

# Differential Secretome And Secreted Interactome of APOE2, E3 and E4

Dhanuddara Mohottalage, Janice Mayne and Daniel Figeys



uOttawa

## Introduction

### Apolipoprotein E (ApoE)

Apolipoprotein E is involved in lipid metabolism

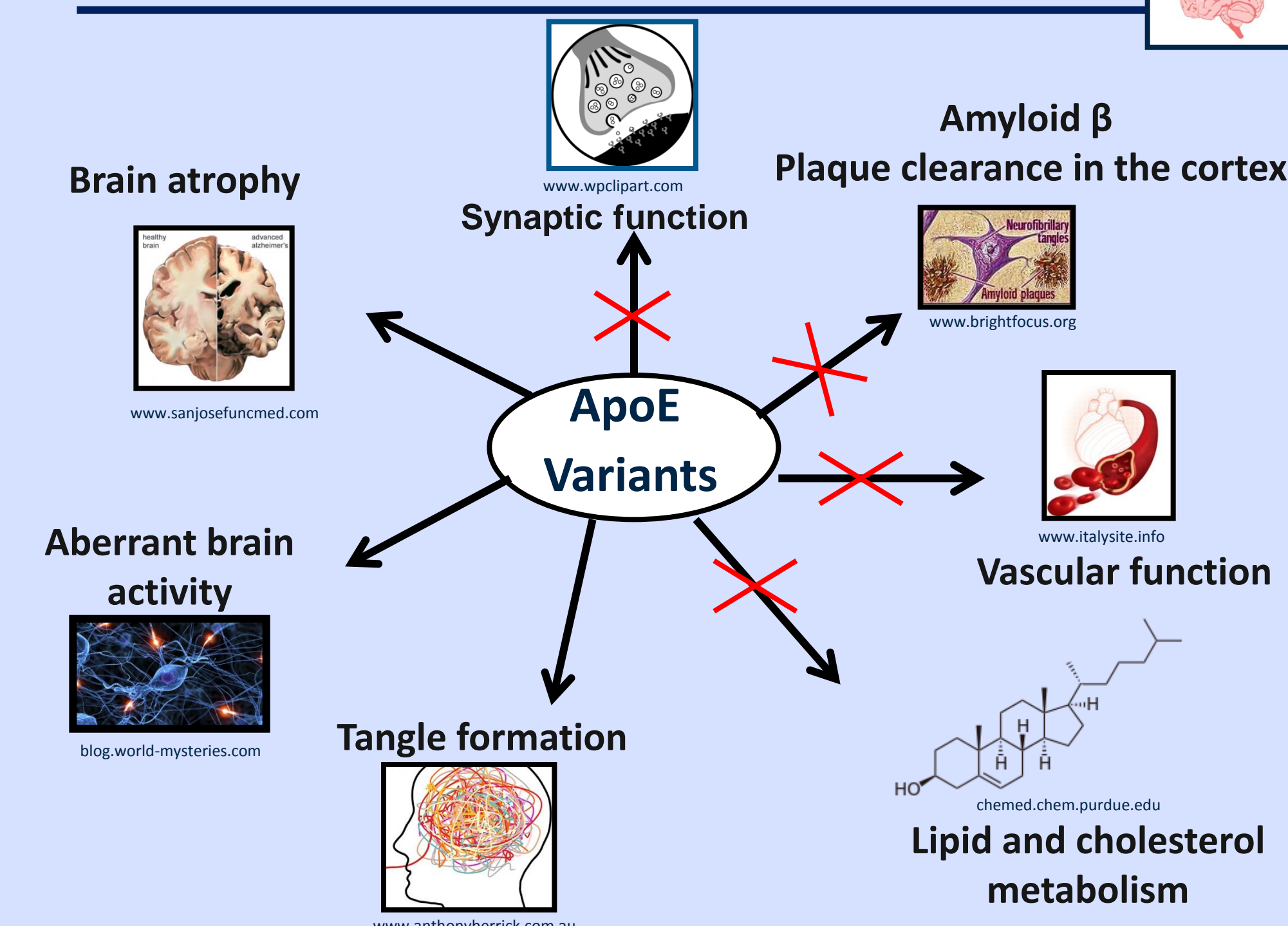
ApoE is a polymorphic gene in humans with three major allelic variations *E2*, *E3* (wildtype) and *E4*<sup>1</sup>

The *E4* variant differs from *E3* isoform by an amino acid substitution (Cys112Arg) and *E2* by amino acid substitution (Arg158Cys)

An allele inheritance of ApoE4 variant increases the risk of developing Alzheimer's disease while ApoE2 variant may provide some protection.

