Breakout Session Report
Cell Viability

- Cell Preparation for Viability Measurement
  - Cell clumping
  - Debris
  - What can be measured?
- Fraction of cells or total in well measurement
- Measurement of viability after thawing
  - Freezing/thawing issues can occur
  - DMSO removal/characterization is common among labs

Cell Clumping

- It appears that cell clumping is a problem with many cell types.
  - May be due to cell adherence
  - May be due to DNA release after cell rupture. Potentially viable cells become part of the clump. Addition of Dnase would help reduce this, but once clumping starts, cell counting and viability will not be accurate.
- Cells that produce aggregates as part of the culture process, e.g. ES or iPS embryoid bodies, would be difficult to obtain reliable and reproducible cell counts and viability measurements.
- To obtain reliable viability measurements, a single cell suspension in the absence of debris would be required, otherwise too many errors would be introduced.

Cell Debris

- Viability can be significantly affected by the presence of cell debris.
- To accurately measure viability, the cells would have to be “clean” of debris to avoid false positive results.
- Cell washing and/or cell fractionation may be needed.

Viability after Thawing

- Method of cryopreservation affects cell viability.
- Thawing protocol can affect viability, cell count, recovery, yield.
- DMSO is toxic to cells.
- Cells can clump.
- Considerable amount of cell debris.
- At each step, viability can be affected.

What can be Measured?

- Is viability based on the whole intact cell functionality, e.g. metabolic viability measurements?
- Is viability a dye exclusion method? If so:
  - What is the basis of the viability measurement: stained individual nuclei or total cells (total nuclei)?
  - How are these distinguished?
- Are multiple fluorophores useful?
- Are the metrics for dye exclusion reliable and reproducible.
- What is being measured: live or dead cells?

Reporting issues

- Writing down “what you did” is not always enough.
- Evidence of what you did means measurement. What evidence do you have that it went OK.
- Centrifugation speed and time? Are you right? What do you expect? How do you make it reproducible?
- Can you measure something that validates a previous process? This is useful to report.

What Measurement?

- Instrument readout
  - Metabolic
  - Color/Visual
  - Fluorescence/Visual
  - Calibrated, standardized, validated
- Sample preparation/handling
  - Fresh: adherent, non-adherent
  - Frozen
- Do different viability measurements need to correlate with each other and if not, which one can be trusted?