Users Manual

Pool & Superpool Matrix Pooling Technology

For BAC Library (or Fosmid Library)

Three Plate Superpools
Matrix Plate Format Comprised of
Three Superpools per 96-well Plate (Round II PCR)

For Superpool Systems constructed after January 1, 2008

Manual Version June 2009
February 18, 2003 **edited September 2018**

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File: 3PlateP&SPMatrixUserManual14.doc

Overview

The Superpooling and Matrix Pooling Technology has been designed to improve the efficiency and the robustness of PCR-based screening of BAC Libraries. The Matrix System reduces the PCR experiments by more than 50%! Thus the determination of the plate and well containing a BAC clone of interest is achieved by only half of the amount of PCR experiments normally required with traditional plate/row/column pooled BAC Libraries. In this Matrix Pool System, two PCR bands are required to confirm a positive hit, allowing the identification system to compensate for false positive bands or missing bands (false negatives).

Each Pool & Superpool kit is custom built for the specific needs of the researcher. In the Three Plate Matrix System, each Superpool is composed of three 384-well plates so 1,152 individual BAC clones per Superpool. The number of BAC clones in the library determines the total number of Superpools needed. The kit comes with two identical sets (A & B) of plates comprised of the Superpool Plates and the Matrix Pools Plates with the extracted DNA from independently grown, then separately pooled BAC clones.

Library screening is performed in two separate rounds: 'Round I PCR' & 'Round II PCR'.

Round I PCR is performed on the Superpool Collection Plate. The results from Round I PCR will identify which Superpool(s) contains the BAC clone(s) with the sequence of interest (there may be more than one Superpool identified). The researcher may choose to pursue one or more positive hits from Round I PCR.

The Round II PCR is then performed on the Matrix Pools Plate(s) for the specific Superpool identified in Round I PCR. The Matrix Pools are comprised of Plate Pools, Row Pools and Column Pools (PRC). There is a total of 21 Matrix Pools for each Superpool:

- Three Plate Pools (PP)
- Eight Matrix Row Pools (MRP)
- Ten Matrix Column Pools (MCP)

Round II PCR requires ONLY 21 PCR experiments plus controls (for each positive hit pursued from Round I PCR). The results from Round II PCR should allow the researcher to identify the plate and well position of a single positive hit. In comparison, a traditional plate/row/column pooling strategy, Round II PCR screening WOULD require 43 PCR reactions plus controls.

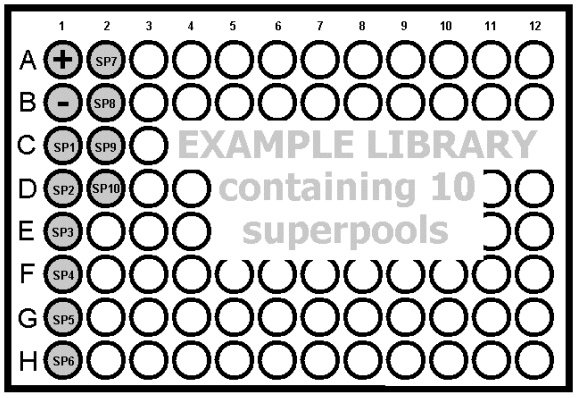
The interpretation of positive hits also called "BAC clone library plate & well deconvolution", from Round II PCR (screening of the Matrix Pools) is done, by comparing the positive hits seen on the electrophoresis results to the Matrix Pool Keys in this manual. The Keys are necessary to provide the location (plate, row and column) of all positive clones from Round II PCR. The BAC clone deconvolution can also be performed using our online system:
http://puffer.ampliconexpress.com/
The online system is compatible with CSV files for high throughput analysis of PCR results.

GRAPHICAL OVERVIEW

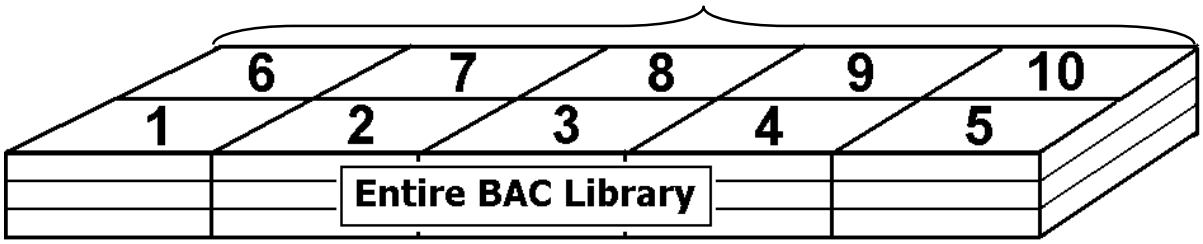
The researcher will receive two sets of three identical Superpool Collection Plates, which will be used for Round I PCR.

Each of the six Superpool Plates will provide enough template for at least 500 PCR experiments (A1, A2, A3, B1, B2, B3 is 6 x 500 = 3,000 Round I PCRs).

After viewing Round I PCR electrophoresis results, the researcher will determine which Superpool(s) to screen in Round II PCR.

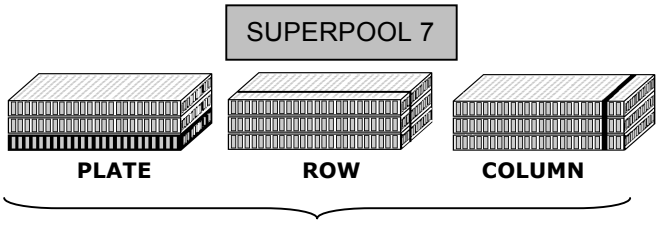


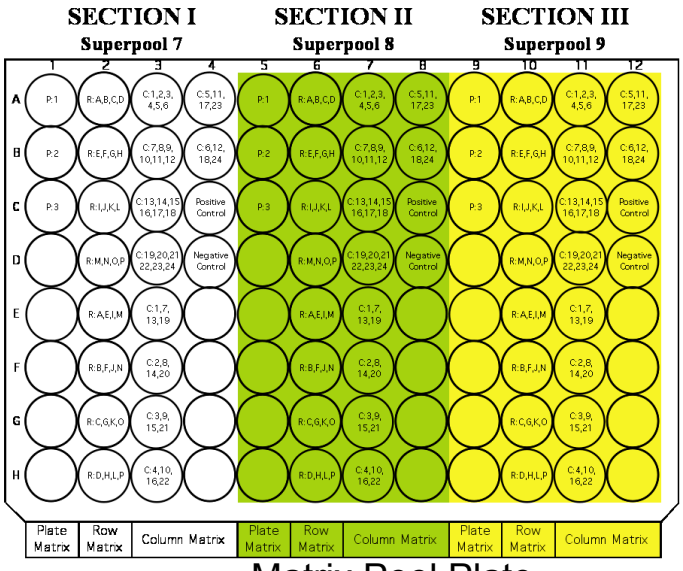
Library Code Superpool Collection Plate Copy #1
Library plates 001-030



(Superpool 7 is used in the screening example starting on p.14)

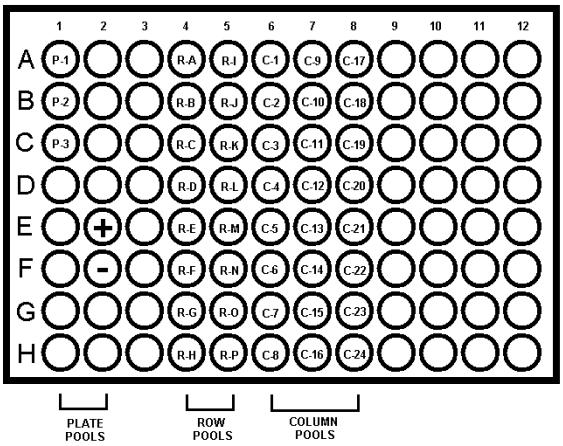
The BAC library is separated into sequential Superpools of three 384-well plates.
Each Superpool of 3 plates is further separated into 3 Plate pools, 16 Row pools and 24 Column pools (PRC).





Matrix Pool Plate

Plate Row Column (PRC) Plate
For Superpool 7



The PRC pooled DNA is further combined onto Matrix Pool Plates.
The researcher will receive two identical Matrix Pool Plates (Set A & Set B) for each Superpool to perform Round II PCR.

Contents of Kit (Biological Materials and Documentation Supplied)

All plates (Superpool & Matrix) should be stored at -20ºC. It is best to reduce the number of freeze/thaw cycles as much as possible. If the plates are going to be used at least one time per week, they should be refrigerated rather than frozen to reduce the freeze/thaw cycles.

