Integrating Multi-plate Comparison and Profile Map Algorithms to Identify Mouse Monoclonal Antibody Hits in Antibody Discovery

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Overview:

Mouse IgG Type and Titer assay is a no-wash assay, with a wide dynamic range requiring no sample dilution. The simple mix-and-read workflow simultaneously measures five endpoints: 1. Antibody isotype. 2. IgG quantity per isotype. 3. Total IgG secretion level. 4. Cell count. 5. Cell health. The high-content data enables quick differentiation between monoclonal and polyclonal wells; determine IgG quantity for qualifying stable clones and for downstream functional assays; provide isotype information to facilitate PCR-mediated gene cloning; and monitor cell proliferation and health in the original cell cloning plate before gene cloning.

As part of the integrated solution, ForeCyt™ software provides the powerful data mining tools for large screening data sets, locating polyclonal wells for exclusion and identifying wells in which monoclonal antibodies reach user-defined thresholds of IgG quantitation. ForeCyt's Panorama feature provides interactive and visual multi-plate data analysis including profile maps with user-specified IgG criteria to quickly discover monoclonal antibody hits, and line graphs for hit-ranking, not only at the plate level, but also at the screening campaign level. The integrated IntelliCyt solution of Mouse IgG Type and Titer assay with ForeCyt's data analysis reduces the time for researchers to make actionable cloning decisions from high-content antibody readouts and intuitive visualization.

Introduction:

Monoclonal antibodies are the fastest-growing class of therapeutic agents with success in treating a wide range of diseases such as cancer, cardiovascular disease, autoimmune disorders and infectious disease. Most therapeutic antibody candidates are initially generated from mouse hybridomas or primary B cell clones after antigen immunization. In the antibody discovery workflow (Figure 1), primary screens identify clones with specific attributes (i.e. binding specificity, cross species reactivity, selectivity and affinity). Potential candidates from the screen are assessed for a variety of critical parameters such as IgG isotyping, antibody quantification, and cell number/health which is vital information for lead molecule generation.

Quantification of mouse antibody from cell culture supernatant is traditionally assessed using enzyme-linked immunosorbent assay (ELISA). ELISA is a time consuming, single-endpoint assay, often requiring sample dilution and multiple washes. Additionally, separate IgG isotyping and cell count/health assays are performed to provide the scientific insight for downstream cloning. IntelliCyt disrupts this traditional workflow with a novel solution: Mouse IgG Type and Titer assay, a patented high throughput, multiplexed assay coupled with IntelliCyt's ForeCyt data analysis software. This platform streamlines antibody discovery, identifying optimal clones for downstream processes.
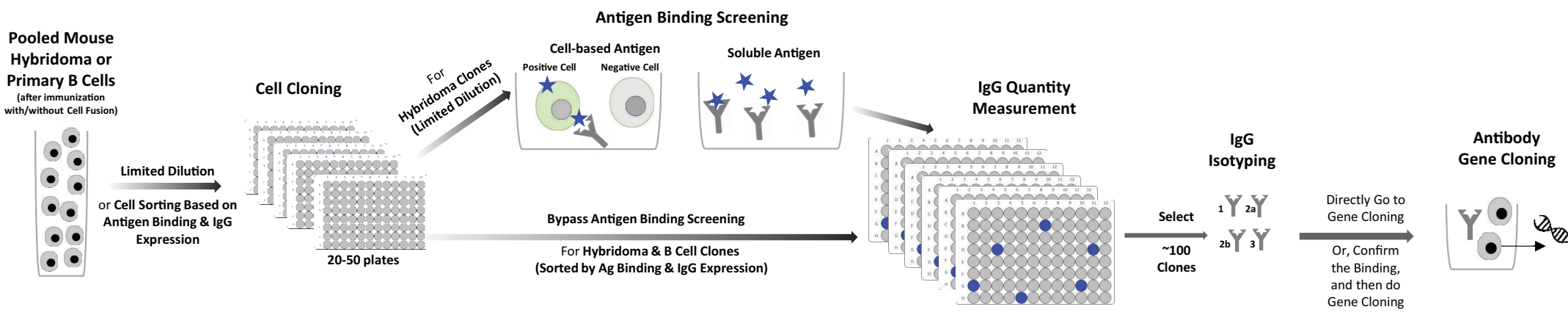

