Users Manual

Pool & Superpool Matrix Pooling Technology

For BAC Library (or Fosmid Library)

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

For Superpool Systems constructed after January 1, 2008

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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 Seven Plate Matrix System, each Superpool is composed of seven 384-well plates so 2,688 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 23 Matrix Pools for each Superpool:

- Five Matrix Plate Pools (MPP)
- Eight Matrix Row Pools (MRP)
- Ten Matrix Column Pools (MCP)

Round II PCR requires ONLY 23 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 47 PCR reactions plus controls.

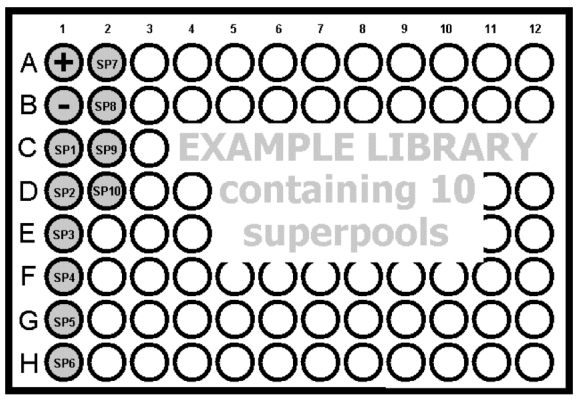
The interpretation of positive hits also called "BAC clone library plate & well deconvolution", from Round II PCR (screening of the Matrix Pools) is done, by comparing the positive hits seen on the electrophoresis results to the Matrix Pool Keys in this manual. The Keys are necessary to provide the location (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

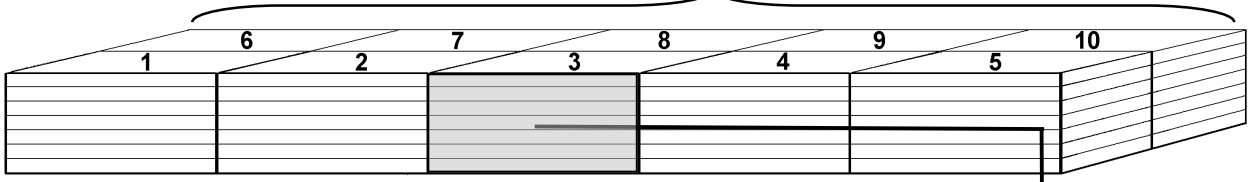
The researcher will receive two sets of three identical Superpool Collection Plates, which will be used for Round I PCR.

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 001-70



Entire BAC Library

(Superpool 3 is used in the screening example starting on p.13)

The BAC library is separated into sequential Superpools of seven 384-well plates.
Each Superpool of 7 plates is further separated into 7 Plate pools, 16 Row pools and 24 Column pools (PRC).

SUPERPOOL 3

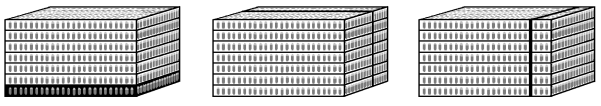
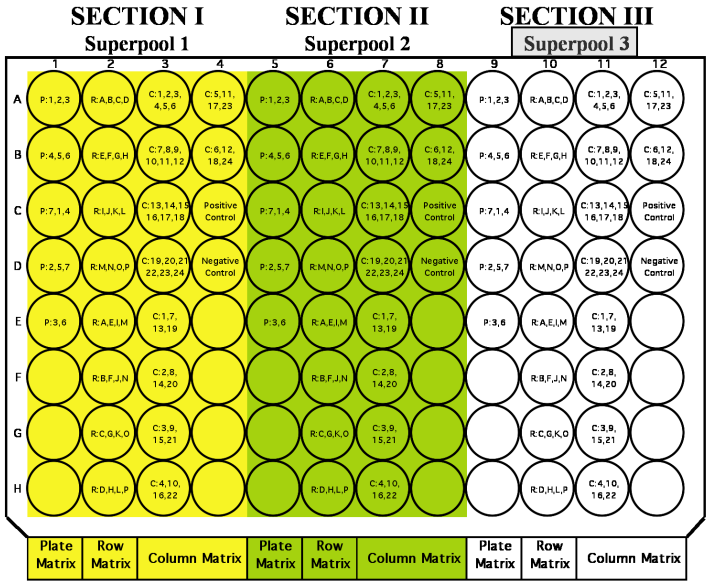
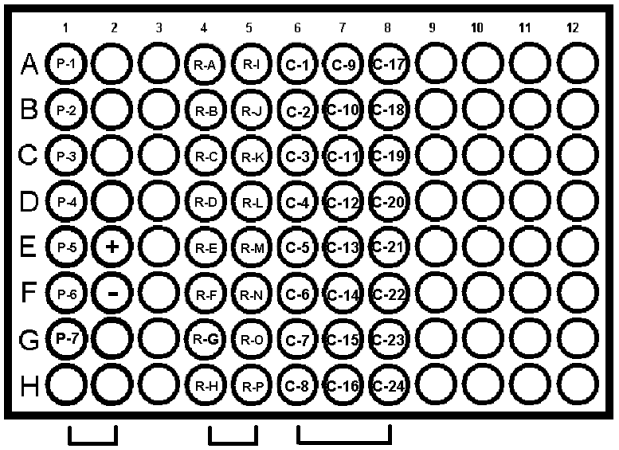


Plate Row Column (PRC) Plate for Superpool 3



Matrix Pool Plate



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 a 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 will also make 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 19) 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 10 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 one of the reserve plates 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 bands "positive hits" (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 23 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 Matrix Plate containing the Superpool 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 the 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 orientation and refreeze (–20°C) the Matrix Plate DNA template.
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 bands "positive hits".

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

30. Compare the positive Matrix Plate Pool (MPP) gel bands to the Matrix Plate Pools Key on page 29. Record all possible intersections of the gel bands on the Matrix Plate Pools Key. These intersections are the plate(s) pools with the hit(s). Alternately, use the gel banding patterns on page 29 to identify the original source plate(s) location.
31. Compare the positive Matrix Row Pools gel bands to the Matrix Row Pools Key on page 30. 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 30 to identify the original source row(s) location.
32. Compare the positive Matrix Column Pools gel bands to the Matrix Column Pools Key on page 31. Record all possible intersections of the gel bands on the Matrix Column Key. These intersections are the column(s) pools with the hit(s). Alternately, use the gel banding patterns on page 31 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, look at the Superpool Plate Key on pages 20-26 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 "7-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 up 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 ample 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 templates 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 672 different clones but some have 1,152 clones in each matrix pool and others have only 448 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 47 reactions for each Superpool instead of the 23 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+} (2mM final concentration)
1 µL TAQ Polymerase (~1-5 Units)
9 µL H2O

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 seven consecutive 384-well plates from a BAC library (see Superpool Plate Key starting on page 20). 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 2,688 BAC clones from the first seven plates of the BAC library (Plate 001 to Plate 007).

Superpool SP-2 has the DNA of all the 2,688 BAC clones from the second seven plates of the BAC library (Plate 008 to Plate 014).

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 starting on page 20 of this document for exact details.

Matrix Pools:
To each Superpool corresponds a set of 23 Matrix Pools. Each set of Matrix Pools is aliquoted into two identical **Matrix Pool Plates** to help reduce the risk of contamination. The Matrix Pools of Superpool #1 are named as follow:

Matrix Plate Pools 1MPP-A1 through 1MPP-E1 for the 5 wells that contain the matrix of plates 001-007 in Superpool #1. Matrix Plate Pools contain 1,152 or 768 clones. See the Matrix Plate Pool Key on page 29 for exact composition of each well.
(Wells A1 –D1 have 3 different plates x 384 wells/plate=1,152 clones per Matrix Plate Pool)
(Well E1 has 2 different plates x 384 wells/plate=768 clones per Matrix Plate Pool)

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 672 clones. See the Matrix Row Pool Key on page 30 for exact composition of each well.
(7 different plates x 4 different rows x 24 row wells/plate=672 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 31 for exact composition of each well. The Matrix Column Pools in wells A3 through D3 have 672 clones (7 different plates x 6 different columns x 16 column wells/plate=672 clones per Matrix Column Pool). The Matrix Column Pools in wells E3 through B4 contain 448 clones (7 different plates x 4 different columns x 16 column wells/plate=768 clones per Matrix Row Pool).

