

# Uncovering the function of a novel gene involved in neurodegeneration

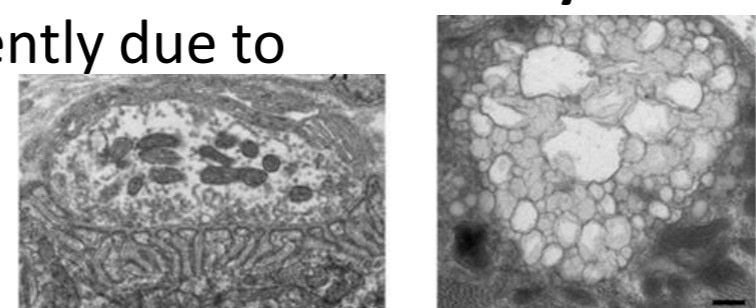
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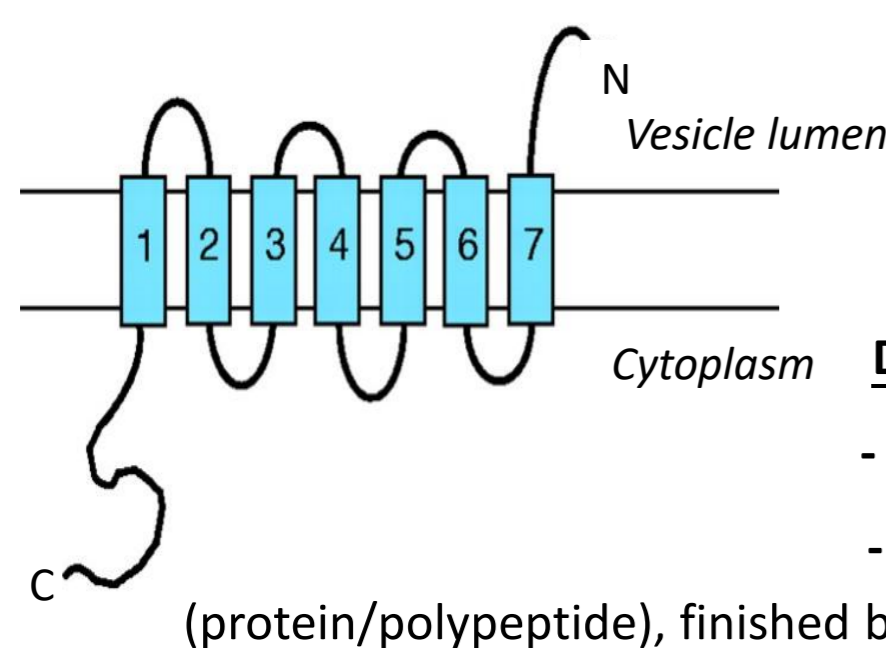
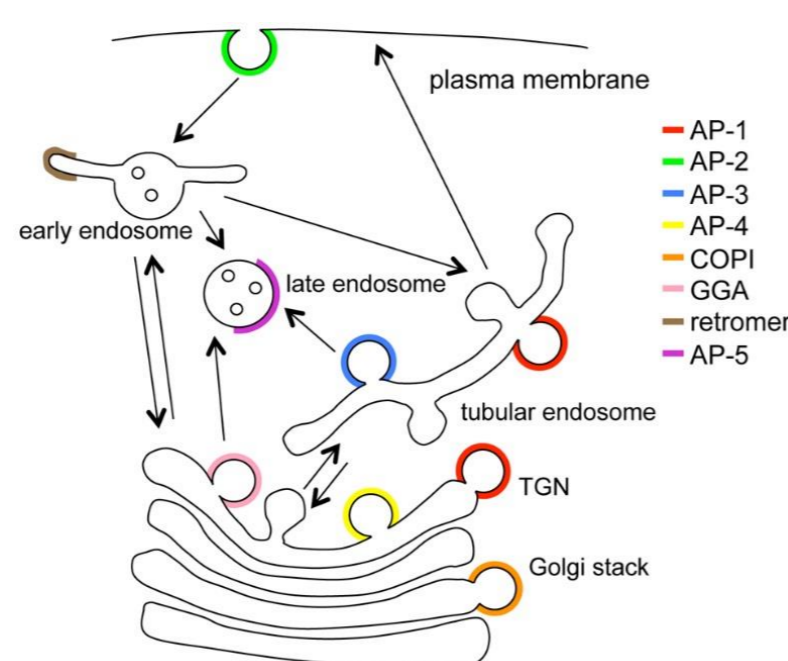
## Neurodegeneration and Tmem184b

**Dementia** consist of a spectrum of disorders, where neurodegeneration affects memory, comprehension, learning capacity, language, and sometimes emotional control and motivation. The **mechanisms by which neurons die in neurodegeneration** is frequently due to **accumulation of protein or lipid clusters**, but the mechanisms are poorly understood.



