Cloning and expression of fluorescently labeled α-synuclein in *Escherichia coli*

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Introduction

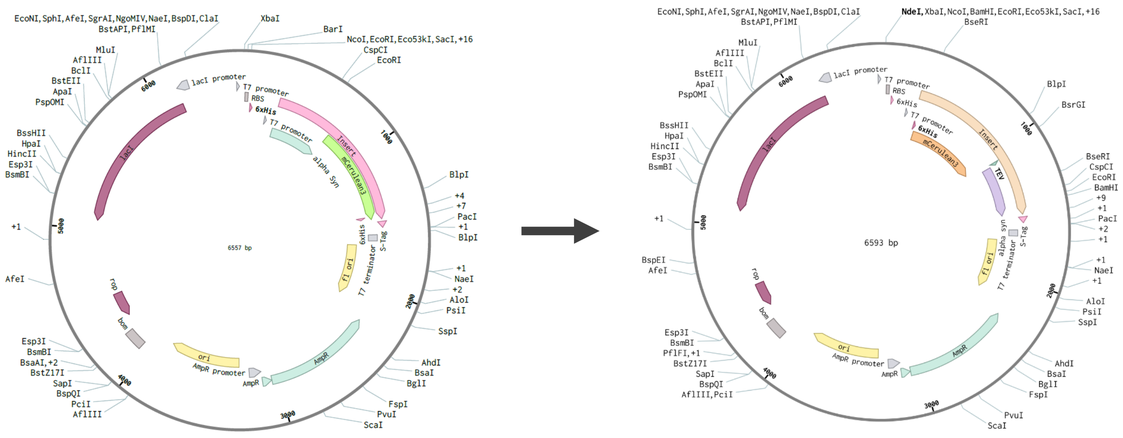
- Understanding Parkinson's and other related neural diseases requires a large amount of pure α-synuclein to conduct *in vitro* experiments.
- Fluorescent proteins are an indispensable tool in research for many purposes (e.g. *in vivo* localization studies, studies of protein interactions, …).
- The mCerulean3 – α-synuclein fusion was already shown to express with a very high yield in *E. coli.*

Objectives

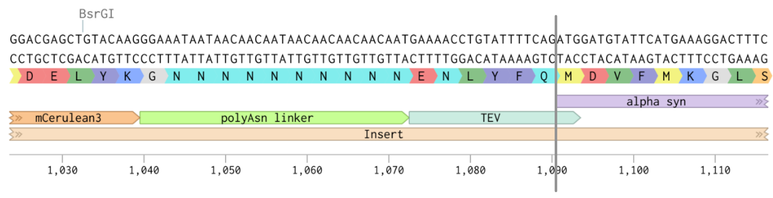
- Creating a vector for the **expression of α-synuclein** N-terminally fused with **His_6-mCerulean3** through a polyasparagine linker and a **TEV proteolytic site**.
- Expression of the chimeric protein in *E. coli* BL21(DE3).
- Proteolysis by TEV protease.

Methods

- **α-synuclein** and **mCerulean3** were amplified from the commercial vector **pDUET-1-alpha-synuclein-mCerulean3-His6** by **PCR**.
- A **bridge region** was either synthesized from primers or added to the ends of the previous PCR products by another round of PCR.
- The fragments were assembled into the final gene by **SOEing PCR**.
- The gene was **subcloned into the pET-20b vector**, then **cloned into the starting vector backbone (pDUET-1)**.



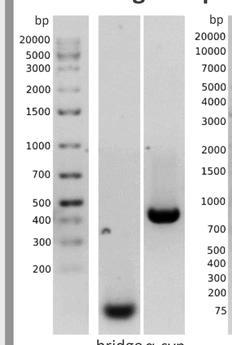
- Recombinants were screened for by **colony PCR** and the DNA isolated by a commercial kit (from ***E. coli* DH5α**), then **sequenced**.
- The obtained plasmids were transformed into ***E. coli* BL21(DE3)**, then expressed and purified by **IMAC**.
- The obtained proteins were proteolyzed by **TEV protease**, releasing **His6-tagged mCerulean3** and **untagged α-synuclein**.



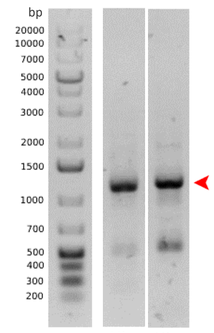
Results

- Three vectors were successfully created: one with the chimeric gene in pET-20b (possibility of periplasmic expression), two with the chimeric gene in pDUET-1 (His_6 and His_8).
- The protein was successfully expressed (~2 mg/liter of medium), purified by IMAC and cut by TEV protease.