Each Pool & Superpool kit comes with the following items:
1. Instruction Manual with detailed examples, graphics, Superpool Key and Matrix Pools Keys, (Matrix Plate, Matrix Row and Matrix Column).
2. SIX identical sets of Superpool DNA (on 96-well plates) for the Round I PCR. These are called "Set A1, Set A2, Set A3, Set B1, Set B2, Set B3". The Superpools are aliquoted and delivered on SIX 2 mL 96-well plates. This helps reduce the risk of contaminating the entire collection.
3. TWO identical sets of Matrix Plate, Matrix Row and Matrix Column DNA Pools for each Superpool (delivered on TWO 1 mL 96-well plates) to be used in Round II PCR. These are called "Set A and Set B". Remember, each Superpool will have a corresponding section of a Matrix Pool Plate 96-well plate (two aliquots to help reduce the risk of contamination).
4. Two identical 2.0 mL tubes each containing 1.0 mL of both PCR control primers for the positive PCR control at a concentration of 10 µM for each primer. There is enough primer mix for about 1,000 positive PCR control reactions.
5. Amplicon Express also made a Set C of the entire resource to use for technical support and will store it at -20ºC for 1 year.
6. Clone deconvolution can be performed using the Keys (starting Page 20) or the online system: http://puffer.ampliconexpress.com/

Note: the resources should be stored at -20ºC until needed (and stored at 4ºC when in use). We recommend placing Set A and Set B plates in different -20ºC freezers (in case of freezer failure).

There is ~6,000 µL (~6.0 mL) of Superpool DNA supplied for each Superpool (~1,000 µL aliquoted on SIX identical 2 mL volume 96-well plates), enough for approximately 3,000 Round I PCR experiments (BAC library screenings). These are called "Set A1, A2, A3, B1, B2, B3".

There is ~1,600 µL (~1.6 mL) of DNA supplied for each of the Matrix Pools (~800 µL (~0.8 mL) aliquoted on two identical 1 mL 96-well plates), enough for approximately +500 Round II PCR experiments on each Superpool. Matrix Pools are used for exact clone deconvolution from Round I screenings. These Matrix Pools are called "Set A and Set B".

For technical support please contact Robert Bogden bogden@ampliconexpress.com
or call 1-509-332-8080 (9am to 5pm US Pacific Time).

Quick Start Users Guide

Checklist For Using This Kit:

Initial steps

1. Identify the 'sequence of interest' by a variety of methods (i.e. similarity to other genes, or organisms, etc.).
2. Design robust PCR primers to amplify the sequence of interest (i.e. no hairpins, matching annealing temperatures and minimal duplex formation).
3. Perform BLAST search for known sequences using the primer sequences to identify potential problems (i.e. primers designed on a repetitive element).
4. Have newly designed PCR primers manufactured, we recommend: http://www.idtdna.com/PrimerQuest/Home/Index

Preliminary Steps

5. Test amplification with above primers on genomic DNA and run positive and negative genomic controls (chosen by the researcher) and other controls for identifying proper Mg^{++} concentration and other PCR reagents according to the directions supplied with each.
6. Identify appropriate PCR annealing temperature, amplification, cycling and electrophoresis conditions that generate the appropriate band on gel electrophoresis (see page 11 for suggested PCR conditions for the included PCR amplification controls).
7. Once a robust PCR protocol has been determined and tested on genomic DNA and controls are favorable, the researcher is ready for screening the Superpool Collection Plate.
8. Make a PCR master mix with information gained in step 5 and tested in step 6 to screen the Superpool DNA. Note- there are 8 Superpools in the following example plus controls.

IT IS IMPORTANT TO KEEP THE **PCR SET-UP AREA** COMPLETELY SEPARATE FROM ALL AMPLIFIED PCR PRODUCTS.

Superpool Screening

9. Prepare the PCR set-up area with careful attention to insure NO contamination of the Superpool Collection Plate can occur. If the wells are accidentally contaminated with genomic template, the pooled DNA source has been irreversibly contaminated, the complete plate must be discarded, and the reserve plate used.
10. Thaw the Superpool Collection Plate and keep it on ice.
11. Spin down Superpool Collection Plate to remove any liquid on the mat (lid). Carefully remove the mat and be certain that the mat and the plate are not contaminated.
12. Prepare PCR tubes using the PCR master mix prepared in step 8 and the Superpool Collection Plate DNA template and any additional controls needed.
13. Carefully reapply the mat paying close attention to the mat label and orientation and refrigerate (4°C) or refreeze the Superpool Collection Plate DNA template (-20°C).
14. Run the PCR experiment for the appropriate cycles determined in step 6.

Gel Electrophoresis of Superpool Collection Plate PCR Experiment

15. Prepare appropriate gel for observing PCR products of interest (usually 0.8 to 2.0 % agarose).
16. Load gel with ladder and PCR products.
17. Electrophoresis with standard conditions determined in step 6.
18. Stain gel and record which Superpool(s) have "positive hits" bands (sequences of interest).

IT IS IMPORTANT TO KEEP THE **PCR SET-UP AREA** COMPLETELY SEPARATE FROM ALL AMPLIFIED PCR PRODUCTS.

Superpool of interest Screening

19. Prepare a PCR master mix (like in step 8) for 21 reactions plus controls to follow one Superpool positive hit. If additional Superpool hits are to be tracked, then make sufficient PCR master mix for all interested Superpools.
20. Clean the PCR set-up area with careful attention to assure NO contamination of the Superpool or Matrix plates can occur. If the wells are accidentally contaminated with genomic template or Superpooled template, the pooled DNA source has been irreversibly contaminated and the complete plate must be discarded and the (one) reserve plate used.
21. Thaw the Superpool Matrix Plate identified in step 18 and keep it on ice.
22. Spin down Superpool Matrix Plate to remove any liquid on the mat (lid). Carefully remove the mat and be certain that the mat and plate are not contaminated.
23. Prepare PCR tubes using the PCR master mix prepared in step 19 and the Superpool Matrix Plate DNA template (thawed in Step 21) and any additional controls needed.
24. Carefully reapply the mat paying close attention to the mat label and orientation and refrigerate (4°C) or refreeze the Superpool Collection Plate DNA template (-20°C).
25. Run the PCR experiment for the appropriate cycles determined in step 6.

Gel Electrophoresis of Superpool Matrix Plate PCR Experiment

26. Prepare appropriate gel for observing PCR products of interest (usually 0.8 to 2.0 % agarose.
27. Load gel with ladder and PCR products.
28. Electrophoresis with standard conditions determined in step 6.
29. Stain gel and record which Matrix Plate Pool wells have "positive hits" bands.

Interpret the Matrix gel data to identify the specific Plate, Row and Column

30. Compare the positive Plate Pool (PP) gel bands to the Plate Pools Key on page 23. Use the gel banding patterns or Plate Pool Key on page 23 to identify the original source plate(s) location.

31. Compare the positive Matrix Row Pool (MRP) gel bands to the Matrix Row Pools Key on page 24. Record all possible intersections of the gel bands on the Matrix Row Pools Key. These intersections are the row(s) pools with the hit(s). Alternately, use the gel banding patterns on page 24 to identify the original source row(s) location.

32. Compare the positive Matrix Column Pool (MCP) gel bands to the Matrix Column Pool Key on page 25. Record all possible intersections of the gel bands on the Matrix Column Pool Key. These intersections are the column(s) pools with the hit(s). Alternately, use the gel banding patterns on page 25 to identify the original source column(s) location.

Final identification of the Clone of Interest (If ONLY 1 Plate, 1 Row, and 1 Column are identified during matrix interpretation)

33. Use the Superpool hit data recorded in step 18 and the Plate Pool data recorded in step 30 and look at the Superpool Key on page 21 to identify the number of the library plate of interest.
34. Use the Row Pool data and Column Pool data determined in steps 31 and 32 to identify the clone well location on the library plate identified in step 34.

Feel free to use our online BAC clone deconvolution system:
http://puffer.ampliconexpress.com/
Following the online link: choose "Online Clone Search", then under the Preset Configuration select "3-Plate-Superpool-3-Plate-Matrix" and then enter the Superpool # and the PCR results (Data Pool) on the Graphical User Interface (bold band, weak band, no band etc.). CSV files can also be uploaded for high throughput analysis.

Final identification of the Clones of Interest (If MULTIPLE Plate, Row and Column hits are identified during matrix interpretation)

35. Use the data recorded in steps 30, 31 and 32, and write down all the possible Plates, Rows and Columns the clones could possibly be located.
36. The ambiguities can be resolved in the clone confirmation test.

Clone Confirmation Test

37. Prepare antibiotic media for growing the individual clones identified in steps 34 and 35.
38. Carefully remove the clone of interest from the BAC Library Source Plate and inoculate media prepared in step 37. The BAC Library Source Plates are not included in the Pool & Superpool Kit; if you do not have a copy of the BAC library, please contact Robert Bogden <bogden@ampliconexpress.com> to obtain a copy. Many researchers have a variety of methods for aseptically removing a single clone from 384 well plates without thawing the entire plate. These methods include sterile toothpicks, a sterilized loop that is still warm enough to melt the top of the well of interest, and a number of other individual preferences.
39. Grow the clones and make a confirmatory test with the primer pair on lysed cells to be certain that the clone of interest has been located. We also recommend including another clone in this confirmation test that is not identified as a hit for a negative control.

Troubleshooting FAQ

1. Can I use smaller volumes of template or reduced PCR reaction volumes?

We have tested for sample template at 35 rounds of PCR. Allowing for some template degradation with multiple freeze/thaw cycles, we would not recommend less than 2 µL of template. We have included sufficient template pools for about 3,000 PCR primer pair screenings of the Superpool Collection Plates and about +500 screenings of the Matrix Plates. Some researchers have reduced the volume with good success but we do not offer technical support or troubleshooting support for template volumes below 2 µL (or final PCR volumes below 20 µL).