Recently, a **novel gene named TMEM184b** has been identified as a **potential mediator of neurodegeneration**. The protein encoded by the TMEM184b gene is predicted to **localize to membranes**, suggesting that it is important for membrane homeostasis in neurons.

Its **C-terminus**, loose in the cytoplasm, encodes a **specific signal (a dileucine motif)** that is known to **control the localisation** of membrane proteins.



### Definitions:

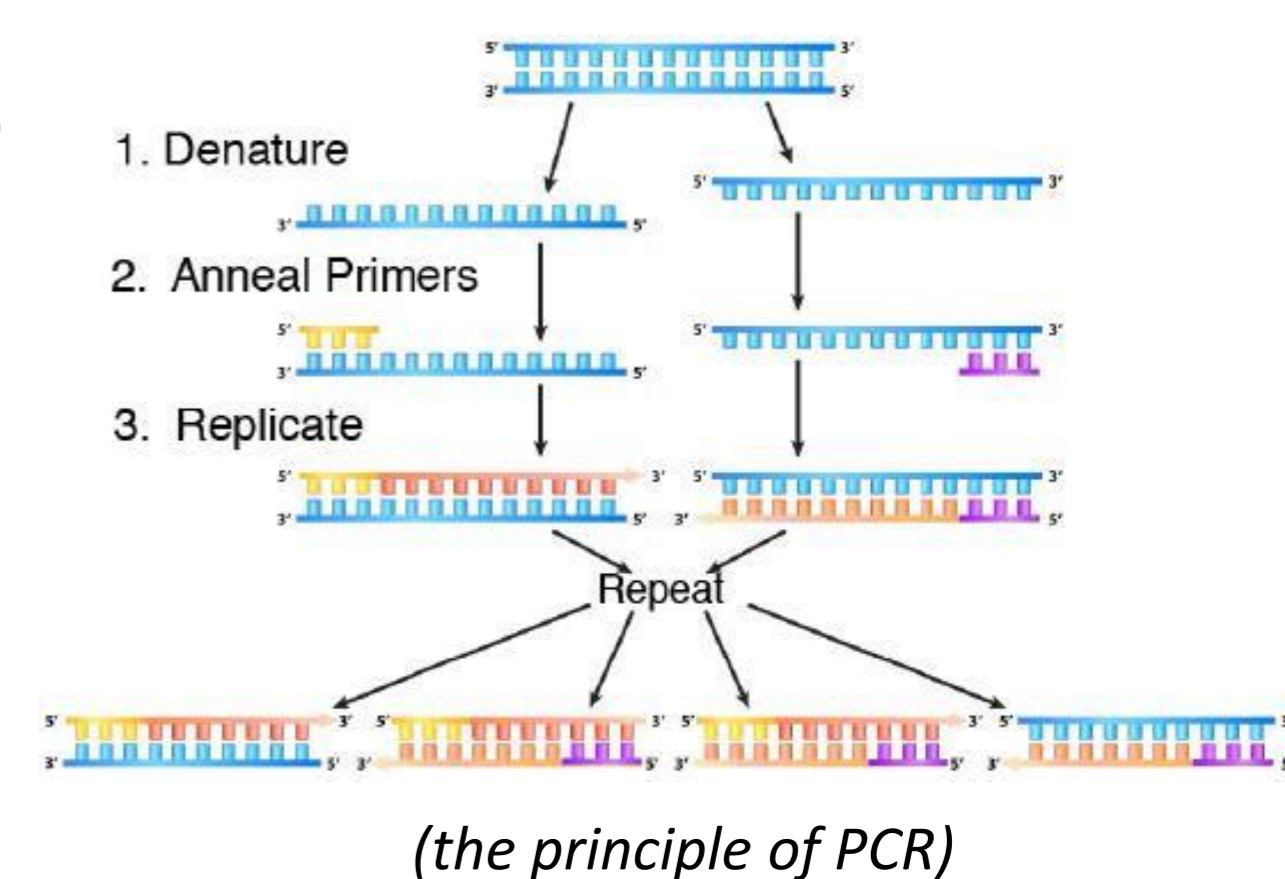
- **Amino acids** which form proteins.
- **C-terminus** is the end of an amino acid chain (protein/polypeptide), finished by a free carboxyl group (-COOH).

