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TurboID Proximity Labeling of a Protocadherin Protein to Characterize Interacting Protein Complex

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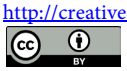

Abstract

The study of the neuron has always been a fundamental aspect when it came to studying mental illnesses such as autism and depression. The protein protocadherin-9 (PCDH9) is an important transmembrane protein in the development of the neuron synapse. Hence, research on its protein interactome is key to understanding its functionality and specific properties. A newly discovered biotin ligase, TurboID, is a proximity labeler that is designed to be able to label and observe transmembrane proteins, something that previous methods struggled with. The TurboID method is verified in HEK293T cells and primary cultured mouse cortical neurons. Results have proven the validity of the TurboID method in observing PCDH9-interacting proteins.

Keywords

TurboID, PCDH9, Proximity Labeling, Protein Interactome, Synapse Development

1. Introduction

The protein PCDH9 (protocadherin9) is a part of the protocadherin protein family, which is part of the cadherin superfamily of proteins. Protocadherins (Pcdhs) exist in two forms throughout Kingdom Animalia: there are non-clustered Pcdhs that exist in multiple zones of the genome; and clustered Pcdhs that come in three specific gene clusters named α, β, γ [1]. As a transmembrane protein, Pcdhs contain three domains: extracellular, transcellular, and intracellular [2]. Pcdhs serve a particularly important role in the development of synapses: Pcdh

genes are activated and expressed throughout the developmental phase of the synapse, and various subsets of the Pcdhs are expressed differently in each neuron. This results in myriads of potential forms of cell surfaces that can arise from different combinations and expressions of different Pcdhs, potentially influencing how synapses grow into their functional form [3]. Because of their importance in synapse development, Pcdhs are integral to the study of neurodevelopmental and neurological illnesses: specifically looking at PCDH9, it is able to cause "abnormal cortical development, abnormal long-term social and object recognition, hyperactivity, and abnormal sensorimotor functions in PCDH9 KO mice", and expressed in various brain regions. Therefore, it is likely involved in various neural functions including fear extinction, optokinetic response (OKR), cognitive function, and sensory processing [4].

To study the role of PCDH9 protein, information needs to be gathered on its interactome. A recent breakthrough in proximity labeling methods has yielded a new biotin ligase, TurboID that is very well suited for investigating PCDH9. This new proximity labeler was engineered using yeast-directed evolution, and it has the property of faster labeling kinetics while still maintaining non-toxic labeling conditions that make biotin ligases ideal for in vivo applications [5]. Traditional methods like BioID have been extensively used, but are limited by low activity and long labeling times [6]. The neuronal synapse has traditionally been a difficult area to investigate, a challenge that TurboID was designed to overcome. As aforementioned, BioID is limited by its very low or undetectable activity in subcellular, membrane-bound compartments. TurboID has a much higher affinity for biotin, making it a much more active labeler in comparison—it is "the most active biotin ligase-based method" [5].

This project aims to investigate the protein interactome of PCDH9 in the neuronal synapse using the proximity labeling method of TurboID. This new method can overcome the difficulties of traditional proximity labeling methods, and help identify key proteins that play important roles in neurodevelopmental and neurological diseases.

2. Methods

2.1. DNA Assembly

2.1.1. DNA Construction

The map of the pUltra-PCDH9-TurboID construct is built using the SnapGene application and the NEBuilder website. The HA-tagged TurboID gene, along with the PCDH9 gene is cloned into the pUltra plasmid (**Figure 1**).

2.1.2. PCR

After obtaining the plasmid containing PCDH9 and TurboID, PCR needs to be carried out so that its quantity reaches a certain amount that is needed by the experiment. Firstly, the plasmid containing PCDH9 and TurboID genes needs to be diluted using ddH_2O to a concentration of 100 ng/μl.

