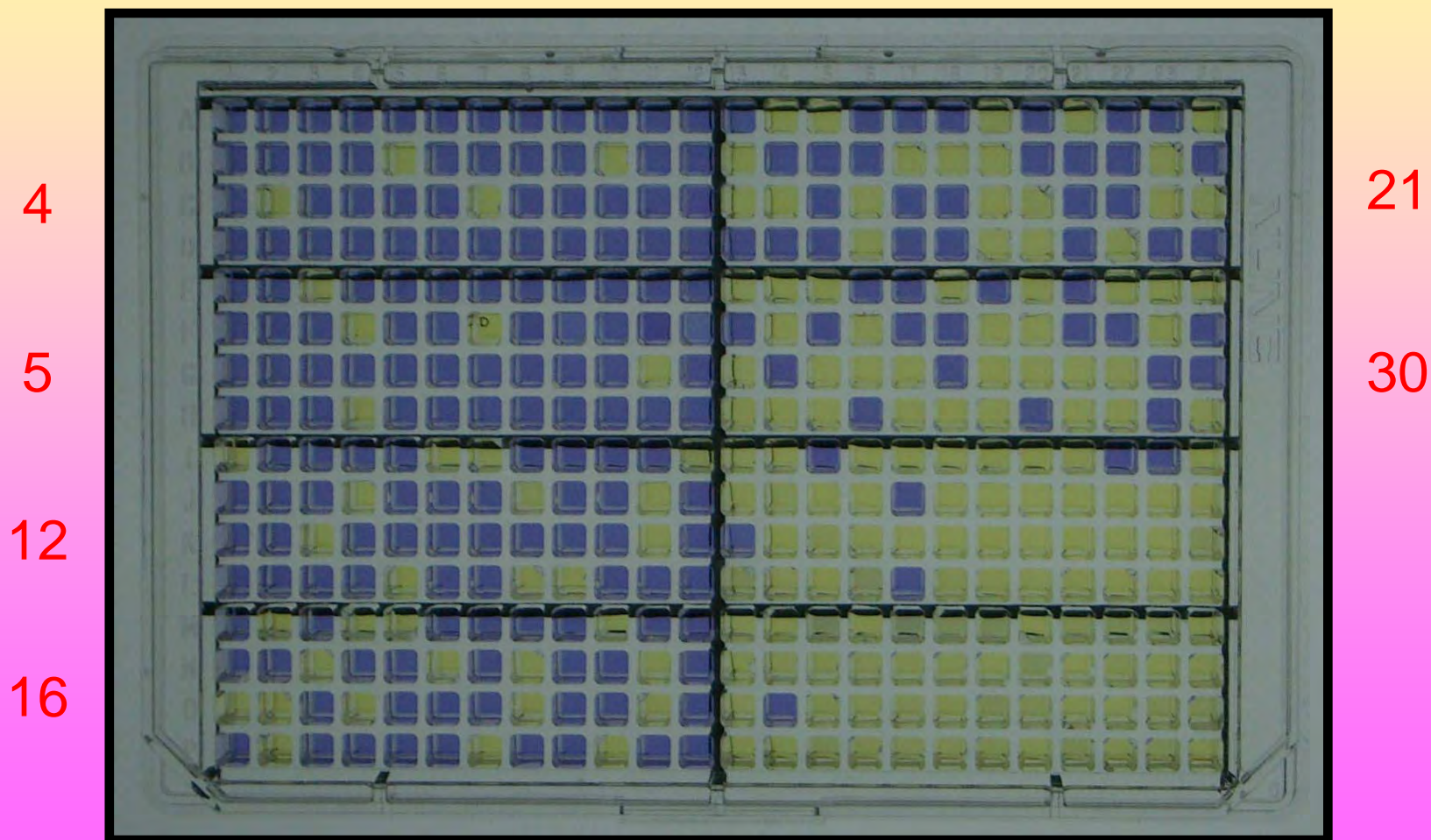


His+ revertants can form colonies in the wells. The bacterial metabolism changes the pH of the media which is followed by a colour change from purple to yellow.



