

- antibodies produced by differentiated B-cells
- recombination results in B-cell lineages producing distinct antibodies
- serum contains antibodies representing many lineages
- difficult to isolate B-cells of defined specificity
- cannot grow B-cells in culture

Kohler and Milstien (1975)

- fused B-cells with myeloma cells → stable **hybridoma**
- hybridoma secretes **monoclonal antibody**

Monoclonal Antibodies

- **Advantages**
 - unlimited supply
 - defined reagent
- **Disadvantages**
 - single epitope
 - time consuming

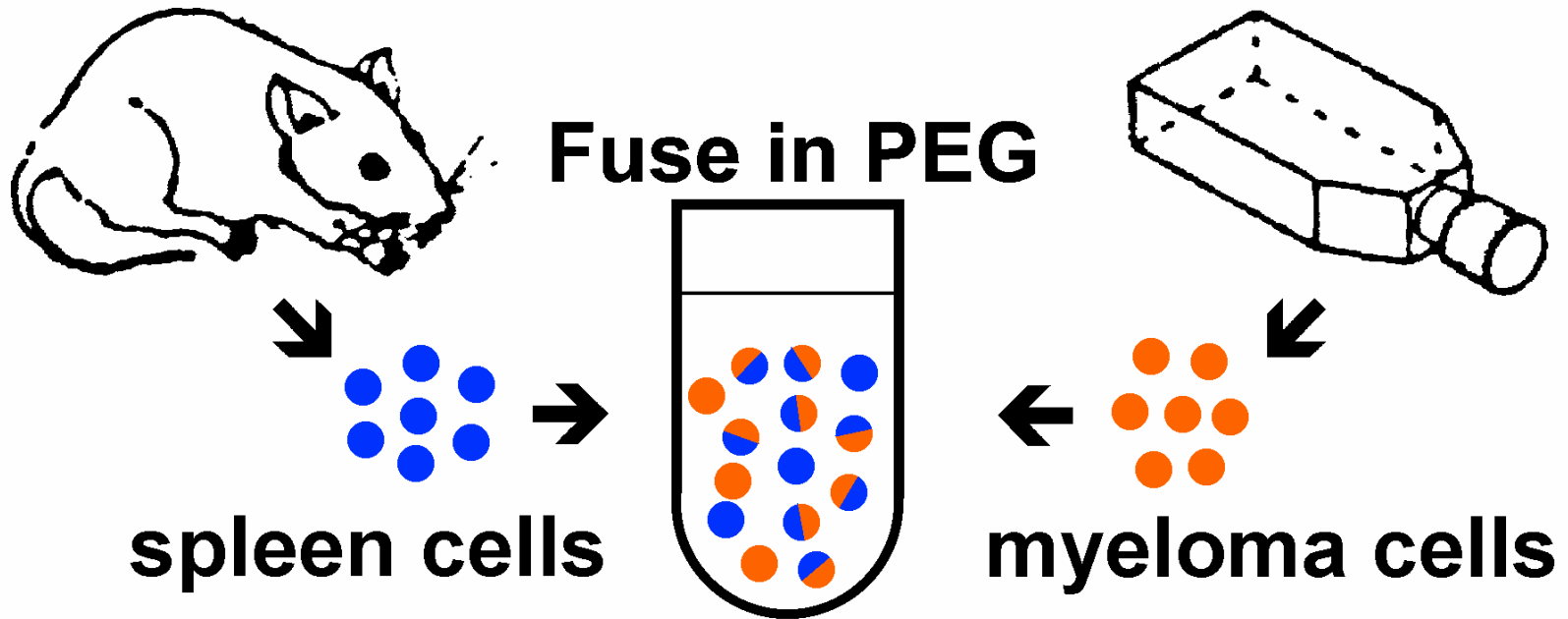
Preparation

- 1 Immunization*
- 2 Fusion
- 3 Selection
- 4 Screening & Cloning
- 5 Production

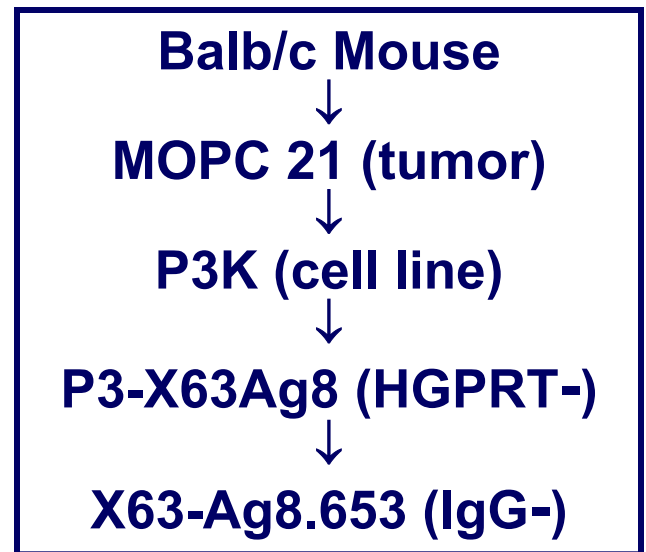
-
- **rodents, especially mice, are animal of choice**
 - best characterized myeloma lines are from mice
 - match strains for ascites production
 - **possible to use crude antigens**

