Hybridoma Cell Line Development Workflow Anti-species IgG-FL Primary antibody Cells or beads with antigen Hybridoma fusion Transfer of colony to ClonePix 2 System, Hybridoma Accurate and **Pre-Fusion Myeloma Growth Medium and** Hybridoma Growth Medium (with HT) & Semi-Solid Selection and Cloning gentle picking destination plates Hybridoma Expansion Medium (without HT) Hybridoma Expansion Medium Medium (with and without HAT), (without HT), Hybridoma Fusion Medium at 1:1 ratio CloneDetect Reagent CloneSelect Imager Culture myeloma cells in Scale up clones producing desired Clone screening and selection in pre-fusion growth medium. Monitor growth of antibodies and wean off HT selection. semi-solid medium. Prepare splenocytes for fusion. picked clones. **Functional** Scale-Up & Wean **Pre-Fusion** Fusion **Clone Selection Clone Picking Clone Stability Expand** Characterization Hybridoma Fusion Medium, ClonePix 2 System, SpectraMax i3x or other Hybridoma Expansion Medium Hybridoma Fusion Recovery Medium, Hybridoma Growth Medium (with HT) Molecular Devices microplate readers (without HT) Hybridoma Polyethylene Glycol for Cell Fusion Perform secondary screening Expand clones producing desired Pick selected, high-producing clones antibodies (e.g. bioreactors or from semi-solid medium and transfer to on clone supernatants Fuse splenocytes and (e.g. ELISA). large flasks). liquid medium. myeloma cells. VIEW EVERY GROWTH CURVE IN EVERY WELL 3% Day 1

