# Transfection and induction of FLAG-HA tagged contruct into cell culture:SC:Graveley

Revision as of 18:07, 13 June 2012 by NLWashington (Talk | contribs | block)
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## Protocol Text

[edit] [rich edit]

FLAG-HA constructs were prepared according to Construction of FLAG-HA expression clones:SC:Graveley for a specific target. Plasmid DNA was then prepared using the PureLink™ HQ Mini Plasmid Purification Kit [Invitrogen K2100-01], and eluted with 50ul sterile water into a 1.5ml DNA LoBind microfuge tube (Eppendorf). The DNAs were quantified and assayed for A260/A280 ratio >1.8 using a NanoDrop spectrophotometer (ThermoFisher). Drosophila S2R+ cells were grown at 25°C in non-tissue culture treated polystyrene flasks (Corning Incorporated). Each clone was transiently transfected into a single 54ml culture of Drosophila S2R+ cells (1 x 106 cells per ml) grown in a T-150 flask in Schneider's Drosophila Media (GIBCO 21720) with 10% heat-inactivated fetal bovine serum (GIBCO 26140079). Twelve micrograms of each DNA was combined with 300ul of Effectene (Qiagen) following the manufacturer's protocol. Twenty four hours after transfection, expression of the tagged protein was induced with 0.35mM CuSO4. This level of CuSO4 has been tested to induce a low-to-medium level of recombinant protein expression for a majority of representative clones. Cells were grown for 24 hours after induction.

## Validation Form

[edit] [rich edit]

(This section to be completed by Project Bioinformatics contact. Toggle the 'help' link below, or contact your DCC Liaison with questions.)

## Protocol "Transfection and induction of FLAG-HA tagged contruct into cell culture:SC:Graveley" (Version 4)

Protocol Type: mged-protocol:Treatment, mged-protocol:grow ?

Input type: mged-char:cell_line [cell line] ?

Ouput type: obi-biomaterial:cell culture ? obi-biomaterial:cell cult...

Short Description: Plasmid DNA was then prepared using the PureLink? HQ Mini Plasmid Purification Kit [Invitrogen K2100-01], and eluted with 50ul sterile water into a 1.5ml DNA LoBind microfuge tube (Eppendorf). The DNAs were quantified and assayed for A260/A280 ratio >1.8 using a NanoDrop spectrophotometer (ThermoFisher). Drosophila S2R+ cells were grown at 25°C in non-tissue culture treated polystyrene flasks (Corning Incorporated).

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URL: ?

References: ?

Species: D. melanogaster ?

Lab: Graveley, Brenton ?

Update Restore

Autogenerated minimum SDRF template

Please use this page's permanent link when referencing it in data submission (e.g. in the IDF):
http://wiki.modencode.org/project/index.php?title=Transfection_and_induction_of_FLAG-HA_tagged_contruct_into_cell_culture:SC:Graveley&oldid=78429
IE Users: Right-click and choose 'Copy Shortcut' to copy the permalink URL to the clipboard.

Categories: Protocol | Celniker Group Protocol