## The project so far

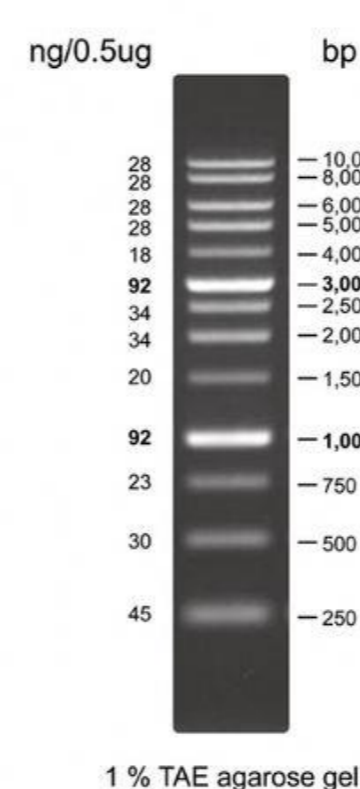
Transfer the original gene sequence **Tmem184b** into bacteria through a bacterial transformation and an overnight culture. **Purified the DNA** with a mini prep.

**The steps of cloning** (process done with DTmem184b this Summer):

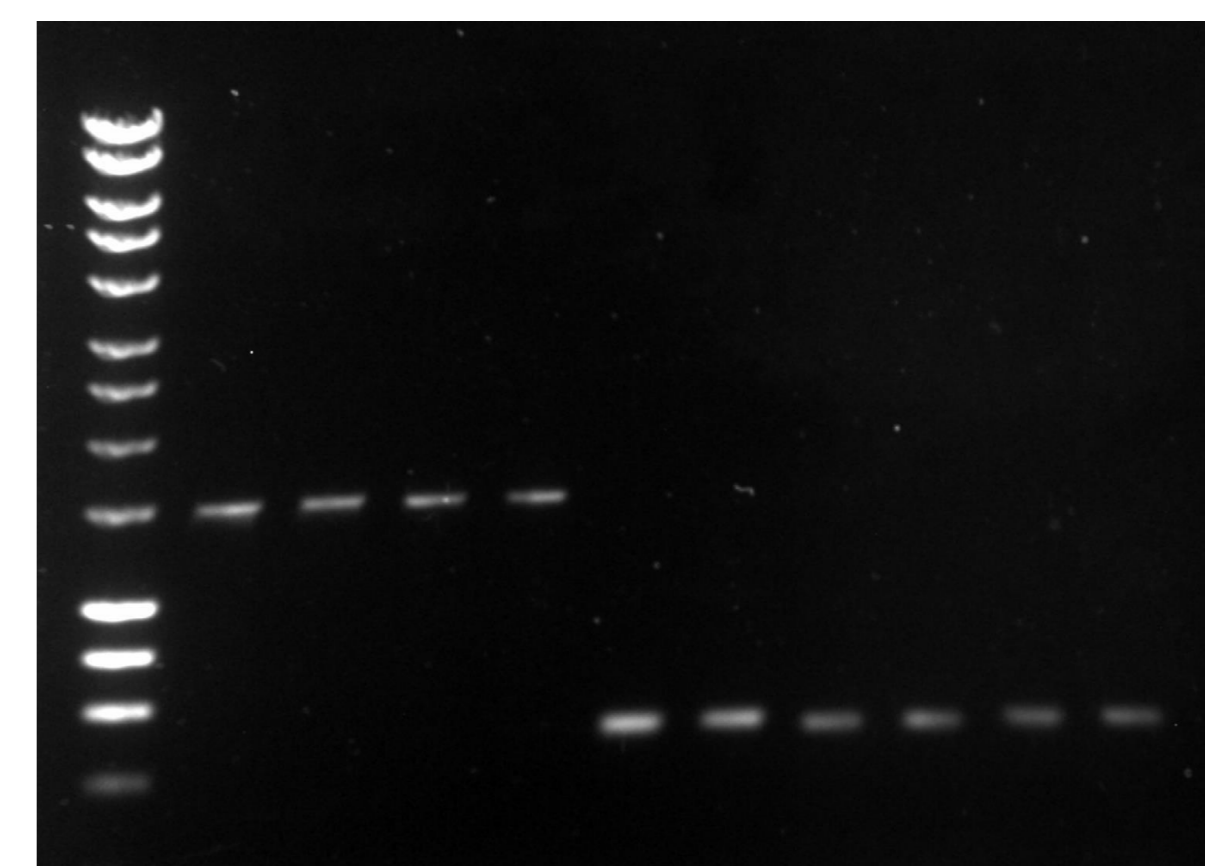
- Design primers
- PCR (Polymerase Chain Reaction)
- Double digest
- PCR purification
- Gel cut out
- Gel purification
- Ligation



