1.0 Purpose
The purpose of this modified SOP 2.5 is to detail the procedure for dissociating Spheres formed from NSCLC cell lines.

2.0 Scope
SOP 2.5 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Description</th>
<th>Storage Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Cell Strainer (BD Falcon)</td>
<td>40uM Nylon</td>
<td>Supply Area</td>
</tr>
<tr>
<td>2.0</td>
<td>50-ml Steriflip (Millipore)</td>
<td>0.22uM</td>
<td>Supply Area</td>
</tr>
<tr>
<td>3.0</td>
<td>HBSS (without FBS)</td>
<td>Hank’s Balanced Salt Solution</td>
<td>Cold Storage (026-380C)</td>
</tr>
<tr>
<td>4.0</td>
<td>0.25% Trypsin</td>
<td>Serine Protease</td>
<td>Freezer #2 (026-328S-A)</td>
</tr>
<tr>
<td>5.0</td>
<td>RPMI 1640/10%FBS</td>
<td>Complete Medium</td>
<td>Cold Storage (026-380C)</td>
</tr>
<tr>
<td>6.0</td>
<td>5-ml Flow tube (BD Falcon)</td>
<td>Polystyrene Round-Bottom Tube</td>
<td>Supply Area</td>
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<tr>
<td>7.0</td>
<td>DAPI in HBSS (without FBS)</td>
<td>1:1000</td>
<td></td>
</tr>
</tbody>
</table>

4.0 Procedure

Collection of Spheres
4.1 Collect the Spheres with a 40uM Nylon Cell Strainer and a 50-ml Conical tube. Use the sidewall or corner of Strainer to decrease loss of Spheres.
4.2 The Culture Medium contains the single cells. Spin at 1000 rpm for 5 minutes. Filter the Supernatant with a 50-ml Steriflip and save as the conditioned medium for future use.
4.3 Turn the Strainer up-down and wash the Spheres on the sidewall or corner of Strainer with 10ml HBSS (without FBS) into a 50-ml Conical tube.
4.4 Spin at 1000 rpm for 5 minutes and Remove supernatant.
4.5 Wash the pellet with 10ml HBSS.
4.6 Spin at 1000 rpm for 5 minutes and Remove supernatant.

Trypsin Digestion
4.7 Add 3-5ml of Trypsin pre-warmed at 37°C.
4.8 Incubate for 5 minutes at Room Temperature or 3 minutes at 37°C.
4.9 Up-down the Sphere digestion with a glass pipette and Pass the Sphere digestion for 3-5 times through a 22-gauge Needle with Flat-end.
4.10 Add 5ml of RPMI 1640/10%FBS to inactivate Trypsin.
4.11 Spin at 1000 rpm for 5 minutes and Remove supernatant.
4.12 Wash the pellet with 10ml HBSS and Spin at 1000 rpm for 5 minutes.
4.13 Re-suspend the pellet with 3ml HBSS and transfer into a 5-ml Flow tube.
4.14 Spin at 1000 rpm for 5 minutes and Remove supernatant.
4.15 Re-suspend the pellet with 3ml HBSS and Repeat 4.12 step.
4.16 Add 1ml DAPI in HBSS (without FBS).

5.0 Applicable References
The original SOP 2.5.