Figure 1. Traditional Lead-identification Workflow in Antibody Discovery. Researchers need to run multiple separate assays to measure IgG quantity and isotyping, cell health/proliferation before downstream functional assay and gene cloning.
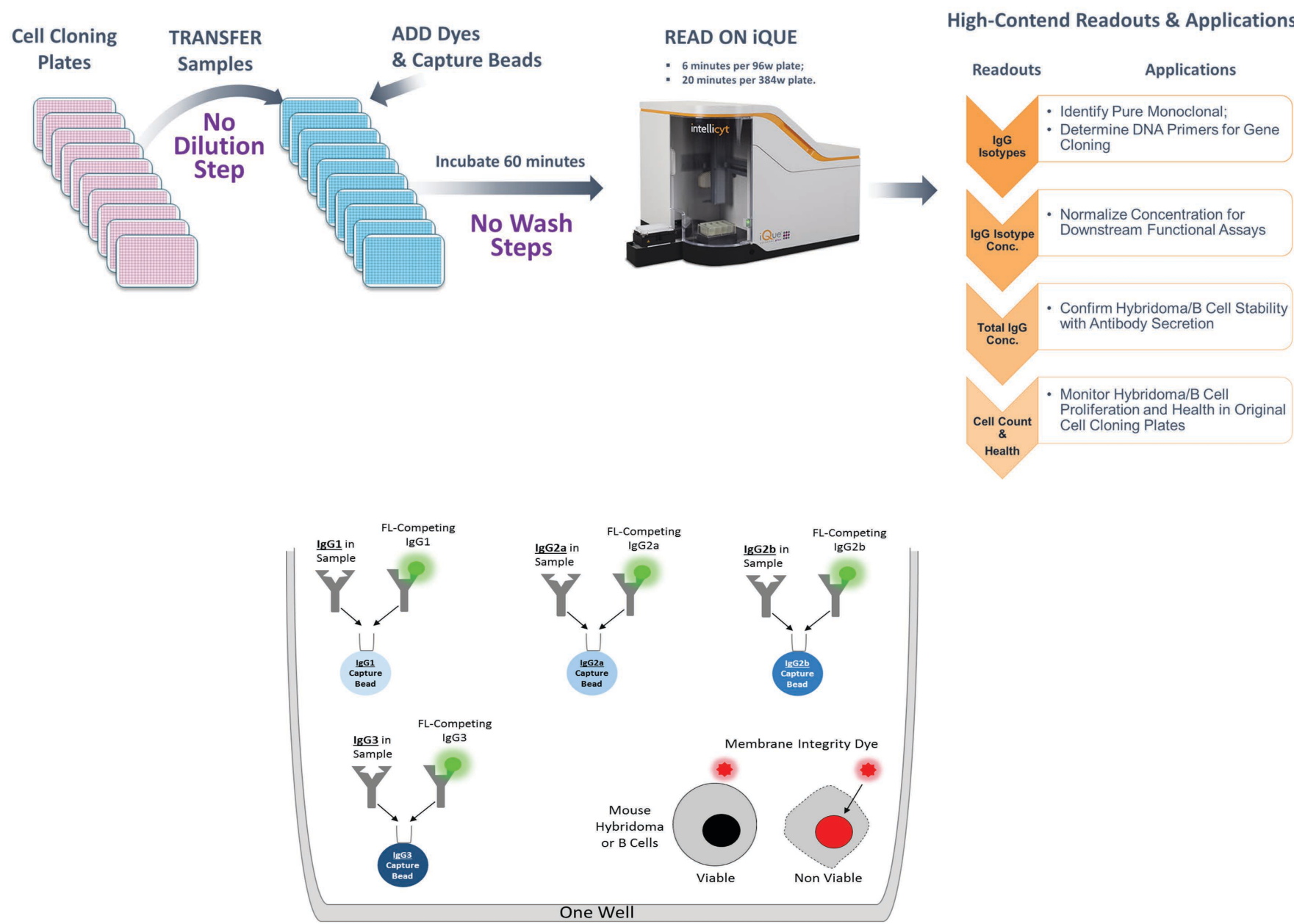
Methods:

Figure 2. Assay Innovation: Streamlined Assay Workflow with Multiplex Biochemistry.

