# Automate Tissue Processing with STEMprep™ for Versatile Sample Preparation

Grace F.T. Poon[1], Alice Liang[1]*, Alistair Lee Chenery[1]*, Jenna Thompson[1]*, So Ra Lee[1]*, Jason Dixon[1], Ryan Molina[1], Payam Zachkani[1], Andrew Nobles[1], Bilal El yassem[1], Chris Ryan[1], Marc Delorme[1], Rodrigo Martins[1], Martin O'Keane[1], Andy Kokaji[1], Allen C. Eaves[1,2], Sharon A. Louis[1], and Frann Antignano[1]

[1]STEMCELL Technologies Inc., Vancouver, Canada; [2]Terry Fox Laboratory, BC Cancer, Vancouver, Canada. *These authors contributed equally to this work.

## INTRODUCTION

Tissue environments are specialized to support distinct functions. Analyzing cell interactions and gene expression within tissues is essential for advancing tissue research and understanding disease mechanisms. Tissue samples must first be processed with protocols that preserve cell viability, yield, and functionality. However, achieving an optimal balance between enzymatic and mechanical dissociation for tissues can be time-consuming and technically demanding. To address this, we developed the STEMprep™ Tissue Dissociation System to efficiently generate single cells from tissue samples while maintaining cell function in downstream applications. This system features an instrument with integrated temperature control and tissue-specific dissociation programs, specialized sample tubes, and kits for enzymatic digestion. Our results demonstrate high cell viability and yield for mouse CT26 and B16 tumours, as well as normal brain, liver, lung, and spleen tissues. STEMprep™-processed samples are compatible with subsequent EasySep™ cell isolation and remain functional in cell-specific downstream assays. High-quality RNA can also be extracted directly using a homogenization protocol, enabling gene analysis workflows. The STEMprep™ Tissue Dissociation System streamlines tissue processing compared to manual procedures, enhancing consistency, throughput, and efficiency to accelerate tissue research.

## METHODS

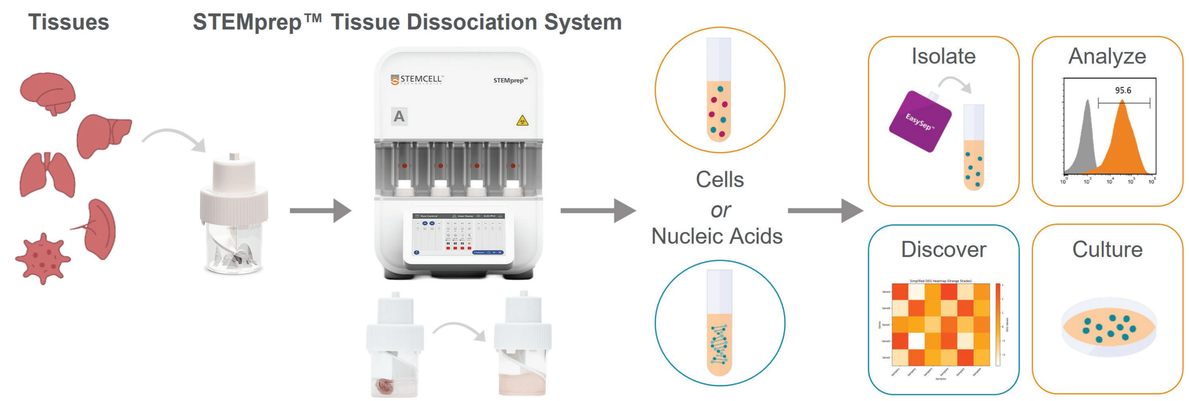



**FIGURE 1. STEMprep™ System: Streamlined Tissue Dissociation for Diverse Downstream Applications**

The STEMprep™ Tissue Dissociation System offers a versatile workflow for generating single-cell suspensions or tissue homogenates from a variety of mouse tissues for diverse downstream applications. STEMprep™-processed cells can be used for cell separation, cell culture, flow cytometry, and other assays.

