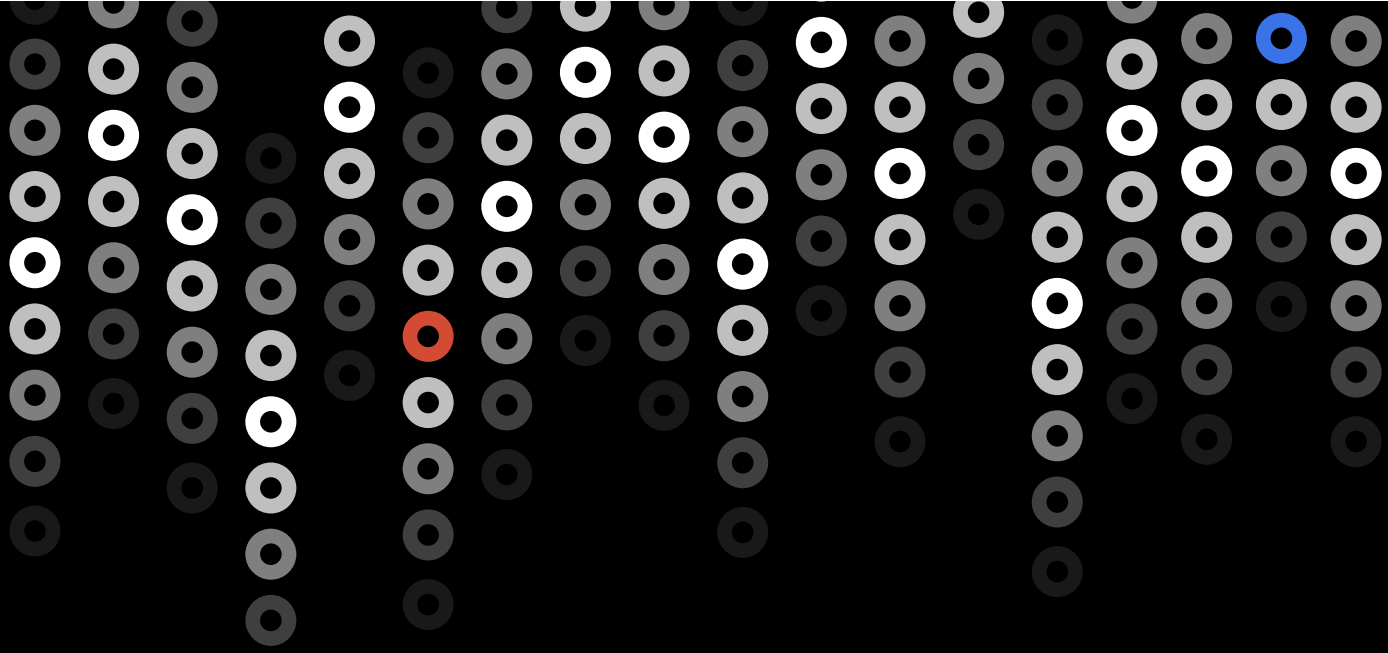


YOUR GROUND CONTROL IN HIGH-THROUGHPUT BIOLOGY

WHITE PAPER

DG-WP0004 - Rev A - White Paper

MICROFLUIDICS-ASSISTED ANTIBODY DISCOVERY

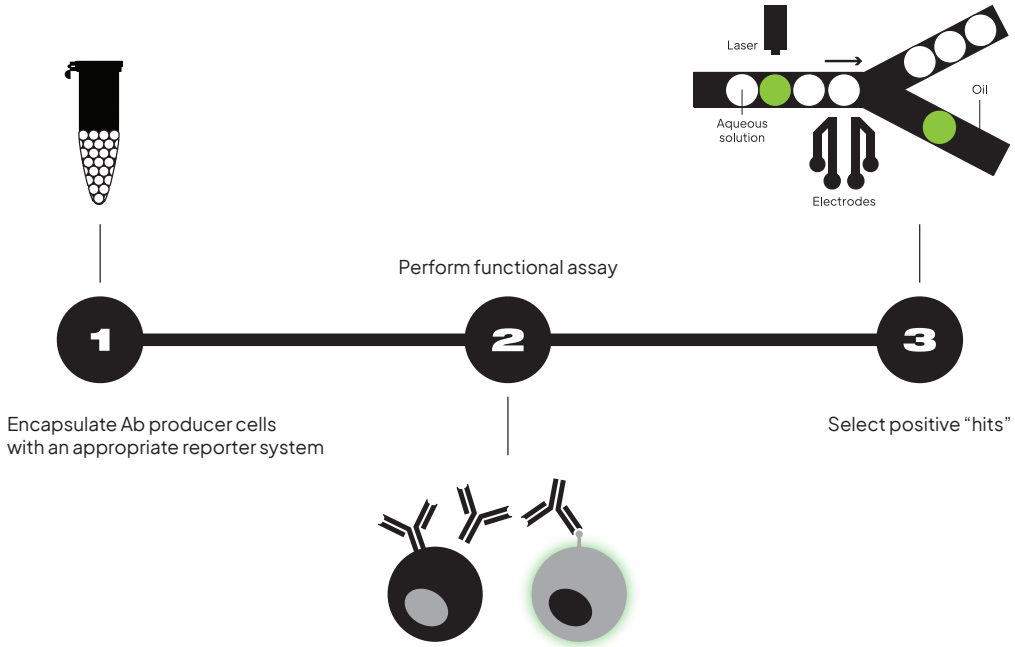
Droplet Genomics

DROPLETGENOMICS.COM

Microfluidics-assisted antibody discovery

SUMMARY:

- Conventional antibody screening workflows are severely limited in terms of throughput, cost, and assay design.
- Droplet microfluidics offers massive parallelization together with the ability to interrogate extracellular biomolecules without losing the phenotype-genotype linkage.



INTRODUCTION

Antibodies have become the fastest-growing class of biopharmaceuticals used against diseases ranging from allergies and infections to cancer. The development of immunotherapies requires detailed knowledge of immune signaling, immune cell migration, and immune repertoires, and the majority of these details can be addressed only on a single-cell level, e.g., stimulation of T-cells has been known to generate a large variety of signaling events; recombination events in B- and T-cells create extremely heterogeneous populations[1].

Technologies to screen antibodies face certain limitations: conventional hybridoma screens typically are slow and costly and allow screening only a few thousand clones. Moreover, the plate-based format depends on cell proliferation to obtain sufficient amounts of antibodies for testing. Thus, it is hard to avoid immortalization (which correlates with the loss in diversity) and apply such screens to primary cells. Display methods, such as phage, yeast, or ribosome display, are able to screen larger immune diversity, however, they typically test only for binding to recombinant

antigens and may involve unnatural pairing of heavy- and light-chain genes[2]. Furthermore, functional characterization of purified antibodies following display-based selection is mandatory. All in all, the workload for antibody selection and characterization can be colossal, with only a moderate chance to obtain clones with optimal functional activity. Water-in-oil microdroplets, serving as small individual reaction vessels, allow not only massive parallelization without the substantial inflation of costs but also enable direct screening for function rather than binding, which increases the overall chance of success of the antibody screening campaign.

Most antibody-secreting cells that are interrogated by microfluidics-assisted screens are plasma cells obtained from rodent immunization; among others, memory B cells, plasmablasts from human peripheral blood, and secretion libraries are used. Within droplet emulsion, antibodies retain their native chain pairing that can be subsequently analyzed by next-generation sequencing (NGS) and sequence information then used for antibody subcloning[3].

