4-26-2019

Cloning, Sequencing, Expression and Characterization of an Alzheimer's-Specific Monoclonal Antibody

Anna K. Jones
University of Missouri-St. Louis, akh9r6@mail.umsl.edu

Thao Pham

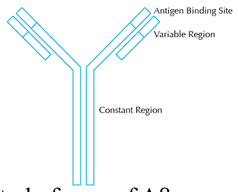
Cloning, sequencing, expression and characterization of an Alzheimer's-specific monoclonal antibody

Anna K. Jones, Thao Pham and Michael R. Nichols, Ph.D.

Department of Chemistry and Biochemistry, University of Missouri-St. Louis

Introduction

A hallmark of Alzheimer's Disease (AD) is the aggregation of misfolded amyloid-beta (Aβ) proteins, which form soluble intermediates that eventually accumulate into fibrillar plaques. A common form of Aβ is Aβ42. Previous data in the Nichols Laboratory has shown that soluble protofibrillar Aβ species is a highly active and proinflammatory form.



Antibodies are a way to target and study forms of Aβ. Monoclonal antibody (mAbSL) recognizes the soluble intermediates of Aβ at a high affinity.

Objectives

- To clone and sequence DNA fragments of antibodies from rabbits immunized with Aβ42 protofibrils.
- To visualize qualitatively (dot blot) and quantitatively (ELISA) that the antibody was successfully secreted from cells and selective for Aβ42.
- To affinity purify the antibodies from cell expression.
- To test selectivity for Aβ42 PF of purified antibodies.

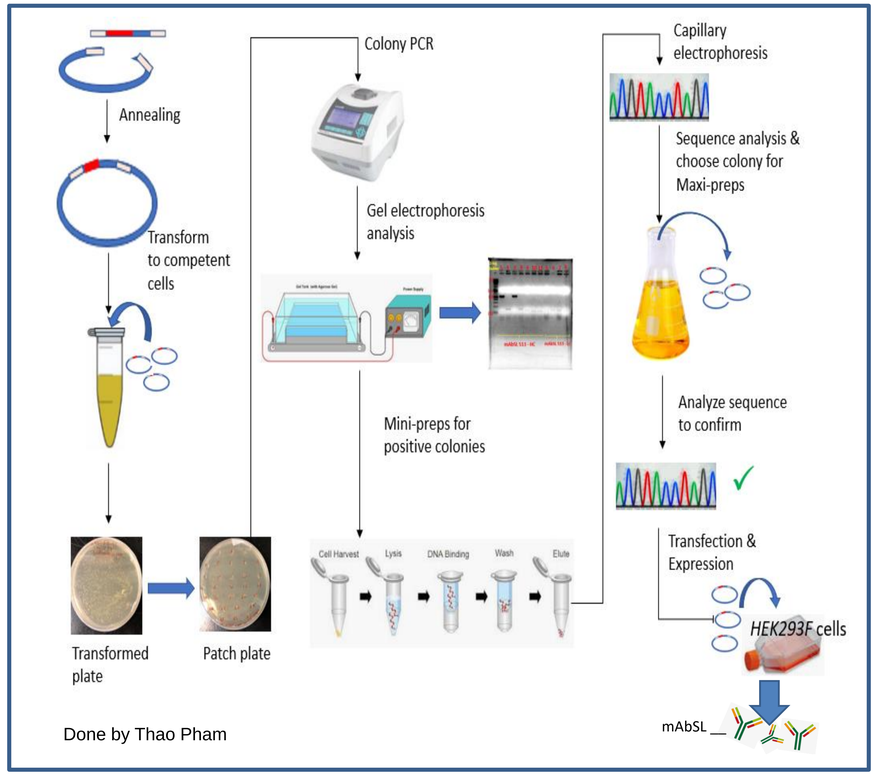
Methods

Cloning Fragments into Linearized Vectors: In PCR tube add 1 µL 5XPhusion HF buffer, 2 µL linearized vector, 1 µL assembled fragment and 1 µL ddH_20 place into thermocycler. Transform 2 µL of reaction into 50 µL E. coli cells. Put on ice 30 min, heat shock in 42°C water bath for 1 min, put back on ice 1 min. Add 1 mL super optimal broth and shake at 225 rpm at 37°C 1 hr. Spin cells at 5000 x g for 10 minutes, remove 900 µL supernatant, resuspend pellet in remaining SOC media, plate cells on LB-amp plate. Incubate for 24 hrs at 37°C. Next day, make patch plate.

