APPENDIX A

ADDITIONAL RESULT FOR CHAPTER III: OUTPUT RESULT FROM THE

ANALYSIS OF VH OF ANTI-C2 MONOCLONAL ANTIBODY USING

'AMPHI' SOFTWARE


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VHC2-Dharshan
SEQ. LENGTH: 118 AMINOACIDS

  BLOCK      MID POINT OF BLOCKS   THETA  I(THETA)  HEL    A1     A2
             ---------------------
           RES NO.  RES.     HYD.

              1     ASP     -.770
              2     VAL     1.220
              3     GLN     -.220
              4     LEU     1.700
              5     GLN     -.220
   1- 11      6     GLU     -.640   170.    6.53    0     .59    .40
   2- 12      7     SER     -.040   150.    4.17    0    1.05    .56
   3- 13      8     GLY      .000   100.    5.22    1    2.36   1.22
   4- 14      9     PRO      .720   100.    4.86    1    2.14   1.35
   5- 15     10     ASP     -.770   145.    2.73    0    1.32   1.65
   6- 16     11     LEU     1.700   115.    3.32    1    1.31   1.91
   7- 17     12     VAL     1.220   135.    3.36    1    1.19   2.36
   8- 18     13     LYS     -.990   115.    4.71    1    1.45   2.06
   9- 19     14     PRO      .720   110.    5.88    1    1.67   2.43
  10- 20     15     SER     -.040   120.    5.73    1    1.03   2.03
  11- 21     16     GLN     -.220   120.    3.91    1     .85   1.59
  12- 22     17     SER     -.040   180.    4.49    0     .65   1.13
  13- 23     18     LEU     1.700   180.    3.84    0     .79    .81
  14- 24     19     SER     -.040   165.    3.54    0     .45    .27
  15- 25     20     LEU     1.700   165.    3.69    0     .32    .27
  16- 26     21     THR      .260   160.    4.12    0     .37    .54
  17- 27     22     CYS     1.540   165.    4.50    0     .24    .44
  18- 28     23     THR      .260   165.    4.47    0     .19    .43
  19- 29     24     VAL     1.220   160.    4.67    0     .19    .29
  20- 30     25     THR      .260   155.    4.18    0     .14    .21
  21- 31     26     GLY      .000   145.    3.50    0     .38    .78
  22- 32     27     TYR      .960   150.    2.93    0     .39    .71
  23- 33     28     SER     -.040   155.    1.62    0     .65   1.33
  24- 34     29     ILE     1.800   125.    2.24    1     .93   1.75
  25- 35     30     THR      .260   180.    3.86    0     .56   2.02
  26- 36     31     SER     -.040   180.    3.95    0     .49   2.13
  27- 37     32     ALA      .310   180.    5.86    0     .32   1.17
  28- 38     33     TYR      .960   130.    4.52    1     .33   1.36
  29- 39     34     ASN     -.600   130.    6.43    1     .58   1.58
  30- 40     35     TRP     2.250   130.    4.42    1     .51   1.18
  31- 41     36     HIS      .130   130.    6.01    1     .97   1.68
  32- 42     37     TRP     2.250   135.    4.60    1    1.14   1.67
  33- 43     38     ILE     1.800   120.    6.12    1    1.26   1.84
  34- 44     39     ARG    -1.010    75.    5.27    0    1.26   1.32
  35- 45     40     GLN     -.220     0.    5.83    0     .99   1.31
  36- 46     41     PHE     1.790    80.   10.46    1    1.48   1.08
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37- 47    42   PRO    .720    85.    9.56   1   1.45    .88
38- 48    43   GLY    .000   140.    7.66   0    .97    .83
39- 49    44   ASN   -.600    55.    6.45   0    .79    .70
40- 50    45   LYS   -.990   150.    6.46   0    .69    .75
41- 51    46   LEU   1.700   150.    6.01   0    .43    .90
42- 52    47   GLU   -.640   120.    4.51   1    .63   1.56
43- 53    48   TRP   2.250   120.    4.72   1    .65   1.72
44- 54    49   MET   1.230   125.    6.83   1    .44   2.03
45- 55    50   GLY    .000   135.    6.18   1    .29   1.33
46- 56    51   TYR    .960   125.    5.72   1    .80   2.17
47- 57    52   ILE   1.800   120.    3.52   1   1.24   1.84
48- 58    53   SER   -.040     0.    3.34   0    .87   1.16
49- 59    54   TYR    .960   130.    1.92   1    .31   1.19
50- 60    55   ASN   -.600    40.    1.72   0    .34   1.26
51- 61    56   GLY    .000   130.    2.30   1    .19   1.15
52- 62    57   THR    .260   180.    2.81   0    .14    .99
53- 63    58   THR    .260   130.    1.61   1    .43   2.21
54- 64    59   SER   -.040   180.    2.90   0    .70    .76
55- 65    60   TYR    .960   180.    4.21   0    .96    .64
56- 66    61   ASN   -.600   165.    3.48   0   1.02    .59
57- 67    62   PRO    .720   165.    4.36   0    .38    .16
58- 68    63   SER   -.040   180.    6.60   0    .87    .20
59- 69    64   LEU   1.700   170.    6.21   0   1.06    .25
60- 70    65   LYS   -.990   180.    8.08   0    .38    .24
61- 71    66   SER   -.040   170.    7.11   0    .24    .27
62- 72    67   ARG  -1.010   160.    6.77   0    .34    .76
63- 73    68   ILE   1.800   165.    5.60   0    .60    .57
64- 74    69   SER   -.040    55.    6.52   0    .59    .53
65- 75    70   ILE   1.800    50.    4.64   0    .35    .47
66- 76    71   THR    .260   160.    3.52   0    .55    .81
67- 77    72   ARG  -1.010   180.    3.61   0    .60    .65
68- 78    73   ASP   -.770     0.    4.86   0    .71    .59
69- 79    74   THR    .260    80.    6.90   1   1.17    .33
70- 80    75   SER   -.040     0.    8.46   0    .57    .29
71- 81    76   LYS   -.990    15.    8.29   0    .20    .13
72- 82    77   ASN   -.600    30.    6.09   0    .26    .17
73- 83    78   GLN   -.220    30.    6.33   0    .42    .33
74- 84    79   PHE   1.790    40.    7.39   0    .30    .35
75- 85    80   PHE   1.790    40.    7.24   0    .33    .39
76- 86    81   LEU   1.700    50.    7.16   0    .52    .61
77- 87    82   GLN   -.220    45.    4.20   0   1.08    .86
78- 88    83   LEU   1.700   115.    3.58   1   1.03   1.50
79- 89    84   ASN   -.600     0.    4.37   0    .78    .61
80- 90    85   SER   -.040   135.    4.91   1    .35   1.23
81- 91    86   VAL   1.220   145.    3.65   0    .13    .80
82- 92    87   THR    .260   130.    2.61   1    .98   1.55
83- 93    88   THR    .260    75.    2.30   0    .84   1.27
84- 94    89   GLU   -.640    55.    2.64   0    .42    .95
85- 95    90   ASP   -.770    45.    2.33   0    .46    .49
86- 96    91   THR    .260    30.    4.16   0    .24    .18
87- 97    92   ALA    .310    35.    3.28   0    .64    .23
88- 98    93   THR    .260    40.    4.20   0    .80    .46
89- 99    94   TYR    .960    35.    4.96   0    .48    .23
90-100    95   TYR    .960    35.    5.19   0    .22    .14
91-101    96   CYS   1.540    35.    4.94   0    .18    .27
92-102    97   ALA    .310    30.    6.20   0    .38    .11
93-103    98   ARG  -1.010    35.    6.32   0    .43    .20
94-104    99   ASN   -.600    35.    9.30   0    .70    .24
95-105   100   ASP   -.770    30.   11.37   0    .62    .10
96-106   101   GLU   -.640    40.   10.24   0    .79    .17
97-107   102   ARG  -1.010    35.    8.97   0    .74    .27
98-108   103   ALA    .310    20.   10.85   0   1.10    .33
99-109   104   TRP   2.250    30.    8.73   0   1.14    .60
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100-110   105   PHE   1.790   30.    8.08   0   1.06    .55
101-111   106   ALA    .310   85.    7.55   1   1.50    .29
102-112   107   TYR    .960   90.    5.65   1   2.02    .68
103-113   108   TRP   2.250   85.    3.99   1   1.62   1.27
104-114   109   GLY    .000   80.    3.76   1   1.04    .92
105-115   110   GLN   -.220  130.    3.14   1    .76   1.44
106-116   111   GLY    .000   75.    2.98   0    .64   1.21
107-117   112   THR    .260   55.    3.40   0    .41   1.03
108-118   113   LEU   1.700   55.    3.68   0    .52   1.12
          114   VAL   1.220
          115   THR    .260
          116   VAL   1.220
          117   SER   -.040
          118   ALA    .310
```

PREDICTED AMPHIPATHIC SEGMENTS