– **No Sample dilution.** Cell culture samples can be transferred directly into assay plates

– **No wash.** Fluorescence background is low and there is no need to wash the assay plate

– **Shorten time from samples to data.** Assay handling time is about 15 minutes; incubation time is 60 minutes. The acquisition time is about 6 minutes per 96-w and 20 minutes per 384-w plate. Total time from samples to data is less than 100 minutes

– **Accelerate actionable decision process.** The total time from the acquired data to the final hit-finding for clone decision, may take minutes per plate and less than 1 hour for a screening campaign

– **Multiplex biochemistry:** 4 capture beads for precise quantification of 4 IgG isotypes (1, 2a, 2b, 3). Traditional singleplex assay cannot precisely measure IgG quantity for each isotype, due to isotype-dependent affinity difference. In addition, live/dead discrimination, and total cell counts are measured in the same assay

Samples and Reagent

Two sample types were analyzed. The first was a mixture of mouse hybridoma cells and supernatant assessed in a single 96-well plate. The second analysis was supernatant from mouse primary B cell samples in a multi-plate screening study. The Mouse IgG Type and Titer kit (IntelliCyt, a Sartorius brand) was used to analyze the mouse IgG isotype and quantity and as well as cell count/cell health (for hybridoma sample). The assay was run according to the assay kit manual with a streamlined workflow (Figure2).

Data Acquisition and Analysis Workflow

The assay kit includes a plug-and-play USB drive template which automates data acquisition and analysis to streamline the analysis process. The template includes the plate design, sampling protocol, and gates to automatically generate standard curves, heat maps and other key metrics. After data acquisition, heat maps and metrics will automatically populate to the corresponding data points. ForeCyt Panorama performs multiplate analysis including monoclonal hit identification, and the creation of a profile map which detects the quantity thresholds of four mouse IgG isotypes defining wells containing single clone with stable secretion of monoclonal antibody. Line graphs created in Panorama ranks all user defined hits for each isotype.

References:

[1] S. Kreye, et al. GlycoExpress™: A toolbox for the high yield production of glycooptimized fully human biopharmaceuticals in perfusion bioreactors at different scales.
Publication No.: SBI1557-e160401, 2016

Results:

Integration of IntelliCyt Mouse IgG Type and Titer Assay with ForeCyt Data Analysis Leads to Quick Visualization of Hybridoma Hits at Plate Level

