# Chapter 15

# Selection of Antibody Fragments by Yeast Display

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## Abstract

The critical need for renewable, high-quality affinity reagents in biological research, as well as for diagnostic and therapeutic applications, has required the development of new platforms of discovery. Yeast display is one of the main methods of in vitro display technology with phage display. Yeast display has been chosen by numerous groups to refine both affinity and specificity of antibodies because it enables fine discrimination between mutant clones of similar affinity. In addition, the construction of display libraries of antibody fragments in yeast permit to sample the immune antibody repertoire more fully than using phage. This chapter gives an updated overview of the available systems of yeast display platforms and libraries, followed up by technical descriptions of selection methods of antibody fragments by yeast display.

**Key words:** Yeast display scFv libraries, pYD, pAGA2, Magnetic sorting, Flow sorting, Panning, Homologous recombination, p416 BCCP

## 1. Introduction

Hybridoma technology has reliably provided monoclonal antibody reagents for the past 35 years. Yet, this technology is ridden by inherent limitations such as the dependence on animal immunization and thus could not achieve in vivo generation of antibodies against lethal toxins or pathogenes, tolerogenic molecules, or targets retained in secretory pathways. In contrast, the use of in vitro display technologies enables the identification of such affinity reagents (for reviews *see* (1–3)). For example, recombinant antibodies (scFv) binding to the envelope protein of West Nile virus were identified by yeast display (4, 5), as well as antibodies recognizing all four serotypes of Botulinum neurotoxins for rapid detection in clinical samples (6). Yeast can be used not only to display proteins, peptides, allergens (7), and even glycans (8, 9) but also to display naïve, immunized, or synthetic repertoires of various affinity reagents, belonging or not to the superfamily of immunoglobulins.

Patrick Chames (ed.), *Antibody Engineering: Methods and Protocols, Second Edition*, Methods in Molecular Biology, vol. 907,
DOI 10.1007/978-1-61779-974-7_15, 

The yeast display method was first described in 1997 by Boder and Wittrup (10). The unique capability of this approach for very fine discrimination between mutant clones of similar affinity became rapidly evident (11). Because fine discrimination is critical for rapid assessment of affinity maturation, the selection of antibody fragments by yeast display has been chosen by numerous groups to refine both affinity and specificity. Superior quality of reagents was obtained, even for difficult targets such as small molecules (haptens), including fluorescein for which Boder and colleagues isolated antibody fragments with a monovalent antigen-binding affinity of 48 fM (12). Yeast display platform was also shown to be useful for the identification of affinity reagents binding to inorganic material surfaces, enabling the study of mechanisms governing interfacial interactions and the generation of material-specific reagents (13). Furthermore, the direct comparison of yeast and phage display using the same scFv cDNA library derived from HIV-1 immune patients, and screened by the same selecting antigen (HIV-1 gp120), demonstrated that yeast display sampled the immune antibody repertoire more fully than phage display (14).

Yeast display has been trusted by many groups to improve the affinity and specificity of recognition reagents (15). Simple methods for creating diversity are based on the amplification by error-prone PCR or on the use of an *E. coli* mutator strain to mutagenize an antibody fragment (10, 16). Such methods have improved the affinity of scFv variants for TCR (17) and of Fab up to 10-fold after selection by flow cytometry analysis (18), with an increase of potency of 238-fold for a bivalent immunotoxin scFv (19). However, to increase the specificity by several hundred folds, directed evolution is required (for review, see (20)). The yeast display method also facilitates directed evolution as shown in various groups, including Shusta and colleagues who evolved single-chain T cell receptors (scTCR) to enhance stability and soluble expression, which correlated with improved resistance to thermal denaturation and intracellular processing (21); Weaver-Feldhaus and colleagues who reported an increase of specificity of more than 300-fold for a single-domain (VH) clone directed to Calmodulin (22); and very recently Orcutt et al. who published an increase of specificity of 1,000-fold for a DOTA-chelate-binding scFv (23). Directed evolution has also been used to engineer stabilized variants of a single-chain form of the class II region of the major histocompatibility complex (MHC) I-A(g7) (24).

The first published and most widely used system of yeast display is based on the fusion of the yeast cell surface gene encoding the a-agglutinin yeast mating adhesion receptor (AGA2) in N-terminal of the fragment to display at the cell surface of *Saccharomyces cerevisiae* (*S. cerevisiae*). This receptor stabilizes cell–cell interactions and facilitates fusion between mating "a" and α haploid yeast cells. It is composed of Aga1 and Aga2 proteins that bind to each other

during secretion through two disulfide bonds. After secretion, Aga1 attaches covalently to b-glucan of the yeast cell wall, thus permitting the display on the yeast cell surface of an Aga2-fused protein (10). The fusion to Aga2 has also been used to display transient or unstable complexes that can dissociate during a long induction. The "STUCKED" method, for Stabilization of Transient and Unstable Complexes by Engineered Disulfide, was developed to cost- and time-effectively study quaternary structures. The protein complex, including scFv, is displayed on the yeast surface as described in (25), with one subunit expressed as a fusion with Aga2 and the other one expressed as a soluble form. A cysteine residue is introduced in each subunit to form an intersubunit disulfide. The engineered interchain disulfide helps stabilizing the protein complex (26). The same principle of yeast display has also been used to create very large combinatorial Fab libraries by mating. To do so, Weaver-Feldhaus and colleagues amplified the heavy ($V_H$) and first constant regions (CH1) as well as the variable light chains ($V_\kappa$) from B cells of non-immunized donors. The constant light chain was amplified from the $C_k$ gene (accession number P01834). Cloning by homologous recombination was performed by cotransformation of YVH10 yeast strain with $V_\kappa$ fragments and the linearized vector pPNL30 for yeast secretion; pPNL30 vector was derived from pPNL9 vector after the addition of $C_k$. Similarly, JAR300, the mating partner of YVH10, was cotransformed with $V_H$ fragments and linearized vector pPNL20 for yeast display, derived from pCTcon after the addition of CH1. Although the transformation yields were relatively modest, the yeast mating of YVH10 secreting $V_k + C_k$ with JAR300 displaying $V_H$ + CH1, could generate a very large library of displayed Fab (25).

Yeast display vectors based on Aga2–Aga1 interaction are available. pYD vector has been widely used, including for the display of nanobodies (VHH) (27) that are functional antibodies devoid of light chains, found in Camelids (Bactrian camels, dromedaries, and llamas) (28) and in some cartilaginous fish (29). These single domain antibodies binds antigens without requiring domain pairing and are remarkably resistant against extremes pH and chaotropic agents, making them especially suited for oral immunotherapy (for review, *see* (30)). The pYD vector has been further modified by several groups. For examples, Wang and colleagues modified the fusion side of Aga2 to scFv in pYD1, and reported that the fusion of Aga2 in C-terminus of the scFv using their new vector called pYD5 improves the expression of some scFv (31). Van den Beucken et al. have reengineered pYD1 to make it suitable for Fab expression, by adapting to yeast the most frequently used format for Fab display on phage (32). The resulting pTQ3 vector contains two expression cassettes driven by identical inducible promoters, one for each variable chain, and enables display and affinity maturation of heterodimeric Fab antibodies. The tandem expression on

S. cerevisiae surface of the variable heavy chain ($V_H$) fused to Aga2 and of the soluble light chain ($V_L$) is galactose-driven (18).