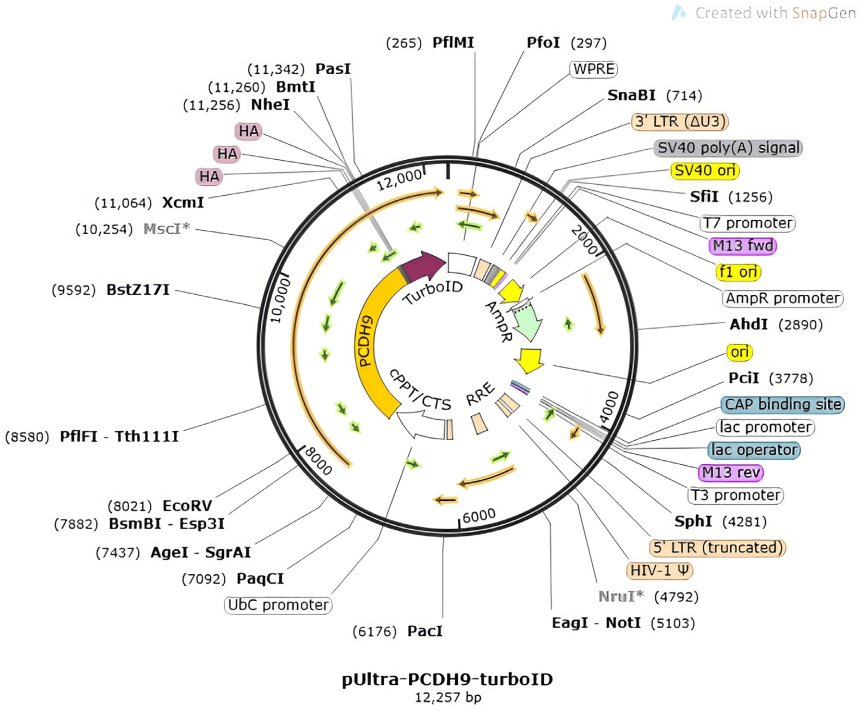

Figure 1. Construction of pUltra-PCDH9-TurboID Plasmid Gene.

The PCR solution requires the following (**Table 1**):

The PCR process is then conducted using a PCR machine. The programming of the PCR is as follows: (**Table 2**).

3. DNA Construct Verification

3.1. DNA Gel Electrophoresis

Agarose gel is used in this process: 20 - 40 ml TAE solution is used, along with a certain quantity of agarose based on the concentration desired. 0.6% was used in this experiment: 20 × 0.6% = 0.12 grams of agarose. The solution is heated until the agarose is completely dissolved (cannot be observed with the naked eye), and DNA dye is added at a ratio of 1:20,000—1 μl for 20 ml of TAE. The final solution is molded by pouring it into a container with a specific shape and size, containing the appropriate brush. After 30 - 60 minutes, the solution should have solidified into an agarose gel.

After the gel has solidified, it is removed from the container and placed into the electrophoresis tank, submerged under TAE solution. The products from PCR are used in 5 ul quantities; DNA loading solution is added at a 1:5 ratio, which means 1 ul is added to the 5 ul of PCR products for a total of 6 μl. Each 6 μl unit is added to a slot on the agarose gel—the slots are made through the brush attached to the container. The first slot is for the DNA marker, which acts as a ruler for the PCR products to compare. The remaining slots are for the 6 μl PCR product-DNA loading solution mixes.

After all, solutions are added, the power source is activated, and the electrophoresis is run for 30 - 40 minutes at 30 volts.

Table 1. Composition and amount of PCR solution.

Composition	Volume
ddH_2O	18 μl
DNA buffer	25 μl
DNA mix	1 μl
upstream primer	2 μl
downstream primer	2 μl
DNA polymerase	1 μl
DNA template	1 μl

*The end solution should have a volume of 50 μl.

Table 2. PCR procedure and corresponding factors.

1	95˚C	30 s
2	95˚C	15 s
3	65.3˚C	15 s
4	72˚C	1 min
5	72˚C	5 min
6	4˚C	5 min

When the electrophoresis process is finished, the gel is removed and observed under a GelImager to confirm that the construct is of the correct length. The PCDH9-TurboID gene should have a length of around 3000 base pairs, which can be identified using the marker.

3.2. DNA Gel Recovery

The agarose gel used in step 2.1 is placed under a UV light source and makes the DNA strand within visible. A blade is used to cut off the largest DNA piece—the amount of gel removed should be minimized. The DNA gel piece should then be stored inside an EP tube. (The following steps are based on the Vazyme DNA recovery kit; a separate kit may also be used)

After DNA electrophoresis, the DNA gel should be cut off from the gel, and placed in an EP tube. Heat a mixture of DNA gel and 1 volume of buffer GDP at 50˚C for 7 - 10 minutes. Transfer the solution 700 μl at a time into a collection tube containing a mini filtration column. Centrifuge the tube at 12,000 rpm for 1 minute. Discard all filtrate, and add 300 μl of GDP buffer to the filtration column. Place at room temperature for 60 s, then centrifuge at 12,000 rpm for 60 s.

Discard all filtrate, and add 300 μl of GDP buffer to the filtration column. Centrifuge the solution at 12,000 rpm for 1 minute. After discarding the filtrate, centrifuge the empty tube at 12,000 rpm for 2 minutes. Place the column in an EP tube, and add 25 μl of ddH_2O to the center of the column membrane. After 2 min, centrifuge at 12,000 rpm for 1 minute. Store EP tube at −20˚C.

3.3. Gibson Assembly

The DNA from step 2.2 is tested for its concentration.

