gibco®
by life technologies™

An Introduction to Stem Cell Maintenance

Publication Part Number MAN0006676 **Revision Date** 22 August 2012

Introduction

Pluripotent Stem Cells (PSCs) can divide indefinitely, self-renew, differentiate and functionally develop into almost any cell in the body, given the right conditions. There are several kinds of pluripotent stem cells:

- **Embryonic Stem Cells:** Isolated from the inner cell mass of the blastocyst stage of a developing embryo. These early cells were destined to create a fetus following implantation.
- **Embryonic Germ Cells:** Derived from aborted fetuses. These early cells were destined to become sperm and eggs.
- **Embryonic Carcinoma Cells:** Isolated from certain types of fetal tumors.
- **Induced Pluripotent Stem Cells:** Generated via ectopic expression of one or more genes to reprogram an adult somatic cell.

PSCs are generally maintained on a layer of feeder cells for many passages without any compromise to proliferation, pluripotency or differentiation potential. Feeder cells are usually murine embryonic fibroblasts (MEF), which must be irradiated or chemically treated to inactive them (noted as iMEF) prior to culturing with PSCs. Alternatively, PSCs can be maintained in feeder-free conditions using specialized media systems on a matrix-coated tissue culture surface. This introduction will discuss the feeder-dependent and feeder-free culture of human ESCs and iPSCs (noted as hESCs and hiPSCs), which will be referred to as PSCs.

Feeder-based medium is comprised of basal medium supplemented with 15–20% KnockOut™ Serum Replacement (KSR) and other additives. Feeder-free medium is either Conditioned Medium (CM), which is comprised of feeder-based medium that has been conditioned on iMEFs and therefore can be used to grow PSCs in the absence of feeders, or commercially available media that support feeder-independent growth of PSCs.

Helpful Tips and Tricks

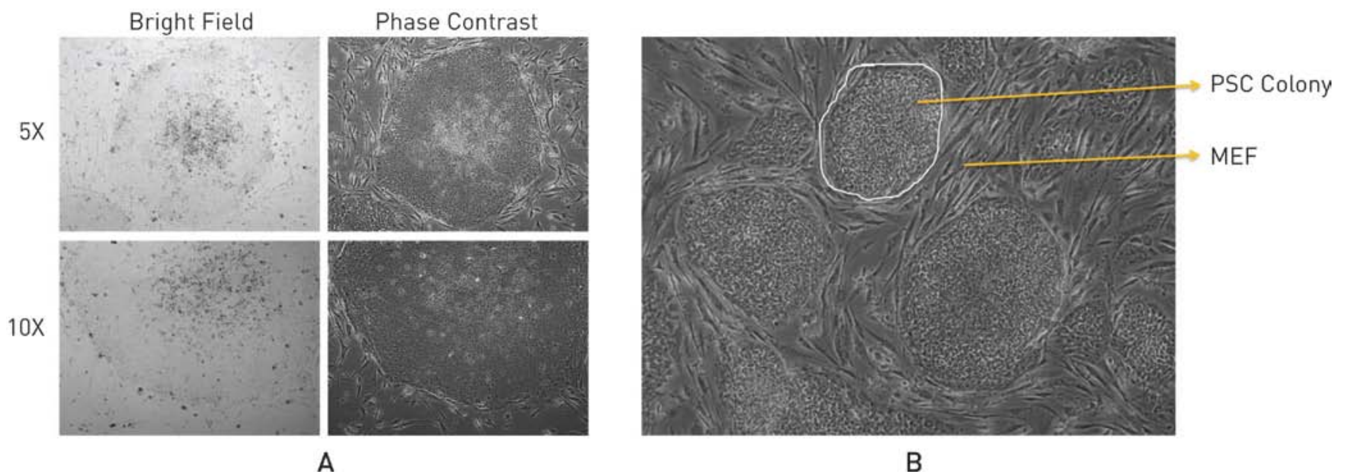
- General maintenance of PSC cultures requires daily removal of spent media and replenishment with fresh PSC medium. It is crucial to add fresh bFGF, aseptically on a daily basis, to the pre-warmed media prior to adding to the cells. Daily visual inspection of cell morphology is highly recommended for proper growth and for the removal of any differentiating colonies via manual dissection.
- As daily maintenance of the PSC cultures is required, it is helpful to develop an optimal working schedule. PSCs should be split every 3–4 days, based on colony size and distribution. Hands-on experience and a keen eye are most important in PSC culture. To avoid spontaneous differentiation of the colonies, **do not**:
  - allow colonies to overgrow and touch each other
  - over incubate the colonies in enzyme, when passaging them
  - passage huge colonies

