



Antibody reagents: new advances, new immunological insights

Background

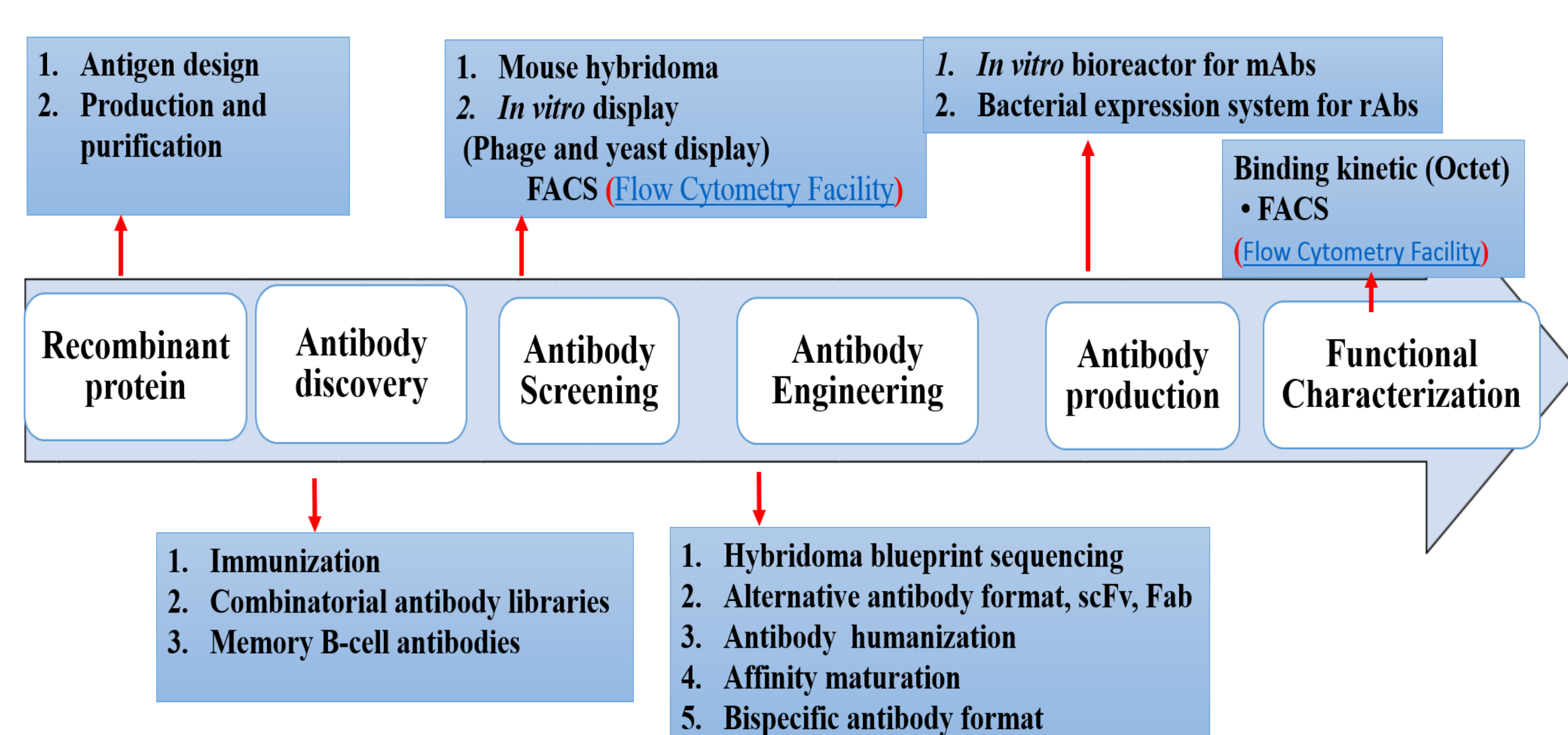
The Antibody Engineering and Technology (AbET) core is the antibody research core in the School of Medicine and University of Virginia Cancer Center. The AbET core provides current technologies and tailored solutions for antibody discovery and antibody engineering for research, development of novel biomarkers, diagnostics and therapeutic applications. The main focus of the core is to generate novel high-affinity monoclonal antibodies and provide techniques to engineer antibodies and related molecules.

AbET core mission is to provide investigators access to current antibody engineering technologies to create the future's most powerful molecules at the University of Virginia as well as research institutions and biomedical companies outside the University community.

