Data Collector – DNA + Protein Workflow v3

*Ensure Tapestri Instrument is updated with v3 firmware before use*

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| **Chapter** | **Step** | **Question** | **Image/Data** |
| **1 a |**Prepare Cell Suspension | **A |**After first centrifugation step | Cell pellet visible?o **Yes** o **No** | [15 mL tube with cell pellet] |
| **B |**After final washing step | Cell pellet visible?o **Yes** o **No** | [15 mL tube with cell pellet] |
| **C |**First quantification | Cell concentration quantifiable?o **Yes** o **No** | [Cell suspension on slide, exported from imager]  |
| **D |**Quantification prior to cell staining | Cell concentration between 20,000 and 30,000 cells/µL in at least 40 µL?o **Yes** o **No** | [Cell suspension on slide, exported from imager, 5X dilution]  |
| **1 b |**Stain and Wash Cells | **E |**After first AOC washing step | Cell pellet visible?o **Yes** o **No** | [15 mL tube with cell pellet] |
| **F |**After second AOC washing step | Cell pellet visible?o **Yes** o **No** | [15 mL tube with cell pellet] |
| **G |**After third AOC washing step | Cell pellet visible?o **Yes** o **No** | [15 mL tube with cell pellet] |
| **H |**Final quantification after cell dilution  | Cell concentration between 2,800 and 3,200 cells/µL?o **Yes** o **No** | [Cell suspension on slide, exported from imager]  |
| **2 |**Encapsulate Cells | **A |**After reagent loading | Bubbles in any of the reagents?o **Yes** o **No** | [Top view of DNA cartridge w/o gasket applied] |
| **B |**After encapsulation program finished | Emulsions fully intact?o **Yes** o **No** | [Emulsion-safe tube w/ encapsulated cells] |
| **C |**After encapsulation program finished | Remaining cell suspension volume [reservoir 2] < 10 µL?o **Yes** o **No** | [Measure volume] |
| **D |**When removing oil | Total volume ~100 µL in the tube?o **Yes** o **No** | [Emulsion-safe tube w/ encapsulated cells and oil removed] |
| **3 |**Lyse and Digest Cells | **A |** Before thermal cycling | Thermal cycling protocol correct?o **Yes** o **No** | [Thermal cycling protocol image] |
| **B |** Before thermal cycling | PCR skirt used?o **Yes** o **No** | [Thermal heat block image] |
| **C |** After thermal cycling | Emulsions fully intact?o **Yes** o **No** | [Emulsion-safe tube w/ encapsulated cells] |
| **4 |**Barcode Cells | **A |**After reagent loading, priming | Bubbles in any of the reagents?o **Yes** o **No** | [Top view of DNA cartridge w/o gasket applied] |
| **B |**After reagent loading, barcoding | Bubbles in any of the reagents?o **Yes** o **No** | [Top view of DNA cartridge w/o gasket applied] |
| **C |**After barcoding program finished | Emulsions fully intact and evenly distributed?o **Yes** o **No** | [8x emulsion-safe tubes w/ barcoded cells]  |
| **D |**After barcoding program finished | Record remaining Barcoding Bead volume [reservoir 7]o **Yes** o **No** | [Measure volume] |
| **E |**After barcoding program finished | Record remaining Barcode Mix volume [reservoir 8] | [Measure volume] |
| **F |**When removing oil | Total volume ~100 µL in each tube?o **Yes** o **No** | [8x emulsion-safe tubes w/ barcoded cells and oil removed] |
| **5 |**Targeted PCR Amplification | **A |**Before thermal cycling | Thermal cycling protocol correct?o **Yes** o **No** | [Thermal cycling protocol without tubes, started with remaining time visible] |
| **B |**Before thermal cycling | PCR skirt used?o **Yes** o **No** | [Thermal heat block image] |
| **C |**After thermal cycling | Emulsions fully intact?o **Yes** o **No** | [8x emulsion-safe tubes w/ barcoded cells]  |
| **6 |**Emulsion Breakage | **A |**After emulsion breakage | Emulsions fully broken?o **Yes** o **No** | [8x emulsion-safe tubes w/ broken emulsions]  |
| **7 |**Cleanup PCR Products | **A |**After enzymatic cleanup | Sample spun down and transferred to new tube after enzymatic cleanup? o **Yes** o **No** | [Sample after transfer to new tube]  |
| **8 |**PCR Target Library | **A |**After quantification w/ Qubit (Final Libraries) | DNA concentration within spec? DNA: > 2.0 ng/µLProtein: > 1.0 ng/µLo **Yes** o **No** | [Qubit reading] |
| **B | (Optional)**Quantification w/ Qubit (Targeted PCR - DNA Library) | DNA concentration recorded? o **Yes** o **No** | [Qubit reading] |
| **C |**Bioanalyzer or TapeStation Results | Expected peak at ~460 bp (DNA library) and ~250 (protein library) with few (<5%) off-target fragments (e.g., primer dimers)?o **Yes** o **No** | [Fragment analyzer results] |