It is thought to be involved in the amyloid- $\beta$  brain plaque deposition formed in Alzheimer's patients and affects glial mediated inflammatory responses in the brain<sup>1</sup>

### ApoE Impacts onset of Alzheimer's disease and familial dysbetalipoproteinemia<sup>2</sup>



### Alzheimer's Disease

Progressive neurodegenerative disorder that is the most common form of dementia<sup>3</sup>

as of 2013, 500,000 Canadians are affected by this disease<sup>13</sup>

It results in severe memory loss and hinders everyday activities

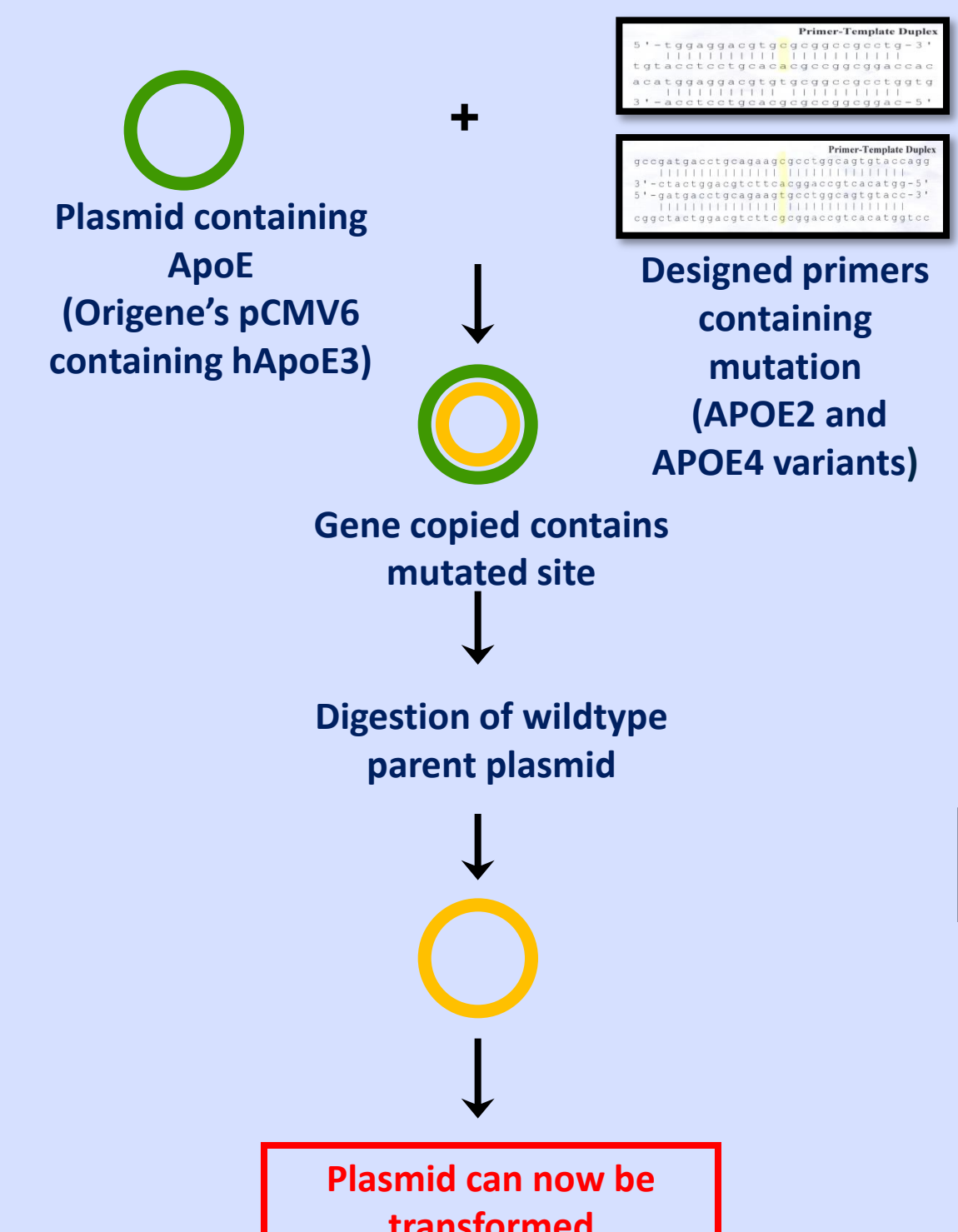
The *E4* variant is the largest known genetic risk factor for late-onset of Alzheimer's disease<sup>4</sup>