FUNCTIONAL SCREENING ASSAYS

Early examples of microfluidics-assisted functional screening in the context of antibody discovery interrogated single hybridoma cells. Debs et al. demonstrated the successful selection of hybridoma cells secreting antibodies that inhibit angiotensin converting enzyme (ACE-1) by sorting out weakly fluorescent droplet population (=inhibition of ACE-1 occurred) from strongly fluorescent droplets (=no inhibition, ACE-1 processed its fluorogenic substrate)[4].

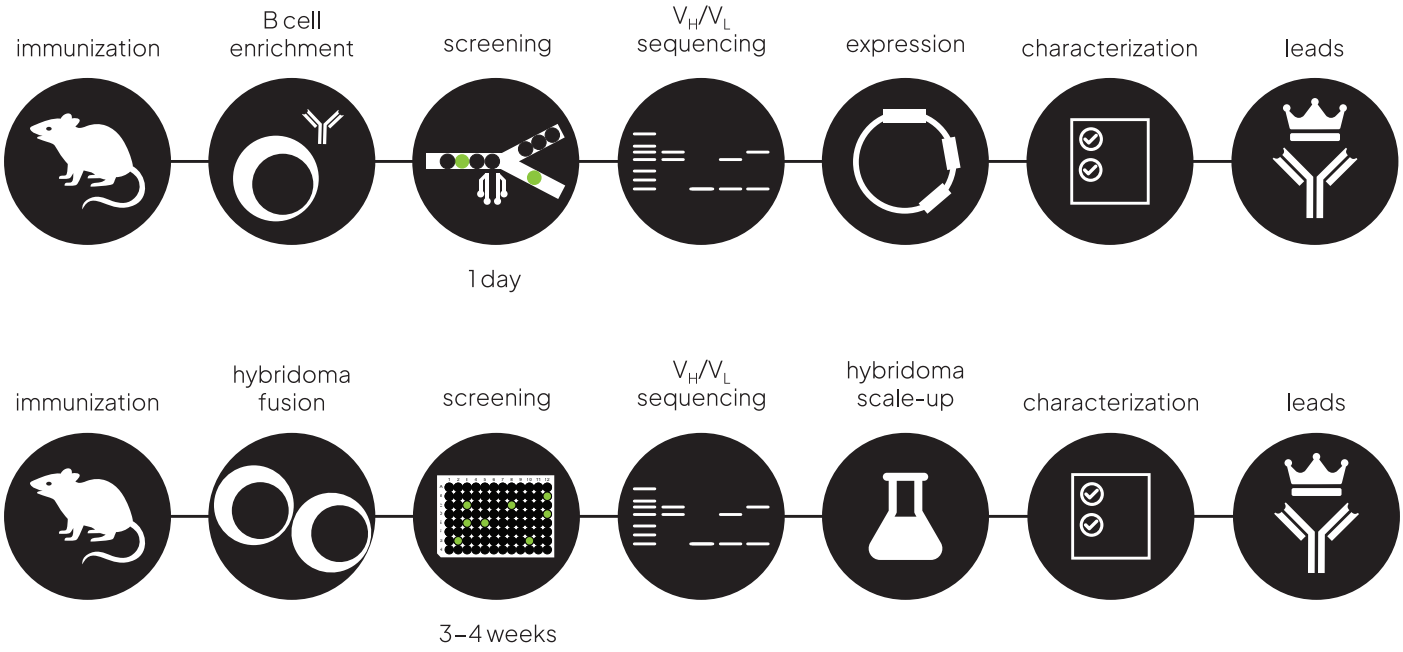


Figure 1. The overview of antibody discovery strategies. The top panel depicts microfluidics-assisted screening of primary cells; the bottom panel shows the conventional hybridoma workflow. Droplet-based screening streamlines the selection process, moreover, allows for selection based on function rather than binding as in the case of ELISA.

Nonetheless, droplet-based screening applies to non-immortalized primary cells as well, as no cell proliferation is required for this approach, and low amounts of antibodies can be detected given the small volume of droplets.