- Generally, a manageable 7-day schedule for PSC culture is employed as follows:
  - **Monday:** Feed existing PSC cultures and make iMEF plates
  - **Tuesday**: Split PSC cultures onto iMEFs prepared Monday. A 1:3 or 1:4 split, meaning one dish passaged into 3 dishes or 4 dishes, respectively, is good for maintenance.
  - **Wednesday:** Feed PSC cultures.
  - **Thursday:** Feed existing PSC cultures and make iMEF plates (unless you have remaining iMEFs from Monday, which may be used at this time).
  - **Friday:** Split PSC cultures onto iMEFs. A 1:4 or 1:5 split is good for maintenance over the weekend.
  - **Saturday/Sunday:** Feed PSC cultures.

    Optional: If cells cannot be fed both weekend days, you may skip a single day and just feed your cultures an additional 1–2 mL of media the day before.

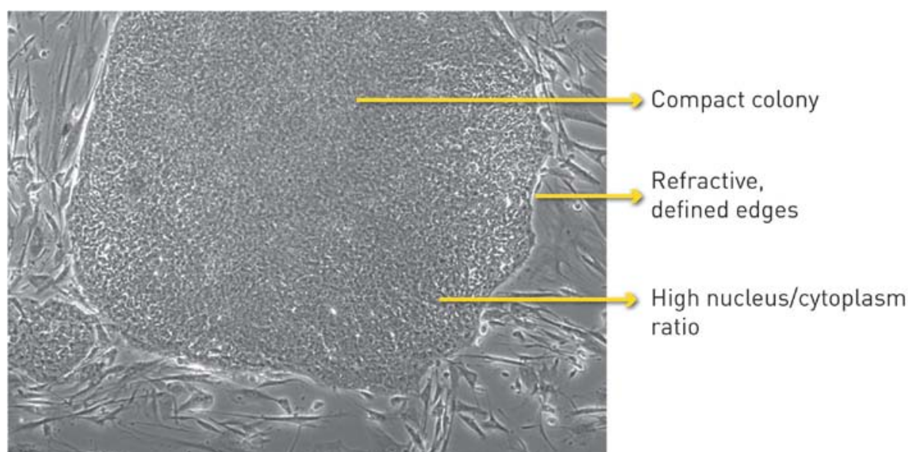
Using the Correct Microscope

Figure 1 A microscope with a 5X Objective and Phase contrast or DIC is ideal to observe a PSC colony morphology (panel A). Traditionally cultured hESCs or hiPSCs appear as compact colonies surrounded by fibroblast shaped feeder cells (iMEF) (panel B).



Recognizing Correct Morphology

Figure 2 H9 ESC cultured on iMEF. PSCs grow in a compact colony formation with very well defined borders. PSCs have a high nucleus-to-cytoplasm ratio and the colonies grow in a 3-dimensional radial pattern.



Deviation in PSC Morphology with Differentiation

Figure 3 PSC colonies that are beginning to differentiate show loss of defined edges and the emergence of large differentiated cells. The central core remains compact and it is possible to rescue this colony by scrapping out the differentiated cells at the edges.

