Standard Operating Procedure for the DK U54 Cooperative Center of Excellence in Hematology (CCEH) Hypoxia Core at the Indiana University School of Medicine

NOTE: The below methodology may require some changes in the future as we learn more from our work and the work of others. **Any live animal work performed in the Hypoxia Core must be approved on PI IACUC Protocol.**

Hypoxic Mouse Bone Marrow Infusion: Bone Marrow Transplant

Materials:

NOTE: Our chambers are set to 3% O2 unless otherwise directed. It is important to always include extra supplies other than just what you calculated needing. Additional material cannot be added into chamber at time of experiment as it will not have been acclimated to the lower O_2 levels. All hardware and reagents (e.g. culture media, antibodies, etc.) must be pre-equilibrated to hypoxia chamber at least 18 hours before the chamber is to be used for experiments. Be advised that very small volumes left to equilibrate overnight might result in loss of volume due to the evaporation caused by the moving gasses.

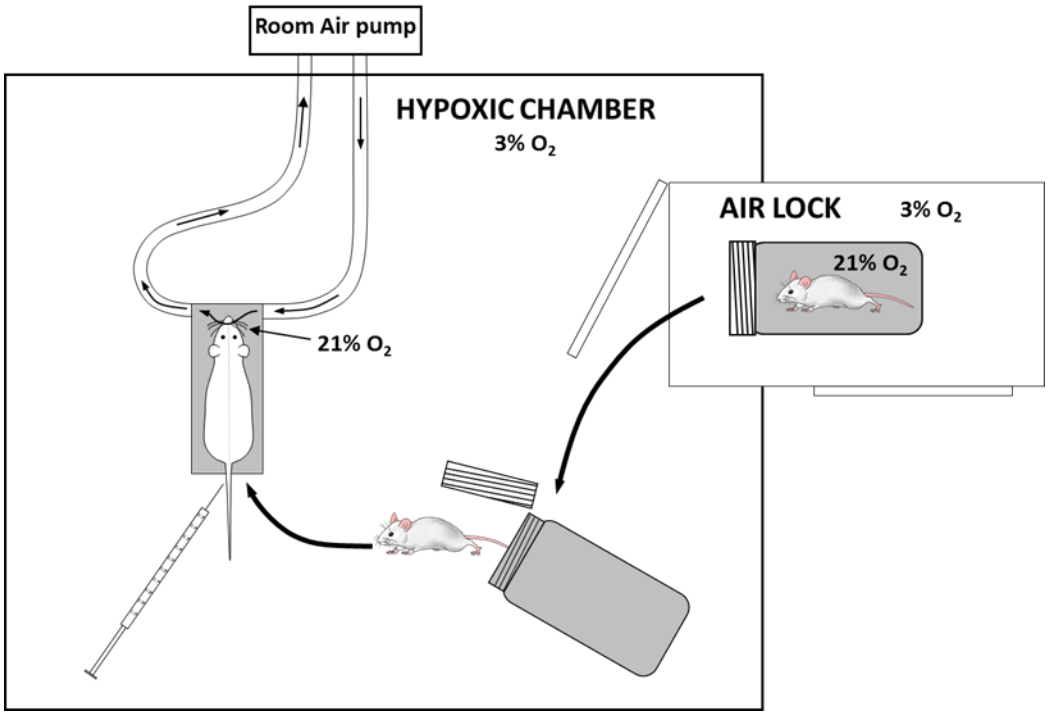
- Cells to be infused: cell concentration should allow for desired dose to be administered in volume of 0.2ml.
- Syringes: 1ml syringe
- Animal restraint fitted with aeration cone (not equilibrated)
- Transfer container (not equilibrated)

Procedure

1. Warm recipient mice via either warming pad or heat lamp per IACUC protocol.
2. Place mouse into transfer chamber containing ambient air.
3. Quickly, transfer mouse into chamber by placing in air lock and purging lock
   It is not necessary to wait for the 60 second purge cycle to complete before moving mouse into main chamber.
4. Move mouse into main chamber.
5. Transfer mouse from transfer container to restraint device and immediately start aeration pump (see figure).
6. With tail extended outside of restrain exposed to hypoxic environment, disinfect with alcohol wipe and inject 0.2ml of desired dose via one of the lateral tail veins.
7. Remove needle and apply pressure on injection site for a few seconds.
8. Quickly transfer mouse back to transfer container, out of chamber, and back to its cage.
9. Proceed to next animal.

Comments/Notes:

- Warming animals: special care must be taken not to overheat animals. Keep close watch on cage during this time
- All steps moving mice in and out of chamber are abbreviated when using live animals (e.g. airlock purge). Therefore, you must watch chamber O_2 concentrations closely to begin a chamber purge if necessary.
- Even though the aeration cone has an inlet and outlet hose, some leakage does occur into the chamber which may cause O_2 concentration to rise. Therefore, you must watch chamber O_2 concentrations closely to begin a chamber purge if necessary.



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