

# **Production of polyclonal and monoclonal antibodies**

# Monoclonal Antibodies

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# Monoclonal Antibodies

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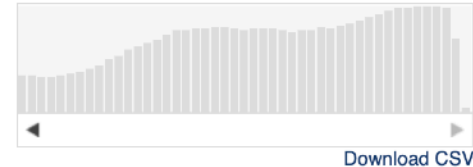
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Chiu ML et al. Curr Opin Struct Biol. (2016)

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Byzova NA et al. Bioconjug Chem. (2017)

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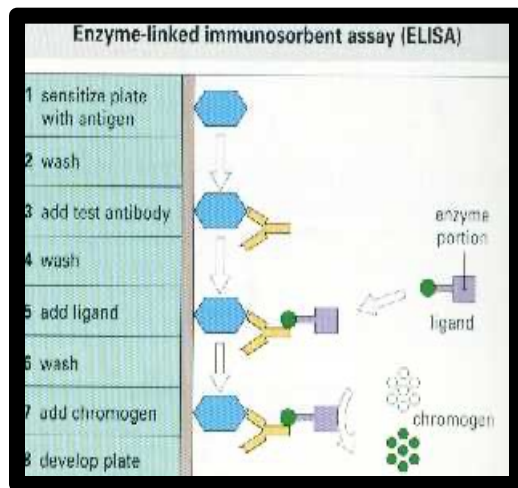
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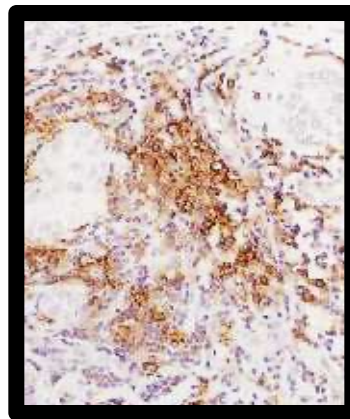
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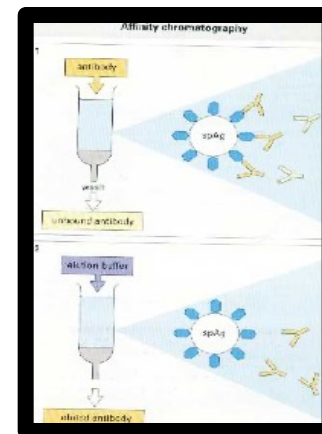
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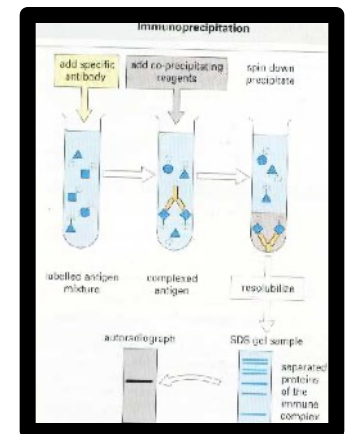
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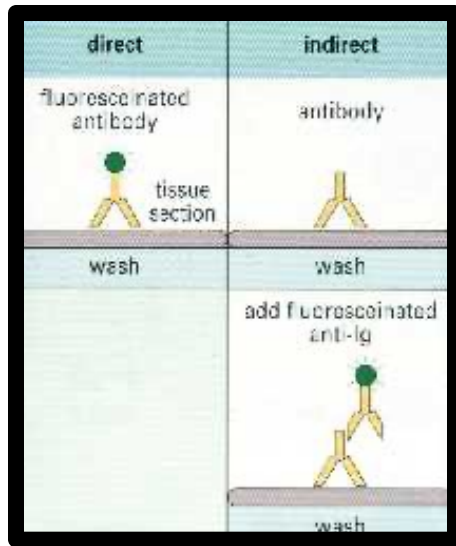


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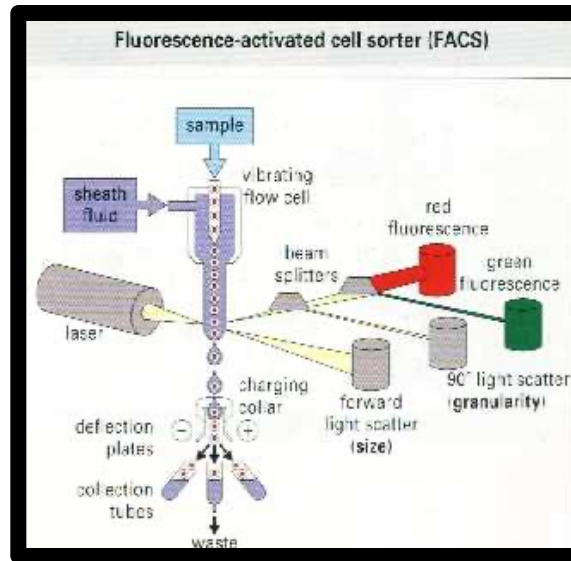


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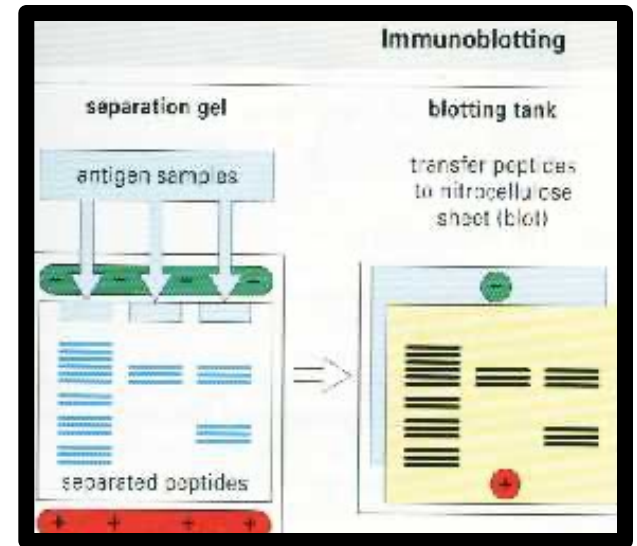
# Use of monoclonal antibodies



Immuno-  
fluorescence



Flow cytometry



Immunoblotting

# Antibody Production

## Polyclonal:

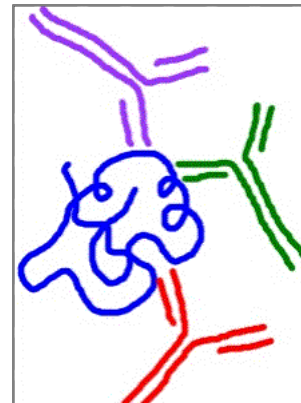
Antibodies are collected from sera of exposed animal,