Antibody reagent

- Antibodies are essential affinity reagents in basic research, biotechnology, and diagnostics as well as the fastest growing class of therapeutics.
- Recombinant antibody libraries pools containing billions of unique antibodies:
 - More effectively isolate affinity reagents that recognize antigens of interest,
 - The sequence of each antibody can be decoded by sequencing of the linked DNA,
 - The selections are performed under controlled, *in vitro* conditions that can be tailored to suit the demands of the antigen.
- Recombinant methods continue to evolve improve, and new applications for recombinant antibodies:
 - An alternative antibody format platforms,
 - Recombinant antibodies are also modular, because they can be fused genetically to additional domains to endow additional functions, such as fluorescence to track protein localization and trafficking.
- By combining vast stores of genomic data based antigens with combinatorial antibody libraries, it is possible to target the proteome with a comprehensive set of recombinant affinity reagents that would enable:
 - The rapid elucidation of protein function,
 - The identification of key protein-protein interactions that are involved in disease states such as biomarkers.

Value added services



<https://med.virginia.edu/antibody-engineering-technology/>

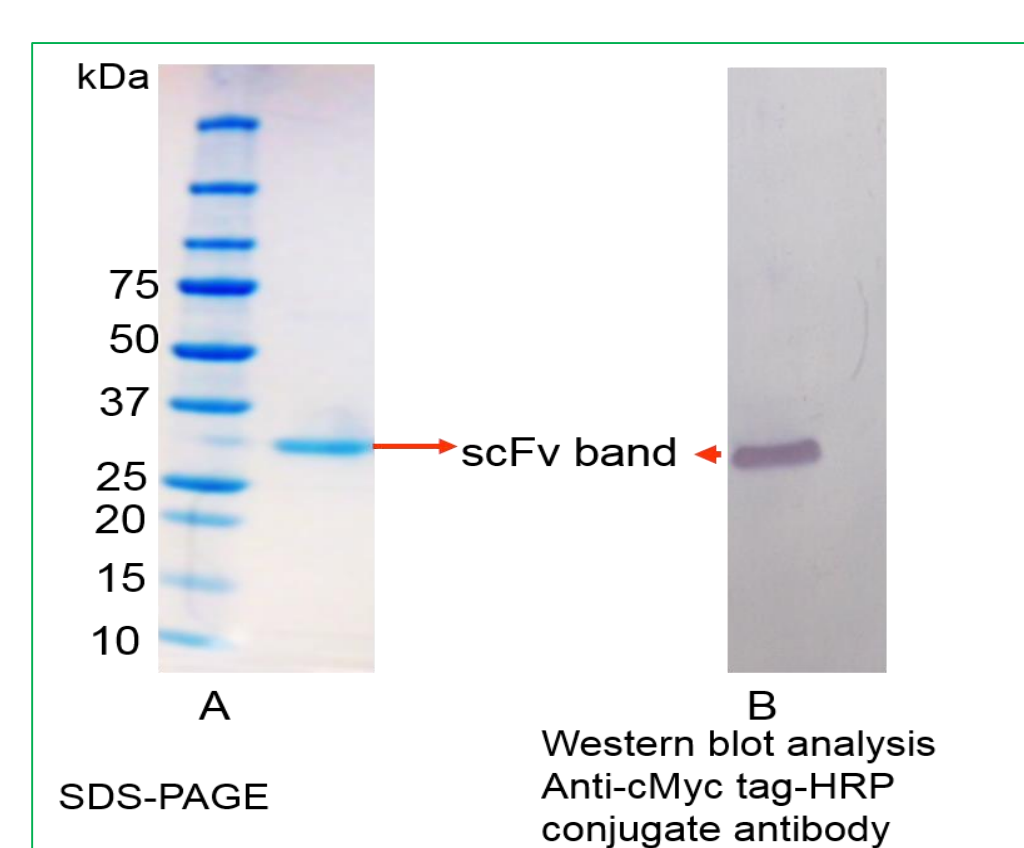
Antibody production and purification



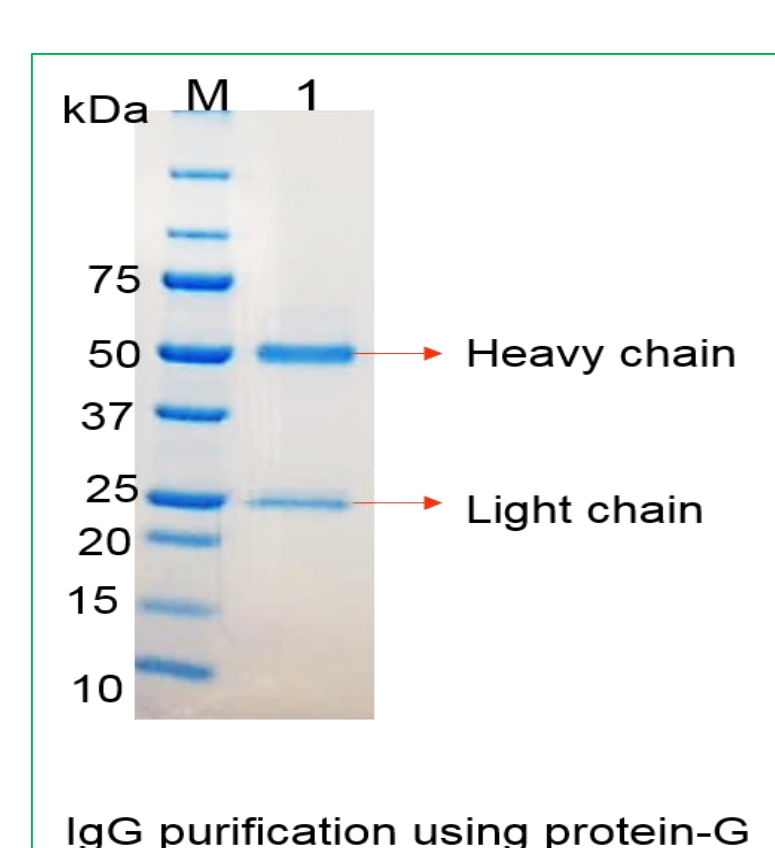
Bioreactor



FPLC



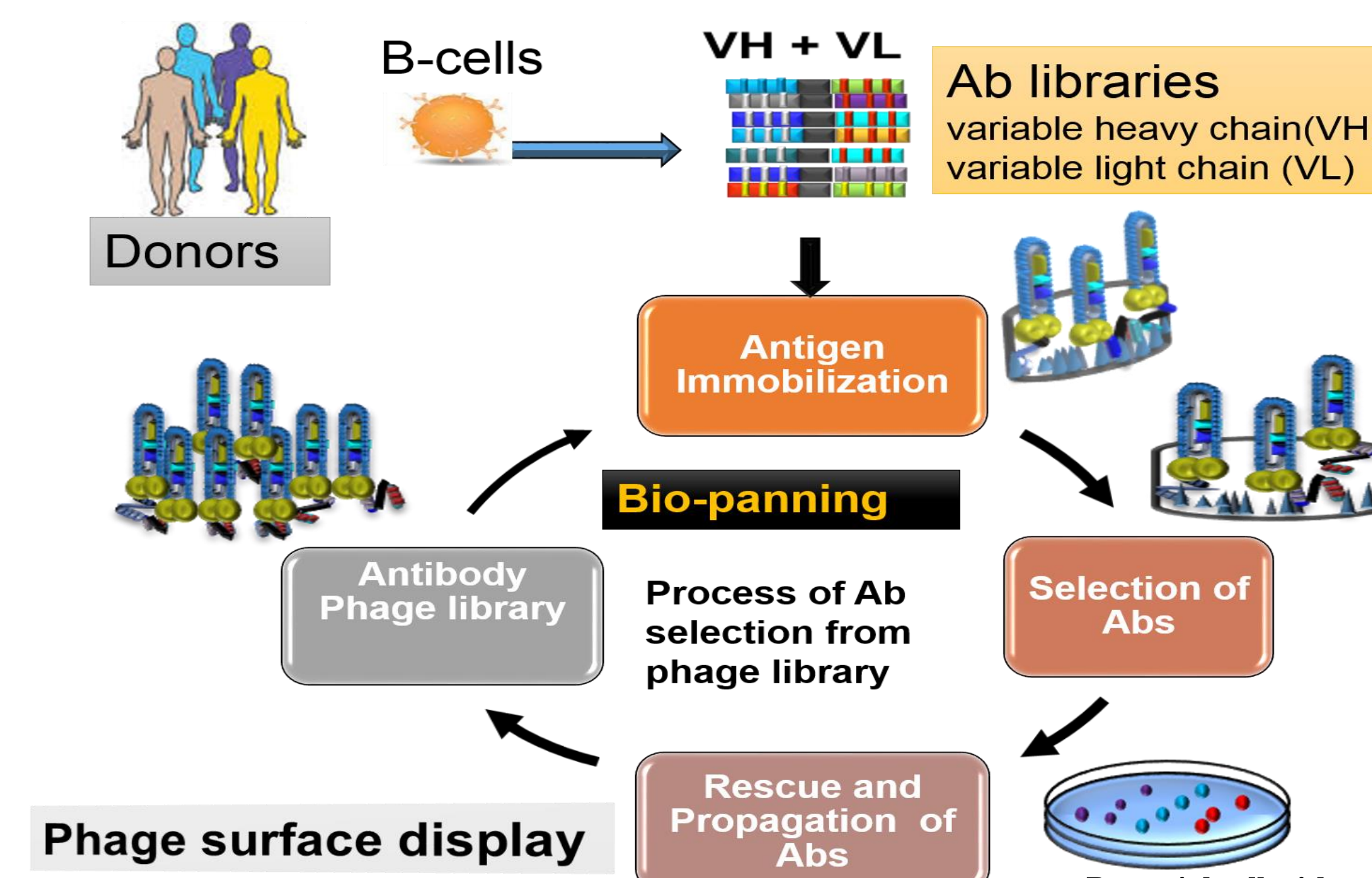
Recombinant antibody fragment



Antibody discovery

Combinatory antibody libraries

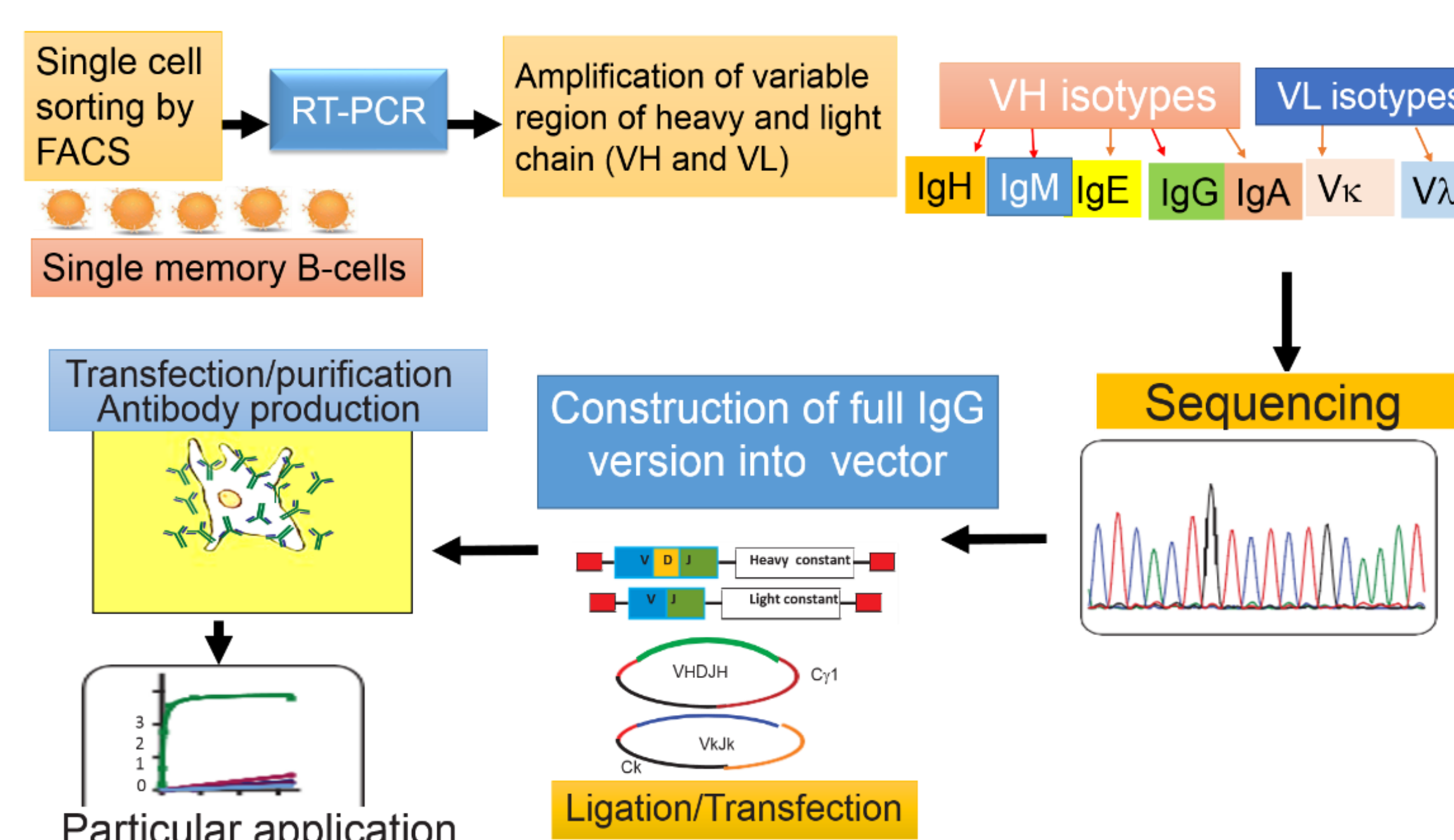
In vitro immune repertoires and selection methodologies that can be used to derive antibodies with or without the direct immunization of a living host.



Overview of antibody library production and selections using phage display.

