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X-tremeGENE™ HP DNA Transfection Reagent Protocol for transfection of SH-SY5Y cells

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SH-SY5Y cells were transfected with a pcDNA3.1 vector plasmid. Stable transfection was performed using the XtremeGENE reagent for 72 hours, and 400 µg/ml G418 antibiotics used as the selection marker . Colonies were selected and expanded for routine culture in growth media supplemented with G418.

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- This is a genetic manipulation procedure and as such must have been approved by the GM Committee to cover the plasmid, inserted DNA, cells to be transfected and person performing the experiment.
- Only specific rooms are covered for these experiments (see the departmental safety codes of practice).
- Make sure you are familiar with these safety documents, and in particular the protocols relating to spillages and decontamination.

1 **Protocol from: https://www.sigmaaldrich.com/GB/en/technical-documents/protocol/cell-culture-and-cell-culture-analysis/transfection-and-gene-editing/xtghp-general-protocol**

Cell Preparation for Transfection

2 Plate SH-SY5Y cells in 10mm^{2}dish approximately 24 hours before transfection, making sure cells are at optimal concentration (70–90 % confluency).

Transfection

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1. Allow X-tremeGENE™ HP DNA Transfection Reagent, DNA and diluent (Opti:MEM®I Reduced Serum Medium or serum-free medium) to warm to +15 °C to +25 °C, and vortex gently.
2. Place diluent in a sterile tube.
3. Add plasmid DNA (2 µg) . Gently pipette up and down to mix.
4. Add 2 µL X-tremeGENE™ HP DNA Transfection Reagent to the diluted DNA.
5. Vortex the mixture.
6. Incubate for 15 minutes at +15 °C to +25 °C.
7. Add transfection complex to the cells in a dropwise manner.
8. Gently shake or swirl the wells or flasks to ensure even distribution over the entire plate.
9. Incubate cells for 72 hours before replacing with complete SH-SY5Y culture media (dx.doi.org/10.17504/protocols.io.bp2l617jzvqe/v1) supplemented with 400 µg/ml G418 antibiotics and incubated at 37°C and 5% CO_2 for selection.

Culturing transfected SH-SY5Y cells

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1. Growth medium with selection reagent was replaced every 2-3 days.
2. After ensuring all mock transfections had died, colonies from the transfected cells were selected with sterile cloning cylinders.
3. Colonies were transferred to 6 well plates and maintain in growth medium containing G418.
4. Once confluent, cells were trypsinised and expanded into a 10mm^{2}dish in 10 mL normal growth media.
5. Clones were frozen down or pelleted for characterisation.

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