- or -

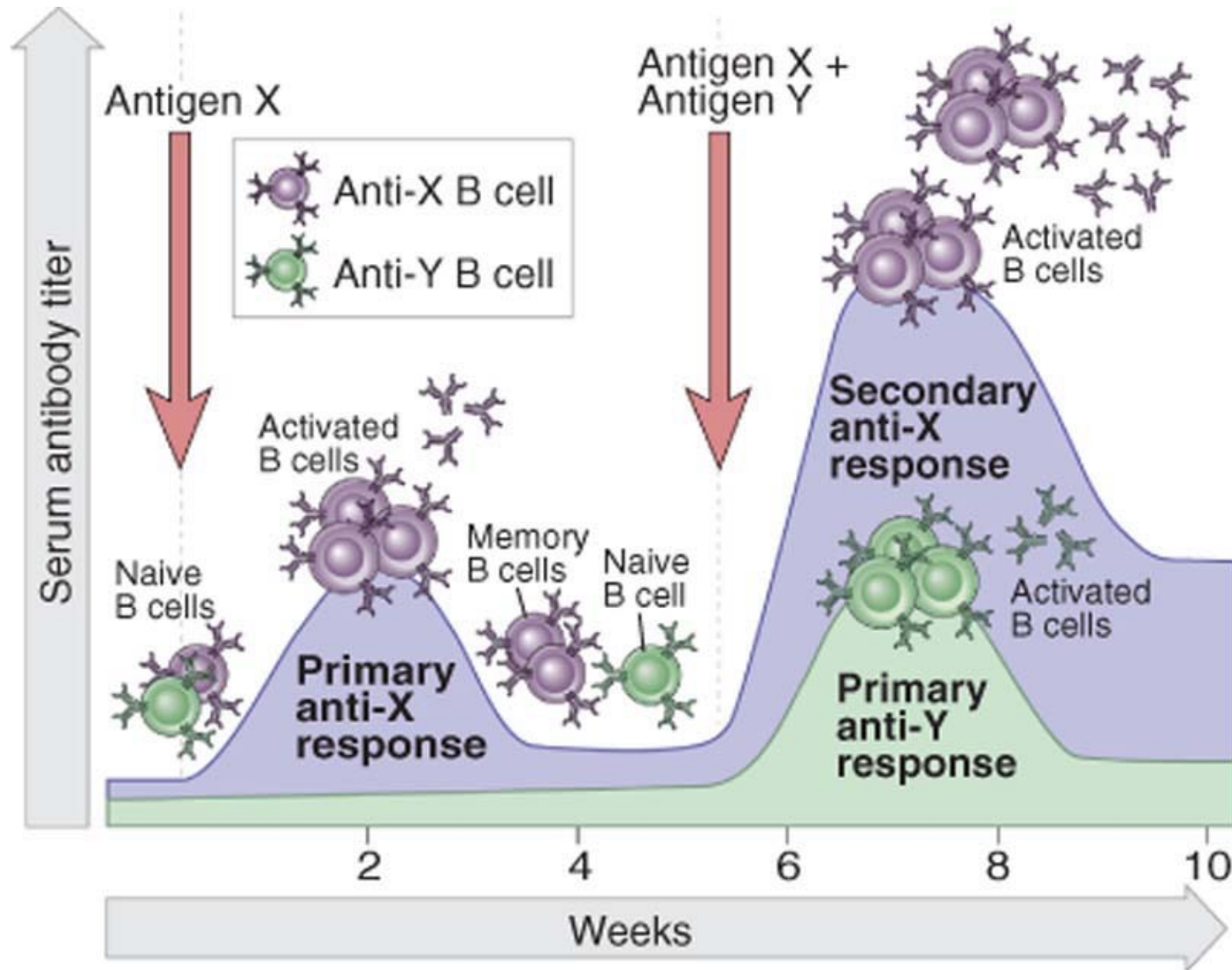
a combination of monoclonal colonies is combined.

Can be any animal: **Rabbit**, **Goat**, Horse, Rat, Sheep, etc...

Suite of antibodies recognizing multiple antigenic sites of injected biochemical.



# Timing for Ab production



# Disadvantages of Polyclonal Antisera

- Antiserum is composed of a mixture of **high** and **low** affinity antibody populations
- Antiserum is composed of a **mixture of antibodies** with different specificities - not all recognize the target of interest
- If the animal has had an infection, antibodies against the infecting organism will be present - can lead to “**non-specific**” **binding**
- **Quantity** of antiserum is **limited** by amount of serum and **life of immunized animal**.
- **Antigen must be pure.**

# Advantages of Polyclonal Antisera

- Antiserum recognizes many different epitopes on the target
- Can usually be used for many different research procedures  
(Immunohistochemistry, immunofluorescence, immunoprecipitation, ELISA, precipitation assays, functional assay)
- Can be affinity purified to eliminate the non-specific binding antibodies

# Production of monoclonal antibodies

One of the most important example of induction and selection of stable cell mutant and cell fusion is the production of **hybridoma**

Hybridomas are hybrid of cells obtained fusing lymphocytes and a tumor cell line obtained from multiple myeloma.

The aim is to obtain a stable clone of lymphocytes in order to obtain in vitro, for long time and in high amount, monoclonal antibodies that usually lymphocytes produce after their differentiation to plasma cells.



&



Georges Köhler  
(1946-1995)

Cesar Milstein  
(1927-2002)

*Nature Vol. 256 August 7 1975*

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**Continuous cultures of fused cells  
secreting antibody of predefined specificity**

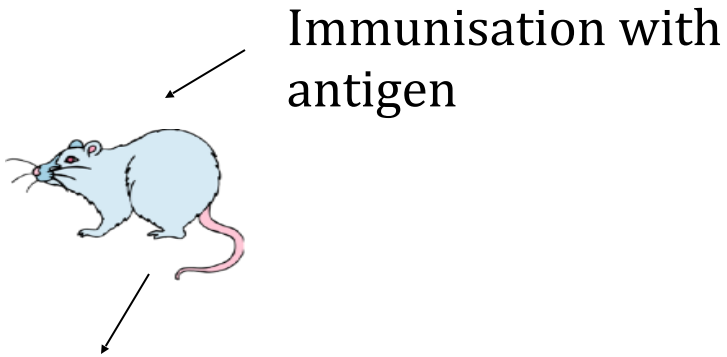
Method to produce monoclonal antibodies -1975

Nobel Prize - 1984

# **How to make a monoclonal antibody**

1. Immunize mice
2. Test the serum
3. Purify lymphocytes from the spleen
4. Perform a fusion
5. Screen the fusion for the right cells
6. Grow the hybridomas
7. Harvest the antibody
8. Concentrate and purify the product

