Antibody Epitope Mapping using Shotgun Mutagenesis

Epitope Mapping

Characterizing the binding sites of monoclonal antibodies (MAbs) on target antigens, their 'epitopes', can aid in the discovery and development of new therapeutics, vaccines, and diagnostics. Direct methods for locating and distinguishing epitopes, such as co-crystallography, are often impractical and cannot readily be employed for membrane protein targets such as GPCRs and ion channels. Identification and mapping of MAb epitopes is therefore commonly achieved using site-directed mutagenesis. Epitope mapping by this approach involves systematically introducing residue substitutions along the target protein, and then determining the effect of each mutation on MAb recognition. Each point-mutation must be individually sequence-verified, expressed in cells, and assayed for altered expression and reactivity, so conventional mutational mapping is usually not practical for comprehensive analysis of an entire protein. Using a high-throughput "Shotgun Mutagenesis" approach for structure-function mapping, Integral Molecular is able to rapidly construct comprehensive epitope maps across the entire sequence of even difficult target proteins, enabling both linear and conformational epitopes to be identified.

Shotgun Mutagenesis

Shotgun Mutagenesis uses a proprietary high-throughput cellular expression technology that enables the expression and analysis of large libraries of mutated target proteins within eukaryotic cells. Every residue in a protein is individually mutated, usually to multiple other amino acids, in order to assay changes in function. Entire mutation libraries can be repeatedly expressed and assayed, making it possible to map the complete epitopes of dozens of different antibodies in a short amount of time. Proteins are expressed within standard mammalian cell lines, so even difficult proteins that require eukaryotic translational or post-translational processing can be mapped.

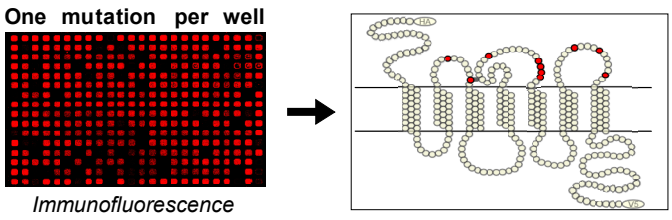

Figure 1. Shotgun Mutagenesis Mapping of MAb Epitopes. To map epitopes, the immunoreactivity of each MAb is tested against a library of protein mutants arrayed in 384-well microplates. Fluorescence intensity in each well is imaged and quantified to determine the reactivity of each MAb with each mutant clone. Critical residues identified using Shotgun Mutagenesis are shown shaded in red on a diagram of the target protein.

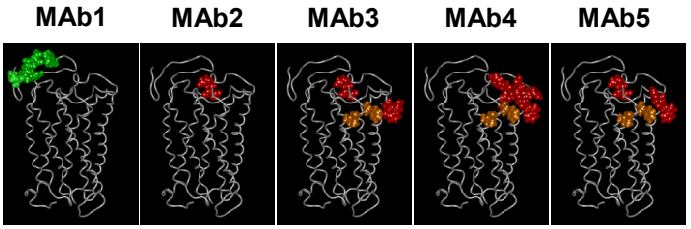

Figure 2. Epitope mapping of CCR5 MAbs. The critical residues that compose the epitopes of five commercially available MAbs are shown mapped on a three-dimensional model of the receptor.

Epitope Mapping of CCR5 MAbs

Shotgun Mutagenesis was used to map the binding epitopes of five commercially available MAbs on the chemokine receptor CCR5. A library of eukaryotic expression plasmids coding for point mutations along the entire 1 kb CCR5 gene was prepared, and each clone was expressed in HEK-293 cells. Epitope tags at the N-terminus and C-terminus of the CCR5 construct enabled measurement of surface expression and full-length translation of each clone to verify correct folding, trafficking, and expression. Immunofluorescence was used to detect MAb reactivity with each clone in the mutation library (**Figure 1**). Clones that were fully expressed but that were no longer reactive with the MAbs contained amino acid mutations at critical residue positions that are required for formation of the epitopes. To visualize how the epitopes are formed in the three-dimensional structure of the protein, critical amino acids were mapped onto a structural model of CCR5 (**Figure 2**). All critical residues identified by Shotgun Mutagenesis for each of the antibodies were localized to the extracellular regions of the receptor. Shotgun Mutagenesis identified all of the critical residues identified by others in previous site-directed mutagenesis studies, as well as additional critical residues not previously identified.

Contact Us

Shotgun Mutagenesis mapping, including comprehensive mutation of user-specified genes, data collection, and structural analyses, are provided to customers on a fee-for-service basis. For more information contact us at:

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