We used a 1:2 ratio for the vector and gene in the Gibson assembly. We wanted to assemble three genes: the PCDH9 gene, the TurboID gene, and the pUltra plasmid gene. The volumes calculated are 0.95 μl, 0.2 μl, and 1.41 μl respectively, to reach the specific weight requirements for the genetic material. ddH_2O is added until the total volume of the solution reaches 5 μl. The mixture is heated in a PCR machine at 50˚C for 1 hour, then cooled down at 4˚C for 5 - 10 minutes.

3.4. Transformation

The stable 3 competent cell is used in this transformation process, because of its ability to reduce recombination. The experiment will involve viral transduction in later steps, which have a high base level of mutations.

The stable 3 cells are collected from the −80˚C refrigerator and 10 μl of Gibson assembled DNA product from step 2.3 is added. The mixture is left on ice for 30 minutes, then heated at 42˚C for 30 s, then returned to ice for 2 minutes. 300 μl of liquid LB is added for bacterial nutrition. The mixture is cultured in a 37˚C environment for 30 - 45 minutes, then added to solid LB. The agar plate is then placed in a 37˚C storage chamber, placed upside-down for 12 - 16 hours.

3.5. Colony Culturing

After the 12 - 16 hours phase of step 2.3 has elapsed, the agar plate is removed from the 37˚C environment.

4 - 6 10 ml tubes are prepared. Add 3 ml of LB with ampicillin content into each tube.

Use an Eppendorf pipette tip to gently touch a colony on the solid LB, then place the tip into the LB solution. Repeat until each tube contains a single tip from a different colony. Place the tubes into a 37˚C lab shaking machine overnight (15 - 18 hours)

3.6. Plasmid Extraction

(The following steps are based on the Magen Hipure plasmid micro kit; a separate kit may also be used).

The tubes are removed from the shaking device after 12 hours. 1.5 ml of solution from each tube is added to an EP tube. Centrifuge for 1 minute at 13,000 rpm. Repeat for the remaining 1.5 ml solution.

Remove all solutions in the EP tube. 200 μl of buffer P1 is added, and the solution is vortexed until it turns milky. Add 200 μl of buffer P2, and rotate the EP tubes so that it fully mixes. 300 μl of buffer P3 is added, and the EP tubes are rotated to mix the solution. Centrifuge for 10 minutes at 13,000 rpm. Prepare spin columns, attached to collection tubes.

Add the solution in the EP tubes into the spin columns, then centrifuge for 1

minute at 13,000 rpm. Dispose of the collection tube filtrate, then add 600 μl of buffer PW2 and centrifuge the spin columns at 13,000 rpm for 1 minute. Remove the filtrate, and centrifuge at 13,000 rpm for 1 minute. Place the spin columns in 1.5 ml EP tubes. Add 20 - 30 μl of pre-heated ddH_2O to the spin column, and centrifuge at 13,000 rpm for 1 minute. After discarding the spin columns, the content in the EP tubes contains the desired plasmids.

3.7. Enzyme Cleavage Identification

To confirm that the Gibson assembly process (step 2.3) has successfully linked the DNA fragment of TurboID, PCDH9, and pUltra, we use enzymatic cleavage.

The results of plasmid extraction are then tested for DNA concentration. 2 μl of plasmid solution is needed for a DNA concentration of 100 - 200 ng/μl. The plasmid is added to a mixture that contains 0.2 μl of EcoRI enzyme, 1 μl of cut smart buffer, and ddH_2O. The total mixture should have a volume of 10 μl, and the volume of ddH_2O should be adjusted accordingly. The mixture is placed in a 37˚C incubator for 4 hours.

Gel electrophoresis is conducted, identical to step 2.1, except that the content added to the gel slots are the mixtures concocted as above.

The results are observed under a GelImager, and a positive result for the Gibson assembly should include DNA fragments with base pair lengths of 1497, 1974, and 8752.

4. Cell Culturing

4.1. Culturing of 293T Cell

For the transfection part of the TurboID-PCDH9 experiment, the HEK293T cell was used. It should be grown at 37˚C using a cell culture medium that contains fetal bovine serum. The cultured batch is then placed in a 6 or 12-well plate.

The next step—transfection of plasmids—should take place once the density of the HEK293T reaches 70% - 80%.

4.2. Plasmid Transfection

A transfection mix is needed to conduct the transfection. The mix involves 2 parts.

Firstly, 1.2 μg of plasmid needs to be added—this can be calculated using DNA density. DPBS solution is added until the total volume is equivalent to 100 μl. In practice, DPBS should be added first.

A second mix of 100 μl PEI + DPBS mix is needed. This mix is placed on a vortex machine for 10 s, then placed still for 2 - 10 minutes. Afterward, the two mixes are added together to form a final 200 μl solution. The transfection solutions are added drop by drop to their respective wells. The wells are then stored in a 37˚C cell storage chamber.