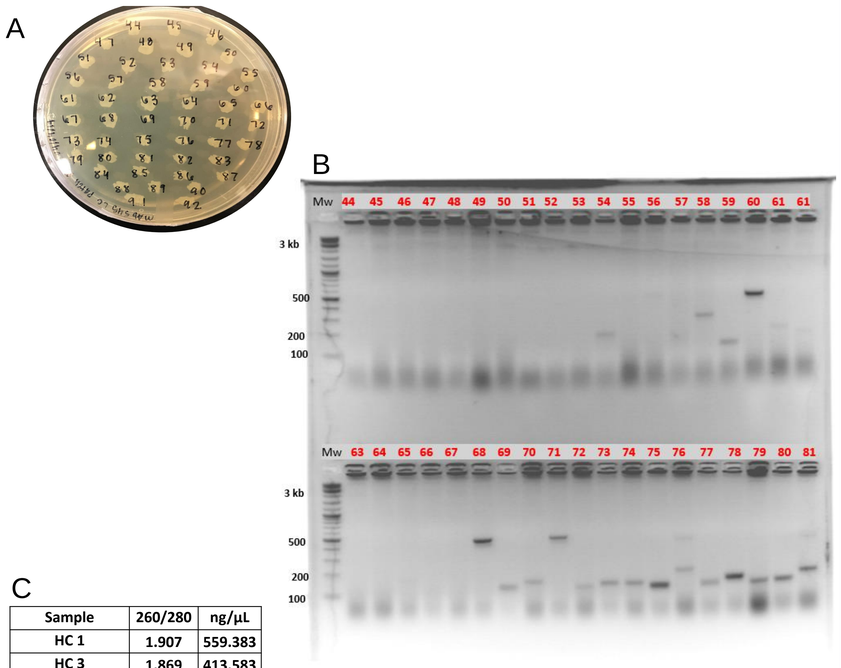
Colony PCR: In PCR tube, add PCR master mix, forward and reverse primers and nuclease free water. Select a single colony from patch plate and add to tube. Thermocycler used for 90 min to do PCR. Pour 2% agarose gel + EtBr into gel caster, solidify. Remove comb, load 2-log molecular weight. Add 6X loading dye to finished PCR tubes. Load sample into wells. Run at 100V for 45 min. Image gel.

Indirect ELISA: Aβ42 protofibrils range of 320nm-0nm were adsorbed to a 96-well plate for 1 hour at 4°C. With washing between each step, blocking buffer was added & incubated 1 hr. Primary antibody mAb 108 or mAb 113 (1:1000 dilution) were added & incubated 1 hr. Anti-rabbit IgG-horseradish peroxidase (HRP) antibody conjugate added & incubated 1 hr. TMB substrate was added & incubated 10 mins. Sulfuric acid was added to stop reaction.

Dot Blot: On a nitrocellulose membrane, 1.7 µL of cell media were pipetted, adsorbed for five minutes. Membrane was covered and gently shaken with 5 mL blocking buffer for 1 hr. With washes occurring 3X between each step, the membrane was treated with 5 mL of antibody diluent for 1 hour, 1:1000 dilution anti-Rabbit IgG-HRP antibody for 1 hr, and Pierce ECL western blotting substrate for 1 min. The membrane was pressed against film & the film was developed.



Done by Thao Pham



C

Sample	260/280	ng/µL
HC 1	1.907	559.383
HC 3	1.869	413.583
HC 6	1.867	153.283
LC 4	1.841	293.283
LC 5	1.886	184.483
LC 14	1.861	243.883

Figure 1: A) Patch plate of cloned bacteria colonies. All colonies are used for a selection process of clones that contain the correct length insert. **B)** After colony PCR, a 2% agarose gel of the samples is ran to find clones with a positive insert of DNA fragment. With at least three positives a mini prep of the plasmid DNA is done to determine if the positives colonies on the gel contain the same sequence of plasmid for the light or heavy chain of the antibody. **C)** DNA quantification is determined before sequencing.