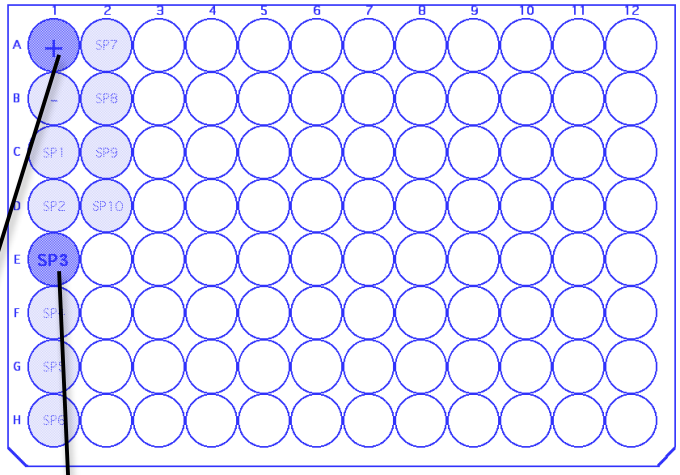
Please see page 29-31 for the exact composition of each well in the Matrix Pools Plate.
Remember that each Superpool has its own Section of a 96-well plate of Matrix Pools.

Example Clone Identification (Round I PCR):

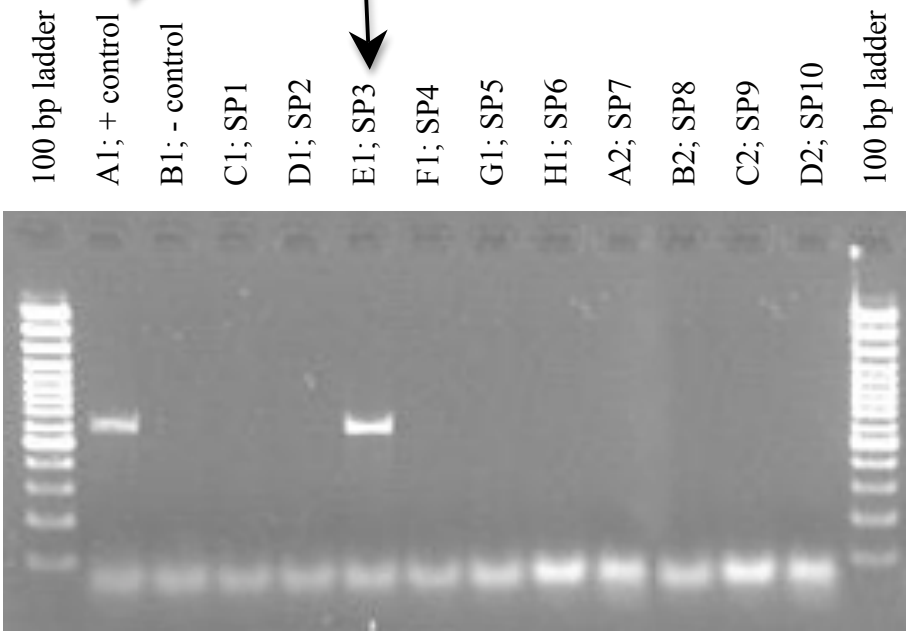
SCREENING THE SUPERPOOLS

In this example, using a 10-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 3 (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 3 Matrix Pool Plate Section.

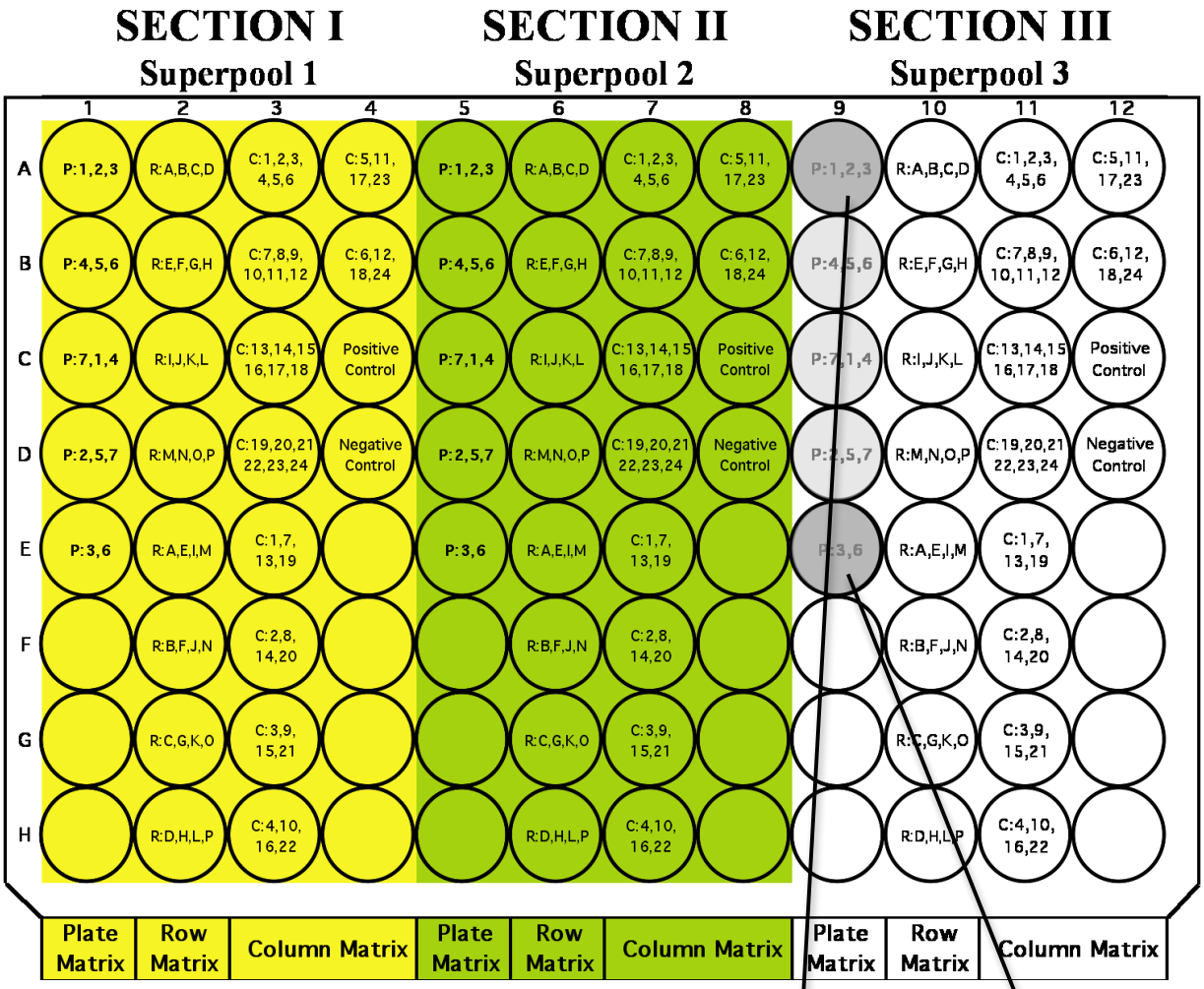


Library Code Superpool Collection Plate Copy #1
Library plates 001-070



Example Clone Identification (Round II PCR):