(*In this experiment, there are 4 groups—a TurboID group without PCDH9, the C terminal of PCDH9, the N terminal of PCDH9, and a pUltra control

group.)

4.3. Biotin Addition

TurboID requires the addition of biotin as an activator to produce biotin-5'-AMP, to mark surrounding proteins. In this experiment, biotin is added 48 hours after the original transfection has occurred, and a 2-hour timeframe is given for TurboID to act.

As aforementioned, each of the 4 groups has 3 separate wells, with 0 μl, 100 μl, and 500 μl of biotin added respectively. The wells should be then placed in a 37˚C environment.

5. Protein Extraction and Analysis

5.1. Cellular Protein Extraction

After the 2 hours from the biotin addition have elapsed, take the wells containing the cells, and remove the existing solution within. Wash repeatedly with PBS.

All the following steps are conducted on ice. Add 250 μl of RIPA buffer, and spread it evenly across the well using an Eppendorf pipette. Use the pipette to transfer the protein precipitate into an EP tube. Repeat this step for each well. Wait for ten minutes, keeping the EP tubes submerged in ice. Then use a 4˚C centrifuge for the EP tubes at 12,000 rpm for 5 minutes. Add the streptavidin beads solution to the EP tubes. Store overnight on a rotating device at 4˚C.

5.2. Western Blot (WB)

The WB gel electrophoresis for this batch of protein products uses an 8% acrylamide gel. A 15-slot comb is used. Once the western blot gel is set up in a running buffer, the proteins in the EP tubes extracted from the cells are removed from the ice. 16 μl of the protein is used for each slot, meaning that the 5x SDS sample buffer required is 4 μl based on a 4:1 ratio.

A total of 12 slots are used up in this experiment, and the three remaining spots are for the WB markers that act similarly to DNA markers in agarose gel electrophoresis—they are a ruler by which the proteins can be compared.

After all the solutions are added, the electrophoresis power source is activated at 90 V for 30 minutes, then increased to 110 V for 1 - 2 hours. The machine is deactivated once the solution is run down to the bottom of the gel.

6. Virus Packaging

6.1. Transfection

15 cm dish:

The DNA mix that needs to be transfected into the cell is as follows: 22 μg lentiviral gene carrier, 15 μg helper plasmid e.g. psPAX2, 5 μg pMD2G coat protein, 2 μg pAdVantage vector. The plasmids above were transfected into HEK293T cells in a dish by PEI transfection reagent.

6.2. Purification

At 48 and 72 hours after the transfection, the viral medium is collected and stored at 4˚C. A chondroitin sulfate solution is needed: mix 64 mg of protamine sulfate, 14 g of NaCl, 20 ml of 10*PBS (pH 7.4), and 80 ml of ddH_2O. The solution is adjusted to pH 7.0 - 7.2, filtered through 0.22 μm, and stored at 4˚C.

The viral medium collected is centrifuged at 4˚C, 800 g for 10 minutes, then transferred to a new 50 ml test tube. Mix chondroitin sulfate and viral medium at a 1:3 ratio. Shake at 4˚C for 16 - 20 hours. Centrifuge at 4˚C, 4000 g for 60 minutes. Remove all medium solution, add neurobasal medium at 1% of the volume of the medium, vortex, and store at −80˚C.

7. Results

7.1. Plasmid Design and Construction

To make the construct express the PCDH9-3XHA-TurboID gene, the NEBuilder website was used to obtain the Gibson assemble a strategy to clone 3XHA-TurboID and PCDH9 gene into pUltra plasmid simultaneously. An ampicillin resistance gene coupled with ampicillin-positive LB cultures is used for screening of positively ligated colonies (**Figure 2(a)**). 3XHA-TurboID and PCDH9 genes are obtained by PCR amplification from a plasmid template that contains these genes. Gibson assembly is then carried out to add the 3XHA-TurboID and PCDH9 gene onto the pUltra plasmid. The transformation process used Stable 3 competent cells to minimize viral mutations in preparation for viral transfection. The E. coli bacteria were cultured using Amp+ LB followed by plasmid extraction. Restriction Enzyme cleavage and Sanger sequencing were conducted to verify the construct with correctly inserted DNA fragments (**Figure 2(b)**).

1) This is the result of the enzymatic cleavage after the plasmid extraction of the Gibson Assembly product. *The leftmost lane is DNA markers, and the sizes they represent are 500, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 8000, and 10,000 base pair (bottom to top). Based on the DNA fragment pattern after restriction enzyme digestion, these 3 plasmids all contain correctly inserted 3XHA-TurboID and PCDH9 genes.

