Teratoma Assays
Brigitte Arduini, version 1, 2013-Jul-10

The original protocol (from Human Embryonic Stem Cells: The Practical Handbook (chapter by Noggle, Spagnoli and Brivanlou); published 2007-Jul-23) and subsequent modifications were communicated to me by Scott Noggle.

The ability for hES cells to generate teratomas (Keller G, 2005; Spagnoli FM and Brivanlou AH, 2006) in immuno-compromised mice is used as a diagnostic criteria for bona fide embryonic stem cells. In this in vivo assay, hES cells are engrafted into immuno-compromised adult mice in various tissues to generate teratomas. The resulting tumors are routinely analyzed by istology for the various derivatives of the three primary germ layers. With the exception of the host vasculature within the tumor, the teratomas are predominantly derived from the hES cell graft (Gertow et al., 2004). In the case of the vasculature, it was noted that both human graft-derived cells and host-derived mouse cells can contribute to the vessel structures. Frequently, other differentiated and organized tissue can be found in the tumors. This can include, for example, neural tissue and retinal pigmented epithelium, muscle, cartilage, bone, and epithelial cells of the endoderm and ectoderm. However, many of these tissues may be immature and definitive identification of the mature tissue can be difficult. The assistance of a trained pathologist in evaluating the tissues is highly recommended.

Teratomas can be generated at various sites in adult SCID mice by subcutaneous, intraperitoneal or intramuscular injection, implantation under the kidney capsule or beneath the testis capsule (Pera et al., 2003; Przyborski, 2005). As the site of implantation may also influence the growth and differentiation of the teratoma (Przyborski, 2005; Cooke et al., 2006), it is recommended that several sites be tested to access the developmental potential of the hES cells. The strain of SCID mice may also make a difference in the success of engraftment (Przyborski, 2005). NOD-SCID mice are probably the best recipients, followed by the SCID-beige strain. The protocols for subcutaneous, intraperitoneal, and intramuscular injection are similar and have the advantage of being technically simple to perform and do not require surgical manipulation of the mice.

Procedure
Typically, 6- to 8-week-old male NOD-SCID mice are used for injections.

Begin with a 60mm matrigel-coated plate, approximately 60-80% confluent with hESCs in maintenance medium. hESCs are harvested as for passaging (see Enzymatic Passaging, Protocol 1), except:

1. After centrifugation, resuspend the cell pellet in hESC growth medium (100 µl per injection).
2. Transfer cells to a chilled cryovial on ice.
3. Mix with an equal volume of thawed Matrigel stock solution and maintain on ice until the time of injection.
4. Load hESCs into a syringe prior to fitting with a large gauge needle.
5. Inject cells immediately: 0.2ml per mouse, usually five mice total. Injection sites may include beneath the skin on the dorsal rear flank (subcutaneous), into the abdomen (intraperitoneal) or into the muscle of a single real leg (intramuscular).

6. Monitor mice and injections sites weekly for 6 – 22 weeks. The mice should be weighed weekly and watched for signs of infection during the incubation period.

7. Teratomas can be recovered by dissection with surrounding tissue and usually arise between six and eight weeks after engraftment. Tumors are fixed in formalin and sent for histological examination by a pathology service. Alternatively, they can be embedded for cryosectioning and processed for immunohistochemical detection of germ layer markers.