



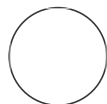
APR 03, 2023

Constructs and generation of stable cell lines

In 2 collections

michela.deleidi¹

¹German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany



Federico Bertoli

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.rm7vzbr98vx1/v1

Protocol Citation: michela.deleidi 2023. Constructs and generation of stable cell lines. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.rm7vzbr98vx1/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
 We use this protocol and it's working

Created: Apr 01, 2023

Last Modified: Apr 03, 2023

PROTOCOL integer ID:
 79874

ABSTRACT

Protocol used to generate stable Flp-In T-REx-HEK 293 cell lines expressing WT or mutant GCase (E326K or L444P) as a V5-FLAG-tagged protein using a tetracycline-inducible system.

- 1 Constructs were purchased from IDT (gBlocks Gene Fragments) and subcloned into pcDNA5/frt/to (Thermo Fisher Scientific, # V652020).

- 2 For the generation of V5-FLAG-GCase lines, the tag was positioned at the N-terminus, three aa after the cleavage site of the leader sequence. These three amino acids are repeated after the tag to ensure that the GBA1 sequence is intact.
- 3 To ensure proper cleavage, the V5-FLAG tag was inserted 12 bp after the cleavage site, and this 9 bp were repeated after the tag. To avoid interference with proper protein folding, we employed the short V5 sequence (IPNPLLGLD).
- 4 Site-directed mutagenesis was performed according to the manufacturer's protocol (Agilent, QuickChange II XL), and base pair exchange was confirmed by Sanger sequencing.
- 5 Flp-In 293 T-Rex cells(Thermo Fisher Scientific, #R78007) were grown in media composed of Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich), 10% fetal bovine serum (Gibco), 1% GlutaMax (Gibco) supplemented with 100 µg/ml Zeocin (InvivoGen) and 15µg/ml blasticidin (InvivoGen).
- 6 Inducible Flp-In T-Rex 293 cells were generated according to the manufacturer's protocol (Thermo Fisher Scientific).
- 7 The selection was performed with DMEM supplemented with 15µg/ml blasticidin and 100 µg/ml hygromycin B Gold (InvivoGen) 48 h after transfection and continued until the expression of the gene of interest was induced by treating the cells with 50 ng/ml doxycycline hyclate (Sigma–Aldrich) for 48 h.