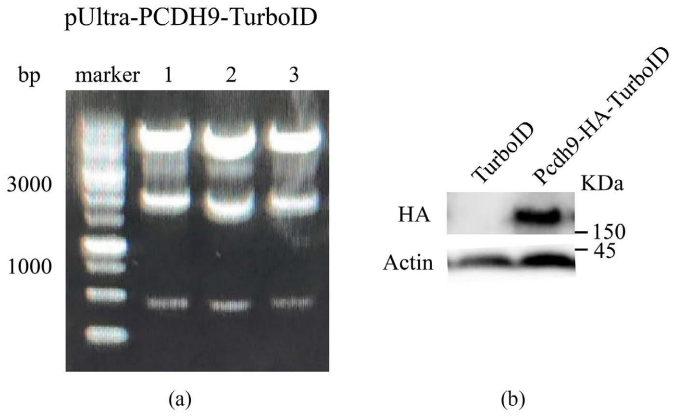

Figure 2. pUltra-PCDH9-Turboid plasmid construction

2) The PCDH9-TurboID construct was verified via transfection into HEK293T cells and the following western blot analysis with an antibody against the HA tag.

7.2. TurboID Construct Validation in HEK293T Cells

The plasmids confirmed for a positive result are then transfected into HEK293T cells. Firstly, 1.2 μg of plasmid needs to be added. DPBS solution should be added to make a total volume of 100 μl (In practice, DPBS should be added first). A second mix of 100 μl PEI + DPBS mix are needed. This mix is placed on a vortex machine for 10 s, then placed still for 2 - 10 minutes. Afterward, the two mixes are added together to form a final 200 μl solution. The final mix is added drop by drop to the separate wells depending on their content. The wells are stored away in a 37˚C environment.

After 2 days, the cells are retrieved from the storage chamber. To extract the proteins, PBS is used to wash the cells, then they are lysed. The proteins were run through a Western Blot, and the results are as follows:

After being washed with horseradish peroxidase, four groups are compared to each other. The PCDH9-TurboID group has yielded the most proteins out of this WB run. Under 200 μM of biotin, the most proteins were observed on average. In the 500 μM group, the PCDH9-TurboID and TurboID-PCDH9 groups demonstrated similar protein results, potentially hinting at a similarity between the C and N terminals of the PCDH9 protein (Figure 3).

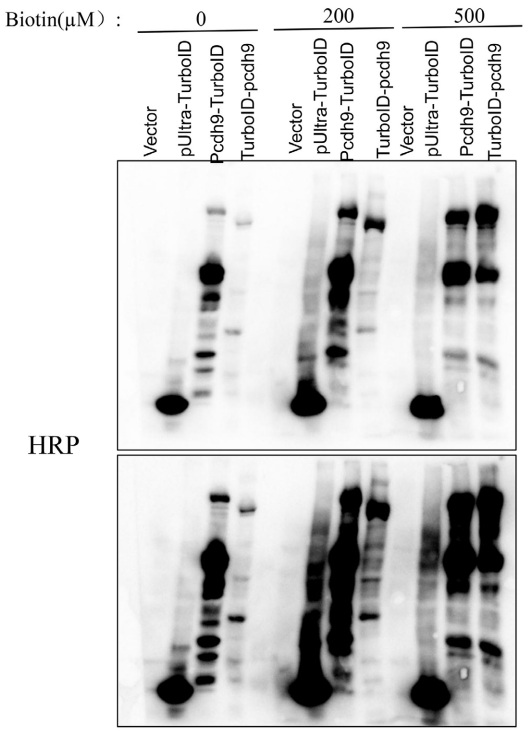

Figure 3. Characterization of biotinylation activity by PCDH9-TurboID. HEK293T cells stably expressing PCDH9-TurboID and TurboID-PCDH9 were treated with 200 μM or 500 μM biotin for 2 hours. Whole-cell lysates were analyzed by streptavidin-HRP blotting.

7.3. TurboID Biotinylation in Cultured Neurons

After neuronal transfection for 3 days, immunofluorescence is conducted to testify to the effectiveness of the TurboID construct. The results are as follows (**Figure 4**):

The staining results show that without biotin (0 μM), the fluorescence levels of proteins labeled with streptavidin-555 are particularly low. In contrast, neuron addition of 200 μM and 500 μM Biotin exhibited marked biotinylation efficacy, which was demonstrated by the strong level of signal released after incubation with Streptavidin-Alexa fluor 555, which binds with biotinylated proteins.