Novel antibody-related structures such as lamprey variable lymphocyte receptors (VLRs) and Adnectins, a new family of therapeutic proteins based on the tenth fibronectin type III domain (33), have also been displayed on yeast. The display of a VLR library was achieved through fusion to the yeast Flo 1p surface anchor (34). While phage display of major histocompatibility complex proteins (MHC) has proven to be extremely challenging, yeast display enabled assembly and display of MHC heterodimers with entirely noncovalent interchain interactions. But, although yeast display MHC could bind to peptides, they were not able to stimulate antigen specific T cells (35), and efforts in understanding MHC functional structure and stabilization are still ongoing (36). Yeast surface display of T cell receptor (TCR) using pYD was undertaken using three or two TCR domains, Vα, Vβ, and Cβ (scTCR) or Vα linked to Vβ (scTv), respectively. Stabilization of yeast display scTv could be achieved by mutating the interface of the Vα- and Vβ-chains and the Vβ-chain framework region (37), while the addition of a disulfide bond allowed stable display of scTCR on yeast (38).

Limitations have been reported regarding the display of heterologous proteins on *S. cerevisiae*, including N-linked glycosylation and, to a lesser extent, core disulfides (39). Several alternative platforms for yeast display on *S. cerevisiae* and on *Pichia pastoris* have been developed during the past decade. Alternative yeast display strategies of antibody fragments on *S. cerevisiae* include 1/the coexpression of the scFv fused to the adapter GR1 and of the genetically integrated adapter GR2 fused to the outer wall protein Cwp2, which permits the pairwise interaction of the scFv-GR1 and GR2-Cwp2 fusions in the yeast Cells and results in scFv display (40), and 2/the use of the classical streptavidin–biotin interaction to display in vivo biotinylated antibodies (41) at avidinated yeast cell surface (Secretion and Capture Technology (SECANT) platform) (42).

Finally, tools for display on *P. pastoris*, a yeast strain that compares favorably to *S. cerevisiae* in terms of yield and of posttranslational modifications (43, 44), have been recently developed. Jacobs et al. first adapted the Aga2-based display technology in *P. pastoris* (45). Later, Su and colleagues used the Sed1 anchor system under the control of the alcohol oxidase 1 promoter (pAOX1) to display *Candida antarctica* lipase B (CALB) and obtained large amounts of hydrolytic activity of the displayed CALB (more than 220 U/g dry cells after 120 h of culture) (46). The same year, Rychaert et al. fused an immune library of camelid nanobodies directed against Green Fluorescence Protein to the C-terminal part of the *S. cerevisiae* alpha-agglutinin gene (SAG1) for display on *P. pastoris* (47). Finally in 2011, Jo et al. published the use of the gene TIP1 encoding the glycosylphosphatidylinositol (GPI)-anchored protein

of *S. cerevisiae* as an anchoring motif to fuse to Human lactoferrin cDNA (hLf). The fused hLF was biologically active at the surface of *P. pastoris*, as demonstrated by the antibacterial activity of the intact recombinant yeast cells and cell lysates against target microorganisms (48).

These examples underline the remarkable potential of yeast display technology for a wide range of applications. However, phage display is often preferred to yeast display as a method of antibody selection. This bias in favor of phage is probably based in part on habits, as the yeast display platform appeared more recently (10) than the phage display one (49). But the lack of yeast sorting expertise and of adequate equipment, as well as the low transformation efficiency of yeast to compare with bacteria, may also have played an important role (for review see (50)). The goal of this chapter is to give an updated overview of the available systems of yeast display libraries of antibody fragments and vectors, followed up by technical advices to make the selection of antibody fragments by yeast display more accessible to the general user.

## 2. Materials

### *2.1. Available Libraries and Vectors for Selection of Antibody Fragments by Yeast Display*

1. Naïve yeast display library of antibody fragments (scFv): Display via Aga1–Aga2 interaction, created by Feldhaus and colleagues in the vector pPNL6 under the control of a galactose-driven promoter, and transformed in the yeast host strain EBY100 that contains a chromosomal integrant of the AGA1 gene (51). The library is available for free distribution through the Pacific Northwest National Laboratory (PNNL), Richland, WA, http://www.sysbio.org/dataresources/singlechain.stm, pending material transfer agreement (MTA).
2. TTP yeast display library of antibody fragments (scFv): We also built up a library on the same principle but from peripheral B cells of a patient with thrombotic thrombocytopenic purpura (TTP), an autoimmune disease, using the vector pAGA2 (52) that enables homologous recombination to convert yeast display scFv into site-specific biotinylated secreted scFv (biobodies (41)). This paired yeast-display/secretory scFv library platform permits to overcome the loss of binding activity observed with other system of expression upon conversion of yeast display clones into soluble fragments (22). The TTP yeast display scFv library, as well as the vector for yeast display is available upon request to N. Scholler (naths@mail.med.upenn.edu), pending MTA with the University of Pennsylvania, Philadelphia, PA.

3. Vectors for antibody fragment selection. The company Invitrogen commercializes the vector pYD1 (or pCTCON2 (53)) that permits yeast display based on AGA1–AGA2 binding.

The display vector pAGA2 and its companion vector for yeast secretion (p416-BCCP) (52) are available upon request to N. Scholler (naths@mail.med.upenn.edu), pending MTA with the University of Pennsylvania, Philadelphia, PA.

### 2.2. Material for Selection of Yeast Display Recombinant Antibodies

#### 2.2.1. General Materials

1. Yeast display strain: EBY100 (Invitrogen).
2. Yeast secretion strain: YVH10 available upon request at http://www.sysbio.org/dataresources/singlechain.stm, pending MTA with PNNL.
3. For yeast secretion of site-specific biotinylated proteins: BIRA-BJ5475 available upon request to N. Scholler (naths@mail.med.upenn.edu), pending MTA with the University of Pennsylvania.
4. Biotinylated peptides.
5. Recombinant proteins, purified native proteins or cell lysates, and corresponding control antigen.
6. Chemical biotinylation kit.
7. In vivo biotinylation (in vivo biotinylation in yeast available upon request to N. Scholler (naths@mail.med.upenn.edu), pending MTA with the University of Pennsylvania); medium and/or smallTube-o-dialyzer (Genotech #786-142-15K and #786-141-4k).
8. Growth media: SD-CAA and SD-CAA agar for EBY100, YVH10 and diploid; SD-CAA+TRP for transformed YVH10; SD-CAA+URA for BIRA-BJ5475; YEPD for diploid (Table 1).
9. Induction media: SGR-CAA for EBY100; SGR-CAA+TRP for transformed YVH10; YEPGR for diploid (Table 2).
10. Mating media: YEPD; YEDP agar (Table 3).
11. PBE buffer: Phosphate buffer saline supplemented with 5 g/l of BSA fraction V and 10 mM EDTA, pH 8.
12. Incubator shaker at 30°C.
13. Incubator shaker at 20°C.
14. Rotator.

