

EVALUATION AND REDESIGN: HOW MANUFACTURERS CAN IMPROVE PROCESSING

Contract manufacturers can help the biotechnology industry refine production processes and increase efficiency, says Robert Fleischaker of Vista Biologicals

It is frequently assumed that the relationship between the contract manufacturer and the biotechnology industry is relatively simple. In principle the contract manufacturer is provided with a cell line and a process for downstream recovery and purification. The manufacturer then carefully follows that process and subsequently returns a purified product. While on occasion events do follow this script, more often than not the process, as presented to the contract manufacturer, has deficiencies which need to be addressed and corrected at this point in the product cycle. As a result, the initial role of the contract manufacturer often becomes one of process evaluation and redesign. An example of the extent of the service that can be required to do the job correctly is the focus of this article.

Defining problem areas

Vista Biologicals was asked to produce and purify an antibody that was being evaluated for use as a radiopharmaceutical for imaging purposes. There were many difficulties with the process:

- the product was an IgM and the purification process was not well defined;
- the productivity of the cell line was not stable and it also produced an IgG contaminant;
- the productivity of the cell line was poor at best;
- the cell line would not grow in any of the serum-free media to which we had access.

Consequently the purification problem was largely one of separating small amounts of IgM from a large excess of IgG, which is difficult because the large molecular weight of IgM (about 900,000 vs. 180,000 daltons) tends to favor the adsorption of IgG in many chromatography processes.

Our development effort was defined by focusing on achieving three goals:

- to eliminate the production of the IgG contaminant and screen for a cell line that stably produces IgM;
- to develop a cell line that grew in a serum-free or preferably a protein-free media;
- to develop a purification process that provided antibody of sustainable purity and was prepared using buffers that did not interfere with the chemistry of the isotope labelling process.

Implementing solutions

The cell line was a hybridoma that had been prepared using one of the original fusion partners developed by Kohler and Milstein. The original fusion partners secreted an IgG and have long since been replaced with fusion partners that do not secrete IgG. Cytofluorometric analysis confirmed that not only did the cell line we were provided with produce both IgM and IgG, but that with time production of the IgM was lost, while the cell continued to produce IgG. This information suggested that if the prob-

ABSTRACT

During the early stages of cell line production a contract manufacturer often experiences deficiencies in the process provided. An initial role of the contract manufacturer then becomes one of process evaluation and redesign. When Vista Biologicals were presented with a faulty antibody purification process they took investigative steps to set it right.

lem of the cell line's instability was to be solved, the genetic information coding for the IgG would have to be eliminated. We accomplished this task by refusing the cell line with a non-secreting fusion partner (653 PF) that had been adapted to grow in a protein-free culture media (RPMI supplemented with 1% Vitacyte). Because of the hybridoma cell line's inability to grow in protein-free media, we were able to select only cells that contained genes from both the original B cell line and from the new non-secreting partner by plating the cells after fusion with PEG (polyurethane glycol) in 96 well plates containing protein-free media with HAT (hypoxanthine, aminopterin and thymidine).

From these cells we selected several single clones and tested them for production of IgM. Those that showed growth in protein-free media and were positive for antigen specific IgM production were analyzed for IgM and IgG production by cytofluorometric analysis and electrophoresis (see figures). The polyacrylamide gel electrophoresis (PAGE) shows that not only had the IgG been eliminated but it also points out how little protein other than IgM was present in the protein-free media. The long term stability of the cell line was further enhanced by successively re-isolating the new cell line by clonal selection two additional times. Cytofluorometric analysis was again used to confirm that subpopulations of non-producing cells had not developed and that the population was relatively homogenous.

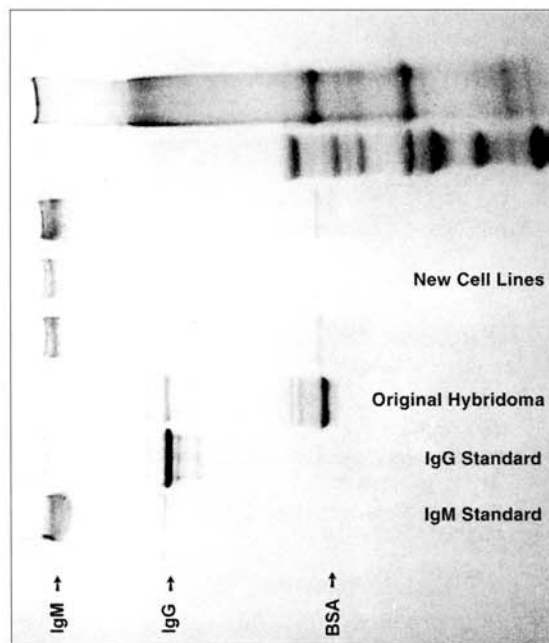


Fig. 1

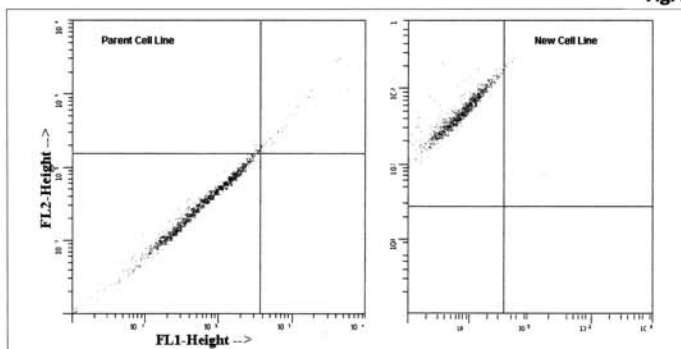


Fig. 2

Achieving success

Subsequently, a three-step purification process was developed. It consisted of concentration of the tissue culture media via ultra-filtration (UF, 30k mwt cut off) and adsorption on a weak anion-exchange (PEI) resin. The IgM is contained in the flow-through while the column functions to bind phenol red from the tissue culture media and strongly acid materials (e.g., DNA and lipopolysaccharides). The flow-through is then diafiltered via UF (500k mwt cut off) into a low ionic strength buffer and adsorbed on strong anion-exchange (Q) resin. The IgM is captured on the resin and is subsequently eluted by a step increase in ionic strength. The material is concentrated via UF (500 mwt cut off) and applied to a sizing column where it appears just after the void volume. The final material shows single band on PAGE. In addition, the process was developed so that onyl glycine, gly-gly, maltose and sodium chloride are used in formulating the buffers. We have avoided the use of TRIS, bis TRIS-propane, phosphate, sucrose, borate, EDTA and other agents which would interfere with the radiolabeling chemistry.

The use of backfusion accomplished several goals: the nonspecific production of IgG by the cell line was eliminated, the stability of the cell line was improved and the cell line was transferred into a protein-free media. The latter is significant as it not only simplified the development of a suitable purification process, but simplifies the validation of virus removal and increase the acceptance of the product in foreign markets.

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