2. All the Superpools are showing hits, what can I do and what could this mean?

Check the positive and negative genomic controls, if the PCR primers are not stringent enough, there can be broad amplification. Try increasing the PCR annealing temperature. If this does not help, the primer sequences could be part of a repeating element or other common sequence that would have multiple copies in the genome.

Blast your primers against databases to look for possible common sequence homology.

You could also have genomic DNA contamination in your Superpool Collection Plate.

3. The bands are not all the same intensity, does that mean something is wrong?

There are a number of factors that influence amplification efficiency. Most matrix pools have 288 different clones but some have 384 clones in each matrix pool and others have only 192 clones (See the Matrix Keys sections for clone counts). If the Key indicates the bands would be from one specific plate, it is likely that you have found a hit. If they would indicate different source plates, it could be that PCR amplification parameters in Quick Start User Guide step 6 are not as robust as initially thought.

4. There are 3 bands in one of my matrix gels, what could this mean?

It could mean that there are two clones that have been identified as hits. Use the Keys to identify what two sources they came from. There could be as many as 4 clones identified with 5 bands if they all were in one matrix pool and then they would have to be in four other separate matrix pools. You should see more than 2 bands in other matrices as well. Follow Quick Start Users Guide steps 35, 36, 37 and 38 for multiple hits resolution.

5. I still cannot resolve the issue of 3 bands in only one of my matrix gels, what can I do?

Follow the Clone Confirmation Test in Quick Start User Guide steps 37, 38 and 39. You can also use our online clone-screening program at: http://puffer.ampliconexpress.com/

If the issue remains unresolved, call technical support for further suggestions.

6. Is there any way to screen all the Superpools in one round of PCR with fewer PCR experiments than running all the Superpool collection and all the Superpool Matrix Plates at the same time?

Yes, please call technical support for additional information.

7. I think I may have contaminated my plates, is there any way to check?

Rerun an earlier primer pair that did not have hits in the questionable Superpool or a primer pair that had correctly been screened.

8. The Matrix Keys are too confusing to follow each time I have a hit, what can I do?

There are two options; the first is to call technical support for pointers and a phone tutorial. Please email a list of at least 10 hits that you have found but have had difficulty in resolving. The second solution is to do more PCR experiments by using our standard PCR system where there are 43 reactions for each Superpool instead of the 21 reactions in the Matrix system for the same Superpool.

9. I have used up all of my Superpool Collection stock. Can I get more?

Yes, we are able to supply additional pooled stocks at a discounted price for original purchasers of our Pooling and Superpooling System. Please contact technical support for further information and costs.

For technical support please contact Robert Bogden bogden@ampliconexpress.com
or call 1-509-332-8080 (9am to 5pm US Pacific Time).

Screening Recommendations

We highly recommend researchers develop a robust PCR protocol for proper amplification of positive and negative controls before screening the Superpool DNA. There is a positive control DNA template included in all plates (Superpools and Matrix Pools). The positive control DNA templates are at the same relative concentrations as the corresponding pools. The positive control primers have a 57 ºC annealing temperature and produce a 607 bp.

The positive PCR control primers are:
AM001-C12-M13-F (20 mer) Td=57.3 5' ATATAATGCAAAAAGTGGCT 3'
AM001-C12-M13-R (20 mer) Td=57.6 5' GTAATGACCCTTTTCTCTCC 3'

A recommended PCR protocol for amplifying the positive control template follows:
2 µL DNA positive control from Superpool plate or a Matrix Pool plate
2 µL Primers for positive control (both primers are in the tube and give a 607 bp product)
2 µL 10X PCR buffer and dNTP's (supplied with TAQ polymerase, use as directed)
4 µL [10mM] Mg^{2+} (2 mM final concentration)
1 µL TAQ Polymerase (~1 Unit)
9 µL H_2O

20 µL final volume

Thermocycler Conditions:
Step 1 94ºC for 120 seconds
Step 2 94ºC for 30 seconds
Step 3 57ºC for 30 seconds
Step 4 72ºC for 60 seconds
Step 5 Go to Step 2 for 35 cycles total
Step 6 72ºC for 600 seconds
Step 7 Hold at 4ºC

Internal Standard:

As part of our Quality Assurance protocol, we choose a missed well in one of the Superpools of each BAC Library and replace the missed well with a Positive Control BAC clone. This is the same BAC clone that we use for our positive control. The Superpool, plate and well location of this positive control is different for each library. This positive clone is a BAC clone of approximately 130 Kb from *Anaplasma marginale*. If you have a positive hit from your primers of interest in the QA control well identified for your library, the hit is not from your organism. Please contact Technical Support if you identify a hit from your primers on this QA control BAC clone.

Detailed Description of Pools & Superpools:

The system consists of a collection of multiple Superpools and their corresponding sets of Matrix Pools. The screening starts with the Superpools in Round I PCR and determines which set of corresponding Matrix Pools to screen during Round II PCR. The Superpools and Matrix Pools are prepared independently and provided in duplicated sets of 96-well plates.

Superpools:
Each Superpool consists of three consecutive 384-well plates from a BAC library (see Superpool Plate Key page 21). The DNA is prepared after growing EACH BAC CLONE separately (to avoid growth competition between BAC clones), then combining the 1,152 cultures into one large-scale BAC DNA Prep. The Superpool of BAC DNA is then aliquoted onto two sets of 3 identical 96-well plates: the **Superpool Plate**.

Superpool SP-1 has the DNA of all the 1,152 BAC clones from the first three plates of the BAC library (Plate 001 to Plate 003).

Superpool SP-2 has all the DNA of all the 1,152 BAC clones from the second three plates of the BAC library (Plate 004 to Plate 006).

This naming continues for the entire library. The total number of Superpools is determined by the total number of clones in the BAC library.
Please see the Superpool Plate Key on page 21 of this document for exact details.

Matrix Pools:
To each Superpool corresponds a set of 21 Matrix Pools. Each set of Matrix Pools is aliquoted into two identical **Matrix Pools Plates** to help reduce the risk of contamination. The Matrix Pools of Superpool #1 are named as follow:

Plate Pools: 1PP-A1 through 1PP-C1 for the 3 wells that contain the matrix of plates 1-3 in Superpool #1. Matrix Plate Pools contain 384 clones. See the Matrix Plate Pool Key on page 23 for exact composition of each well.

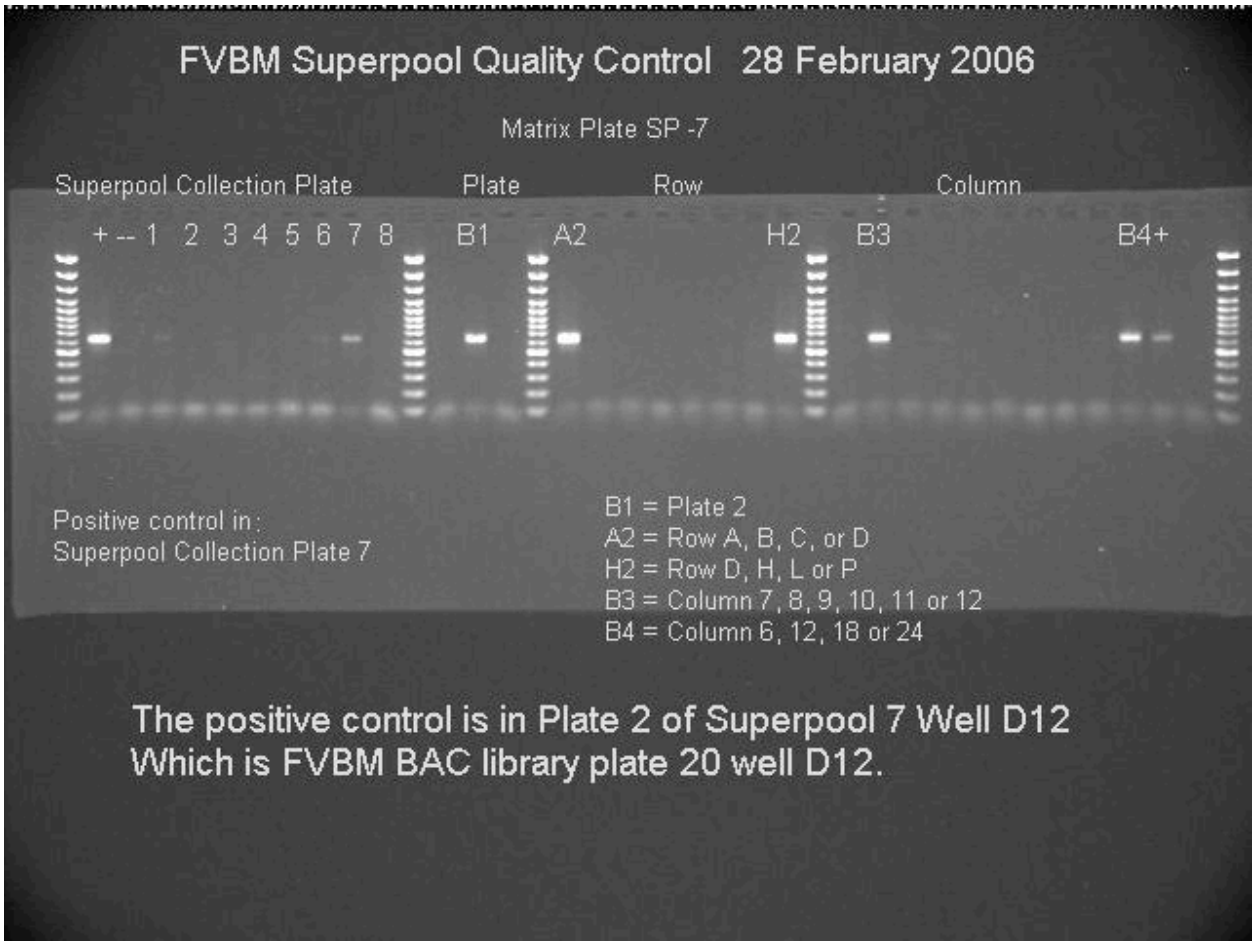
Matrix Row Pools: 1MRP-A2 through 1MRP-H2 for the 8 wells that contain the matrix of rows A-P in Superpool #1. Each Matrix Row Pool contains 288 clones. See the Matrix Row Pool Key on page 24 for exact composition of each well.
(3 different plates x 4 different rows x 24 row wells/plate=288 clones per Matrix Row Pool)