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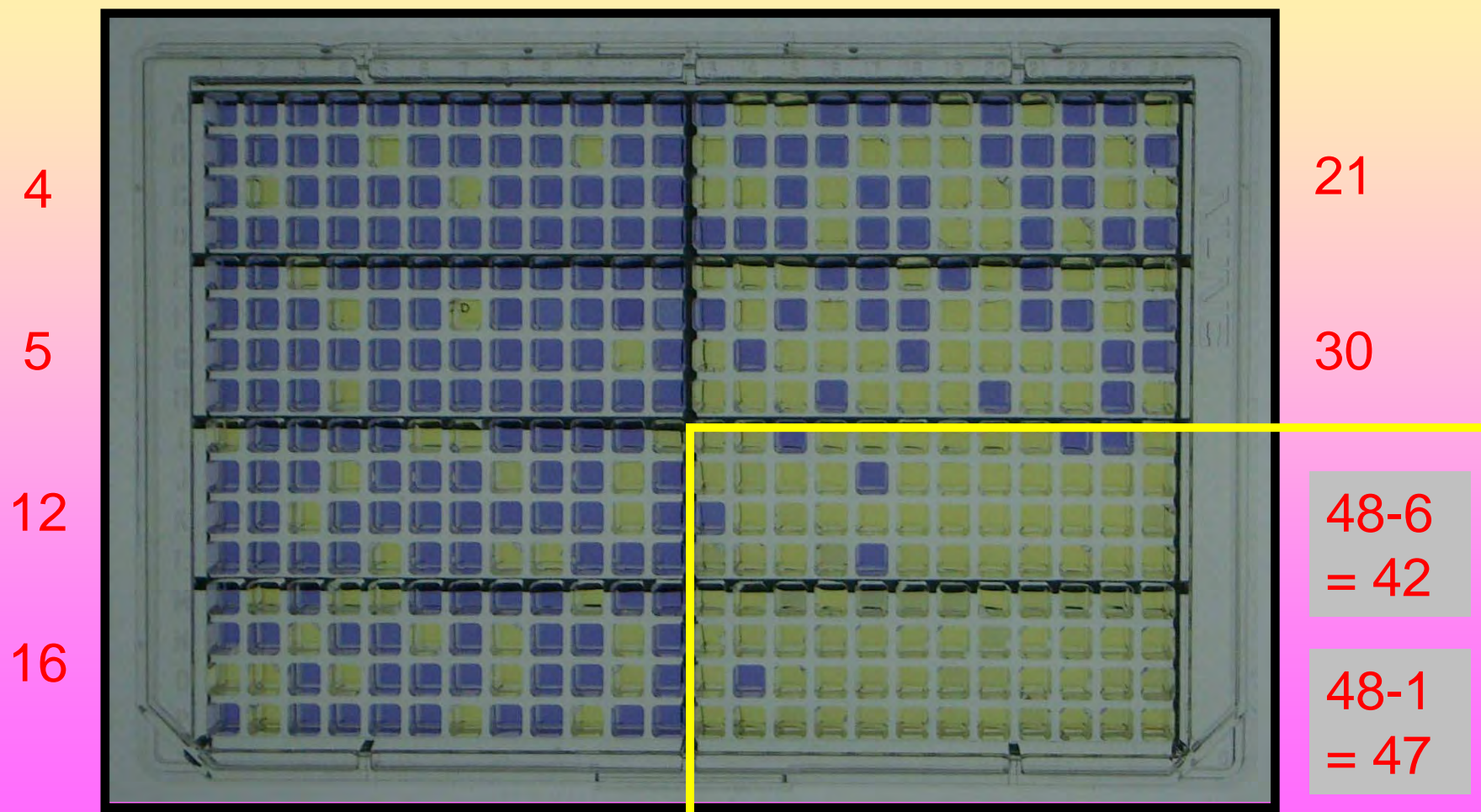
Count the number of yellow wells in each area corresponding to a chemical dilution or a control.



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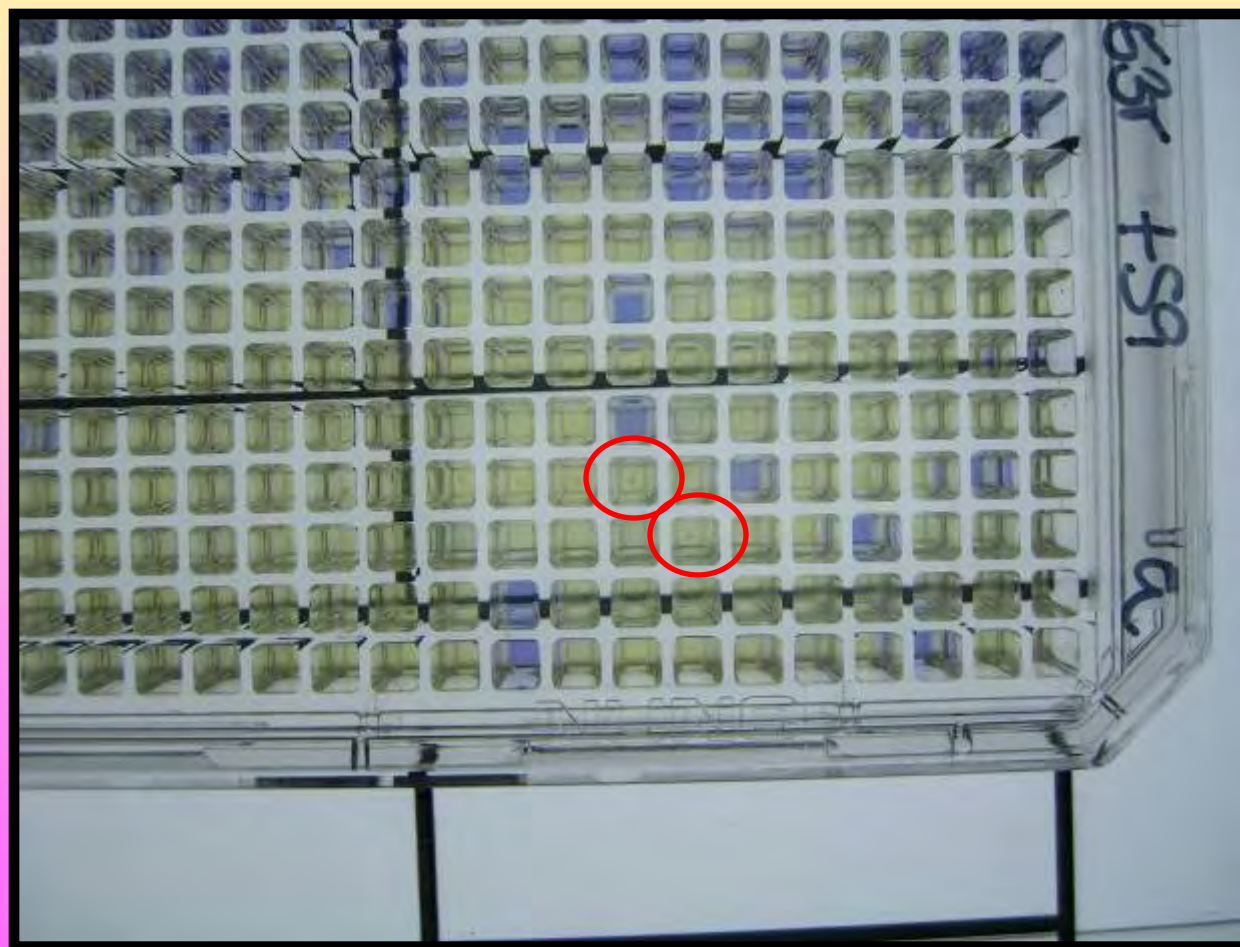


