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RNAi in cell culture:SC:Brenton

Revision as of 22:41, 4 September 2009 by NLWashington (Talk | contribs | block)
(diff) ← Older revision | Current revision (diff) | Newer revision → (diff)

Protocol Text [edit] [rich edit]

Enter your protocol text here

Notes [edit] [rich edit]

Optional notes or comments can be added here

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 "RNAi in cell culture:SC:Brenton" (Version 5)

Protocol Type: mged-protocol:compound_based_treatment, obi-process:nucleic acid ? obi-process:nucleic acid ...

Input type: SO:RNAi_reagent [dsRNA], mged-char:cell_line ?

Ouput type: mged-material:total_RNA [RNA extract] ?

Short Description: RNA interference was performed essentially as described previously (Park JW, Parisky K, Celotto AM, Reenan RA, Graveley BR. Identification of alternative splicing regulators by RNA interference in Drosophila. Proc Natl Acad Sci U S A. 2004, 101(45): 15974-15979. Park JW, Graveley BR. Use of RNA interference to dissect the roles of trans-acting factors in alternative pre-mRNA splicing. Methods, 2005, 37(4): 341-344). S2-DRSC cells were cultured with Schneider's medium plus 10% heat-inactivated FCS at 27 °C. One day prior to dsRNA treatment, cells were split into six-well culture dishes at a density 1 × 106 cells/ml. Immediately prior to the addition of dsRNA, the culture medium was replaced with fresh Schneider's medium without FCS, followed by the addition of 20 ?g of each dsRNA directly into the FCS free medium and the cells incubated for 5-hours at 27 °C. After incubation with the dsRNA, 10% FCS was added back to cell culture. After 2 days, a second dose of 20 ?g of dsRNA was added to each well in the same manner as described above and the cells incubated for 2 additional days after the re-addition of 10% FCS. After of the dsRNA treatment, total RNA was isolated using Trizol reagent (Invitrogen) according to the manufacturer?s directions. For each targeted gene, two parallel dsRNA treatments and total RNAs preparations were performed independently. Untreated S2-DRSC cells were used as reference. To monitor the level of mRNA depletion, primer sets (Table 2) that amplify regions of the targeted mRNAs outside of the ?

URL: ?

References: ?

Species: D. melanogaster ?

Lab: ?

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=RNAi_in_cell_culture:SC:Brenton&oldid=23643
IE Users: Right-click and choose 'Copy Shortcut' to copy the permalink URL to the clipboard.

Categories: Protocol | Celniker Group Protocol