Here we use one 96-well plate assay with mouse hybridoma samples as an example (Figure 3). The template included in the kit automatically generated four isotype-specific standard curves after sample acquisition. All IgG information is automatically populated in the heat maps and dot plots. Next, a Panorama view of all IgG isotypes in the samples wells were shown in an overlay line graph. The mouse hybridoma samples with IgG isotype and quantity information are displayed in the line graph for monitoring and quality-control of the screening results at the single plate level. From the acquired data to the full data analysis for a single plate may only take minutes.
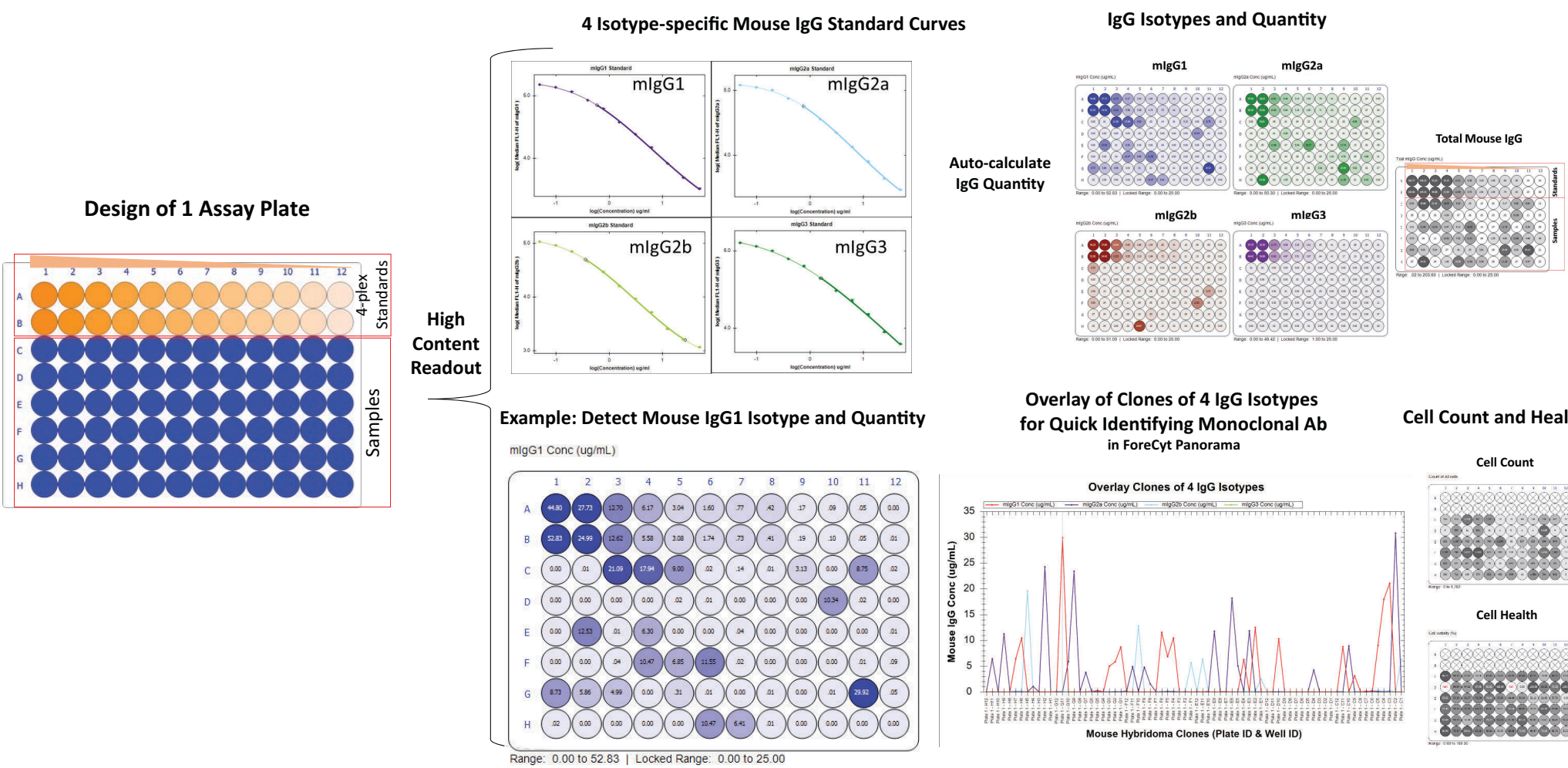

Figure 3. Demonstration Data Analysis of High-content Readouts in IntelliCyt Mouse IgG Type and Titer Assay with Mouse Hybridoma Samples. ForeCyt software quickly provides the IgG isotyping and clone purity information in an overlay line graph using the Panorama function. Cell count and health/viability is displayed in heat maps or in a data grid.

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Multi-plate Data Acquisition and Analysis Workflow for a Screening Campaign Enables the Global Identification of Monoclonal Hits