```
    MID POINTS  ANGLES       AS
      OF BLOCKS
-------------------------------------

  K P   8-  9   100.-100.     4.5
    P  11- 16   110.-135.    12.4
    P  33- 38   120.-135.     9.3
       47- 52   120.-135.    10.7
      106-110    80.-130.     7.6

    NO. OF PREDICTED BLOCKS :   25
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APPENDIX B

ADDITIONAL RESULT FOR CHAPTER III: OUTPUT RESULT FROM THE

ANALYSIS OF VL OF ANTI-C2 MONOCLONAL ANTIBODY USING

'AMPHI' SOFTWARE

```
VLC2Dharshan
SEQ. LENGTH: 119 AMINOACIDS

  BLOCK     MID POINT OF BLOCKS   THETA  I(THETA)  HEL   A1    A2
          ---------------------
          RES NO.  RES.    HYD.

            1      ASP    -.770
            2      VAL    1.220
            3      VAL    1.220
            4      MET    1.230
            5      THR     .260
  1- 11     6      GLN    -.220   180.    3.18     0    .44   .49
  2- 12     7      THR     .260    50.    2.47     0    .18   .32
  3- 13     8      PRO     .720   175.    2.12     0    .27   .27
  4- 14     9      LEU    1.700   160.    2.84     0    .38   .15
  5- 15    10      THR     .260   170.    3.53     0    .36   .16
  6- 16    11      LEU    1.700   175.    4.06     0    .47   .46
  7- 17    12      SER    -.040   160.    3.97     0    .61   .63
  8- 18    13      VAL    1.220   160.    3.65     0    .68   .52
  9- 19    14      THR     .260   165.    4.02     0    .46   .53
 10- 20    15      ILE    1.800   170.    2.39     0   1.40   .73
 11- 21    16      GLY     .000   180.    2.56     0   1.14  1.29
 12- 22    17      GLN    -.220   130.    3.27     1    .60  2.25
 13- 23    18      PRO     .720   130.    3.91     1    .21  1.91
 14- 24    19      ALA     .310   180.    4.00     0    .86  1.26
 15- 25    20      SER    -.040   135.    4.15     1    .85  1.26
 16- 26    21      ILE    1.800   160.    3.33     0    .25   .46
 17- 27    22      SER    -.040   155.    4.61     0    .23   .45
 18- 28    23      CYS    1.540   155.    3.50     0    .26   .50
 19- 29    24      LYS    -.990   175.    3.18     0    .53   .95
 20- 30    25      SER    -.040    45.    4.32     0    .61   .48
 21- 31    26      SER    -.040   180.    4.99     0    .93   .87
 22- 32    27      GLN    -.220   105.    4.59     1   1.74  1.32
 23- 33    28      SER    -.040    50.    5.12     0   1.08   .94
 24- 34    29      LEU    1.700    45.    3.78     0    .98   .53
 25- 35    30      LEU    1.700    40.    3.30     0    .99   .40
 26- 36    31      ASP    -.770    40.    3.70     0   1.19   .45
 27- 37    32      SER    -.040    45.    4.65     0   1.64   .50
 28- 38    33      ASP    -.770    40.    6.21     0    .99   .31
 29- 39    34      GLY     .000    45.    6.12     0   1.18   .37
 30- 40    35      LYS    -.990   180.    5.59     0    .97   .72
 31- 41    36      THR     .260    10.    7.13     0   1.10   .81
 32- 42    37      TYR     .960    20.    7.37     0    .74   .52
 33- 43    38      LEU    1.700    25.    6.35     0    .86   .46
 34- 44    39      ASN    -.600    85.    7.04     1    .95   .14
 35- 45    40      TRP    2.250    85.    6.67     1   1.11   .26
 36- 46    41      LEU    1.700   150.    4.13     0    .98   .42
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37- 47    42    LEU    1.700    30.    3.86    0    1.14    .46
38- 48    43    GLN    -.220    30.    4.16    0    1.00    .26
39- 49    44    ARG   -1.010    80.    4.38    1     .84    .28
40- 50    45    PRO     .720     0.    6.92    0     .62    .54
41- 51    46    GLY     .000   100.    6.02    1    2.25    .80
42- 52    47    GLN    -.220   105.    7.60    1    2.36   1.83
43- 53    48    SER    -.040   100.    6.32    1    2.41   1.05
44- 54    49    PRO     .720    95.    4.36    1    1.69    .76
45- 55    50    LYS    -.990    25.    5.13    0    1.11    .86
46- 56    51    ARG   -1.010    30.    5.58    0    1.37    .90
47- 57    52    LEU    1.700    35.    5.66    0    1.41   1.33
48- 58    53    ILE    1.800    40.    7.93    0    1.37    .79
