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A General Guide To Generate Different Humanized Mouse Models

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ABSTRACT

This protocol details how to create humanized mouse models from NSG mice. Four different variants of humanized mice can be generated based on whether or not the native thymus is retained or if a human thymus piece is transplanted. Which type of humanized mouse is desired depends on the goals for the experiment. See our protocols on NSG mouse thymectomy, human CD34+ cell isolation, and human fetal thymus preparation for more details on some of the steps in this protocol ("Thymectomy procedure to remove native thymus of NSG mice", "Human CD34+ cell isolation from fetal liver, and fetal thymus preparation", "CD34+ isolation from human bone marrow").

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1 NOD-scid common gamma chain knockout (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) (NSG) and NSG-(K^b D^b)^{null} (IA)^{null}(NSG MHC KO) mice are obtained from the Jackson Laboratory or bred in-house and housed in a specific pathogen-free microisolator environment. Adult mice are used at 6–10 wk of age.

2 Discarded human fetal thymus and liver tissues of gestational age 17–20 wk are obtained without identifiers from Advanced Bioscience Resources (ABR, Alameda, CA). Informed consent from women choosing to donate fetal remains for research are obtained by ABR.

3 Fetal thymus fragments are cryopreserved in 10% dimethyl sulfoxide (DMSO) and 90% human AB serum (Atlanta Biologicals).

4 Fetal livers (FL) are cut into small pieces and incubated in **5 mL** of **0.1 mg/ml** liberase (Liberase™ Research Grade, 05401119001, Roche) for 20 minutes in a **37 °C** CO_2 incubator. Every 5-7 minutes, the samples are vortexed and pipetted up and down to facilitate the digestion process. Digested cells are washed and $CD34^+$ cells (referred to as HSCs) are positively sorted with magnetic cell sorting (MACS) (Miltenyi Biotec). From each FL, we harvest 10-30 million $CD34^+$cells with a purity of 70-90%. $CD34^+$ HSCs are also cryopreserved in 10% DMSO and 90% human AB serum.

5 Four types of HIS mice can be generated with human FL-derived $CD34^+$HSCs: HIS mice with no thymus, with a native mouse thymus, with a human thymus graft, or with both human and mouse thymi. To generate HIS mice with no thymus, NSG mice are thymectomized and 2 weeks later, sublethaly irradiated (100cGy) and injected i.v. with 1-2x10^5 human FL-derived $CD34^+$HSCs. HIS mice with a native mouse thymus are generated similarly, except they were not thymectomized. Similar to the first group, HIS mice with a human thymus graft are thymectomized. Two weeks later, the mice are injected i.v. with HSCs and transplanted with autologous (to human HSCs) human fetal thymus fragments measuring about 1 mm^3 under the kidney capsule. HIS mice with both human and mouse thymi are generated similar to the HIS mice with a human thymus, except they were not thymectomized.

6 To ensure that pre-existing T cells in the transplanted donor human thymus are not able to persist, we freeze and thaw the thymus tissues and also pipett them up and down several times before transplantation to release as many thymocytes as possible. To further deplete passenger thymocytes that might migrate to the periphery, an anti-human CD2 antibody is injected to the mice in 2 weekly doses (400µg/mouse, intraperitoneally).

7 HIS mice humanized at the neonatal stage are generated by injecting FL HSCs into neonates 1-2 days after birth (intra-liver, 10^5 HSCs in 50µl PBS injected using a 28-gauge needle). Neonatal mice receive half of the irradiation dose (50cGy) that the adult mice receive (100cGy).

8 To generate Personalized Immune (PI) HIS mice with a human thymus graft, thymectomized NSG mice aresublethally irradiated (100cGy) and injected i.v. with 2x10^5 bone marrow (BM)-derived $CD34^+$ HSCs from an adult donor. Two weeks after thymectomy, the mice are transplanted with human fetal thymus fragments (matched to the BM HSCs for a class I and a class II HLA allele) measuring about 1 mm^3 under the kidney capsule. Similar to generation of mice with fetal HSCs, measures are applied to deplete the pre-existing thymus graft-derived T cells.

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