University of Michigan Comprehensive Cancer Center	SOP-BCR-3.5	**Cell Passage**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 09/24/98 Revised: 7/13/12

1.0 Purpose

The purpose of SOP 3.5 is to provide details on how to split cells

2.0 Scope

SOP 3.5 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	HBSS	Hank's Balanced Salt Solution	Cold Storage (026-380C)
2.0	Trypsin/EDTA	Cell detachment	Freezer #2 (026-328S-A)
3.0	FBS	Fetal Bovine Serum	Freezer #2 (026-328S-A)

4.0 Procedure

4.1 **Before splitting:** (Note: volumes may vary depending on culture equipment used--adjust as necessary)

- Take out trypsin, HBSS and medium place in water bath and warm to 37° C
  - *Take out desired number of new culture flasks/plates for cells. (If using collagen coated plates, take out of freezer to warm and rinse once with 2mL of HBSS)*
- Label new culture equipment with cell line, passage #, medium type and date
- Add 15 mL of medium to each fresh flask/plate.
- For cells grown in serum free medium, add 2% FBS to the fresh flask/plate (this is for cell attachment)

4.2 **Splitting**:

- Aspirate medium from the cells to be split
- Rinse flask with 5-10 mL HBSS, aspirate HBSS.
- Add 3 mL of warm 0.25%Trypsin/EDTA and place flasks/plates in incubator for approximately 1-2mins.
  - *This step is to detach the cells from the bottom, if not completely detached, whack the sides of flask firmly with the palm of your hand.*
- Check that all cells have been detached by viewing them under a microscope.
- When complete, add 10mL of HBSS with 2%FBS mixture to the flask. This will stop the trypsin from further digestion.
- At this point the cells should be moving and rolling off of the dish, pipette the media up and down, thus ensuring complete detachment of all cells.
- After cells are detached, pipette entire contents of flask into a 15 mL centrifuge tube.
- Place tube in centrifuge and spin at 1000xG for 5mins. A pellet should develop, discard supernatant and resuspend in approx 4mL of HBSS (This amount will vary depending on the volume of the cell pellet). Split this mixture equally amongst the new flasks.
- Plate cells according to the split ratios indicated, these may be modified once you feel comfortable with the cell lines you are working with.

5.0 Applicable References

6.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/13/12	CL	Updated room locations