

Materials List for

# Electroporation-Mediated Delivery of Cas9 Ribonucleoproteins and mRNA into Freshly Isolated Primary Mouse Hepatocytes

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## Materials

| Name                                                                 | Company                   | Catalog Number | Comments                      |
|----------------------------------------------------------------------|---------------------------|----------------|-------------------------------|
| <b>Equipment</b>                                                     |                           |                |                               |
| 0.2 mL PCR 8-tube FLEX-FREE strip, attached clear flat caps, natural | USA Scientific            | 1402-4700      |                               |
| 6-well Collagen Plates                                               | Advanced Biomatrix        | 5073           |                               |
| accuSpin Micro 17R                                                   | Fisher Scientific         | 13-100-675     |                               |
| All-in-one Fluorescent Microscope                                    | Keyence                   | BZ-X810        |                               |
| Analog Vortex Mixer                                                  | VWR                       | 97043-562      |                               |
| ART Wide BORE filtered tips 1,000 µL                                 | ThermoFisher Scientific   | 2079GPK        |                               |
| Automated Cell Counter                                               | Bio-Rad Laboratories      | TC20           |                               |
| Blue Wax Dissection Tray                                             | Braintree Scientific Inc. | DTW1           | 9" x 6.5" x 1/2"+F21          |
| Cell scraper/lifter                                                  | Argos Technologies        | UX-04396-53    | Non-pyrogenic, sterile        |
| Conical tubes (15 mL)                                                | Fisher Scientific         | 339650         |                               |
| Conical tubes (50 mL)                                                | Fisher Scientific         | 14-432-22      |                               |
| Cotton applicators                                                   | Fisher Scientific         | 22-363-170     |                               |
| Curved scissors                                                      | Cooper Surgical           | 62131          |                               |
| Disposable Petri Dishes                                              | Falcon                    | 351029         | 100mm,sterile                 |
| Disposable Petri Dishes                                              | VWR                       | 25373-100      | 35mm, sterile                 |
| Epoch Microplate Spectrophotometer                                   | BioTek Instruments        | 250082         |                               |
| Falcon Cell strainer (70 µm)                                         | Fisher Scientific         | 08-771-2       |                               |
| Forceps                                                              | Cooper Surgical           | 61864          | Euro-Med Adson Tissue Forceps |
| IV catheters                                                         | BD                        | 382612         | 24 G x 0.75 in                |
| IV infusion set                                                      | Baxter                    | 2C6401         |                               |
| MyFuge 12 Mini Centrifuge                                            | Benchmark Scientific      | 1220P38        |                               |
| Needles                                                              | Fisher Scientific         | 05-561-20      | 25 G                          |

|                                                         |                          |             |                                                                         |
|---------------------------------------------------------|--------------------------|-------------|-------------------------------------------------------------------------|
| Nucleofector 2b Device                                  | Lonza                    | AAB-1001    | Program T-028 was used for electroporation in mouse hepatocytes         |
| Peristaltic Pump                                        | Masterflex               | HV-77120-42 | 10 to 60 rpm; 90 to 260 VAC                                             |
| Precision pump tubing                                   | Masterflex               | HV-96410-14 | 25 ft, silicone                                                         |
| Primaria Culture Plates                                 | Corning Life Sciences    | 353846      | Nitrogen-containing tissue culture plates                               |
| Serological Pipets (25 mL)                              | Fisher Scientific        | 12-567-604  |                                                                         |
| Syringes                                                | BD                       | 329464      | 1 mL, sterile                                                           |
| T100 Thermal Cycler                                     | Bio-Rad Laboratories     | 1861096     |                                                                         |
| Water bath                                              | ALT                      | 27577       | Thermo Scientific Precision Microprocessor Controlled 280 Series, 2.5 L |
| <b>Reagents</b>                                         |                          |             |                                                                         |
| Alt-R S.p. Cas9 Nuclease V3                             | IDT                      | 1081058     |                                                                         |
| Beckman Coulter AMPure XP, 5 mL                         | Fisher Scientific        | NC9959336   |                                                                         |
| CleanCap Cas9 mRNA                                      | Trilink Biotechnologies  | L-7606-100  |                                                                         |
| CleanCap EGFP mRNA                                      | Trilink Biotechnologies  | L-7201-100  |                                                                         |
| Corning Matrigel Matrix                                 | Corning Life Sciences    | 356234      |                                                                         |
| DMEM                                                    | ThermoFisher Scientific  | 11885076    | Low glucose, pyruvate                                                   |
| Ethanol 70%                                             | VWR                      | 71001-652   |                                                                         |
| Fetal bovine serum                                      | Thermoscientific         | 26140-079   |                                                                         |
| Hepatocyte Maintenance Medium (MM)                      | Lonza                    | MM250       |                                                                         |
| Hepatocyte Plating Medium (PM)                          | Lonza                    | MP100       |                                                                         |
| Mouse Albumin ELISA Kit                                 | Fisher Scientific        | NC0010653   |                                                                         |
| Mouse/Rat Hepatocyte Nucleofector Kit                   | Lonza                    | VPL-1004    |                                                                         |
| OneTaq HotStart DNA Polymerase                          | New England Biolabs      | M0481L      |                                                                         |
| PBS 10x pH 7.4                                          | Thermoscientific         | 70011-044   | No calcium or magnesium chloride                                        |
| Percoll (PVP solution)                                  | Santa Cruz Biotechnology | sc-500790A  |                                                                         |
| Periodic acid                                           | Sigma-Aldrich            | P7875-25G   |                                                                         |
| Permout Mounting Medium                                 | VWR                      | 100496-550  |                                                                         |
| QuickExtract DNA Extraction Solution                    | Lucigen Corporation      | QE05090     |                                                                         |
| Schiff's fuchsin-sulfite reagent                        | Sigma-Aldrich            | S5133       |                                                                         |
| Trypsin-EDTA (0.25%)                                    | ThermoFisher Scientific  | 25200056    | Phenol red                                                              |
| Vybrant MTT Cell Viability Assay                        | ThermoFisher Scientific  | V13154      |                                                                         |
| <b>Perfusion Solution 1 (pH 7.4, filter sterilized)</b> |                          |             | Stable at 4 °C for 2 months                                             |
| EBSS                                                    | Fisher Scientific        | 14155063    | Complete to 200 mL                                                      |
| EGTA (0.5 M)                                            | Bioworld                 | 40520008-1  | 200 µL                                                                  |
| HEPES (pH 7.3, 1 M)                                     | ThermoFisher Scientific  | AAJ16924AE  | 2 mL                                                                    |
| <b>Perfusion Solution 2 (pH 7.4, filter sterilized)</b> |                          |             | Stable at 4 °C for 2 months                                             |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O (1.8 mM)           | Sigma                    | C7902-500G  | 360 µL of 1 M stock                                                     |
| EBSS (no calcium, no magnesium, no phenol red)          | Fisher Scientific        | 14-155-063  | Complete to 200 mL                                                      |
| HEPES (1 M)                                             | ThermoFisher Scientific  | AAJ16924AE  | 2 mL                                                                    |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O (0.8 mM)           | Sigma                    | 30391-25G   | 160 µL of 1 M stock                                                     |
| <b>Perfusion Solution 3</b>                             |                          |             | Prepared fresh prior to use                                             |
| Solution 2                                              |                          |             | 50 mL                                                                   |

|                                           |                                                                     |            |                                |
|-------------------------------------------|---------------------------------------------------------------------|------------|--------------------------------|
| Liberase                                  | Roche                                                               | 5401127001 | 0.094 Wunsch units/mL          |
| <b>Mouse Anesthetic Cocktail</b>          |                                                                     |            |                                |
| Acepromzine                               |                                                                     |            | 0.25 mg/mL final concentration |
| Ketamine                                  |                                                                     |            | 7.5 mg/mL final concentration  |
| Xylazine                                  |                                                                     |            | 1.5 mg/mL final concentration  |
| <b>Software</b>                           | <b>URL</b>                                                          |            |                                |
| Benchling                                 | <a href="https://www.benchling.com/">https://www.benchling.com/</a> |            |                                |
| ImageJ                                    | <a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a> |            |                                |
| TIDE: Tracking of Indels by Decomposition | <a href="https://tide.nki.nl/">https://tide.nki.nl/</a>             |            |                                |