When many positive wells are present, it is easier to count the blue negative wells and calculate the difference to 48 wells.



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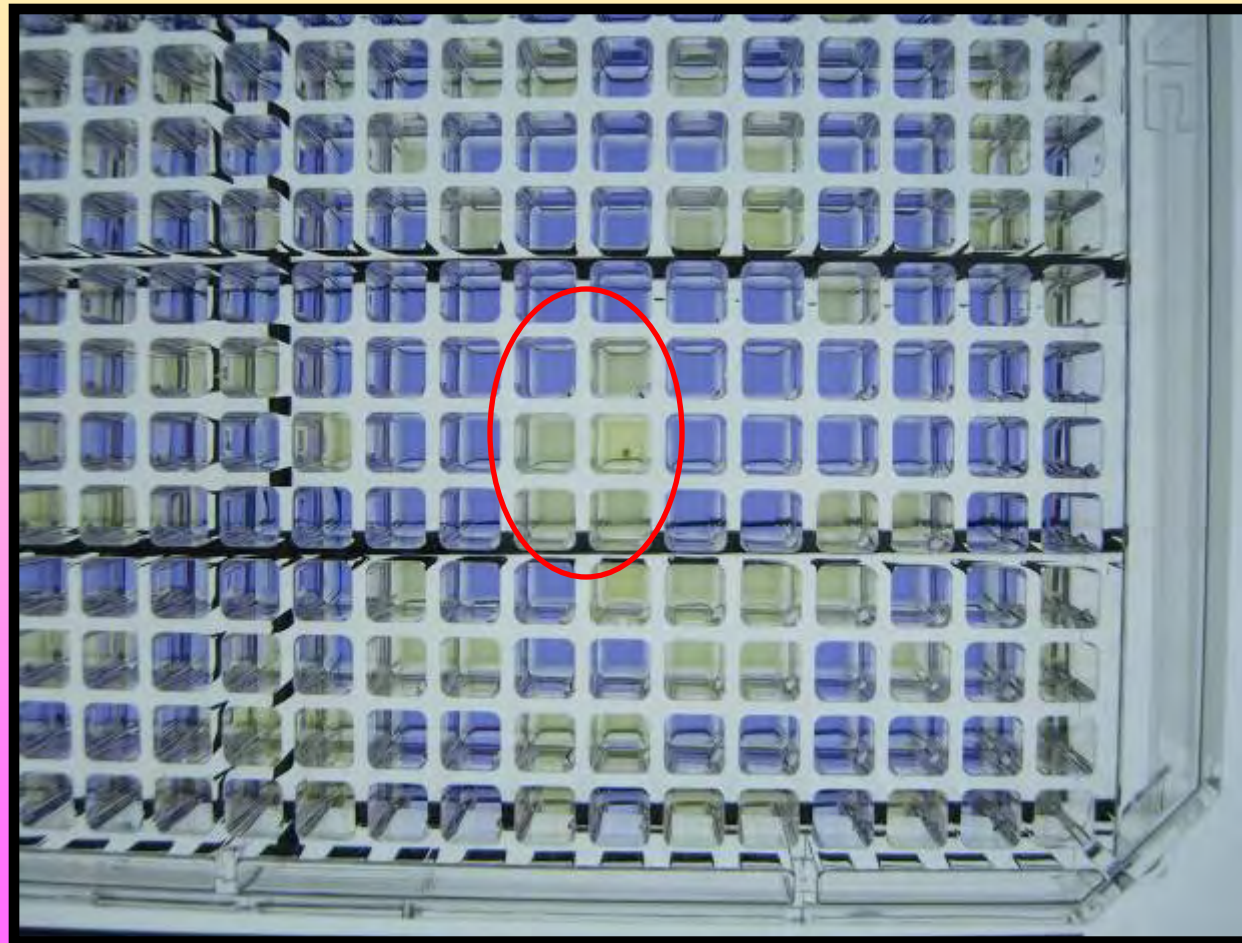
Note that colonies are often visible as small greyish dots. They can help to identify positive wells.