## RESULTS

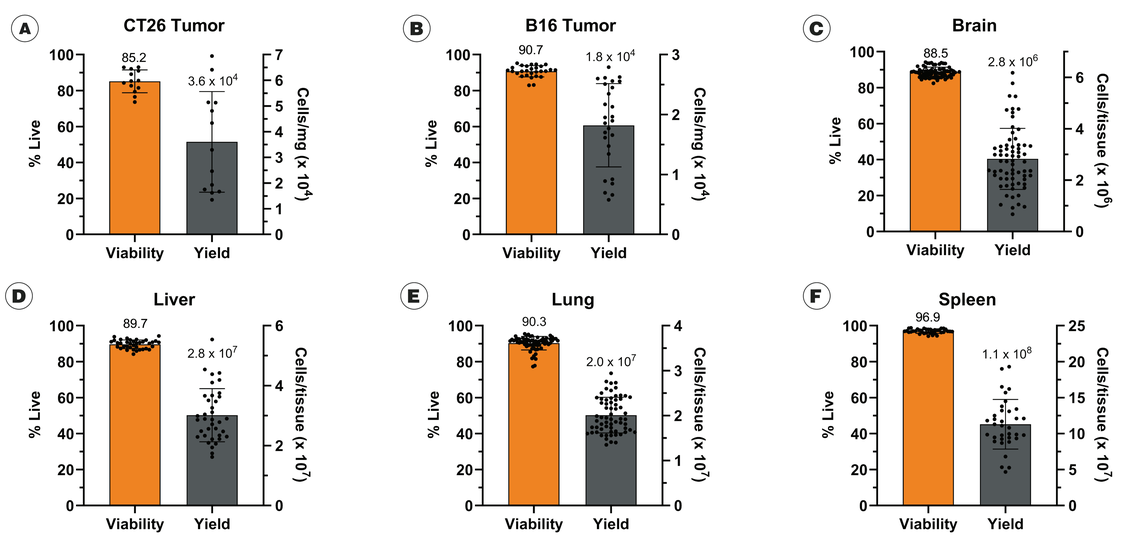



**FIGURE 2. STEMprep™ System Enables Reliable Automated Mouse Tissue Dissociation**

Mouse tissues were dissociated into single-cell suspensions using the STEMprep™ Tissue Dissociator and STEMprep™ tissue-specific kits. Viability and yield of single-cells suspensions generated from **(A)** CT26 colon carcinoma tumors (n = 13), **(B)** B16 melanoma tumors (n = 28), **(C)** brain (n = 68), **(D)** liver (n = 37), **(E)** lung (n = 64), and **(F)** spleen (n = 35). Primary solid tumors were generated by subcutaneous injection of tumor cells into the flanks of mice. Tumor, liver, and lung samples were treated with ammonium chloride solution to lyse red blood cells. Brain samples were processed with 18% OptiPrep™ (10.8% w/v iodixanol) to remove myelin and cell debris prior to analysis. Cell viability and yield following STEMprep™ processing were assessed by flow cytometry. Data are presented as mean ± SD.

