**Table 3. Detailed analysis of published conditions for *DUX4* mRNA detection**

| Material or step in the procedure | Present study | Hewitt *et al.* (1), Lyle *et al.* (2) | Yip and Picketts (3) | Winokur *et al.* (4) | Osborne *et al.* (5) | Alexiadis *et al.* (6) |
|---|---|---|---|---|---|---|
| Transfected cells | C2C12 (D4Z4 + *pLAM*) | NA | C2C12 (D4Z4 concatemer no *pLAM*) | NA | NA | NA |
| Myoblasts | Differentiating (more abundant RNA than in proliferating cells) From control and FSHD | NA | Proliferating and differentiating C2C12 | NA (biopsies) | NA (biopsies) | Proliferating (?) From control and FSHD |
| Expression study | RT-PCR | Screening of cDNA libraries | RT-PCR | Microarray | Microarray | RT-PCR |
| RNA extraction | NucleoSpin RNAII (Macherey-Nagel) or Aurum Total RNA Mini kit (BioRad)* | _ | Guanidinium salt | TRIzol | TRI-Reagent [refers to Welle *et al.* (18)] | TRIzol |
| DNase treatment | From the kit: directly on RNA bound to the silica membrane (* + 1 unit/µg of DNase I Amplification grade (Invitrogen) **+ 10 units of RNase inhibitor (Fermentas) 10 mM DTT, 15** | _ | _ | | | RQ1 (Promega) 1 unit/10 µg RNA 60 min 37°C or DNaseI Amplification grade |

| Material or step in the procedure | | Present study | Hewitt *et al.* (1), Lyle *et al.* (2) | Yip and Picketts (3) | Winokur *et al.* (4) | Osborne *et al.* (5) | Alexiadis *et al.* (6) |
|---|---|---|---|---|---|---|---|
| | | **min RT** | | | | | (Invitrogen) 3 units/3 µg RNA 45 min RT |
| RT | Primer | **DUX4-specific primer** | _ | Random hexamers | Oligo dT-[T7] | oligo-dT-[T7] [refers to Welle *et al.* (19)] | Random hexamers |
| | denaturing step | 2-4 µg of fresh RNA (immediately after extraction and DNase-treatment) + primer + dNTPs 5 min 65°C, 1 min on ice | _ | 5 µg | | | 10 and 3 µg? |
| | enzyme | 200 units of SuperScript III (Invitrogen) per tube, on ice final reaction volume: 20µl | _ | Superscript RT (Invitrogen) | Superscript choice (Invitrogen) | ? | SuperScript III (Invitrogen) |
| | T°/timing | 55°C, 1 h Inactivation: 70°C 15 min | _ | 42°C 50 min (?) | ? | ? | 50°C 50 min (?) |
| RNase H treatment | | **20 min 37°C (2.5 units)** | _ | ? | | | ? |
| PCR primers | | Forward: *DUX4* sequence in D4Z4; Reverse: *DUX4* sequence in pLAM | | | *DUX4* sequence in D4Z4 | | *DUX4* sequence in D4Z4 |

| Material or step in the procedure | Present study | Hewitt *et al.* (1), Lyle *et al.* (2) | Yip and Picketts (3) | Winokur *et al.* (4) | Osborne *et al.* (5) | Alexiadis *et al.* (6) |
|---|---|---|---|---|---|---|
| PCR | **8-12 µl** of cDNA Touch-down Platinium *Pfx* DNA polymerase **with the PCRx enhancer solution (final concentration 3X)** | _ | 2 µl of cDNA (?) | | | 2 µl of cDNA (?) |

The question marks in brackets refer to missing indications in the studies or basic procedures of the RT instruction manuals. NA, not applicable.