Tapestri® Genotype Multiplexing

IMPORTANT *This protocol describes the necessary steps to multiplex up to three **unrelated** samples upstream of Encapsulation on the Tapestri® instrument. Germline variants are used for sample demultiplexing using the Tapestri Pipeline. For accurate demultiplexing, each sample must comprise at least 0.1% of the pooled cell suspension.*

DNA-only Applications

NOTE *Follow the Tapestri® Single-Cell DNA v3 User Guide, PN MB05-0017, Steps 1.1–1.8 (through cell resuspension in DPBS, before centrifugation). Perform Steps 1.2–1.5 for each sample one at a time and store on ice, then process all samples in parallel.*

1. **Quantify the cells and assess viability** using an automated cell counter or hemocytometer following best practices and the manufacturer's instructions.

   NOTE *The recommended input for cell encapsulation is 100,000 cells (30,000 minimum). It is recommended to use 150,000 cells in Step 2 below (50,000 minimum), to account for cell loss.*

2. **Pool cells** from up to three samples for a **total of 150,000 cells** in a new **1.5 mL DNA LoBind** tube. *If needed, use a 15 mL conical tube to accommodate larger volumes.*

   NOTE *If using a diploid cell line as a control for CNV measurement, add enough diploid cells to constitute 5% of the final pool.*

3. Centrifuge the multiplexed cell suspension at **400 x g for 5 minutes** at 4 °C in a swinging bucket.
4. **Place cells on ice** and proceed **immediately** to *Step 1.9* of the *Tapestri® Single-Cell DNA v3 User Guide, PN MB05-0017.*

DNA + Protein Applications

NOTE *Follow the Tapestri® Single-Cell DNA + Protein v3 User Guide, PN MB05-0018, Steps 1.1–1.16 (through cell resuspension in Cell Staining Buffer). Perform Steps 1.3–1.9 for each sample one at a time and store on ice, then process all samples in parallel.*

1. **Quantify the cells and assess viability** using an automated cell counter or hemocytometer following best practices and the manufacturer's instructions.

   NOTE *The recommended input for cell staining is 1M cells (400,000 minimum). It is recommended to use 1.5M cells in Step 2 below (600,000 minimum), to account for cell loss.*

2. **Pool cells** from up to three samples for a **total of 1.5M cells** in a new **1.5 mL DNA LoBind** tube. *If needed, use a 15 mL conical tube to accommodate larger volumes.*

   NOTE *If using a diploid cell line as a control for CNV measurement, add enough diploid cells to constitute 5% of the final pool.*

3. **Place cells on ice** and proceed **immediately** to *Step 1.17* of the *Tapestri® Single-Cell DNA + Protein v3 User Guide, PN MB05-0018.*