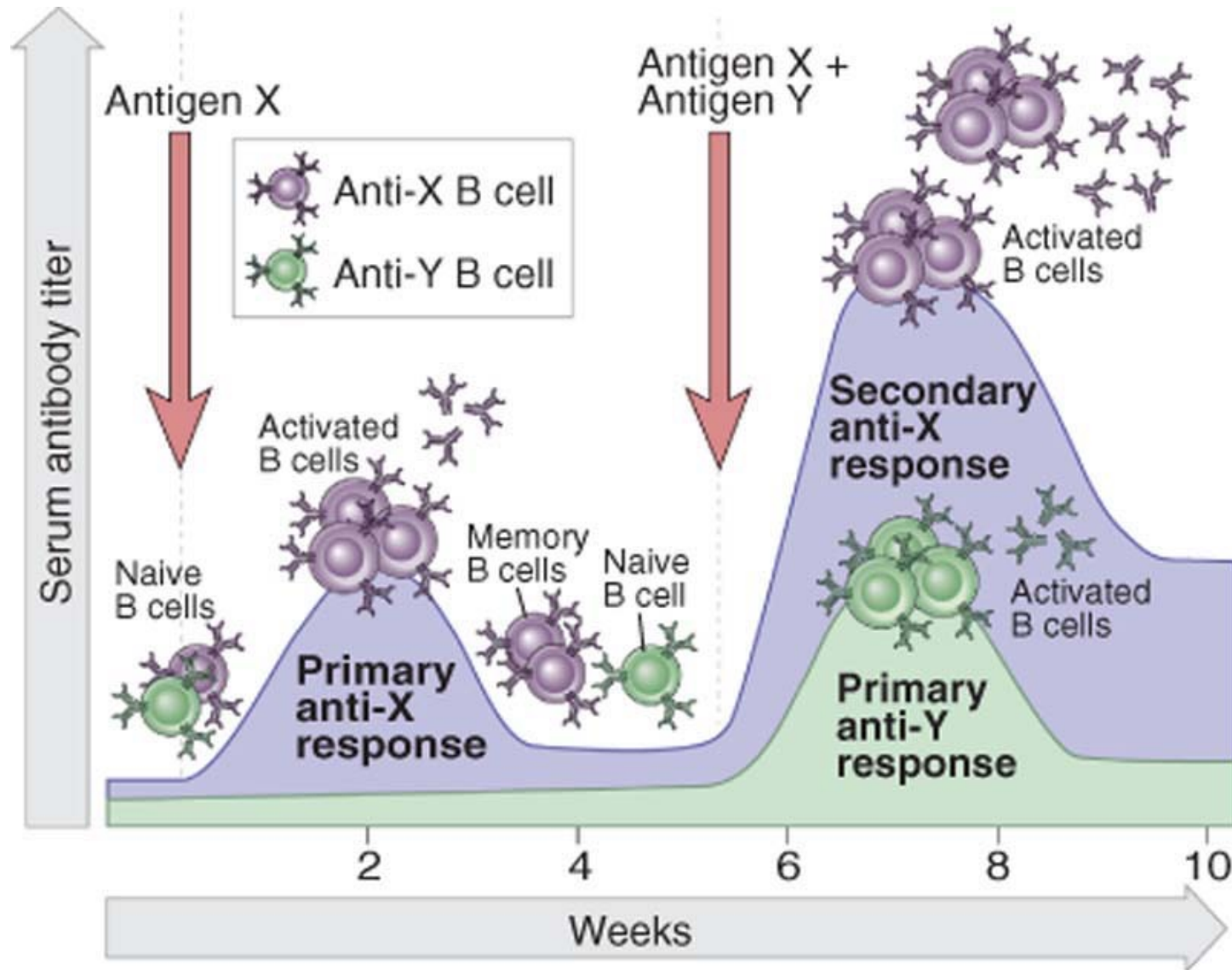
# Production of Monoclonal Antibodies



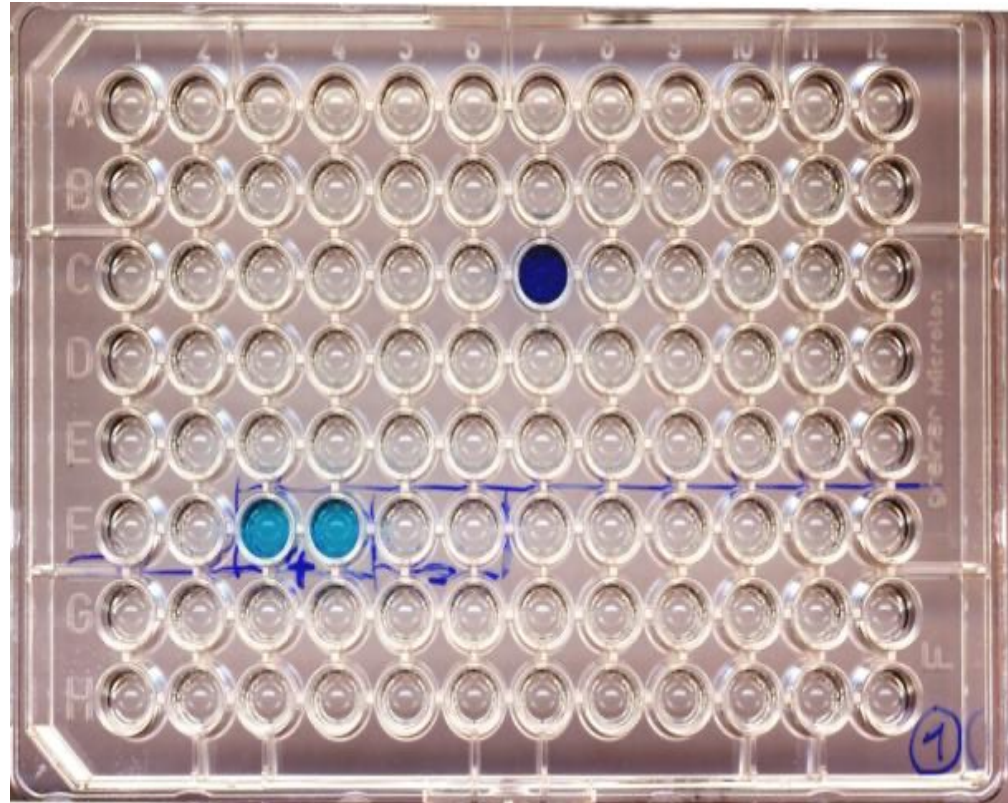
# Immunize the mice - Inoculation

- The mice are aseptically inoculated with the antigen combined with an adjuvant.
- Inoculations are done either **sub-cutaneously** or **intra-peritoneally**.
- Normal dose per mouse is between **20 and 100 micrograms** of protein.
- Inoculations are performed every 14 to 21 days.

# Timing for Ab production



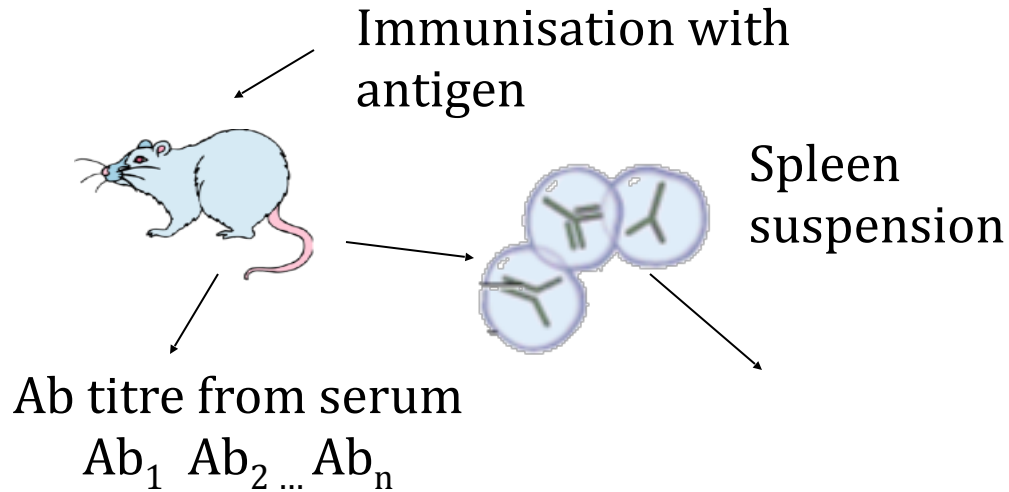
# Test the serum – In the lab



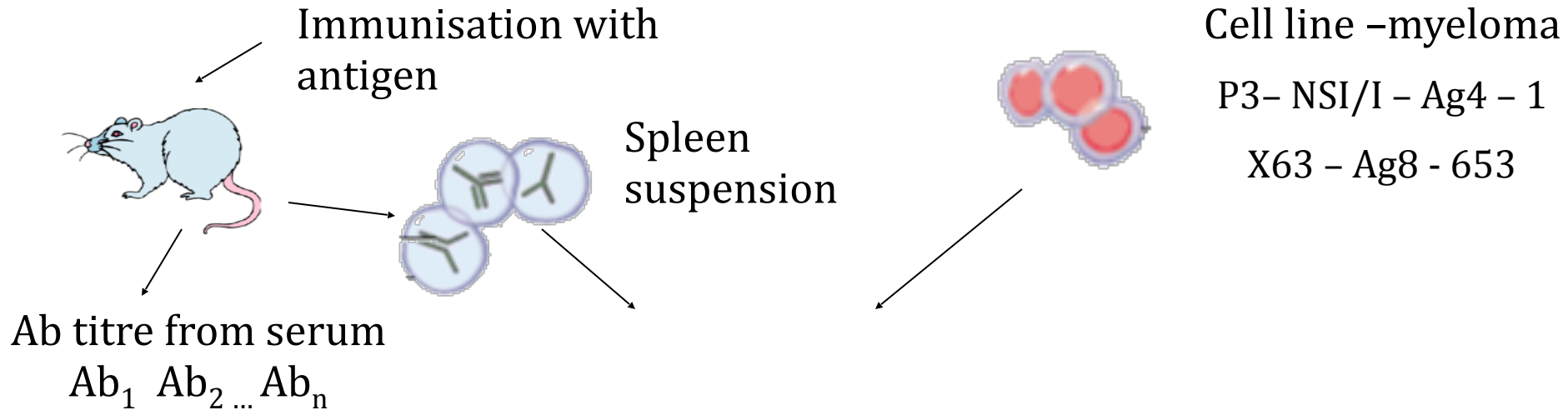
# Test the serum - Decision time

- When the serum titer of the mice has reached a plateau, an additional ELISA test is performed to determine the predominant *isotype* present. The two *isotypes* that are most common in mouse serum are IgG and IgM.
  - A *fusion* is done when the IgG level is high and the IgM level is low.
- Sometimes additional testing is done (Western blots, immunofluorescence) to determine whether the serum response is specific for the selected antigen.
  - The mouse with the strongest, most specific response is chosen for the fusion.

