IMPORTANT RULES
FOR SUCCESSFUL CELL CULTURING

1. Keep the cryovials in dry ice for transport & temporary storage
   - Store the cryovials long-term in liquid nitrogen
   - **DO NOT** expose the cryovial to ambient temperature

2. Use the right kind of tissue culture ware
   - Use flasks, dishes and plates cell culture-treated for optimal cell adhesion
   - Brands can vary, so you may wish to test and compare: If experiencing issues, contact the plate manufacturer or CAI for recommendations
   - Coat vessels with attachment factors or ECM components when indicated
   - **DO NOT** use un-treated or un-coated plates for adherent cells

3. Thaw the cryovials at 37°C, and remove before fully thawing
   - Thaw cryovials in a 37°C water bath and take out after approximately 60 seconds
   - There should still be a few ice crystals left after thawing
   - **DO NOT** over-thaw the cryovials

4. Use media & subculture reagents at or below room temperature
   - **DO NOT** preheat culture/growth medium, trypsin/EDTA, HBSS, or trypsin neutralizing solution to 37°C in an incubator or water bath

5. Use proper cell detachment solutions and technique
   - Use Cell Applications, Inc. Trypsin/EDTA, with the correct concentration
   - Knock the cells loose when the cells round up: Do not wait for the cells to detach by themselves
   - **DO NOT** over-trypsinize the cells