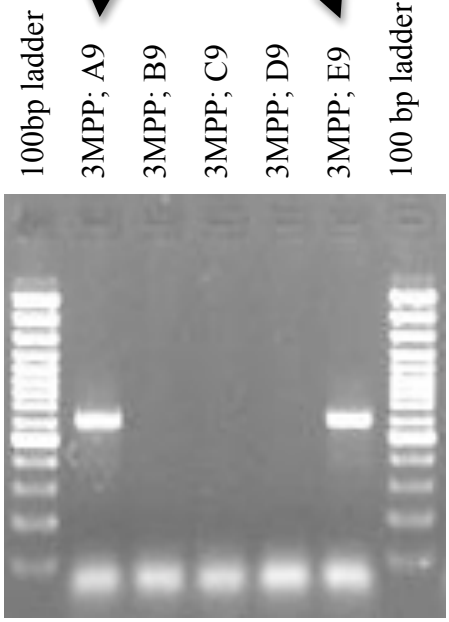
SCREENING THE MATRIX PLATE POOLS



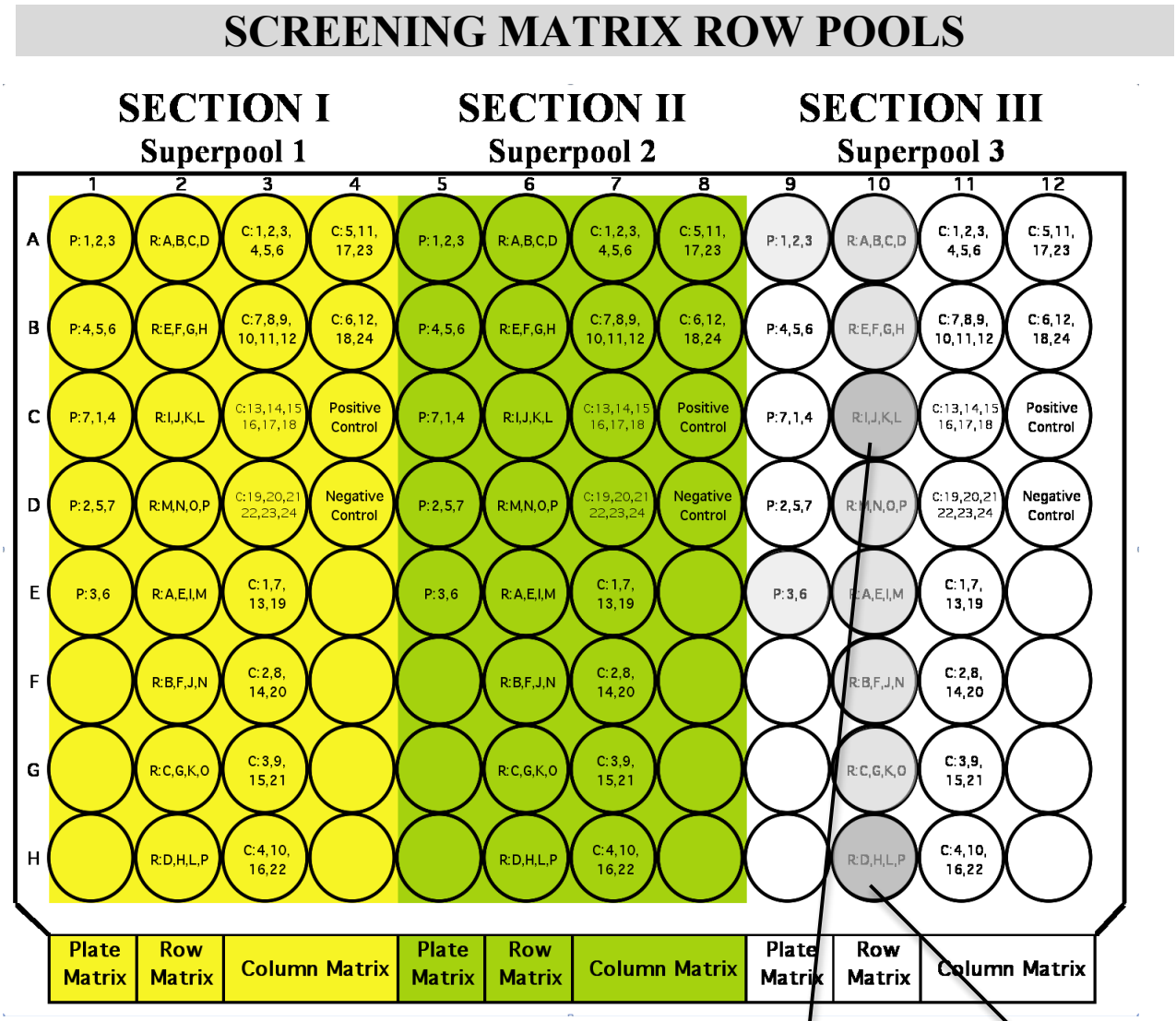
The results of Round II PCR gel electrophoresis for the SP-3 Matrix Plate Pools show a positive hit in wells A9 and E9 of the SP-3 Matrix Plate Pools.

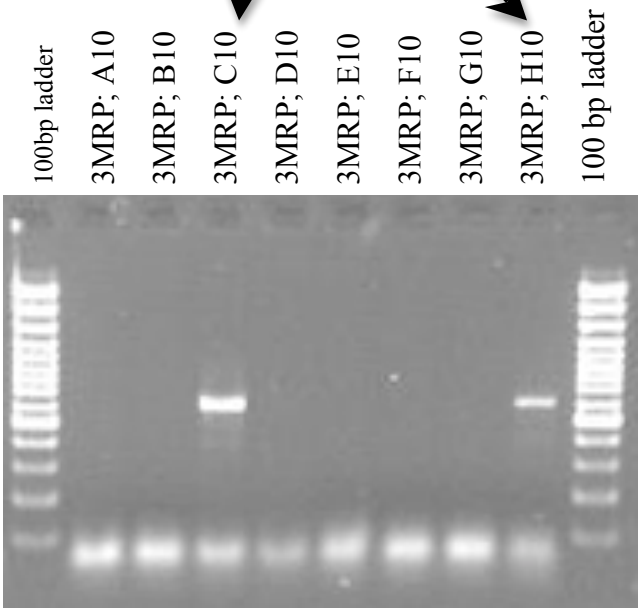
The Matrix Plate Loading pattern and the Matrix Plate Pools Key (page 28) show that the clone of interest is in Matrix Plate Pool 3.

The actual Library plate corresponding to SP-3 Plate 3 position is Library plate #017 (see Superpool Plate Key on page 20).



Example Clone Identification (Round II PCR):





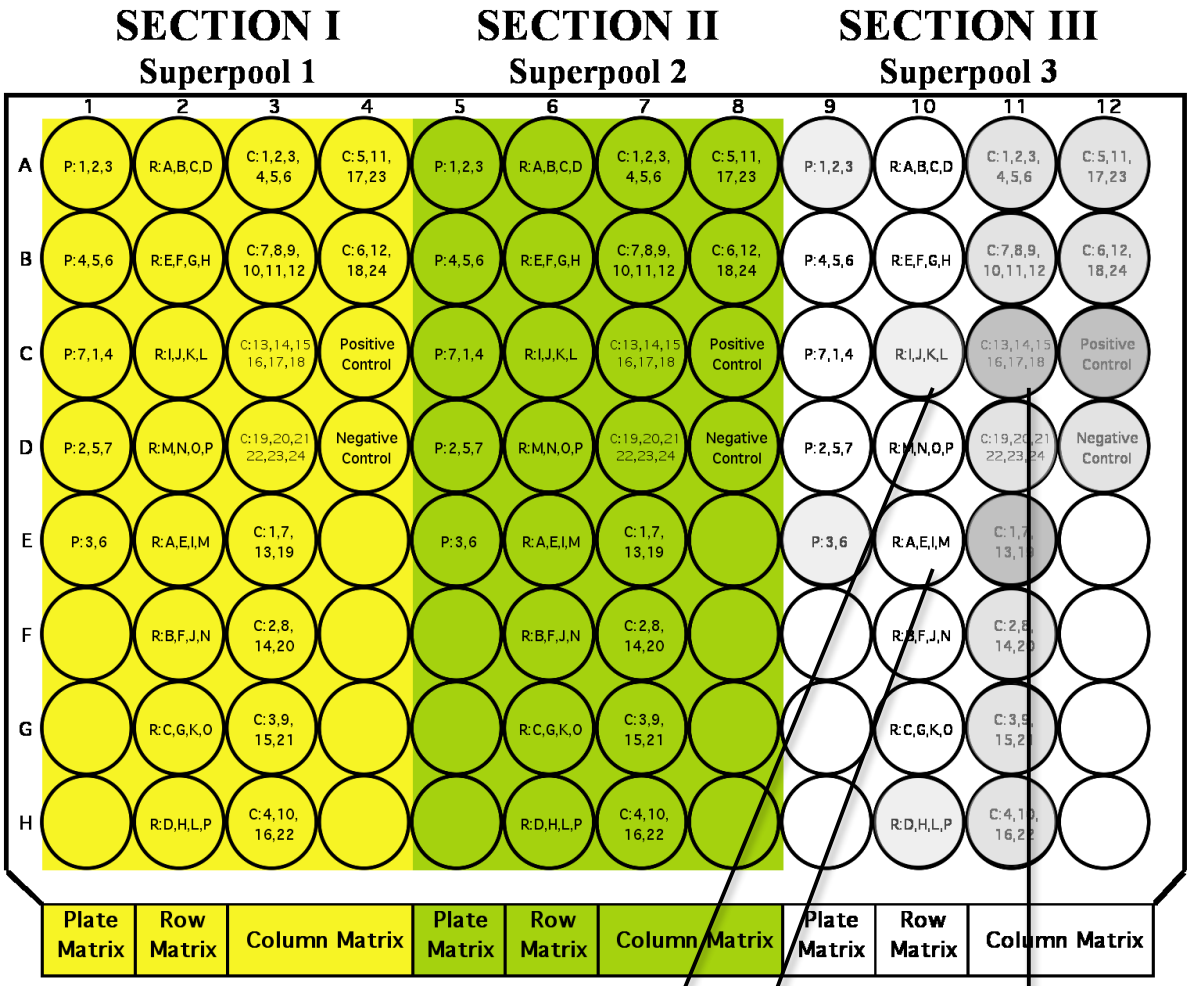
The results of Round II PCR gel electrophoresis for the SP-3 Matrix Row Pools show a positive hit in wells C10 and H10 of the SP-3 Matrix Row Pools.

The Matrix Plate Loading pattern and the Matrix Row Pools Key (page 29) show that the clone of interest is in Row L.