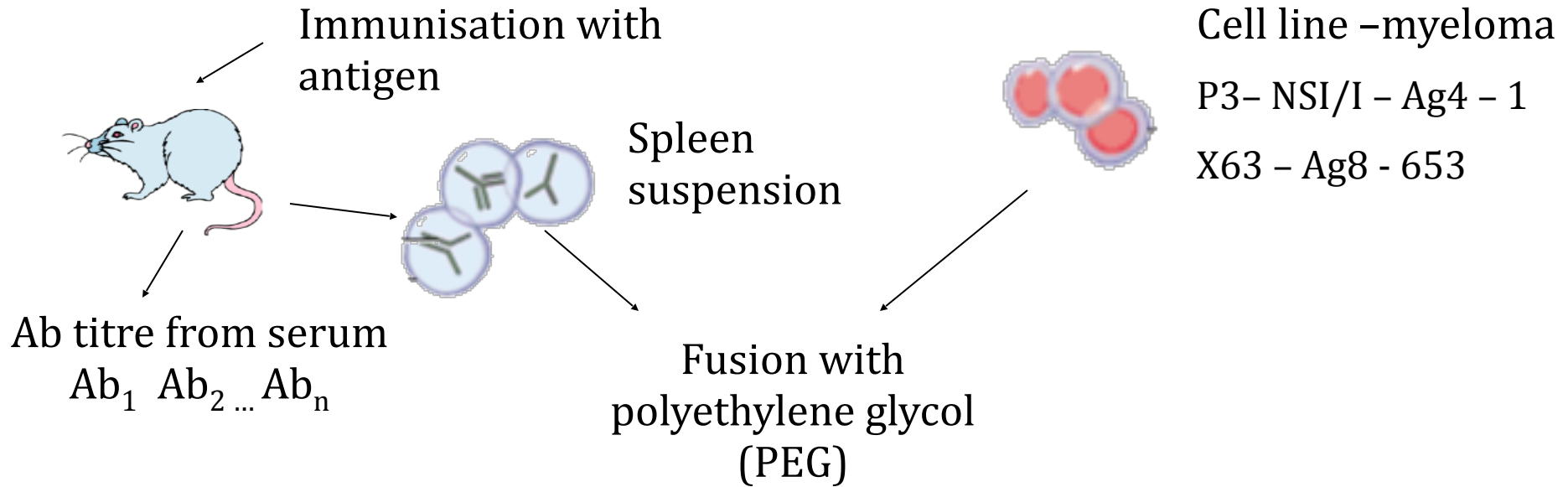
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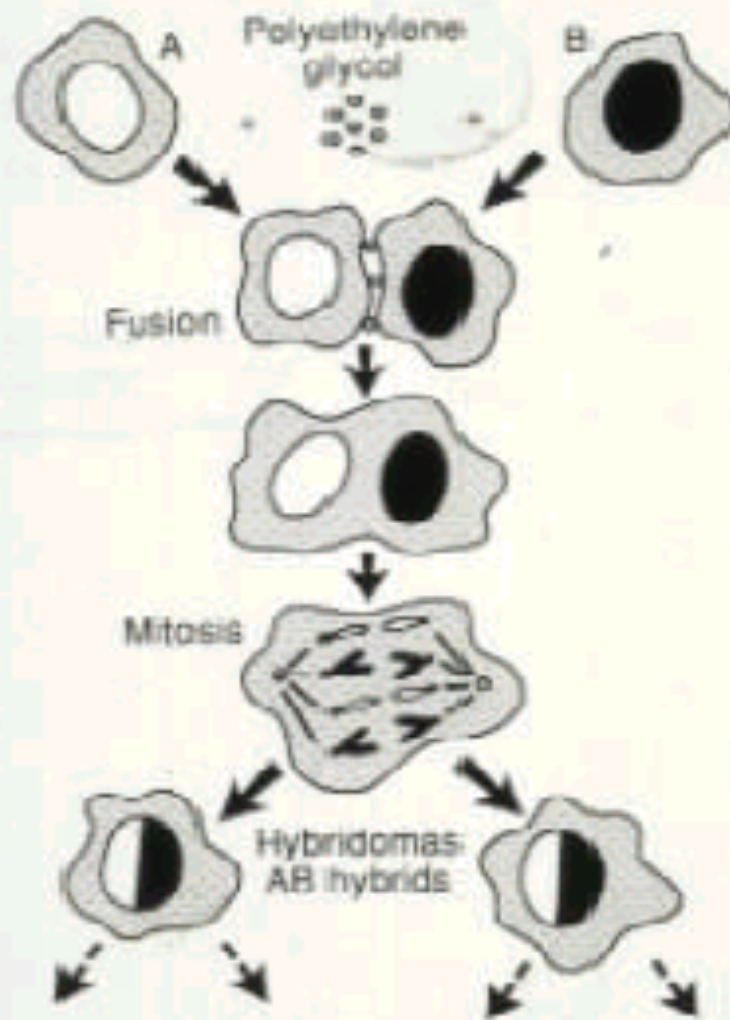


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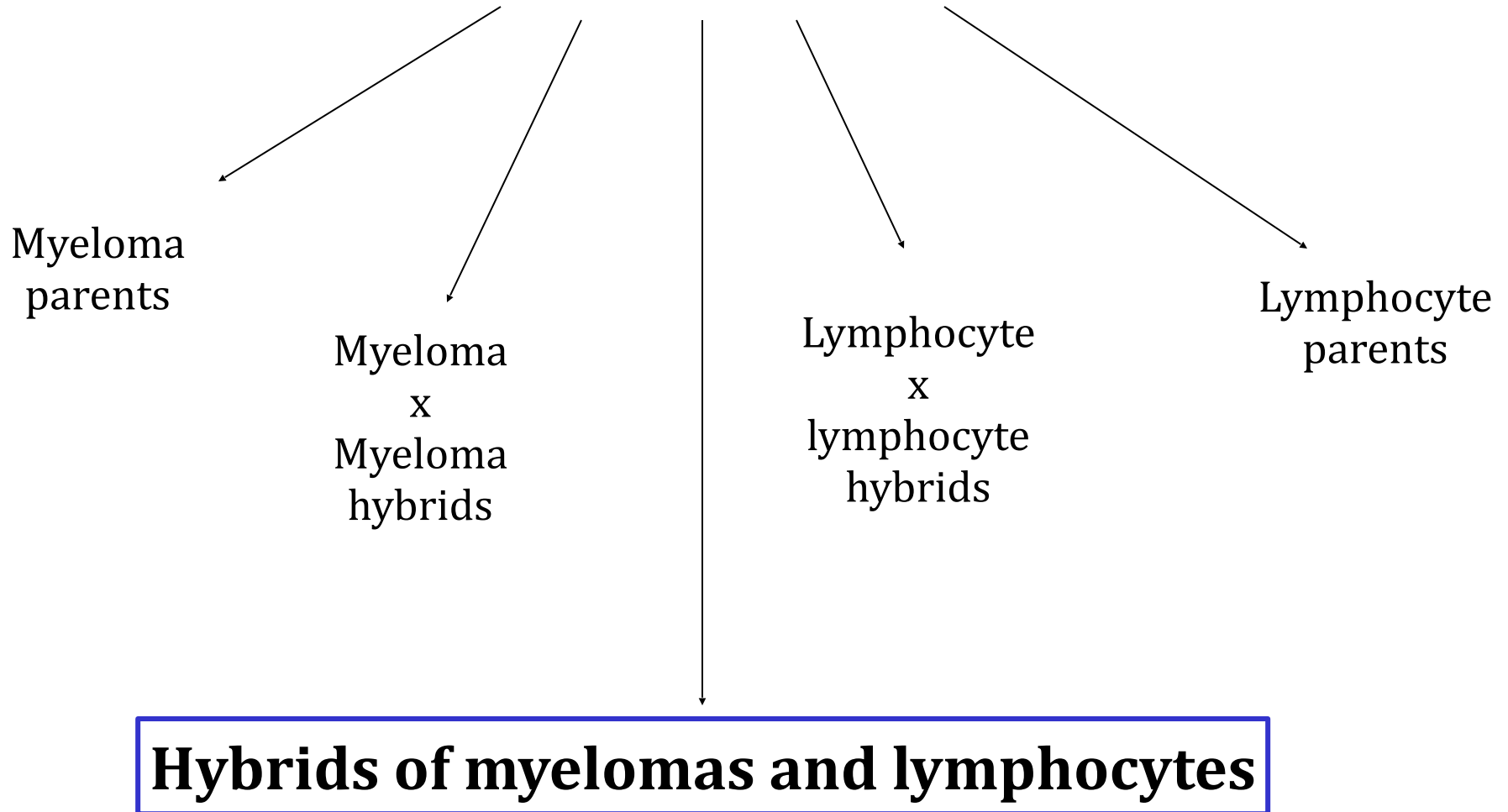