49- 59    54    TYR     .960    50.    7.78    0    1.84    .91
50- 60    55    LEU    1.700   105.    7.07    0    1.19    .98
51- 61    56    VAL    1.220   110.    6.96    0    1.15   1.48
52- 62    57    SER    -.040    20.    5.36    0    1.13    .92
53- 63    58    LYS    -.990    25.    4.21    0    1.05    .96
54- 64    59    LEU    1.700    90.    3.81    1    1.51    .56
55- 65    60    ASP    -.770    95.    5.81    1    2.26    .63
56- 66    61    SER    -.040    90.    4.75    1    1.90   1.04
57- 67    62    GLY     .000    90.    6.06    1    2.18    .99
58- 68    63    VAL    1.220    90.    6.60    1    2.22    .93
59- 69    64    PRO     .720    80.    6.32    1    1.94   1.01
60- 70    65    ASP    -.770    80.    4.22    1    1.86   1.04
61- 71    66    ARG   -1.010    85.    3.64    1    2.62   1.20
62- 72    67    PHE    1.790    90.    3.28    1    2.52   1.36
63- 73    68    THR     .260   100.    3.06    1    2.41   1.19
64- 74    69    GLY     .000   100.    2.26    1    2.16   1.85
65- 75    70    SER    -.040   110.    2.33    1    1.20   1.27
66- 76    71    GLY     .000   180.    5.34    0     .63   1.26
67- 77    72    SER    -.040   165.    2.63    0     .48    .95
68- 78    73    GLY     .000   180.    3.13    0     .06    .30
69- 79    74    THR     .260   180.    4.75    0     .38    .17
70- 80    75    ASP    -.770   180.    7.32    0     .68    .24
71- 81    76    PHE    1.790   175.    8.93    0     .48    .42
72- 82    77    ALA     .310   160.    7.66    0     .74    .60
73- 83    78    LEU    1.700   160.    8.30    0     .98    .96
74- 84    79    LYS    -.990   155.    9.39    0     .74   1.04
75- 85    80    ILE    1.800   160.    8.21    0     .68   1.02
76- 86    81    ARG   -1.010   150.    8.15    0     .53    .57
77- 87    82    ARG   -1.010   140.    9.17    0     .17   1.46
78- 88    83    VAL    1.220   140.   11.71    0     .35   1.27
79- 89    84    GLU    -.640   135.    9.09    1    1.09   1.92
80- 90    85    ALA     .310   140.    8.25    0     .68   1.22
81- 91    86    GLU    -.640   140.    6.37    0     .38   1.01
82- 92    87    ASP    -.770   150.    5.18    0     .38    .52
83- 93    88    LEU    1.700   145.    4.57    0     .11    .80
84- 94    89    GLY     .000     0.    6.77    0     .15   1.00
85- 95    90    VAL    1.220    30.    3.89    0     .45   1.31
86- 96    91    TYR     .960     0.    4.80    0     .52   1.26
87- 97    92    TYR     .960   180.    3.24    0     .41    .95
88- 98    93    CYS    1.540    55.    2.62    0     .55   1.37
89- 99    94    TRP    2.250    45.    3.65    0     .58   1.25
90-100    95    GLN    -.220    45.    2.29    0     .45    .94
91-101    96    GLY     .000    60.    3.33    0     .36   1.21
92-102    97    THR     .260    65.    4.10    0     .32    .92
93-103    98    HIS     .130    70.    4.69    0     .60   1.05
94-104    99    PHE    1.790    80.    3.19    1     .81   1.34
95-105   100    PRO     .720   105.    2.05    1    1.67   1.19
96-106   101    HIS     .130    85.    2.81    1    2.03    .69
97-107   102    THR     .260    85.    2.12    1    2.33   1.06
98-108   103    PHE    1.790   100.    3.57    1    2.14   1.03
99-109   104    GLY     .000   105.    4.13    1    1.60   1.39
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100-110   105   GLY    .000   180.   3.86   0   .69   2.04
101-111   106   GLY    .000   180.   5.83   0   .35   1.22
102-112   107   THR    .260   180.   7.61   0   .51    .82
103-113   108   LYS   -.990   135.   4.85   1   .58   1.03
104-114   109   LEU   1.700   160.   5.91   0   .14    .33
105-115   110   GLU   -.640   160.   5.99   0   .14    .37
106-116   111   ILE   1.800   160.   6.13   0   .16    .31
107-117   112   LYS   -.990   180.   5.62   0   .04    .57
108-118   113   ARG  -1.010   180.   6.38   0   .04    .59
109-119   114   LYS   -.990    50.   5.72   0   .10    .77
          115   SER   -.040
          116   THR    .260
          117   LEU   1.700
          118   THR    .260
          119   GLY    .000
```