Matrix Column Pools: 1MCP-A3 through 1MCP-B4 for the 10 wells that contain the matrix of columns 1-24 in Superpool #1. See the Matrix Column Pool Key on page 25 for exact composition of each well. The Matrix Column Pools in wells A3 through D3 have 288 clones (3 different plates x 6 different columns x 16 column wells/plate=288 clones per Matrix Column Pool). The Matrix Column Pools in wells E3 through B4 contain 192 clones (3 different plates x 4 different columns x 16 column wells/plate=192 clones per Matrix Row Pool).

Please see pages 23-25 for the exact composition of each well in the Matrix Pools.
Remember that each Superpool has its own corresponding set of Matrix Pools on a Section of one of the Matrix Pools Plates.

For technical support please contact Robert Bogden <bogden@ampliconexpress.com> or call 1-509-332-8080 (9am to 5pm US Pacific Time).

The following pages will step you through the process of clone identification. Each step will refer to one section of the complete gel photo seen here.

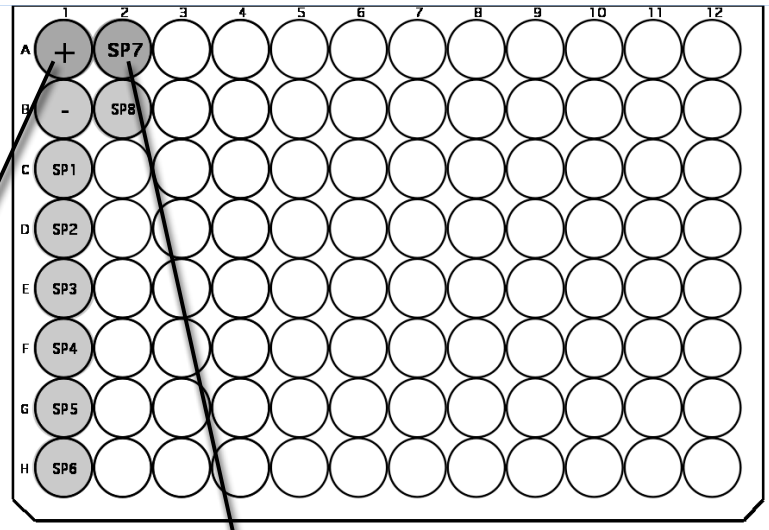


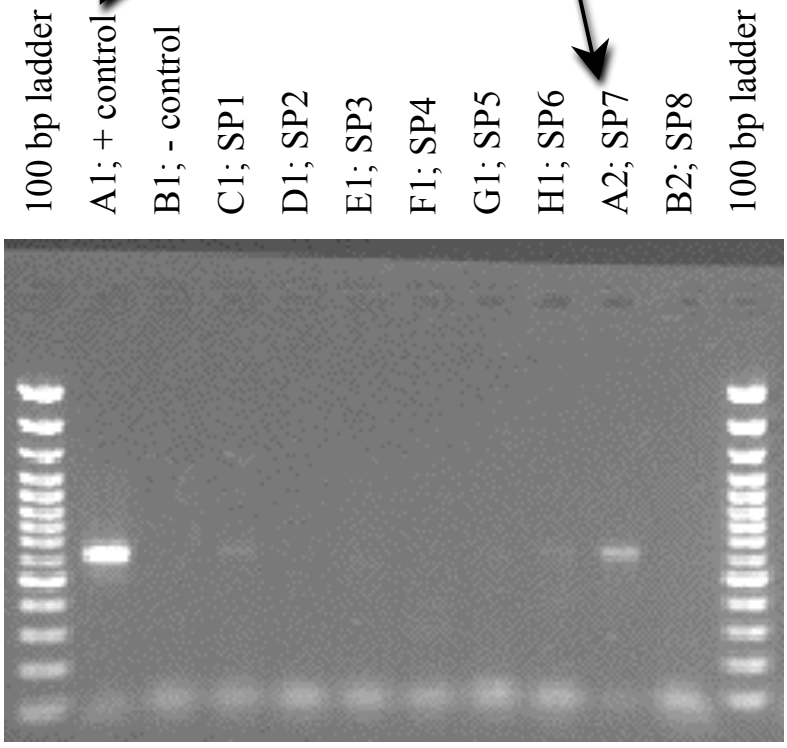
Example Clone Identification (Round I PCR):

SCREENING THE SUPERPOOLS

In this example, using an 8-Superpool library, the Round I PCR gel results show "hits" in the positive control (using positive control primers with a 607 bp product) and Superpool 7 (to find the plate and well location of our QA control). This QA control is placed in all libraries to provide a Quality Assurance test and to enable researchers to test the Pool and Superpool method on a known control.

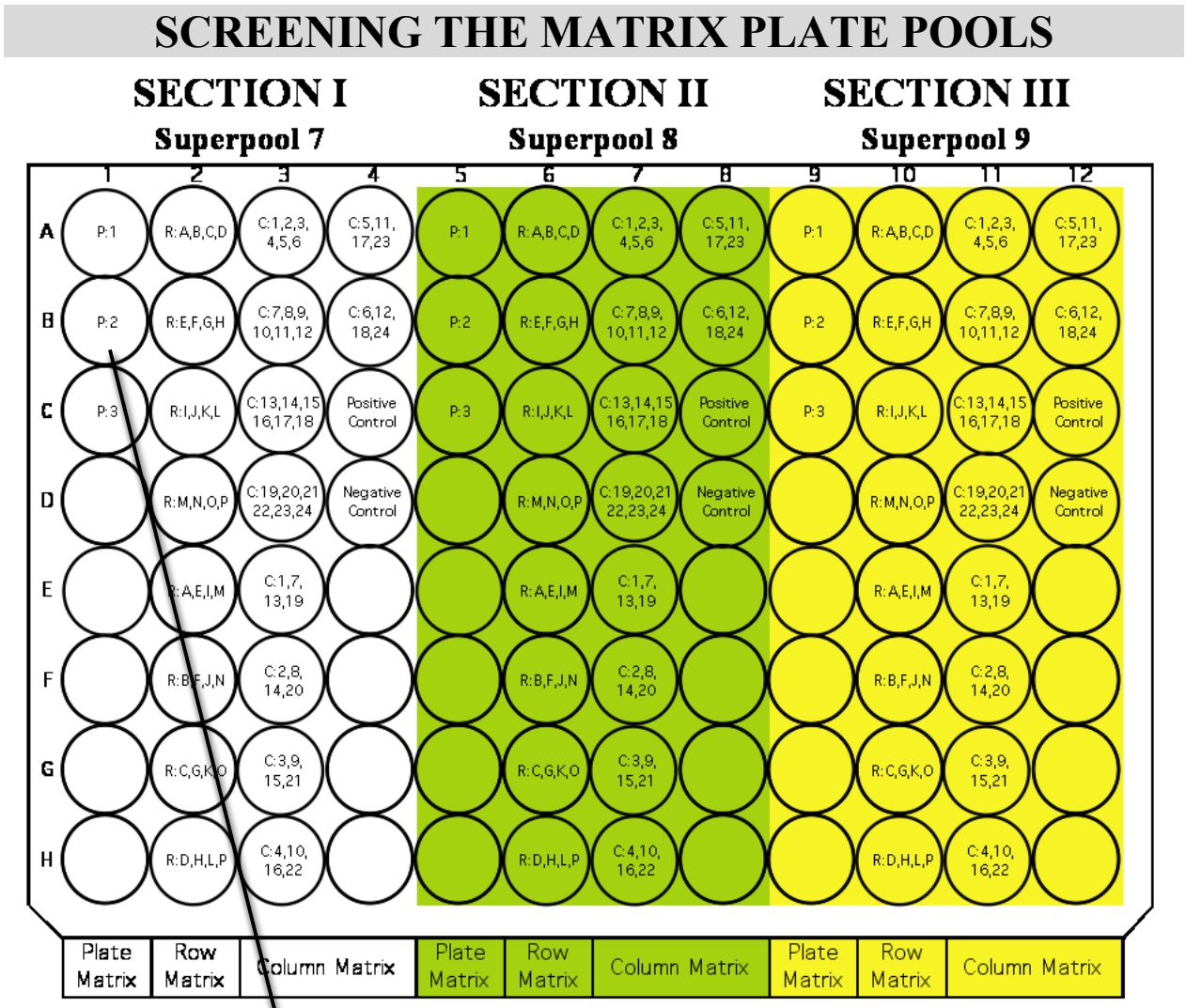
The researcher will now proceed with Round II PCR on the Superpool 7 Matrix Pool Plate Section.

