

Materials List for:

Multi-exon Skipping Using Cocktail Antisense Oligonucleotides in the Canine X-linked Muscular Dystrophy

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URL: <https://www.jove.com/video/53776>

DOI: [doi:10.3791/53776](https://doi.org/10.3791/53776)

Materials

| Name | Company | Catalog Number | Comments |
|--|---------------|----------------|---|
| 2'OMePS Transfection of Dog Myoblasts | | | |
| 3 ml 6-well plates | IWAKI | 5816-006 | |
| Dulbecco's modified Eagle's medium (DMEM) | Gibco | 11965-092 | |
| Fetal bovine serum (FBS) | HyClone | SH30071.01 | |
| Penicillin | Sigma-Aldrich | P4333 | 200 U/ml |
| Lipofectin | Invitrogen | 18292-011 | Total volume of 100 ml in opti-MEM media at a ratio of 2:1 for lipofectin. 10 ml lipofectin for 5 mg RNA. |
| 2'OMePS | Eurogentec | | Ex6A (GUU GAUUGUCGGACCCAGCUCAGG), Ex6B (ACCUAUGA CUGUGGAUGAGAGCGUU), and Ex8A (CUUCCUGG AUGGCUUCAUGCUCAC). |
| Horse Serum | Gibco | 16050-114 | 2% |
| streptomycin | Sigma-Aldrich | P4333 | 200 µg/ml |
| Bovine serum albumin (BSA) | Sigma-Aldrich | A9418 | |
| Insulin | | | |
| Morpholino transfection of dog myoblasts | | | |
| All material from MePS Transfection of Dog Myoblasts for culturing | | | |
| Antisense morpholinos | Gene-tools | | Ex6A(GTTGATTGTCGGACCCAGC TCAGG), Ex6B(ACCTATGACTGTGGATGAG AGCGTT), Ex8A(CTTCCTGGATGGCTTCAAT GCTCAC) Dilute to a final volume of 100 L in opti-MEM media. 120–200 mg/kg of morpholinos at 32 mg/ml in saline |
| Endo-Porter | Gene-tools | | |
| guanidinium thiocyanate-phenol-chloroform | Invitrogen | 15596-018 | 1 ml/plate |
| RNA Extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR) | | | |
| guanidinium thiocyanate-phenol-chloroform | Invitrogen | 15596-018 | 1 ml/plate |
| Chloroform | Sigma-Aldrich | P3803 | 200 µl |

| | | | |
|---|--------------------------|----------------|---|
| 1.5 ml Tubes | Eppendorf | 22363204 | |
| Centrifuge | Beckman-Coulter | | |
| 75% Ethanol | Sigma-Aldrich | 34852 | |
| UV Spectrometer | | | |
| Forward primer in exon 5 | Invitrogen | | CTGACTCTTGGTTTGATTGGA 1.5 µl 10 µM |
| Reverse primer in exon 10 | Invitrogen | | TGCTTCGGTCTCTGTCAATG 1.5 µl 10 µM |
| dNTPs | Clontech | 3040 | |
| One-Step RT-PCR kit | Qiagen | 210210 | |
| Thermo-cycler | Scinco | | |
| Complementary DNA (cDNA) Sequencing | | | |
| Gel extraction kit | Qiagen | 28704 | |
| Centrifuge | | | |
| Terminator v3.1 Cycle Sequencing Kit | Applied Biosystems | 4337454 | |
| Intramuscular injections or open muscle biopsy | | | |
| Surgical Tools | | | Scissors, scalpel, needle, surgical thread |
| Vet Ointment | | | |
| Iodophors | webtextiles | 12190-71-5 | |
| chlorohexidine | Peridex | 12134 | |
| Surgical Drapes | | | |
| Scrubs | | | |
| Facial Mask | | | |
| Surgical Gloves | | | |
| Head Covering | | | |
| Thiopental sodium | Mitsubishi Tanabe Pharma | | 20 mg/kg |
| Isoflurane | Abbott laboratories | 05260-05 | 2-3% |
| Antisense morpholinos | Gene-tools | | Ex6A(GTTGATTGTCGGACCCAGC TCAGG), Ex6B(ACCTATGACTGTGGATGAG AGCGTT), Ex8A(CTTCCTGGATGGCTTCAAT GCTCAC) Dilute to a final volume of 100 L in opti-MEM media. 120–200 mg/kg of morpholinos at 32 mg/ml in saline |
| 27 G Needles | TERUMO | SG3-2325 | |
| 50 ml syringe | TERUMO | SG2-03L2225 | |
| buprenorphine hydrochloride | | | |
| Tongue Depressor | | | |
| cephalexin | | 15 to 30 mg/kg | |
| cefazolin | | 15 to 30 mg/kg | |
| buprenorphine | | 0.01 mg/kg | |
| buprenorphine hydrochloride | 0.02 mg/kg | | |
| Systemic Injections | | | |
| Syringe infusion pump | Muromachi | | |
| 22 G Indwelling needles | TERUMO | SG3-2225 | |

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|---|------------------------------|-----------------------|---|
| 27 G Needles | TERUMO | SG3-2325 | |
| 50 ml syringe | TERUMO | SG2-03L2225 | |
| Saline | Ohtsuka-Pharmaceutical | 28372 | |
| Clinical Grading of Dogs | | | |
| Video Camera | | | |
| Stop watch | | | |
| Magnetic resonance imaging (MRI) | | | |
| 3 Tesla MRI I | | | |
| 18 cm diameter/18 cm length human extremity coil | | | |
| Muscle sampling and preparation (necropsy) | | | |
| Thiopental sodium | Mitsubishi Tanabe Pharma | | 20 mg/kg |
| Isoflurane | Abbott laboratories | 05260-05 | 2-3% |
| Tragacanth gum | | | 10-20 ml |
| Liquid Nitrogen | | | |
| Cork Discs | Iwai-kagaku | 101412-806 | |
| Dry Ice | | | |
| Tweezers | | | |
| Poly-L-lysine-coated slides | Fisher | 22-037-216 | |
| Cryostat Microsystem | Leica | | cm1900 |
| Immunohistochemistry | | | |
| DYS1 | Novocastra | NCL-DYS1 | 1:150 dilutions |
| DYS2 | Novocastra | NCL-DYS2 | 1:150 dilutions |
| Alexa 594 goat antimouse IgG1 | Invitrogen | A-21125 | 1:2,500 dilutions |
| Alexa 594 goat antimouse IgG2 | Invitrogen | A-11005 | 1:2,500 dilutions |
| DAPI | Invitrogen | D1306 | Contains mounting agent |
| Goat Serum | Invitrogen | 10000C | 15% |
| PBS | | | |
| Moisture chamber | Scientific Devise Laboratory | 197-BL | |
| Chamber slide | Lab-tek | 154453 | |
| Cover Glasses | Fisher | 12-540A | |
| Hydrophobic barrier pen | | | |
| Fluorescent microscope | | | 594 nm at 20X magnification. |
| Western Blotting | | | |
| Distilled Water | | | |
| Hand Homogenizer | | | |
| 2× Laemmli SDS-loading buffer | | | 0.1 M Tris-HCl (pH 6.6), 2% (w/v) SDS, 2% (0.28 M) beta-mercaptoethanol, 20% glycerol, 0.01% bromophenol blue |
| SDS gels | Bio-Rad | 161-1210 | 5% resolving |
| SDS gels | Invitrogen | Invitrogen, WG1601BOX | 3-8% |
| PVDF membrane | GE | 10600021 | |
| Methanol | | | |
| Running buffer (10×) | | | 250 mM of Tris-Base, 1,920 mM of Glycine |
| Running buffer (1×) | | | 10% 10× buffer, 20% methanol |

| | | | |
|--|-------------------|-------------|--|
| 0.05% PBS/Tween 20 (PBST) | | | 2,000 ml 3× 200 ml for washing |
| PBST/5% milk powder | | | 100 ml |
| Protein Assay Kit | BCA | T9650 | |
| Tween 20 | Sigma | P5927 | |
| Urea | Sigma | U5378 | |
| Beta Mercaptoethanol | Millipore | ES-007-E | |
| SDS | Sigma | L3771 | |
| Tris-Acetate | Sigma | | |
| Tris HCl | Sigma | T3253 | |
| Glycerol | Sigma | G8773 | |
| Loading/sample buffer for Western blotting | NuPage Invitrogen | NP007 | |
| NaCl | Sigma | S3014 | |
| PMSF | Sigma | P7626 | |
| Protease cocktail inhibitor | Roche | 11836153001 | |
| Cathode Buffer | | | 0.025 M Tris base + 40 mM 6-aminocaproic acid + 20% Methanol |
| Anode Buffer | | | 0.03 M Tris Base + 20% Methanol |
| Concentred Anode Buffer | | | 0.3 M Tris base + 20% Methanol |
| desmin antibody | Abcam | ab8592 | |
| DYS1 | Novocastra | NCL-DYS1 | 1:150 dilutions |
| ImageJ Software | | | |