#### 2.2.2. Specific Reagents and Equipment for Magnetic Sorting

Magnetic Beads

1. Streptavidin MicroBeads (Miltenyi).
2. Anti-biotin MicroBeads (Miltenyi).
3. Anti-c-myc MicroBeads (Miltenyi).

## Table 1
## Growth media

| | g/l |
|---|---|
| *SD-CAA: Defined growth medium for EBY100 (yeast display), nontransformed YVH10, or diploid yeast (biotinylated protein secretion)* | |
| [a]Casamino acids, acid hydrolyzed casein (low sodium chloride and iron concentrations) | 5 |
| [b]Dextrose anhydrous (crystalline granules) | 20 |
| [a]Yeast nitrogen base w/o amino acids and ammonium sulfate | 1.7 |
| [a]Ammonium sulfate (enzyme-grade) | 5.3 |
| [c]Phosphate buffer sodium phosphate dibasic heptahydrate $Na_2HPO_4$-$7H_2O$ | 10.2 |
| Sodium phosphate monobasic $NaH_2PO4$-$H_2O$ | 8.6 |
| *SD-CAA + TRP: Defined growth medium for transformed YVH10 (protein secretion)* | |
| [a]Casamino acids, acid hydrolyzed casein (low sodium chloride and iron concentrations) | 5 |
| [b]Dextrose anhydrous (crystalline granules) | 20 |
| [a]Yeast Nitrogen Base w/o amino acids and ammonium sulfate | 1.7 |
| [a]Ammonium sulfate (enzyme-grade) | 5.3 |
| [c]Phosphate buffer | |
| Sodium phosphate dibasic heptahydrate $Na_2HPO_4$-$7H_2O$ | 10.2 |
| Sodium phosphate monobasic $NaH_2PO4$-$H_2O$ | 8.6 |
| [b]L-Tryptophan 0.8 % (reagent grade, ≥98 % TLC, MW 204.23) | (1:100) |
| *SD-CAA + URA: Defined growth medium for BIRA-BJ5475* | |
| [a]Casamino acids, acid hydrolyzed casein (low sodium chloride and iron concentrations) | 5 |
| [b]Dextrose anhydrous (crystalline granules) | 20 |
| [a]Yeast nitrogen base w/o amino acids and ammonium sulfate | 1.7 |
| [a]Ammonium sulfate (enzyme-grade) | 5.3 |
| [c]Phosphate buffer | |
| Sodium phosphate dibasic heptahydrate $Na_2HPO_4$-$7H_2O$ | 10.2 |
| Sodium phosphate monobasic $NaH_2PO4$-$H_2O$ | 8.6 |
| [b]Uracil 0.2 % (reagent grade, ≥99 % TLC, MW 112.09) | (1:100) |
| *SD-CAA agar: For plating EBV100, YVH10, or diploid yeast* | |
| [a]Casamino acids, acid hydrolyzed casein (low sodium chloride and iron concentrations) | 5 |
| [b]Dextrose anhydrous (crystalline granules) | 20 |
| [a]Yeast nitrogen base w/o amino acids and ammonium sulfate | 1.7 |
| [a]Ammonium sulfate (enzyme-grade) | 5.3 |
| [c]Phosphate buffer | |
| Sodium phosphate dibasic heptahydrate $Na_2HPO_4$-$7H_2O$ | 10.2 |
| Sodium phosphate monobasic $NaH_2PO4$-$H_2O$ | 8.6 |
| [d]Agar | 10 |

[a]Autoclave together for 15 min

[b]Filter-sterilize, aliquot, and store as concentrated solutions [Dextrose (10×, 200 g/l), Tryptophan (100×, 0.8 %), Uracil (100×, 0.2 %)] for no more than 3 months at −20°C

[c]Autoclave separately for 15 min and mix after cooling to avoid precipitation

[d]Autoclave separately as a concentrated solution (4×) and mix soon after autoclaving (for example, 250 ml of 4× solution (40 g/l) per liter of medium). Avoid bubbles

*Note*: All media are supplemented with 1 % Penicillin–streptomycin

**Table 2**
**Induction Media**

| | g/l |
|---|---|
| *SGR-CAA: Induction medium for yeast display on EBY100* | |
| [a]Casamino acids, acid hydrolyzed casein (low sodium chloride and iron concentrations) | 5 |
| [b]Sugars | |
| Dextrose anhydrous (crystalline granules) | 1 |
| D(+) Raffinose pentahydrate | 20 |
| D-Galactose (+), anhydrous | 20 |
| [a]Yeast Nitrogen Base w/o amino acids & ammonium sulfate | 1.7 |
| [a]Ammonium sulfate (enzyme-grade) | 5.3 |
| [c]Phosphate buffer | |
| Sodium phosphate dibasic heptahydrate $Na_2HPO_4$-$7H_2O$ | 10.2 |
| Sodium phosphate monobasic $NaH_2PO4$-$H_2O$ | 8.6 |
| *YEPGR + TRP: Induction medium for secretion of antibody fragments by YVH10* | |
| [a]Yeast base | |
| Yeast extract | 10 |
| Peptone | 20 |
| [b]Sugars | |
| Dextrose anhydrous (crystalline granules) | 1 |
| D(+) Raffinose pentahydrate | 20 |
| D-Galactose (+), anhydrous | 20 |
| [b]L-Tryptophan 0.8 % (reagent grade, ≥98 % TLC, MW 204.23) | (1:100) |
| *YEPGR: Induction medium for secretion of biotinylated antibody fragments by diploid* | |
| [a]Yeast base | |
| Yeast extract | 10 |
| Peptone | 20 |
| [b]Sugars | |
| Dextrose anhydrous (crystalline granules) | 1 |
| D(+) Raffinose pentahydrate | 20 |
| D-Galactose (+), anhydrous | 20 |

[a]Autoclave together for 15 min
[b]Filter-sterilize, aliquot, and store as concentrated solutions (10× for sugars and 100× for L-Tryptophan 0.8 %) for no more than 3 months at –20°C
[c]Autoclave separately for 15 min and mix after cooling to avoid precipitation
*Note*: All media are supplemented with 1 % Penicillin-streptomycin

Equipment for Magnetic Separation

1. AutoMACS™ Pro Separator Starter Kit (Miltenyi) and/or QuadroMACS™ Starting Kit, (Miltenyi), that includes a magnetic separation unit for four samples (QuadroMACS),
2. A magnetic stand (MACS MultiStand) and 25 LS Columns for enrichment.
3. LD columns for magnetic depletion (Miltenyi).
4. Cell strainers (Fisher).

**Table 3**
**Mating media**

| | g/l |
|---|---|
| *YEPD: Rich growth medium for YVH10 Mating medium for Tf-YVH10 x BIRA-BJ5475* | |
| [a]Yeast base | |
| Yeast extract | 10 |
| Peptone | 20 |
| [b]Dextrose anhydrous (crystalline granules) | 20 |
| *YEPD agar: Mating plates (Tf-YVH10 x BIRA-BJ5475)* | |
| [a]Yeast base | |
| Yeast extract | 10 |
| Peptone | 20 |
| [b]Dextrose anhydrous (crystalline granules) | 20 |
| [c]Agar | 10 |

[a]Autoclave together for 15 min
[b]Filter-sterilize, aliquot, and store as a concentrated solution (10×, 200 g/l) for up to 3 months
[c]Autoclave separately as a concentrated solution (4×) and mix soon after autoclaving (for example, 250 ml of 4× solution (40 g/l) per liter of medium). Avoid bubbles
*Note*: All media are supplemented with 1 % Penicillin–streptomycin

*2.2.3. Specific Reagents and Equipment for Selection by Flow Sorting*

1. Cell sorter.
2. Anti-c-myc antibody (9E10, Santa Cruz Biotechnology).
3. Alexa Fluor® 488 F(ab′)2 fragment of goat anti-mouse IgG (H + L) (Invitrogen).
4. Phytoerythrin-labeled streptavidin (BD Pharmingen).

*2.2.4. Specific Reagents and Equipment for Selection by Panning*

1. Cell lines with and without surface expression of the antigen of interest.
2. Surface biotinylated cell lysates from cell lines with and without surface expression of the antigen of interest.
3. $C0_2$ incubator at 37°C.
4. Sterile hood for cell culture.
5. Collagen type I-coated 6-well plates.

*2.2.5. Specific Reagents and Equipment for Cloning by Homologous Recombination*

1. Heated dry bath.
2. Incubator with rotator at 30°C.
3. DNA electrophoresis apparatus.
4. Thermocycler (PCR machine).
5. MasterPure Yeast DNA Purification Kit (Epicentre).
6. QIAprep Spin Miniprep Kit (250) (Qiagen).
7. QIAquick gel extraction Kit (Qiagen).

