SOP SCB IPS 021_v1	Title: **Induction of teratoma formation for in vivo differentiation**

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OBJECTIVE

Test the in vivo pluripotency of a human stem cell line with the formation of teratomas in SCID mice.

MATERIALS AND EQUIPMENT

- mTeSR-1 (Stem Cell Technologies, Ref.85851)
- Phosphate Buffer saline 1x (PBS, Gibco, Ref.10010-015)
- EDTA 0.5M (Life Technologies, Ref. AM9260G)
- Microcentrifuge tubes (Sigma Aldrich, Ref.T3406)
- Insulin syringe. (Afora, Ref. ZXIE1)

PROCEDURES

NOTE: All steps must be performed under aseptic conditions within a class II safety cabinet.

1. Culture the hPSC in a p10 plate until reaches around 90% confluency.
2. Remove the cells with EDTA as described in **SOP SCB 015_v1** and divide the medium with the cells in two 15 ml. tubes.
3. Centrifuge at 1000 rpm for 5 minutes
4. Discard the supernatant and resuspend with 0,5 ml of mTesR-1 medium.
5. Transfer the cells to 2 microcentrifuge tubes.
6. Centrifuge the cells at 200G x 2 minutes at room temperature.
7. Load a syringe of insulin, connected with a 25G needle, with the supernatant leaving the pellet in the tube (40 µl approximately)
8. Resuspend the pellet lightly and aspirate it with the syringe.
   Then, inject this 40 µl of pellet in the desired place of a SCID Mouse (One sample will be used for one selected place):

   - Intra-muscular, in the gastrocnemius muscle of the right leg.
   - Subcutaneous, in the interscapular area. (In the subcutaneous area it´s possible to inject about 150 µl)
   - Intratesticular, in the right testicle (Inject while holding lightly the testis through the scrotum. Stop the injection as soon as an increase in pressure in the testis is felt).

9. After 8-12 weeks, the animals will be sacrificed, and the samples will be collected.
10. Process the samples for conventional histology or by immunohistochemistry techniques.