Antibody Humanization Guide

Antibody Engineering
ALPHA LIFETECH INC.

1. Introduction

Within each V domain (VL and VH) there are three non-consecutive complementarity-determining regions (CDRs) loops or hypervariable regions, that directly interact with an antigen. For this reason, CDRs and adjoint regions contain the most important information regarding antigen-specificity in a given molecule. This knowledge has led to the development of one of the most popular methods of antibody humanization, CDR-grafting, which takes parental complementarity determining regions (CDR) into human framework (FR) regions. Thus, reducing the risk of the molecule being recognized as foreign by the patients' immune system. Parental antibody specificity and affinity are conserved thanks to the preservation of residues implicated in antigen binding.

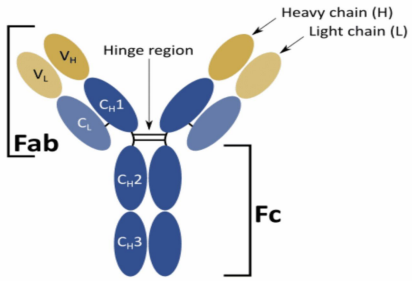

Figure 1. Common Antibody Structure

Humanized antibodies constitute the majority of today' s approved therapeutic antibodies. Humanization is important for reducing the immunogenicity of monoclonal antibodies derived from xenogeneic sources (commonly rodent) and for improving their activation of the human immune system. Since the development of the hybridoma technology, a large number of rodent monoclonal antibodies with specificity for antigens of therapeutic interest have been generated and characterized.

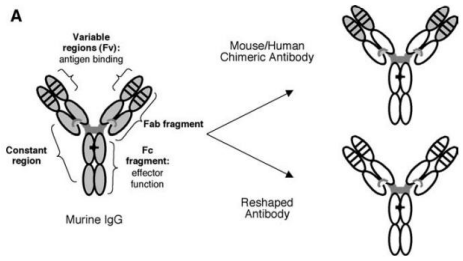

Figure 2. Antibody humanization and CDR Grafting

2. Antibody Humanization Workflow

1. Homology Modeling of the Murine Variable Region (homology modeling methods)
2. Human Acceptor Framework Selection
3. CDR Grafting and Expression of Reshaped Antibodies
4. Binding Analysis and Framework Back-mutations

3. Tips for Antibody Humanization

1. For the expression of antibodies as full IgGs, eukaryotic vectors that provide the light- and heavy-chain constant domains are available from AERES Biomedical, London, UK (SuperVector Expression System) and Lonza Biologics, NH, USA (pCON vectors). For expression as single-chain Fv fragments (scFv), common prokaryotic vectors that allow periplasmic protein expression can be used.
2. In the homology approach, a structural template(s) is chosen based on sequence homology and length. Conflflicts between amino acid side-chains are then resolved by energy minimization. This approach is especially useful for the β-sheet framework and CDR loops with known canonical structures (see Subheading 1.1.). In contrast, the conformational search approach seeks to build CDR conformations ab initio by generating a large number of theoretically possible conformations by varying the dihedral angles of the peptide backbone. The predicted loop conformation is obtained after energetic evaluation of the conformers. This is useful for CDRs that do not belong to any of the canonical classes and those that are poorly modeled by homology alone.
3. V-BASE and IMGT are online databases featuring human immunoglobulin germline sequences.
4. The Fv expression cassettes are initially synthesized as half-length inserts and sequenced before the two halves are stitched together. This allows the early detec-154 Lotion of PCR artifacts, which are likely at the numerous primer junctions, and avoids the subsequent need to sequence a large number of clones for the correct full-length cassette sequence.
5. Design overhanging primers that are partially complementary to the 5′- and 3′-ends of the murine VL and VH gene for the introduction of additional sequences.
6. The shufflfling of the murine and reshaped VL/VH domains and subsequent analyses of the hybrid constructs will reveal whether the reshaped antibody retains the binding affifinity and specifificity of the murine antibody and, if not, the CDR grafting of which domain (VL, VH, or both) has resulted in the change in binding characteristics.

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