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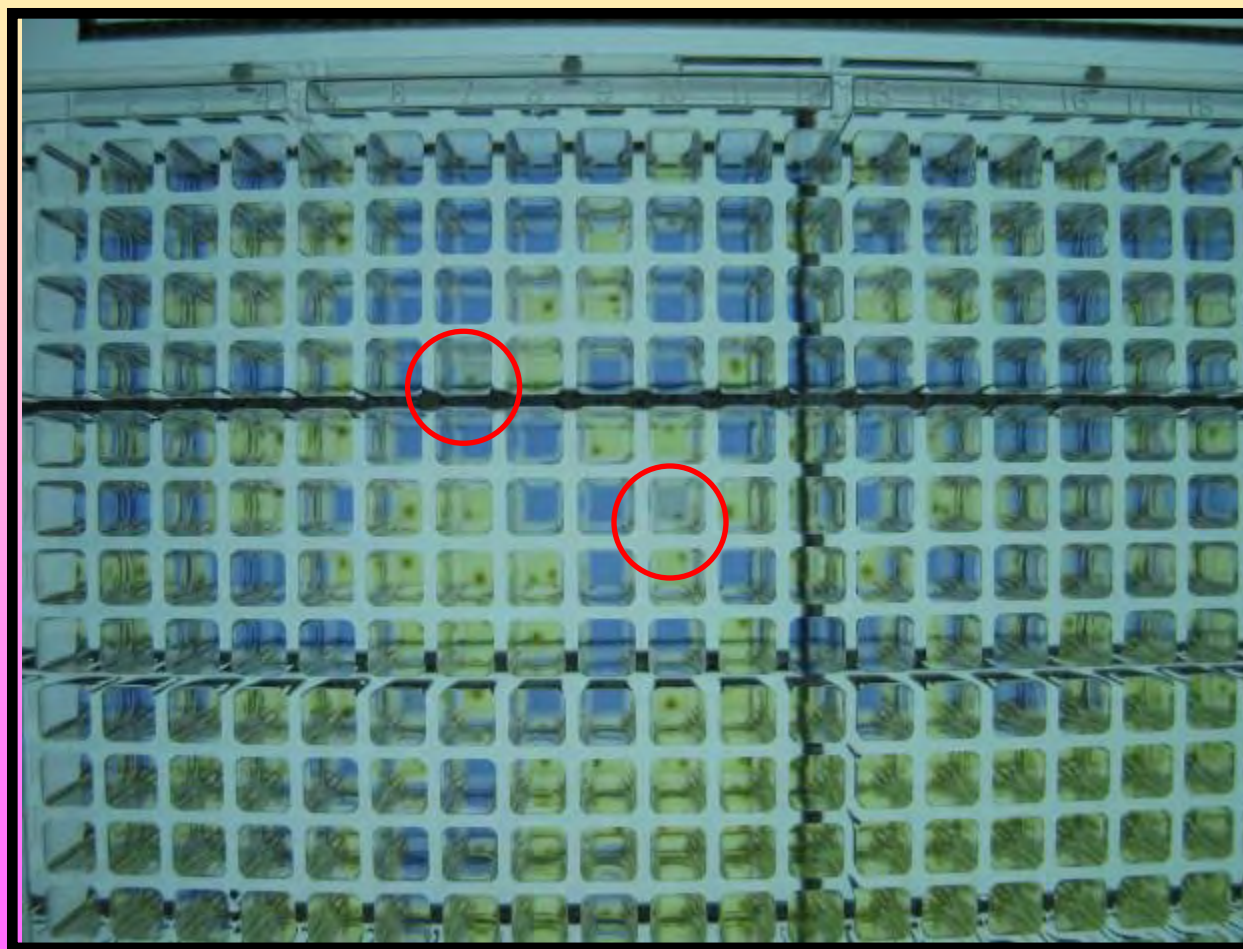


As these colonies are often not so clearly visible, the change of color should be used as the prime indicator of bacterial growth.



