URSA: ultra rapid selection of antibodies from an antibody phage display library

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M13-based antibody phage display libraries are increasingly being utilized to isolate antibodies with a particular affinity, specificity, and avidity for a variety of targets (1–9). Selection of target binders usually involves a series of steps that include the overnight propagation and subsequent purification of the phage between rounds of selection (10–15). A relatively small amount of purified phage sample is then used as input for the next round of

selection. Typically, three to five rounds of selection are performed, and this process takes 1–2 weeks to complete. Here we describe an alternate selection method, termed Ultra Rapid Selection of Antibodies (URSA). In this method, only phage produced from the first one or two extrusions after bacterial infection are used as input phage for the subsequent round of selection without purification. The extrusion of phage typically occurs 20–

30 min after the initiation of infection, with approximately 2000 phage/cell being produced in the first extrusion and 100–200 phage/cell at each subsequent extrusion (16). Because the input phage are extruded in the presence of the target, the standard overnight propagation and phage purifications are avoided. This method allows three to five rounds of selection to be performed in a single day.

We have generated a Fab-displaying phage library by combining human heavy chain and light chain repertoires into an M13-like phage vector (GenBank® accession no. AY754023) resulting in a library comprising 2.0×10^9 clones. This library was used for all selection campaigns. Initially, three parallel experiments were performed to determine the optimal incubation times and temperatures required to produce the best fraction of output over input (FOI). Table 1 illustrates the results from the time and temperature optimization experiment using streptavidin (Sv) as a target. The largest increase in FOI from rounds 1 to 3 was produced using Condition 1 (37°C for 20 min plus 30°C for 25 min).

The optimized URSA method is depicted in Table 2. After selections against Sv, phage clones generated from individual transformants were isolated, propagated, and purified. Sv-binding clones were subjected to a Fab-phage enzyme-linked immunosorbent assay (ELISA) and phage that yielded a signal 3-fold over background were considered to be positive binders or hits. The hit rate percentage, 10%, was similar to results we obtained for this target in a standard selection method (data not shown).

Fluorescein isothiocyanate (FITC) selections were carried out essentially as described for the Sv experiment with the following exceptions. Biotinylated bovine serum albumin (BSA)-FITC (250 nM) was incubated with 2.0×10^{11} phage for 60 min at room temperature. This target-phage complex was added to 100 µL Dynal M280 beads (Dynal Biotech, Oslo, Norway) in 2% milk phosphate-buffered saline (MPBS) and incubated for 30 min at room temperature. Fab sequences from phage clones yielding a signal 3-fold over background in the target ELISA

Table 1. Summary of Colony-Forming Units and Fraction of Output Over Input Data From Optimization Experiment

	Round 1	Round 2	Round 3
	cfu (FOI)	cfu (FOI)	cfu (FOI)
Condition 1 37°C for 20 min + 30°C for 25 min	5.8×10^7 (1.95×10^{-4})	8.0×10^7 (2.7×10^{-4})	5.1×10^7 (1.7×10^{-4})
Condition 2 37°C for 45 min	5.1×10^5 (8.8×10^{-3})	1.2×10^6 (1.5×10^{-2})	7.5×10^5 (1.2×10^{-2})
Condition 3 30°C for 45 min	7.3×10^4 (1.4×10^{-1})	5.4×10^4 (4.5×10^{-2})	4.8×10^4 (6.3×10^{-2})

cfu, colony-forming units; FOI, fraction of output over input.

Table 2. URSA Protocol

1. Block 100 µL streptavidin (Sv)-coated Dynal M280 magnetic beads with five volumes 2% (w/v) milk phosphate-buffered saline (MPBS) for 30 min.
 2. Wash beads with one volume 0.1% (v/v) Tween® 20 in 1× PBS (PBST).
 3. Incubate 3×10^{11} Fab-displaying phage and beads in five volumes 0.1% (v/v) Tween 20 in MPBS (MPBST) for 30 min at room temperature.
 4. Remove unbound phage and wash beads three times in PBST.
 5. Add 500 µLs XL-1 Blue-MRF^c cells (Stratagene, La Jolla, CA, USA) grown to an A_{600} of 0.50.
 6. Incubate bacteria/phage mixture at 37°C for 15 min and then add isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM.
 7. Incubate for an additional 5 min at 37°C and then incubate in a 30°C shaker for 25 min.
 8. Remove bacteria and wash beads seven times with PBST.
 9. Repeat steps 5 through 8 for three to five rounds of selection.

were subsequently cloned into the soluble Fab (sFab) expression vector. After expression in *Escherichia coli*, the sFabs were purified from cell supernatants by overnight batch binding using protein A affinity chromatography. sFab ELISAs were performed to determine the fraction of sFabs that specifically bound to the target antigen. Interestingly, the URSA FITC selection produced over a 7-fold increase in the percent of specific target binding sFabs when compared to a previous selection using standard methodologies. Eleven out of 96 clones produced ELISA positive results, and all 11 Fabs were unique by sequence analysis. In a separate experiment using a metalloprotease target, MMP26, we found that URSA and standard selection methods yielded similar hit rates. Fab sequences from phage clones from both selection campaigns were formatted into full-length immunoglobulin G (IgG) and performed similarly in subsequent in vitro Matrigel™ (BD Biosciences, San Jose, CA, USA) cell invasion assays (manuscript in preparation).

Future experimentation to determine the robustness of this method may include the use of different library types and targets. One could envision the use of this method for phagemid-based antibody libraries. The method would be similar to the one outlined here for the phage library, except that it would be necessary to first determine when Fab-phage are initially extruded after helper phage addition. In the protocol listed in Table 2, 2.0×10^{10} helper phage would be added after the 37°C incubation for 15 min, and the phage-bacteria mixture would be incubated for the time required to produce an initial extrusion of phage. Use of this method with targets that may be degraded during this procedure would require the use of newly prepared target-coated beads in the subsequent rounds of selection. Between infection and the first extrusion, the infected cells can be transferred to a new vessel containing fresh target beads. Beads coated with a second type of target would allow for selection of cross-reactive Fabs. Beads coated with a different protein or ligand could also be used to subtract out Fabs having undesired binding.

Using only the phage from the initial viral extrusions allowed for a significant decrease in the time needed to perform selections. In our hands, the data generated from three target selections shows that the URSA technique, when compared to standard methods, produced equivalent or superior yields of Fab binders. By using the URSA method or a modified version of this method, laboratories regularly performing target selections may benefit from the decreased amount of time necessary to produce therapeutic candidates.

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COMPETING INTERESTS STATEMENT

The authors declare that they are all employed by Dyax.

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