Index

A

Activated cell sorting 218, 262, 339
ADCC 20, 146, 325, 562
Affinity maturation 32, 102, 293–307, 309–319, 324, 337–338, 353–355, 358–360, 371–372, 374–375, 383
Aggregating V_H 211, 212
Angiogenin 471–472, 476–477, 485–486
Antibiotics
  ampicillin 64, 72, 77, 85, 95, 133, 136–139, 141, 149, 156, 176–179, 183, 193–194, 199, 203, 219, 223–226, 231, 234–236, 296, 311, 312, 342, 379, 381, 407, 408, 413, 420, 492–494, 514, 534
  carbenicillin 105–108, 115–117, 119, 121–122, 126, 162–169, 245, 246, 250, 280, 282, 283, 356, 357, 367, 369–371
  kanamycin 77, 85, 95, 106, 107, 117, 119, 126, 133, 136, 138–139, 162, 163, 165, 167, 169, 170, 176, 179, 193, 200, 204, 246, 251, 252, 296, 312, 320, 356, 357, 366, 368, 370, 493, 494
  tetracycline 106, 107, 116–120, 124–126, 148, 162–168, 312, 356, 366, 512, 514
Antibody
  affinity maturation 32–33, 102, 293–307, 309–319, 324, 337–338, 353–354, 358–360, 371, 374–375
  engineering 337–351
  fragments 146–147, 161–162, 187–212, 218, 242, 274, 279–280, 293–295, 310, 316–320, 323–324, 337–338, 370, 472, 483, 510, 515
  internalization 438
  library 33, 63, 86, 96, 130, 134, 140, 146, 148, 150, 176, 179, 341, 342, 350, 354, 359, 360, 373
  production 324, 402, 492, 503, 509–515
  repertoire 63, 101–102, 110, 261–277
  therapy 2, 11, 32
Antibody-based candidate therapeutics 19, 21, 81–82
Antibody-derived scaffold 22–23
Antigen capture 31–61
Antigen-specific 21, 23, 130, 175–185, 189, 217, 261–263, 275, 464
Anti-M13 phage antibody 133, 139, 163, 171, 177, 182, 194, 205, 296, 297, 305, 357, 369, 370
*ara*BAD promoter 287
Auristatin 446, 448–449, 456, 458
Avastin 4, 11, 12, 19, 21

B

Bacillus megaterium 509–515
Backbone 159, 274, 310, 458–459, 487, 534, 536, 539, 540–541, 560
Bacterial culture 141, 148, 151, 152, 157
Bacteriophage 146, 148, 153, 192, 211, 299, 301, 373, 423
BAFF receptor 3, 354
Bio-panning 161–174
Bioreactor 488, 501, 504–507, 510
Biotinylated antigen 74, 107, 120, 124, 245, 248, 249, 253, 257, 310, 317, 318, 345, 349, 368–369, 375
Bispecific antibody 377–403
BxPC-3 ..... 547–548, 551–555

C

Calicheamicin 6, 11, 425, 446, 448–450, 456–459, 465
Camelid V_HHs 187, 188, 189–190, 324
Cancer therapy 161, 377, 471, 499
CD22 ..... 16, 293–295, 302–305, 338–345, 351, 425–441, 446, 448, 450, 471–488
cDNA
  storage 62–65, 89, 97, 102, 111–114, 134, 135, 140, 219, 227, 411
  synthesis 77, 84, 88, 111, 130, 134, 406, 410–411
CDR loops 355
CDRs 65, 66, 82–84, 89, 96, 109, 190, 297, 354–355, 359–360, 372, 373, 409
Cell display
  mammalian 337–351
  yeast 338
Cell lines
  293T 37, 339
  A431 ..... 162, 166–167, 171–172

(*continued*)
BS-C-1 .... 34, 38
BT549 147
CHO 502–506
COS-7 .... 381, 394
HeLa 34, 38
MDAMB231 147
MDAMB453 147
SUM52PE 147
SUM159PT 147
Cell selection 145–159
Chimeric heavy-chain antibodies (cHCAbs) 323–335
Chromatography 37, 48–52, 55, 127, 149–150, 177, 189–190, 205, 207–209, 213, 379, 396, 403, 427, 433, 440–441, 454, 457, 465, 474–475, 482–483, 492, 493, 535
Combination index 546, 553, 554
Common epitopes 12, 70, 82, 102, 110, 124, 162, 176, 189, 197, 294, 302, 325, 338, 339, 342, 377, 406, 408–409, 414–417, 422–423, 478, 492, 560
Competent cells 71–72, 77, 85, 95, 98, 133, 136, 139, 149, 163, 173, 192, 211, 229–230, 234, 237, 245, 251, 295, 326, 330, 357–358, 366, 374, 392, 408, 419, 493, 494, 496, 534
Complete mini protease inhibitor 43
Computer 295, 552, 554, 570, 577, 578
Conformation 302, 354, 355
Conjugate 445–465
Cross-reactive antibodies 146, 353–376
Cross-species binding 353–376
Cytotoxicity 20, 145, 325, 377, 378, 425–442, 446, 449–450, 457–458, 477, 486, 562–563