7.4. Viral Transfection Verification

To verify the transduction efficiency of the packaged virus, we added 1 ul and 5 μl of the control virus pUltra-GFP with a green fluorescent protein to HEK293T cells, respectively. Fewer cells were observed to be infected with 1 μl of pUltra-GFP after 72 hours, while a much higher level of infection efficiency was seen with 5 μl of pUltra-GFP (**Figure 5(a)**). In addition, we co-infected primary neurons in vitro with pUltra-GFP and found that the virus had infected neurons on the fifth day (**Figure 5(a)**). The expression of PCDH9-TurboID was verified by Western blot after collecting neurons on the tenth day. The results showed that PCDH9-TurboID had been expressed in neurons through virus infection (**Figure 5(b)**). These results prove that our virus packaging method is valid and successful.

Figure 6 is a workflow diagram that briefly summarizes the steps taken in this experiment.

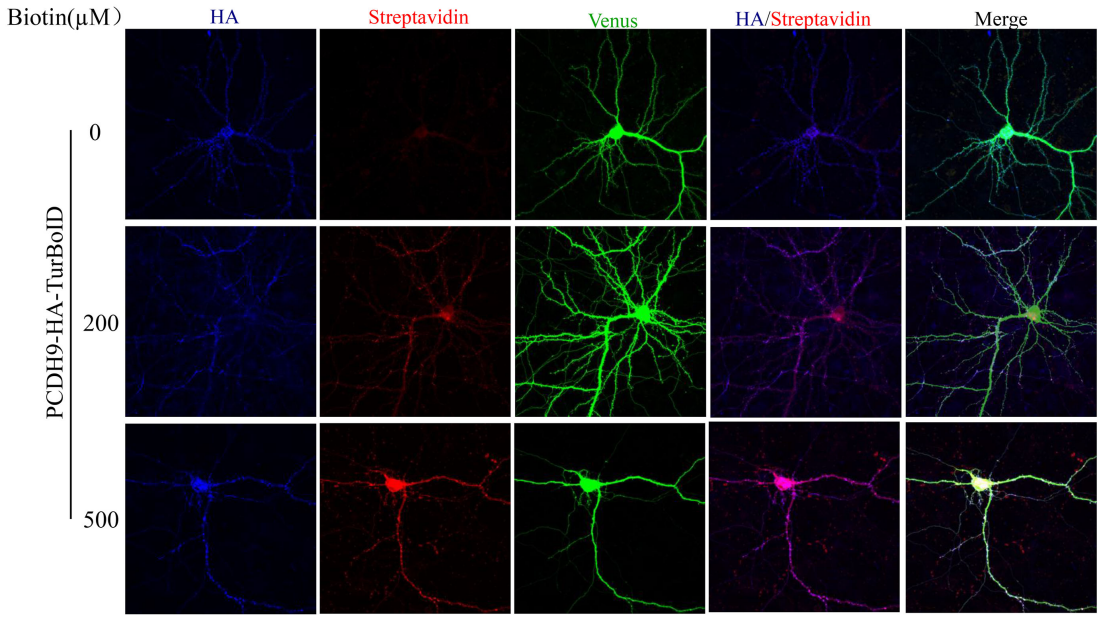

Figure 4. Confocal fluorescence imaging of primary neurons expressing PCDH9-3XHA-TurboID. Cells were treated with 200 μM or 500 μM biotin for 2 hours (+Biotin), and untreated cells (−Biotin) were used as a control.

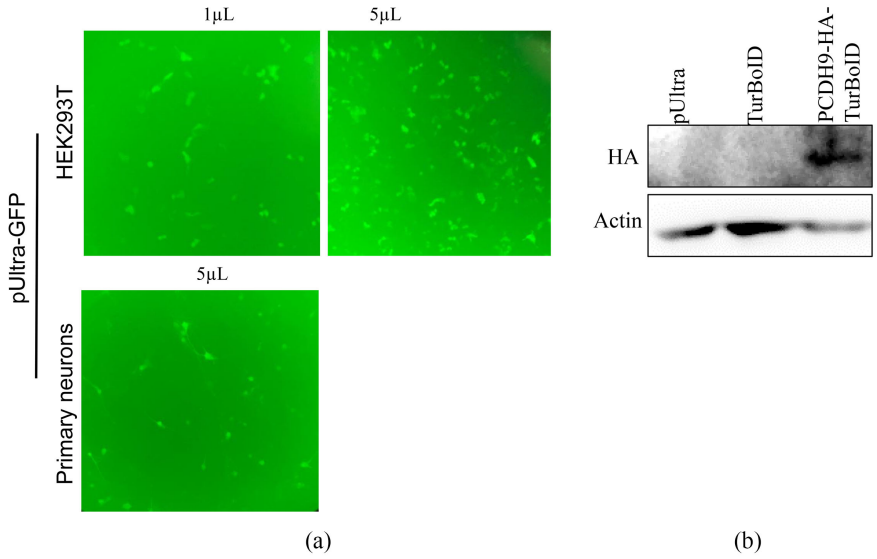

Figure 5. Virus infection efficiency verification. (a) Fluorescence images of HEK293T cells and primary neurons after pUltra-GFP virus infection, respectively; (b) Western blot validation after pUltra-GFP virus infection of primary neurons.