PREDICTED AMPHIPATHIC SEGMENTS

```
    MID POINTS   ANGLES        AS
      OF BLOCKS
--------------------------------------

    P  17- 18   130.-130.     4.2
    P  46- 49    95.-105.     8.7
       59- 70    80.-110.    24.8
  K P  99-104    80.-105.    11.1

      NO. OF PREDICTED BLOCKS :   24
```

APPENDIX C

COMPONENTS OF cDNA, PCR AND LIGATION MIXTURE

cDNA Mix

Components	Volume
RT buffer (10X)	2.0 µl
MgCl (25 mM)	4.0 µl
DTT (0.1 M)	2.0 µl
RNaseOUT	1.0 µl
SuperScript III RT	1.0 µl

PCR Mix

Components	Volume
Buffer (10X)	5.0 µl
dNTP (10 mM)	1.0 µl
MgCl (25 mM)	3.0 µl
Taq DNA Polymerase Recombinant	1.0 µl
DNA	2.0 µl
Forward primer	2.0 µl
Reverse primer	2.0 µl
Sterile water	34.0 µl

Ligation Mix

Components	Volume
Salt solution	1.0 µl
pCR2.1-TOPO vector	1.0 µl
DNA	2.0 µl
Sterile water	2.0 µl

APPENDIX D

COMPONENTS OF DIGESTION MIXTURES

Digestion mixture 1

Components	Volume
Buffer 2	5.0 µl
BSA	0.5 µl
*EcoR*V	0.5 µl
*Nhe*I	0.5 µl
Sterile water	40.0 µl

Digestion mixture 2

Components	Volume
Buffer 3	5.0 µl
BSA (100X)	0.5 µl
*EcoR*V	0.5 µl
*Sal*I	0.5 µl
Sterile water	40.0 µl

