

# Antibody Discovery by Deep Mining of Immune Repertoires: A demonstration of the HiFiBio CelliGO™ Platform versatility and robustness with Tetanus Toxoid antigen as a case study

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The CelliGO™ technology is a fully integrated antibody discovery engine that is based on single droplet microfluidics, to achieve high throughput, single cell, function-based screening of antibody-secreting primary B cell populations.

We present a case study using B cells derived from a tetanus toxoid (TT) immunized mouse. By applying the CelliGO platform, we easily generated several hundreds of highly diverse anti TT specific antibodies. The recovered antibody repertoire covers more than 10 V-genes families, and includes several examples of clonal expansion. Antibodies have been cloned and transiently expressed as human IgG. More than 90% of the expressed antibodies showed specificity to TT. Our efficient and streamlined workflow can isolate and confirm specificity towards a diverse set of target specific antibody repertoires within 4-5 weeks.

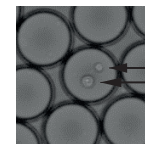
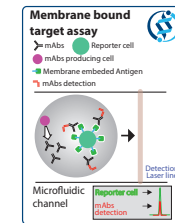
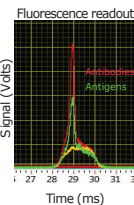
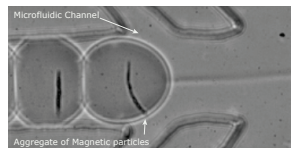
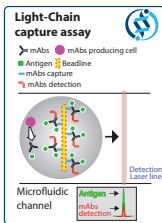
## Detection of antigen-specific Antibody secretion in droplets

### 1- Single-cell droplet based bio-assays

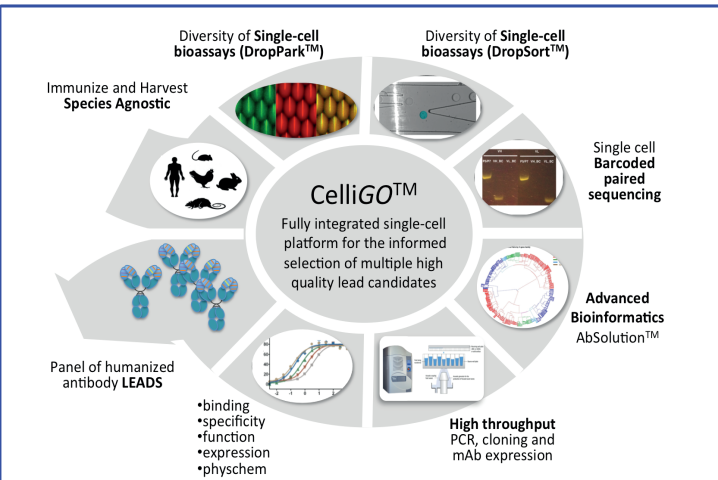
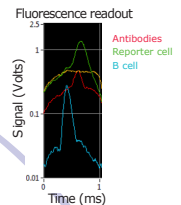
We have designed, implemented and validated droplet based microfluidics bio-assays to detect single cell monoclonal antibody secretion and select the droplets containing cells expressing antibodies specific to the antigen of interest. The droplet volume is compatible with cell survival for extended time and achievement of high secretion rate within minutes of incubations. Our platform is compatible with any primary cell species.

Our bio-assays uses combination of fluorescently labeled reagents to identify, analyse and sort droplets containing antigen and function-specific antibody secreting cells (internalization, agonist/antagonist activities, etc...)

### 2- Identification of mAb specific to soluble antigen



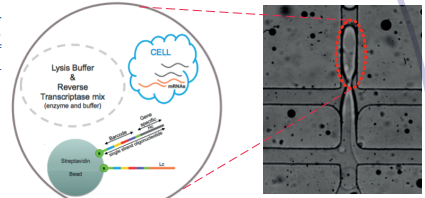
Droplet  
Antibody-secreting cell  
Reporter cell



## Diversity of Paired Sequences

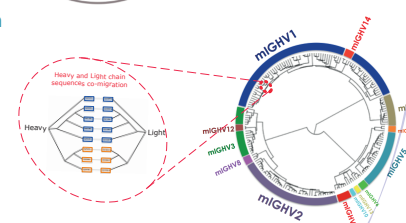
### 1- Droplet based Single cell VH/VL pairing

We have developed our proprietary single cell barcodes for the recovery of naturally paired VH and VL sequences.



### 2- Clonal expansion

Phylogenetic tree of paired sequences V-genes families shows recovery of > 10 V-genes families; for which some pairs highlight co-migrating heavy and light chain variable sequences.



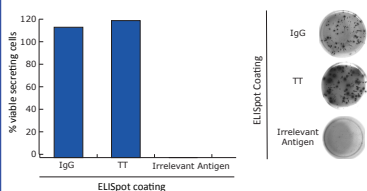
These clusters reflect the efficiency and the sensitivity of our platform for deep mining into the B cell repertoire.

## Characterization and Validation

From >1000 distinct antibodies recovered from 2 animals, compressed into > 300 unique antibodies (based on VDJ recombination), 41 have been selected for rapid cloning and ELISA. Above 90% of tested mAbs confirmed binding.

### 1- Sorted cell viability/specificity

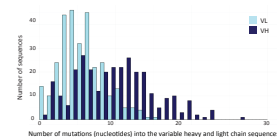
We monitored viability, antibody secretion and antibody specificity of sorted cells by ELISpot.



~100 sorted cells were analyzed by ELISpot, in wells pre-coated with either α-mouse IgG, TT antigen or irrelevant antigen.

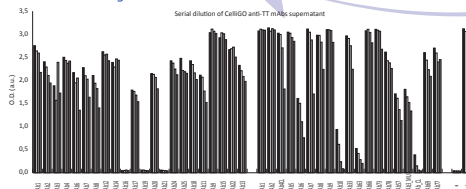
### 2- Affinity maturation

Our antibodies have undergone affinity maturation into the secondary lymphoid organs of the animal.



### 3- Binding validation (ELISA)

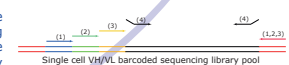
Antigen-based ELISA was used to assess specificity of the cloned antibodies to the target.



## High Throughput Cloning and Expression of Ab candidates

### Rapid, Automated Paired VH/VL Cloning

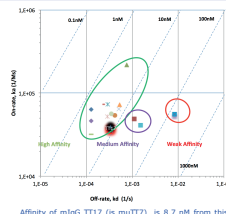
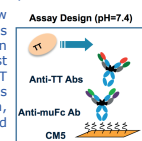
Dial-out based PCR of paired sequences from the sequencing library and subsequent in frame cloning into expression vector are automated to achieve high throughput cloning and expression of antibody candidates into human IgG1 backbone.



### 4- Biacore SPR

15 mAbs isolated from CelliGO have been analyzed by Biacore SPR.

The data show that Tetanus Toxoid can bind to most of the anti-TT antibodies with high, medium and low affinities.



## HiFiBio CelliGO™ Platform versatility supports discovery of antibodies targeting a diversity of epitopes

CelliGO accommodates a wide range of flexible in-droplet bioassays based on interaction with recombinant antigens, bacterial and cell transmembrane targets, and on functional assays (identification of internalizing, agonist and antagonist antibodies).

Our platform is able to rapidly identify potent antibodies, to efficiently mine immune repertoires of wild-type and transgenic rodents, successfully select and recover target specific cross species reactive antibodies.

We thank Ginger Shen (Pfizer, Cambridge, MA) for the Biacore SPR analysis of the anti-TT antibodies.