Monoclonal Antibody Generation

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Common Antibody Formats

**IgG Immunoglobulin**
MW ~150 kDa

**Fab**
MW ~50 kDa

**Single-chain Fv (scFv)**
MW ~25 kDa
Monoclonal Antibody Generation Methods – Overview

1) Phage Display
(in vitro)

2) Animal Immunization & Hybridoma
(in vivo)

3) Single B-Cell Isolation
(ex vivo)
Antibody Generation *in vitro* – Phage Display

**Phage display.** A scFv library is generated from variable heavy and light chain regions isolated from human B cells. The library is displayed on the pIII pilus of bacteriophage particles. For panning, the phages are incubated with the target antigen. Non-specific binders are washed away, then binders are eluted, amplified, and used for subsequent rounds of panning. Individual clones can be isolated following multiple cycles of panning for further screening and analysis.

Other *in vitro* methods include yeast display, ribosome display, and mammalian display.
Antibody Generation *in vivo* – Immunization Strategies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description/Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunogen</td>
<td>DNA, Peptides, Proteins, Cells</td>
</tr>
<tr>
<td>Carrier</td>
<td>Proteins, Nanoparticles, Viral Vectors, Virus-Like Particles, Exosomes</td>
</tr>
<tr>
<td>Animal Species</td>
<td>Mouse, Rat, Rabbit, Hamster, Goat, Sheep, Llama, Alpaca, Chicken etc.</td>
</tr>
<tr>
<td>Strains</td>
<td>Various strains of a species respond differently to each target</td>
</tr>
<tr>
<td>Adjuvants</td>
<td>Increase level of immune response to target</td>
</tr>
<tr>
<td>Dose</td>
<td>Low vs. high dose changes antibody diversity and affinity</td>
</tr>
<tr>
<td>Frequency</td>
<td>Spacing between immunizations influences antibody diversity and affinity</td>
</tr>
<tr>
<td>Administration Route</td>
<td>Intramuscular, subcutaneous, intraperitoneal, intrasplenic</td>
</tr>
<tr>
<td>Prime/Boost Regimen</td>
<td>Homologous vs. heterologous prime-boost regimens</td>
</tr>
</tbody>
</table>

Immunization strategies are highly individual and target-specific
**Antibody Generation *in vivo* – Hybridoma Technology**

**Hybridoma generation and screening.** Mice are immunized multiple times with the desired antigen. B cells recovered from the spleen are fused with myeloma cells for immortalization. Hybridoma are grown in HAT medium (hypoxanthine, aminopterin, thymidine) for selective survival. Antibodies secreted by the hybridoma are screened for their reactivity against the antigen in binding and functional assays. Selected hybridoma are further expanded in order to obtain monoclonal antibodies with the desired specificity.

*Adapted from Loureiro et al, Biomolecules 5, 1783-1809 (2015)*
# Antibody Generation – *in vivo* vs. *in vitro*

<table>
<thead>
<tr>
<th>Method</th>
<th>Pros</th>
<th>Cons</th>
</tr>
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</table>
| Animal Immunizations    | • Wide array of species and immunization methods  
                         | • *In vivo* selection and affinity maturation  
                         | • Transgenic mice with human antibody repertoire available | • Difficult for integral membrane proteins  
                         |                                                                      | • May be challenging to break immunological tolerance |
| Display Methods         | • Multiple options for display platform and antibody formats  
                         | • Rapid identification of low-affinity binders  
                         | • Works for many different types of targets | • Time-consuming to generate high-affinity antibodies  
                         |                                                                      | • Selected antibody may not fulfill manufacturability criteria |

Method of antibody generation is determined based on the characteristics of the target and the antibody selection criteria for binding & function
**Single B Cell Technologies**

**B cell immortalization.** Human B cells can be immortalized by transformation with Epstein Barr Virus. Transformed B cells are plated at limited dilution and expanded, followed by screening for antibodies that bind the desired antigen.

**Single-cell expression cloning.** If an antigen bait is available, antigen-specific B cells can be isolated by flow cytometry. Alternatively, plasmablasts activated by a recent immunization contain a high frequency of antigen-specific cells that can be used for single cell expression cloning.

These techniques allow for “deep interrogation” of antibody repertoires, but require high-throughput cloning and screening capabilities.

*Adapted from Wilson & Andrews, Nat Rev Immunol 12, 709-719 (2012)*
Antibody Generation – Screening Assays

**Primary Binding**
- Solid-phase capture assays (e.g. ELISA)
- Bead-based assays (e.g. AlphaLISA®, Luminex®)
- Flow cytometry

**Affinity**
- Surface plasmon resonance (biosensor) for soluble proteins
- Binding to target on cells

**Mechanistic Assays**
- Protein interaction assays using fluorimetric readout

**Functional Assays**
- Enzymatic assays or cell-based reporter assays
- Readout technologies: enzymatic activity, luciferase, fluorophore, secretion of a soluble factor, cell proliferation, apoptosis etc.