In this example, screening of mouse primary B cell clones was performed (Figure 4), which illustrates the same accelerated work flow as in the hybridoma screen. Using the Panorama feature, the user-defined quantity criteria of IgG subtype quickly filters out the polyclonal antibody wells, and the monoclonal antibody wells with a low level of IgG. Only the monoclonal antibody wells with IgG isotype concentrations above user-defined threshold are populated in the line graph. The data analysis for a whole screening campaign to identify antibody clones to move into downstream processing/cloning requires about one hour.
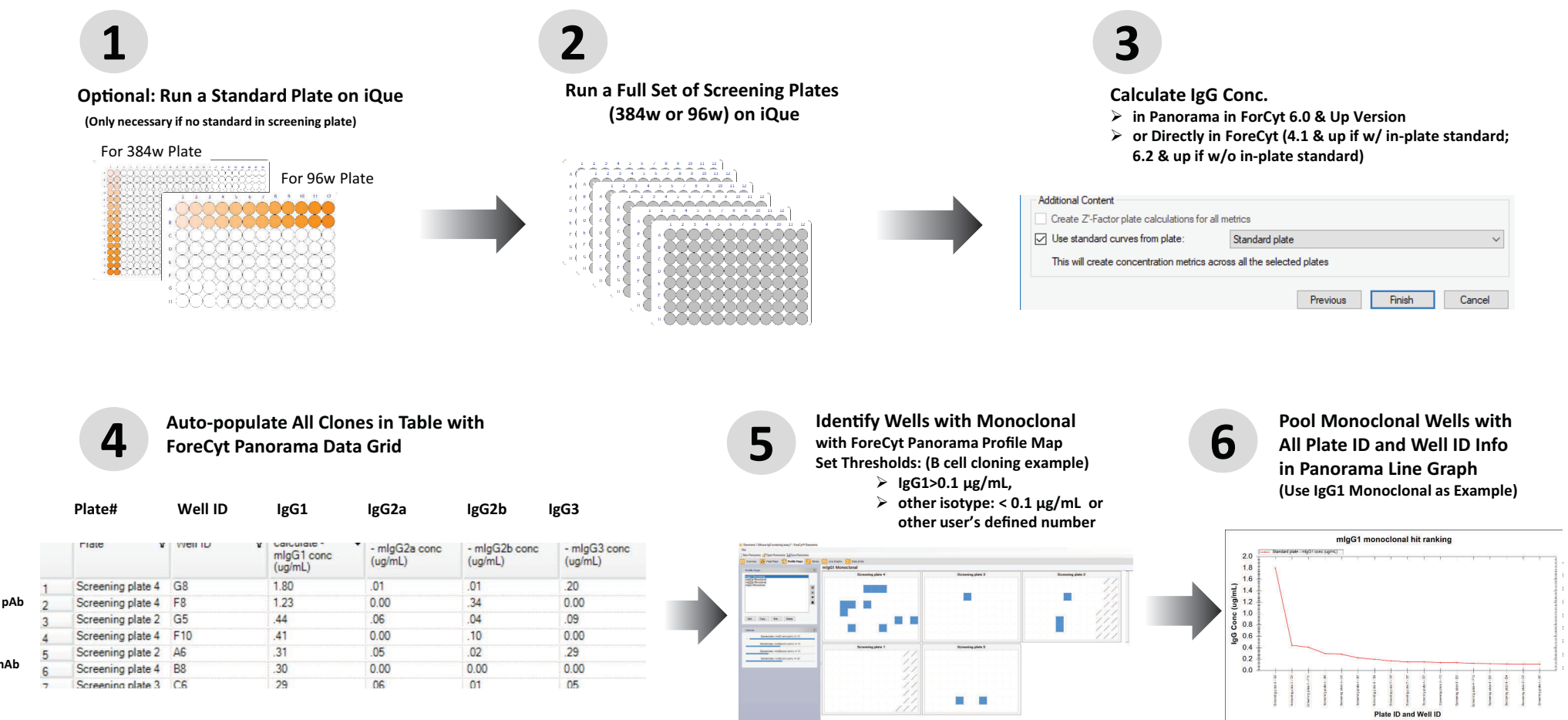

Figure 4. Data Acquisition and Analysis Workflow in a Screening Campaign. An example screening of mouse primary B cell clones. IgG quantitation may occur at plate-level or at the screening campaign level, depending on whether the standards are assigned to each plate or not. The monoclonal but not polyclonal wells will be pooled at the campaign level to provide plate-ID and well-ID information for all the clones that meet user's criteria as specified in the profile map algorithm in ForeCyt Panorama.

Conclusion:

The Mouse IgG Type and Titer Assay is a novel assay platform for mouse antibody discovery that disrupts the traditional time-consuming workflow. The iQue Screener platform provides high throughput, rapid sampling for a screening campaign. ForeCyt quickly transformed the large raw data sets into rich visual context by applying the multi-plate Panorama analysis algorithms. This allows researchers to quickly identify the critical mouse antibody clone attributes required for actionable decisions that ultimately result in robust antibody discovery.

The novel assay features are:

– **Multiplex Assay:** 4 beads precisely measure IgG quantity of 4 isotypes; cell/beads mixture assay

– **Full competition Assay:** very low assay background

– **Wide Dynamic Range:** 0.1-50 µg/mL mouse IgG quantitation

– **High-content Readouts:** polyclonal/monoclonal; isotype; isotype IgG quantity; cell count; cell health

– **Software Integration:** ForeCyt Panorama is integrated for multi-plate hit finding and visualization

– **Streamlined Workflow:** No wash and no sample dilution; Total time from sample to data: less than 100 minutes; Total time from the screening data to the actionable cloning decision: less than 1 hour for a screening campaign