D

Daudi
cells 302, 305, 430, 438, 441
human lymphoma 429
Deimmunization 405–423
DELFIA 281–285, 288
Denaturing of antigen 67, 90, 92, 134, 161, 190, 191, 201, 202, 207, 212, 228, 234, 281, 283, 324, 385–386, 389, 390, 393, 411, 417, 423
Diabody 378
Disulfide 109, 195, 280, 427, 431–434, 440, 441, 446–449, 451–458, 464–465, 510
Diversity 81–98, 354–355, 359–360, 373
DNA 35, 63–64, 91–96, 113–116, 122, 139, 155–156, 187–212, 222–237, 266–275, 279, 282, 295, 314–315, 328–332, 350–351, 412–414, 418–421, 445–446, 477–479, 492–493, 510, 515, 574, 577
Domain antibody 81, 188, 534, 536, 537
Dynabeads 107, 120, 133, 177, 180, 312

E

Electroporation 64, 71–73, 77, 85, 95, 98, 106, 125, 133, 134, 140, 183, 192, 193, 223, 225, 237, 238, 245, 251, 282, 295, 301, 314, 326, 330, 360, 473, 479, 496, 506
ELISA 121–122, 127, 133, 138–139, 169–170, 177, 181–182, 194, 204–205, 209, 210, 296, 303–305, 318–319, 357, 369–370, 374, 382, 395–396, 398, 400, 492, 501, 520, 528
Envelop 175–185
Epidermal growth factor 11, 12, 162, 378
receptor 545–557
Epitope 12, 70, 82, 102, 110, 124, 162, 176, 189, 197, 294, 302, 325, 338, 339, 342, 377, 406, 408–409, 414–417, 422–423, 478, 492, 560
Error-prone PCR 310, 311, 313–314, 319, 320, 354
Eukaryotic expression 34, 387, 388, 472

F

Fab 4, 5, 13, 17, 81–98, 101–127, 161–174, 263, 274, 275, 280, 357–358, 370–371, 423, 492–496, 510
Fab library 129–141
FACS 217, 218, 224, 231, 234–235, 238–239, 248–249, 262–263, 267, 268, 275–276, 297, 305–306, 339, 341, 344, 346, 347–349, 351, 436, 475, 483, 484
Fc receptors 20
FDA approval 4, 6, 8, 14–18, 217
Ficoll-Paque Plus 82, 86, 130
FileMaker 569–579
Flow cytometry 145, 147, 148, 152–153, 241–258, 297, 305, 307, 339, 341, 342, 344, 348, 483
Fluorescence 153, 157, 171, 218, 238, 243, 248, 256, 257, 262, 281, 339, 345, 346, 484
FRs (framework regions) 81, 82, 83, 84, 89, 353, 354
Full-length IgG antibody 217–239, 277, 378
Fusion protein 471–488

G

Gemtuzumab ozogamicin. *See* Mylotarg®
Glycoprotein 22, 31–56, 175, 185, 426, 492, 518, 560, 565
gp41....................33, 175–184, 518
Grafting 23, 81
Gram-positive bacteria 509–510

H

HB2151 ....133, 139, 183, 492, 493, 494–495
HEK293 expression system....................325–326
HEK-293T....................337–341, 344, 347–351
Helper phage
  M13KO7 ....64, 73, 85, 95, 133, 136, 138, 176, 178, 179, 181, 182, 191, 193, 194, 201, 204, 493, 494
  VCS-M13....................64, 73
Hendra virus 33, 42, 491–492
HER2....................5, 14, 145–147, 324, 446
Herceptin 1, 5, 12, 14, 19, 145, 324
High throughput 189, 218
High-throughput screen....................241–258
HIV....................175–185
Human....................61–79, 81–98, 129–141, 161–174, 261–277, 559–565
Humanization....................1, 33, 102, 110, 354, 405–423, 465
Humanized single-chain Fv 471–488
Humira....................1, 5, 11, 12, 19, 21, 129

I

IC_{50}....................23, 357, 370, 371, 372, 438, 441, 449–450, 458, 472, 486, 519, 520, 522, 524–526
IgG1....................13–18, 21, 22, 24, 135, 221, 274, 326, 329, 331, 371, 381–388, 426, 447, 450, 452, 455, 500
IgM....................16, 23, 130, 135
Imidazol....................139, 150, 157, 194, 195, 207, 474, 483, 487, 488, 493, 495, 511, 513, 515, 535, 536
Immunogenicity....................20, 146, 242, 293–294, 405, 426, 471, 560
ImmunoRNase....................471–488
Immunotoxin 307, 426, 431, 471
In vitro antibody affinity maturation....................293–307, 337
Inclusion bodies 325
Inhibition ELISA....................499–507
Inhibition of infection 517–529