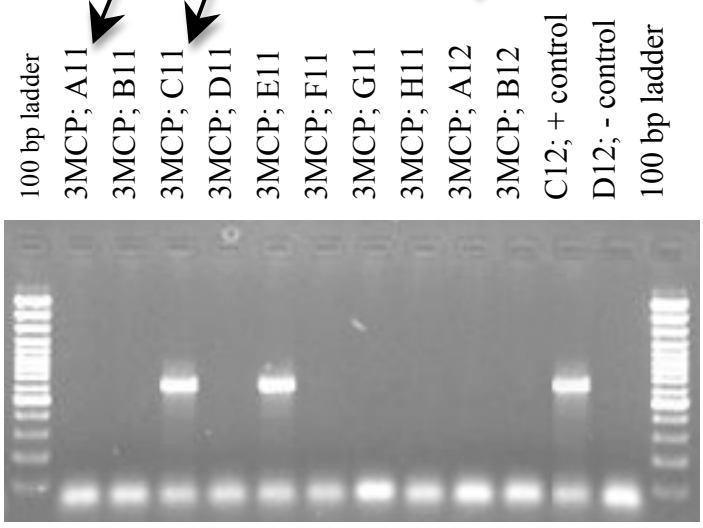
This result, combined with the Round II Plate Pool result, show the clone of interest is in: Library plate #017, Row L.

Example Clone Identification (Round II PCR):

SCREENING THE MATRIX COLUMN POOLS



The results of Round II PCR gel electrophoresis for the SP-3 Matrix Column Pools show a positive hit in wells C11, E11 and C12 of the SP-3 Matrix Column Pools.

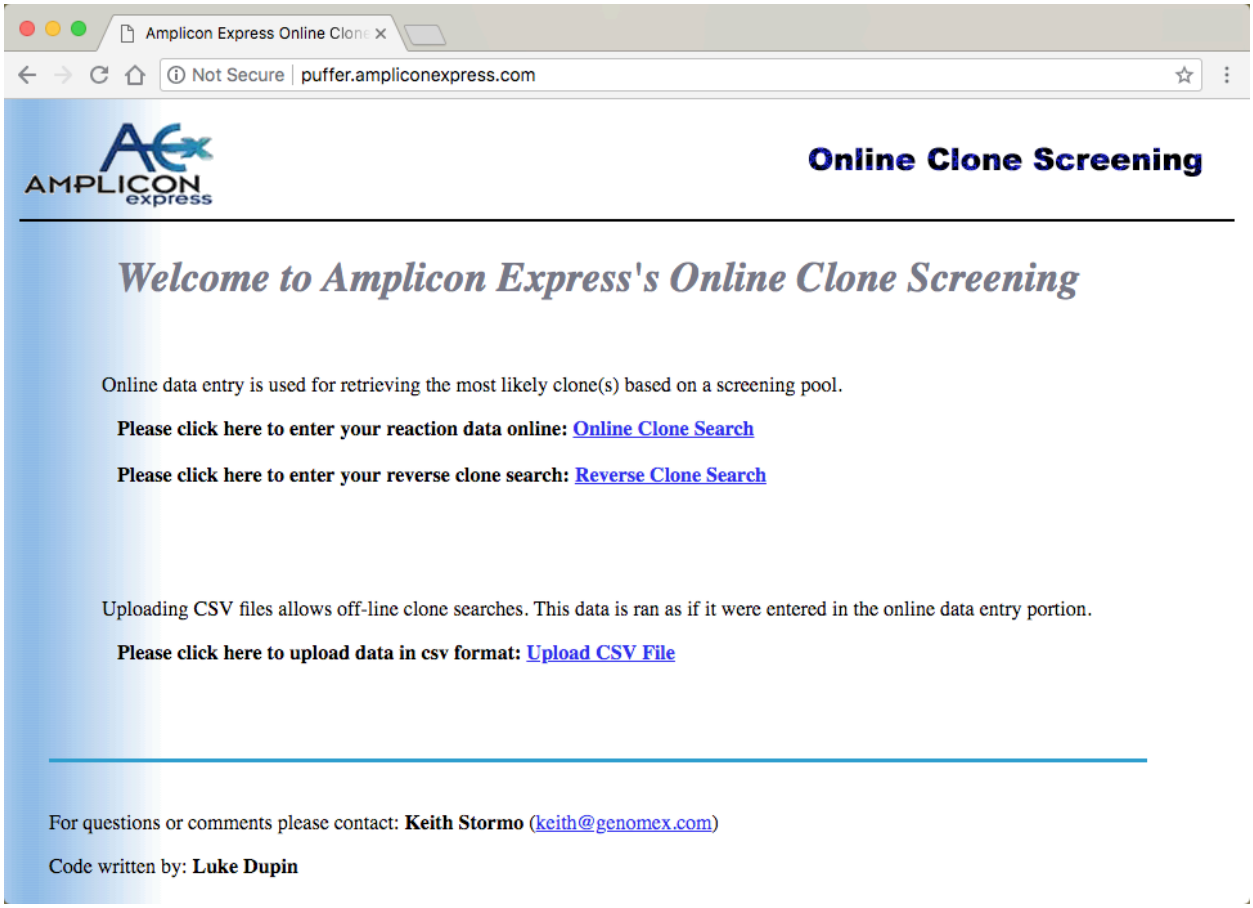


The Matrix Plate Loading pattern and the Matrix Column Pools Key (page 30) show that the clone of interest is in Column 13.

This result, combined with the Round II Plate and Row Pool results, show the clone of interest is in:
Library plate #017, Row L, Column 13.

Online Clone Identification System

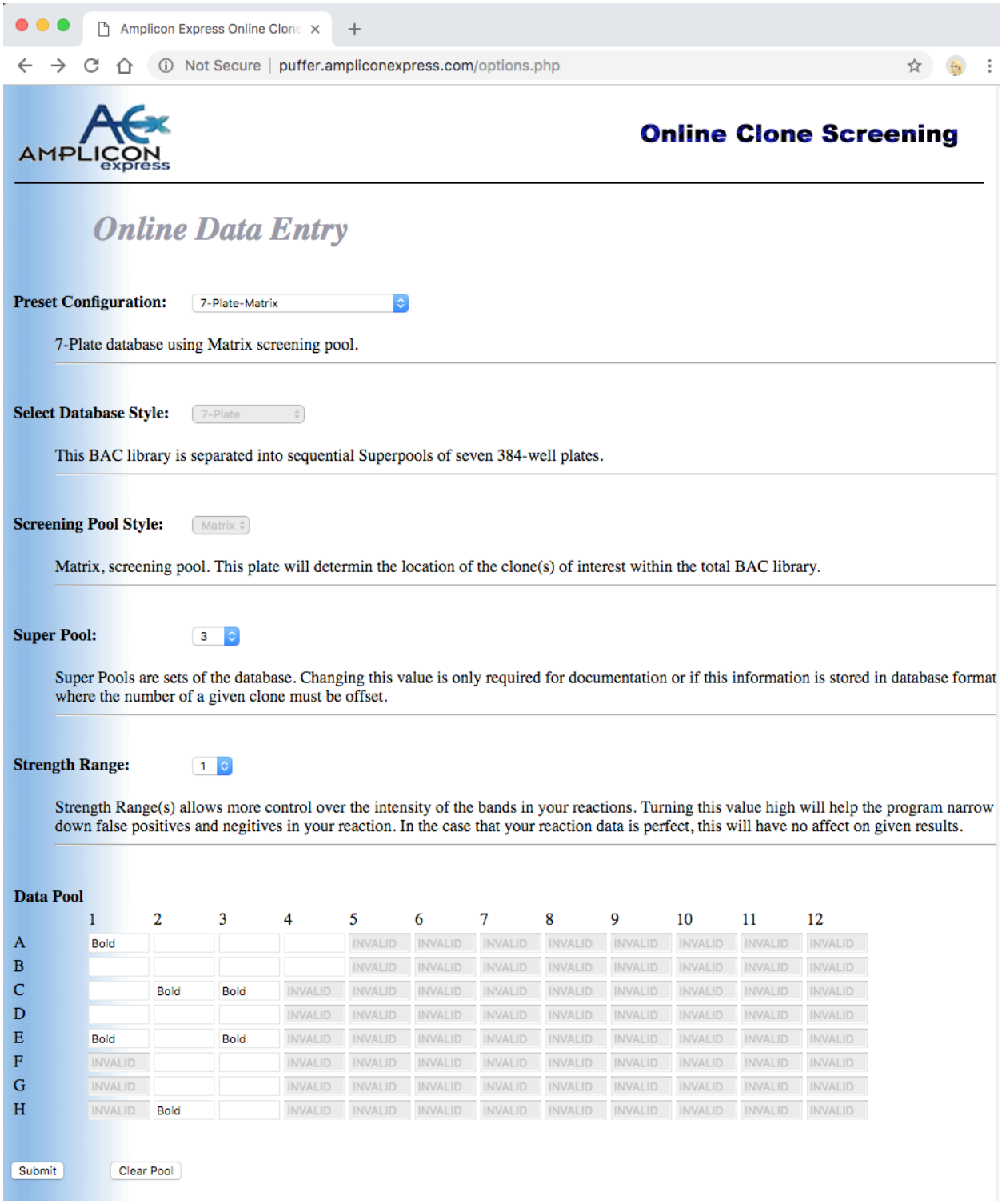
The **Amplicon Express** clone-screening tool is found at:
http://puffer.ampliconexpress.com/