8. Restriction enzymes EcoR1 and Sal1.
9. Platinum Taq DNA polymerase (Invitrogen).
10. dNTPs Master Mix.
11. *Primers for scFv conversion* from yeast display to yeast secreted:
    5′-shuffling primer: 5′-ggt tct ggt ggt gga ggt tct ggt ggt ggt gga tct ggt ggt ggt ggt tct gct agc-3′
    3′-shuffling primer: 5′-gag acc gag gag agg gtt agg gat agg ctt acc gtc gac caa gtc ttc ttc aga aat aag ctt-3′.
12. *Sequencing primers for soluble scFv*: Forward 5′-ggc atg aac cat caa cac c-3′; Reverse 5′-tag gga cct aga ctt cag g-3′.
13. 1M LiAc pH7.5: dissolve 102 g/l of Lithium Acetate in water. Adjust pH to 7.5. Filter-sterilize.
14. 10× TE pH7.5: Mix 0.1 mM Tris (12.1 g/l of 1 M Tris) and 0.01 M EDTA (3.7 g/l of 0.5 M EDTA, pH 8). Adjust pH to 7.5 with HCl. Filter-sterilize.
15. 1× LiAc/TE: Mix 1:10 dilutions of above in water (100 mM LiAc pH 7.5 and 1× TE pH 7.5).
16. 40 % PEG/0.1 M LiAc/1× TE pH 7.5: mix 400 g/l of Poly(Ethylene Glycol) MW3350 with 100 ml of 1 M LiAc and 100 ml of 10× TE pH 7.5 in water. Filter-sterilize.
17. Denatured salmon sperm DNA (10 mg/ml).

## 3. Methods

### 3.1. Amplification of the Yeast Display Library and Display of Antibody Fragments

1. Thaw one frozen aliquot of the library at room temperature and resuspend into 1 l of SD-CAA.
2. Grow at 30°C with agitation (250 rpm), overnight (ON). After 16–20 h, the absorbance at 600 nm should read between 1 and 2 ($A_{600} = 1$ corresponds at $10^7$ yeast/ml).
3. Proceed to induction immediately, or store the culture up to 3 weeks at 4°C (see Note 1). The display of antibody fragments is induced by culture of the grown library in presence of galactose (Table 2).
4. Induce at room temperature (22–24°C, RT) with agitation (250 rpm), ON. $A_{600}$ must be 0.5 after yeast resuspension in SGR-CAA, and 1–1.5 after ON culture (see Note 2).
5. Pellet induced yeast by centrifugation, resuspend in 50 ml of PBE, and pellet again (see Note 3). The pellet should measure about 5 ml.
6. Resuspend the pellet in an equal volume of PBE. Proceed to sorting/panning immediately, or store the induced library up to 2 weeks at 4°C (see Note 4).

### 3.2. Antigen Preparation

The antigen and the appropriate control should be biotinylated with the same protocol. Because the quantity of available antigen is often limiting, we recommend using Pierce kit for biotinylation followed up by dialysis in tube for small volume sample (Tube-o-dialyzer). The biotinylation should be validated by western blot, probed with a HRP-labeled streptavidin. Alternatively, the protein can be expressed by yeast diploid to permit site-specific biotinylation, as described in Subheading 3.7.

### 3.3. Selection by Magnetic Sorting

#### 3.3.1. First Round of Magnetic Sorting

1. Add 50 nM to 1 μM of biotinylated antigen to the 10 ml of induced yeast. Incubate at room temperature for 30 min with rotation, then place on ice for 5 min.
2. Prepare ice-cold PBE (see Note 5).
3. Pellet induced the complexes yeast/antigen by centrifugation; discard supernatant; resuspend in 50 ml of ice-cold PBE. Repeat the sequence three times.
4. Resuspend the washed pellet with 5 ml of ice-cold PBE and add 200 μl of Streptavidin-coated magnetic beads. Incubate at 4°C for 10 min and mix gently the suspension by inversion every 2 min.
5. Add 40 ml ice-cold PBE. Pellet by centrifugation and discard supernatant. Resuspend the sample in 50 ml ice cold PBE and keep on ice during the whole sorting procedure.
6. Pass yeast through a cell strainer to avoid clumps and proceed immediately to magnetic sorting.

#### 3.3.2. Second Round of Magnetic Enrichment

Proceed as for the first round with the following modifications

1. Add 50 nm to 1 μM of biotinylated antigen to the 5 ml of yeast.
2. After incubation with the antigen, resuspend in 2.5 ml of PBE instead of 10 ml, and add 100 μl of anti-biotin magnetic beads (see Note 6).
3. At the end of the second round of magnetic sorting, yeast can be grown as previously described, or resuspended within 4 days after the magnetic sorting in 0.5 ml of ice-cold PBE for staining by flow cytometry analysis and/or sorting.

Magnetic sorting can then be done equally well manually or using an automated bead capture system (AutoMACS). In case multiple samples are to be treated simultaneously, AutoMACS is the preferred method.

Manual Enrichment

1. Install the LS column on the magnetic stand and pre-wet the column with 1 ml of ice-cold PBE.
2. Add 7 ml of sample and let it go through the column by gravity.

3. Remove the column from the magnetic stand to rearrange the beads and put it back in the magnet.
4. Rinse with 1 ml ice-cold PBE.
5. Repeat the steps 2–4 until the entire sample passed through the column:
6. Once the entire sample has been run through the column, rinse it three times with 3 ml ice cold PBE. Discard the effluent.
7. Remove the column from magnet and set it over a clean 15 ml tube.
8. Add 7 ml of ice cold PBE and push the yeast through with the column piston.
9. Pellet by centrifugation and resuspend in 10 ml of SD-CAA.
10. Evaluate the enrichment by plating on SD-CAA plates three serial dilutions ($10^2$; $10^3$ and $10^4$) of 10 μl of eluted yeast.
11. Resuspend the rest of the yeast in 200 ml of SD-CAA and grow at 30°C with agitation until $A_{600} = 1–2$.
12. Pellet by centrifugation, resuspend in 200 ml SGR-CAA to obtain $A_{600} = 0.5$, and induce with agitation in SGR-CAA at RT ON, until $A_{600} = 1$, as in Subheading 3.1, step 2.
13. Pellet by centrifugation, wash three times and resuspend with ice cold PBE to obtain a final volume of 5 ml.

Manual Depletion

Magnetic depletion with LD columns can be used to eliminate cross reactive clones.

1. Pass cell through a cell strainer to avoid clumps.
2. Install the LD column on the magnetic stand. Pre-wet the column with 0.5 ml of ethanol 70 % and rinse with 3 ml of ice-cold PBE.
3. Place a clean 15 ml tube under the column. Add 7 ml of sample and let it go through the column by gravity.
4. Collect the effluent and rinse the column three times with 3 ml ice-cold PBE.

Because depletion can increase the relative number of yeast that do not display any antibody fragments, it is recommended to follow up this procedure by an enrichment using anti-c-myc beads to select the yeast that display scFv (see Note 7).

Automatic Enrichment

1. Turn on autoMACS and run the “Clean program.”
2. Chose the separation protocol “Possel” for sorting of antibody fragments binding to novel antigens, and “Possel 2” for sorting of antibody fragments binding to tags such as c-myc.

3. Place a 50 ml tube filled with 20 ml of SD-CAA under the "pos1" port to collect eluted cells
4. Place an empty 50 ml tube under the "neg1" port to collect the flow through.
5. Place the yeast incubated with a biotinylated antigen under the intake port and proceed to the separation.

### 3.4. Selection by Flow Sorting

This procedure is to be performed immediately after a magnetic enrichment. Because the antigen has been already added during the magnetic sorting, there is no need to add more antigens.

