Criteria for optimal stem cell maintenance

Ready for passaging - ~70% confluency

Cells from ideal single cell trituration

Consistent maturity

- Morphology
 - High degree of "Stemness" well defined edges, uniform/smooth "lake-like" middle. Avoid colonies with spontaneous differentiation.
 - Loss of stem-ness can be confirmed by testing for high levels of human Stem Cell markers (Oct4, Nanog, SSEA3/4) by flow or immunostaining
- Seeding density and confluence avoid passaging over-confluent monolayers. Aim for 70-85% confluence.
- Trituration/resuspension Ensuring consistency in single cell suspension will result in colonies of similar size and maturity for passaging
- Maturity Aim for a majority of mature colonies -well packed colonies with small, smooth cells in the middle at d3-d4

Criteria for optimal stem cell maintenance *Things to avoid*

Non ideal trituration – clumping and inconsistent





Over-confluent – do no use



Criteria for optimal stem cell maintenance *Things to avoid*

Compromised morphology



- Bad colonies: signs of stress/differentiation shown through presence of 'larger,' flat/different-looking cells
- Do not use since the prevalence of bad colonies (despite the presence of a few good colonies) will negatively affect the whole well

Criteria for optimal stem cell maintenance *Things to avoid*



Cells not fed for <u>one</u> day, and see these warning flags/signs of stress:

• Spiky, flat, different morphology on all borders

Cell seeding and colony growth timeline



Confluency-time of passaging depends on cells seeded

500K cells seeded on D0

Day 3



Not ready for passaging - ~50% confluency

Day 4



Ready for passaging - ~85% confluency



Ready for passaging - ~70% confluency





Overconfluent - Do not use

1 million cells seeded on D0

Example of banked cell lines – range of morphologies

Two lines with typically ideal morphology – representative of most lines

DSP (AICS17)



Tubulin (AICS12)

MYH10 (AICS24)



Act B (AICS16)

Two lines with typically less ideal morphology – some spiky cells observed at the edge of colonies