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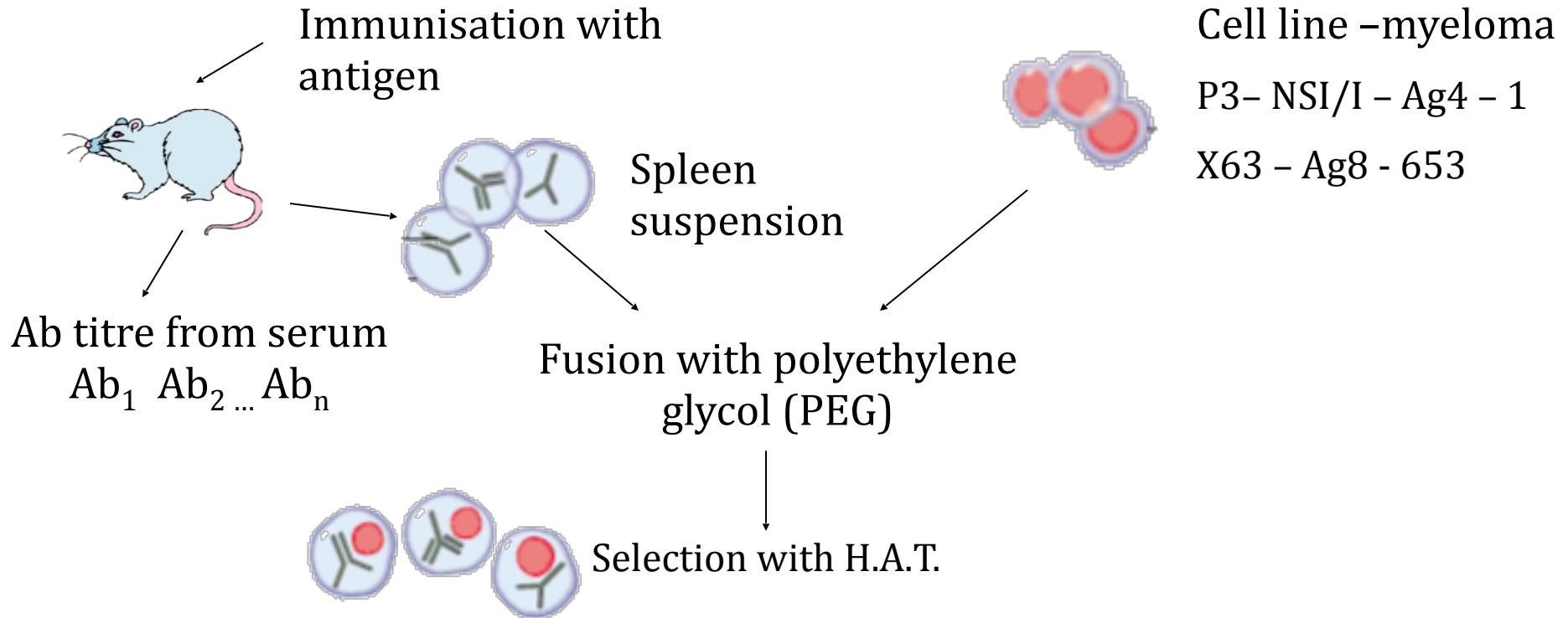
# Cell types present after fusion



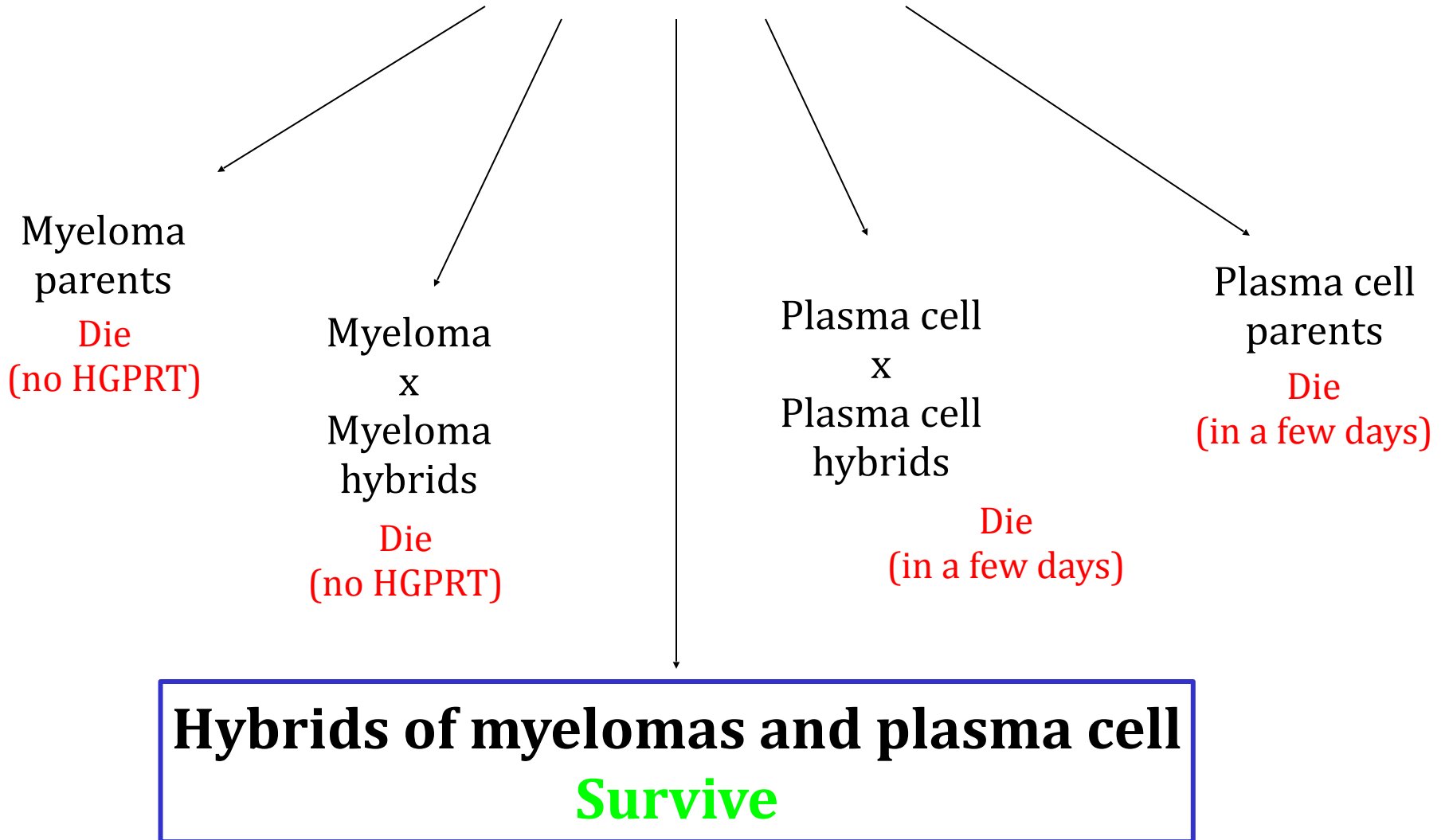
# What does HAT do?

- Myeloma cell fusion partners were selected for **the loss of the ability to synthesize hypoxanthine-guanine phosphoribosyl transferase (HGPRT)**
- HGPRT enables cells to synthesize purines using an extracellular source of hypoxanthine as a precursor.
- Normally, the absence of HGPRT is not a problem because cells have an alternate biochemical pathway (termed the rescue pathway) they use to synthesize purines. The rescue pathway allows myeloma cells to divide normally.
- **The rescue pathway is inhibited by aminopterin.** In the presence of aminopterin, HGPRT is essential for survival.
- HAT contains:
  - Hypoxanthine
  - Aminopterin
  - Thymidine
- HAT is selective for fused, (hybridoma) cells because:
  - Unfused myeloma cells cannot grow because they lack HGPRT
  - Unfused normal spleen cells cannot grow indefinitely because of their limited life span.
- In hybridomas the spleen cell partner supplies HGPRT and the myeloma partner is immortal because it is a cancer cell, overcoming the growth block of spleen cells.