#### 3.4.1. Staining Protocol After Magnetic Enrichment

1. Remove 10 μl from the 0.5 ml of eluted yeast and set aside on ice as negative control #1.
2. Add 1:100 dilution of anti c-myc antibody to the 0.5 ml of eluted yeast and incubate on ice for 30 min.
3. Pellet yeast by centrifugation in a microfuge at maximum speed for 10 s.
4. Wash the pellet with 1 ml of ice-cold PBE and resuspend with 0.5 ml of ice-cold PBE.
5. Remove 10 μl of c-myc labeled sample and set aside as negative control #2.
6. Add 1:200 dilution of 488 anti-mouse Ig and 1:100 dilution of PE labeled Streptavidin to the samples.
7. Add only 1:200 dilution of 488 anti-mouse Ig to the negative controls #1 and #2.
8. Incubate sample and controls on ice for 30 min.
9. Wash once with ice-cold PBE and resuspend sample and controls in 100 μl ice-cold PBE.

#### 3.4.2. Staining Protocol After Induction

In contrast with the previous method, here the antigen is added during the incubation. Antigen-specific avidity of the yeast display antibody fragments can be calculated by varying the concentration of antigens (53) (see Note 8).

1. Pellet yeast by centrifugation after induction.
2. Wash in PBE and resuspend with 0.5 ml of PBE in a 1.5 ml microtube.
3. Remove two aliquots of 10 μl for negative controls and set aside on ice.
4. Add 1:100 dilution of anti c-myc antibody and 50 nM to 1 μM of antigen.
5. Add only 1:100 of anti-c-myc antibody to control #1.
6. Incubate sample and control on ice for 30 min.
7. Pellet cells for 10 s, wash once with ice-cold PBE and resuspend sample and control in 100 μl ice-cold PBE.

8. Add 1:200 of 488 Alexa fluor anti-mIg antibody and 1:100 dilution of PE labeled streptavidin to the sample and both controls.
9. Incubate on ice for 30 min.
10. Wash once with ice-cold PBE and resuspend sample and controls in 100 µl ice-cold PBE.

#### *3.4.3. Flow Sorting*

It is critical to calibrate the compensation using the negative control and the control positive for the expression of antibody fragments (Fig. 1a–f).

1. Sort the double positive yeast in the upper right quadrant (Fig. 1g, h).
2. Sorted yeast should be collected in YEPD and plated on SD-CAA agar.
3. After 2 days scrap the colonies and induce in SGR-CAA for 24 h at RT.
4. Analyze antigen specificity by flow cytometry as described in Subheading 3.4.3. When more than 80 % of the yeast display scFv bind to the antigen of interest (Fig. 1i), the selected yeast display sub-library is converted to soluble form as described in Subheadings 3.6 and 3.7.

### *3.5. Selection of Yeast Display Recombinant Antibodies by Panning*

Although panning has been extensively used for screening phage display and despite the fact that yeast display possesses the necessary attributes for successful panning, including low levels of nonspecific interaction and multivalent display (54), few authors have published work related to yeast display panning on cells. Using a system model of yeast displaying anti-fluorescein scFv, Wang et al. first demonstrated that successful selections required as few as 1,700 fluorescein ligands per cell, and a three-round enrichment ratio of $10^6$ was possible (54). The same team further used yeast panning to identify scFv that bind to the plasma membranes of brain endothelial cells (55).

1. Induced yeast at tenfold excess of the library size.
2. Resuspended the induced library in PBE.
3. Distribute at $5 \times 10^7$ yeast/cm$^2$ onto cell monolayer immobilized on collagen-coated 6-well plates.
4. Incubate yeast with cells for 2 h at 4°C (see Note 9).
5. Harvest supernatants and add 1 ml of ice-cold PBE in each well.
6. Rock the plates 25 times.
7. Harvest supernatants and add 1 ml of ice-cold PBE in each well.

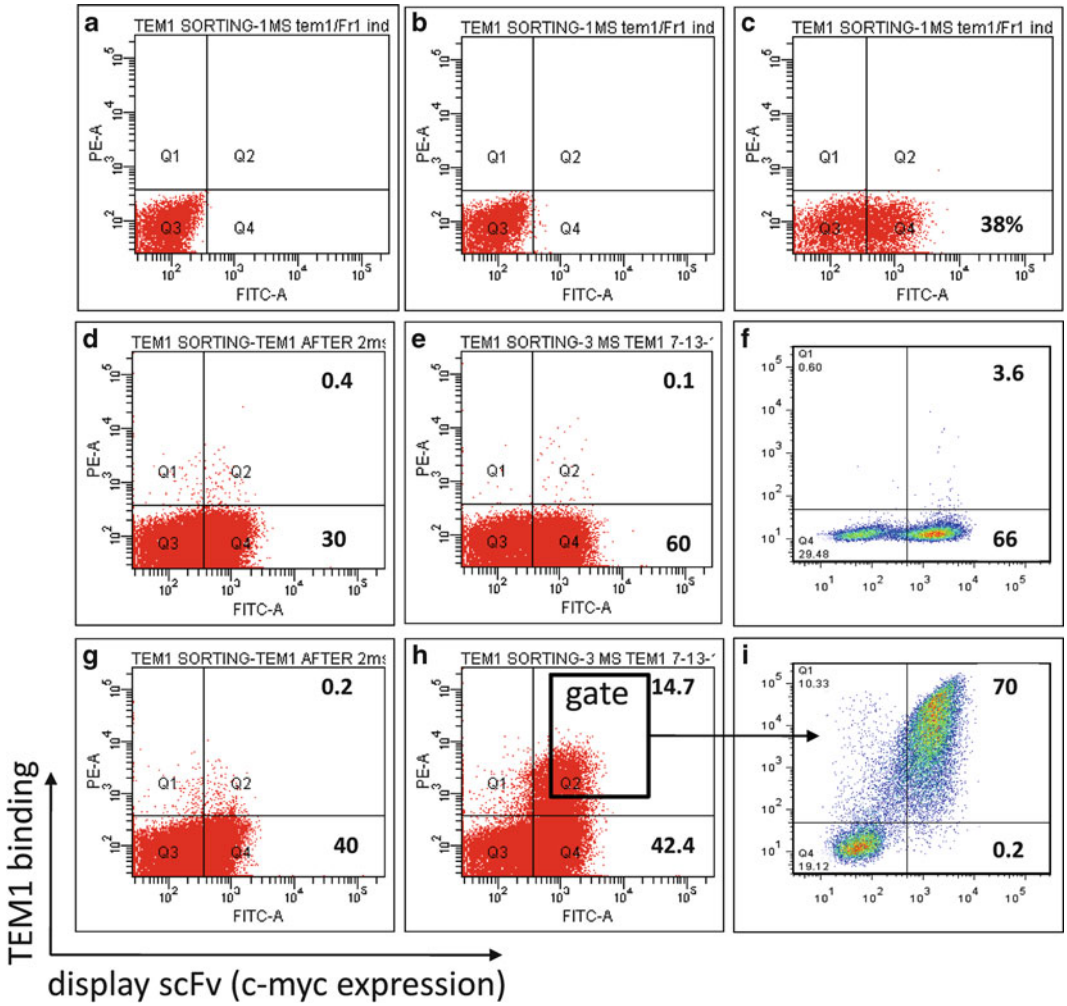


Fig. 1. Selection of anti-TEM1 yeast display scFv by magnetic and flow sorting. Flow cytometry analysis of yeast-display scFv before (**a**–**c**) or after sorting (**d**–**i**). A yeast-display scFv library was enriched for scFv binding to 50 nM of biotinylated TEM1 protein by two (**d**, **g**) or three (**e**, **h**) rounds of magnetic sorting, followed by one round of flow sorting (**f**, **i**) gated on Q (**h**). TEM1 binding to yeast-display scFv was measured by staining with 50 nM of biotinylated TEM1 protein and anti-c-myc antibody detected by PE-labeled streptavidin and 488 Alexa anti-mIg (**g**–**i**). As positive controls for scFv display and control of non-specific binding to streptavidin, yeast were incubated with anti-c-myc antibody detected 488 Alexa anti-mIg, and PE-labeled streptavidin (**c**–**f**). As negative controls, yeast were not stained (**a**) or stained with second reagents only (**b**).