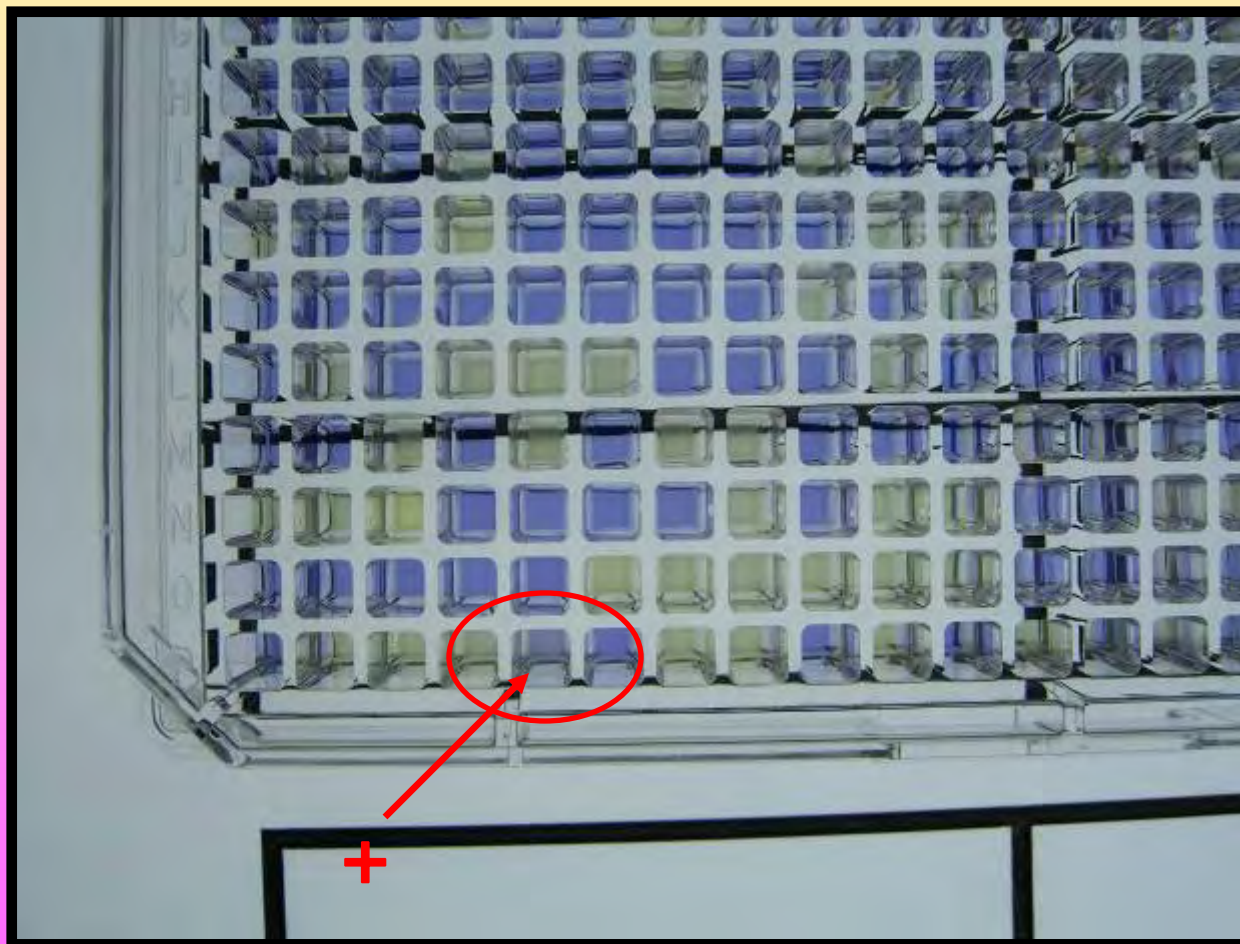
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Note that the intensity of the yellow color can vary....



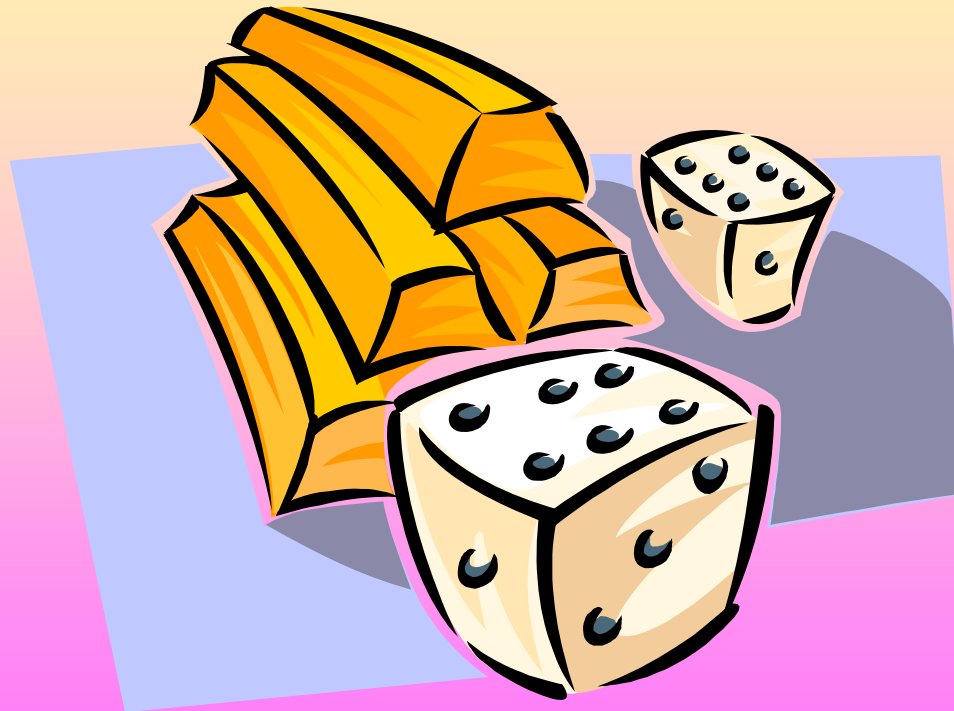
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....and even the slightest deviation from the negative purple color should be counted as positive!



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Data Evaluation



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Record the number of positive wells for each sample....

TA98 # Positive Wells -S9

Concentration	Plate 1	Plate 2	Plate 3
0			
1			
2			
3			
4			
5			
6			
+			

TA100 # Positive Wells -S9

Concentration	Plate 1	Plate 2	Plate 3
0			
1			
2			
3			
4			
5			
6			
+			

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Record the number of positive wells for each sample....