# Production of Monoclonal Antibodies



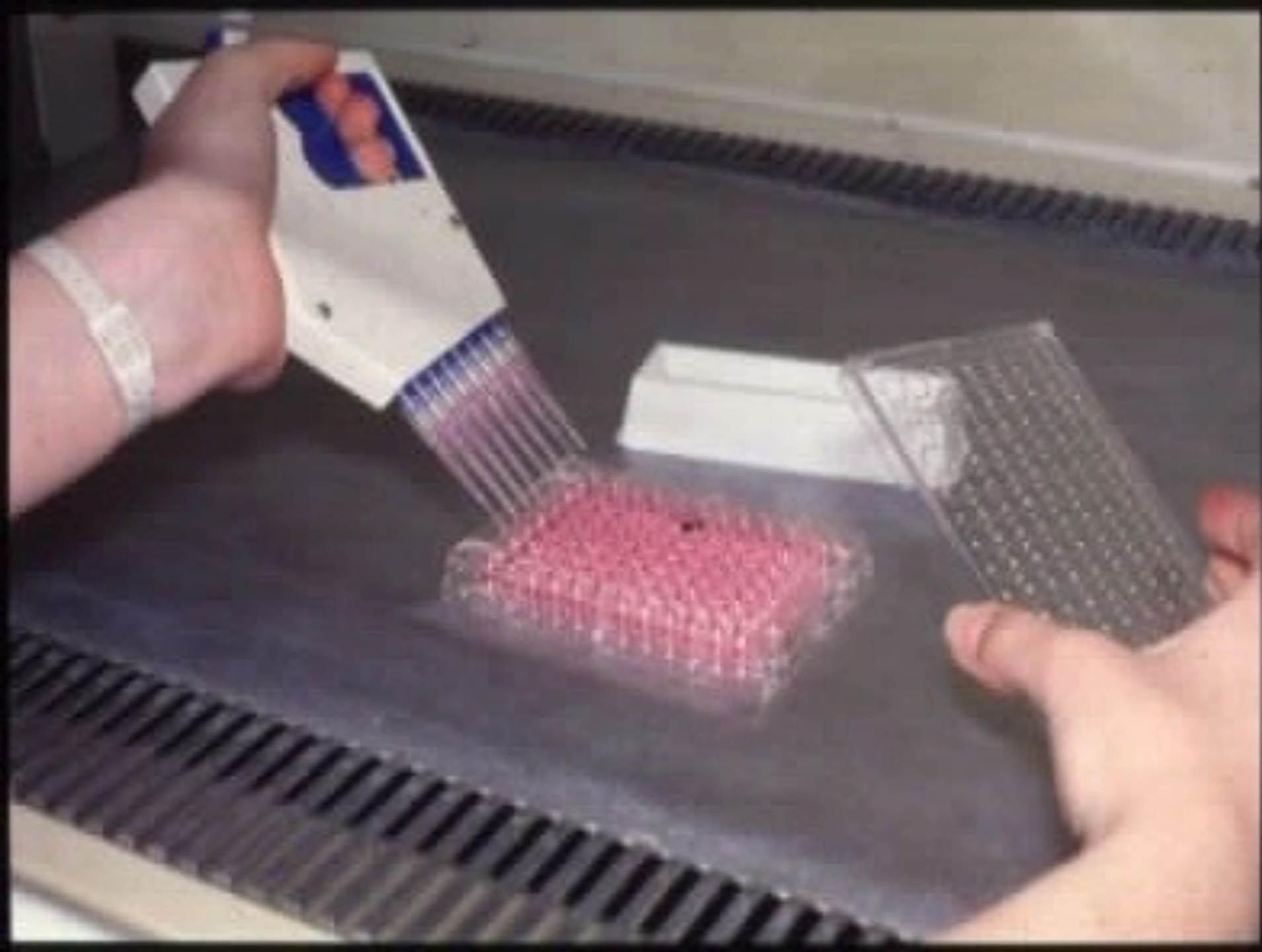
# Cell types present after fusion



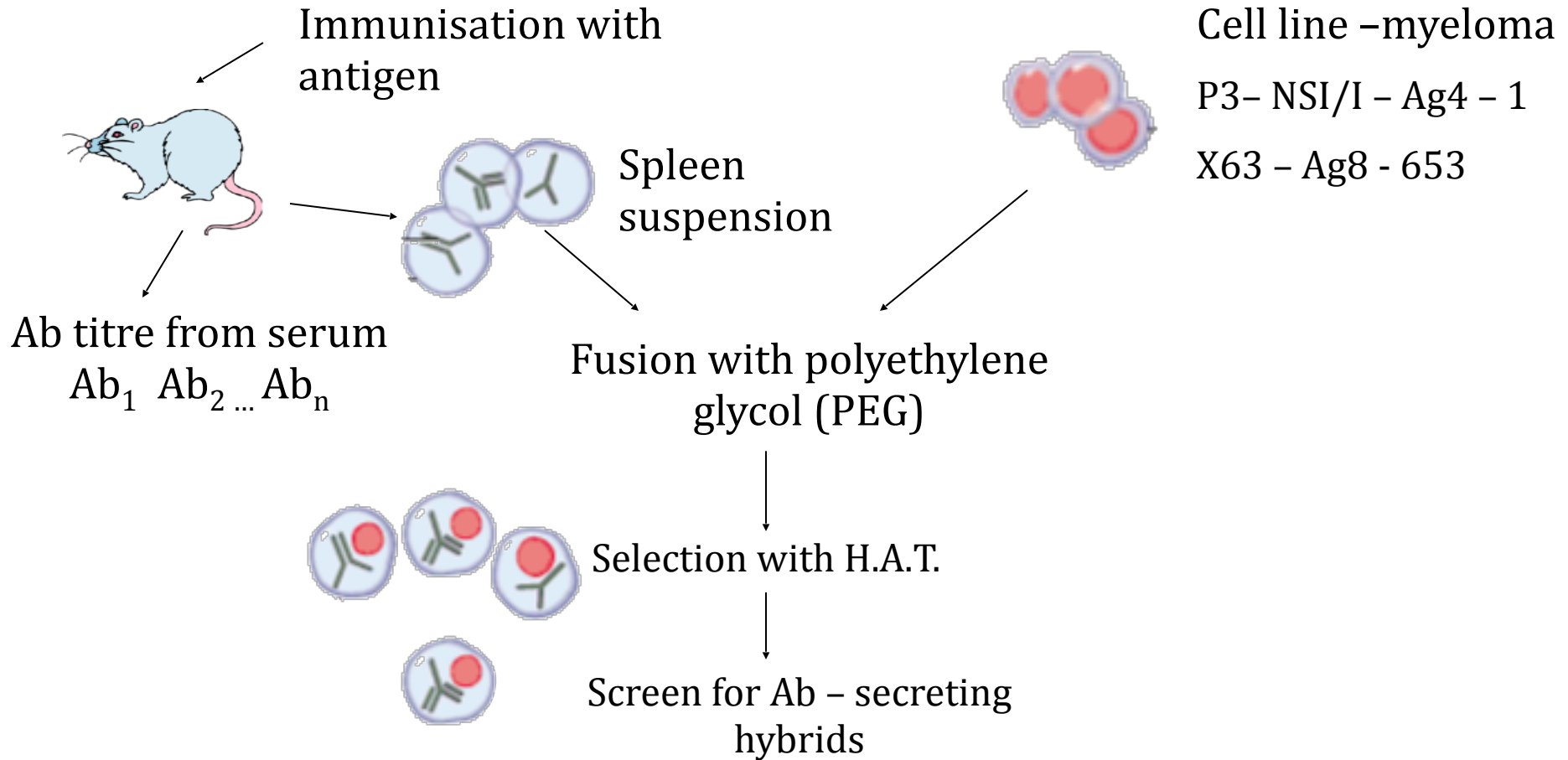
# Perform a fusion - Growing the cells (hybridomas)

- Cells are grown in a 37° C incubator.
- Cells are kept in an atmosphere of about 5% CO<sub>2</sub>.
- The cells are fed after 7 days of incubation.
- The cells are checked for growth after 10 days of incubation.