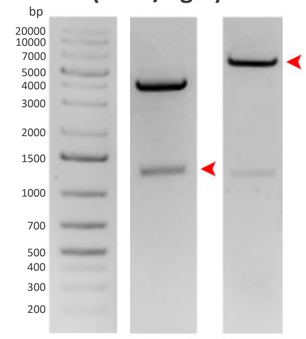
Starting PCR products



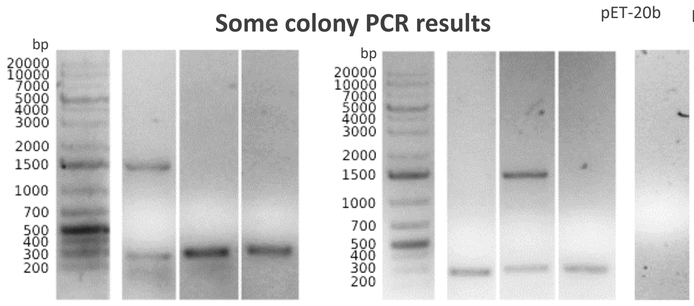
SOEing PCR products



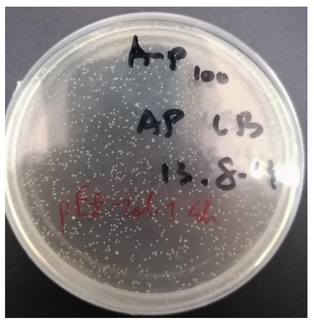
Restriction products (NdeI/BglII)



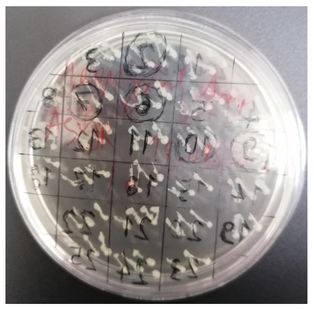
Some colony PCR results



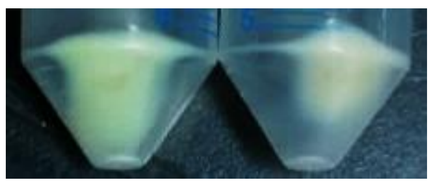
Agar plate with transformants



Colony PCR agar plates



Bacterial pellet after expression

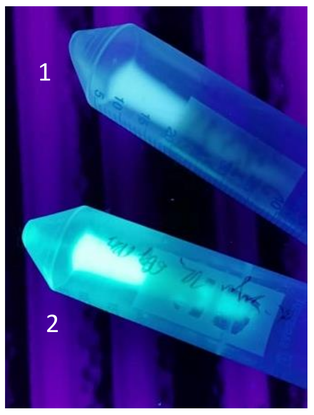


E. coli transformed with the construct | *E. coli* transformed with a non-fluorescent protein

Proteolysis mixture under UV

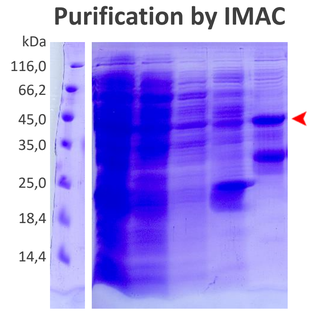
Protein + TEV | Protein | TEV

Bacterial pellet under UV



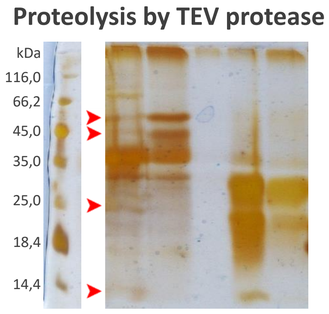
1– *E. coli* expressing a non-fluorescent protein
2– *E. coli* expressing the chimeric protein

Purification by IMAC



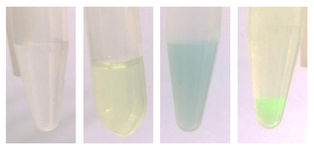
1 – Applied 2 – Flow-through
3 – Wash with Tris-NaCl buffer
4 – Elution with Tris-NaCl 50 mM imidazole buffer
5 – Elution with Tris-NaCl 300 mM imidazole buffer

Proteolysis by TEV protease



1 – Protein + TEV protease
2 – Protein 3 – TEV protease
4 – TEV positive substrate + TEV protease
5 – TEV positive substrate

IMAC



Buffer | Starting mixture | Matrix | Matrix with bound protein

Future steps

- Protein confirmation by MS
- Western blot confirmation
- Checking protein localization
- Expression optimization
- Proteolysis optimization
- Further purification
- Conformational analysis
- Interactions with other proteins

Acknowledgement

The authors acknowledge the support of the Science Fund of the Republic of Serbia (PROMIS project LEAPSyn-SCI, grant no. 6039663) and by the Ministry of Education, Science and Technological Development, the Republic of Serbia (Contract No. 451-03-68/2022-14/200168).