8. Rotate the plates five times.
9. Harvest supernatants and add 1 ml of ice-cold PBE in each well.
10. Rotate the plates five times.
11. Harvest supernatants and add 1 ml of ice-cold PBE in each well.

12. Rotate the plates ten times.
13. Resuspend the washed cell/yeast in SD-CAA and pool all the wells together in 50 ml of SD-CAA.
14. Evaluate the enrichment by plating on SD-CAA plates three serial dilutions ($10^2$; $10^3$ and $10^4$) of 10 μl of eluted yeast.
15. Resuspend the rest of the yeast in 200 ml of SD-CAA and grow at 30°C with agitation until $A_{600} = 1–2$.
16. Pellet by centrifugation, resuspend in 200 ml SGR-CAA to obtain $A_{600} = 0.5$, and induce with agitation in SGR-CAA at RT ON, until $A_{600} = 1$, as in Subheading 3.1.2.
17. Pellet by centrifugation, wash three times and resuspend with ice cold PBE to obtain a final volume of 5 ml.
18. Proceed to more rounds of panning until 70–80 % of the yeast bind to the cells of interest (Fig. 1i).
19. Convert the selected sub-library of yeast display into soluble form.

### *3.6. Conversion of an Antigen-Specific Yeast Display scFv Sublibrary into Secreted scFv*

#### *3.6.1. Yeast Cell Lysis*

Pellet by centrifugation the yeast from a saturated 5–10ml culture ($A_{600} > 8$) and discard the supernatant.

1. Resuspend in 0.3 ml of yeast lysis solution (MasterPure Yeast DNA Purification Kit).
2. Incubate the lysate at 65°C for 15 min, then on ice for 5 min.
3. Pellet by centrifugation 10 min at 3,000 × *g* and discard the supernatant.
4. Thoroughly resuspend the pellet in 250 μl of buffer P1 (Qiagen miniprep kit).
5. Add 250 μl of buffer P2 and mix by inversion ten times.
6. Incubate at RT for 5 min.
7. Add 350 μl of buffer P3 ad mix by inversion ten times.
8. Centrifuge at maximum speed for 15 min.
9. Transfer the supernatant into the Qiagen plasmid mini columns.
10. Apply 750 μl of PE buffer onto the column and centrifuge at maximum speed for 30 s.
11. Transfer the filter column in a clean tube.
12. Apply 40 μl of TE in the center of the column and incubate at 55°C for 10 min in a dry bath.
13. Elute by centrifugation at maximum speed for 1 min.
14. Quantify the eluted plasmid DNA.

*3.6.2. Preparation of the scFv fragments*

1. PCR-amplify yeast extracted DNA using the 5′ and 3′ shuffling primers. Set up the PCR Mix as follow:

| |
|---|
| 26.5 µl $H_2O$ |
| 5 µl 2 mM dNTPs |
| 5 µl 2.5 µM of 5′ shuffling primer |
| 5 µl 2.5 µM of 3′ shuffling primer |
| 5 µl 10× Taq HiFi Buffer |
| 2 µl 50 mM $MgSO_4$ |
| 0.5 µl Platinum Taq HiFi |
| 1 µl (100 ng) of eluted plasmid DNA |

   Cycling conditions: 95°C 5 min; (95°C 30 s; 55°C 45 s; 72°C 2 min)×35 ; 72°C 15 min.
2. Run PCR product on 0.8 % agarose gel 30 min, 120 V.

Excise band and clean up with QiaQuick Gel Extraction kit.

*3.6.3. Preparation of the Secretion Vector p416-BCCP*

1. Double digest 5 µg of p416-BCCP with EcoR1/Sal1 (enough for 100 transformations) as follow:

| |
|---|
| 5 µl p416 Gal1 BCCP (1 mg/ml stock) |
| 2.5 µl EcoR1 (20,000 U/ml) |
| 0.5 µl Sal1 (100,000 U/ml) |
| 10 µl NEB Buffer 3 |
| 1 µl BSA |
| 81 µl NF $H_2O$ |

2. Incubate at 37°C for 2 h.
3. Run on 0.8 % agarose gel for 1.5 h.
4. Excise the linearized vector, avoiding supercoil.
5. Purify with QiaQuick gel extraction kit.
6. Elute with 50 µl EB buffer.

The cloning by homologous recombination is then achieved by the cotransformation of YVH10 with the linearized p416-BCCP vector and the PCR fragments elongated with recombination sequences.

*3.6.4. Preparation of Yeast YVH10 Strain*

1. Inoculate YVH10 from the freezer culture into 5 ml of YEPD (Table 3).
2. Grow overnight on the roller at 30°C ON until to $A_{600} > 8$.
3. Store the culture at 4°C for up to 2 months.

4. Use a fresh ON culture at a 20× dilution to inoculate a 200 ml flask with 40 ml of 2× YEPD.
5. Shake at 30°C for 3–5h until the cell density ranges between $5 \times 10^6$ and $2 \times 10^7$ yeast/ml. Transformation efficiency falls off sharply beyond those limits.
6. Transfer the cultures to centrifuge tubes and pellet by centrifugation.
7. Discard the supernatant and resuspend the cells in 10 ml of sterile water.
8. Pellet by centrifugation and discard the supernatant.
9. Resuspend in 1× LiAc/TE using 1 ml for each transformation that will be done. Let sit at RT for at least 10 min.

*3.6.5. Cotransformation (See Note 10)*

1. Transfer 1 ml of LiAc-treated YVH10 to microfuge tubes.
2. Centrifuge 1 min at 6,000 × *g*.
3. Discard the supernatant carefully and put the pellets on ice.
4. Layer over the pellet cold 40 % PEG/0.1M LiAc/1× TE. *Do not* resuspend.
5. Layer on top 20 µl of freshly denatured salmon sperm DNA (10 mg/ml).
6. Layer on top 0.5 µl of EcoR1/Sal1-cut p416 BCCP (100 µg/ml) and 10 µl of gel purified PCR fragments.
7. Vortex exactly 15 s to resuspend the pellet.
8. Heat shock at 42°C for 30–45 min.
9. Pellet by centrifugation for 5 min at 6,000 × *g* in microfuge (see Note 11).
10. Resuspend the pellet in 500 µl of 2× YEPD by vortexing (Table 3).
11. Put the microfuge tubes into 10 ml tubes and install them on the roller 3–16 h at 30°C.
12. Pellet by centrifugation and resuspend in 200 µl of sterile $H_2O$.
13. Spread 0.1 ml of undiluted and 1:10 and 1:100 dilutions to pick individual colonies on SD-CAA + TRP plates (Table 1).
14. Incubate at 30°C for 48–72 h.
15. Pick individual colonies and induce at RT with shaking for 48–72 h in YEPGR + TRP (Table 2).

Transformed colonies secrete scFv tagged with Flag, V5, and HIS6.

### *3.7. Site-Specific Biotinylation by Yeast Mating*

Yeast mating is a convenient method of introducing two different plasmids into the same host cells. We use this protocol for site-specific biotinylation of secreted scFv.