The interest in direct screening of primary cells is rising alongside the development of ingenious functional and cell-based screening assays that might be performed in conjunction with single-cell sequencing of heavy- and light-chain genes. Droplet-based systems are highly flexible, the only prerequisite for assay design is the correlation of the functional response with the change of the fluorescent signal. Several reported examples include[5]:

- tracking of opsonization

…detection of acidification of THP1–derived macrophages using pH-reactive dyes

- tracking of internalization of antibodies

…detection of a higher concentration of internalized labeled antibodies in reporter cells as compared to antibodies on the cell surface

- triggering the expression of GFP through an antibody-mediated intracellular signaling

…agonist antibody triggers TNRF1 which in turn activates NFkB; the expression of GFP that is controlled by NFkB-responsive promoter is used as a reporter.

Notably, the fluorescent signal must not necessarily occur inside the reporter cell or on the cell surface. Droplet sorting ensures the maintenance of compartmentalization throughout the whole screening procedure, thus genotype-phenotype connection will not be lost even if the target analyte will not be physically associated with the cell. This is in contrast to the fluorescence-activate cell sorting (FACS) approach that is suitable only for intracellular or surface-bound biomolecules. An illustrative example of the solution-phase analysis is the detection of antibody secretion on the basis of fluorescence resonance energy transfer (FRET) between appropriately labeled pairs of secondary antibodies[3].

CANCER IMMUNOTHERAPY

Next-generation immunotherapies are promising a paradigm shift in cancer treatment. Bispecific antibodies, such as bispecific T-cell engagers (BiTE), were shown to trigger anticancer immunity[6]. They are unnatural biologics that are engineered to recognize two different epitopes either on the same or different target antigens. Bispecific antibodies exhibit unique therapeutic modes of action, such as binding to a T cell-specific molecule and to a tumor-associated antigen which in turn leads to the physical linking of T cell to a tumor cell, ultimately stimulating T cell activation, tumor killing, and cytokine production. The main bottleneck in the selection of optimal bispecific antibodies for clinical use has been the necessity to narrow down the pool of candidates due to the complexity and inefficiency of conventional antibody discovery methods and candidate generation using validated monospecific antibodies. Notably, the construction of a functional bispecific antibody from two pre-characterized antigen binders is not straightforward because binding does not always translate to the expected functional activity. Therefore, the conventional design process relies on trial-and-error empiric testing.

Cost-efficient ultra-high-throughput screening allows the analysis of unbiased combinatorial libraries. Segaliny et al. have demonstrated the efficiency of the droplet microfluidics-based approach for the discovery of functional bispecific antibodies by screening libraries of >1M cells in a single run, even when functional clones were present at <<1% abundance. Microfluidics-assisted functional screening is sought to ultimately pave the way for the rational design of bi- or multispecific antibodies through gaining a deeper fundamental understanding of the relationships between their sequence, structure, and function[7].

PRACTICAL CONSIDERATIONS

How do I isolate individual clones after droplet sorting? Droplet sorting is a rapid process that typically operates at a rate of ~300 Hz. The speed of cell dispensing into plate wells does not exceed 1 Hz due to technical constraints. Speed-related incompatibility makes it difficult to seamlessly integrate both processes. To isolate individual cells for further colony expansion, a sorted cell population should be recovered from the emulsion and dispensed using a separate dedicated device.

What do I need to do to determine V_H-V_L pairs? The transcriptomes of selected functional antibody-producing cells might be profiled using single-cell RNA library preparation techniques. Cell barcoding preserves the cognate V_H/V_L pairing information.

CONCLUSIONS

- The droplet format allows to design and run functional screening assays on a massive scale.
- As no cell proliferation is required for droplet-based functional assays, non-immortalized primary cells can be interrogated directly.
- Combined with single-cell RNA sequencing library preparation, screening of single antibody-producing cells might provide information on cognate pairs of heavy- and light-chain genes of functional antibodies.

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For technical assistance, contact
Droplet Genomics support.

+370 619 78075
support@dropletgenomics.com

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