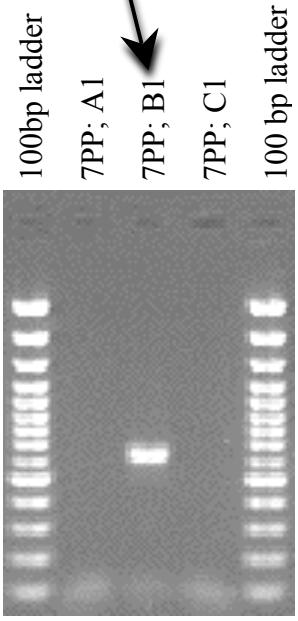




"Superpool Collection Plate" Far-left section of the photo on page 13.

Example Clone Identification (Round II PCR):





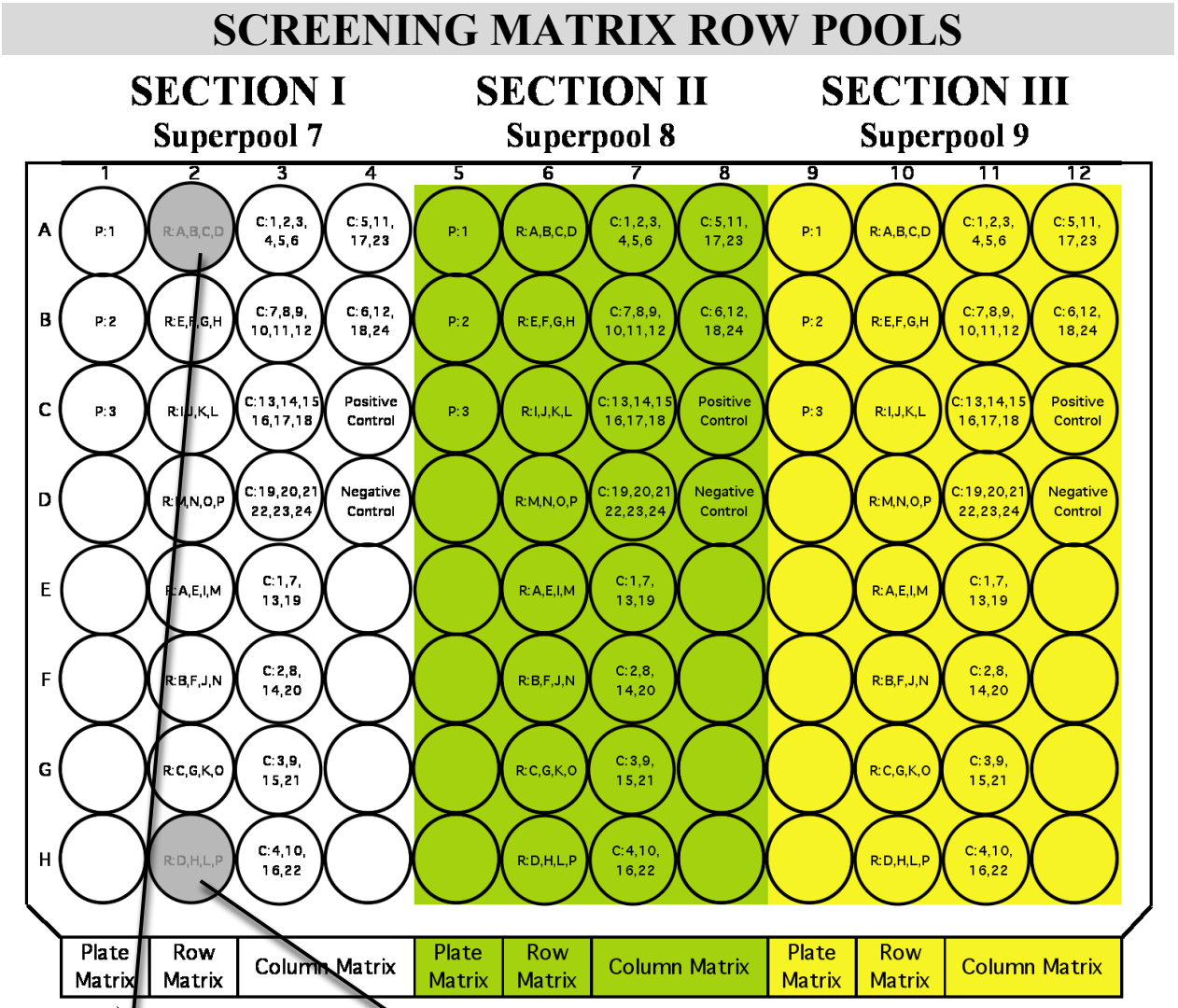
The results of Round II PCR gel electrophoresis for the SP-7 Plate Pools show a positive hit in well B1 of the SP-7 Plate Pools.

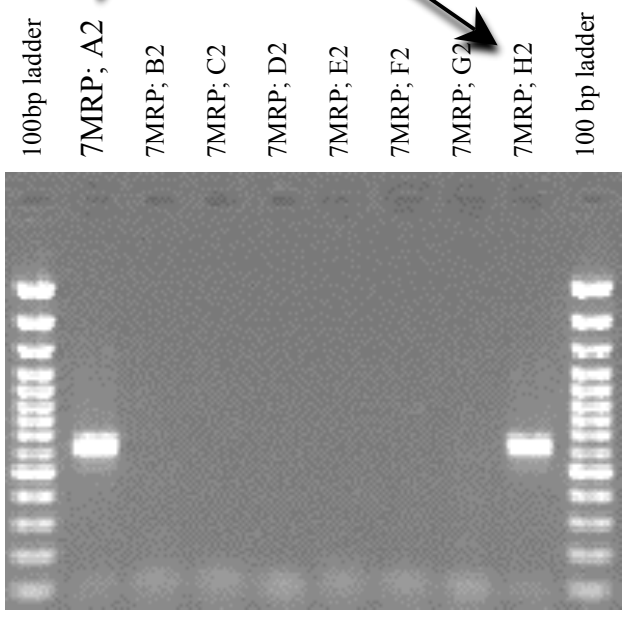
The Matrix Plate Loading pattern and the Plate Pool Key (page 23) show that the clone of interest is in Plate Pool 2.

The actual Library plate corresponding to SP-7 plate 2 position is Library plate #020 (see Superpool Key on page 21).

"Plate" left-middle section of the photo on page 13.

Example Clone Identification (Round II PCR):





The results of Round II PCR gel electrophoresis for the SP-7 Matrix Row Pools show a positive hit in wells A2 and H2 of the SP-7 Matrix Row Pools.