L

Lentil lectin Sepharose™ 4B....................35, 39
Library construction 62, 63, 66, 67, 68, 81–98, 130, 188–189, 191, 195, 237, 282–284, 301, 341, 358
Linker....................55, 67, 69–70, 131, 132, 135, 250, 251, 267, 273, 338, 383, 385, 445–452, 455, 458, 459, 478
Luminex....................243–256
Lysosome 448, 462

M

Magnetic beads....................74, 118, 133, 137, 138, 242
Maytansinoid 446–465
Medium
  2xTY 148, 149, 151, 152, 156, 157, 534, 536
  2YT....................85, 95, 96, 133, 136–139, 141, 176–177, 179–184, 296, 311–318, 356, 366, 369–370, 493–496
  DME-H21....................147
  DMEM-10....................34, 35, 43–47
  RPMI....................147, 428, 437, 438, 451, 465, 475, 519, 547
  SOB 64, 71–72, 77, 223, 225, 229, 230, 311, 314, 546, 555–556
  SOC 64, 72, 77, 106, 115, 116, 192, 199, 223, 225, 230, 234, 245, 251, 296, 311, 314, 341, 347, 388, 392, 407, 413, 419, 493, 494
  YTAG....................64, 72–75, 77, 380, 383
  YTAK 65, 73, 76, 77
Methodology....................146, 176, 180, 569, 571
Microtubule 445–446, 449
MinElute Gel Extraction 105, 106, 113–116
Molecular evolution....................293–307
Monoclonal antibodies
  approved by US FDA 4, 10, 11
  human neutralizing HIV–1 559–565
  rabbit....................101–127
Monoclonal phage ELISA 178, 182–183, 185, 189, 194, 204–205
Multiplex....................241–258, 264, 267–271
Multivalent....................146, 188, 190, 193, 200, 201, 202, 209, 211, 279–280
Mylotarg® 6, 425, 445, 446, 448, 449

N

Naive libraries 161–174
Nanobodies 22
Neutralization 22, 33, 36, 40–41, 55, 74, 177, 226, 237, 277, 517–529, 560, 562–563

Ni-NTA....139, 149–150, 154, 157, 158, 492, 493, 495, 496, 511, 513
Nipah virus....31, 33, 42, 491
NlpA-ZZ....217, 218–219, 223–225, 230–235
NMR relaxation....533–541
Non-Hodgkin's lymphoma....10, 425–442
Non-human primate models....559–565

O

Oligo(dT)....103, 111, 263, 271, 409–410
Oligomerization....31–56
Oligonucleotide-directed mutagenesis....353–376
Onconase....425–442
One-step cloning....129–141
Overlap extension....62, 69, 92–93, 196, 197, 261–277

P

p24 infection....517–529
PA-FITC....217–239
Palivizumab....8, 12, 32, 491
Panning....63, 73–75, 78, 96, 101, 107–108, 110, 117–121, 124, 133, 137, 138–141, 175–185, 189–191, 193, 200, 201, 203–205, 212, 294, 296, 302–307, 310, 312, 316–318, 373
Paramyxovirus....31, 33, 491–492
Passive immunization....559–565
Passively administered antibody....32
pBAD33-NlpA-ZZ....218–219, 223–225, 230–231, 234, 235
PBMC....262, 263, 275, 518–527, 562–563
pC3C....105, 109, 113, 115, 116, 123, 125, 127
pComb3X....82, 83, 94, 135, 136
PCR....61–79, 84–85, 103, 111, 133, 135, 227, 234, 263, 264, 265, 266–277, 311, 313, 389–390, 393, 565
pDFC-scFv11F8....390–392
PEG8000....95–96, 133, 138
Periplasm....109, 127, 141, 156, 157, 170, 206, 217–219, 226, 236–237, 281, 282, 492, 510, 534
Phage
- antibody....145, 147, 148, 150, 153, 158, 176, 179, 242, 243
- display....101–127, 129–141, 187–212, 309–321
  - library....63, 188–191, 196, 200, 202, 211, 219, 301, 383
- ELISA....76, 79, 120–121, 127, 133, 138–139, 169, 172, 177–178, 181–182, 185, 189, 194, 203–205, 212, 296, 303, 304, 371

Phagemid....70–71, 82, 85, 93, 105, 113–114, 494, 496
Plasma cells....124, 262–263, 267, 269
pLCK-scFvA12....391, 392, 394
pMAZ360-IgG....218, 219, 223, 225, 227, 228, 230, 231, 234, 235, 236
pMID-A12....391, 392, 393
pMID-11F8....391, 393
Polymixin B....133, 139, 141
Production systems....509–510
Protective epitopes....560
Protein
- design....9, 17, 18, 279
- dynamics....533–534
- expression....38, 43, 46, 194, 206, 212, 250, 251, 255, 261, 337, 338, 350, 534, 540

pTT5....326, 327, 329, 330–332
Purification....35, 39, 40, 47, 133, 139, 162, 178, 183, 194–195, 205, 207, 226, 236, 328, 333, 383, 386, 393–394, 396, 400–403, 435, 474, 482, 491–497, 501, 505–506, 511, 513, 537

Q

QIAPrep....106, 108, 117, 122, 223, 226, 230, 236, 245, 350, 378, 383

R

Rabbit monoclonal antibodies....101–127
Recombinant
- antibody....218, 338, 492, 507, 510
- phage....187, 492, 494–495
- vaccinia virus....9, 34, 37, 38, 55, 177

Remicade....1, 6, 11, 12, 19, 21
Respiratory syncytial virus (RSV)....2–3, 32, 491–492
Ribonuclease zymogram....484–485
Rituxan....1, 7, 9, 10, 12, 17, 19, 145
RT-PCR....65, 77, 84, 103, 111, 262, 263, 267, 268, 269, 276, 565

S

ScFv....13, 61–64, 67, 69–71, 73–76, 81, 102, 147, 149, 150, 153–159, 245–247, 250–251, 253, 279–288, 299–307, 310, 315, 337–349, 380–392, 394, 396–402, 472, 477–478, 480, 483–485, 488, 510–515, 519, 522, 523, 526, 533
SCID mice....439, 451, 458
Selection system....190, 500
Semi-synthetic library....61
Sequence relational database....569–579
Shark V_{NAR}s....187
SHIV....559–565

Single-chain
antibodies 61–79, 241–258, 337–338
Fab (scFab) 509–515
fragment 377, 510, 514
Fv 61, 146, 279, 299, 378, 380
Single-domain antibody 187, 188
Somatic hypermutation 293–297, 302
Spheroplasts 217, 224, 231–232, 234, 238–239
S-protein 34, 35, 36, 39, 40, 43, 47
Stability design strategies 280
Stable clones 325, 500–504
Streptavidin capture of antigen 74, 118, 310, 317
Subtractive bio-panning 162, 163
Subtractive panning 162, 166, 172
SuperScript III 84, 89, 103, 111
Symplex™ 262–276
Synagis 3, 8, 12, 18, 19, 20, 21, 32
Synergy 545–557
Synthetic library 61, 62, 65, 212

T

Targeted drug delivery 146
T-cell epitope 405–409, 414, 416–417, 422, 423
TG1 ....64, 65, 71, 85, 95, 133, 136, 138, 139, 148, 151, 156, 176, 179, 181, 184, 192, 196, 199, 201, 203, 206, 211, 295, 302, 303, 326, 331–332, 493, 494, 496, 534
Therapeutic antibodies 1–24, 353, 499–507
Thioether 445, 448, 452, 455–458, 465
TNF-alpha 129
Total immunoglobulin 2–3, 61, 82, 102, 110, 130, 227, 262
tRNA assay 485

U

Unfolded scFv 281

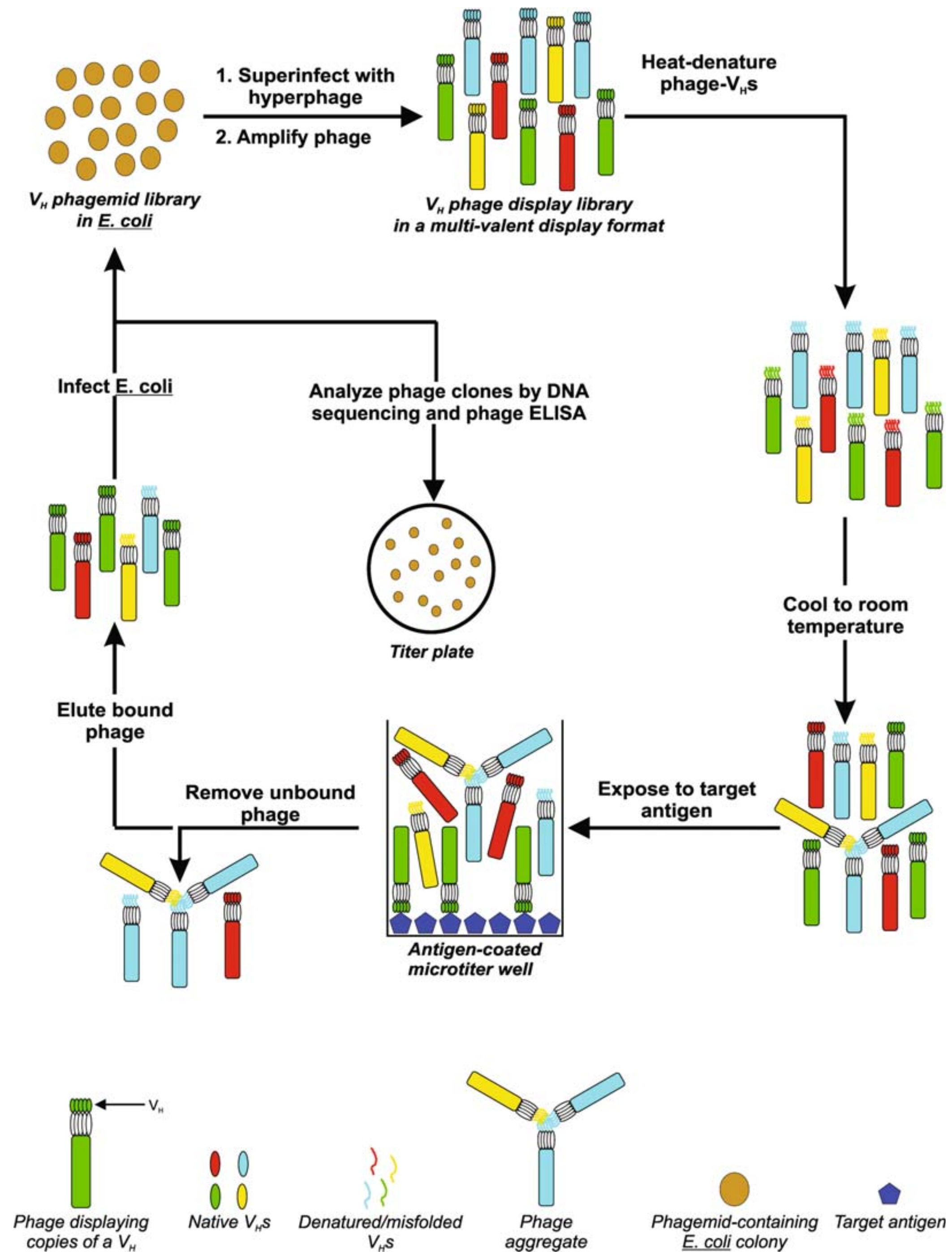
V

Variable heavy (VH) 217, 236, 277, 280, 422, 477
Variable light (VL) 217, 236, 280, 378, 477
Vascular endothelial growth factor 11, 12, 545–557

X

XL1-Blue 64, 65, 71, 105, 106, 107, 115, 116, 118–120, 124, 162, 163–166, 168–169, 174, 223–225, 315, 316, 367, 369, 370, 373, 408, 419, 421

Color Plates

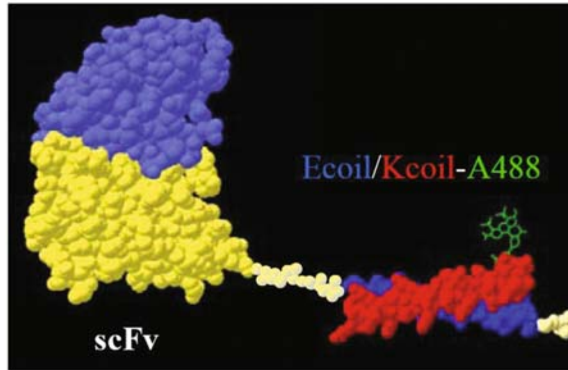


Color Plate 1. The heat-denaturation panning scheme for isolating non-aggregating V_H binders from a phagemid vector-based V_H phage-display library. *E. coli* cells harboring the V_H genes in a phagemid were superinfected with hyperphage to produce and subsequently amplify a V_H phage library in a multivalent display format. The phage library was subjected to

Color Plate 1. (continued) heat denaturation and subsequently cooled to allow for refolding, and hence, recovery of non-aggregating V_Hs. The pool of heated-and-cooled phages were then exposed to the target antigen for binding. Unbound phages (aggregating phage, non-aggregating, non-binding phage, phage displaying misfolded V_Hs) were discarded, and bound phages were eluted. The eluted phages were used to infect *E. coli* cells which were subsequently superinfected for a new round of panning. Several rounds of panning were performed to enrich, for non-aggregating binders. The titer of eluted phage was determined for each round, by plating serial dilutions of the infected cells prior to superinfection. At the end of the panning, identification of binders was initiated by DNA sequencing and phage ELISA of clones from the titer plates. (*See* discussion on p. 201)

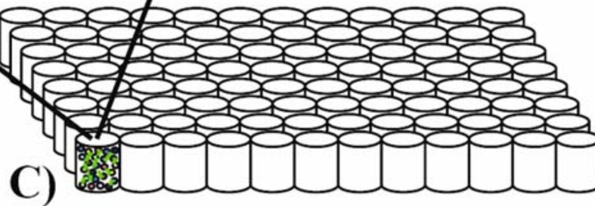
P2 Biotinylated Antigen 1
P3 Biotinylated Antigen 2
P4 Biotinylated Antigen 3
P5 Biotinylated Antigen 4
P6 Biotinylated Antigen 5
P7 Biotinylated Antigen 6
P8 Biotinylated Antigen 7
P9 Neutravidin Bead Only

A)

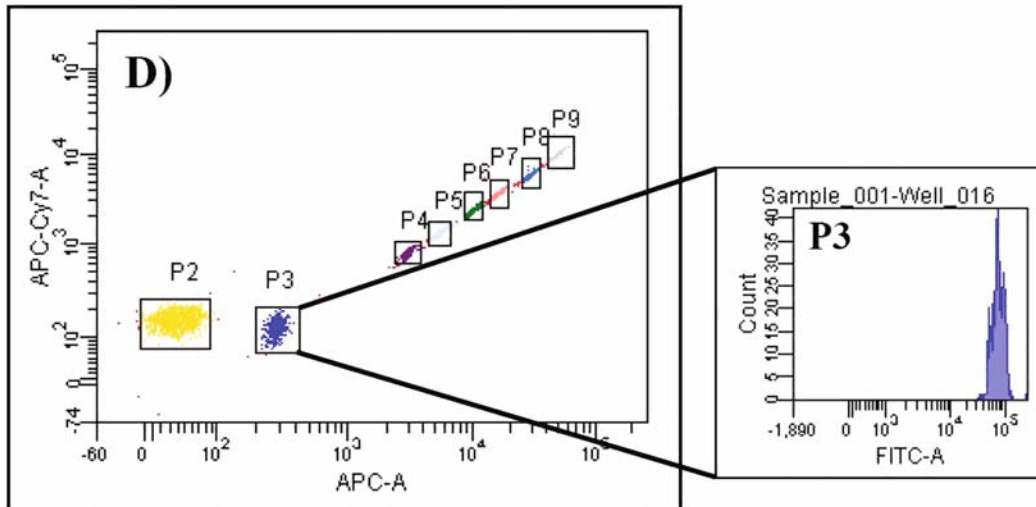


B)