Accumulation of Amyloid- $\beta$  is influenced by APOE genotype<sup>4</sup>

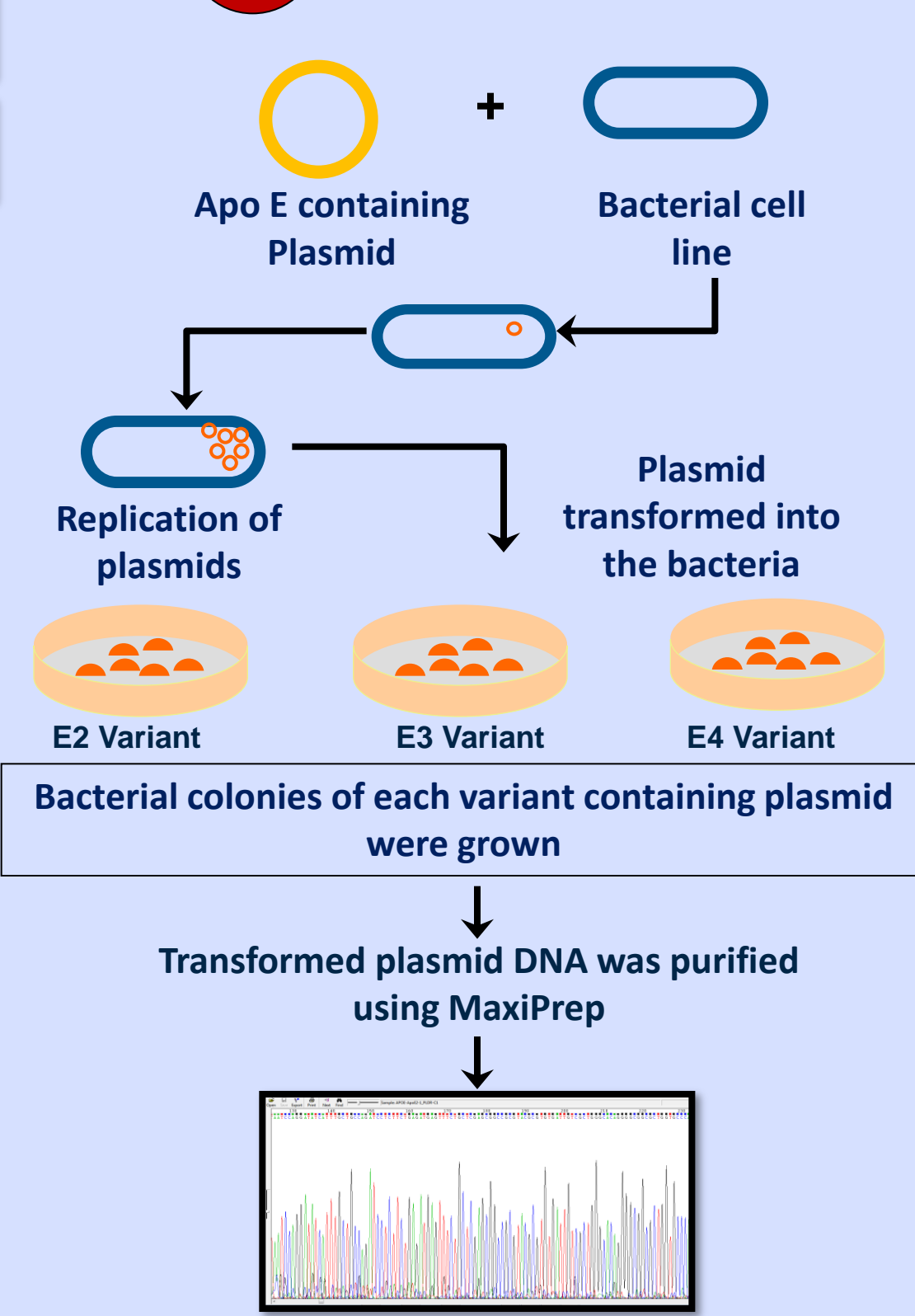
## Abstract

Apolipoprotein E (ApoE) plays a role in removing chylomicrons, very low density lipoproteins and triglyceride-rich lipoprotein components from the bloodstream. The three common allelic variations in the APOE gene include: *E2*, *E3* and *E4* where *E3* is considered to be the normal isoform. The *E2* variant differs by a single amino acid (a.a) substitution (Arg158Cys) and is known to increase familial dysbetalipoproteinemia. The *E4* variant of the ApoE gene differs by a single a.a substitution (Cys112Arg) and is a known risk factor for late on-set Alzheimer's Disease. The exact role of ApoE variants in these disease mechanisms is largely unknown. Proteins that were present in the secretome/secreted interactome will be analyzed in human liver and neural cell lines containing ApoE2 and ApoE4 variants compared to ApoE3. Site directed mutagenesis was used to produce the *E2* and *E4* variants from wild-type ApoE3 containing plasmid. Cell lines were transiently transfected with each. Quantitative spike-in stable isotope-labeling of amino acids in culture (SILAC) reference samples were produced and mass spectrometry analyses will be used for observing quantitative changes in the secretome and various interactors. Through quantitative mass spectrometry, we will determine how the expression of ApoE3 and its variants affects the secreted protein networks of liver and neuronal cell lines. These analyses may reveal how these variants affect lipoprotein metabolism and Alzheimer's disease progression.