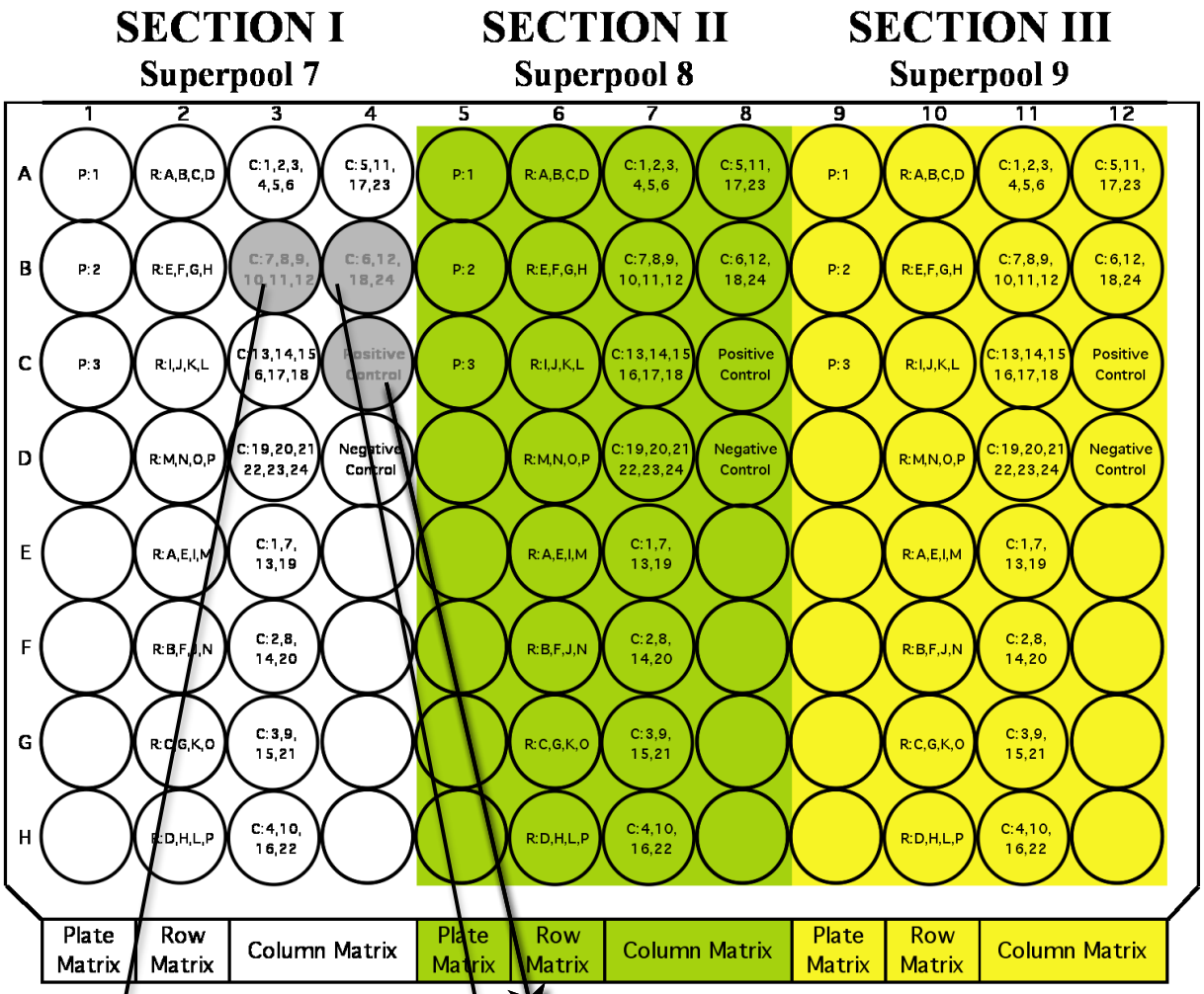
The Matrix Plate Loading pattern and the Matrix Row Key (page 24) show that the clone of interest is in Row D.

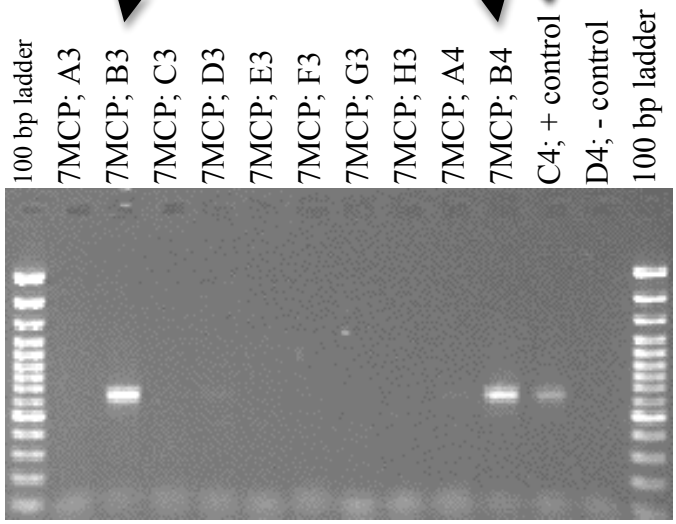
This result, combined with the Round II Plate Pool result, show the clone of interest is in: SP-7 plate 2 Library plate #020, Row D.

"Row" Right-middle section of the photo on page 13.

Example Clone Identification (Round II PCR):

SCREENING THE MATRIX COLUMN POOLS





The results of Round II PCR gel electrophoresis for the SP-7 Matrix Column Pools show a positive hit in wells B3, B4 and C4 of the SP-7 Matrix Column Pools.

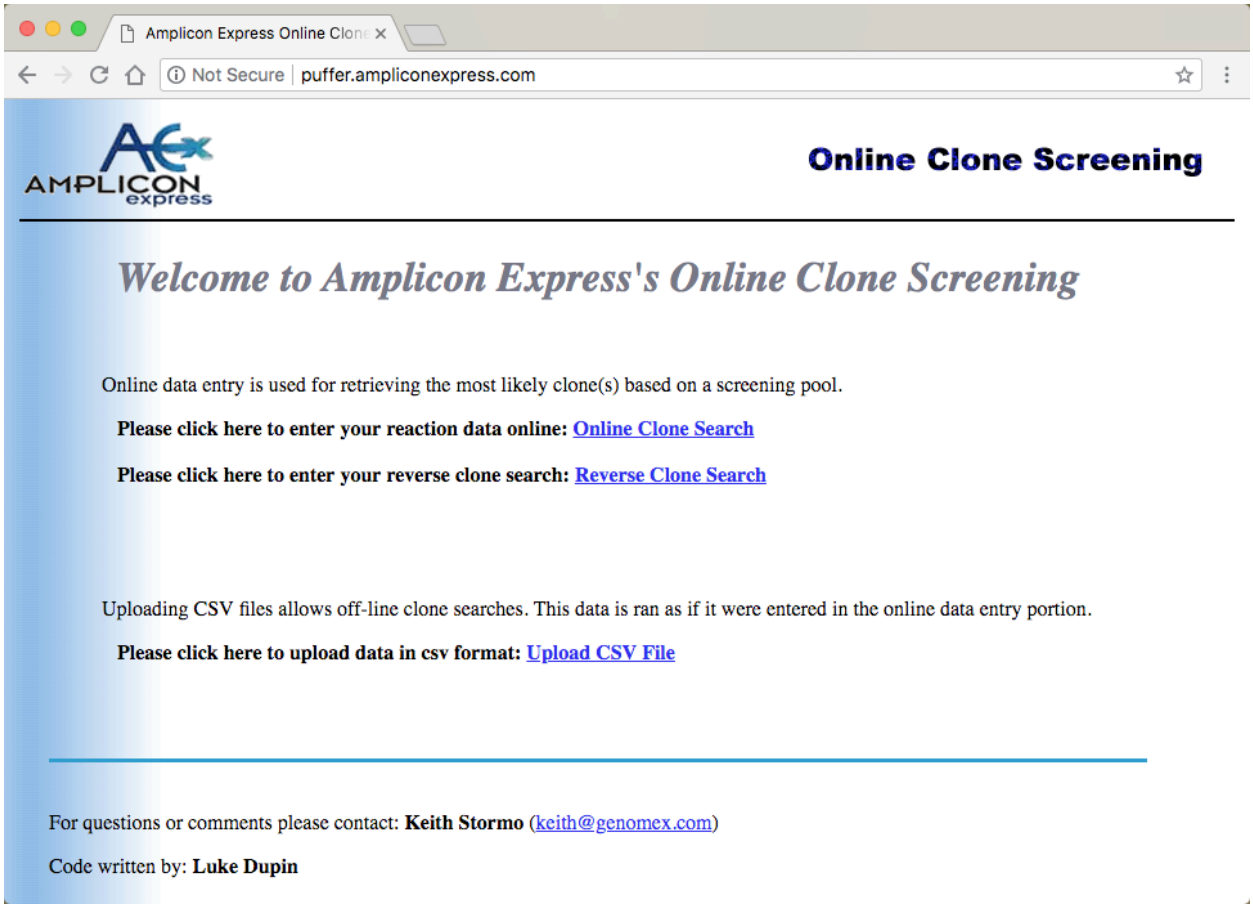
The Matrix Plate Loading pattern and the Matrix Column Key (page 25) show that the clone of interest is in Column 12.

This result, combined with the Round II Plate and Row Pool results, show the clone of interest is in: SP-7 plate 2 Library plate #020, Row D, Column 12.

"Column" Far-middle section of the photo on page 13.