1kb ladder



ladder nbr.1 nbr.2



**Right picture:** the different mutants are run on an agarose gel to see if the restrictive enzymes have cut the sequence at the right length.

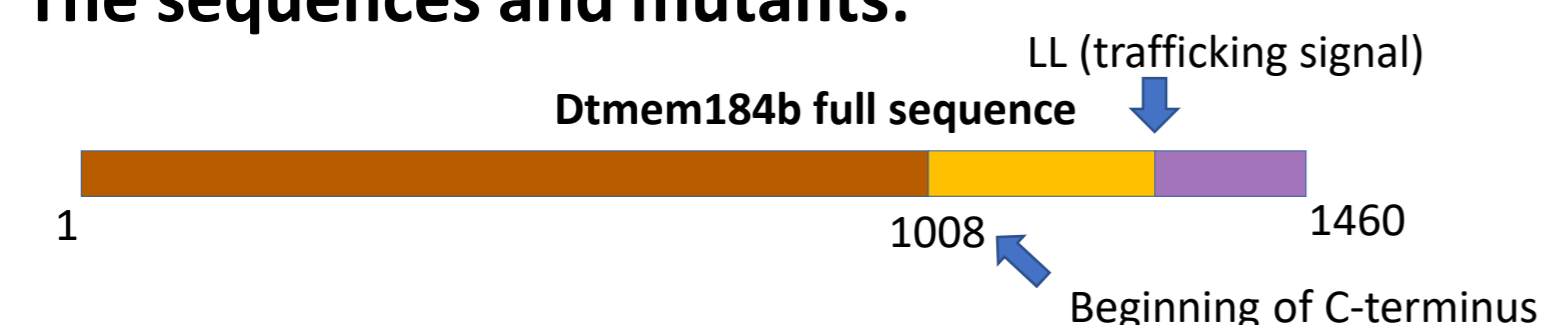
**Left picture:** A 1kb ladder to know the length of the given sequences.

## The idea

**Over express C-terminus to see its influence on cell trafficking.**

- Create sequences/mutants of the original gene
- multiply them → ligate them into plasmids
- put the plasmids into cells
- look at cells behaviors

**The sequences and mutants:**



1. Full length sequence in pEGFP-N1 (~1500 bp)
2. Full length → trafficking motif in pEGFP-N1 (~1500 bp)
3. C-terminus → end of sequence in pEGFP-C1 (~500 bp)
4. C-terminus → end of sequence in pGEX-4T-1 (~500 bp)
5. C-terminus → trafficking motif in pGEX-4T-1 (~500 bp)

**What are plasmids?** Small, circular, double-stranded DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently.

## Summary

- Tmem184b** is a **potential mediator of neurodegeneration** that shows an interesting **C-terminus** with a specific **trafficking signal** (a dileucine motif).
- Dtmem184b **mutants** have been created to **overexpress the C-terminus** and its signal, in order to look closer at its **influence in molecules trafficking**.

**Next Summer:** I will create my **HTmem184b** mutants. I will also **ligate** the sequences to the plasmids and **integrate** these ones into cells to **look at the cells behaviors and trafficking**.

**Impact in the future:** My project will give a **better awareness** into how **TMEM184b** controls membrane homeostasis in neurons and perhaps how these mechanisms are disrupted in neurodegeneration.