Hybridomas are cells which are have been engineered to generate a wished antibody in huge amount. To generate monoclonal antibodies, B-cells are taken from the spleen of animals and they are fused with myeloma tumor cells which grow indefinitely in culture. Monoclonal antibodies are generated in unique cells through a method which is called as hybridoma technology. In the year of 1975, two scientists discovered hybridoma technology and they were Georges Kohler of West Germany and Cesar Milstein of Argentina.

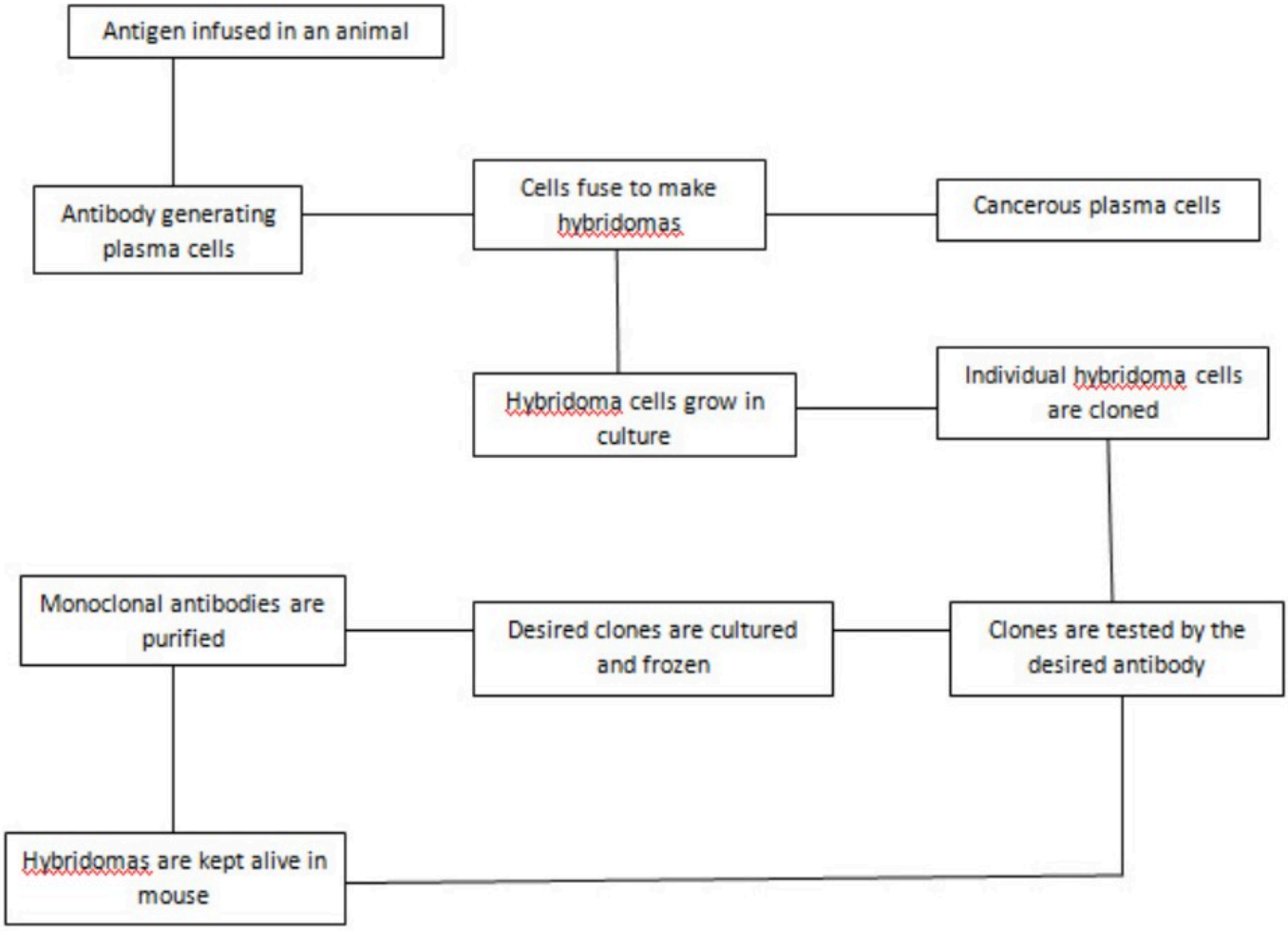
Methodology:

A hybridoma is considered as a harry cell and is generated by the injecting specific antigen to a mouse, also procuring antibody-generating cell from the spleen of mouse and subsequent fusion of this cell is done with a cancerous immune cell which is known as myeloma cell. Thus, the generated hybrid cell can be cloned to generate similar daughter clones. Immune cell product is secreted by daughter cells. Thus, these antibodies come from a single type of cell i.e. a hybridoma cell and they are known as monoclonal antibodies. Benefits of these cells are mentioned below:

- It has ability to combine two distinct types of cells
- Ability to grow continually
- Ability to generate pure antibodies in huge amount

HAT medium i.e. Hypoxanthine Aminopterin Thymidine is used for monoclonal antibodies preparation. Laboratory animals, for example, a mouse was initially exposed to an antigen to which isolation of antibody was done. When splenocytes are separate from a mammal, B cells are fused with immortalized myeloma cells in which HGPRT is lacking. Full form of HGPRT is hypoxanthine-guanine phosphoribosyltransferase gene availing polyethylene glycol or Sendai virus.

Incubation of fuse cells is done in HAT Hypoxanthine Aminopterin Thymidine. Aminopterin which is present in myeloma cells die as they cannot generate nucleotides by de novo or salvage medium prevents the block way which permits for nucleotide synthesis. Therefore, B cells and D cells which are not fused die as they have a short lifespan. B cell myeloma hybrids do not die, they survive as HGPRT gene which is coming from B cells is functional. These cells generate antibodies which are immortal. After this, an incubated medium will be diluted into multiwell plates, as antibodies in a B cell are generated by similar B cell. Because they are generated by same B cell they get directed towards the same epitope. Hence, these are called as monoclonal antibodies. Below is the diagrammatic representation of methodology of hybridoma technology:


Antigen infused in an animal
Antibody generating plasma cells
Cells fuse to make hybridomas
Cancerous plasma cells
Hybridoma cells grow in culture
Individual hybridoma cells are cloned
Monoclonal antibodies are purified
Desired clones are cultured and frozen
Clones are tested by the desired antibody
Hybridomas are kept alive in mouse

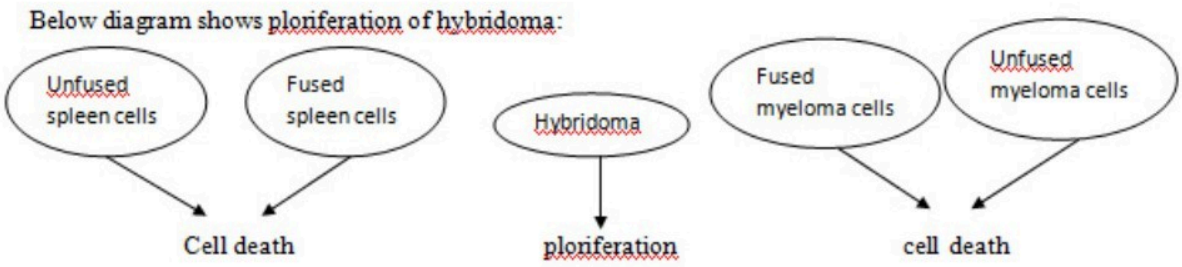


Fig2: Ploriferation of Hybridoma

Improvements in hybridoma technology:

Many efforts were in last 10 to 15 years to enhance yield of monoclonal antibodies availing hybridoma technology. Some of these efforts are mentioned below:

- In the beginning, Sendai virus was used to promote fusion
- Use of myelomas
- Continuous cell line was availed as a partner of fusion for antibody generating B cells
- Feeder layers were used for hybridoma production and optimal growth

Feeder layers consist of following:

- Macrophages which are derived from rat
- Murine peritoneal cells
- Human fibroblasts