Click on the link "Online Clone Search"

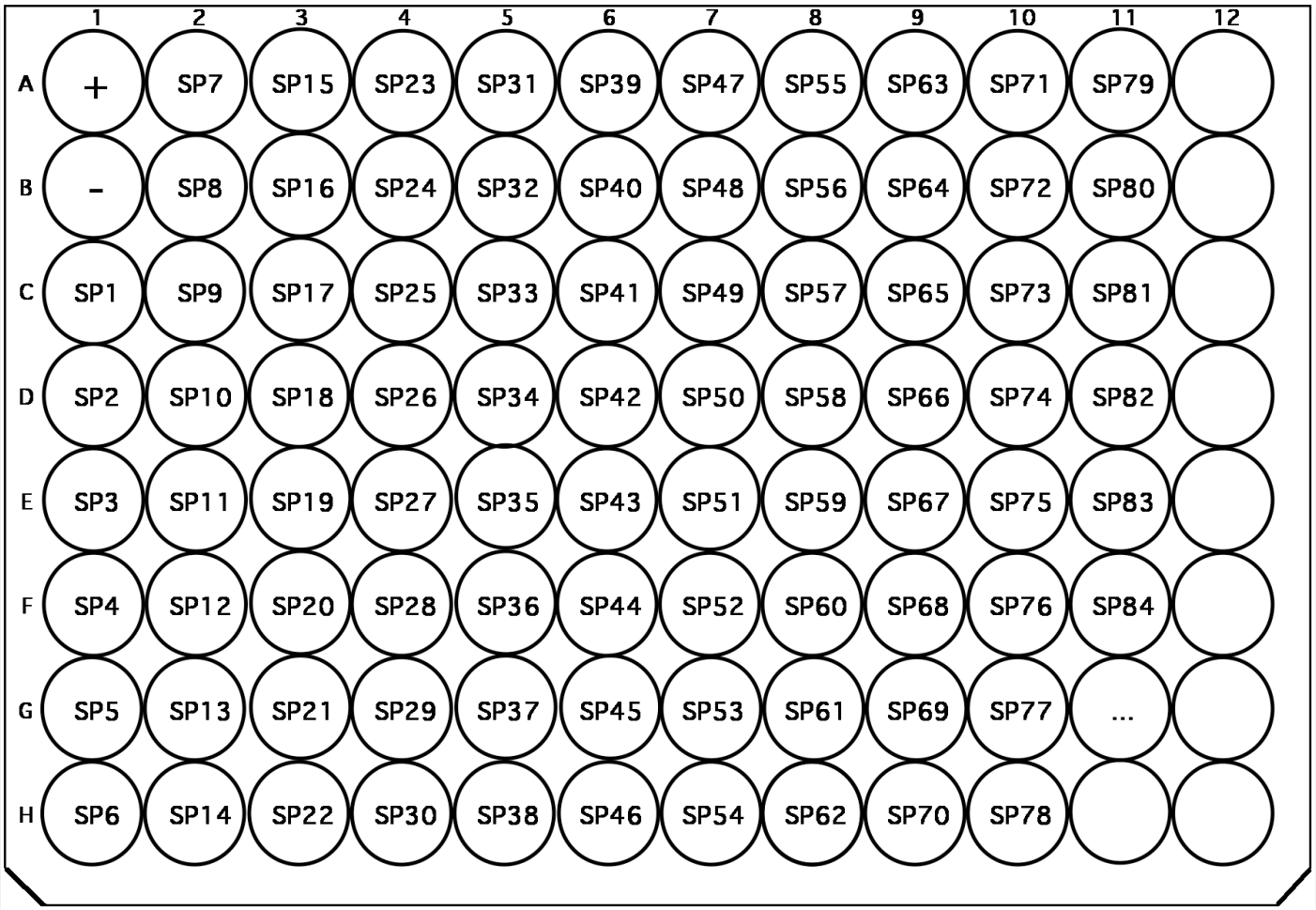
Select Preset Configuration: *7-Plate-Matrix*

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



It will take you to the results page.

Superpool 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 Superpool Plate:

To find the corresponding Source Plate number, look for the Superpool number determined with round I PCR. Then use Round II Matrix Plate Pool results and the Matrix Plate Pool Key, which identified 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
1	4	4
1	5	5
1	6	6
1	7	7
2	1	8
2	2	9
2	3	10
2	4	11
2	5	12
2	6	13
2	7	14
3	1	15
3	2	16
3	3	17
3	4	18
3	5	19
3	6	20
3	7	21
4	1	22
4	2	23
4	3	24
4	4	25
4	5	26
4	6	27
4	7	28
5	1	29
5	2	30
5	3	31
5	4	32
5	5	33
5	6	34
5	7	35
6	1	36
6	2	37
6	3	38
6	4	39
6	5	40
6	6	41
6	7	42
7	1	43
7	2	44
7	3	45
7	4	46
7	5	47
7	6	48
7	7	49

Super pool number	Plate number	Source plate number
8	1	50
8	2	51
8	3	52
8	4	53
8	5	54
8	6	55
8	7	56
9	1	57
9	2	58
9	3	59
9	4	60
9	5	61
9	6	62
9	7	63
10	1	64
10	2	65
10	3	66
10	4	67
10	5	68
10	6	69
10	7	70
11	1	71
11	2	72
11	3	73
11	4	74
11	5	75
11	6	76
11	7	77
12	1	78
12	2	79
12	3	80
12	4	81
12	5	82
12	6	83
12	7	84
13	1	85
13	2	86
13	3	87
13	4	88
13	5	89
13	6	90
13	7	91
14	1	92
14	2	93
14	3	94
14	4	95
14	5	96
14	6	97
14	7	98

Super pool number	Plate number	Source plate number
15	1	99
15	2	100
15	3	101
15	4	102
15	5	103
15	6	104
15	7	105
16	1	106
16	2	107
16	3	108
16	4	109
16	5	110
16	6	111
16	7	112
17	1	113
17	2	114
17	3	115
17	4	116
17	5	117
17	6	118
17	7	119
18	1	120
18	2	121
18	3	122
18	4	123
18	5	124
18	6	125
18	7	126
19	1	127
19	2	128
19	3	129
19	4	130
19	5	131
19	6	132
19	7	133
20	1	134
20	2	135
20	3	136
20	4	137
20	5	138
20	6	139
20	7	140
21	1	141
21	2	142
21	3	143
21	4	144
21	5	145
21	6	146
21	7	147

Super pool number	Plate number	Source plate number
22	1	148
22	2	149
22	3	150
22	4	151
22	5	152
22	6	153
22	7	154
23	1	155
23	2	156
23	3	157
23	4	158
23	5	159
23	6	160
23	7	161
24	1	162
24	2	163
24	3	164
24	4	165
24	5	166
24	6	167
24	7	168
25	1	169
25	2	170
25	3	171
25	4	172
25	5	173
25	6	174
25	7	175
26	1	176
26	2	177
26	3	178
26	4	179
26	5	180
26	6	181
26	7	182
27	1	183
27	2	184
27	3	185
27	4	186
27	5	187
27	6	188
27	7	189
28	1	190
28	2	191
28	3	192
28	4	193
28	5	194
28	6	195
28	7	196

Each individual AEX BAC Library has a unique (copy #) and (abbreviation code) that precede the source plate number on the library plate. For example, 1AF017 denotes copy 1 of our Aquilegia BAC library plate 17. This is also coded on the bar code identifier on the front side of the AEX BAC library plate.