1. Pick one colony of transformed VYH10 and one colony of BIRA-BJ5475. Use only large (2–3-mm), fresh (<2-months old)

colonies from the working stock plates. Place both colonies in one 1.5-ml microcentrifuge tube containing 0.5 ml of YEPD medium (Table 3).

2. Vortex tubes to completely resuspend the cells.
3. Incubate at 30°C ON with shaking at 200 × *g*.
4. Spread 100-µl aliquots of the mating culture on SD-CAA agar plate (Table 1).
5. Incubate at 30°C for 48–72 h.
6. Pick individual colonies and induce at RT with shaking for 2–3 days in YEPGR (Table 3).

Diploid clones secrete site-specific biotinylated scFv.

## 4. Notes

1. Yeast can be pelleted by 5 min of centrifugation at 3,000 × *g* and resuspended in 50 ml of SD-CAA. Before induction, yeast should be freshly passaged.
2. Yeast culture should look opalescent white and have a fresh smell. If the color is yellowish and the smell sour, suspect a contamination and consider thawing another library aliquot. We do not recommend using antibiotic or antifungic early in the screening process, as it can reduce the library diversity.
3. Yeast resuspension can be difficult after centrifugation and necessitate moving the pipette around rather than aspirating up and down.
4. We recommend checking for the percentage of yeast display of antibody fragments before beginning the screening. Proceed by flow cytometry analysis, as described in Subheading 3.4.2. Typically 20–40 % of the yeast library clones display scFv (Fig. 1c); after several rounds of sorting the percentage can increase up to 70 % (Fig. 1f, i)
5. If the antigen is tagged but not biotinylated, it is possible to use a biotinylated anti-tag antibody to retrieve the yeast/antigen complexes using streptavidin-coated magnetic beads.
6. Alternating the type of magnetic beads permits to avoid selecting antibody fragments against secondary reagents.
7. Magnetic selection for yeast display scFv is simply performed by incubating yeast resuspended in 1 ml of ice-cold PBE with 20 µl of anti-c-myc beads, followed up by magnetic enrichment as described in Subheading 3.3.2.
8. Because the number of displayed antibody fragments can vary between yeast from $10^4$ to $10^5$, we do not recommend isolating

the yeast display clones that appear to have the highest avidity. Rather, we recommend selecting pools of yeast display clones that bind to 10–50 nM of antigen and converting these pools into yeast secreted antibody fragments to identify the soluble clones of highest affinity.

9. To reduce the size of the yeast display library for panning, a first round of selection can be performed by magnetic sorting using surface-biotinylated cell lysates at 10 μM.
10. The outgrowth after heat-shock is essential for recovering yeast transformants with inserts. Other critical factors: The cell density at the start of the transformation, the pH (all solutions must be at pH 7.5.), the denaturation of the carrier DNA and the polymerization number of the PEG.
11. Do not overspin. The cells will form clumps that are practically impossible to get rid of.

## References


1. Mondon P, Dubreuil O, Bouayadi K, Kharrat H (2008) Human antibody libraries: a race to engineer and explore a larger diversity. Front Biosci 13:1117–1129
2. Baird CL, Fischer CJ, Pefaur NB, Miller KD, Kagan J, Srivastava S, Rodland KD (2010) Developing recombinant antibodies for biomarker detection. Cancer Biomark 6(5–6): 271–279
3. Bradbury AR, Sidhu S, Dubel S, McCafferty J (2011) Beyond natural antibodies: the power of in vitro display technologies. Nat Biotechnol 29(3):245–254
4. Gould LH, Sui J, Foellmer H, Oliphant T, Wang T, Ledizet M, Murakami A, Noonan K, Lambeth C, Kar K, Anderson JF, de Silva AM, Diamond MS, Koski RA, Marasco WA, Fikrig E (2005) Protective and therapeutic capacity of human single-chain Fv-Fc fusion proteins against West Nile virus. J Virol 79(23): 14606–14613
5. Oliphant T, Nybakken GE, Engle M, Xu Q, Nelson CA, Sukupolvi-Petty S, Marri A, Lachmi BE, Olshevsky U, Fremont DH, Pierson TC, Diamond MS (2006) Antibody recognition and neutralization determinants on domains I and II of West Nile Virus envelope protein. J Virol 80(24):12149–12159
6. Kalb SR, Garcia-Rodriguez C, Lou J, Baudys J, Smith TJ, Marks JD, Smith LA, Pirkle JL, Barr JR (2010) Extraction of BoNT/A, /B, /E, and /F with a single, high affinity monoclonal antibody for detection of botulinum neurotoxin by Endopep-MS. PLoS One 5(8):e12237
7. Borodina I, Jensen BM, Sondergaard I, Poulsen LK (2010) Display of wasp venom allergens on the cell surface of Saccharomyces cerevisiae. Microb Cell Fact 9:74
8. Abe H, Shimma Y, Jigami Y (2003) In vitro oligosaccharide synthesis using intact yeast cells that display glycosyltransferases at the cell surface through cell wall-anchored protein Pir. Glycobiology 13(2):87–95
9. Hamilton SR, Gerngross TU (2007) Glycosylation engineering in yeast: the advent of fully humanized yeast. Curr Opin Biotechnol 18(5):387–392
10. Boder ET, Wittrup KD (1997) Yeast surface display for screening combinatorial polypeptide libraries. Nat Biotechnol 15(6):553–557
11. VanAntwerp JJ, Wittrup KD (2000) Fine affinity discrimination by yeast surface display and flow cytometry. Biotechnol Prog 16(1):31–37
12. Boder ET, Wittrup KD (2000) Yeast surface display for directed evolution of protein expression, affinity, and stability. Methods Enzymol 328:430–444
13. Peelle BR, Krauland EM, Wittrup KD, Belcher AM (2005) Probing the interface between biomolecules and inorganic materials using yeast surface display and genetic engineering. Acta Biomater 1(2):145–154
14. Bowley DR, Labrijn AF, Zwick MB, Burton DR (2007) Antigen selection from an HIV-1 immune antibody library displayed on yeast yields many novel antibodies compared to selection from the same library displayed on phage. Protein Eng Des Sel 20(2):81–90