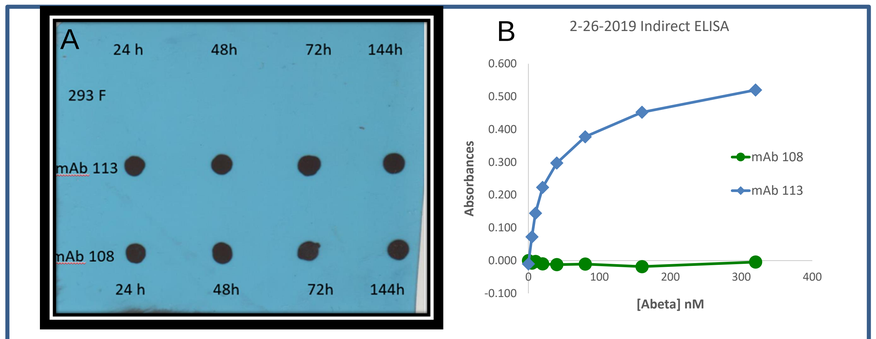


Figure 2: A) HEK 293F cells were transfected with plasmids for mAb 108 and mAb 113. The antibody is secreted into the media and blotted on membrane to test for successful expression. Dotted media is the primary antibody and an anti-rabbit IgG-HRP is secondary antibody. Expression was successful. **B)** Aβ42 protofibrils were plated (320nm-5nm), mAb 108 and mAb 113used as primary antibody, anti-rabbit IgG-HRP used as secondary antibody. Higher affinity binding seen with mAb 113, mAb 108 had little activity in this concentration range.

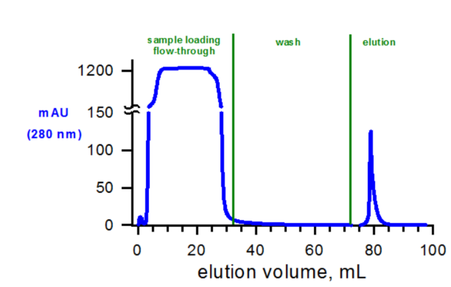
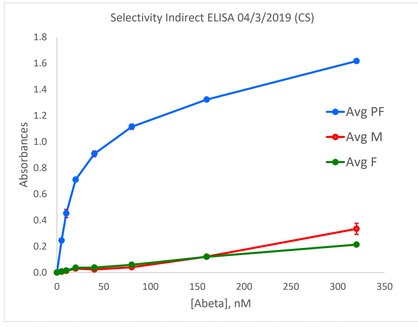


Figure 3: After HEK 293F cellular expression of mAbSL into the media, affinity purification was done at neutral pH (pH 7.4) using a protein G column, which binds tightly to the Fc portion of antibodies. All other extracellular proteins in the media did not bind and were washed out in the flow though. The antibody mAbSL bound to the column and was eluted with an acidic pH buffer (pH 1.8).



Experiment done by Cristina Sinobas Pereira

Figure 4: Purified mAbSL 113 was used in a selectivity ELISA with Aβ42 protofibrils (PF), monomers (M) and fibrils (F). A concentration range of 0-320 nM of isolated Aβ42 PF, M and F were adsorbed to a microwell plate for 1 h. The wells were treated with 0.5 µg/ml of mAbSL 113, followed by an anti-rabbit-IgG-HRP secondary antibody conjugate. The greater absorbance indicates a greater affinity and selectivity for Aβ42 protofibrils by mAbSL 113.

Results, Conclusions & Future Studies

- Cloning does not guarantee that all plasmids will contain the full length DNA insert. Colony PCR and agarose gel analysis is necessary to identify positive, full-length clones for sequencing.
- 2 of the 14 potential mAbSL antibodies have been successfully expressed, purified and tested for selectivity. The light chain or heavy chain of 3 others is complete. 2 other antibodies are in the characterization process.
- Future studies include cloning and sequencing of remaining 7 antibodies, and expression and purification of all 14 mAbSL antibodies.