Key to Superpool Plate (continued):

Super pool number	Plate number	Source plate number
29	1	197
29	2	198
29	3	199
29	4	200
29	5	201
29	6	202
29	7	203
30	1	204
30	2	205
30	3	206
30	4	207
30	5	208
30	6	209
30	7	210
31	1	211
31	2	212
31	3	213
31	4	214
31	5	215
31	6	216
31	7	217
32	1	218
32	2	219
32	3	220
32	4	221
32	5	222
32	6	223
32	7	224
33	1	225
33	2	226
33	3	227
33	4	228
33	5	229
33	6	230
33	7	231
34	1	232
34	2	233
34	3	234
34	4	235
34	5	236
34	6	237
34	7	238
35	1	239
35	2	240
35	3	241
35	4	242
35	5	243
35	6	244
35	7	245

Super pool number	Plate number	Source plate number
36	1	246
36	2	247
36	3	248
36	4	249
36	5	250
36	6	251
36	7	252
37	1	253
37	2	254
37	3	255
37	4	256
37	5	257
37	6	258
37	7	259
38	1	260
38	2	261
38	3	262
38	4	263
38	5	264
38	6	265
38	7	266
39	1	267
39	2	268
39	3	269
39	4	270
39	5	271
39	6	272
39	7	273
40	1	274
40	2	275
40	3	276
40	4	277
40	5	278
40	6	279
40	7	280
41	1	281
41	2	282
41	3	283
41	4	284
41	5	285
41	6	286
41	7	287
42	1	288
42	2	289
42	3	290
42	4	291
42	5	292
42	6	293
42	7	294

Super pool number	Plate number	Source plate number
43	1	295
43	2	296
43	3	297
43	4	298
43	5	299
43	6	300
43	7	301
44	1	302
44	2	303
44	3	304
44	4	305
44	5	306
44	6	307
44	7	308
45	1	309
45	2	310
45	3	311
45	4	312
45	5	313
45	6	314
45	7	315
46	1	316
46	2	317
46	3	318
46	4	319
46	5	320
46	6	321
46	7	322
47	1	323
47	2	324
47	3	325
47	4	326
47	5	327
47	6	328
47	7	329
48	1	330
48	2	331
48	3	332
48	4	333
48	5	334
48	6	335
48	7	336
49	1	337
49	2	338
49	3	339
49	4	340
49	5	341
49	6	342
49	7	343

Super pool number	Plate number	Source plate number
50	1	344
50	2	345
50	3	346
50	4	347
50	5	348
50	6	349
50	7	350
51	1	351
51	2	352
51	3	353
51	4	354
51	5	355
51	6	356
51	7	357
52	1	358
52	2	359
52	3	360
52	4	361
52	5	362
52	6	363
52	7	364
53	1	365
53	2	366
53	3	367
53	4	368
53	5	369
53	6	370
53	7	371
54	1	372
54	2	373
54	3	374
54	4	375
54	5	376
54	6	377
54	7	378
55	1	379
55	2	380
55	3	381
55	4	382
55	5	383
55	6	384
55	7	385
56	1	386
56	2	387
56	3	388
56	4	389
56	5	390
56	6	391
56	7	392

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Key to Superpool Plate (continued):

Super pool number	Plate number	Source plate number
57	1	393
57	2	394
57	3	395
57	4	396
57	5	397
57	6	398
57	7	399
58	1	400
58	2	401
58	3	402
58	4	403
58	5	404
58	6	405
58	7	406
59	1	407
59	2	408
59	3	409
59	4	410
59	5	411
59	6	412
59	7	413
60	1	414
60	2	415
60	3	416
60	4	417
60	5	418
60	6	419
60	7	420
61	1	421
61	2	422
61	3	423
61	4	424
61	5	425
61	6	426
61	7	427
62	1	428
62	2	429
62	3	430
62	4	431
62	5	432
62	6	433
62	7	434
63	1	435
63	2	436
63	3	437
63	4	438
63	5	439
63	6	440
63	7	441

Super pool number	Plate number	Source plate number
64	1	442
64	2	443
64	3	444
64	4	445
64	5	446
64	6	447
64	7	448
65	1	449
65	2	450
65	3	451
65	4	452
65	5	453
65	6	454
65	7	455
66	1	456
66	2	457
66	3	458
66	4	459
66	5	460
66	6	461
66	7	462
67	1	463
67	2	464
67	3	465
67	4	466
67	5	467
67	6	468
67	7	469
68	1	470
68	2	471
68	3	472
68	4	473
68	5	474
68	6	475
68	7	476
69	1	477
69	2	478
69	3	479
69	4	480
69	5	481
69	6	482
69	7	483
70	1	484
70	2	485
70	3	486
70	4	487
70	5	488
70	6	489
70	7	490

Super pool number	Plate number	Source plate number
71	1	491
71	2	492
71	3	493
71	4	494
71	5	495
71	6	496
71	7	497
72	1	498
72	2	499
72	3	500
72	4	501
72	5	502
72	6	503
72	7	504
73	1	505
73	2	506
73	3	507
73	4	508
73	5	509
73	6	510
73	7	511
74	1	512
74	2	513
74	3	514
74	4	515
74	5	516
74	6	517
74	7	518
75	1	519
75	2	520
75	3	521
75	4	522
75	5	523
75	6	524
75	7	525
76	1	526
76	2	527
76	3	528
76	4	529
76	5	530
76	6	531
76	7	532
77	1	533
77	2	534
77	3	535
77	4	536
77	5	537
77	6	538
77	7	539

Super pool number	Plate number	Source plate number
78	1	540
78	2	541
78	3	542
78	4	543
78	5	544
78	6	545
78	7	546
79	1	547
79	2	548
79	3	549
79	4	550
79	5	551
79	6	552
79	7	553
80	1	554
80	2	555
80	3	556
80	4	557
80	5	558
80	6	559
80	7	560
81	1	561
81	2	562
81	3	563
81	4	564
81	5	565
81	6	566
81	7	567
82	1	568
82	2	569
82	3	570
82	4	571
82	5	572
82	6	573
82	7	574
83	1	575
83	2	576
83	3	577
83	4	578
83	5	579
83	6	580
83	7	581
84	1	582
84	2	583
84	3	584
84	4	585
84	5	586
84	6	587
84	7	588

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Key to Superpool Plate (continued):

Super pool number	Plate number	Source plate number
85	1	589
85	2	590
85	3	591
85	4	592
85	5	593
85	6	594
85	7	595
86	1	596
86	2	597
86	3	598
86	4	599
86	5	600
86	6	601
86	7	602
87	1	603
87	2	604
87	3	605
87	4	606
87	5	607
87	6	608
87	7	609
88	1	610
88	2	611
88	3	612
88	4	613
88	5	614
88	6	615
88	7	616
89	1	617
89	2	618
89	3	619
89	4	620
89	5	621
89	6	622
89	7	623
90	1	624
90	2	625
90	3	626
90	4	627
90	5	628
90	6	629
90	7	630
91	1	631
91	2	632
91	3	633
91	4	634
91	5	635
91	6	636
91	7	637

Super pool number	Plate number	Source plate number
92	1	638
92	2	639
92	3	640
92	4	641
92	5	642
92	6	643
92	7	644
93	1	645
93	2	646
93	3	647
93	4	648
93	5	649
93	6	650
93	7	651
94	1	652
94	2	653
94	3	654
94	4	655
94	5	656
94	6	657
94	7	658
95	1	659
95	2	660
95	3	661
95	4	662
95	5	663
95	6	664
95	7	665
96	1	666
96	2	667
96	3	668
96	4	669
96	5	670
96	6	671
96	7	672
97	1	673
97	2	674
97	3	675
97	4	676
97	5	677
97	6	678
97	7	679
98	1	680
98	2	681
98	3	682
98	4	683
98	5	684
98	6	685
98	7	686

Super pool number	Plate number	Source plate number
99	1	687
99	2	688
99	3	689
99	4	690
99	5	691
99	6	692
99	7	693
100	1	694
100	2	695
100	3	696
100	4	697
100	5	698
100	6	699
100	7	700
101	1	701
101	2	702
101	3	703
101	4	704
101	5	705
101	6	706
101	7	707
102	1	708
102	2	709
102	3	710
102	4	711
102	5	712
102	6	713
102	7	714
103	1	715
103	2	716
103	3	717
103	4	718
103	5	719
103	6	720
103	7	721
104	1	722
104	2	723
104	3	724
104	4	725
104	5	726
104	6	727
104	7	728
105	1	729
105	2	730
105	3	731
105	4	732
105	5	733
105	6	734
105	7	735

Super pool number	Plate number	Source plate number
106	1	736
106	2	737
106	3	738
106	4	739
106	5	740
106	6	741
106	7	742
107	1	743
107	2	744
107	3	745
107	4	746
107	5	747
107	6	748
107	7	749
108	1	750
108	2	751
108	3	752
108	4	753
108	5	754
108	6	755
108	7	756
109	1	757
109	2	758
109	3	759
109	4	760
109	5	761
109	6	762
109	7	763
110	1	764
110	2	765
110	3	766
110	4	767
110	5	768
110	6	769
110	7	770
111	1	771
111	2	772
111	3	773
111	4	774
111	5	775
111	6	776
111	7	777
112	1	778
112	2	779
112	3	780
112	4	781
112	5	782
112	6	783
112	7	784

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Key to Superpool Plate (continued):