## 1 Site Directed Mutagenesis with designed primers

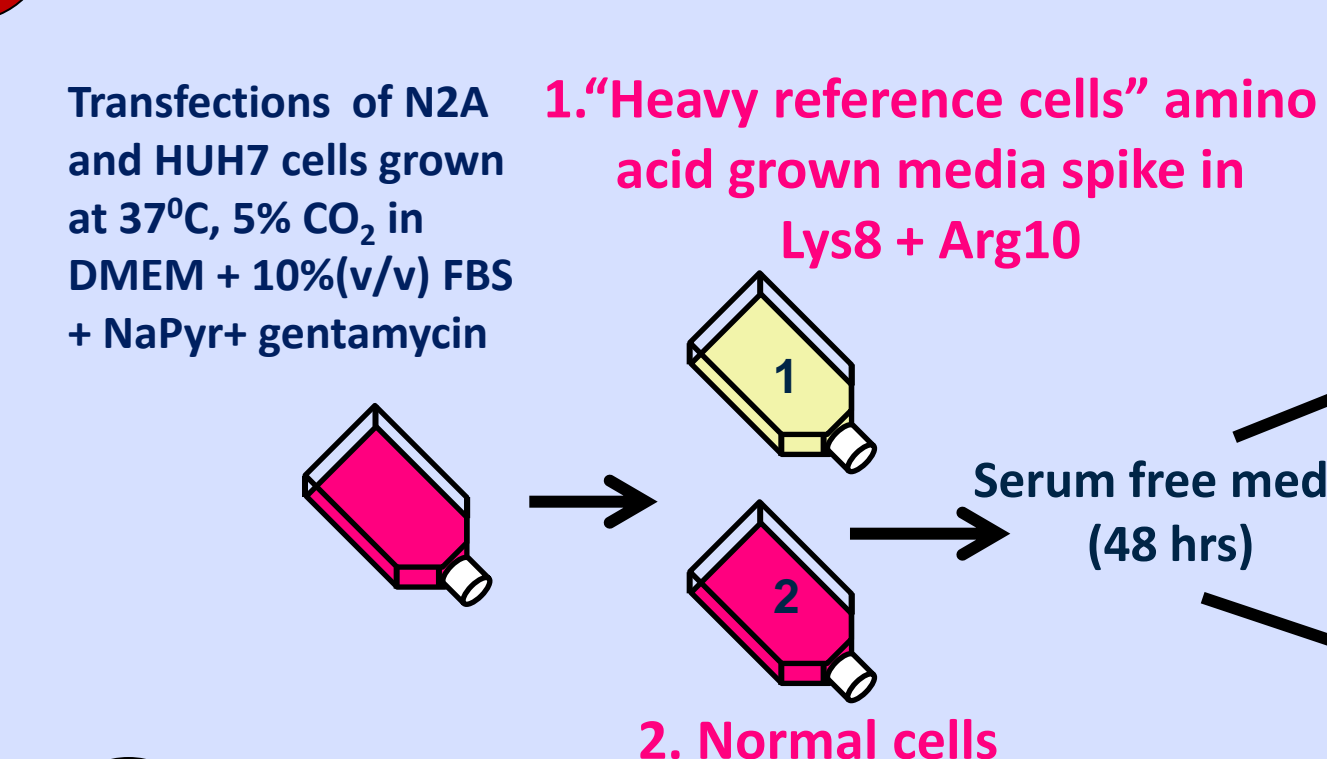


## 2 Transformation

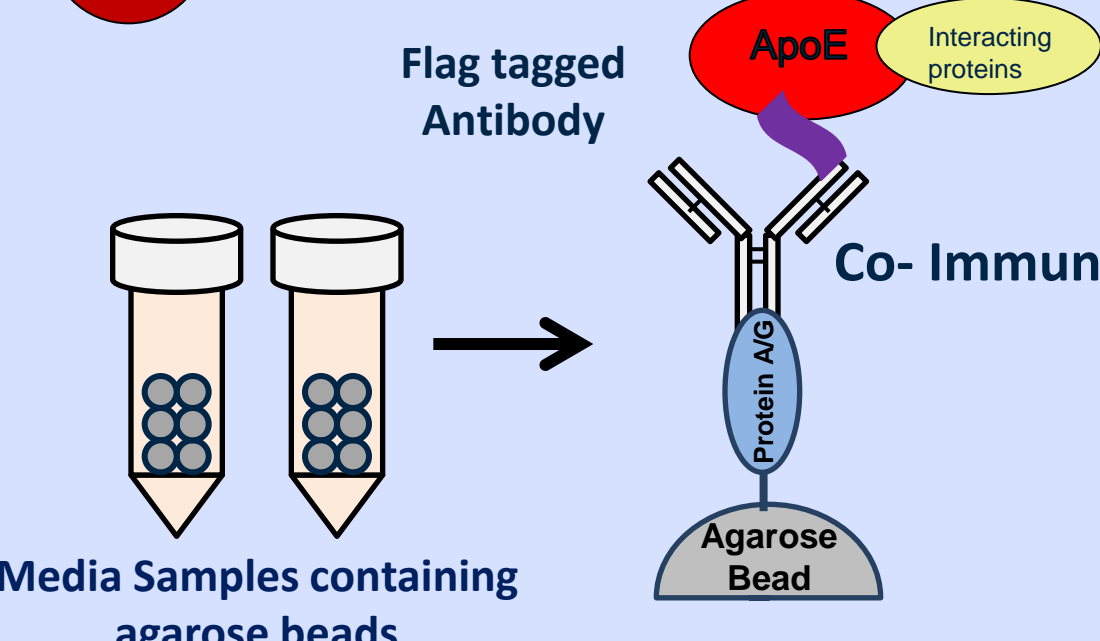


## Experimental Methods

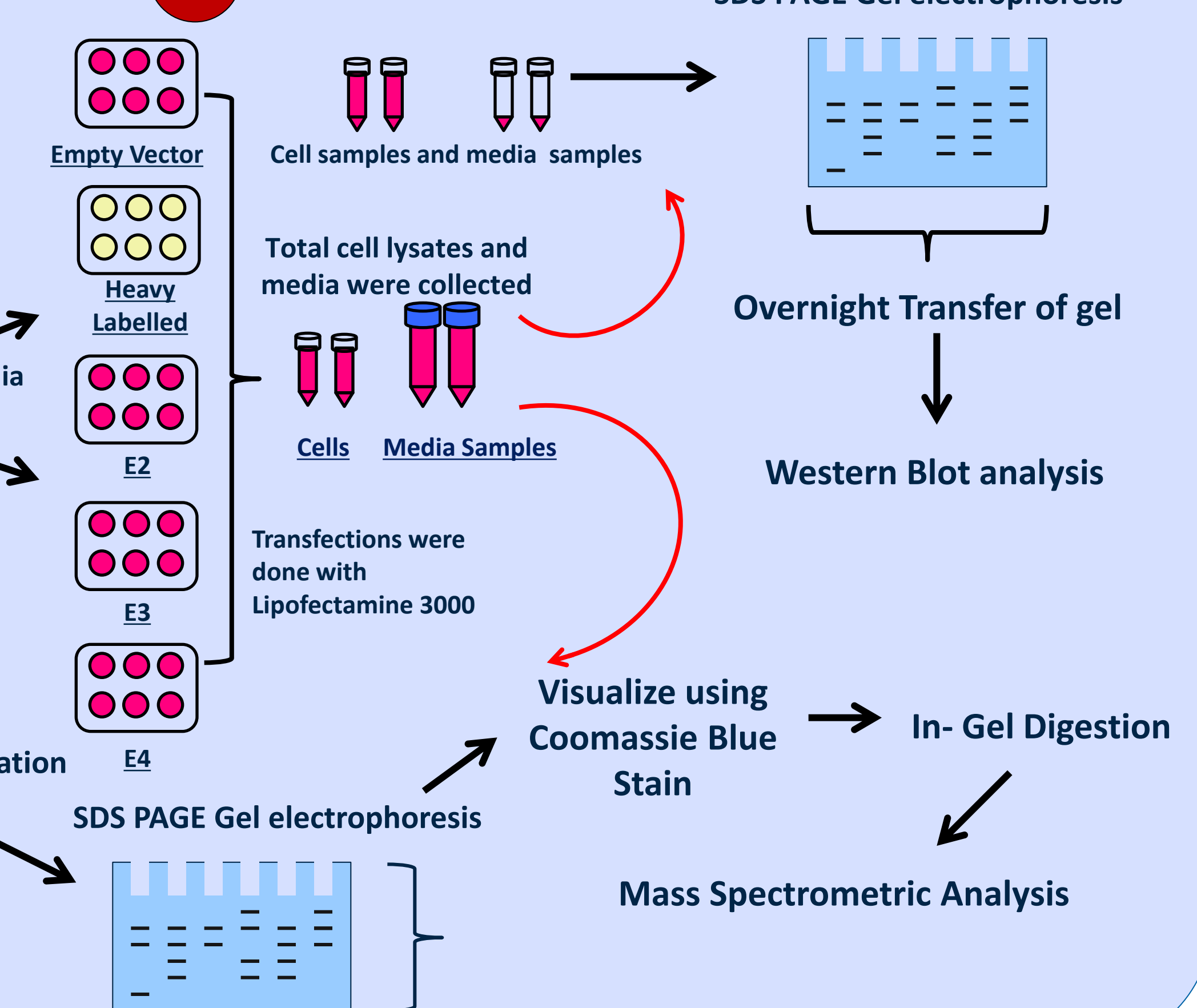
### 3 Cell Culture and SILAC Labeling



### 4a Interactome Analysis



### 4b Secretome Analysis



## Results

### Validation of Successful Transfection using Immunoblotting of total cell lysates and media

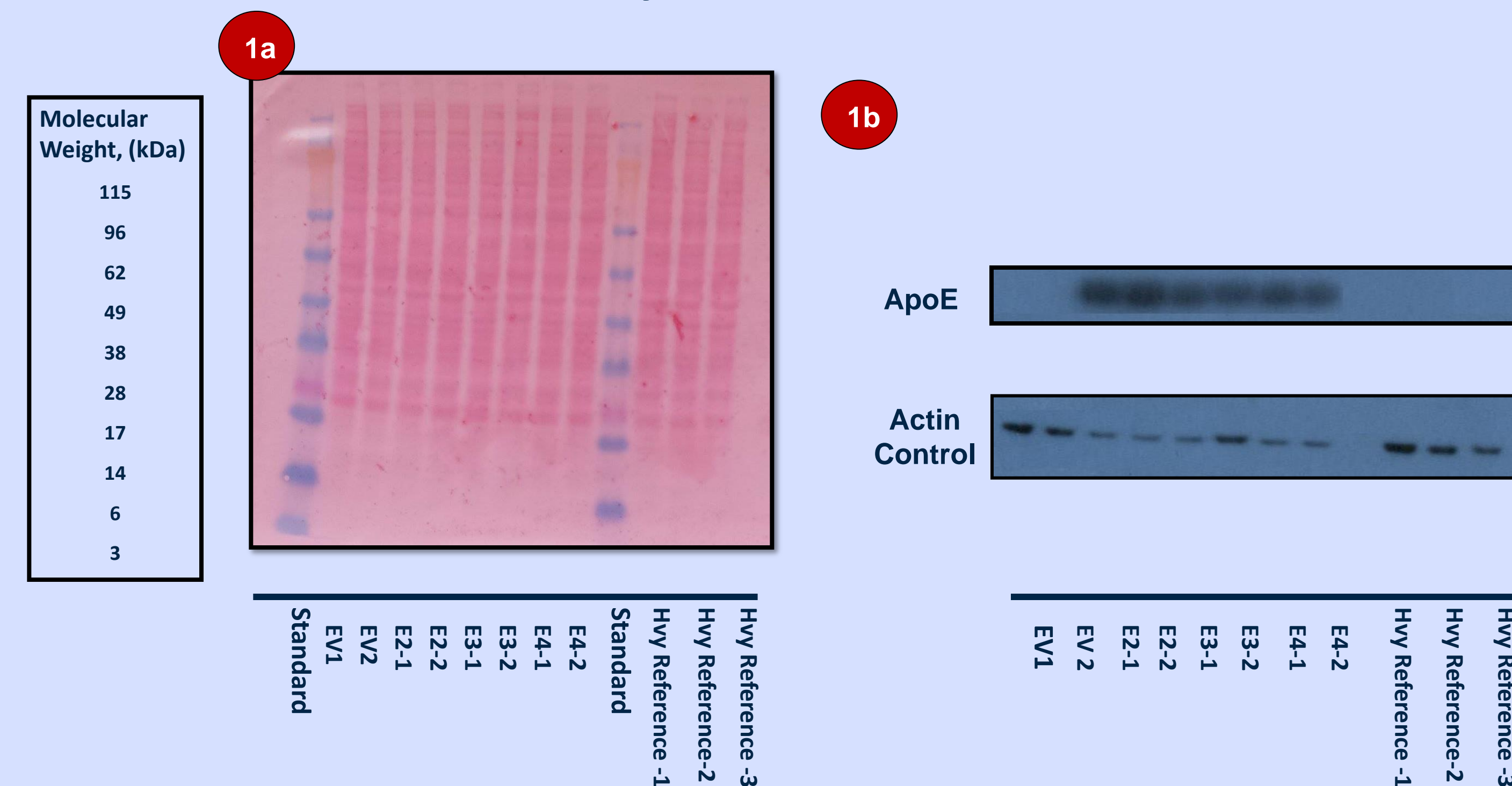


Figure 1: Ponceau method was used to ensure the transfection of the cells were successful. Various proteins of different sizes occupy different spaces on the Gel. Figure 1a illustrates the samples of total cell lysates as well as the media samples in the N2A cells. The empty vector samples (EV) can be used to compare against the bands present on the media samples and total cell lysate samples to ensure the transfections of the ApoE isoforms occurred successfully. Figure 1b illustrates the Western Blotting of the following samples and the Actin is used as a loading control. From the western blot, it is evident that the empty vector does not contain a significant amount of ApoE in comparison to the transiently transfected samples. This showcases that the transfection was effective and that the Empty vector can now serve as a valid control.

