# Hybridoma and monoclonal antibodies

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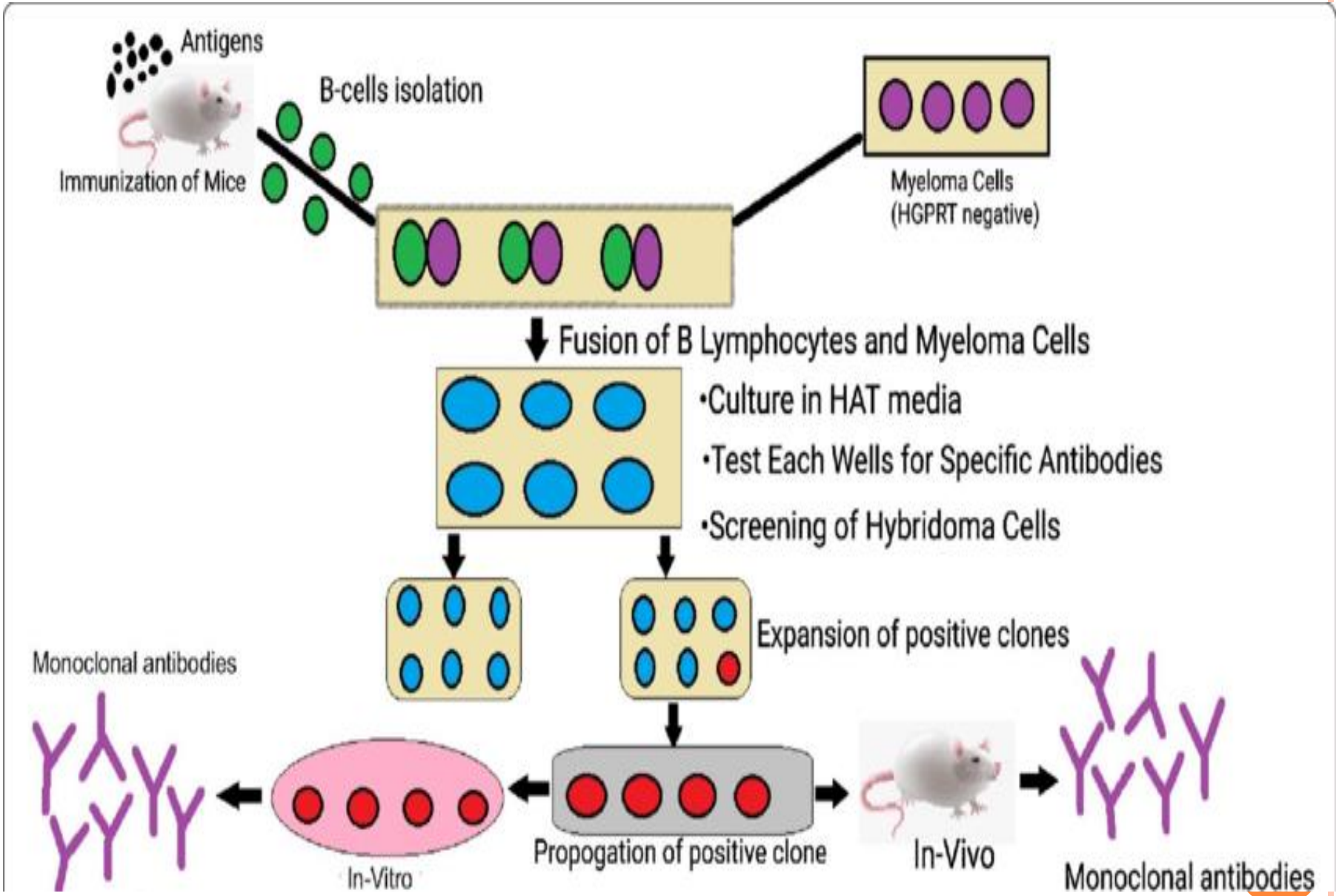
- Hybridoma technology includes formation of hybrid cell lines (hybridomas) by fusing a specific antibody-producing B cell with a myeloma (cancer cell) cell.
- Can grow in tissue culture (*in- vitro*) and have a ability to produce specific antibodies known as monoclonal antibodies (single specificity)
- The term hybridoma was coined by Leonard Herzenberg.
- The production of monoclonal antibodies was invented by Cesar Milstein and Georges J. F. Köhler in 1975.
- They shared the Nobel Prize of 1984 for Medicine and Physiology with Niels Kaj Jerne.

