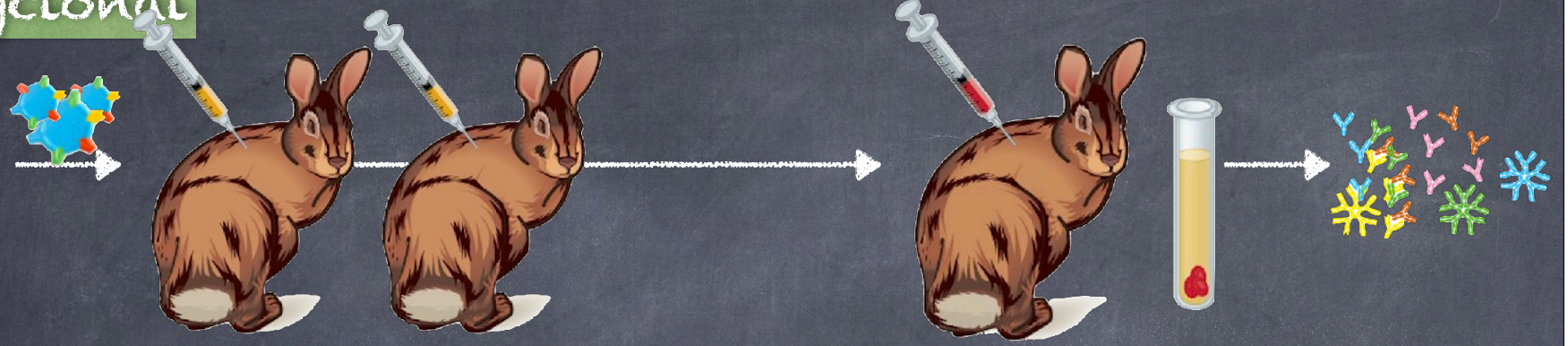
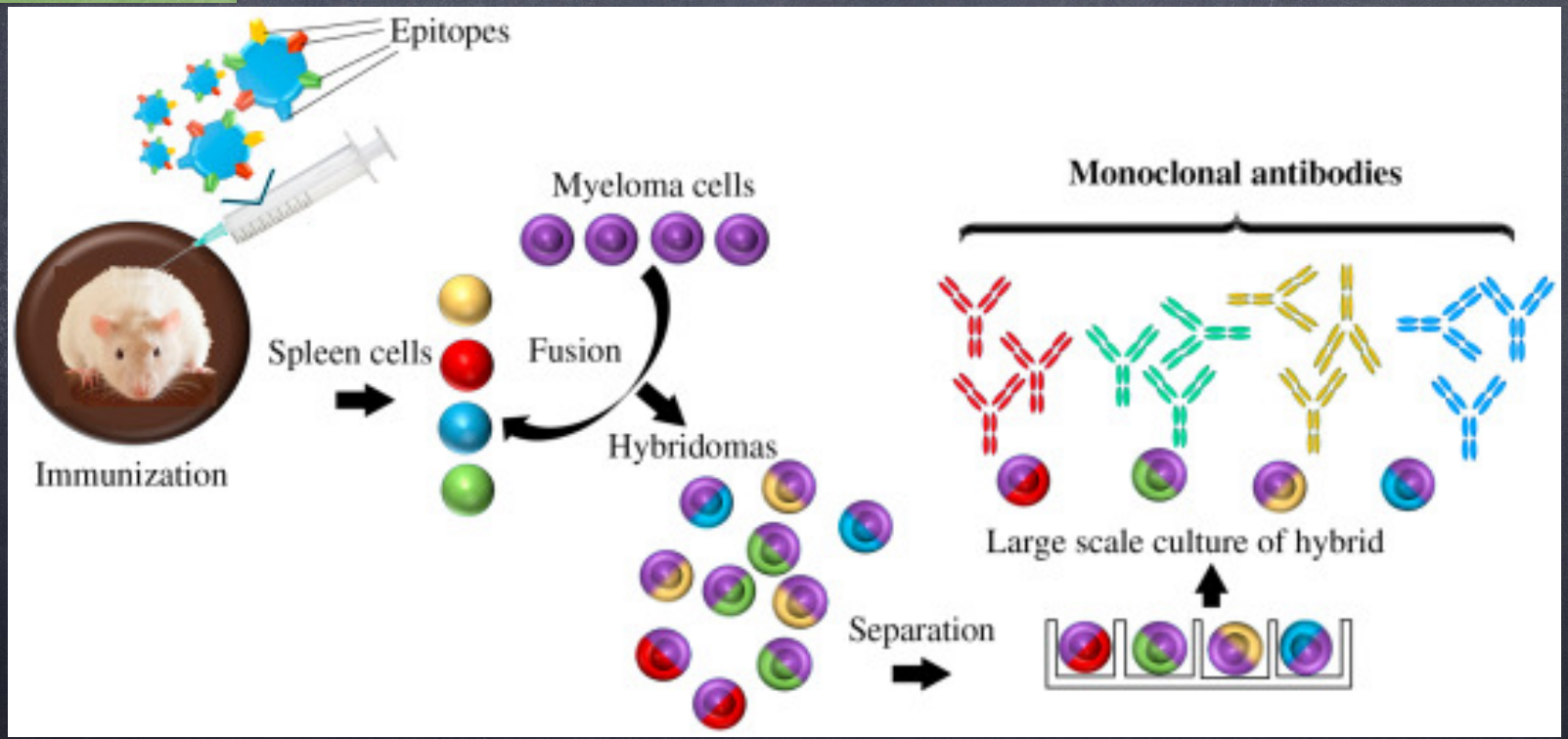
Monoclonal antibodies