## Results

### APOE Co-Immunoprecipitation for Interactome Analysis from Secretome

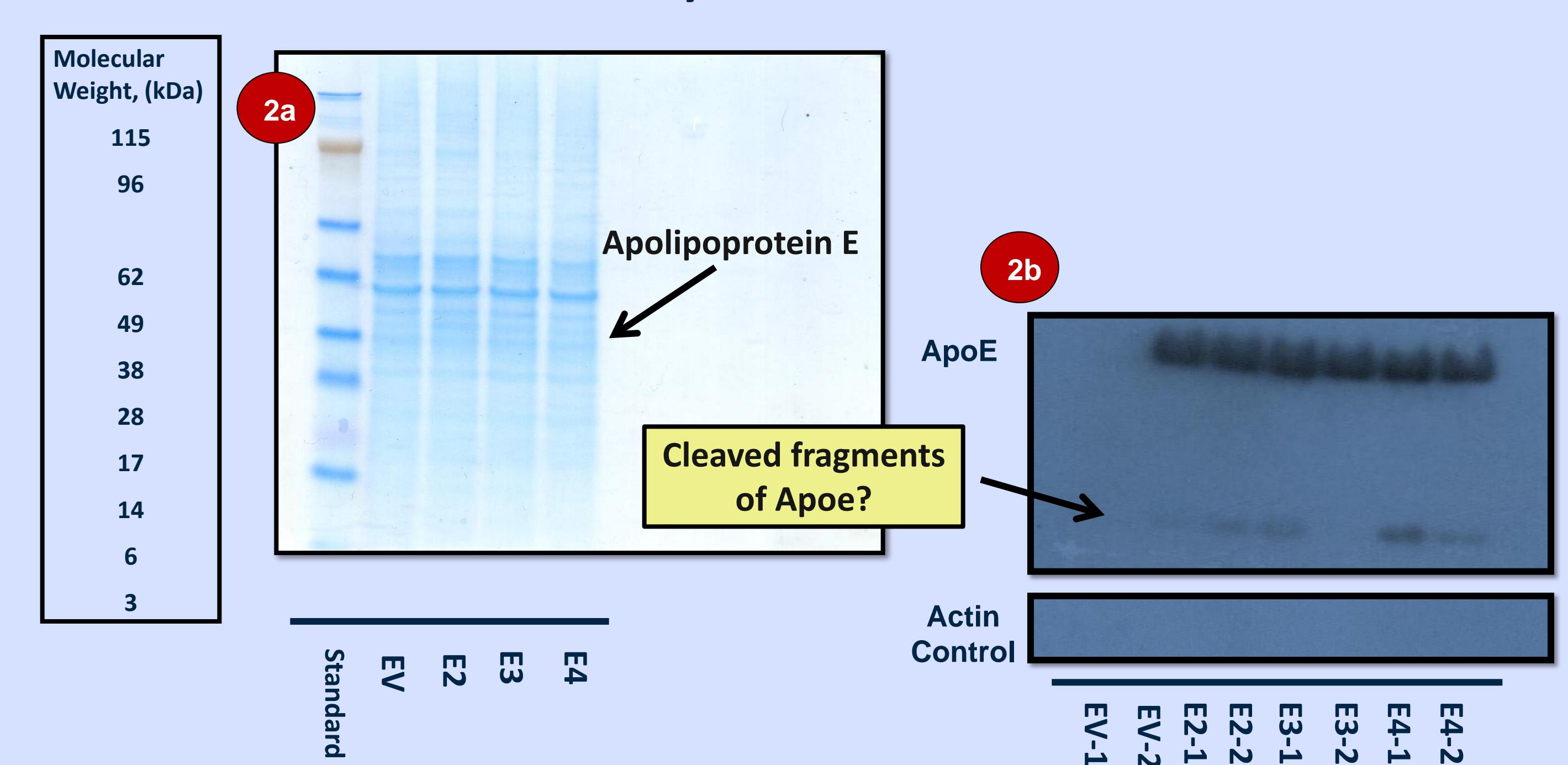


Figure 2: Media collected from N2A cells was used for Co-Immunoprecipitation of ApoE, its variants and interacting proteins. Figure 2a this was done in order to see the presence of APOE variants E2 and E4 against the normal isoform E3. The empty vector is used as a negative control in comparison to the transiently transfected mutant ApoE containing cells. Additionally, the presence of bands of interacting proteins were also observed and will be the proteins of interest when analyzing through mass spectrometry. Figure 2b showcases the western blot for the corresponding samples and the actin control as well for the secretome of the N2A cells. The actin was not present in the samples since the samples were media samples and actin is primarily present intracellularly. Additionally, it is evident that the empty vector does not contain a significant amount of ApoE in comparison to the other transfected samples. This showcases that the transfection was effective and that the empty vector (EV) can now serve as a valid control. Finally, the presence of other proteins lower on the western blot (18-24 kDa) can be attributed to the fact that a compartment ApoE undergoes proteolysis in the extracellular space via a secreted neuronal protease. ApoE is cleaved in neuronal conditioned media by a secreted serine protease. This is also known to be more effective in hippocampal neural cell lines such as the N2A cells.<sup>5</sup>

## Objectives

Discover extracellular interactions of ApoE in human liver and neural cell lines containing ApoE2 and ApoE4 variants compared to ApoE3.

### What are we interested in?

- 1 Secretome: secreted proteins of the cell
- 2 Secreted APOE Interactome: secreted proteins of the cell which interact with ApoE

## Conclusion & Future Work

Western blots confirm the transfection of the variants into cell lines (N2A) and their secretion. In essence, from the Co-Immunoprecipitation it was concluded that there is a clear presence of protein-protein interactions of APOE in the N2A cells. These unknown proteins are interacting with the APOE protein extracellularly as the band on the Co-Immunoprecipitation showcases the presence of these proteins.

### Future Directions

In order to identify the proteins that are interacting with APOE in the secretome, an In-gel digestion will be done followed by mass spectrometry analysis to identify the various protein sequences of the interacting proteins. Additionally, for the secretome analysis, heavy media will be combined at a 1:1 ratio with light media and mass spectrometry analysis will be done to identify proteins that are being differentially secreted by ApoE3 in comparison to control EV and in comparison to ApoE2 and ApoE4 transiently transfected cell lines. Validation of changes will be carried out by immunoblotting and ELISA.

## Acknowledgements

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