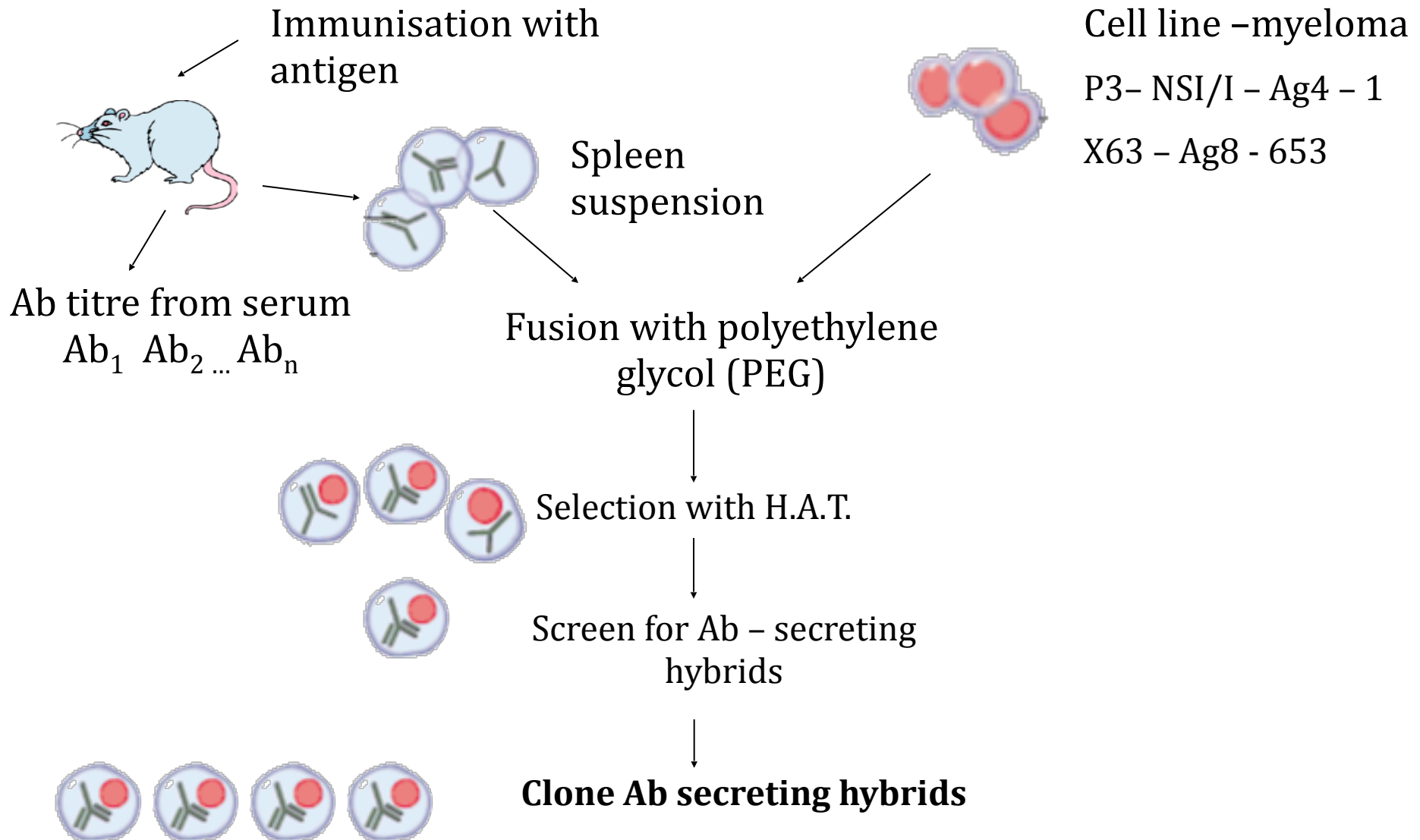




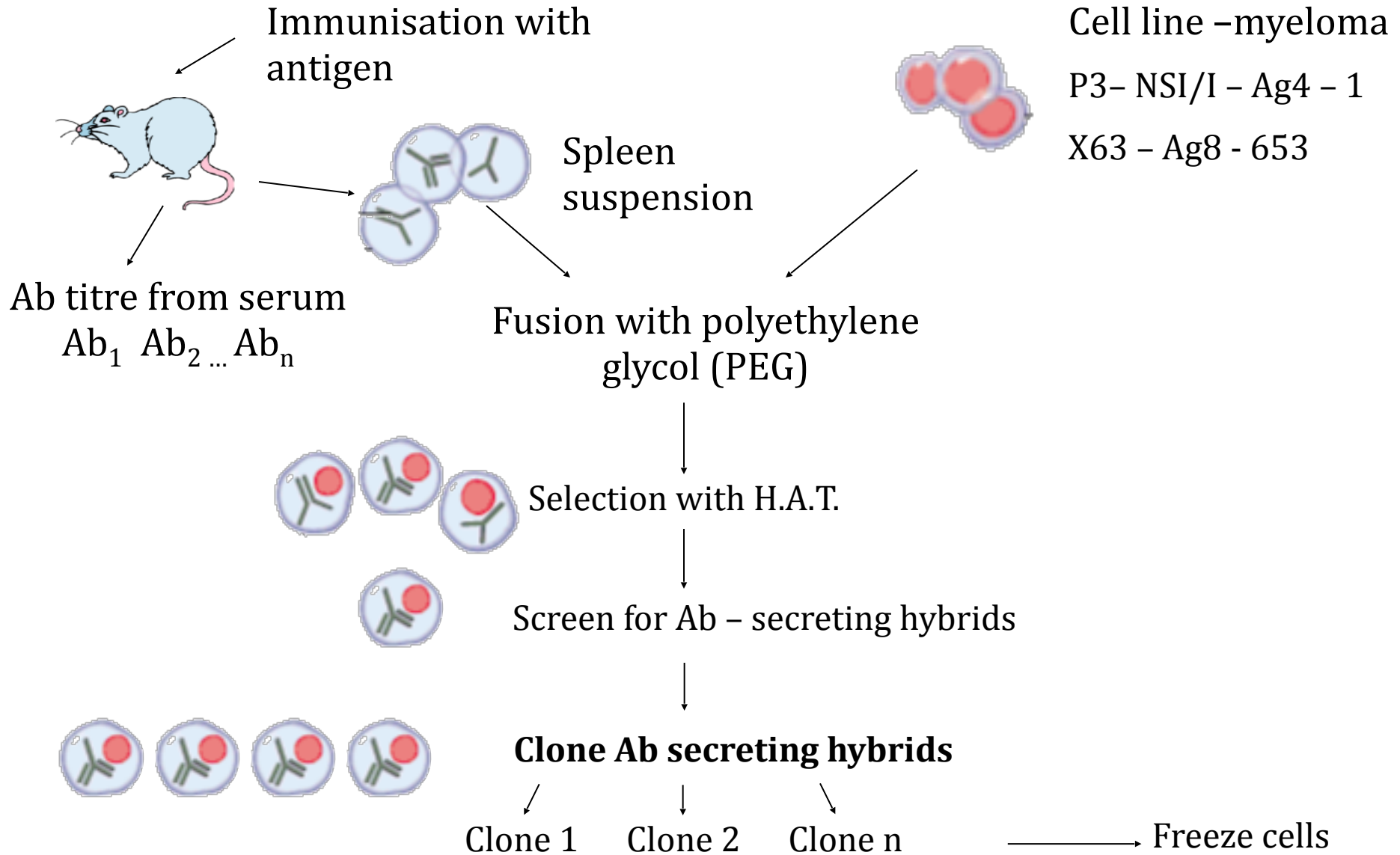
# Production of Monoclonal Antibodies



# Production of Monoclonal Antibodies

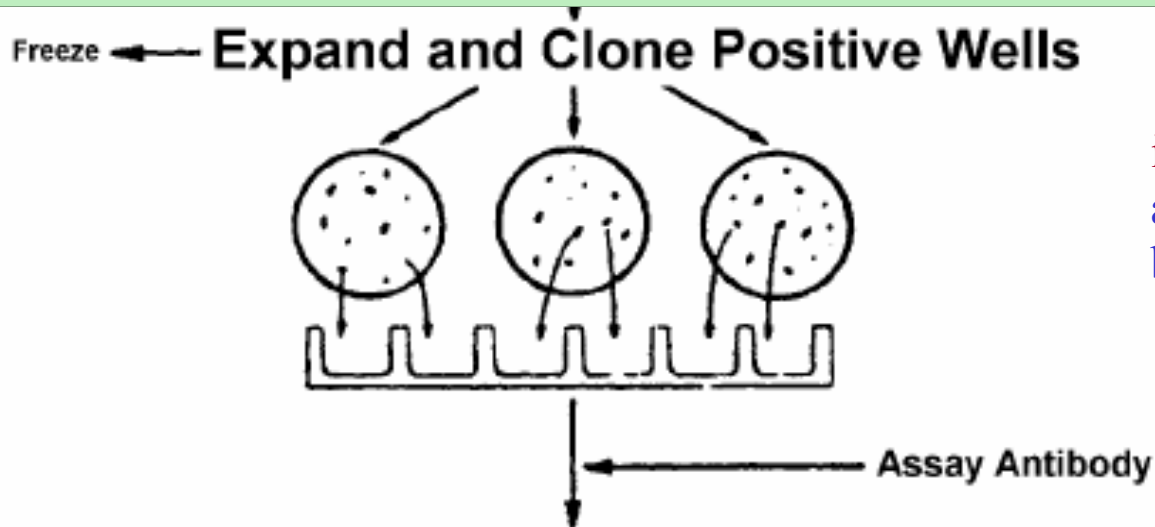


# Production of Monoclonal Antibodies





# Expand in mice or expand in vitro

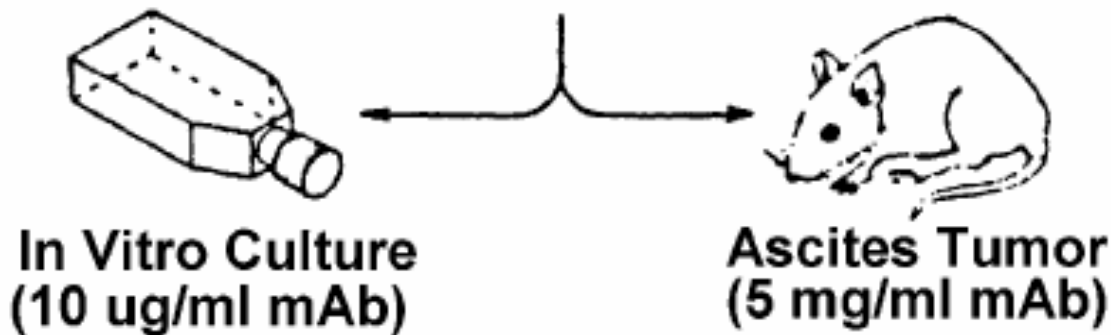


in vitro material is less concentrated  
and contains  
bovine serum

Freeze ← **Reclone Positives**

ascites fluid  
contain high [mAb]  
and minor contamination  
with mouse Ig

**Monoclonal Antibody Production**



# Advantages

1. Possible to select mAbs with the **required specificity**.
2. **Large quantities** of antibodies can be obtained easily.
3. **Pure antibodies** can be obtained more easily.
4. **Indefinite supply**.

# **Disadvantages of Monoclonal Antibodies**

1. Labour intensive.
2. Costly.
3. Longer time span.

# Roller bottles

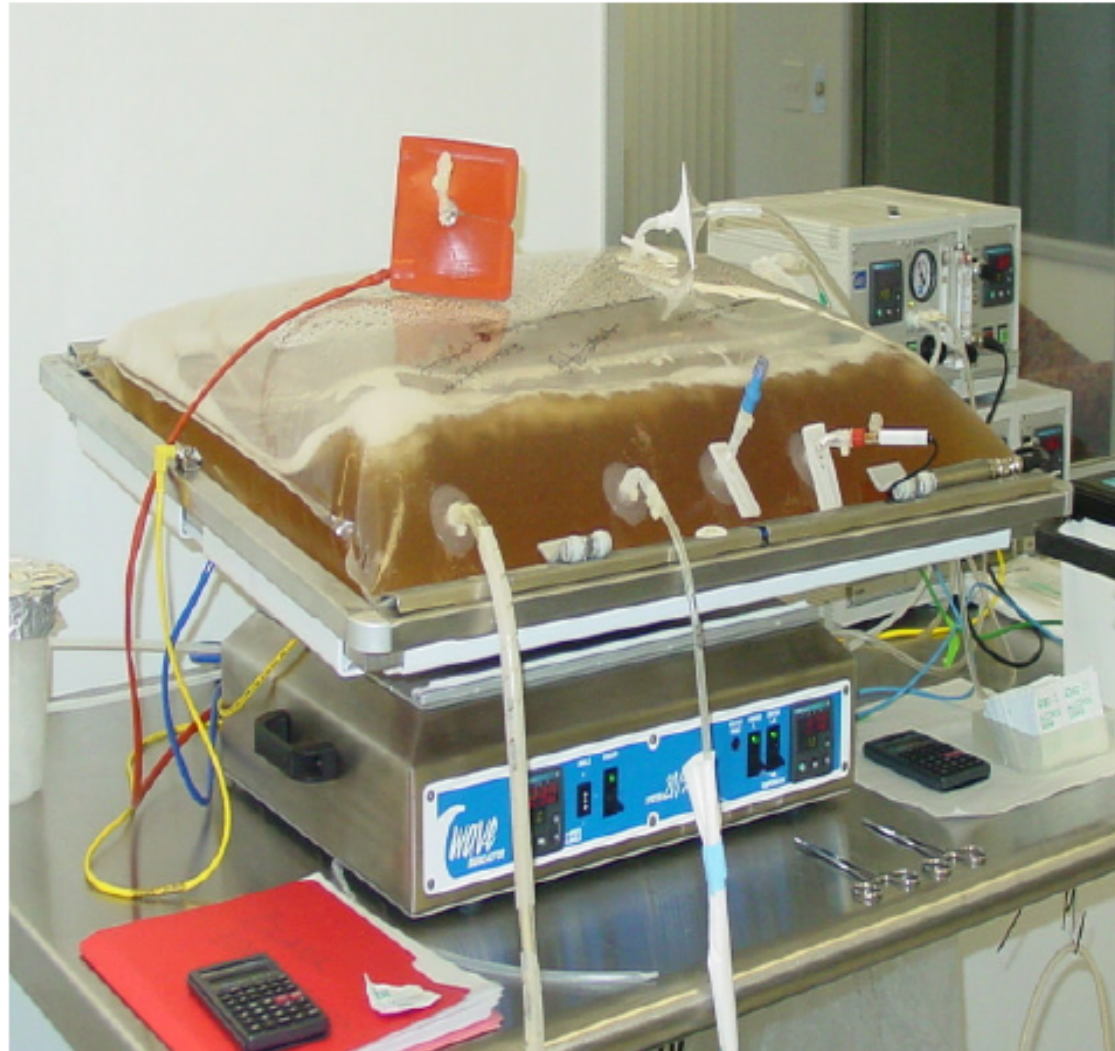
- Old technology, principally for attached cell culture
- Bottle rotates at 1-3 rpm
- Effective OTR related to rotation speed and operating volume
- Scalable by replication



# Bioreactors for Suspension Systems (examples)



# Disposable systems





1-25 L



10-100 L

<http://www.wavebiotech.com/>





20,000 liter mammalian cell fermentor - Lonza Biologics - Portsmouth, NH

## Cost

- Facilities cost \$20-50 million to build
- Antibodies cost \$500-1,000/gram
- High costs present challenges for reimbursement in chronic diseases

# High level production in mammalian cells: the math

Lonza (contract manufacturer) claims = **5.5 g/L yield in 24 days**

**30,000 L** reactor:

30,000 L. X 5.5 g/L = **165 kg in 24 days,**

x 12 = 1,980 kg/year = **2,000,000 g/year**

One mAb dose = 500 mg = **0.5 g**

2,000,000/0.5 = **4 million doses per reactor per year.**

6 doses per patient per year?

4,000,000/6 = **600,000** patients per year per reactor.

At \$10,000 per patient per year → **\$6B** in sales /per 30KL reactor

Not bad!!