Super pool number	Plate number	Source plate number
113	1	785
113	2	786
113	3	787
113	4	788
113	5	789
113	6	790
113	7	791
114	1	792
114	2	793
114	3	794
114	4	795
114	5	796
114	6	797
114	7	798
115	1	799
115	2	800
115	3	801
115	4	802
115	5	803
115	6	804
115	7	805
116	1	806
116	2	807
116	3	808
116	4	809
116	5	810
116	6	811
116	7	812
117	1	813
117	2	814
117	3	815
117	4	816
117	5	817
117	6	818
117	7	819
118	1	820
118	2	821
118	3	822
118	4	823
118	5	824
118	6	825
118	7	826
119	1	827
119	2	828
119	3	829
119	4	830
119	5	831
119	6	832
119	7	833

Super pool number	Plate number	Source plate number
120	1	834
120	2	835
120	3	836
120	4	837
120	5	838
120	6	839
120	7	840
121	1	841
121	2	842
121	3	843
121	4	844
121	5	845
121	6	846
121	7	847
122	1	848
122	2	849
122	3	850
122	4	851
122	5	852
122	6	853
122	7	854
123	1	855
123	2	856
123	3	857
123	4	858
123	5	859
123	6	860
123	7	861
124	1	862
124	2	863
124	3	864
124	4	865
124	5	866
124	6	867
124	7	868
125	1	869
125	2	870
125	3	871
125	4	872
125	5	873
125	6	874
125	7	875
126	1	876
126	2	877
126	3	878
126	4	879
126	5	880
126	6	881
126	7	882

Super pool number	Plate number	Source plate number
127	1	883
127	2	884
127	3	885
127	4	886
127	5	887
127	6	888
127	7	889
128	1	890
128	2	891
128	3	892
128	4	893
128	5	894
128	6	895
128	7	896
129	1	897
129	2	898
129	3	899
129	4	900
129	5	901
129	6	902
129	7	903
130	1	904
130	2	905
130	3	906
130	4	907
130	5	908
130	6	909
130	7	910
131	1	911
131	2	912
131	3	913
131	4	914
131	5	915
131	6	916
131	7	917
132	1	918
132	2	919
132	3	920
132	4	921
132	5	922
132	6	923
132	7	924
133	1	925
133	2	926
133	3	927
133	4	928
133	5	929
133	6	930
133	7	931

Super pool number	Plate number	Source plate number
134	1	932
134	2	933
134	3	934
134	4	935
134	5	936
134	6	937
134	7	938
135	1	939
135	2	940
135	3	941
135	4	942
135	5	943
135	6	944
135	7	945
136	1	946
136	2	947
136	3	948
136	4	949
136	5	950
136	6	951
136	7	952
137	1	953
137	2	954
137	3	955
137	4	956
137	5	957
137	6	958
137	7	959
138	1	960
138	2	961
138	3	962
138	4	963
138	5	964
138	6	965
138	7	966
139	1	967
139	2	968
139	3	969
139	4	970
139	5	971
139	6	972
139	7	973
140	1	974
140	2	975
140	3	976
140	4	977
140	5	978
140	6	979
140	7	980

Each individual AEX BAC Library has a unique (copy #) and (abbreviation code) that precede the source plate number on the library plate. For example, 1AF017 denotes copy 1 of our Aquilegia BAC library plate 17. This is also coded on the bar code identifier on the front side of the AEX BAC library plate.

Key to Superpool Plate (continued):

Super pool number	Plate number	Source plate number
141	1	981
141	2	982
141	3	983
141	4	984
141	5	985
141	6	986
141	7	987
142	1	988
142	2	989
142	3	990
142	4	991
142	5	992
142	6	993
142	7	994
143	1	995
143	2	996
143	3	997
143	4	998
143	5	999
143	6	1000
143	7	1001
144	1	1002
144	2	1003
144	3	1004
144	4	1005
144	5	1006
144	6	1007
144	7	1008
145	1	1009
145	2	1010
145	3	1011
145	4	1012
145	5	1013
145	6	1014
145	7	1015
146	1	1016
146	2	1017
146	3	1018
146	4	1019
146	5	1020
146	6	1021
146	7	1022
147	1	1023
147	2	1024
147	3	1025
147	4	1026
147	5	1027
147	6	1028
147	7	1029

Super pool number	Plate number	Source plate number
148	1	1030
148	2	1031
148	3	1032
148	4	1033
148	5	1034
148	6	1035
148	7	1036
149	1	1037
149	2	1038
149	3	1039
149	4	1040
149	5	1041
149	6	1042
149	7	1043
150	1	1044
150	2	1045
150	3	1046
150	4	1047
150	5	1048
150	6	1049
150	7	1050
151	1	1051
151	2	1052
151	3	1053
151	4	1054
151	5	1055
151	6	1056
151	7	1057
152	1	1058
152	2	1059
152	3	1060
152	4	1061
152	5	1062
152	6	1063
152	7	1064
153	1	1065
153	2	1066
153	3	1067
153	4	1068
153	5	1069
153	6	1070
153	7	1071
154	1	1072
154	2	1073
154	3	1074
154	4	1075
154	5	1076
154	6	1077
154	7	1078

Super pool number	Plate number	Source plate number
155	1	1079
155	2	1080
155	3	1081
155	4	1082
155	5	1083
155	6	1084
155	7	1085
156	1	1086
156	2	1087
156	3	1088
156	4	1089
156	5	1090
156	6	1091
156	7	1092
157	1	1093
157	2	1094
157	3	1095
157	4	1096
157	5	1097
157	6	1098
157	7	1099
158	1	1100
158	2	1101
158	3	1102
158	4	1103
158	5	1104
158	6	1105
158	7	1106
159	1	1107
159	2	1108
159	3	1109
159	4	1110
159	5	1111
159	6	1112
159	7	1113
160	1	1114
160	2	1115
160	3	1116
160	4	1117
160	5	1118
160	6	1119
160	7	1120
161	1	1121
161	2	1122
161	3	1123
161	4	1124
161	5	1125
161	6	1126
161	7	1127

Super pool number	Plate number	Source plate number
162	1	1128
162	2	1129
162	3	1130
162	4	1131
162	5	1132
162	6	1133
162	7	1134
163	1	1135
163	2	1136
163	3	1137
163	4	1138
163	5	1139
163	6	1140
163	7	1141
164	1	1142
164	2	1143
164	3	1144
164	4	1145
164	5	1146
164	6	1147
164	7	1148
165	1	1149
165	2	1150
165	3	1151
165	4	1152
165	5	1153
165	6	1154
165	7	1155
166	1	1156
166	2	1157
166	3	1158
166	4	1159
166	5	1160
166	6	1161
166	7	1162
167	1	1163
167	2	1164
167	3	1165
167	4	1166
167	5	1167
167	6	1168
167	7	1169
168	1	1170
168	2	1171
168	3	1172
168	4	1173
168	5	1174
168	6	1175
168	7	1176

Each individual AEX BAC Library has a unique (copy #) and (abbreviation code) that precede the source plate number on the library plate. For example, 1AF017 denotes copy 1 of our Aquilegia BAC library plate 17. This is also coded on the bar code identifier on the front side of the AEX BAC library plate.

Key to Superpool Plate (continued):

Super pool number	Plate number	Source plate number
169	1	1177
169	2	1178
169	3	1179
169	4	1180
169	5	1181
169	6	1182
169	7	1183
170	1	1184
170	2	1185
170	3	1186
170	4	1187
170	5	1188
170	6	1189
170	7	1190
171	1	1191
171	2	1192
171	3	1193
171	4	1194
171	5	1195
171	6	1196
171	7	1197
172	1	1198
172	2	1199
172	3	1200
172	4	1201
172	5	1202
172	6	1203
172	7	1204
173	1	1205
173	2	1206
173	3	1207
173	4	1208
173	5	1209
173	6	1210
173	7	1211
174	1	1212
174	2	1213
174	3	1214
174	4	1215
174	5	1216
174	6	1217
174	7	1218
175	1	1219
175	2	1220
175	3	1221
175	4	1222
175	5	1223
175	6	1224
175	7	1225

Super pool number	Plate number	Source plate number
176	1	1226
176	2	1227
176	3	1228
176	4	1229
176	5	1230
176	6	1231
176	7	1232
177	1	1233
177	2	1234
177	3	1235
177	4	1236
177	5	1237
177	6	1238
177	7	1239
178	1	1240
178	2	1241
178	3	1242
178	4	1243
178	5	1244
178	6	1245
178	7	1246
179	1	1247
179	2	1248
179	3	1249
179	4	1250
179	5	1251
179	6	1252
179	7	1253
180	1	1254
180	2	1255
180	3	1256
180	4	1257
180	5	1258
180	6	1259
180	7	1260
181	1	1261
181	2	1262
181	3	1263
181	4	1264
181	5	1265
181	6	1266
181	7	1267
182	1	1268
182	2	1269
182	3	1270
182	4	1271
182	5	1272
182	6	1273
182	7	1274