Kcoil: KVSALKEKVSALKEKVSALKEKVSALKEKVSALKEC-A488
Ecoil: EVSALEKEVSALEKEVSALEKEVSALEKEVSALEK

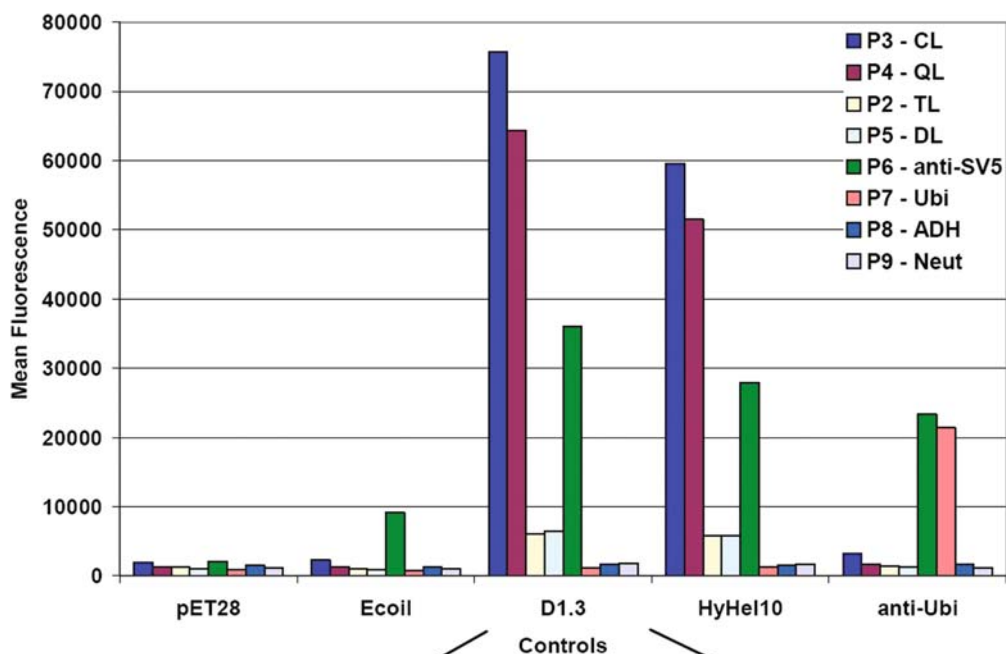


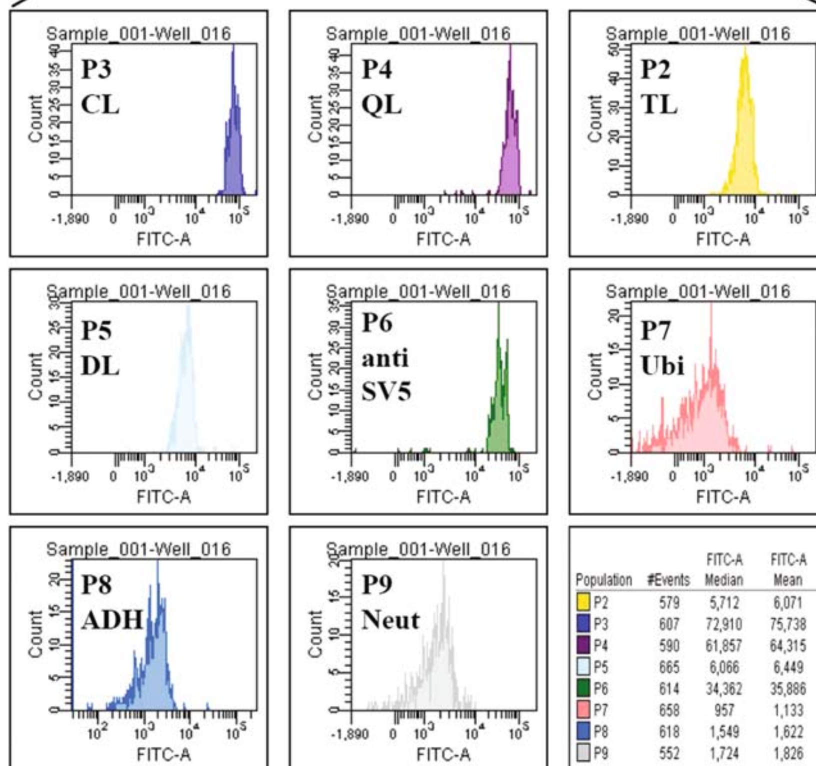
C)



Color Plate 2. (continued)

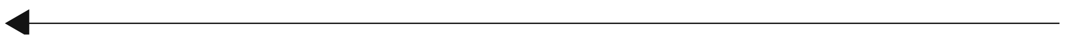
Color Plate 2. Preparation of samples for flow cytometry analysis. (**A**) The eight microsphere multiplex biotinylated antigens were coupled to different colored microsphere sets, facilitated by the biotin–neutravidin interaction. (**B)** Diagram of scFv coiled-coil complex used for screening: scFvs were fluorescently labeled using synthetic Ecoil and Kcoil peptides that form heterodimers. Kcoil labeled with Alexa488 binds to scFv expressed as Ecoil fusions. Incubation of the test scFv binder with the multiplex allows analysis against numerous targets within the same test sample. (**C)** Sample preparation and analysis was carried out in 96-well format. (**D)** The Becton Dickinson LRSII Flow Cytometer was used for data analysis. The microsphere multiplex was separated into gates by excitation using a 633-nm laser through APC-Cy7 (780/60BP) and APC (660/20BP) detectors. The mean fluorescent value of each gate, representing bound labeled scFv, was recorded following excitation using a 488-nm laser through a FITC (530/80BP) detector (Reproduced from **ref.** *(15)* with permission from the American Chemical Society). (*See* discussion on p. 253)



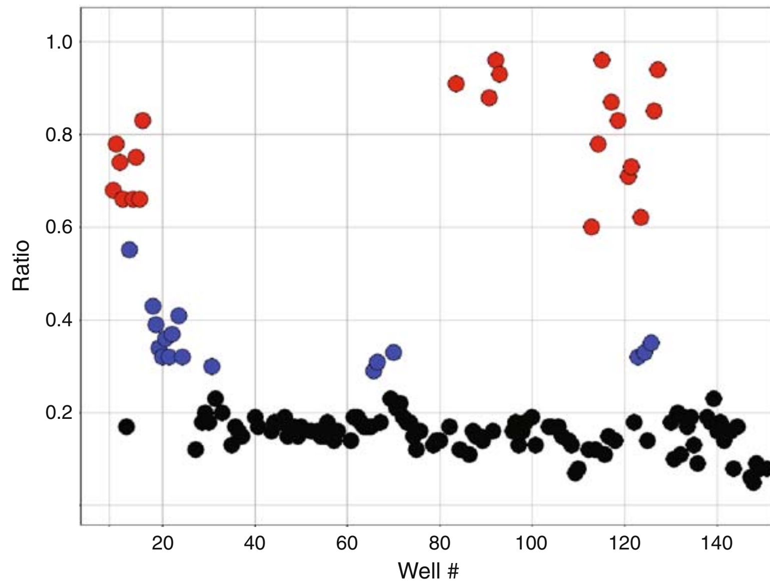


Population	#Events	FITC-A Median	FITC-A Mean
P2	579	5,712	6,071
P3	607	72,910	75,738
P4	590	61,857	64,315
P5	665	6,066	6,449
P6	614	34,362	35,886
P7	658	957	1,133
P8	618	1,549	1,622
P9	552	1,724	1,826