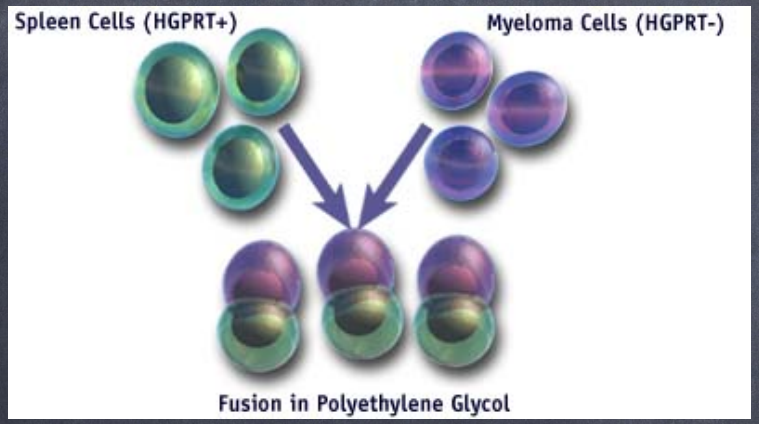
Polyclonal

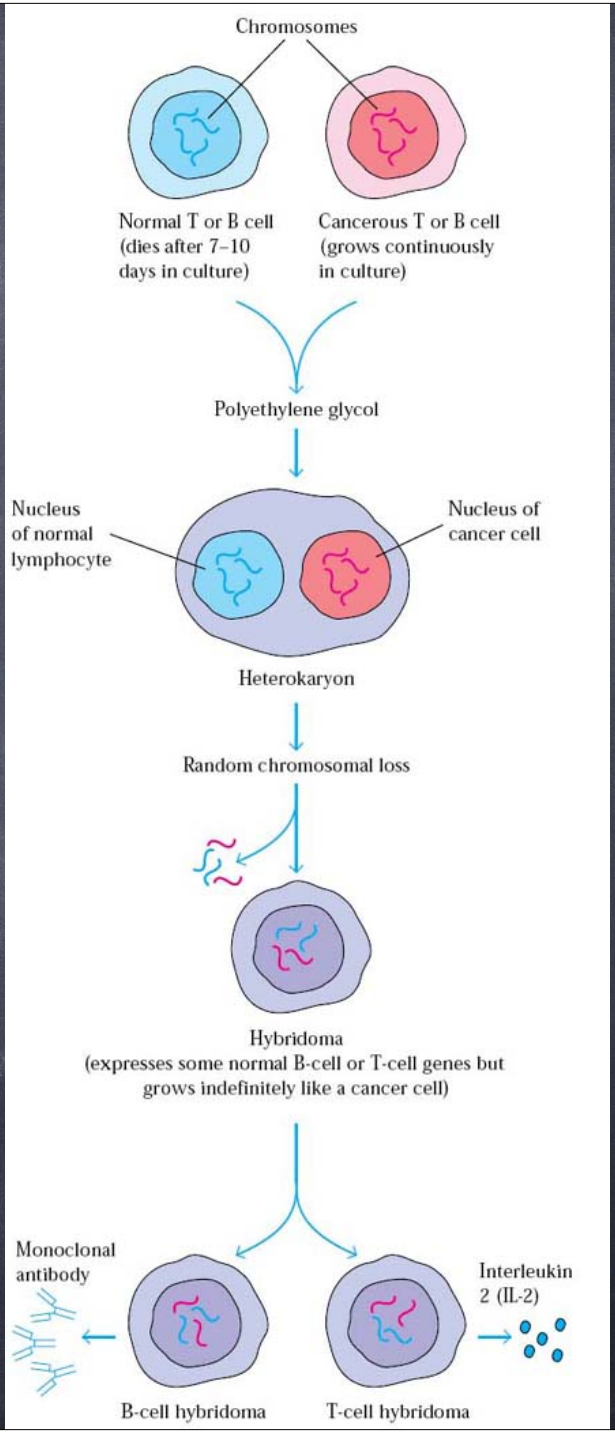


Monoclonal

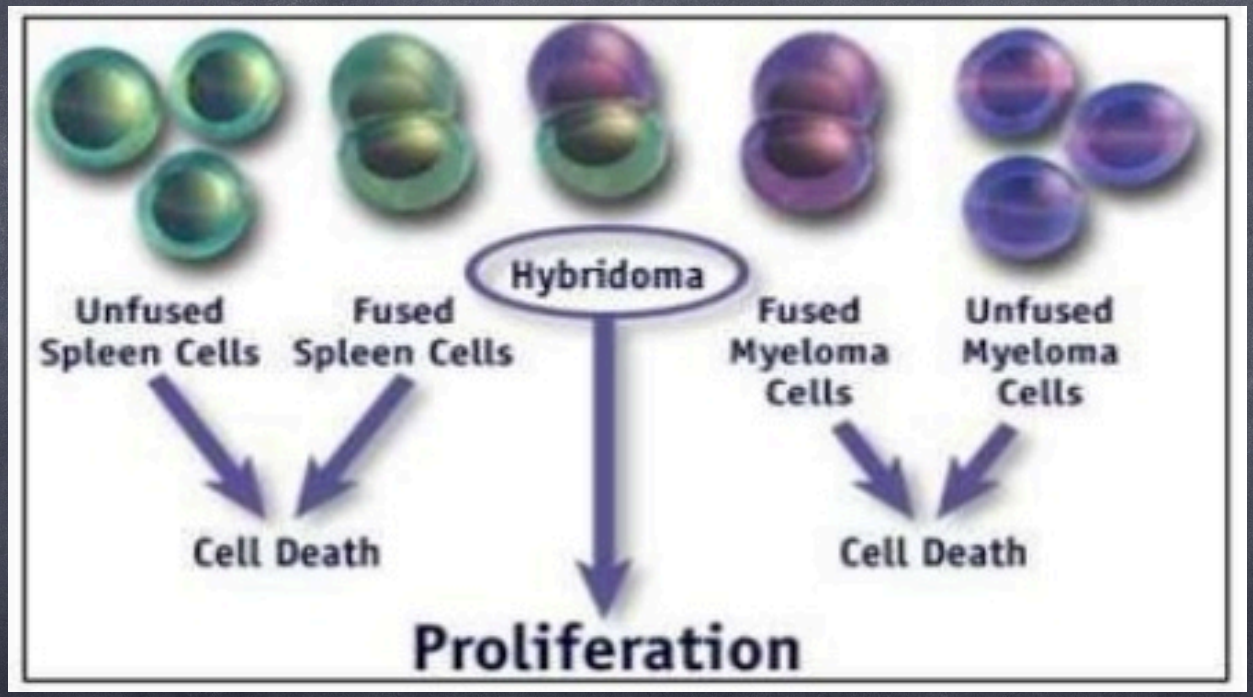


Spleen cells and myeloma cells fusion promoted by PEG





Culture conditions must lead to parental cell death and hybridoma proliferation



HAT selection

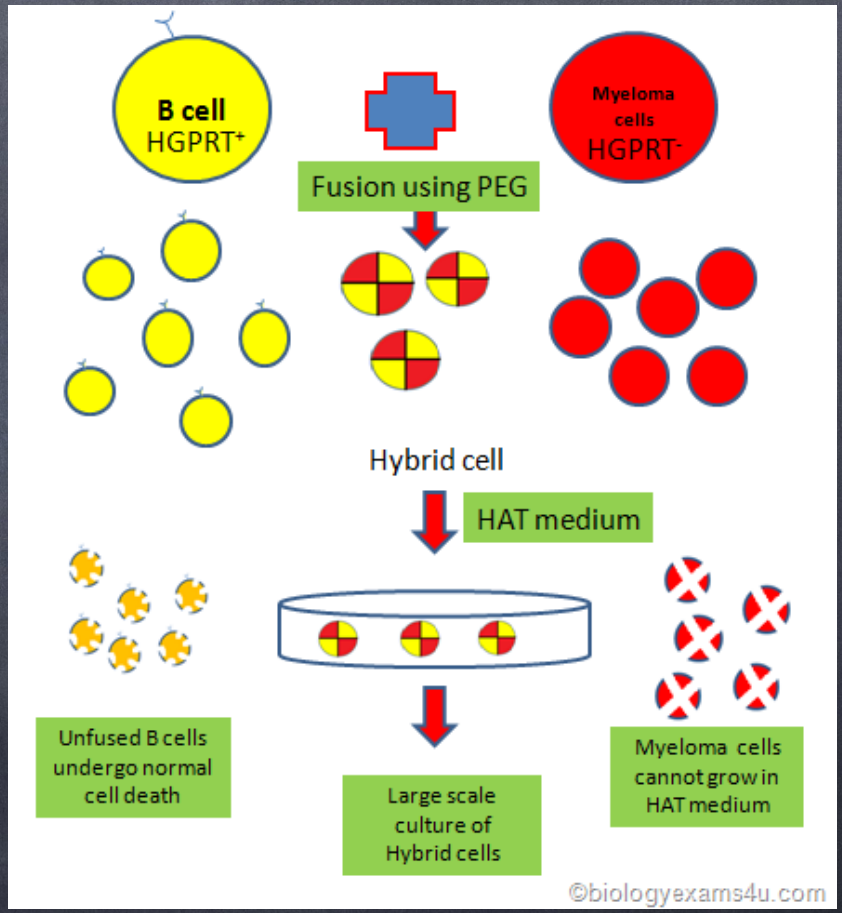
HGPRT- mutant myeloid cells

Hypoxanthine-guanine phosphoribosyltransferase -mutant myeloid cells