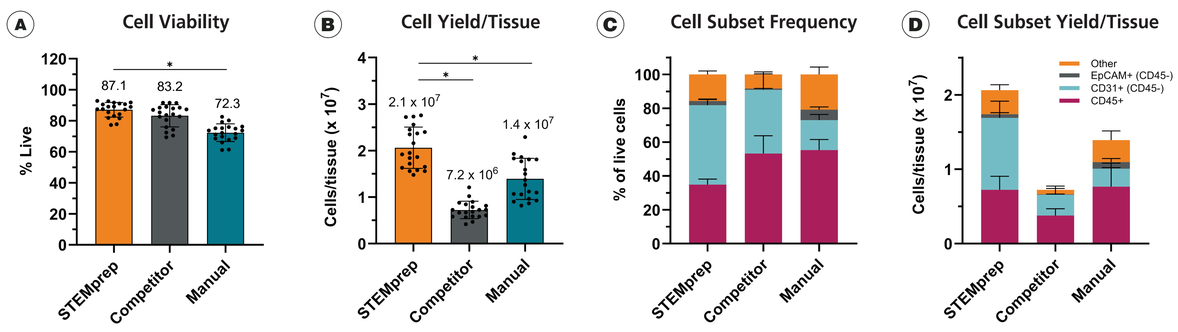



**FIGURE 3. STEMprep™ Lung Dissociation Kit Achieves High Cell Viability and Yield**

Mouse lung tissue processed using the automated STEMprep™ Mouse Lung Dissociation System, a competitor's automated system, or a standard manual method. **(A)** Viability of total nucleated lung cells. **(B)** Yield of viable lung cells per whole tissue. **(C)** Percentages of CD45+ immune and CD45- non-immune cell subsets, including EpCAM+ epithelial and CD31+ endothelial cells after dissociation. **(D)** Yield of lung cell subsets per tissue. Cell viability, total cell yield, and subset composition were assessed by flow cytometry. Red blood cells were lysed with ammonium chloride solution before analysis. Data are presented as mean ± SD (n = 20), * $p < 0.05$ One-way ANOVA with Tukey's multiple comparisons test.

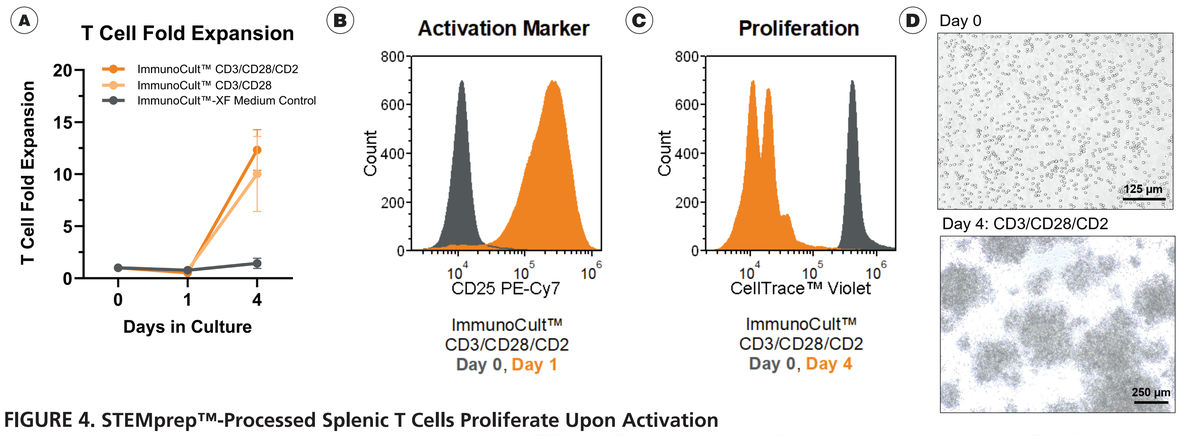
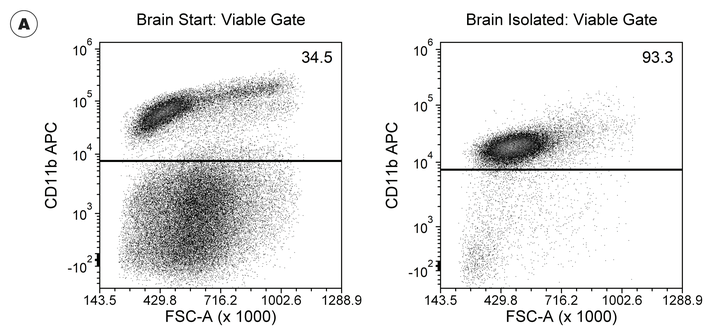



**FIGURE 4. STEMprep™-Processed Splenic T Cells Proliferate Upon Activation**

Mouse spleen tissue was processed using the automated STEMprep™ Mouse Spleen Dissociation System. Splenic T cells were then isolated using EasySep™ Mouse T Cell Isolation Kit. T cells were seeded at 2 x $10^5$ cells in 200 µL of ImmunoCult™-XF T cell Expansion Medium and activated with ImmunoCult™ Mouse T Cell Activators in the presence of IL-2 (50 U/mL) for 4 days. **(A)** Fold expansion of T cells cultured in medium alone or in the presence of ImmunoCult™ activators CD3/CD28 or CD3/CD28/CD2. Flow cytometry analysis of T cells showing **(B)** the expression of T cell activation marker CD25 on day 0 and day 1 after activation and **(C)** the proliferation of CellTrace™ Violet-labelled T cells on day 0 and day 4 of activation. **(D)** Representative light microscopy images of T cell cultures on day 0 and day 4 after activation. Data are presented as mean ± SD (n = 4).



