University of Michigan Comprehensive Cancer Center	SOP-BCR-2.2	**Human Tumor Digestion (from primary tumor) to obtain single cell suspension**	Author: S. Clouthier Approved: M. Wicha	Rev: 2	Issued: 09/24/98 Revised: 8/13/14

1.0 Purpose

The purpose of SOP 2.2 is to provide details on how to obtain a single cell suspension from a primary human tumor.

2.0 Scope

SOP 2.2 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	M-199	Medium 199 cat# 11150-06	Cold Storage (026-380C)
2.0	Collagenase/ Hyaluronidase (10x)	Enzyme cat # 07912	Freezer 2 (026-314S)
3.0	HBSS	Hank's Balanced Salt Solution	Cold Storage (026-380C)
4.0	FBS	Fetal Bovine Serum	Freezer #2 (026-314S)
5.0	40 uM cell strainer	Falcon, cat# 08-771-1	Back stock (026-320S-A)

4.0 Procedure

4.1 Before digestion with tumor collagenase, primary human tumors are cross-cut into small pieces and minced completely until nearly liquid by using scalpels; be sure to cut the tissue and not tear the tissue. You can use tape to hold down the plate while mincing.

4.2 Add collagenase based on amount of tissue. Collagenase: 9 parts medium 199 to every 1 part collagenase/hyaluronidase. Make up 200-250 units of collagenase mix per mL of tumor, total volume adjusted to the size of the tumor, not to exceed 3-4 mL.

4.3 Transfer the cut up tumor in collagenase solution into a 50 mL conical tube.

4.4 Put the 50 mL conical tube into a 37° C shaker or water bath. If in shaker, set motion at 65 rpm for 30 minutes to 1 hour, mixing half way in between. Otherwise, incubate for up to 1 hour in water bath, mixing every 15 minutes.

4.5 Titrate the mixture 20-25 times using a 5 mL syringe with either 18 or 23 gauge needle. The suspension should go through the needle.

4.6 Stop digestion by adding 2% FBS/HBSS mix in at least equal amount of collagenase mix added.

4.7 Cells are then either centrifuged for 30 seconds at 40 g to separate single cells/fibroblasts from organoids (supernatant will contain single cells, pellet organoids) or filtered through a 40 uM nylon mesh cell strainer then centrifuged for 5 minutes at 1000 rpm, supernatant aspirated off, and pellet re-suspended in 2% FBS/HBSS mix.

4.8 Wash twice with 2% FBS/HBSS mix.

4.9 Note: If desired, you can do a differential sedimentation step, to separate the aggregates of cells from the single cells, as described below:

- After first wash with M-199, resuspend cells in 10 mL M-199. Agitation by hand and then let sit for 15 minutes.
- Carefully, remove the liquid from the cells that have settled to the bottom of the tube and set aside.
- Repeat previous two steps once and proceed. Be sure to do a cell count both to the supernatant and the pelleted cells.

4.10 After last wash, resuspend cells in 5 to 10 mL media and/or freeze organoids. Take a 50 µL aliquot to Coulter Count. Count the nuclei so you know how many cells are present.

4.11 Plate the cells in 5% IH at as desired density, usually at 10^6 cells per 35 mm plate. Freeze cells that are not plated at 5-10 x 10^6 cells per ampule in Freezing Media.

5.0 Applicable References

SOP 3.3: Cell Freeze and Thaw
SOP 3.2: Coulter Counting
SOP 4.22: Preparation of Collagenase for tumor digestion

6.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/2/12	CL	Updated room numbers
2.0	8/13/14	TL	Combined with SOP 2.7 since they are the same and lowered digestion time from 2 hours down to 1 hour at most