# ANTIBODIES

| POLYCLONAL | MONOCLONAL |
| --- | --- |
| Each identifying a different epitope | They bind to the same epitope |
| Derived from different B Lymphocytes cell lines | Derived from a single B cell clone |
| Batch to Batch variation affecting Ab reactivity & titre | mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity |
| NOT powerful tools for clinical diagnostic tests | Enable the development of secure immunoassay systems. |


Antigens
Immunization of Mice
B-cells isolation
Myeloma Cells
(HGPRT negative)
Fusion of B Lymphocytes and Myeloma Cells
•Culture in HAT media
•Test Each Wells for Specific Antibodies
•Screening of Hybridoma Cells
Expansion of positive clones
Monoclonal antibodies
In-Vitro
Propogation of positive clone
In-Vivo
Monoclonal antibodies

## Preparation of monoclonal antibodies using hybridoma

- *Immunization / Specific Antigen Inoculation*

  - The first step involves injecting the laboratory animals like rabbits or mice with a selected antigen against which the antibodies are raised through a series of injections over a period of several weeks to stimulate B cell differentiation into plasma B cells and memory B cells.

## Preparation of monoclonal antibodies using hybridoma

- *Isolation of B lymphocytes*

  - Following sacrifice, the spleen is removed in aseptic conditions to isolate the activated B-cells.
  - This procedure is performed using density gradient centrifugation.
  - The presence of antibodies in the serum is identified using methods like ELISA.

## Preparation of monoclonal antibodies using hybridoma

- *Preparation of Myeloma Cell Lines*

  - Few weeks before the cell fusion, metastatic tumor cells are incubated in **8-azaguanine** to get non-functional hypoxanthine-guanine phospho ribosyl transferase (HGPRT) genes in the myeloma cells.
  - Non-functional HGPRT can stop the assembly of nucleotides from the salvage pathway and makes the metastatic tumor cells sensitive to HAT media.

## Preparation of monoclonal antibodies using hybridoma

- *Cell fusion*

  - Cell fusion is the process in which the activated B lymphocytes are fused with HAT-sensitive myeloma cells.
  - This step is performed by centrifugation of freshly obtained activated B-cells with HAT-sensitive myeloma cells in a fusion-promoting media.
  - Polyethylene glycol(PEG) is used in this procedure.

## Preparation of monoclonal antibodies using hybridoma

- *Hybridoma Selection*

  - In the PEG-containing media, cells are fused to form hybridoma cells but even the most efficient fusion method will allow the formation of only about 1 to 2%of fused hybridoma cells.
  - Furthermore, about 1 in 100 cells will be viable hybrid cells. Therefore, there are a number of un fused cells within the media.
  - This step allows the selection of the fused cells from all the un fused cells.

## Preparation of monoclonal antibodies using hybridoma

- *Hybridoma Selection*

  - This is achieved by incubating the cell mixture followed by culturing for 10–14 days in HAT media (a selection media).
  - HAT medium contains Hypoxanthine Aminopterin Thymidine.
  - Aminopterin present in HAT media blocks the power of cells to synthesize nucleotides by the de novo synthesis pathway.
  - Hypoxanthine and deoxy thymidine allow cells with functional hypoxanthine-guanine phospho ribosyl transferase (HGPRT) genes to survive through salvage pathways.

## Preparation of monoclonal antibodies using hybridoma

- *Hybridoma Selection*
  - Due to a limited life span, un fused B cells perish within a few days.
  - Un fused malignant neoplastic cells die as a result of the lack of the hypoxanthine-guanine phospho ribosyl transferase (HGPRT) gene.
  - The presence of aminopterin blocks their ability to synthesize nucleotides through the de novo pathway.
  - Therefore, the remaining viable cells left in the media are the hybrid cells; these hybrid cells have the ability to grow and divide on HAT media.

