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The NIH Protein Capture Reagents Program (PCRP): a standardized protein affinity reagent toolbox

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To the Editor

Selective affinity reagents are essential for characterizing protein levels, localization, and function; they are also important clinical diagnostic tools and therapeutic agents. The biomedical research community needs access to well-characterized, renewable, and high-quality protein affinity reagents. The uneven quality of commercially available research antibodies has recently drawn considerable concern[1,2].

In 2010, the National Institutes of Health launched a pilot program to generate and distribute high-quality affinity reagents against human transcription factors (hTFs) and transcriptional coregulators. The NIH Common Fund Protein Capture Reagents Program (PCRP) production pipeline consists of three core centers: antigen production, traditional monoclonal antibody (mAb) production, and recombinant phage displayed antibody binding fragment (rAb) production. The PCRP differs in important ways from previous efforts to generate collections of affinity reagents for human proteins[3,4]. First, mAbs and rAbs are generated, and these molecules allow for greater ease of characterization and reproducibility than do polyclonal antibodies. Second, individual protein domains and full-length proteins are used as antigens, enriching for rAbs and mAbs that recognize proteins in native conformation. Third, each mAb or rAb is subjected to a high-throughput initial screen of either affinity or specificity; each mAb or rAb is only sent for further validation if it passes this screen. Fourth, each reagent that passes this primary screen is subject to multiple types of secondary validation, including immunoprecipitation (IP) and western blotting (WB). Finally, all reagents are made available to the community at low cost through commercial and not-for-profit sources.

The production pipeline used to generate PCRP affinity reagents is shown in Figure 1. Purified structural domains of individual hTFs are produced at Rutgers University and used to generate both mAbs and rAbs. This antigen supply is supplemented by purified full-length hTFs made at the production sites. rAbs are produced by the Recombinant Antibody Network (RAN) (www.recombinant-antibodies.org). mAbs are produced in a public–private collaboration between the Johns Hopkins University (JHU) and CDI Labs (www.cdi-lab.com)[5,6].

Primary screening for rAbs relies on affinity, and it is performed using competitive ELISA, with only reagents exhibiting $K_d < 50$ nM passed for secondary analysis. Primary screening for mAbs relies on specificity, with IgG-positive mAbs screened against HuProt microarrays, which contain nearly 20,000 unique recombinant full- length human proteins that are expressed and purified from yeast[5]. Antibodies that bind specifically to their expected target are then passed for secondary analysis. Affinity measurements are also made for a subset of mAbs.

Secondary validation for rAbs consists of IP analysis of cell extracts spiked with purified target protein. For mAbs, this validation consists of IP and WB analysis of target proteins expressed in human cell lines using a doxycycline-dependent promoter. Selected reagents are further tested by third-party validation at the National Cancer Institute (NCI), which has

demonstrated good reproducibility for reagents obtained both directly from production labs and from third-party distributors. Over 250 IP-grade mAbs have also been tested for ChIP-Seq. Since this validation pipeline is high throughput, additional user optimization may be necessary for some reagents.

The PCRP has developed a web portal (http://proteincapture.org/) to catalog passing rAbs and mAbs and to make all validation results available. This allows users to select the best reagent for their intended experiment, although further tests and/or optimization may be necessary. PCRP reagents are distributed at low cost through the University of Iowa Developmental Studies Hybridoma Bank (DSHB) (http://dshb.biology.uiowa.edu/), CDI Laboratories (www.cdi-lab.com), and a number of third-party commercial distributors. DNA constructs for each rAb can also be obtained through the DNASU plasmid repository (https://dnasu.org/). Direct links to distributors are available on the PCRP portal.

The PCRP has produced a combined total of over 1,500 rAbs and mAbs (Supplementary Table 1). The use of these well-characterized, renewable affinity reagents is likely to reduce costs and improve reproducibility for studies of hTF function. Furthermore, we hope that this effort serves as a template for future undertak ings aimed at developing specific affinity reagents for the rest of the human proteome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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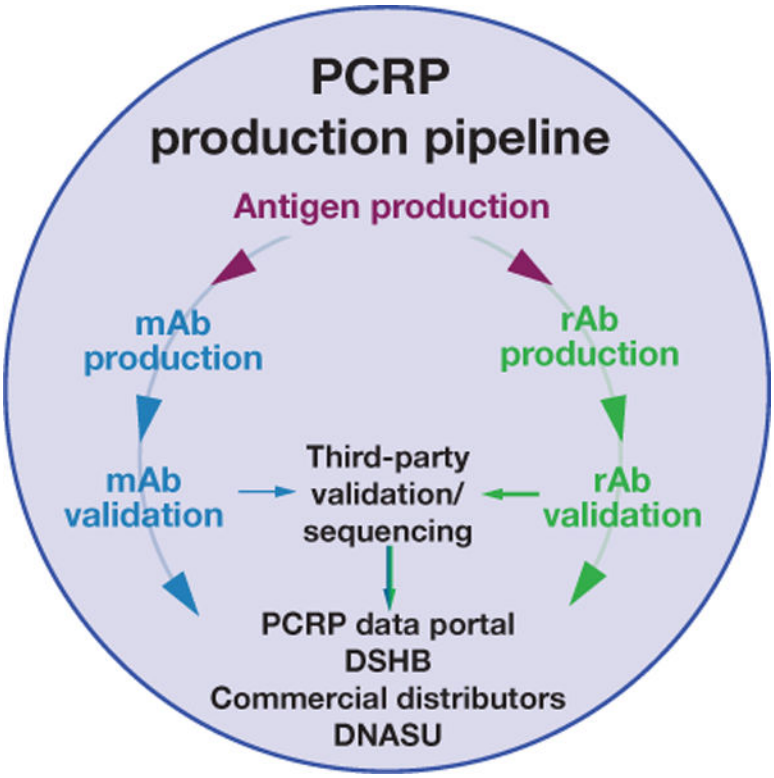

Figure 1.
Flowchart showing pipeline for rAb and mAb generation and validation. All validation data are available at http://proteincapture.org.