TRANSGENIC CORE
Grant Boilerplate

CRISPR/Cas9 mRNA Microinjection

The CHOP Transgenic Core will create the genetically unique strain proposed in this grant application. The CRISPR/Cas9 mRNA will be injected directly into the cytoplasm of fertilized zygotes. This is accomplished with the aid of a DMI 3000B Leica Inverted Microscope mounted on a TMC Anti-Vibration Table. The manipulation and injection of the zygotes are performed using Leica left and right Manual Manipulators. The mRNA is loaded into the ultra-fine injection needle, created using a Sutter P-97 Puller. The opposite end of the instrument holder is connected to the Eppendorf FemtoJet Microinjector. The FemtoJet, with its built-in compressor, provide the regulated force necessary for slow controlled expulsion of the construct though the injection needle, into the pronuclei of the zygotes. The surviving zygotes are then surgically transferred, using the surgical MZ6 Leica microscope, into the reproductive tract of 0.5day pseudo-pregnant surrogate females. Resulting offspring are screened for integration of the mRNA sequence. Mice identified with the correct sequence are considered the founders, or F0, these will then be bred to expand the line for experimental use.

CRISPR/Cas9 RNP Electroporation

The CHOP Transgenic Core will create the genetically unique strain proposed in this grant application. The CRISPR/Cas9 RNP will be electroporated with 0.5day gestation zygotes. The electroporation is performed using a Gene Pulser Electroporator and 1mm cuvettes. This procedure allows the RNP complex to pass through the chemically thinned zona pellucida, and penetrate the fertilized zygote where it incorporates into the mouse genome. Shortly after electroporation the zygotes are surgically transferred, using the surgical MZ6 Leica microscope, into the reproductive tract of 0.5day pseudo-pregnant surrogate females. Resulting offspring are screened for integration of the RNP sequence. Mice identified with the correct sequence are considered the founders, or F0, these will then be bred to expand the line for experimental use.

DNA Microinjection

The CHOP Transgenic Core will create the novel transgenic strain proposed in this grant application. The exogenous recombinant DNA will be injected directly into the pronuclei of fertilized zygotes. This is accomplished with the aid of a DMI 3000B Leica Inverted Microscope mounted on a TMC Anti-Vibration Table. The manipulation and injection of the zygotes are performed using Leica left and right Manual Manipulators. The linear DNA is loaded into the ultra-fine injection needle, created using a Sutter P-97 Puller. The opposite end of the instrument holder is connected to the Eppendorf FemtoJet Microinjector. The FemtoJet, with its built-in compressor, provide the regulated force necessary for slow controlled expulsion of the construct though the injection needle, into the pronuclei of the zygotes. The surviving zygotes are then surgically transferred, using the surgical MZ6 Leica microscope, into the reproductive tract of 0.5day pseudo-pregnant surrogate females. Resulting offspring are screened for integration of the transgene. Mice identified with the correct sequence are considered the founders, or F0, these will then be bred to expand the line for experimental use.

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Embryonic Stem Cell Microinjection

The CHOP Transgenic Core will create the novel gene targeted mice discussed in this grant application. Chimeric mice will be produced by introducing Embryonic Stem (ES) Cell clones, containing the specific gene targeted mutation, into the blastoceol cavity of E3.5 day embryos, also known as blastocysts. This is accomplished using a DMI 3000B Leica Inverted Microscope mounted on a TMC Anti-Vibration Table and a Sutter P-97 Puller. The manipulation and injections of the blastocysts are performed using an Eppendorf Cell Tram air microinjector, which positions and secures the blastocysts during the microinjection process and an Eppendorf Cell Tram oil apparatus, which provides control while picking up and injecting the ES cells. The injected blastocysts are cultured 1-3 hours and then surgically transferred, using the MZ6 Leica microscope, into the uterus of a 2.5 d.p.c. pseudo-pregnant recipient mother mouse. Approximately 10 days after birth the pups are identified for level of cell contribution, which is apparent by the coat color differences. The fur of chimeric heterozygous pups will be a mix of the host blastocyst strain and the ES cell strain. Mice with the highest amount of cell-derived fur will be selected for future breeding to establish the colony.

Mouse Line Rederivation

The CHOP Transgenic Core will re-establish cryopreserved mouse strains, and eliminate pathogens, as proposed in this grant application. Lines archived using sperm will be revived using In-Vitro Fertilization (IVF). This procedure requires the use of the Cook MINC benchtop incubator and MZ6 Leica Stereo Microscopes. Fertilized zygotes generated from the IVF procedure are surgically transferred into the reproductive tract of pseudo-pregnant surrogate female mice using the surgical MZ6 Leica microscope. Resulting offspring will be genotyped and used for future expansion of the strain and ultimately experimental use. Strains that were previously archived with embryos will be revived by slowly thawing and rehydrating before being surgically transferred into the reproductive tract of pseudo-pregnant surrogate female mice using the surgical MZ6 Leica microscope. Resulting offspring will be genotyped and used for future expansion of the strain and ultimately experimental use.

Embryo Cryopreservation

The CHOP Transgenic Core will cryopreserve embryos to archive mouse strains generated in this grant application. A MZ6 Leica Stereo Microscope will be used for the microdissections and harvesting the preimplantation stage embryos. The embryos are then cultured overnight in a Cook MINC benchtop incubator that provides a stable, humidified, gas environment. The following day, viable 2-cell embryos are cryopreserved using the Bio-Cool IV Controlled Rate Freezer, which slowly reduces the temperature at half of a degree per minute until it reaches a pre-programmed set point. The straws, containing the frozen embryos, are then carefully transferred to a dewar containing liquid nitrogen. From there they are transferred to the MVE XC47/11 unit for long term storage.

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Sperm Cryopreservation

The CHOP Transgenic Core will cryopreserve sperm to archive valuable mouse strains created in this grant application. A MZ6 Leica Stereo Microscope will be used during microdissection of the epidydidmides and to assess the motility and concentration of the sperm. The sperm suspension and straws are kept warm during the whole process using a Cook MINC benchtop incubator. The straws are heat sealed and stored in the vapor phase of liquid nitrogen for a short period before being transferred to the MVE XC47/11 liquid nitrogen unit for long-term storage.