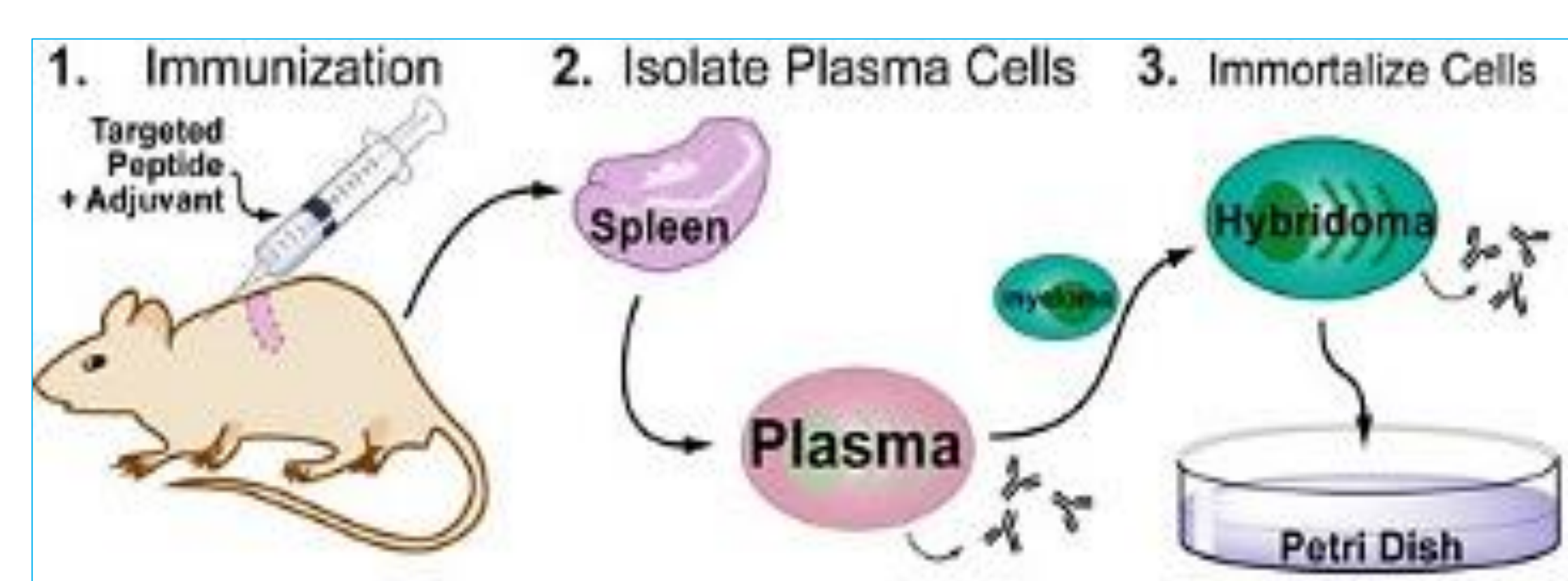
Memory B cell antibodies

Antibody gene libraries from human patients or immunized animals.



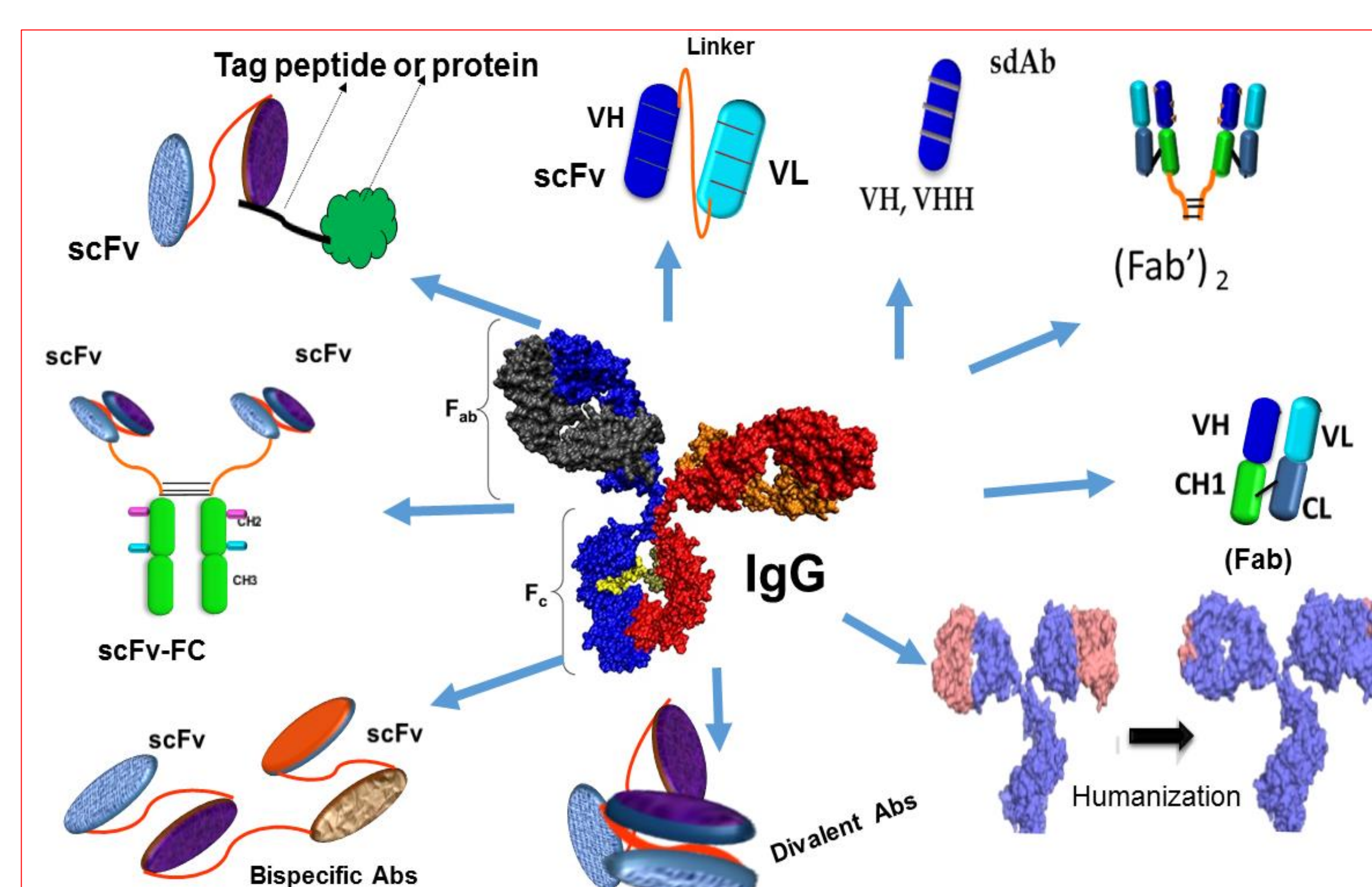
Overview of methodology used for single B-cell antibody gene isolation and recombinant antibody production.

