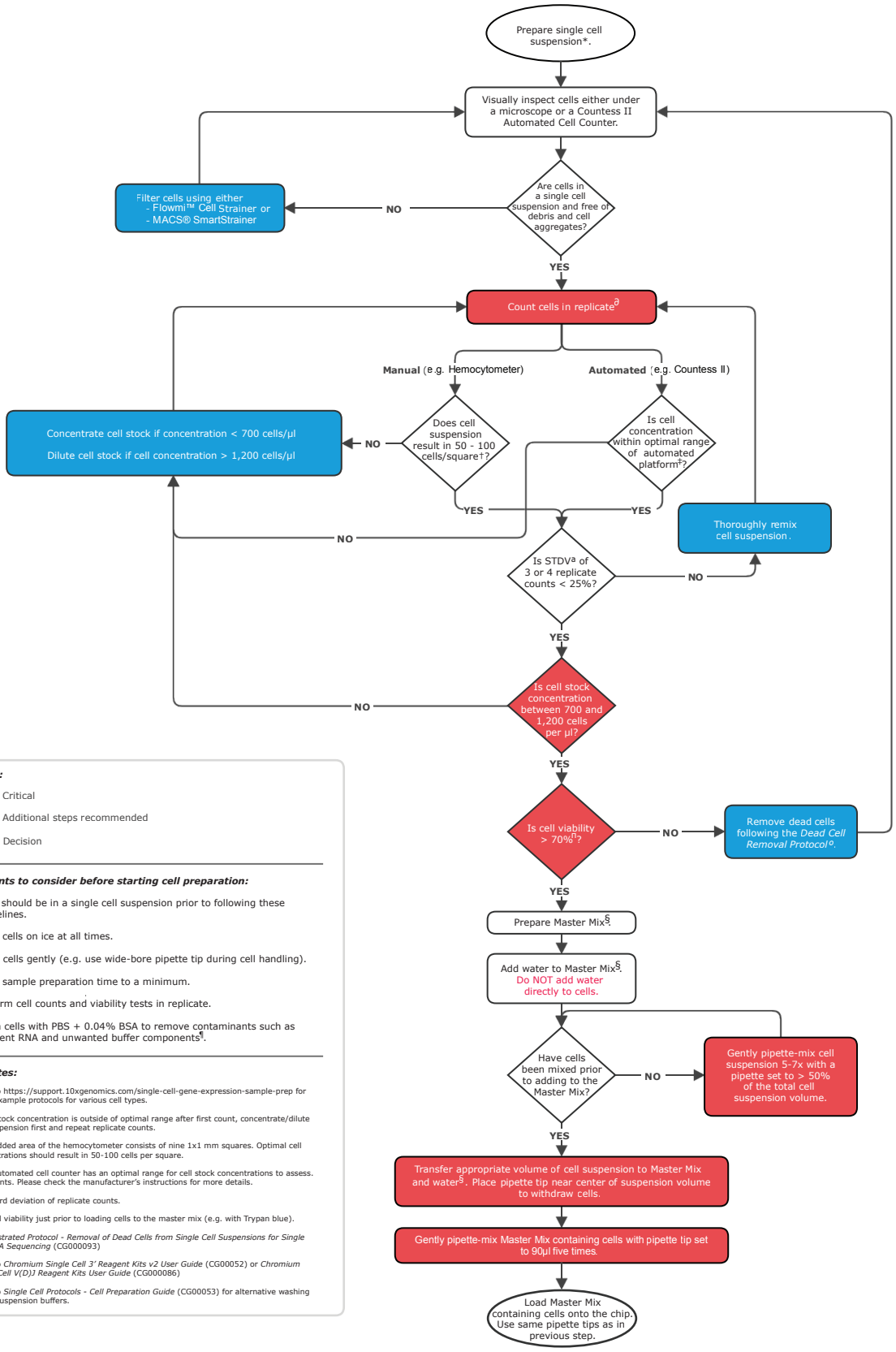
Chromium™ Single Cell Applications

Guidelines for Optimal Sample Preparation

10x GENOMICS®



Legend:

- Critical
- Additional steps recommended
- Decision

Key points to consider before starting cell preparation:

1. Cells should be in a single cell suspension prior to following these guidelines.
2. Keep cells on ice at all times.
3. Treat cells gently (e.g. use wide-bore pipette tip during cell handling).
4. Keep sample preparation time to a minimum.
5. Perform cell counts and viability tests in replicate.
6. Wash cells with PBS + 0.04% BSA to remove contaminants such as ambient RNA and unwanted buffer components¶.

Footnotes:

* Refer to https://support.10xgenomics.com/single-cell-gene-expression-sample-prep for more example protocols for various cell types.

∂ If cell stock concentration is outside of optimal range after first count, concentrate/dilute cell suspension first and repeat replicate counts.

† The gridded area of the hemocytometer consists of nine 1x1 mm squares. Optimal cell concentrations should result in 50-100 cells per square.

‡ Each automated cell counter has an optimal range for cell stock concentrations to assess. cell counts. Please check the manufacturer's instructions for more details.

ª Standard deviation of replicate counts.

n Test cell viability just prior to loading cells to the master mix (e.g. with Trypan blue).

° *Demonstrated Protocol - Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing* (CG000093)

§ Refer to *Chromium Single Cell 3' Reagent Kits v2 User Guide* (CG00052) or *Chromium Single Cell V(D)J Reagent Kits User Guide* (CG000086)

¶ Refer to *Single Cell Protocols - Cell Preparation Guide* (CG00053) for alternative washing and resuspension buffers.