Digestion mixture 3

Components	Volume
Buffer 3	5.0 µl
BSA (100X)	0.5 µl
*Pvu*I	1.0 µl

Digestion mixture 4

Components	Volume
Buffer 1	5.0 µl
BSA (100X)	0.5 µl
*Age*I	0.5 µl
Sterile water	40.5 µl

Digestion mixture 5

Components	Volume
Buffer 3	5.0 µl
BSA (100X)	0.5 µl
*BsiW*I	0.5 µl

Digestion mixture 6

Components	Volume
Buffer 4	5.0 µl
BSA (100X)	0.5 µl
*Nhe*I	0.5 µl
*NgoMI*V	0.5 µl
Sterile water	40.0 µl

Digestion mixture 7

Components	Volume
Buffer 4	5.0 µl
BSA (100X)	0.5 µl
*NgoMI*V	0.5 µl
Sterile water	40.5 µl

Digestion mixture 8

Components	Volume
Buffer 3	5.0 µl
BSA (100X)	0.5 µl
*Not*I	0.5 µl
Sterile water	40.5 µl

Digestion mixture 9

Components	Volume
Buffer 4	5.0 µl
BSA (100X)	0.5 µl
*NgoMI*V	0.5 µl
*BstB*I	0.5 µl

APPENDIX E

CLONEPIX FL SYSTEM

Source: Molecular Devices, USA

APPENDIX F

ÄKTAPRIME PLUS SYSTEM

Source: GE Healthcare, USA

APPENDIX G

ETHICAL CLEARANCE LETTER FOR THE USE OF MONKEYS

UNIVERSITY OF MALAYA

K U A L A   L U M P U R

The Leader In Research and Innovation

PEJABAT KETUA

14 Julai 2010

Suba Darshanan
Jabatan Fisiologi
Fakulti Perubatan
Universiti Malaya

Tuan,

IMMUNOGENICITY EVALUATION OF HUMANIZED ANTIBODIES FOR COLORECTAL AND OVARIAN CANCER DIAGNOSIS OR/ AND THERAPY IN NON-HUMAN PRIMATES

Dengan sukacitanya Jawatankuasa Etika Penjagaan dan Penggunaan Haiwan, Fakulti Perubatan, Universiti Malaya telah meluluskan permohonan untuk penyelidikan tersebut di atas.

No rujukan etika: FIS/14/07/2010/SD (R)

Sila ambil perhatian bahawa nombor rujukan etika yang diberi adalah sah untuk tempoh **dua tahun sehingga 13 Julai 2012.**

Sekian, terima kasih.

Yang benar,

Dr. Haji Azizuddin Bin Haji Kamaruddin
Ketua
Pusat Haiwan Makmal
Fakulti Perubatan
Merangkap Setiausaha Jawatankuasa Etika Penjagaan dan Penggunaan Haiwan

SK   :   Puan Zura Syazleena Hamizan
              Setiausaha MCRC
              Pejabat Dekan
              Fakulti Perubatan

APPENDIX H

LIST OF KITS

Kit	Supplier
One Shot Mach1-T1 Chemically Competent *E. coli* (cat. no.: C8620-03)	Life Technologies, USA
QIAEX gel extraction (cat. no.: 20021)	Qiagen, Germany
QIAfilter Plasmid Maxi (cat. no.: 12263)	Qiagen, Germany
QIAprep spin Miniprep (cat. no.: 27106)	Qiagen, Germany
QIAquick gel extraction (cat. no.: 28704)	Qiagen, Germany
QIAquick PCR purification (cat. no.: 28104)	Qiagen, Germany
RNeasy Mini (cat. no.: 74104)	Qiagen, Germany
Superscript III First strand Synthesis System (cat. no.: 1800080-051	Life Technologies, USA
Taq DNA Polymerase Recombinant (cat. no.: 11615-010)	Life Technologies, USA
TOPO-TA cloning (cat. no.: K4500-01)	Life Technologies, USA

APPENDIX I

LIST OF EQUIPMENT

Equipment	Supplier
Äktaprime Plus system	GE Healthcare, USA
AlphaImager 2200	ProteinSimple, USA
ClonePix FL system	Molecular Devices, USA
CO_2 incubator	Binder, Germany
Coolcell	Biocision, USA
Inverted microscope	Nikon, USA
Mini 50 VP model	Major Science, USA
Nanophotometer	Implen, Germany
Peltier Thermal Cycler-100	MJ Research, USA
Synergy HT multi-mode microplate reader	BioTek, USA
Vivascience Vivaflow 200	Sartorius, USA

APPENDIX J

LIST OF SOFTWARE

Software	Source
AMPHI	Centre of Molecular Immunology, Cuba
Bioedit	http://www.mbio.ncsu.edu/BioEdit/BioEdit.zip
IgBLAST	http://www.ncbi.nlm.nih.gov/igblast/

LIST OF CONSUMABLES

Consumables	Supplier
1 kb DNA ladder	Promega, USA
100 bp DNA ladder	Promega, USA
6-well plates (cat. no.: 3516)	Corning, USA
ABTS	Roche, Germany
Agarose	1st BASE Laboratories, Malaysia
Ampicillin	Life Technologies, USA
Antibiotic/antimycotic (cat. no.: 15240)	Life Technologies, USA
Anti-human capture antibody conjugated to fluorescein isothiocyanate (FITC) (cat. no.: K8200)	Molecular Devices, USA
Anti-human IgG antibody (AHIgG) (cat. no.: I2136)	Sigma-Aldrich, USA
Anti-human IgG conjugated to peroxidase enzyme (cat. no.: A6029)	Sigma-Aldrich, USA
Anti-mouse detection agent-FITC (cat. no.: K8220)	Molecular Devices, USA
Bicistronic UCOE expression vector (CET1019AD)	Milipore, USA
*BstB*I	New England Biolabs, USA
Calf Intestinal Alkaline Phosphatase (CIAP)	Life Technologies, USA

Complete Freund's adjuvant (CFA)	Sigma-Aldrich, USA
Cryotubes (cat. no.: 377224)	Thermo Fisher Scientific, USA)
Dulbecco's modified eagle medium (DMEM)	Biochrom, Germany
E. coli Fast-Media agar with zeocin (cat. no.: fas-zn-s)	InvivoGen, USA
E. coli Fast-Media broth with blasticidin (cat. no.: fas-bl-l)	InvivoGen, USA
E. coli Fast-Media broth with blasticidin (cat. no.: fas-bl-s)	InvivoGen, USA
E. coli Fast-Media broth with zeocin (cat. no.: fas-zn-l)	InvivoGen, USA
*Eco*RV	New England Biolabs, USA
Ethidium bromide	Sigma-Aldrich, USA
Fetal bovine serum (cat. no.: S0615)	Biochrom, Germany
FuGENE HD transfection reagent	Roche, Germany
Full range Rainbow marker	GE Healthcare, USA
Glutamax (cat. no.: 35050)	Life Technologies, USA
HiTrap Protein A HP 1 ml column	GE Healthcare, USA
Hybridoma-SFM (cat. no.: 12045)	Life Technologies, USA
IgG Elution Buffer with a pH of 2.8 (cat. no.: 21004)	Thermo Fisher Scientific, USA
Immuno 96 micro well solid plates (cat. no.: 442404)	Thermo Fisher Scientific, USA
Incomplete Freund's adjuvant (IFA)	Sigma-Aldrich, USA
*I-Sce*I	New England Biolabs, USA
LB-agar	Lennox, USA
LB-broth	Lennox, USA

Leibovitz's L-15 medium	Sigma-Aldrich, USA
L-histidinol dihydrochloride	Sigma-Aldrich, USA
Lipofectamine 2000 (cat. no.: 11668027)	Life Technologies, USA
Monocistronic expression vectors, pFUSe2-CLIg-hk	Invivogen, USA
Monocistronic expression vectors, pFUSE-CHIG-hG1	Invivogen, USA
Monocistronic UCOE expression vector (CET1019AS)	Milipore, USA
*NgoMI*V	New England Biolabs, USA
*Nhe*I	New England Biolabs, USA
*Not*I	New England Biolabs, USA
NS0 cells	ATCC, USA
Opti-MEM reduced-serum medium (cat. no.: 51985034)	Life Technologies, USA
*Pvu*I	New England Biolabs, USA
*Sal*I	New England Biolabs, USA
Semi-solid media for hybridomas/myelomas (cat. no.: K8600)	Molecular Devices, USA
Serum-free cell freezing media containing DMSO (cat. no.: C6295)	Sigma-Aldrich, USA
SFM4CHO media (cat. no.: SH30518)	Thermo Fisher Scientific, USA)
Supercoiled DNA ladder (cat. no.: 15622-012)	Life Technologies, USA

SW1116 cell line (cat. no.: CCL-23)	ATCC, USA
Synthechol (cat. no.: S5442)	Sigma-Aldrich, USA
T4 DNA ligase	Promega, USA
T75 flasks (cat. no.: 156472)	Thermo Fisher Scientific, USA)
Triple flasks	Thermo Fisher Scientific, USA)
Trypan blue dye	(Biochrom, Germany).

APPENDIX L

LIST OF PUBLICATIONS

1. **ELECTRONIC JOURNAL OF BIOTECHNOLOGY**

    2011, Volume: 14, Issue: 2

    http://dx.doi.org/10.2225/vol14-issue2-fulltext-7

    Suba Dharshanan, Heilly Chong, Cheah Swee Hung, Zulkeflie Zamrod & Nazlee Kamal

    Title: Rapid automated selection of mammalian cell line secreting high level of humanized monoclonal antibody using ClonePixFL system and the correlation between exterior median intensity and antibody productivity.

    (Manuscript accepted and published)

2. **ASIA PACIFIC JOURNAL OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY**

    2011, Volume: 19, Issue: 2, Page 63-71

    http://www.msmbb.org.my/apjmbb/html192/192b.pdf

    Suba Dharshanan, Heilly Chong, Cheah Swee Hung & Zulkeflie Zamrod

    Title: Application of cytotechnology techniques: a case study for production, purification and characterization of humanized antibody secreted by NS0 transfectoma

    (Manuscript accepted and published)

3. **SCIENTIFIC RESEARCH AND ESSAY**

   Suba Dharshanan, Heilly Chong, Cheah Swee Hung & Zulkeflie Zamrod

   Title: Development of humanized monoclonal antibodies by logical approach: characterization, functional studies *in vitro* and immunogenicity studies *in vivo* in non-human primates

   (Manuscript submitted)

4. **CYTOTECHNOLOGY**

   Suba Dharshanan, Heilly Chong, Cheah Swee Hung & Zulkeflie Zamrod

   Title: Stable expression of humanized anti-C2 monoclonal antibody in NS0 and CHO cells using pFUSE and UCOE expression system

   (Manuscript submitted)

LIST OF PRESENTATIONS

1. **BIOPROCESS INTERNATIONAL CONFERENCE AND EXHIBITION, BEIJING, CHINA.**

   7-8 SEPTEMBER 2009

   Title: Humanization of anti-C2 antibody and the fast selection of high-antibody producing clones using an automated system.

   Abstract:

   Murine anti-C2 antibody recognized epitopes expressed in ior C2 antigen, a glycoprotein complex expressed in human malignant colorectal cells. The anti-C2 antibody was humanized using a novel method developed and licensed from Center of Molecular Immunology, Cuba. An automated system was used for rapid selection of the high-producing clones.

2. **IMMUNOTHERAPY AND CANCER SEMINAR BY CARIF, SUBANG JAYA, MALAYSIA.** (Invited speaker)

   11 JUNE 2010

   Title: Humanized monoclonal antibodies targeting receptors.

3. **XV INTERNATIONAL SCIENTIFIC CONGRESS CNIC, HAVANA, CUBA.**

   28 JUNE-1 JULY 2010

   Title: High throughput selection of high-producing mammalian cell line using a rapid automated clonal selection method.

Abstract:

The selection of high-producing mammalian cell lines represents a bottleneck in process development for the production of biopharmaceuticals. Traditional methods are time consuming, low probability of monoclonality and significantly limited by the number of clones that can be feasibly screened. Here, we describe the use of ClonePix[TM] FL (a high-throughput automated colony picking system) to screen and pick high producer clones secreting humanized anti-C2 monoclonal antibody (hC2-mAb) from over 100,000 clones in less than 1 hour. Transfected NS0 cells expressing hC2-mAb are immobilized in semi-solid medium and incubated for 1-2 weeks to form colonies. Secreted hC2-mAbs are retained in the proximity of its corresponding colony due to medium viscosity and it can be visualized by the addition of fluorescently labeled capture antibody. When measuring secretion by fluorescence, data from white light and fluorescent images are merged and the system can exclude high-producing colonies that might be close to non-producing colonies, thus preventing contamination with non-producing cells. The highest producing colonies are identified and picked automatically by pre-programmed software for downstream applications. 100,000 clones were screened and only 271 (~0.27%) clones were above threshold value of 1000 fluorescence unit (FU). Out of those 271 clones, 217, 51 and 3 clones were between 1000-2000 FU, 2000-3000 FU and 3000-4000 FU respectively with the highest FU equal to 3840. The high-throughput ability of ClonePix system enables the screening high number of clones to give a reasonable probability of finding high-producing clones which are extremely rare.

4. **RESEARCH AND DEVELOPMENT SEMINAR SERIES, INNO BIOLOGICS SDN BHD, NILAI, MALAYSIA**. (Invited speaker)

14 FEBRUARY 2011.

Title: Development and characterization of humanized anti-c2 monoclonal antibody.

Abstract:

Three versions of humanized anti-C2 monoclonal antibodies (hum-C2 mabs) were developed and transfected into NS0 cells. Instead of limiting dilution cloning method, Clone Pix FL system was used to screen and pick high producer transfectomas secreting hum-C2 mabs. In order to obtain hum-C2 mabs that are free from contaminating exogenous proteins present in serum, the high-producer transfectomas were adapted to serum-free media and cultured in triple flasks. The hum-C2 mabs in the culture supernatant were purified using HiTrap Protein A column and Äktaprime Plus system. The purified hum-C2 mabs was then analyzed by SDS-PAGE under reducing and non-reducing conditions. Under reducing conditions, only two bands were stained with approximately 50 kDa and 25 kDa which correspond to the heavy chain and light chain respectively. Under non-reducing conditions, a higher molecular weight band with an apparent molecular mass of 150 kDa was observed. Two versions of hum-C2 mabs were then immunized intradermically in *Macaca fascicularis* on days 0, 14, 28, and 42. Blood was collected prior to the first immunization and then every 14 days. The antibody response which measured by ELISA shows that both versions of the hum-C2 mabs induces lower antibody response in monkeys compared to mouse-C2 mabs. A cell-based ELISA done also shows that the engineered hum-C2 mabs were still able to bind to C2 antigen expressed by SW1116 colorectal carcinoma cells. However, since the productivity of hum-C2

mabs were low (~6 mg/L), currently hum-C2 mabs are being developed using different expression vectors to significantly improve the yield of antibody.