Mouse hybridoma technology



Overview of methodology used for mouse hybridoma technology for construction of monoclonal antibody.

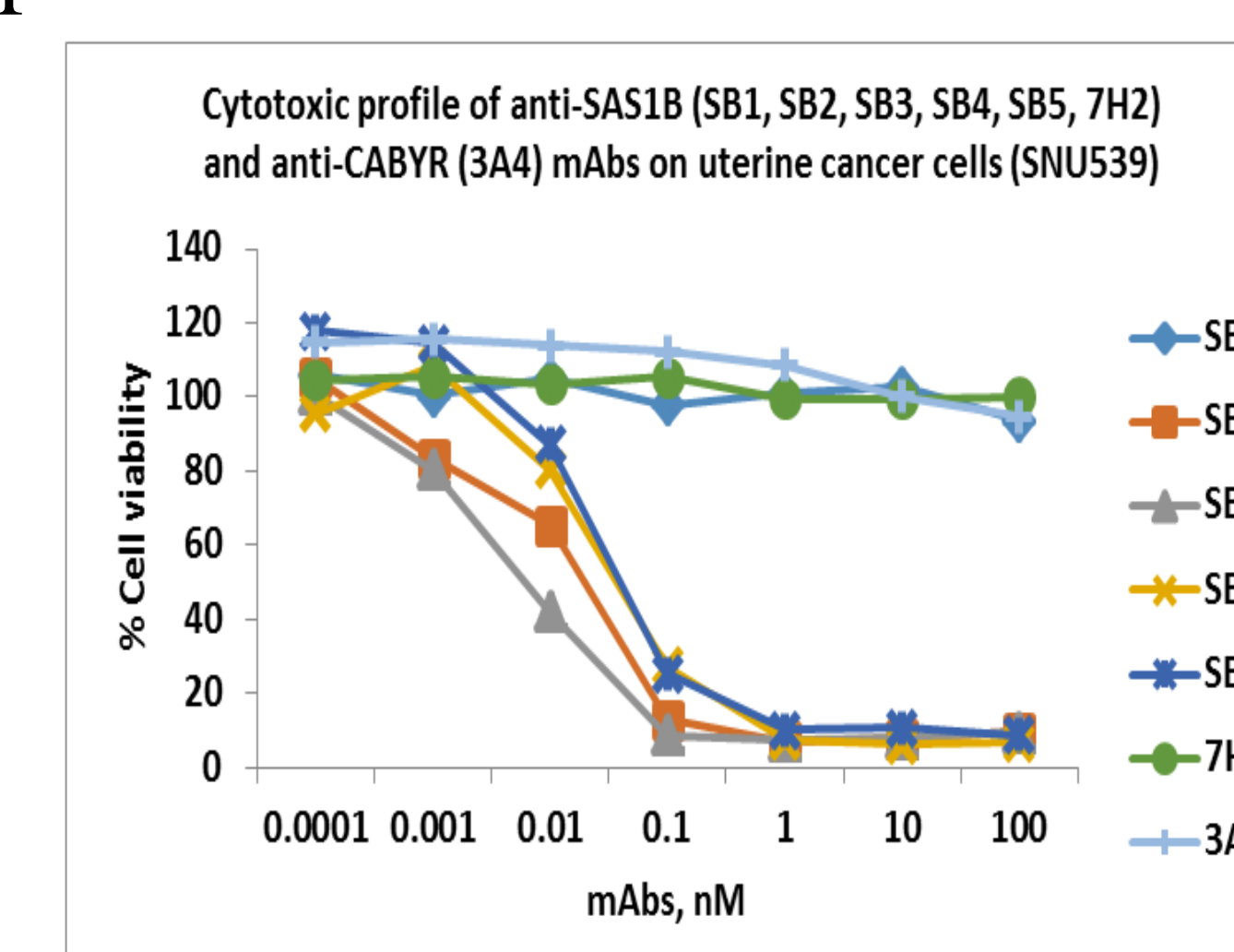
Antibody engineering



Schematic representation of different antibody formats, showing intact 'classic' IgG molecules alongside humanized IgG, single chain fragment variable (scFv-Fc) immunoglobulins. A