## Preparation of monoclonal antibodies using hybridoma

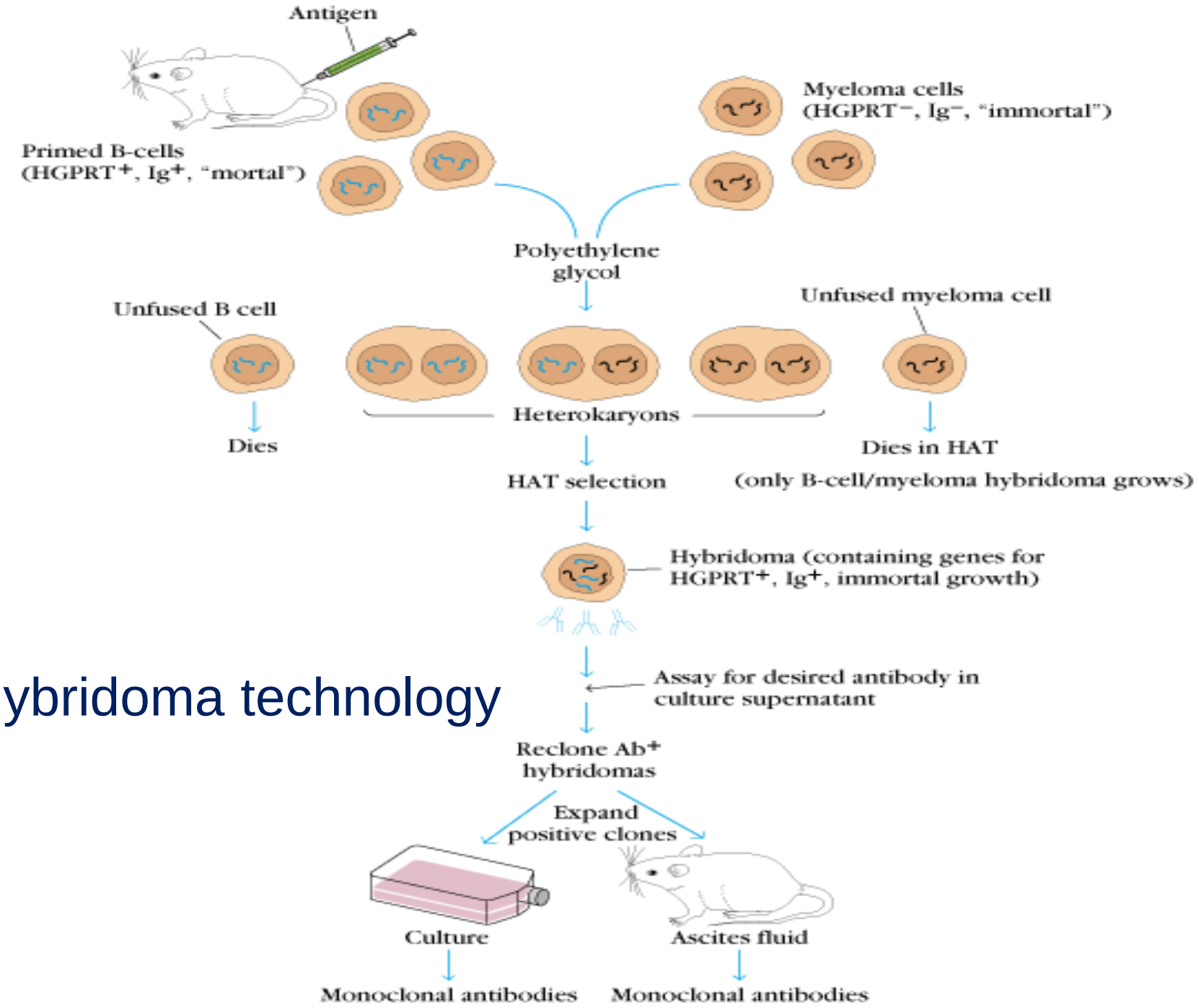
- *Screening of Hybridoma Cells*

  - HAT-selection hybridoma cells are transferred to ELISA plates, where each well houses a single hybridoma cell.
  - This is achieved using the limiting dilution method.