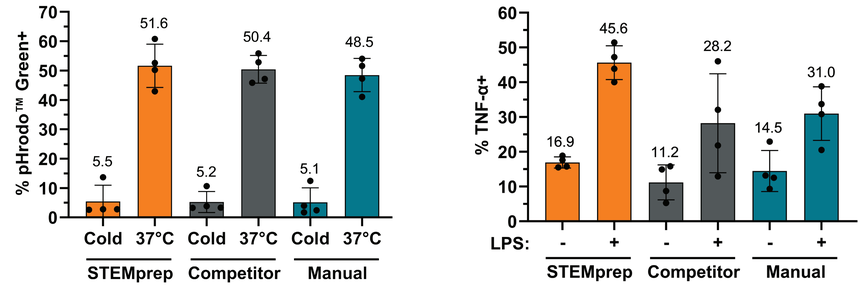




**FIGURE 5. STEMprep™-Processed Mouse Microglia Are Phagocytic and Produce Cytokines upon Activation**

Mouse brain tissue was processed using the automated STEMprep™ Mouse Brain Dissociation System, a competitor's automated system, or a manual dissociation method. **(A)** Following myelin removal, microglia were isolated from the single-cell suspensions using EasySep™ Mouse CD11b Positive Selection Kit II. **(B)** The isolated CD11b+ cells were incubated for 2 hours in the presence of pHrodo™ Green-conjugated E. coli BioParticles™ at 2 - 8°C (Cold) or 37°C. The fluorescence of phagocytosed BioParticles was measured by flow cytometry. **(C)** Intracellular flow cytometry staining of TNF-α production by microglia cultured overnight in the presence of 3 µg/mL Brefeldin A and treated with (+) or without (-) 100 ng/mL lipopolysaccharide (LPS). Data are presented as mean ± SD (n = 4).

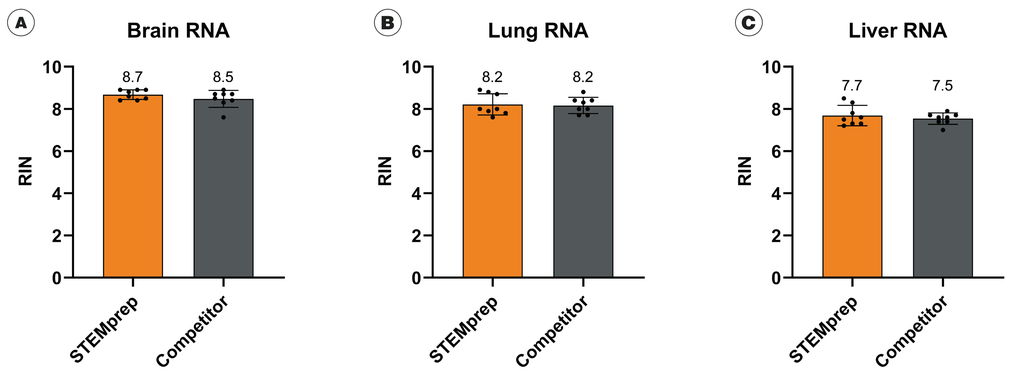



**FIGURE 6. Direct Tissue Homogenization Yields High-Quality RNA**

RNA integrity number (RIN) of RNA extracted from mouse **(A)** brain, **(B)** lung, or **(C)** liver tissues. Tissues were homogenized using the STEMprep™ Tissue Dissociator or a competitor's automated system. RNA was subsequently extracted from the resulting homogenates by EasySep™ Total Nucleic Acid Extraction Kit (with DNase I treatment). The quality of the extracted RNA was assessed using the 2100 Bioanalyzer Instrument and Agilent RNA 6000 Nano Kit. Data are presented as mean ± SD (n = 8).

## Summary

- Excellent cell viability and yields can be achieved from mouse tissues processed with the STEMprep™ System.
- STEMprep™-processed splenic T cells and brain CD11b+ microglia are functional in culture.
- High-quality RNA can be extracted from mouse tissues processed with the STEMprep™ homogenization protocol.