High-throughput screening of mouse B cells with Xdrop® for antibody discovery

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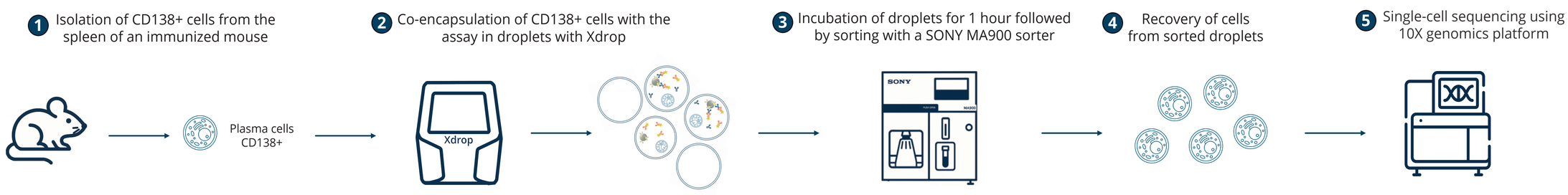
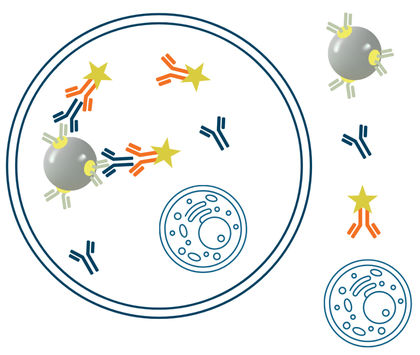
Introduction

Efficient antibody discovery requires high-throughput screening of antibody-secreting cells (ASC). Traditional methods relying on hybridomas or memory B cells are labor-intensive and risk missing rare, high-affinity clones.
In this study, we present a new Xdrop® protocol that generates **millions of droplets in minutes**, enabling rapid functional screening of plasma B cells from immunized animals.

Xdrop single-cell functional analysis workflow



Antibody discovery assay



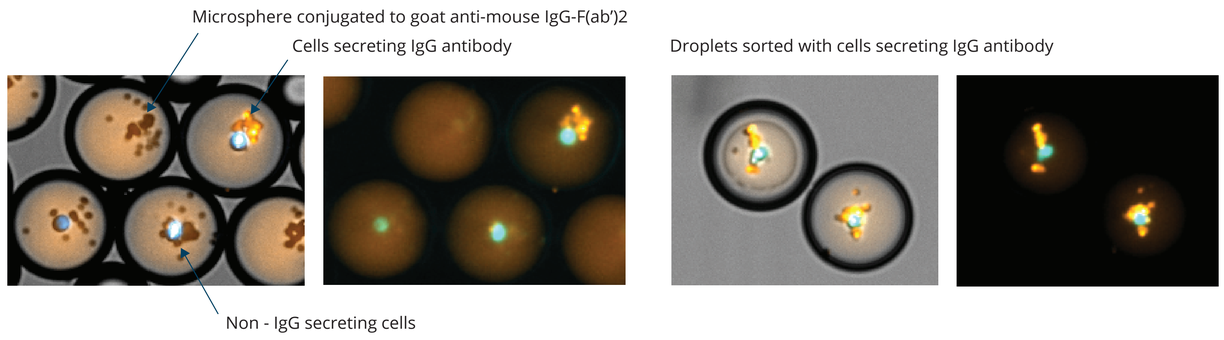
Microsphere conjugated to Goat anti-mouse IgG-F(ab')2 (IgG screening) *or* Human **TNF-α** (TNF-α screening)

Secreted **IgG** antibodies (IgG screening) *or* **TNF-α** antibodies (TNF-α screening)

Detection antibody (Goat Anti-mouse **IgG-Fcγ**- AF594)

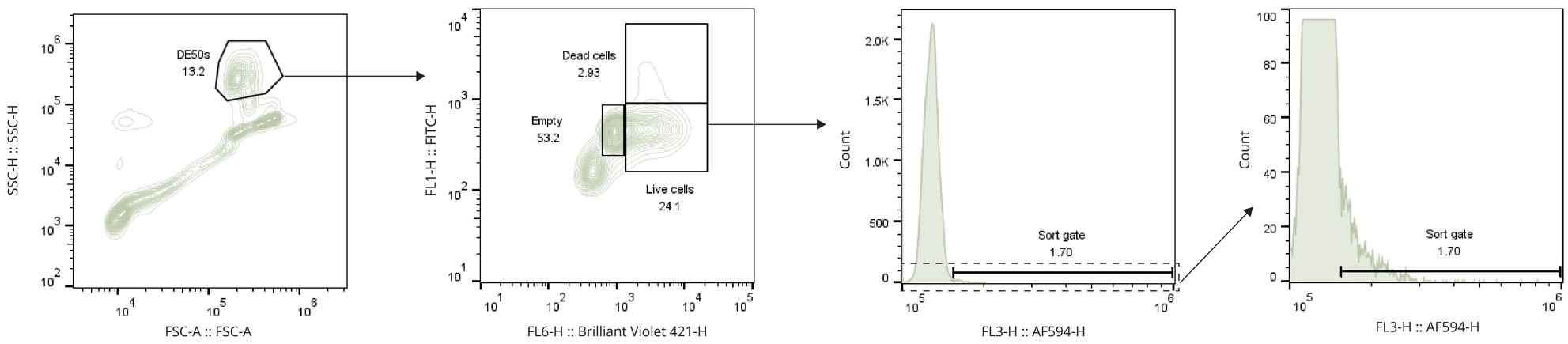
Mouse plasma cells (CD138+)
- stained with CellTrace Violet
- Sytox Green included in droplets