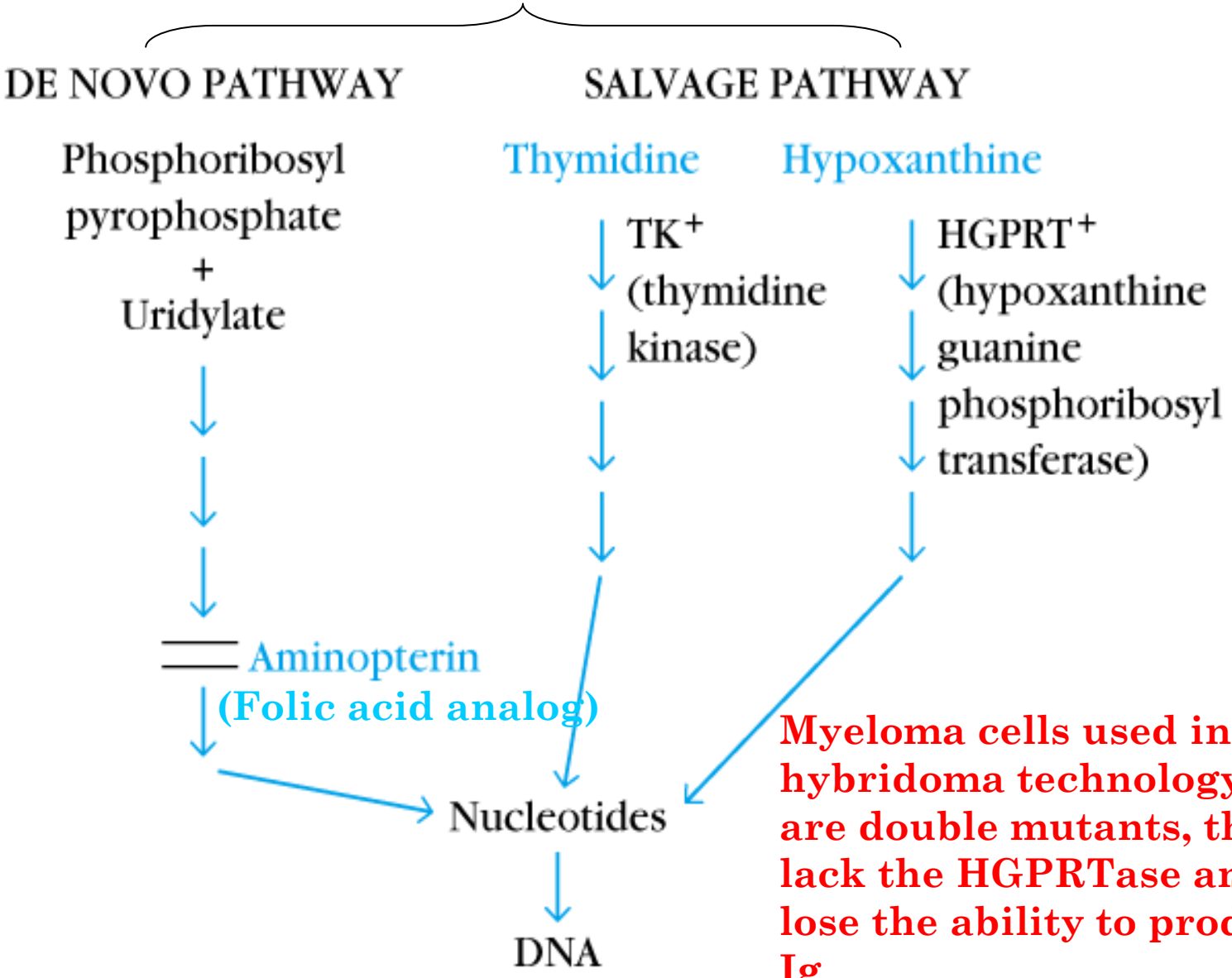
- *Cloning and propagation of hybridoma cell*

  - Hybridomas producing desired antibodies are selected and are then transferred into large culture vessels or flasks
  - The hybridoma cell lines are cultured using in vivo or in vitro methods.

# Hybridoma technology



Two different pathways to synthesis nucleotide in mammalian cells




**Myeloma cells used in hybridoma technology are double mutants, they lack the HGPRTase and lose the ability to produce Ig**

- Myeloma cells are immortal but lack the HGPRT (Hypoxanthine Guanine Phospho Ribosyl Transferase) gene and
- Aminopterin in the HAT medium causes myeloma cells death, as they cannot produce nucleotides by the de novo or salvage medium blocks the pathway that allows for nucleotide synthesis.
- B- cells have short life span but con produce antibody

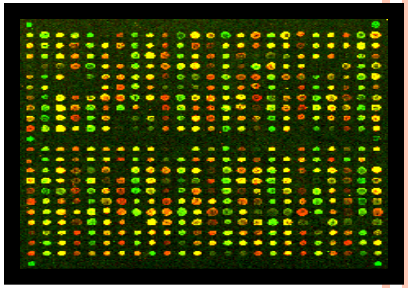
# Applications of Monoclonal Antibodies

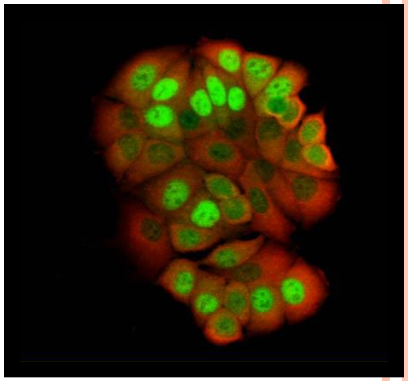
1. Diagnostic Applications.
   Biosensors & Microarrays.

2. Therapeutic Applications.
   Transplant rejection (Muronomab-CD3).
   Cardiovascular disease (Abciximab).
   Cancer (Rituximab).
   Infectious Diseases (Palivizumab).
   Inflammatory disease (Infliximab).

3. Clinical Applications.
   Purification of drugs, Imaging the target.

4. Future Applications.
   Fight against Bioterrorism.



# Human Monoclonal Antibodies

- Production of human monoclonal antibody
  - There are numbers of technical difficulties
    - The lack of human myeloma cells to exhibit immortal growth, be susceptible to HAT selection, to not secrete antibody, and support antibody production in the hybridoma made with them
    - Human B cell sometimes have immortality
    - That is the difficulty of readily obtaining antigen-activated B cells
- To culture human B cells in vitro to produce human monoclonal antibody
  - Transplant human cells with immune response into SCID mice (lack a functional immune system)

# Thank You

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