5. **WEB SEMINAR, MOLECULAR DEVICES, HAMPSHIRE, ENGLAND.**

22 JULY 2011. (Invited speaker)

Title: Using high throughput automated selection to increase discovery of rare, high producing mammalian cell lines.

Abstract:

The selection of high-producing mammalian cell lines represents a bottleneck in process development for the production of biopharmaceuticals. Traditional methods are time consuming; significantly limiting the number of clones that can be feasibly screened and can result in low probability of monoclonality. Here, we describe the use of ClonePix™ to screen and pick high producer clones secreting humanized anti-C2 monoclonal antibody from 100,000 clones in a short duration. The ability of ClonePix to screen high numbers of clones in less time increases the probability of finding high-producing clones that are extremely rare.

6. **POSTGRADUATE SEMINAR, NATIONAL UNIVERSITY OF MALAYSIA, BANGI, MALAYSIA.** (Invited speaker)

10 OCTOBER 2011.

Title: Cancer immunotherapy: humanized monoclonal antibodies.

7. **16th Biological Sciences Graduate Congress, NATIONAL UNIVERSITY OF SINGAPORE, SINGAPORE.**

12-14 DECEMBER 2011.

Title: Rapid and high expression of humanized monoclonal antibodies in mammalian cells.

Abstract:

Humanized monoclonal antibodies (mAbs) are widely used for cancer immunotherapy due to their reduced immunogenicity and high specificity. Production of these valuable biopharmaceuticals often requires the use of mammalian cells, which is an expensive process with usually disappointingly low yields and long development times. Therefore four technologies were integrated to circumvent the limitations of conventional methods for the expression of humanized mAbs. First, T-cell epitope humanization technology was used to replace the conventional method of reducing the immunogenicity of murine mAbs, which often requires some amino acids from murine frameworks to be back mutated to recover its binding capacity. Conversely, T-cell epitope humanization method only judiciously and minimally substitutes potential immunogenic mouse residues in order to reduce its immunogenicity without compromising the binding capacity. Second, although by PCR mutagenesis, it is possible to humanize immunogenic mouse residues, however due to the high error-rate of Taq DNA polymerase, a large number of samples are required for DNA sequence confirmation. Thus, synthetic genes coding the humanized variable domains were used instead. Third, an automated colony picker ClonePix FL system was used to identify and isolate high producing transfectomas secreting humanized mAbs. The limiting dilution cloning method which is commonly applied for this purpose, is time-consuming, has low probability of monoclonality and is significantly limited by the number of clones that can be feasibly screened. In contrast, the high-throughput, rapid and automated nature of ClonePix FL system, allows the screening of large number

of cells in a short period of time with also an increased in the probability of obtaining rare and precious high-producing clones. Finally, the choice of expression vectors is also a crucial factor for a high and stable expression of humanized antibodies. In summary, the integration of four technology platforms allows the rapid and high expression of humanized mAbs.

APPENDIX N

PUBLICATION: CHAPTER 4

APPENDIX O

PUBLICATION: CHAPTER 5

APPENDIX P

PUBLICATION: CHAPTER 6

APPENDIX Q

CERTIFICATE OF AWARD

APPENDIX R

INVITATION TO WRITE A CHAPTER IN LAB-PROTOCOL BOOK:

METHODS IN MOLECULAR BIOLOGY

(HUMANA/SPRINGER PUSLISHING GROUP)

From: Fischer, Nicolas

Sent: vendredi 13 juillet 2012 11:52

To: suba.dharshanan@innobiologics.com

Subject: Invitation to contribute to a new edition of 'Monoclonal Antibodies'

Dear Dr Dharshanan,

As a specialist in your field of research, I am pleased to invite you to contribute a chapter to the new edition of 'Monoclonal Antibodies' that I have the pleasure to co-edit with Dr. Vincent Ossipow. This chapter could be entitled 'Hybridoma subcloning using solid matrices' or you can propose another title that you would find to be more appropriate.

This book will be part of the lab-protocol based 'Methods in Molecular Biology' series, published by Humana Press (part of the Springer publishing group). Over 750 volumes have been published in the series to date. Full details of all the Methods series can be found at Humana's web site http://www.springer.com/series/7651. The Series is prestigious, well established and each chapter appears in PubMed and on the Web of Science.

If you accept this proposal, I will send you detailed explanations. The deadline for draft submission is October 31th 2012.

I would like to stress the fact that the aim is to present routinely used protocols in a defined format, which requires much less work than contributing a more comprehensive discursive chapter. I have attached a previous chapter as an example for your consideration.

I hope that you will be able to contribute to this exciting new edition that aims at being a key reference for people working with monoclonal antibodies. If you would like to suggest a colleague/collaborator/post-doc as a potential author or co-author, this is perfectly possible as well.

You are welcome to contact me at any time should you have any question.

Kind regards,

Nicolas Fischer.

_____

Nicolas FISCHER, PhD

Head of Research Department

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