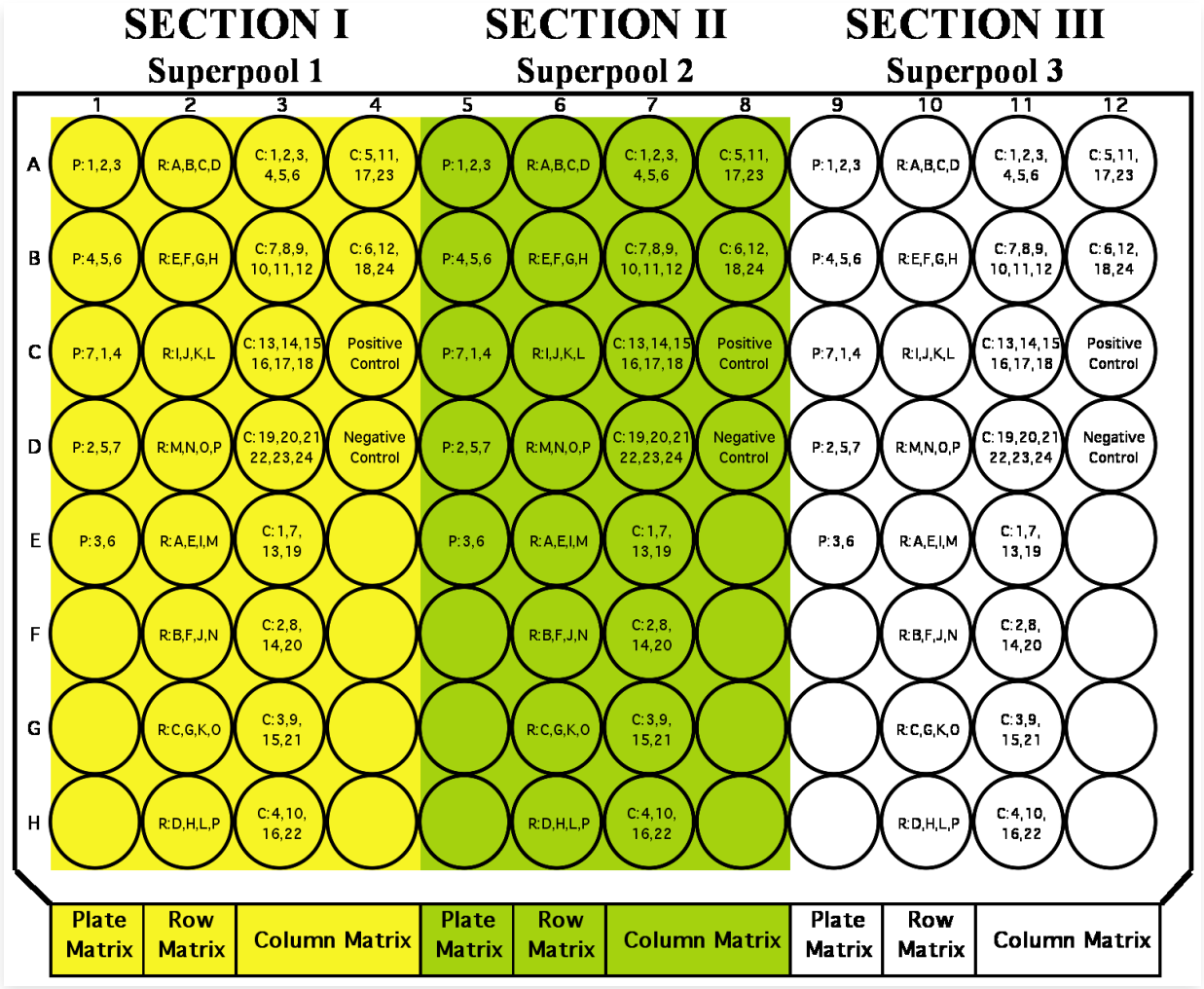
Super pool number	Plate number	Source plate number
183	1	1275
183	2	1276
183	3	1277
183	4	1278
183	5	1279
183	6	1280
183	7	1281
184	1	1282
184	2	1283
184	3	1284
184	4	1285
184	5	1286
184	6	1287
184	7	1288
185	1	1289
185	2	1290
185	3	1291
185	4	1292
185	5	1293
185	6	1294
185	7	1295
186	1	1296
186	2	1297
186	3	1298
186	4	1299
186	5	1300
186	6	1301
186	7	1302
187	1	1303
187	2	1304
187	3	1305
187	4	1306
187	5	1307
187	6	1308
187	7	1309
188	1	1310
188	2	1311
188	3	1312
188	4	1313
188	5	1314
188	6	1315
188	7	1316
189	1	1317
189	2	1318
189	3	1319
189	4	1320
189	5	1321
189	6	1322
189	7	1323

Super pool number	Plate number	Source plate number
190	1	1324
190	2	1325
190	3	1326
190	4	1327
190	5	1328
190	6	1329
190	7	1330
191	1	1331
191	2	1332
191	3	1333
191	4	1334
191	5	1335
191	6	1336
191	7	1337
192	1	1338
192	2	1339
192	3	1340
192	4	1341
192	5	1342
192	6	1343
192	7	1344
193	1	1345
193	2	1346
193	3	1347
193	4	1348
193	5	1349
193	6	1350
193	7	1351
194	1	1352
194	2	1353
194	3	1354
194	4	1355
194	5	1356
194	6	1357
194	7	1358
195	1	1359
195	2	1360
195	3	1361
195	4	1362
195	5	1363
195	6	1364
195	7	1365
196	1	1366
196	2	1367
196	3	1368
196	4	1369
196	5	1370
196	6	1371
196	7	1372

Each individual AEX BAC Library has a unique (copy #) and (abbreviation code) that precede the source plate number on the library plate. For example, 1AF017 denotes copy 1 of our Aquilegia BAC library plate 17. This is also coded on the bar code identifier on the front side of the AEX BAC library plate.

Keys to Matrix Pool Plates 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 7 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 1-3 Matrix Pool (7) Plate Set A

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

AF SP1-3 MATRIX POOL PLATE SET A

SECTION I SP-1	SECTION II SP-2	SECTION III SP-3
(Col 1-4)	(Col 5-8)	(Col 9-12)

All the 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. *Section I* is used in the following examples but the matrix pattern is the same for every Section. For example, for *Section II*, the letters (rows) remain the same but the numbers (columns) shift to 5 since the Matrix Plate pools from *Section II* are located in wells A5-E5. The rows are pooled across and the columns are pooled down. By looking at the location of positive hits on the electrophoresis gel in the construction matrix, the identity of the plate(s) that contain the clone of interest can be determined.

Section I

MATRIX PLATE POOLS	C1	D1	E1
A1	1	2	3
B1	4	5	6
C1	7	7	

An alternate point of view is to look at all of the possible gel banding patterns that could possibly be formed. The shaded bands show all potential patterns and the black bands show the example from our gel photos indicating a hit in plate 3.

Superpool Plate Pools pooled together to form the Matrix Plate Pools. Matrix Plate Pool wells A1-D1 have 1152 clones and E1 has 768 clones. Matrix Pool Plate, plate wells ran on a gel.

MATRIX PLATE POOLS KEY	P:1,2,3	P:4,5,6	P:7,1,4	P:2,5,7	P:3,6
Source Plate	A1	B1	C1	D1	E1
P-1					
P-2					
P-3					
P-4					
P-5					
P-6					
P-7					

Key to Matrix Row Pools:

The Row Pools from one Superpool are put into a matrix as shown below. As with the Matrix Plate Pools (previous page), the following are examples from *Section I*. For *Section II* and *Section III*, the numbers would change to 6 and 10 respectively. The rows are pooled across and the columns are pooled down. By looking at the location of positive hits on the electrophoresis gel in the construction matrix, the identity of the plate(s) that contain the clone of interest can be determined.

Section I

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 possibly 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 L.

Superpool Row Pools pooled together to form the Matrix Row Pools. All Matrix Row Pools have 672 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. As with the Matrix Plate and Row Pools (previous two pages), the following are examples from *Section I*. For *Section II* and *Section III*, the numbers would change to 7 and 8 (section II) and 11 and 12 (*Section III*). The rows are pooled across and the columns are pooled down. By looking at the location of positive hits on the electrophoresis gel in the construction matrix, the identity of the plate(s) that contain the clone of interest can be determined.

Section I

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 possibly 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 column 13.

Superpool Column Pools pooled together to form the Matrix Column Pools. Wells A3-D3 have 672 clones and wells E3-B4 have 448 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: