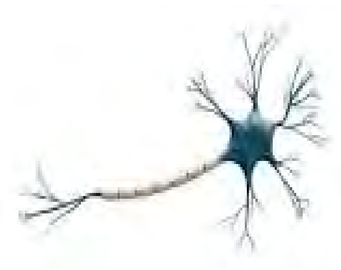
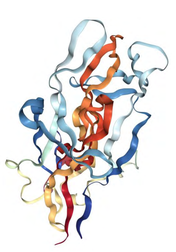
In-house production of brain-derived neurotrophic factor (BDNF) for differentiation of SH-SY5Y cells into matured neurons

Chonticha Saisawang[1,*], Suphansa Priewkhiew[1], Chutikorn Nopparat and Piyarat Govitrapong[1,2]

Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom 73170, Thailand
Chulabhorn Graduate Institute, Chulabhorn Royal Academy, 54 Kamphaeng Phet 6 Road Lak Si, Bangkok 10210, Thailand
*e-mail: chonticha.sai@mahidol.ac.th

Rationale

"As the commercially available BDNF can be a continuous expense we have produced a more economical alternative by cloning, expressing and purifying a recombinant BDNF protein in our laboratory and then comparing it to commercially available one"

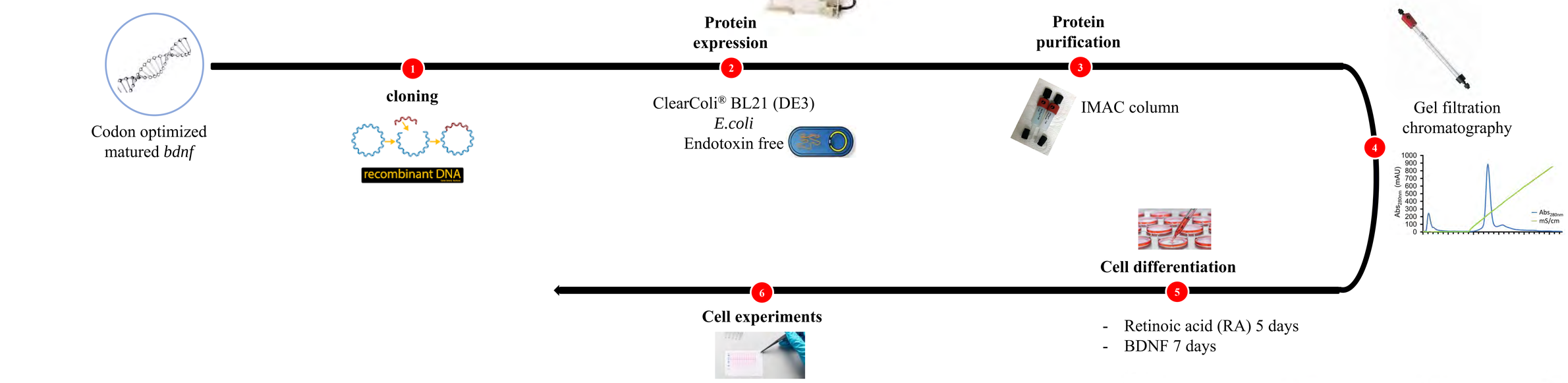
Methodology

Protein production

357 bp of synthetic mature *bdnf* was amplified by PCR and cloned into pET21b vector. The recombinant DNA was verified by DNA sequencing before transforming into expression host. Since the purpose of this project is to use BDNF to differentiate the cell, therefore the ClearColi® BL21 (DE3) endotoxin-free host was selected. This *E.coli* strain was engineered with a modified LPS that does not trigger the endotoxic response in human cells. The protein was expressed at 18°C overnight. The cells were lysed by sonication. The supernatant was applied onto immobilized metal affinity chromatography (IMAC). The partially purified protein was polished up by gel filtration column (Superdex™ 200 increase 10/300 GL).

Cell differentiation

SH-SY5Y cells was culture in DMEM/F12 with appropriate supplements. At 70% cell confluency, the differentiation process was started by adding 10 µM retinoic acid (RA) for 5 days with medium change at day 3. On day 5 cells were washed 3 time with DMEM/F12 media without FBS and incubated in this medium containing 50 ng/mL BDNF for 7 days. Medium was replaced on day 3 and 6.



Results

The recombinant BNDF was eluted out from gel filtration column as a single peak corresponds to 18 KDa monomer BDNF protein. However the pure protein was shown as multimeric form on denaturing SDS-PAGE.

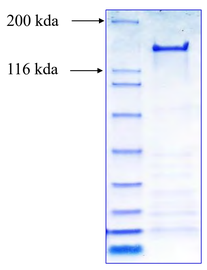


Fig 1: The purity of in-house BDNF protein
1 ug of purified BDNF protein was run on 4-20% Mini-Protean®TGX ™ Precast Protein gel (Bio-Rad) and stained with coomasie blue. Lane 1 is broad-range protein marker (Bio-Rad)

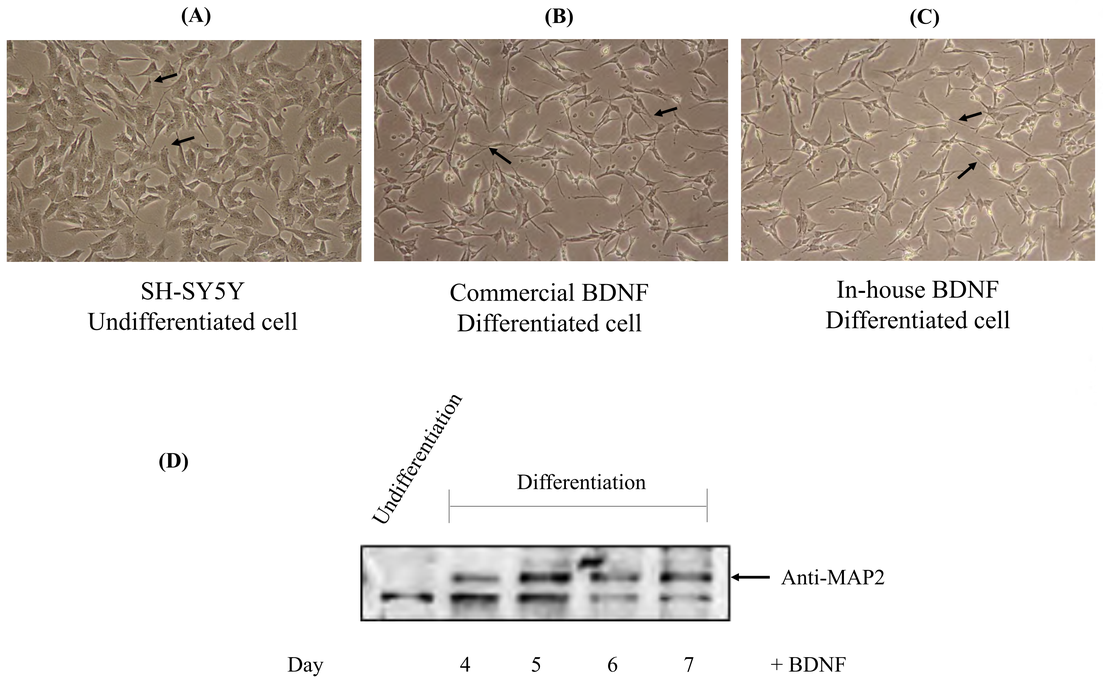


Fig 2: Cell morphology observed under inverted phase-contrast microscopy
(A) Undifferentiated SH-SY5Y cells, arrows show short cellular projection. (B,C) Differentiated neuronal cells after sequential incubation with 10µM retinoic acid for 5 days and 50 ng/ml of commercial and in-house BDNF, respectively for 7 days, arrows show extension and neurite outgrowth. (D) Time course of days 4, 5, 6 and 7 differentiation using in-house BDNF with detection by western blot of microtubule associated protein 2 (MAP2) protein expression as the marker for mature neuronal cells.

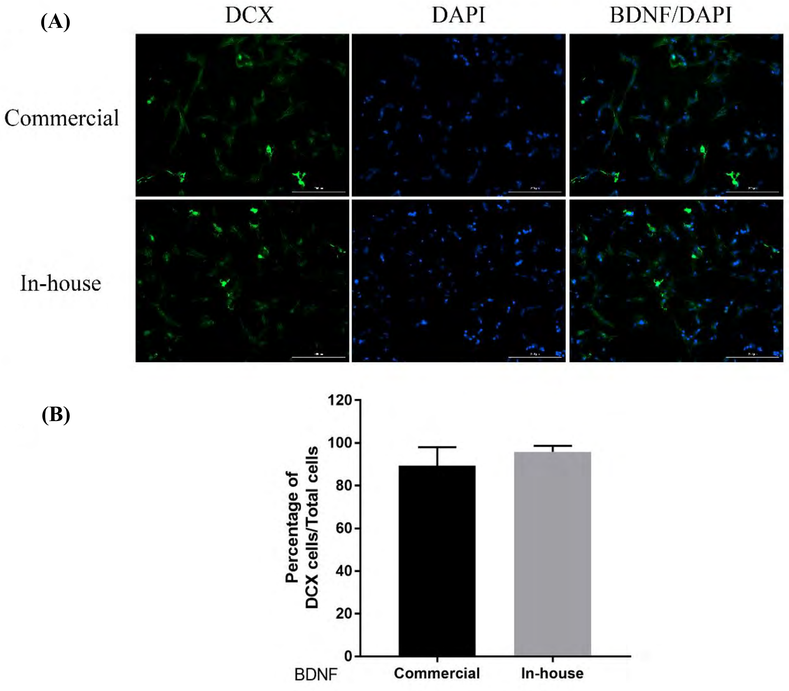


Fig 3: Comparison of commercial and In-house BDNF in neural differentiation. (A) Representative images of the doublecortin (DCX)-stained cells and DAPI-stained cells. DCX stained cells are shown in green and DAPI stained cells are shown in blue. These images were obtained at 10× magnification using a Fluoview FV300 Microscope. (B) Counting of differentiation neurons with DCX immunostaining. The total number of cells and the number of cells containing DCX-stained cells were quantified in each group. The percentage of DCX positive cells in each group compared with the total cell. The data are expressed as the means ± S.E.M. unpaired t test was performed for statistical analysis.

Discussions

We have successfully produced the recombinant brain-derived neurotrophic factor (BDNF) that is economically friendly for in-house use.

Acknowledgement

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