variety of antibody fragments are depicted, including Fab, scFv, single-domain antibody (sdAb) VH, VHH and multimeric formats, such as bispecific-scFv, diabodies, enzymatic conjugated Tag- scFv.

Science highlights from AbET core

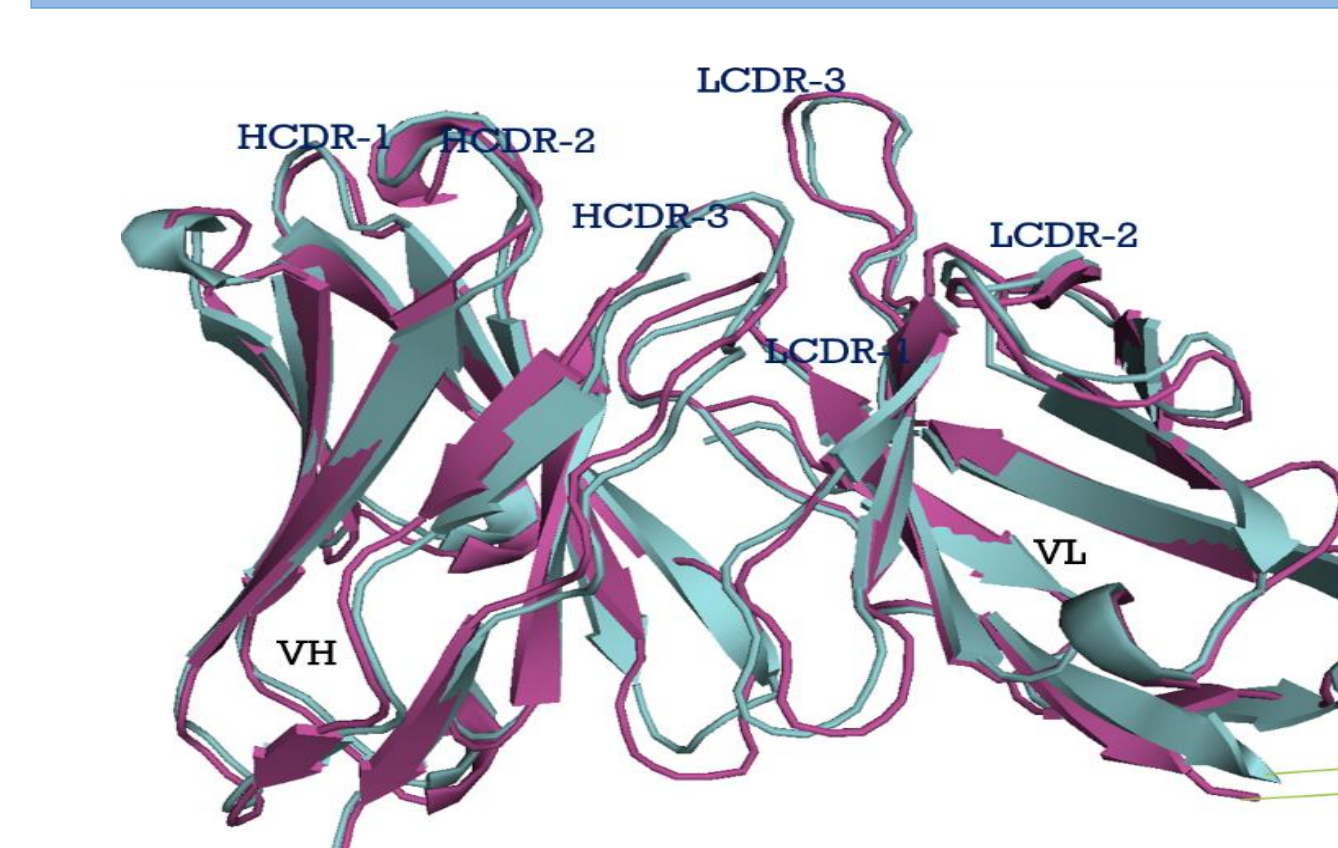
Hybridoma construction and monoclonal antibody production with a focus on cancer research .