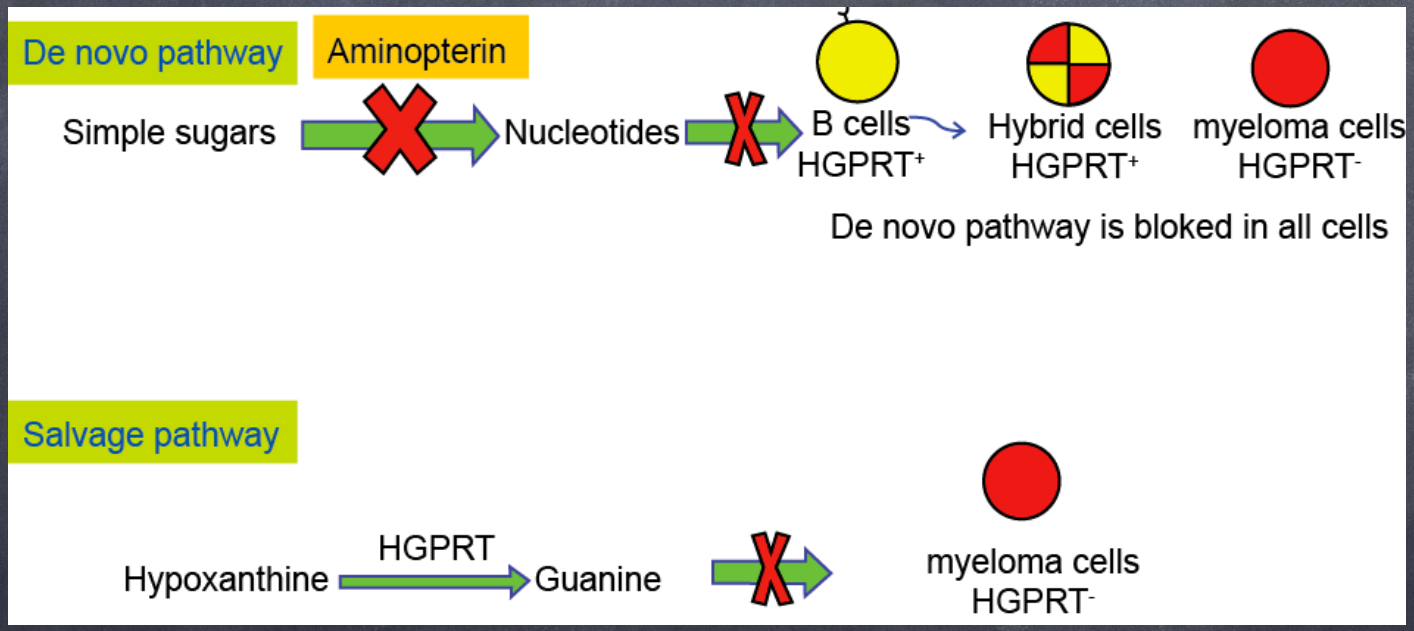
PEG: Polyethylene glycol

HAT medium contains:

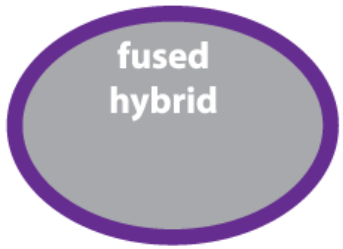
- Hypoxanthine
- Aminopterin
- Thymidine



Molecular basis of hybridoma selection in HAT medium



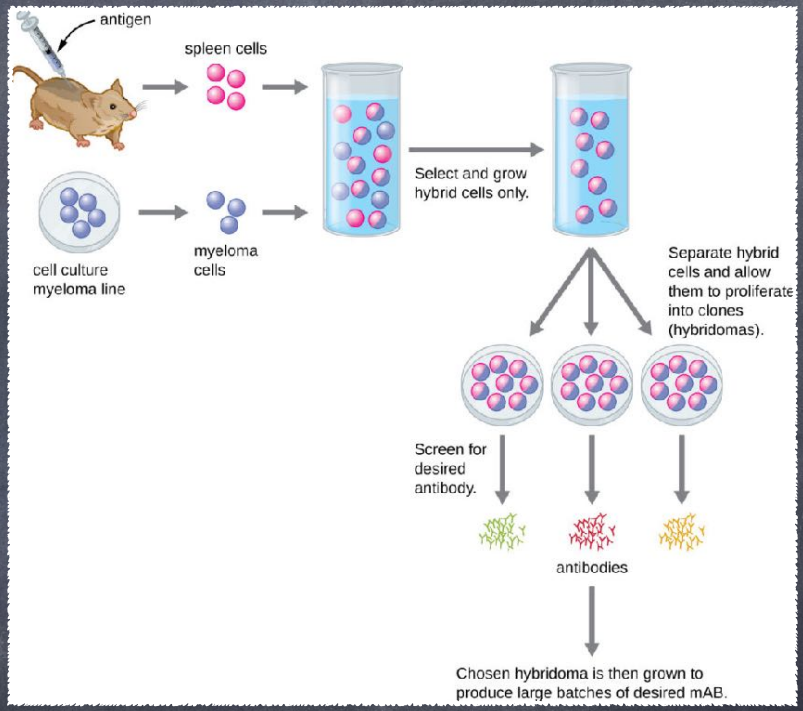
Alternative mutant myeloid cells

	TK -	TK+/TK -	TK +
Genotype:*			
Cell type:			
HAT fate:	**DIES**	**SURVIVES**	**DIES**
Explanation:	**Unable to synthesize DNA:** (1) Thymidine kinase* mutation causes a loss-of-function in the "salvage" pathway and (2) Aminopterin blocks "De novo" pathway.	**Immortal and restored DNA synthesis:** (1) Immortality from plasmacytoma and (2) rescued ability to synthesize DNA due to restored thymidine kinase* function.	**Mortal:** (1) Functional DNA synthesis, but (2) eventually dies because of limited number of replication cycles

**HGPRT (hypoxanthine-guanine phosphoribosyltransferase) mutants can be used in place of TK (thymidine kinase) mutants*

Monoclonal antibody production flowchart

- No needs for antigen purification
- Requires myeloma cells
- HAT selection
- Screen for the desired clones



Western Blotting

also called immunoblotting

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Scientific questions:

- Protein Molecular Weight?
- Expression level?
- Activation or Modification state?