Image cytometry

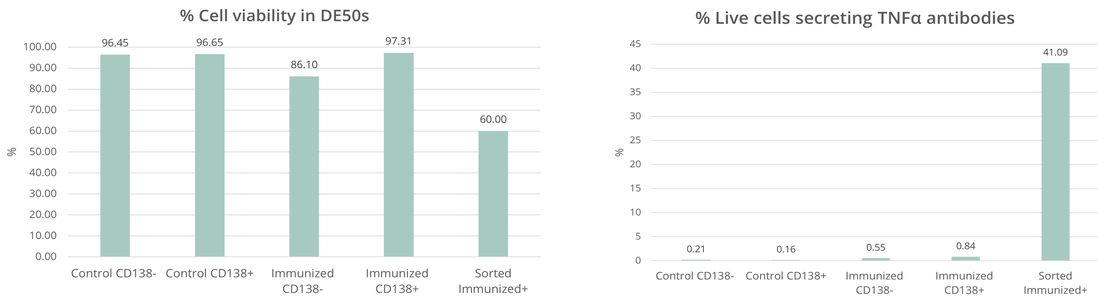


Images obtained with Xcyto®5 (ChemoMetec)

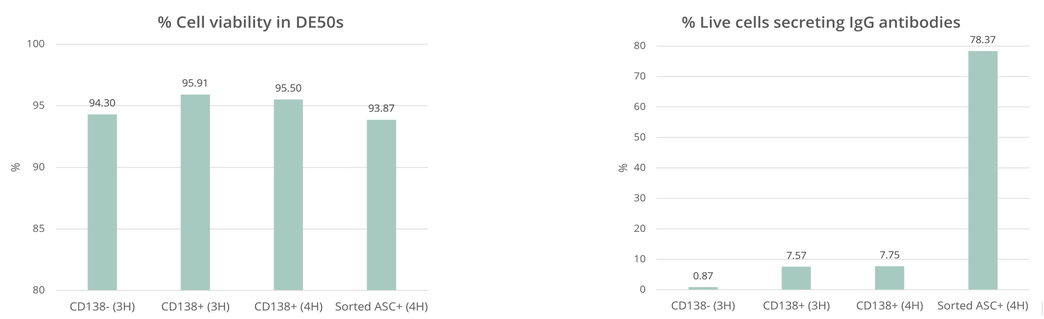
Droplet sorting



TNF-α antibody screen*



IgG screen*



Numbers from two screenings

Mouse	TNF-α 1x immunization*	Naïve
Screen	TNF-α +	IgG +
Total number of splenocytes	146,000,000	150,000,000
Purified CD138+ cells §	931,000	5,900,000
Encapsulated CD138+ cells	279,000	600,000
Droplets generated	~1,000,000	~1,000,000
Total number of droplets analysed	613,875	375,695
Droplets with live cells	145,034	106,778
Sorted out	1,126	1,793

* Day 0: subcutaneous immunization with vaccine composed of 25 µg TNF-α in 0.9% saline and 0.5 mg Alhydrogel. Day 11: intraperitoneal booster with vaccine containing only 25 µg TNF-α in 0.9% saline. Day 14: collection of spleen
§ CD138 MicroBead bound cells passed once (Naïve) or twice (TNF-α) over MS Columns (Miltenyi).

* Data obtained with image cytometry (Xcyto®5, ChemoMetec)

Conclusion

The droplet-based screening of antibody secreting B cells presented here detects secretion of antibodies within droplets, recovery of viable specific antibody producing cells, and hence supports paired V(D)J sequencing. The ability to detect anti-TNF-α production in plasma B cells from a mouse that was only immunized once underlines the sensitivity. The streamlined workflow demonstrated here can be scaled up to accommodate screening of several millions of mouse plasma B cells and can be accomplished within a day. This screening and selection represents a significantly accelerated protocol compared to traditional approaches.

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