Criteria for optimal stem cell maintenance

Cells from ideal single cell trituration

Consistent maturity

Ready for passaging - ~70% confluency

- 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 one day, and see these warning flags/signs of stress:
- Spiky, flat, different morphology on all borders
Cell seeding and colony growth timeline

500K cells seeded on D0

Day 1
Single cell suspension

Day 3
Maturity and consistency in colony size

Max confluence - ready to passage

Day 4
Over-confluent
Do not use

1 million cells seeded on D0
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

1 million cells seeded on D0

Day 3
Ready for passaging - ~70% confluency

Day 4
Overconfluent - Do not use
Example of banked cell lines – range of morphologies

Two lines with typically ideal morphology – representative of most lines

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