15. Siegel RW (2009) Antibody affinity optimization using yeast cell surface display. Methods Mol Biol 504:351–383
16. Low NM, Holliger PH, Winter G (1996) Mimicking somatic hypermutation: affinity maturation of antibodies displayed on bacteriophage using a bacterial mutator strain. J Mol Biol 260(3):359–368
17. Kieke MC, Cho BK, Boder ET, Kranz DM, Wittrup KD (1997) Isolation of anti-T cell receptor scFv mutants by yeast surface display. Protein Eng 10(11):1303–1310
18. van den Beucken T, Pieters H, Steukers M, van der Vaart M, Ladner RC, Hoogenboom HR, Hufton SE (2003) Affinity maturation of Fab antibody fragments by fluorescent-activated cell sorting of yeast-displayed libraries. FEBS Lett 546(2–3):288–294
19. Wang Z, Kim GB, Woo JH, Liu YY, Mathias A, Stavrou S, Neville DM Jr (2007) Improvement of a recombinant anti-monkey anti-CD3 diphtheria toxin based immunotoxin by yeast display affinity maturation of the scFv. Bioconjug Chem 18(3):947–955
20. Chowdhury PS, Wu H (2005) Tailor-made antibody therapeutics. Methods 36(1):11–24
21. Shusta EV, Holler PD, Kieke MC, Kranz DM, Wittrup KD (2000) Directed evolution of a stable scaffold for T-cell receptor engineering. Nat Biotechnol 18(7):754–759
22. Weaver-Feldhaus JM, Miller KD, Feldhaus MJ, Siegel RW (2005) Directed evolution for the development of conformation-specific affinity reagents using yeast display. Protein Eng Des Sel 18(11):527–536
23. Orcutt KD, Slusarczyk AL, Cieslewicz M, Ruiz-Yi B, Bhushan KR, Frangioni JV, Wittrup KD (2011) Engineering an antibody with picomolar affinity to DOTA chelates of multiple radionuclides for pretargeted radioimmunotherapy and imaging. Nucl Med Biol 38(2): 223–233
24. Starwalt SE, Masteller EL, Bluestone JA, Kranz DM (2003) Directed evolution of a single-chain class II MHC product by yeast display. Protein Eng 16(2):147–156
25. Weaver-Feldhaus JM, Lou J, Coleman JR, Siegel RW, Marks JD, Feldhaus MJ (2004) Yeast mating for combinatorial Fab library generation and surface display. FEBS Lett 564(1–2):24–34
26. Lim KH, Madabhushi SR, Mann J, Neelamegham S, Park S (2010) Disulfide trapping of protein complexes on the yeast surface. Biotechnol Bioeng 106(1):27–41
27. Koide A, Gilbreth RN, Esaki K, Tereshko V, Koide S (2007) High-affinity single-domain binding proteins with a binary-code interface. Proc Natl Acad Sci U S A 104(16):6632–6637
28. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, Bendahman N, Hamers R (1993) Naturally occurring antibodies devoid of light chains. Nature 363(6428):446–448
29. Greenberg AS, Avila D, Hughes M, Hughes A, McKinney EC, Flajnik MF (1995) A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature 374(6518):168–173
30. Harmsen MM, De Haard HJ (2007) Properties, production, and applications of camelid single-domain antibody fragments. Appl Microbiol Biotechnol 77(1):13–22
31. Wang Z, Mathias A, Stavrou S, Neville DM Jr (2005) A new yeast display vector permitting free scFv amino termini can augment ligand binding affinities. Protein Eng Des Sel 18(7): 337–343
32. de Haard HJ, van Neer N, Reurs A, Hufton SE, Roovers RC, Henderikx P, de Bruine AP, Arends JW, Hoogenboom HR (1999) A large non-immunized human Fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies. J Biol Chem 274(26):18218–18230
33. Lipovsek D (2011) Adnectins: engineered target-binding protein therapeutics. Protein Eng Des Sel 24(1–2):3–9
34. Tasumi S, Velikovsky CA, Xu G, Gai SA, Wittrup KD, Flajnik MF, Mariuzza RA, Pancer Z (2009) High-affinity lamprey VLRA and VLRB monoclonal antibodies. Proc Natl Acad Sci U S A 106(31):12891–12896
35. Boder ET, Bill JR, Nields AW, Marrack PC, Kappler JW (2005) Yeast surface display of a noncovalent MHC class II heterodimer complexed with antigenic peptide. Biotechnol Bioeng 92(4):485–491
36. Jones LL, Brophy SE, Bankovich AJ, Colf LA, Hanick NA, Garcia KC, Kranz DM (2006) Engineering and characterization of a stabilized alpha1/alpha2 module of the class I major histocompatibility complex product Ld. J Biol Chem 281(35):25734–25744
37. Kieke MC, Shusta EV, Boder ET, Teyton L, Wittrup KD, Kranz DM (1999) Selection of functional T cell receptor mutants from a yeast surface-display library. Proc Natl Acad Sci U S A 96(10):5651–5656
38. Richman SA, Aggen DH, Dossett ML, Donermeyer DL, Allen PM, Greenberg PD, Kranz DM (2009) Structural features of T cell receptor variable regions that enhance domain stability and enable expression as single-chain

ValphaVbeta fragments. Mol Immunol 46(5): 902–916

39. Parthasarathy R, Subramanian S, Boder ET, Discher DE (2006) Post-translational regulation of expression and conformation of an immunoglobulin domain in yeast surface display. Biotechnol Bioeng 93(1):159–168
40. Wang KC, Patel CA, Wang J, Wang X, Luo PP, Zhong P (2010) Yeast surface display of antibodies via the heterodimeric interaction of two coiled-coil adapters. J Immunol Methods 354(1–2):11–19
41. Scholler N, Garvik B, Quarles T, Jiang S, Urban N (2006) Method for generation of in vivo biotinylated recombinant antibodies by yeast mating. J Immunol Methods 317(1–2): 132–143
42. Rakestraw JA, Aird D, Aha PM, Baynes BM, Lipovsek D (2011) Secretion-and-capture cell-surface display for selection of target-binding proteins. Protein Eng Des Sel 24(6):525–530
43. Potgieter TI, Cukan M, Drummond JE, Houston-Cummings NR, Jiang Y, Li F, Lynaugh H, Mallem M, McKelvey TW, Mitchell T, Nylen A, Rittenhour A, Stadheim TA, Zha D, d'Anjou M (2009) Production of monoclonal antibodies by glycoengineered Pichia pastoris. J Biotechnol 139(4):318–325
44. Berdichevsky M, d'Anjou M, Mallem MR, Shaikh SS, Potgieter TI (2011) Improved production of monoclonal antibodies through oxygen-limited cultivation of glycoengineered yeast. J Biotechnol 155(2):217–224
45. Jacobs PP, Ryckaert S, Geysens S, De Vusser K, Callewaert N, Contreras R (2008) Pichia surface display: display of proteins on the surface of glycoengineered Pichia pastoris strains. Biotechnol Lett 30(12):2173–2181
46. Su GD, Zhang X, Lin Y (2010) Surface display of active lipase in Pichia pastoris using Sed1 as an anchor protein. Biotechnol Lett 32(8): 1131–1136
47. Ryckaert S, Pardon E, Steyaert J, Callewaert N (2010) Isolation of antigen-binding camelid heavy chain antibody fragments (nanobodies) from an immune library displayed on the surface of Pichia pastoris. J Biotechnol 145(2): 93–98
48. Jo JH, Im EM, Kim SH, Lee HH (2011) Surface display of human lactoferrin using a glycosylphosphatidylinositol-anchored protein of Saccharomyces cerevisiae in Pichia pastoris. Biotechnol Lett 33(6):1113–1120
49. Smith GP (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 228(4705): 1315–1317
50. Hoogenboom HR (2005) Selecting and screening recombinant antibody libraries. Nat Biotechnol 23(9):1105–1116
51. Feldhaus MJ, Siegel RW, Opresko LK, Coleman JR, Feldhaus JM, Yeung YA, Cochran JR, Heinzelman P, Colby D, Swers J, Graff C, Wiley HS, Wittrup KD (2003) Flow-cytometric isolation of human antibodies from a nonimmune Saccharomyces cerevisiae surface display library. Nat Biotechnol 21(2):163–170
52. Zhao A, Nunez-Cruz S, Li C, Coukos G, Siegel DL, Scholler N (2011) Rapid isolation of high-affinity human antibodies against the tumor vascular marker Endosialin/TEM1, using a paired yeast-display/secretory scFv library platform. J Immunol Methods 363(2):221–232
53. Chao G, Lau WL, Hackel BJ, Sazinsky SL, Lippow SM, Wittrup KD (2006) Isolating and engineering human antibodies using yeast surface display. Nat Protoc 1(2):755–768
54. Wang XX, Shusta EV (2005) The use of scFv-displaying yeast in mammalian cell surface selections. J Immunol Methods 304(1–2): 30–42
55. Wang XX, Cho YK, Shusta EV (2007) Mining a yeast library for brain endothelial cell-binding antibodies. Nat Methods 4(2):143–145