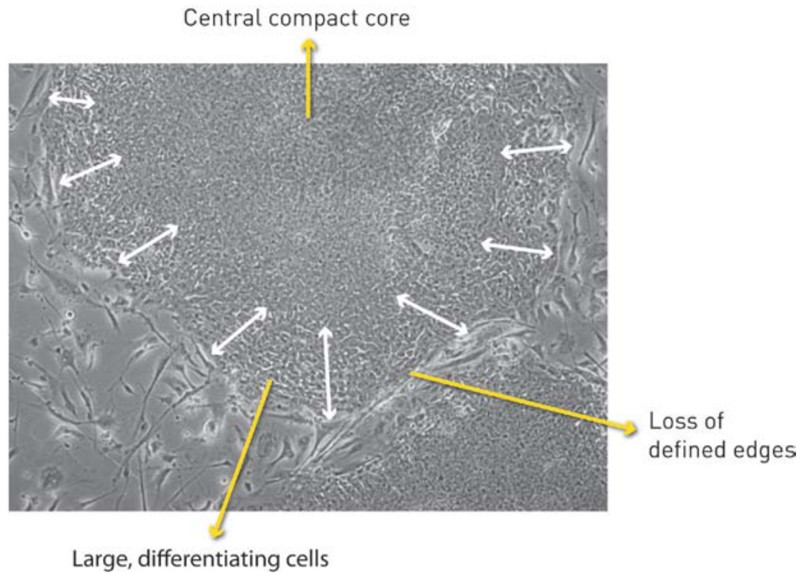
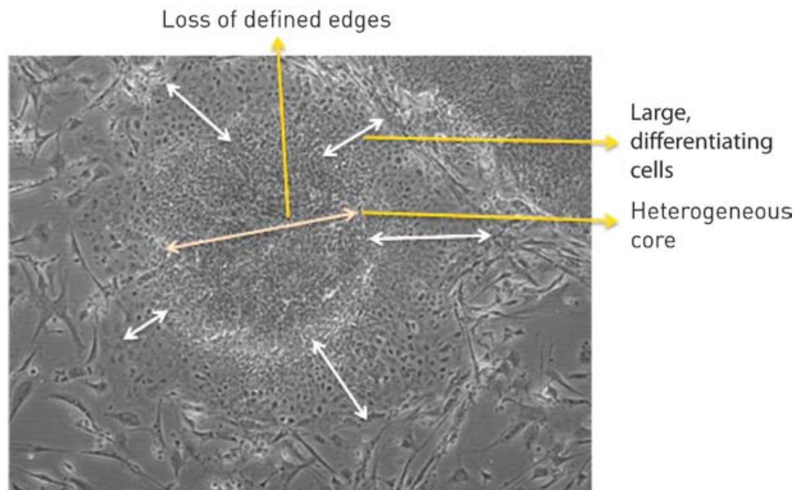


Figure 4 Differentiating colonies show loss of defined edges, possess large differentiated cells and a heterogeneous central core that is not typical of a PSC.



Passaging hPSC Cultures on Feeders

In general, split cultures when the first of the following occurs:

a) iMEF feeder layer is 10 days old; usually differentiation will occur more frequently if MEFs are too old

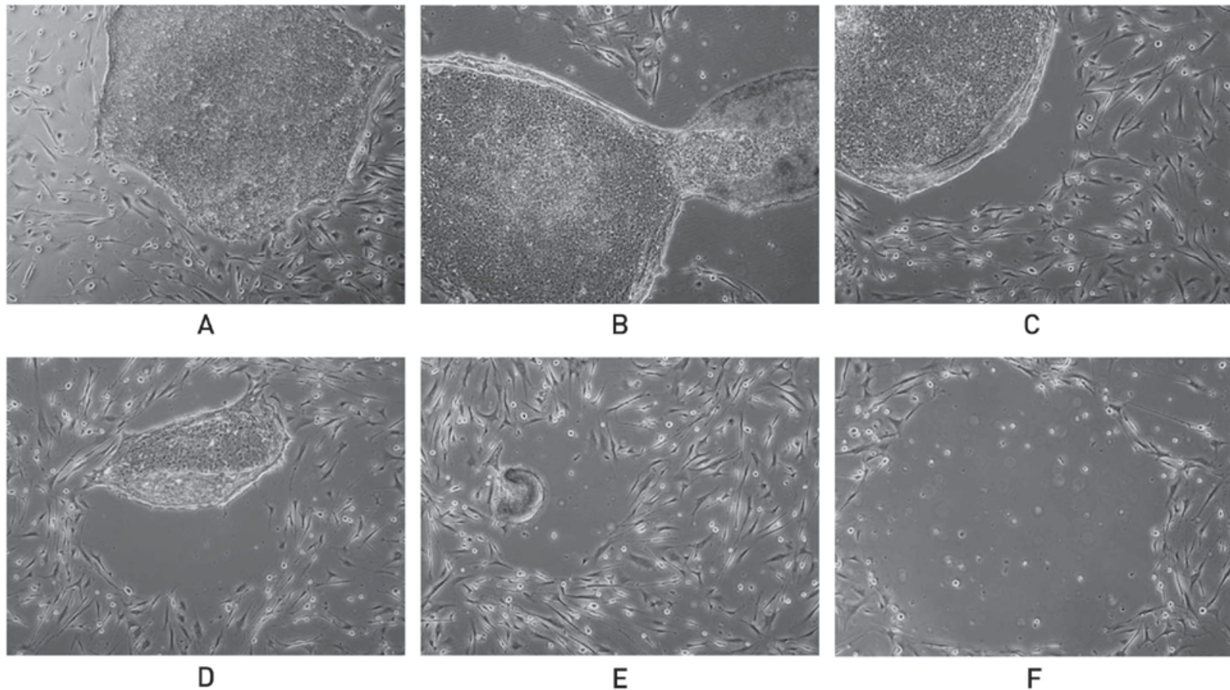
b) PSC colonies are becoming too dense or too large

