ABSTRACT
The generation of hybrid myeloma cell lines for monoclonal antibody production is a fundamental and well established technique. However, the tedious methods required to screen for specific antibodies seen on the hybridomas has varied little over the years. Screening for antibodies directed against cell-surface antigen is particularly problematic. We have developed a robust mix-and-read, cell-based assay for screening hybridoma supernatants using FMAT® system technology. FMAT® system technology employs a macrocortical scanner that images and quantitates both cell- and bead-bound fluorescence. Light generated from a 633 nm helium/neon laser scans an area of 1 mm² with a depth of focus of 6-10 μm from the bottom of each well. The fluorescent debris seen in well 6B5 is discounted by the software.

INTRODUCTION
Monoclonal antibodies are produced by hybrid myeloma or hybridoma cell lines that secrete specific antibodies into the growth media. The screening of hybridoma supernatants for specific antibodies is a critical component of monoclonal antibody generation. Conventional ELISAs, while tedious and time consuming, are sufficient when searching for antibodies directed against soluble antigens. However, screening for antibodies directed against cell surface antigens is often difficult by ELISA. Cell loss during the many wash steps of the procedure is a significant source of variability in a cell ELISA. An alternative to the cell ELISA is flow cytometry, where cell-surface staining by specific antibodies can be detected. However, flow cytometric analysis routinely involves handling and/or analyzing each sample individually in addition to numerous wash and incubation steps. Since thousands of clones are routinely screened, flow cytometry is not a practical option for high-throughput hybridoma screening. A well-suited assay for hybridoma screening would be one that combines the reliability of flow cytometry with the plate format of a cell ELISA and can be performed without wash steps. FMAT® system technology provides a platform that is well suited for hybridoma screening. FMAT® system technology employs a macrocortical scanner that images and quantitates both cell- and bead-bound fluorescence.

MATERIALS AND METHODS
Assay using FMAT® System Technology
Cells expressing the cell surface antigen were grown in tissue culture flasks, collected, washed with screening buffer (PBS, 2% FCS) and resuspended in screening buffer at a concentration of 1×10^6 cells/mL. FMAT® Blue dye-labeled goat anti-mouse IgG(Ig) was diluted to 0.4 μg/mL in screening buffer. To initiate the hybridoma screen, 100 μL of cells, 50 μL of the labeled anti-mouse antibody and 5 μL of hybridoma supernatant were added to each well of 14 FMAT® system 96-well plates. No mixing was required. The final volume of 155 μL per well contained 10,000 cells and 0.13 μg/mL of detection antibody. The plates were scanned after 2 hours of incubation. A well was considered positive if it had a count of over 50 events. A simultaneous screen using control wells (without antigen expression) was performed as described above. Cell ELISA Cells expressing the cell surface antigen were grown in tissue culture flask, collected and plated in 14 96-well plates. After incubating for 48 hours, the tissue culture supernatant was removed and the wells were blocked with 200 μL of screening buffer for 1 hour. The buffer was removed and replaced with 5 μL of hybridoma supernatant and 95 μL of fresh buffer. After 1 hour of incubation, the supernatant was removed and the wells were gently washed 6 times with buffer. Detection antibody (goat anti-mouse IgG(Fc)) was diluted followed by an additional 1 hour incubation. The supernatants were removed, the wells were gently washed 6 times with buffer and substrate was added. The color was allowed to develop for 5-10 minutes before stopping reagent was added. Plates were read using a standard plate reader.

RESULTS
Out of a total of 1,344 wells, 51 were considered positive by FMAT® system technology. The positive wells were further subdivided into those with 10 µg/mL (40% or 520), medium (500-1000; 14 wells), or high (1000-5000) average fluorescence intensity values. Without washing away unbound antibodies, plates were scanned and the positive wells were easily identified. As a proof of concept, we demonstrated that the cell-based assay using FMAT® system technology is the more sensitive and reliable method for hybridoma screening.

CONCLUSION
The hybridoma screening assay for FMAT® system technology was simple to develop, set-up and execute. Unlike the cell ELISA where cells had to be plated 48 hours prior to testing, an FMAT® system technology assay was plated just prior to the screen. This eliminated any edge effects due to uneven cell growth across the plate and reduced the need for a standard curve. Due to the mix-and-read format of the assay, all cell types (suppression or adhesion) can be used without further manipulations. With a cell ELISA, suspension or weakly adherent cells must be fixed by some means to the well surface, thereby precluding adhesion to the wells. As a result, all assays performed using FMAT® system technology are mix-and-read and do not require wash steps. The integrated plate handler allows for walk-away screening for up to 96, 384-, or 1536-well plates.

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