8. Discussion

This investigation uses TurboID, one of the newest inventions of proximity labeling technology to investigate the protein interactome of PCDH9. It was able to effectively combat the difficulties previous biotin ligases faced when mapping transmembrane proteins. At present, we have completed most of the preliminary work in this work (**Figure 6**), and the identification of the interacting proteins of PCDH9 in primary neurons by mass spectrometry will be the focus of our next work.

However, this experiment is still limited and could be improved in the following ways: The control group in this experiment was the pUltra gene and the TurboID gene which did not contain PCDH9. This control group could have been expanded by using a reference genome. This gene should contain the TurboID protein gene and a gene sequence that attaches it to a specific cellular structure. For example, for mapping the proteome of the cytosol-facing endoplasmic reticulum (ER) membrane (ERM), cytosolic TurboID-NES was used as a reference site [6]. As another example, for mapping the interactome of a transcription factor, TurboID fused to a nuclear localization sequence that targets it diffusely throughout the nucleus can be used as a reference [6].

TurboID is subject to a time restraint due to its toxicity when used in long-term experiments. TurboID's high affinity for biotin might overconsume it, leading to cellular dysfunction [7]. Short-term solutions involve adding biotin supplements; in the long term, less toxic methods such as BioID or AirID might be used as alternatives [8].

Such problems identified in this project could reveal potential paths for future researchers to investigate. This experiment mainly addresses the PCDH9 protein directly, and many alternative approaches can be taken.