TA98 # Positive Wells -S9				TA100 # Positive Wells -S9			
Concentration	Plate 1	Plate 2	Plate 3	Concentration	Plate 1	Plate 2	Plate 3
0	4	2	1	0			
0.003125	4	3	6	1			
0.00625	8	6	12	2			
0.0125	25	22	21	3			
0.025	28	33	35	4			
0.05	42	47	44	5			
0.1	47	48	48	6			
+	48	48	48	+			

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....and transfer them to the free Xenometrix Excel Calculation Sheet

	A	B	C	D	E	F	G	H	I	J
1										
2										
3										Enter the negative (solvent) control
4										
5	Compound:									
6	-S9									
7	Conc. 0	Replicate #1	Replicate #2	Replicate #3			Spontaneous			
8							-S9			
9										
10										
11										
12										
13										
14	Pos. Control									
15										
16	Compound:									
17	+S9									
18	Conc. 0	Replicate #1	Replicate #2	Replicate #3			Spontaneous			
19							+S9			
20										
21										
22										
23										
24										
25	Pos. Control									

....and transfer them to the free Xenometrix Excel Calculation Sheet

	A	B	C	D	E	F	G	H	I	J
1										
2										
3										
4										
5	Compound:	CP-1								
6	TA 98 -S9									
7	Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3						
8	0.003	4	3	6						
9	0.006	8	6	12						
10	0.013	25	22	21						
11	0.025	28	33	35						
12	0.05	42	47	44						
13	0.1	47	48	48						
14	2-NF/4-NQO	48	48	48						
15										
16	Compound:	CP-1								
17	TA 98 +S9									
18	Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3						
19	0.003									
20	0.006									
21	0.013									
22	0.025									
23	0.05									
24	0.1									
25	Pos. Control									
26										

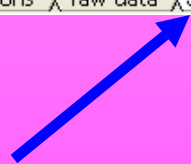
Spontaneous	
TA 98 -S9	
4	
2	
1	

Spontaneous	
TA 98 +S9	



In the “Summary” Tab, the results are calculated automatically, including a “Fold Increase over Baseline” value....

12										
13	CP-1									
14	TA 98 -S9					Assay Date:	21.09.2006			
15	Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
16	0	3	2.33		1.53	3.86				
17	0.003	3	4.33		1.53		1.86	1.12	0.0920	
18	0.006	3	8.67		3.06		3.71	2.24	0.0163	
19	0.013	3	22.67		2.08		9.71	5.87	0.0001	
20	0.025	3	32.00		3.61		13.71	8.29	0.0001	
21	0.05	3	44.33		2.52		19.00	11.48	0.0000	
22	0.1	3	47.67		0.58		20.43	12.35	0.0000	
23	2-NF/4-NQO				3	48.00	0.00			
24										
25	CP-1									
26	TA 98 +S9					Assay Date:	21.09.2006			
27	Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
28	0	0								
29	0.003	0						0.00		
30	0.006	0						0.00		
31	0.013	0						0.00		



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....and results from a T-test.