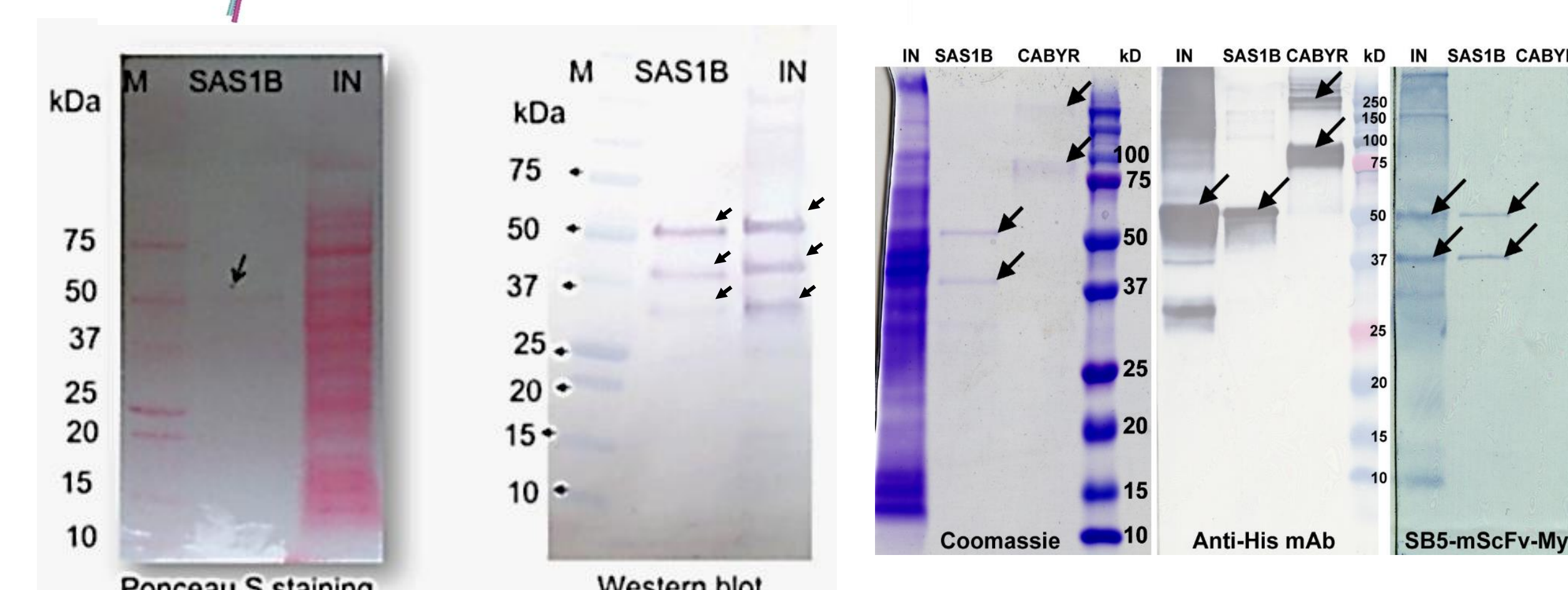
Cytotoxic effect of anti-human SAS1B N-terminus (SB1, SB2, SB3, SB4, SB5) C-terminus 7H2 and anti-CABYR (3A4) mAbs and antibody drug conjugate ADC assay using Fab-anti-mouse IgG Fc conjugated to Duocarmycin DM with cleavable linker on Human Uterine Cancer Cells. This data shows that antibodies: SB2, SB3, SB4 and SB5 to the cell surface protein can deliver the cytotoxic drug to kill the cells.

Courtesy: John C Herr and Arabinda Mandal, Department of Cell Biology

Antibody humanization



Homology modelling of mouse and humanized template sequence using Rosetta homology modelling server.



A: Humanized version antibody B: Mouse version antibody

Western blot analysis mouse scFv humanization scFv and anti-His tag monoclonal antibody. IN: induced E. coli expressing human SAS1B; SAS1B, human: purified, 200 ng; CABYR, human: purified 90 ng. Coomassie: unit gel stained with Coomassie; Anti-His: Anti-His mAb, HRP conjugated; SB5-muscFv-Myc: mouse version mScFv of SB5.Hum2scFv: humanized version of SB5.

Courtesy: John C Herr, Arabinda Mandal and Bhupal Ban Department of Cell Biology and AbET core

Hybridoma sequencing and recombinant antibody

- Patent applications,
- Humanized antibodies,
- Rescue functional antibody gene,
- Authentication (Validation) of hybridoma cell lines,
- Alternatives antibodies formats,

Rescue the functional antibody gene from hybridoma cells.

ELISA test for detection of ghrelin "hunger hormone", is a peptide hormone produced by ghrelinergic cells in the gastrointestinal tract.

Courtesy: Gaylinn, Bruce D. Endocrinology and Metabolism

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