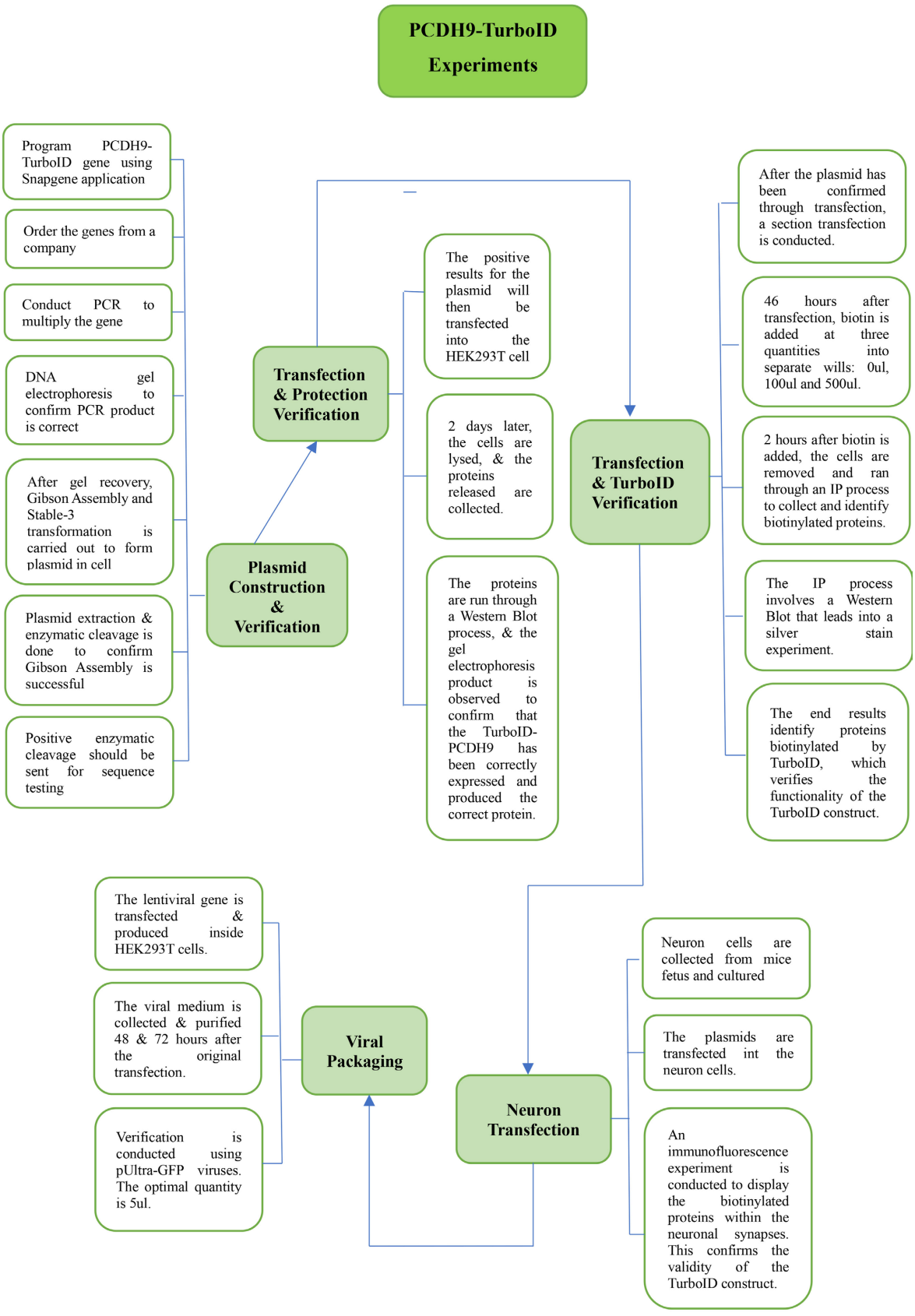

Figure 6. Workflow for the PCDH9-TurboID experiment.

As aforementioned, TurboID becomes much less useful in long-term observations. Because PCDH9 is a protein that mostly interacts in the development stages of the neuronal synapse, which is a process that can take several weeks, this extended period still requires more research [9]. The challenges of this process mainly surround observing PCDH9 for extended periods: currently, it is most effective by using TurboID, however, TurboID cannot create sustained output.

Although PCDH9 has been closely investigated function-wise, there has been a lack of practical studies and research that directly applies this knowledge to the medical area. Further research needs to be done on how the characteristics of PCDH9 can be used in analyzing mental illnesses such as autism and depression [10]. This experiment does hypothesize several potential functions of PCDH9 but has not yet reached a systematic conclusion.

In brief, we used the newly discovered biotin ligase TurboID to design a proximity labeler for the observation of the transmembrane protein PCDH9 in this study. The TurboID method has been verified in HEK293T cells and primary cultured mouse cortical neurons. Our results have proven the validity of the TurboID method in observing PCDH9-interacting proteins.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hulpiau, P. and Van Roy, F. (2009) Molecular Evolution of the Cadherin Superfamily. *The International Journal of Biochemistry & Cell Biology*, **41**, 349-369. https://doi.org/10.1016/j.biocel.2008.09.027

[2] Chen, W.V. and Maniatis, T. (2013) Clustered Protocadherins. *Development*, **140**, 3297-3302. https://doi.org/10.1242/dev.090621

[3] Hamada, S. and Yagi, T. (2006) Protocadherins: Classification, Structures and Function. *Seikagaku*, **78**, 662-671.

[4] Uemura, M., Furuse, T., Yamada, I., *et al.* (2022) Deficiency of Protocadherin 9 Leads to Reduction in Positive Emotional Behaviour. *Scientific Reports*, **12**, Article No. 11933. https://doi.org/10.1038/s41598-022-16106-5

[5] Branon, T.C., Bosch, J.A., Sanchez, A.D., *et al.* (2018) Efficient Proximity Labeling in Living Cells and Organisms with TurboID. *Nature Biotechnology*, **36**, 880-887. https://doi.org/10.1038/nbt.4201

[6] Cho, K.F., Branon, T.C., Rajeev, S., *et al.* (2020) Split-TurboID Enables Contact-Dependent Proximity Labeling in Cells. *Proceedings of the National Academy of Sciences of the United States of America*, **117**, 12143-12154. https://doi.org/10.1073/pnas.1919528117

[7] Zhang, Y., Li, Y., Yang, X., Wen, Z., Nagalakshmi, U. and Dinesh-Kumar, S.P. (2020) TurboID-Based Proximity Labeling for in Planta Identification of Protein-Protein Interaction Networks. *Journal of Visualized Experiments*, No. 159. https://doi.org/10.3791/60728-v

[8] Schopp, I.M., Amaya Ramirez, C.C., Debeljak, J., *et al.* (2017) Split-BioID a Condi-

tional Proteomics Approach to Monitor the Composition of Spatiotemporally Defined Protein Complexes. *Nature Communicationsions*, **8**, Article No. 15690. https://doi.org/10.1038/ncomms15690

[9] Bruining, H., Matsui, A., Oguro-Ando, A., *et al.* (2015) Genetic Mapping in Mice Reveals the Involvement of *Pcdh*9 in Long-Term Social and Object Recognition and Sensorimotor Development. *Biological Psychiatry*, **78**, 485-495. https://doi.org/10.1016/j.biopsych.2015.01.017

[10] Xiao, X., Zheng, F., Chang, H., *et al.* (2018) The Gene Encoding Protocadherin 9 (*PCDH*9), a Novel Risk Factor for Major Depressive Disorder. *Neuropsychopharmacology*, **43**, 1128-1137. https://doi.org/10.1038/npp.2017.241