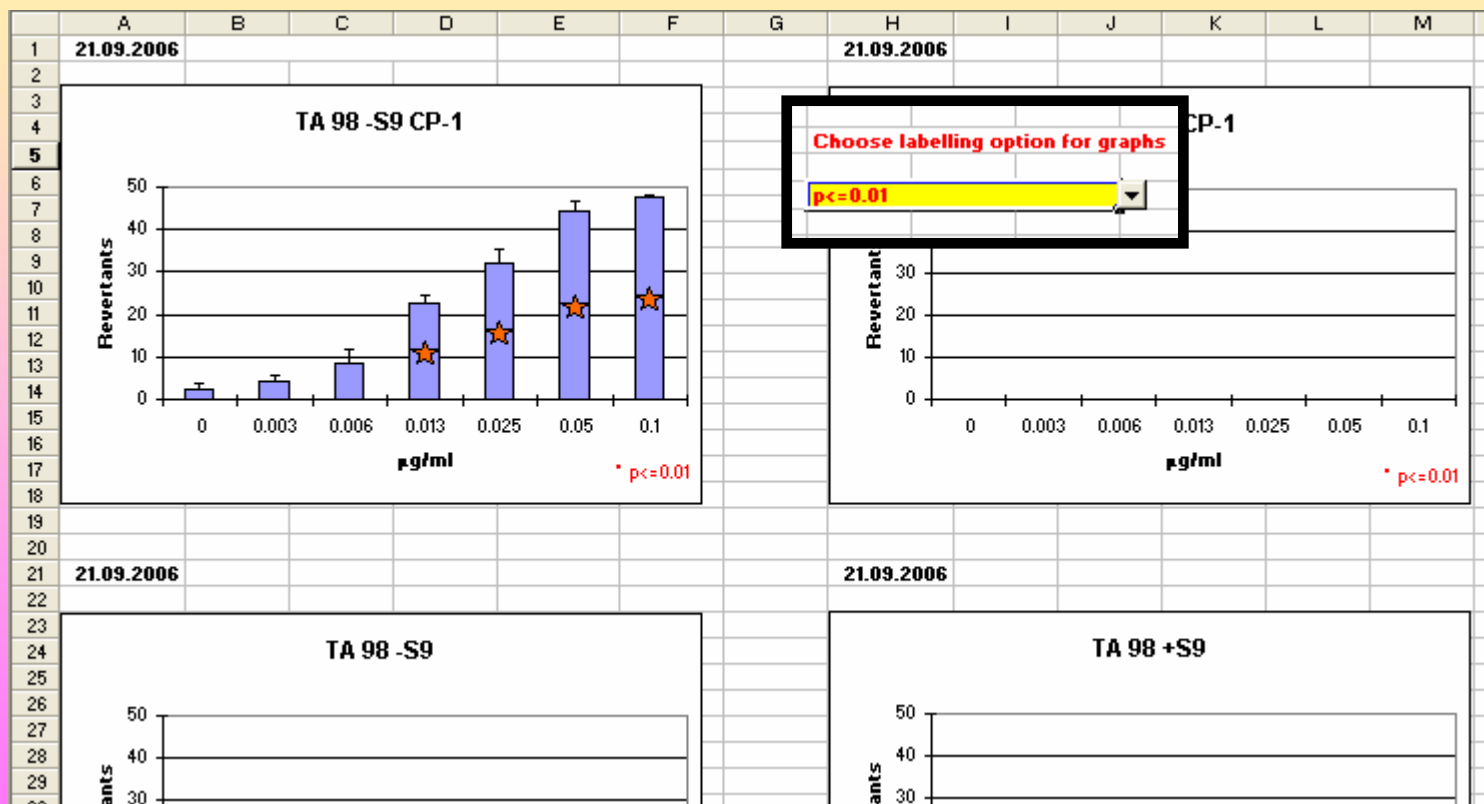
Fold Increases over baseline ≥ 2 and

T-test $p \leq 0.05$ and $p \leq 0.01$ are automatically highlighted red.

12										
13	CP-1									
14	TA 98 -S9					Assay Date:		21.09.2006		
15	Conc. ($\mu\text{g}/\text{ml}$)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
16	0	3	2.33		1.53	3.86				
17	0.003	3	4.33		1.53		1.86	1.12	0.0920	
18	0.006	3	8.67		3.06		3.71	2.24	0.0163	
19	0.013	3	22.67		2.08		9.71	5.87	0.0001	
20	0.025	3	32.00		3.61		13.71	8.29	0.0001	
21	0.05	3	44.33		2.52		19.00	11.48	0.0000	
22	0.1	3	47.67		0.58		20.43	12.35	0.0000	
23	2-NF/4-NQO		3	48.00	0.00					
24										
25	CP-1									
26	TA 98 +S9					Assay Date:		21.09.2006		
27	Conc. ($\mu\text{g}/\text{ml}$)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
28	0	0								
29	0.003	0						0.00		
30	0.006	0						0.00		
31	0.013	0						0.00		

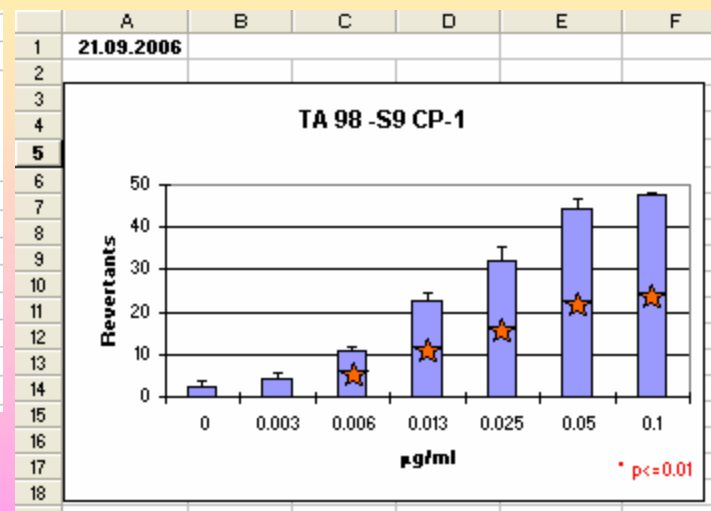
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In the "Graph" tab the results are represented as a Bar Graph with Error Bars and selectable significance indicators.



Based on these data you can decide if the tested compound is mutagenic or not.

CP-1					Assay Date: 21.09.2006			
TA 98 -S9					Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1 sided)
0	3	2.33	Corr. mean	1.53	3.86	1.86	1.12	0.0920
0.003	3	4.33		1.53		3.71	2.24	0.0163
0.006	3	8.67		3.06		9.71	5.87	0.0001
0.013	3	22.67		2.08		13.71	8.29	0.0001
0.025	3	32.00		3.61		19.00	11.48	0.0000
0.05	3	44.33		2.52		20.43	12.35	0.0000
0.1	3	47.67		0.58				
2-NF/4-NQO	3	48.00		0.00				



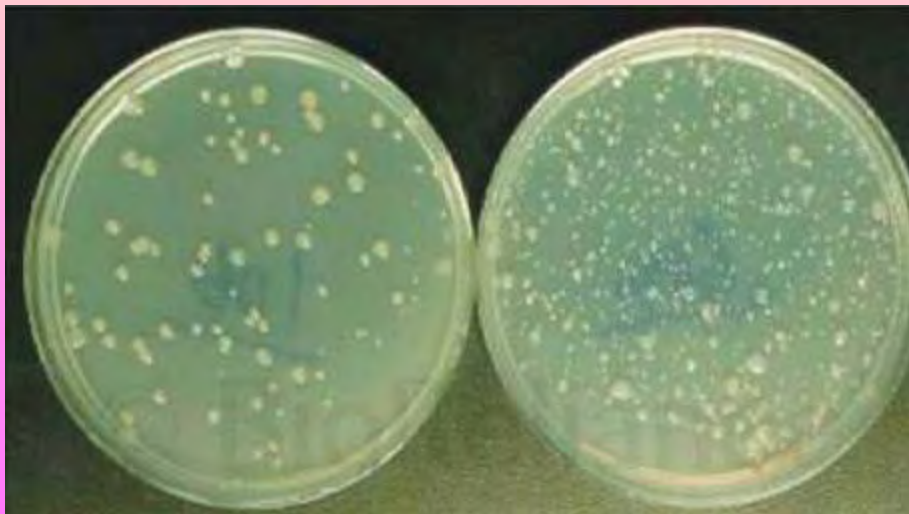
Generally, a “Fold Increase over Baseline” ≥ 2 and a dose effect are considered strong indicators of mutagenicity.

Ease of Result Evaluation Ames MPF / Conventional Ames Test: Colony Counting vs. Colorimetry



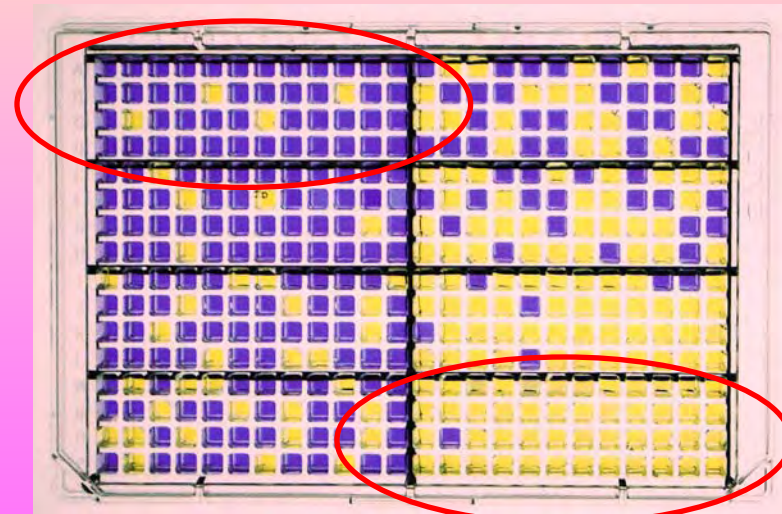
→ Much less tedious counting!

Negative Control = ~ 60



Positive Control:
over 400

Negative Control = 4

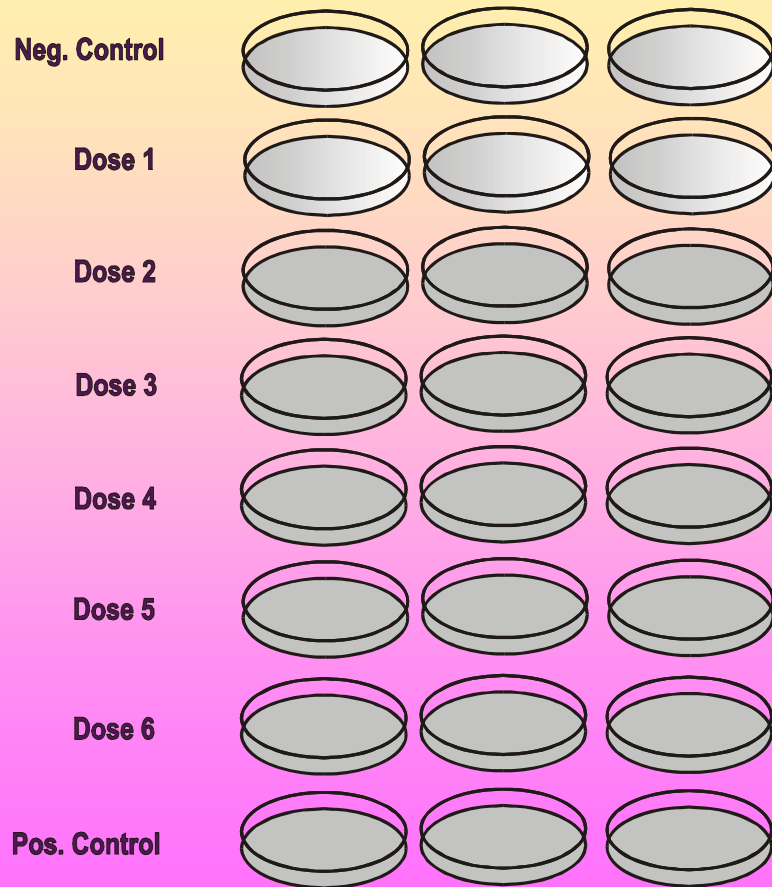


Positive Control:
47

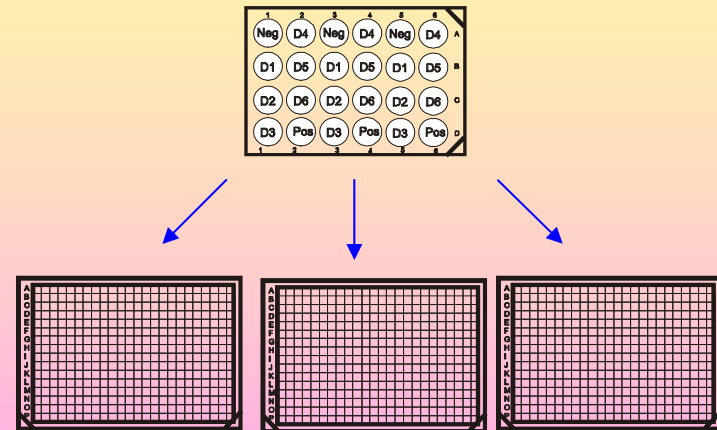
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Ames MPF needs much less plasticware than the conventional Ames Plate Assay!

(Example for 1 test compound, triplicates, 6 dilutions, controls)



24 Petri dishes



- 1 24-well plate
- 3 384-well plates