Protocol

Modification of Antibody Function by Mutagenesis

James R. Dasch and Amy L. Dasch

The ability to “fine-tune” recombinant antibodies by mutagenesis separates recombinant antibodies from hybridoma-derived antibodies because the latter are locked with respect to their properties. Recombinant antibodies can be modified to suit the application: Changes in isotype, format (e.g., scFv, Fab, bispecific antibodies), and specificity can be made once the heavy- and light-chain sequences are available. After immunoglobulin heavy and light chains for a particular antibody have been cloned, the binding site—namely, the complementarity determining regions (CDR)—can be manipulated by mutagenesis to obtain antibody variants with improved properties. The method described here is relatively simple, uses commercially available reagents, and is effective. Using the pComb3H vector, a commercial mutagenesis kit, *PfuTurbo* polymerase (Agilent), and two mutagenic primers, a library of phage with mutagenized heavy and light CDR3 can be obtained.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution’s Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

DNA template

pComb3H is available from Scripps Research Institute, after execution of a Material Transfer agreement (http://www.scripps.edu/mb/barbas/content/pcomb_images/updatepcomb_image.htm). The sequence of *pComb3H* is available at GenBank (AY254174).

DpnI and restriction buffer 4 (New England Biolabs)

Escherichia coli XL1-blue cells, electrocompetent (Agilent)

Mutagenic primers:

LCCDR3RAN8: 5'-CTGCAATGTATTTCTGTCAG(NNK)₈TTCGGTGCTGGGACCAAGGTG-3'
HCCDR3RAN9: 5'-GTCTATTACTGTGCAAGATGG(NNK)₉TACTGGGGCCAAGGCACCAC-3'
where N is any nucleotide, and K = G or T.

Primers are synthesized, 5'-phosphorylated, and purified by polyacrylamide gel electrophoresis.

QuikChange Multi Site-Directed Mutagenesis Kit (Agilent)

This kit contains many of the reagents needed in Step 1.

QIAquick PCR Purification Kit (QIAGEN, #28104)

This kit contains the elution buffer needed in Step 4.

Water, sterile, nanopure

From the Antibodies collection, edited by Edward A. Greenfield.

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Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot093872

Equipment

Electroporation device
Microcentrifuge (Beckman Microfuge 9 or equivalent)
Spectrophotometer
Thermal cycler (PerkinElmer model 9600 or equivalent)
Water bath set at 37°C

METHOD

This approach was described by Kelley and Momany (2003).

1. Prepare ten 50-μL mutagenesis reactions using the QuikChange Multi Site-Directed Mutagenesis Kit:

Reaction buffer (10×)	5 μL
Water, sterile, nanopure	35 μL
Double-stranded DNA template (10–100 ng)	2 μL
Mutagenic primers:	
LCCDR3RAN8 (144 ng/μL)	2 μL
HCCDR3RAN9 (151 ng/μL)	2 μL
dNTP mixture	2 μL
<i>PfuTurbo</i> DNA polymerase (2.5 units/μL)	1 μL
QuikChange Multi enzyme blend	1 μL

2. Perform mutagenesis using the times and temperatures listed below:

Cycle number	Denaturation	Polymerization
1	1 min at 95°C	
2–31	1 min at 95°C	1 min at 63°C
32		8 min at 65°C

3. Digest the DNA with DpnI as follows:

Mutagenesis reaction (from Step 2)	50 μL
NEB restriction buffer 4	6 μL
DpnI (20 units/μL)	2 μL
H ₂ O, distilled	2 μL
Incubate the digest for 1 h at 37°C.	

4. Purify the digested DNA using a spin column. Elute the DNA from the column with a volume of 30 μL of elution buffer.
5. Quantitate the DNA either spectrophotometrically or by comparison to standards using agarose gel electrophoresis.
6. Transform 5 μg of DNA from each reaction into 1300 μL of electrocompetent *E. coli* XL1-Blue cells. The final library consists of phage from ten separate transformations.

On a panel of six test antigens, after three rounds of panning, significant numbers of rec Fab could be obtained in each case. The diversity of the library from this relatively simple process is estimated at 1.16×10^7 .

RELATED INFORMATION

A wide number of mutagenic approaches have been described. Many are based on the seminal work of Kunkel (1985) and thus have been referred to as “Kunkel mutagenesis.” Several improvements to the Kunkel mutagenesis approach have been described. Huovinen et al. (2012) improved the number of mutant transformants by 300-fold over the Kunkel method by using rolling circle amplification.

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This work, like many papers in this field, is the subject of an international patent application (PCT/FI2010/051068). The protocol above was described by Kelley and Momany (2003).

REFERENCES

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