siRNA_METAFECTENE ^{R}SI^{+}

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Materials:
C2C12 muscle cells
Sterile 10-cm and 12-well tissue culture plates
Cfx96 thermocycler

Growth medium:
DMEM 4,5 g Glucose
2 % L-Glutamine
1 % Non Essential Amino Acids
0,1 % Gentamycine
10 % Fetal Calf Serum

Differentiation medium
DMEM 4,5 g Glucose
2 % L-Glutamine
1 % Non Essential Amino Acids
0,1 % Gentamycine
2% Horse Serum

Transfection reagents
METAFECTENE ^{R}*SI* +
Buffer SI+
-siRNAs directed against UBE2B (siUBE2B) or no known protein (sc-siRNA), 20 µM stock

C2C12 cells were grown using growth medium in 10-cm dishes until 50% confluence and then seeded in 12-well tissue culture plates. At 80-90% confluence, C2C12 cells were washed with sterile 1X PBS and differentiated in low-serum differentiation medium for 7 days. C2C12 cells were transfected either at day 0 of differentiation (myoblasts) or at day 5 of differentiation (myotubes). When differentiated, these cells are known to be resistant to transfection using common transfection reagents.

Cell lysis: 48h post transfection

Transfection of siRNA:
- *Experimental*: siRNAs directed against UBE2B (siUBE2B). Two different siRNAs were used in combination. These siRNAs were already known to specifically target UBE2B (experiments not shown realized using the kit N-ter from Sigma).
- *Negative control*: a siRNA with no known target (sc-siRNA, in house design)

Conditions:
SI+ 1X buffer was prepared from 10X stock the day before transfection

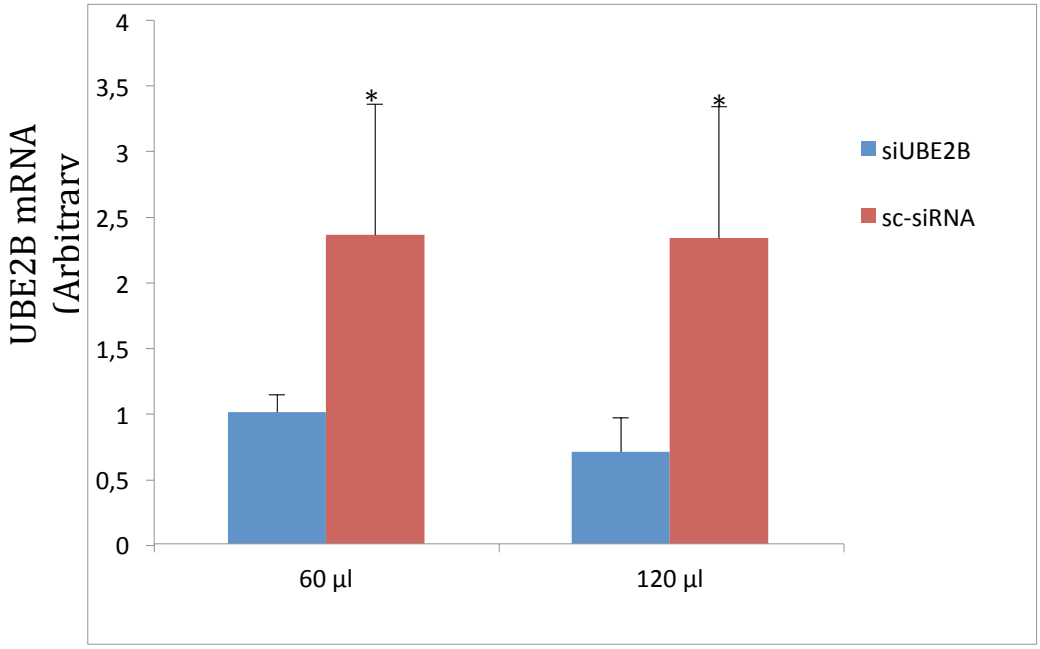
The SI+ buffer, the METAFECTENE $^{R}SI+$ and the siRNAs were put at room temperature. For each condition, the following solutions were prepared in a 1.5 ml Eppendorf**, the reagents were mixed following the order indicated and gentle pipetting was used to mix carefully each reagent added**; two conditions were tested:

1/
- 60 µl SI^+ 1X buffer
- 2.9 µl METAFECTENE S^+
- 5.4 µl siUBE2B (108 pmol) or sc-siRNA

2/
- 120 µl SI^+ 1X buffer
- 5.8 µl METAFECTENE S^+
- 10.8 µl siUBE2B (216 pmol) or sc-siRNA

The lipoplexes obtained were incubated 15 minutes at room temperature. Differentiation medium (900 µl) was then added to the lipoplexes and the mixture was then added to each well.

The amounts are indicated for 1 well but premixes were performed for n = 4. The C2C12 myotubes were maintained at 37°C for 48 h. Myotubes were then washed twice with 1X PBS. TRIzol® (Life technologies) was added in each well (150 µl), cells were scrapped and total RNA was purified according to the manufacturer. qRT-PCR was performed for UBE2B and YWHAZ, HPRT1 and GusB housekeeping genes using the SsoAdvanced SYBRGreen Supermix kit and a Cfx96 thermocycler (BioRad). Calculations were made using the comparative ΔCt method.



Transfection of siUBE2B induced a 57-65% decrease of UBE2B mRNA levels when compared to sc-siRNA controls. This indicates that UBE2B was efficiently knocked down at the mRNA levels.