Complete primer set for amplification and expression of full-length recombinant human monoclonal antibodies from single human B cells

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INTRODUCTION

Background:

B cells are vital in the in the immune response against pathogens due to their production of antibodies that help eliminate pathogens. Monoclonal antibodies (mAbs) are currently used in the treatments of infections, metabolic disorders, and cancer due to their high specificity. Recently, the specificity of antibodies has allowed them to be used for diagnostic purposes as well as in drug conjugation. **Research Question:**

We would like to know if a new primer set can be made that amplifies all the possible immunoglobulin gene rearrangements so that all possible recombinant human monoclonal antibodies can be made from single B cells.

Objective:

To design and verify a primer set that can amplify all possible V(D)J rearrangements so that we can clone and express all possible recombinant monoclonal antibodies from single human B cells.

METHODS

Human Ig Gene Segment Database:

- Sequences of IgH, kappa, and lambda downloaded from NCBI human reference genome.
- MacVector was used to obtain sequences of variable and joining segments.
- Gene segments missed retrieved from IGMT.

Staining and Sorting Single Human B Cells:

- Used flow cytometry to sort the fresh human peripheral blood Leukopak.
- Single B1 cells, naïve B cells, and memory B cells were separated and amplified.

Nested RT-PCR Amplification, Purification, Cloning, and Expression of Ig Transcripts:

- cDNA was made from amplified samples using ProFlex PCR system.
- First round PCR products were used as template to perform amplification with family-specific individual primers of second-round primer set.
- Amplicons made from PCR cloned into suitable mammalian expression vectors.
- Afterwards, transfection and cloning in HEK293T cells, followed by purification with commercially available kit following manufacturer's instructions (Qiagen).
- Concentration of purified mAb estimated with BCA kit and purity was assessed with SDS-PAGE.

Isotype-specific amplification of heavy and light chains from bulk-sorted B cells done for IgA, IgD, IgE, IgM, Ig kappa, and Ig lambda isotypes.

- PCR products analyzed by QIAxcel Advanced system.

RESULTS

Assessment of V and J Gene Coverage

- 112 IgH chain sequences represent 30 (55.55%) of 54 functional genes.
- Ig Kappa chain had 41 functional V kappa segments categorized into 6 subgroups. 22 (53.65%) of 41 V kappa genes with no representation from subgroup 5.
- For Ig Lambda chain, 33 functional V lambda gene segments are classified into 10 subgroups.
- Observed 21 (63.63%) of 33 functional V lambda gene segments.
- All J Lambda genes except for J Lambda 6 is shown in dataset.

Production of Recombinant Human mAbs

- SDS-PAGE analysis of 5 purified mAbs made from human memory B cells and B1 cells shows successful production of recombinant human mAbs.

Nested (RT-)PCR amplification of IgH and IgL chain transcripts from PBMCs using individual second round primers.

- We observed amplification with each functional gene-specific second round primer, with the exception of one primer of Ig Lambda second round sense primer.

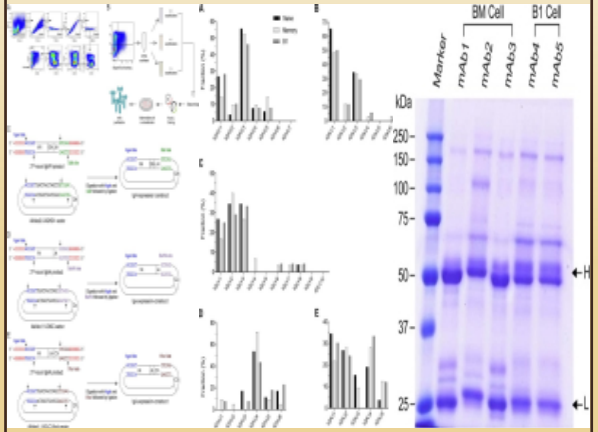

Figure 2: Strategy used to identify B cell subsets and schematic of recombinant mAb production

Figure 3: Joining and variable gene coverage in B cell subsets.

Figure 4: SDS-PAGE analysis of production of recombinant human mAbs.

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CONCLUSION

Accomplished:

- Described complete human Ig primer set designed to permit detailed analysis of human antibody collection.
- Primer can be easily adapted for any full-length antibody expression vector system.
- Method is highly sensitive as it allows amplification of expressed variable regions from the IgH and IgL transcripts from sorted single human B cells.
- Improved design of our primer set allows efficient amplification and production of full -length recombinant human mAbs starting from single sorted B cells.

Research and Clinical Applications:

- Approach can be used to establish clonality in diagnosis of multiple myeloma.
- Can be used to analyze antigen-specific B cell response following infection or vaccination at nucleotide and amino acid sequence level.

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