Immunized Mouse

In Vitro Culture

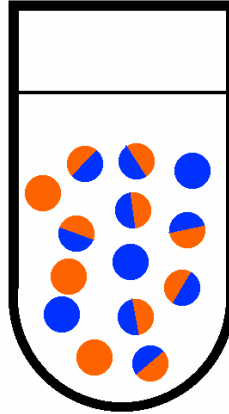


- mouse making desired antibodies
- boost 3-4 days before fusion
- remove spleen (lymph nodes etc) and harvest cells
- mix with myeloma cells in presence of fusogenic agent



HAT Medium:

- Hypoxanthine
(purine salvage)
- Aminopterin
(DHFR inhibitor)
- Thymidine
(pyrimidine salvage)



Nucleotide Metabolism:

- salvage and de novo pathways
- HGPRT essential for purine salvage
- DHFR essential for de novo synthesis

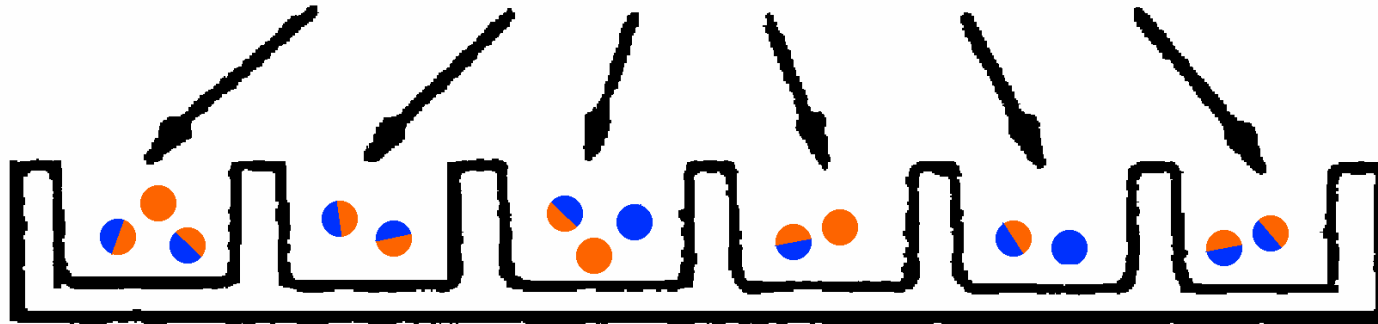




Plate in HAT Medium

HGPRT = hypoxanthine-guanine phosphoribosyl transferase
DHFR = dihydrofolate reductase


Selection in HAT Medium

Myeloma (tumor) Cell  +  Normal Lymphocyte

○ HGPRT deficient
■ immortal

● has HGPRT
□ no growth

Fusion



unfused myeloma



no HGPRT
dies in HAT

unfused lymphocyte



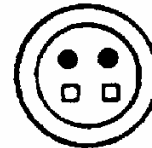
no growth

myeloma + myeloma



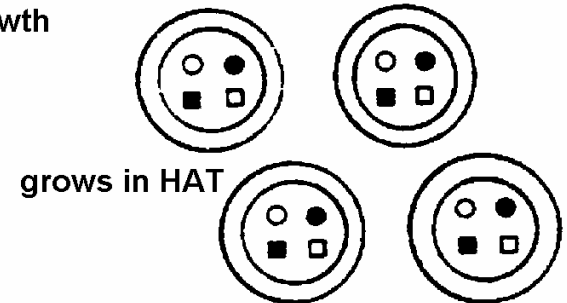
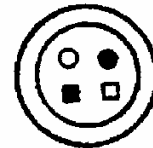
no HGPRT
dies in HAT

lymphocyte + lymphocyte



no growth

myeloma + lymphocyte



grows in HAT

Human mAbs produced by transforming lymphocytes with EBV

Screening/Cloning



↓ HAT Selection



Screening
(assay Ab)

↓ Clone Positives



Re-assay

↓
Re-Clone and Characterize Positives

Cloning Methods

- soft agar
- limiting dilution

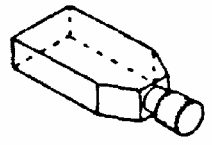
PREPARATION OF MONOCLONAL ANTIBODIES

Immunized Mouse



spleen cells

In Vitro Culture



myeloma cells

FUSION

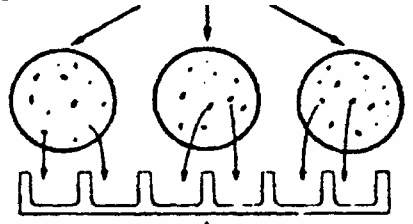


Selection of hybrids in HAT medium

Assay Antibody

Expand and Clone Positive Wells

Freeze

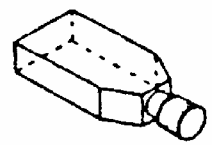


Assay Antibody

Reclone Positives

Freeze

Monoclonal Antibody Production



In Vitro Culture
(10 ug/ml mAb)



Ascites Tumor
(5 mg/ml mAb)

Production

- produce mAbs in vitro or with ascites
- harvest culture media (supernatant)
 - in vitro material is less concentrated and contains bovine serum
- ascites are tumors grown in peritoneal cavity
 - ascites fluid and sera contain high [mAb]
 - minor contamination with mouse IgG

Affinity Purification

- use Ag or epitope to make affinity column
- recover Abs from blots, etc.