c) Increased differentiation occurs

Enzymatic Passaging of hPSCs

Enzymatic passaging of PSCs will vary from cell line to cell line. Some hESC cell lines or hiPSCs may not react in the same manner to enzymatic passaging, and consequently the enzyme's type, concentration, and exposure time must be empirically determined for the particular cell line to be passaged. If the hESC or hiPSC line being cultured is not optimally passaged enzymatically, manual or mechanical passaging must be performed.

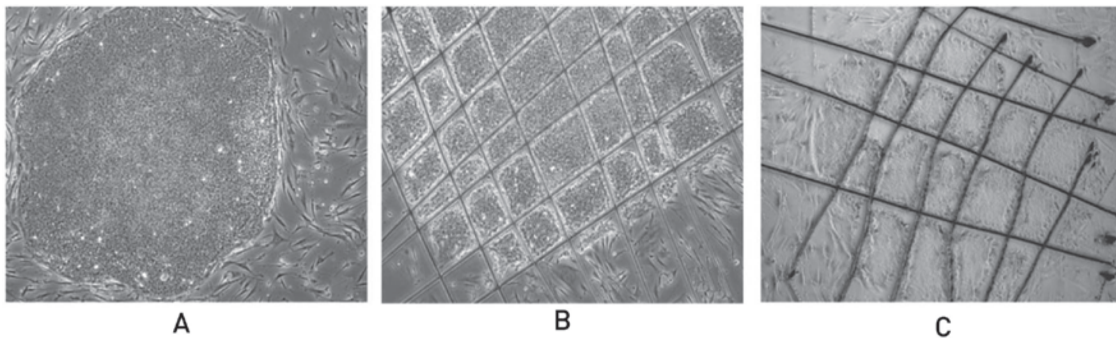
Figure 5 PSC colonies can be harvested in bulk using enzymatic methods such as treatment with Collagenase Type IV for 30–60 minutes. During this time, attached PSC colonies (panel A) curl up and detach from the dish (panels B–E), leaving behind iMEFs (panel F). When the colonies begin to curl up (panels B-C), cell clusters can be gently dislodged with a 5-mL pipette. Care should be taken to not over expose the PSCs to the enzyme as it may affect the efficiency of recovery.



