Expression 3xhisflag tagged Thoc1 protein in murine embryonic fibroblasts

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Introduction:

Thoc1 protein is a component of the recently discovered TREX protein complex involved in transcriptional elongation and RNA processing. Our goal is to purify this complex from cells to understand its function and regulation. To facilitate this, we are expressing a dual affinity tagged version of the Thoc1 protein in murine embryonic fibroblasts. Endogenous, untagged Thoc1 protein will be depleted by Cre mediated gene deletion.

Materials and methods:

Thoc1 3xhisflag tagged expression vector were transfected into MEFs using Metafectene Pro. For comparison, we also transfected the expression vector using Lipofectamine 2000 (Invitrogen) or Fugenge HD (Roche).

Experimental procedures / transfection protocol:

1. Plant $0.67x10^5$ MEF cells / per well in 12 well plate
2. Incubate at 37°C for 5 hours. (We did the same day transfection this time. Confluency was about 90% before transfection. Cells were passenged every day.)
3. Solution A: 1.5ug DNA in 50ul PBS (or in 50 ul Opti-MEM for the other two reagents) , mix well by gentle pipetting.

   Solution B: 2.5ul Metafectene Pro in 50 ul PBS. (or in 50 ul Opti-MEM for the other two reagents) , mix well by gentle pipetting.
4. Combine two solutions by gentle pipetting once and incubate at room temperature for 15-20 min.
5. After incubation, add complex dropwise to the cells, move plate back and forth to mix it well.
6. Incubate at 37°C for 20 hours, and collect for western blot.

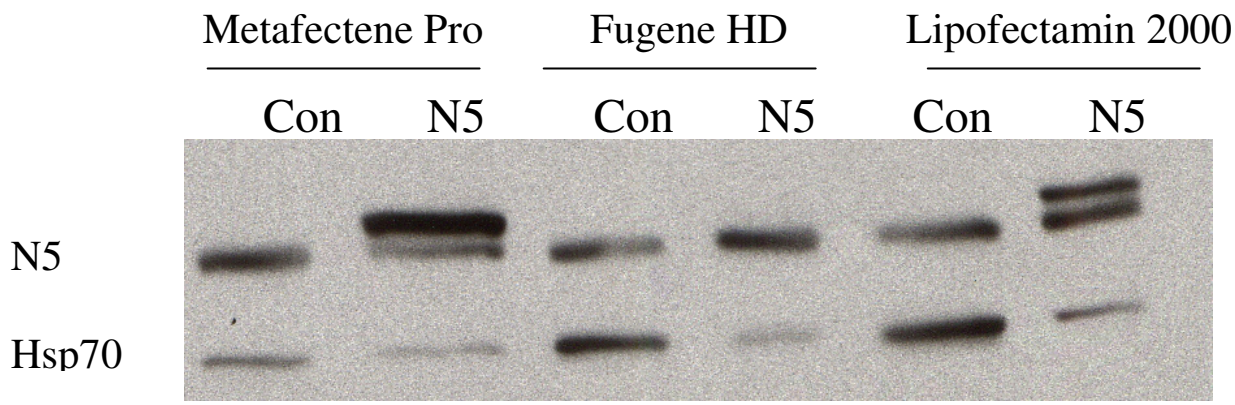
Results and discussion:

Expression levels of the tagged Thoc1 protein were higher using Metafectene Pro than using Fugene HD or Lipofectamine 2000.

Conclusion / summary:

The transfection efficiency of MEFs using Metafectene Pro is superior to two other commonly used and commercially available lipofection reagents.

Appendix:


Metafectene Pro
Fugene HD
Lipofectamin 2000
Con
N5
Con
N5
Con
N5
N5
Hsp70