Online Clone Identification System

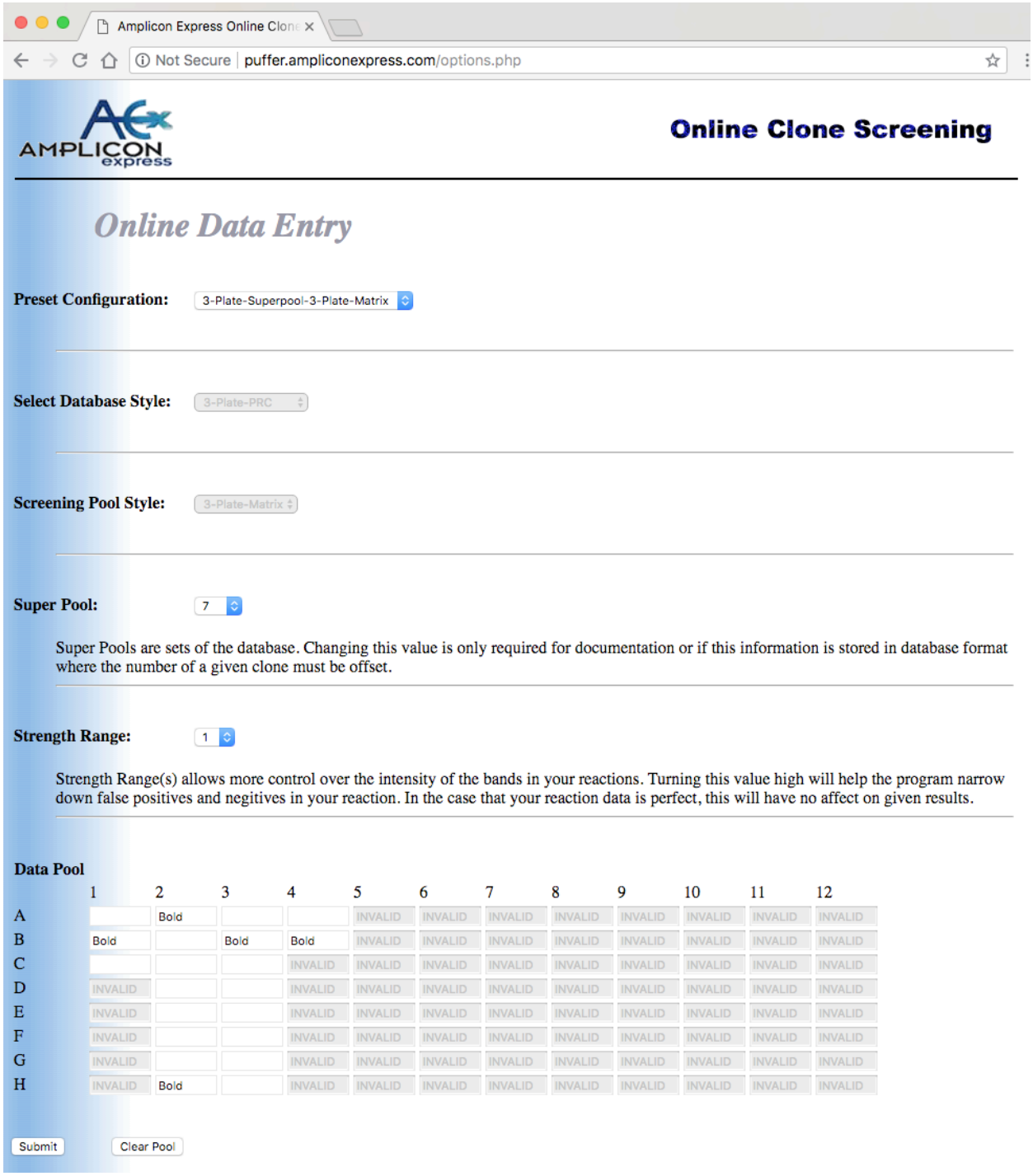
The **Amplicon Express** clone-screening tool is found at:
http://puffer.ampliconexpress.com/



Click on the link "Online Clone Search"

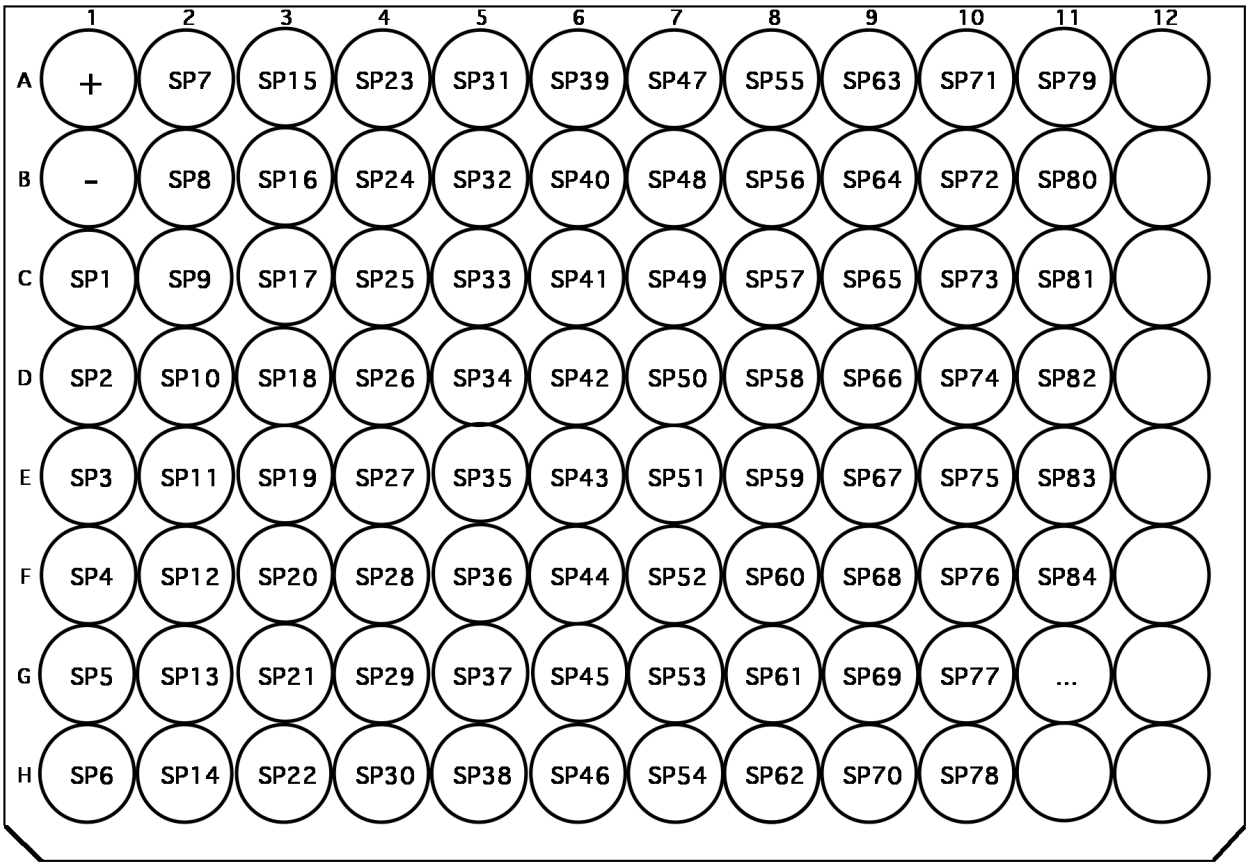
Select Preset Configuration: *3-Plate-Superpool-3Plate-Matrix*

Input your data on this page. Click "*Submit*". Click "OK" on the pop-up.



It will take you to the results page.

Superpools Plate Graphic



Library Code SP 1-84 Superpool Collection Plate Set A1

Spin ~500g before carefully removing mat & use 2 µl / reaction

LIBRARY CODE SP1-84 SUPERPOOL COLLECTION PLATE SET A1

Key to Superpools Plate:

To find the corresponding Source Plate number, look for the Superpool number determined with Round I PCR. Then use Round II Matrix Plate Pools results and the Matrix Plate Pools Key to identify the plate number. These two numbers in the table identify the Source Plate number that corresponds to the plate in your frozen BAC Library containing the BAC clone of interest.

Super pool number	Plate number	Source plate number
1	1	1
1	2	2
1	3	3
2	1	4
2	2	5
2	3	6
3	1	7
3	2	8
3	3	9
4	1	10
4	2	11
4	3	12
5	1	13
5	2	14
5	3	15
6	1	16
6	2	17
6	3	18
7	1	19
7	**2**	**20**
7	3	21
8	1	22
8	2	23
8	3	24
9	1	25
9	2	26
9	3	27
10	1	28
10	2	29
10	3	30
11	1	31
11	2	32
11	3	33
12	1	34
12	2	35
12	3	36
13	1	37
13	2	38
13	3	39
14	1	40
14	2	41
14	3	42

Super pool number	Plate number	Source plate number
15	1	43
15	2	44
15	3	45
16	1	46
16	2	47
16	3	48
17	1	49
17	2	50
17	3	51
18	1	52
18	2	53
18	3	54
19	1	55
19	2	56
19	3	57
20	1	58
20	2	59
20	3	60
21	1	61
21	2	62
21	3	63
22	1	64
22	2	65
22	3	66
23	1	67
23	2	68
23	3	69
24	1	70
24	2	71
24	3	72
25	1	73
25	2	74
25	3	75
26	1	76
26	2	77
26	3	78
27	1	79
27	2	80
27	3	81
28	1	82
28	2	83
28	3	84