Mechanical Passaging of hPSCs

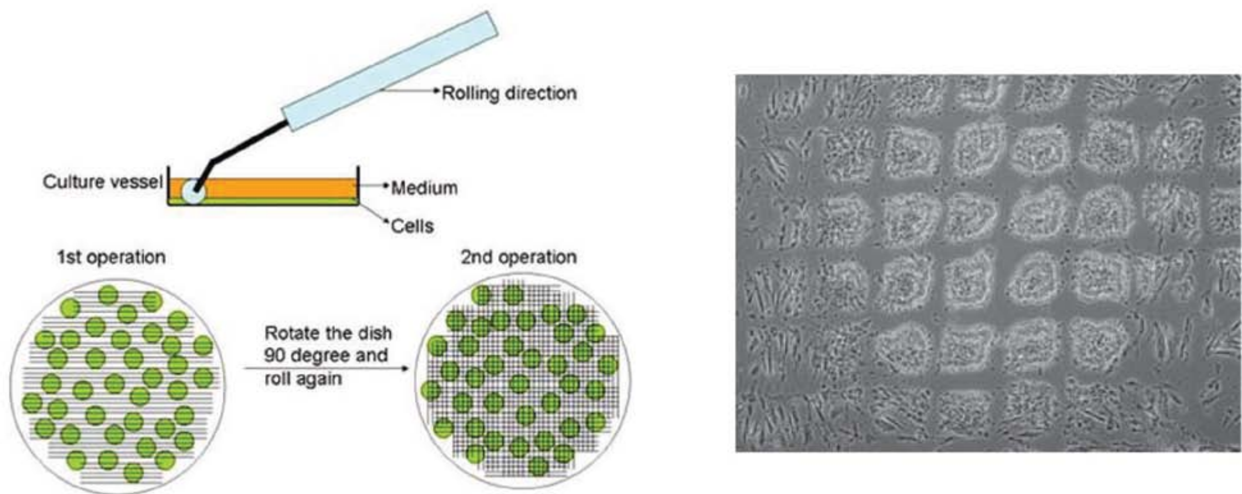
During the culture of PSCs, it may be necessary to manually dissect PSC colonies to either remove undesired portions of a colony or whole colonies, or to break up and passage individual colonies. Traditional tools include a drawn-out glass Pasteur pipette and needles that can be used to dissect individual colonies. In particular, this method is used for maintenance of PSC colonies by removing unwanted differentiated colonies and for manually passaging colonies of PSCs that cannot be passaged enzymatically. Mechanical passaging is also an important tool for hiPSC selection and maintenance.

Figure 6 PSC colonies (panel A) may be manually dissected using a needle and syringe. Straight cuts in horizontal and vertical directions create a checker board pattern of small cell clusters that can be re-seeded for expansion and passaging (panel B). Uneven cuts (panel C) and microscale manipulations make this procedure labor-intensive.



Mechanical Passaging of hPSCs Using the StemPro® EZPassage™ Disposable Stem Cell Passaging Tool

Figure 7 Colonies can be mechanically scored using the StemPro® EZPassage™ tool. The entire dish is scored with two quick movements and the broken-down parts of the colony are transferred to a fresh iMEF-coated dish.



Daily Monitoring of Passaged hPSCs

A typical passage schedule for PSC is as follows:

Day 0: Seed iMEFs on Attachment Factor–coated tissue culture plates or dishes.

Day 1: Seed PSCs on attached iMEF feeder layer.

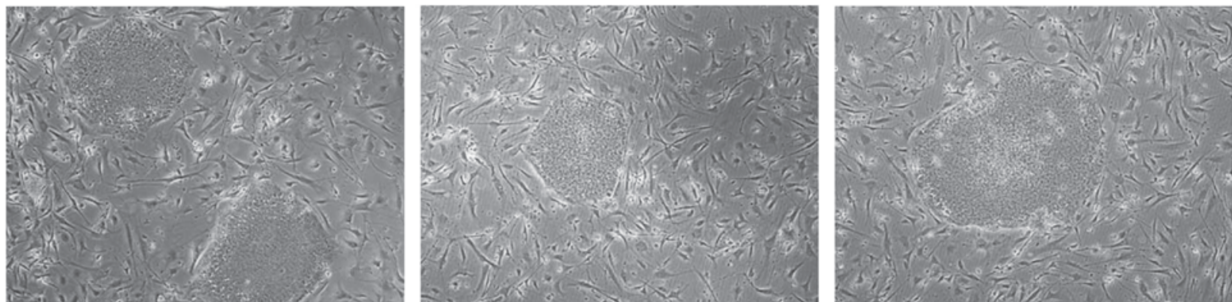
Day 2–4: Change media and monitor for distribution and morphology of attached colonies.

Day 5: Passage PSCs onto iMEF coated dishes.

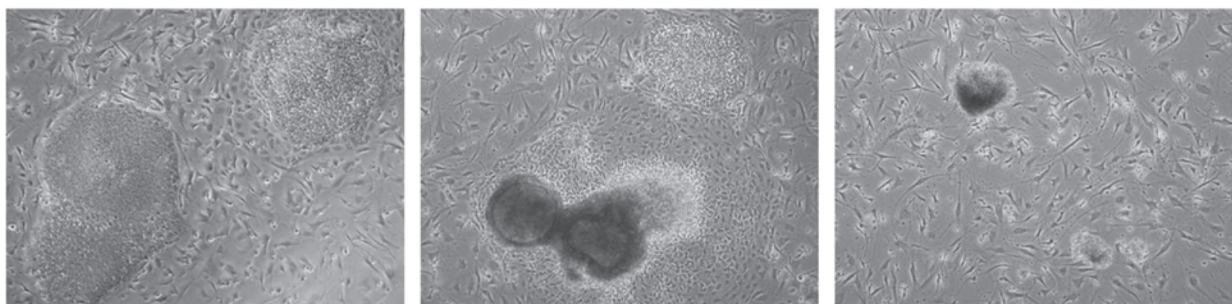
- It is important to remove spent culture media and add fresh media **every day**. Most often, differentiated cells at the edges of each colony can be mechanically removed with a 27-gauge needle before passaging. It is critical to observe the distribution of attached colonies 24 hours post-passage.
- Colonies sometimes can all be clustered towards the middle, close to each other or on top of each other. An even distribution of colonies is critical for the maintenance of undifferentiated colonies during expansion. It is also important to monitor the attachment and morphology of the colonies. In all dishes there will always be some areas with unattached cells or cells that look differentiated. However, the majority of the colonies in the dish must be well-separated, with a pristine morphology.

Day 2

Figure 8 Cells 24 hours post-passage (Day 2). Examples of areas containing colonies with good (top panels) versus bad morphologies (bottom panels).



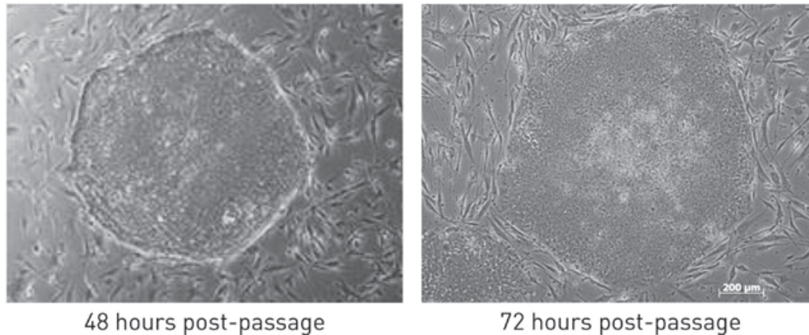
Uniformly sized colonies distantly placed and attached firmly to the iMEF layer



Colonies too close to each other | Differentiated areas | Unattached cells

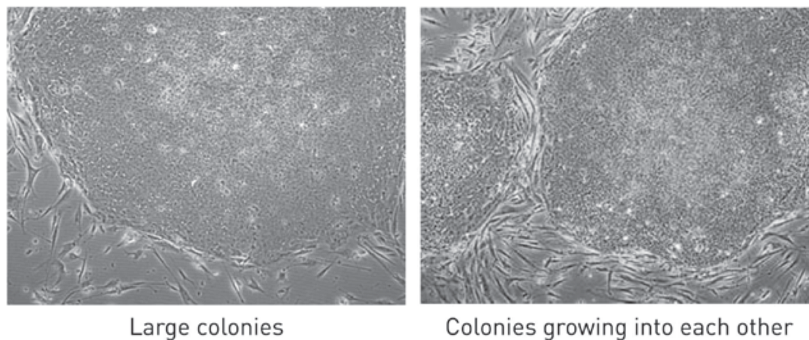
Days 3–4

Figure 9 48 to 72 hours post-passage (Days 3–4). Well-separated, seeded-down colonies continue to expand and grow in size while maintaining their morphology.



Day 5

Figure 10 hPSC colonies are ready for passage 96 hours post-passage (Day 4). Most colonies are large (at 5X magnification they fit the entire visual field) and the neighboring colonies begin to grow into each other.



Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Disclaimer

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

© 2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.
For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com