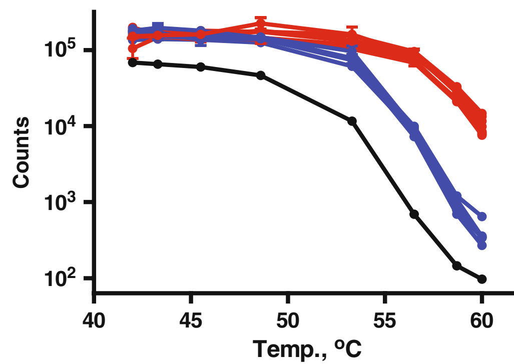
Color Plate 3. (continued)



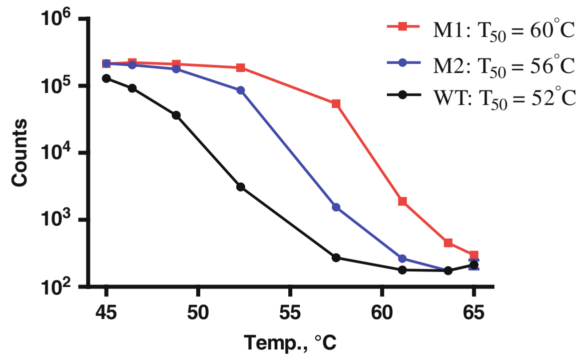
Color Plate 3. Flow cytometry screening data produced for positive control scFvs. The positive control scFvs D1.3, HyHel10, and anti-ubiquitin (anti-Ubi) show specific binding to their respective antigens; chicken lysozyme (CL) and ubiquitin (Ubi). Cross-reactivity with Quail Lysozyme (QL), Turkey Lysozyme (TL), Duck Lysozyme (DL), alcohol dehydrogenase (ADH), and Neutravidin (Neut) is also determined. The anti-SV5 value indicates the level of scFv–Ecoil fusion protein expression. Analysis of BL21 transformants containing pET28 and pEP-Ecoil (Ecoil) vectors demonstrates the insignificant background binding of free Kcoil–A488 to labeled microspheres. *Inset*: Raw flow cytometry data output for D1.3 binding to the microsphere multiplex (*see* **Note 21**) (Reproduced from **ref.** *(15)* with permission from the American Chemical Society). (*See* discussion on p. 253)



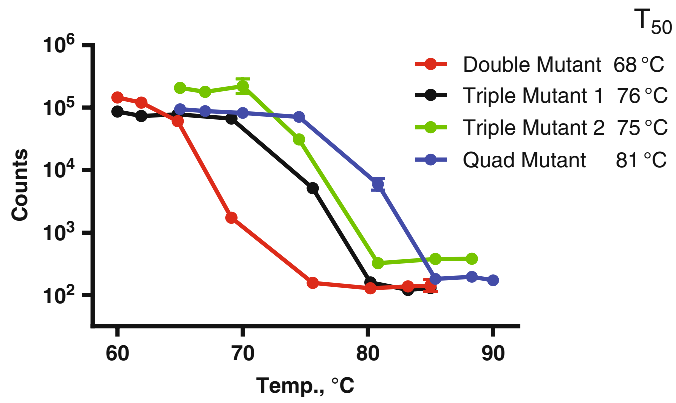
Color Plate 4. Scatter plot of screening data. As described in the test, the data are plotted as a ratio of activity remaining after the thermal challenge. Samples marked in red are likely hits, blue denotes possible hits, and black are non-stabilized. (*See* discussion on p. 284)



Color Plate 5. Confirmation assay. Samples identified in **Fig. 14.1** were challenged over a range of temperature and plated as described in the text. The black curve denotes the behavior of the parental scFv, the blue traces represent weakly stabilized hits, and the red curves are samples derived from cultures containing scFv mutations which are highly stabilized. (*See* discussion on p. 285)



Color Plate 6. T_{50} assay. Sequence-dereplicated mutations were retransformed into the W3110 strain, and individually optimized thermal challenge gradients were used to derive a T_{50} value for each mutation. (*See* discussion on p. 286)



Color Plate 7. Additive effect of combining stabilizing mutations. (*See* discussion on p. 286)