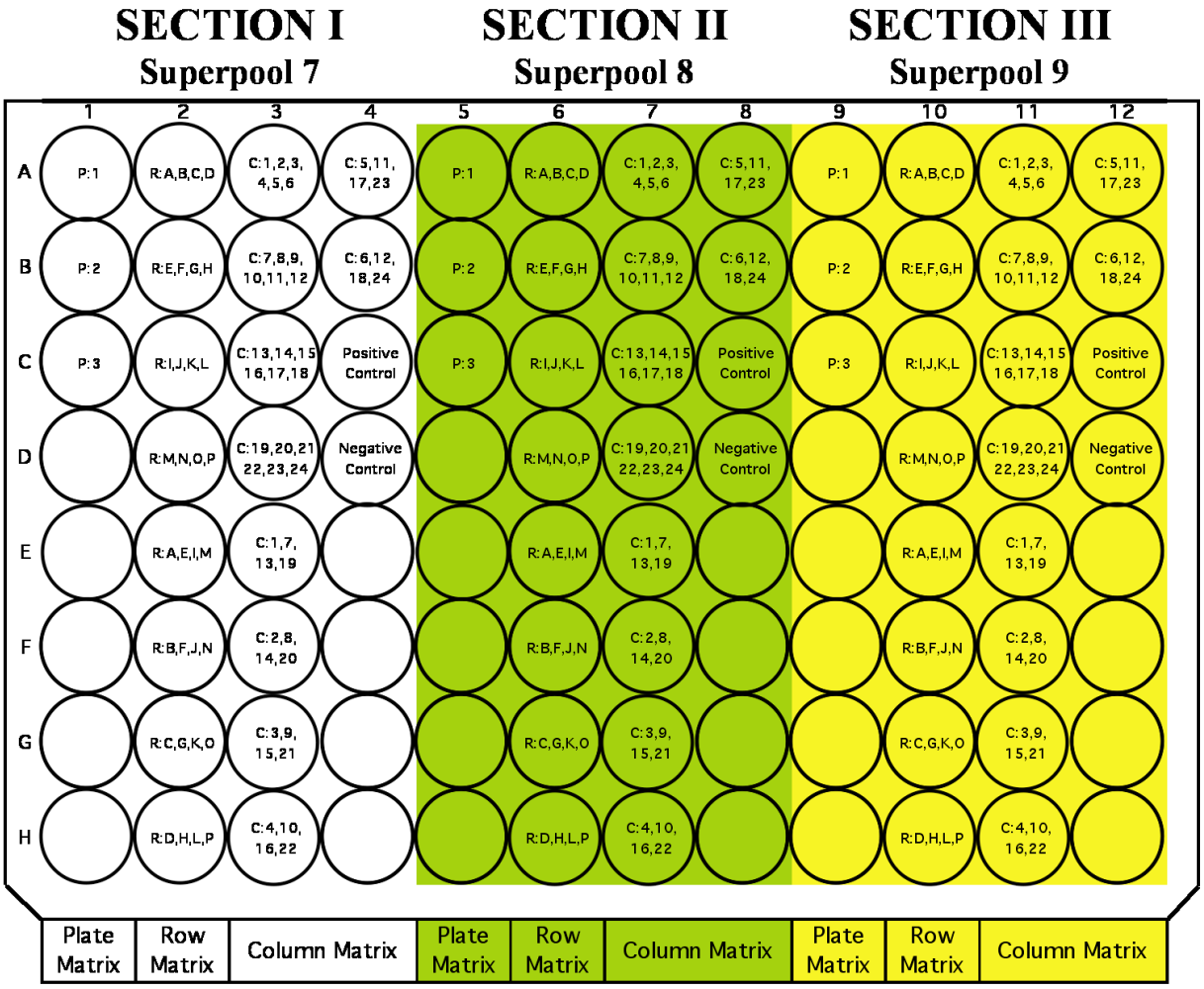
Super pool number	Plate number	Source plate number
29	1	85
29	2	86
29	3	87
30	1	88
30	2	89
30	3	90
31	1	91
31	2	92
31	3	93
32	1	94
32	2	95
32	3	96
33	1	97
33	2	98
33	3	99
34	1	100
34	2	101
34	3	102
35	1	103
35	2	104
35	3	105
36	1	106
36	2	107
36	3	108
37	1	109
37	2	110
37	3	111
38	1	112
38	2	113
38	3	114
39	1	115
39	2	116
39	3	117
40	1	118
40	2	119
40	3	120
41	1	121
41	2	122
41	3	123
42	1	124
42	2	125
42	3	126

Super pool number	Plate number	Source plate number
43	1	127
43	2	128
43	3	129
44	1	130
44	2	131
44	3	132
45	1	133
45	2	134
45	3	135
46	1	136
46	2	137
46	3	138
47	1	139
47	2	140
47	3	141
48	1	142
48	2	143
48	3	144
49	1	145
49	2	146
49	3	147
50	1	148
50	2	149
50	3	150
51	1	151
51	2	152
51	3	153
52	1	154
52	2	155
52	3	156
53	1	157
53	2	158
53	3	159
54	1	160
54	2	161
54	3	162
55	1	163
55	2	164
55	3	165
56	1	166
56	2	167
56	3	168

Each individual AEX BAC Library has a unique (copy #) and (abbreviation code) that precede the source plate number on the library plate. For example, 1FVBM020 denotes copy 1 of our Strawberry BAC library plate 20. This is also coded on the bar code identifier on the left side of the AEX BAC library plate.

Keys to Matrix Pools Plate for Each Superpool

Each **Matrix Pools Plate** is divided in three Sections, each Section containing a set of Matrix Pools with all of the pools generated from the 3 plates that make up each Superpool. The Matrix Pools in each Section (I, II and II) present the same loading pattern for each Superpool.



Aquilegia AF SP 7-9 Matrix Pool (3) Plate Set A

Spin ~500g before carefully removing mat & use 2 µl / reaction

AF SP7-9 MATRIX POOL PLATE SET A

SECTION I SP-7	SECTION II SP-8	SECTION III SP-9
(Col 1-4)	(Col 5-8)	(Col 9-12)

All plates are labeled with Tough Tags in both text and machine readable bar code fonts. The label is affixed to the front of the plate.

The following three pages detail the Keys to interpret the gel electrophoresis bands formed from PCR products generated from the Matrix Pool Plate Pools.

Key to Matrix Plate Pools:

The Plate Pools from one Superpool are put into a matrix as shown below. The identity of the plate(s) containing the clone of interest can be determined by looking at the location of positive hits on the electrophoresis gel in the construction matrix.

	PLATE POOLS
A1	1
B1	2
C1	3

An alternate point of view is to look at all of the possible gel banding patterns that could be formed. The black bands show the example from our gel photo indicating a hit in plate 2.

PLATE POOLS KEY	Matrix Pool Plate, plate wells ran on a gel.		
Source Plate	A1	B1	C1
P-1			
P-2			
P-3			

Key to Matrix Row Pools:

The Row Pools from one Superpool are put into a matrix as shown below. The rows are pooled across and the columns are pooled down. The identity of the plate(s) that contain the clone of interest can be determined by looking at the location of positive hits on the electrophoresis gel in the construction matrix.

MATRIX ROW POOLS	E2	F2	G2	H2
A2	A	B	C	D
B2	E	F	G	H
C2	I	J	K	L
D2	M	N	O	P

An alternate point of view is to look at all of the possible gel banding patterns that could be formed. The shaded bands show all possible potential patterns and the black bands show the example from our gel photos indicating a hit in Row D.

Superpool Row Pools pooled together to form the Matrix Row Pools. All Matrix Row Pools have 288 clones.
Matrix Pool Plate, row wells ran on a gel.

MATRIX ROW POOLS KEY	R:A,B,C,D	R:E,F,G,H	R:I,J,K,L	R:M,N,O,P	R:A,E,I,M	R:B,F,J,N	R:C,G,K,O	R:D,H,L,P
Source Row	A2	B2	C2	D2	E2	F2	G2	H2
R-A								
R-B								
R-C								
R-D								
R-E								
R-F								
R-G								
R-H								
R-I								
R-J								
R-K								
R-L								
R-M								
R-N								
R-O								
R-P								

Key to Matrix Column Pools:

The Column Pools from one Superpool are put into a matrix as shown below. The rows are pooled across and the columns are pooled down. The identity of the plate(s) that contain the clone of interest can be determined by looking at the location of positive hits on the electrophoresis gel in the construction matrix.

MATRIX COLUMN POOLS	E3	F3	G3	H3	A4	B4
A3	1	2	3	4	5	6
B3	7	8	9	10	11	12
C3	13	14	15	16	17	18
D3	19	20	21	22	23	24

An alternate point of view is to look at all of the possible gel banding patterns that could be formed. The shaded bands show all possible potential patterns and the black bands show the example from our gel photo indicating a hit in column 12.

Superpool Column Pools pooled together to form the Matrix Column Pools. Wells A3-D3 have 288 clones and wells E3-B4 have 192 clones. Matrix Pool Plate, column wells ran on a gel.

MATRIX COLUMN POOLS KEY	C:1,2,3, 4,5,6	C:7,8,9, 10,11,12	C:13,14,15, 16,17,18	C:19,20,21, 22,23,24	C: 1,7,13,19	C: 2,8,14,20	C: 3,9,15,21	C: 4,10,16,22	C: 5,11,17,23	C: 6,12,18,24
Source Column	A3	B3	C3	D3	E3	F3	G3	H3	A4	B4
C-1										
C-2										
C-3										
C-4										
C-5										
C-6										
C-7										
C-8										
C-9										
C-10										
C-11										
C-12										
C-13										
C-14										
C-15										
C-16										
C-17										
C-18										
C-19										
C-20										
C-21										
C-22										
C-23										
C-24										

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This page is for custom tools to further resolve multiple hits.

Notes: