Passaging with ReleSR

Equipment:

- Pre-Coated Cell Culture Plates

Reagents/Materials:

- ReleSR (StemCell Technologies, Cat. 05872)
- Cell Culture Media

Procedure:

1. Aspirate media from cells
    a. Optional: Rinse once with 1X dPBS (without CaMg). This helps to remove any excess calcium that might be around.
2. Add ReleSR to the well or plate with cells (needs to be enough to cover the plate):

Plate Type	**Volume of ReleSR to add per plate/well**
6-well	500uL
12-well	300uL
24-well	200uL
96-well	50-100uL
10cm plate	3-5mL

3. After adding ReleSR to the well or plate, aspirate off ReleSR so that the colonies are exposed to a thin film of ReleSR.
4. Place the plate in an incubator for 5-7 minutes.
    a. Incubation time can vary based on cell line, cell culture matrix, and whether differentiation exists. Please see the detailed protocol from StemCell Technologies for more information.
5. After the plate has finished incubating, firmly tap the plate against your hand (or the wall of a BSC) to dislodge colonies from the plate (~30-60 seconds)
6. Resuspend colonies with an appropriate volume of cell culture media (i.e., 1mL for one well of a 6-well, 0.5mL-1mL for one well of a 12-well, 0.5mL for one well of a 24-well, etc.)
7. Dilute cells into a new plate containing cell culture media.
8. Mix cells by moving the plate back and forth and side to side (avoid a circular motion)
    a. It's important to do this immediately after cells have been added to a new plate **and** when the plate is put back into the incubator.
9. Feed cells the next day.

Protocol Updates:

Date	Update Notes