Feasibility of Using snRNA-seq for Multi-Tissue Exploration of Human Parturition.

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Introduction

- **Single cell (sc) RNA-seq** has been previously used for transcriptome profiling of reproductive tissues in attempts to identify molecular signatures of human labour [1-3]. However, (i) needs enzymatic/mechanical dissociation of fresh tissue, (ii) shows cell size bias from use of microfluidics-based technology, and (iii) unable to distinguish between different nuclei in multinucleated cells.
- **Single nuclear (sn) RNA-seq** is suitable for use with frozen tissues, and nuclei size is less problematic for microfluidics-based processing [4].
- **Spatial transcriptomics (ST)** with tissue sections can be used to assess whether changes in cell types of interest are location-specific and dependent on the composition of neighbouring cells in heterogenous tissues [5].

Data from these approaches should be compared to evaluate their limits of data interpretation, and whether they can compensate each other sufficiently to provide a full picture of transcriptional changes across multiple sites within the uterus that collectively contribute to its transition from **non-labour (NL)** to **spontaneous labour (SL)**.

Objective

Assess the quality of sequencing data that can be acquired from study participant-matched biopsies of the **lower segment (LS)** and **upper segment sub-parietalis (USP)** regions of the uterus, along with **decidua parietalis (DP)**, using snRNA-seq, and compare this to scRNA-seq and ST.

Methods

Term gestation singleton pregnant study participants gave written consent (LREC #10/H0801/10) prior to Caesarean section while either NL or SL; n=4 each. All three types of biopsies were collected from each study participant.

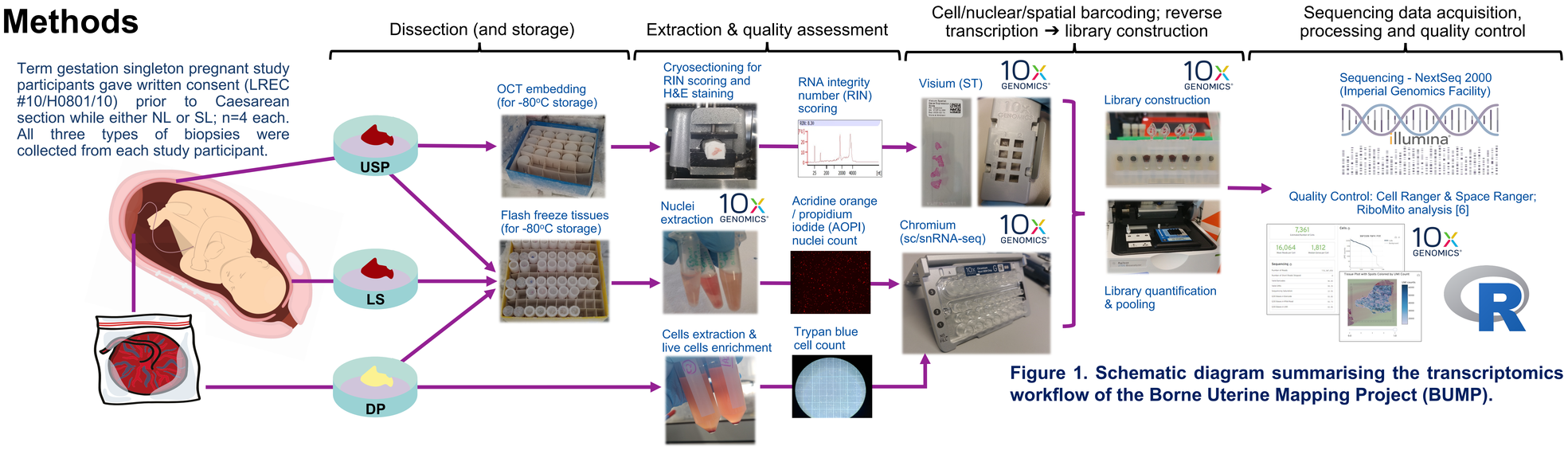


Figure 1. Schematic diagram summarising the transcriptomics workflow of the Borne Uterine Mapping Project (BUMP).

Results

Table 1. Cell Ranger metrics for snRNA-seq and scRNA-seq meet the necessary thresholds to indicate good quality data has been acquired (albeit to different extents). All values presented as median (with min-max range); n=4.

Cell Ranger QC [ideal thresholds]	Nuclei						Cells
	Lower segment (LS)		Upper sub-parietalis (USP)		Decidual parietalis (DP)		
	NL	*SL*	*NL*	*SL*	*NL*	*SL*	*NL*
Estimated # nuclei/cells recovered [5000]	7220 (4680-26392)	4722 (4157-14506)	6314 (5859-9741)	7426 (4605-10296)	7238 (6366-8564)	6262 (2127-7924)	4714 (3307-6345)
Mean # reads per nucleus/cell [20,000]	19328 (16636-21117)	28265 (13034-37258)	19402 (16098-20334)	24972 (18560-26095)	18693 (15422-21315)	24538 (19348-72957)	26183 (22223-41453)
Median # genes per nucleus/cell [≥1000]	1434 (1352-1545)	1409 (709-1636)	1711 (1552-1841)	1651 (1297-1864)	1658 (1300-1790)	1766 (725-2030)	2381 (2047-2503)
Reads in nuclei/cells (%) [≥70%]	62.3 (56.4-74.4)	56.5 (50.5-58.3)	75.0 (71.0-75.4)	66.4 (65.8-68.0)	73.6 (63.7-77.0)	74.4 (25.7-82.1)	90.4 (90.1-91.3)
Reads mapped antisense to genes (%) [sc ≤20%, sn ≤40%]	31.1 (23.8-37.0)	20.0 (17.0-24.1)	24.7 (21.3-35.1)	17.3 (13.4-30.0)	16.1 (12.7-25.2)	6.8 (4.8-25.2)	14.8 (13.9-17.3)

Table 2. Space Ranger metrics also indicate good quality ST sequencing data has been acquired. Median (with min-max range) presented, where the value for each study participant was calculated from two biopsies; n=4.

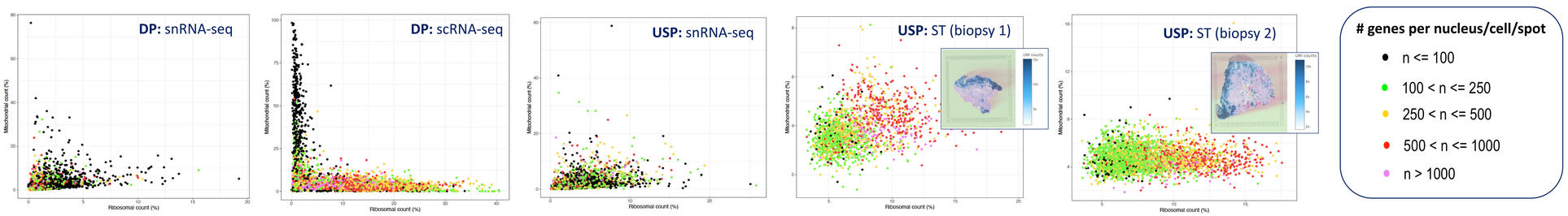
Space Ranger QC [ideal thresholds]	Upper sub-parietalis (USP)	
	NL	*SL*
# spots under tissue	2314 (1579-3053)	2183 (1387-2618)
Mean # reads per spot [20,000]	95890 (50563-119795)	80655 (66468-111901)
Median # genes per spot [≥1000]	2029 (1501-2319)	2089 (1825-2235)
Reads in spots under tissue (%) [≥70%]	88.1 (85.4-92.3)	91.3 (88.7-92.3)
Reads mapped antisense to genes (%) [≤20%]	0.7 (0.6-0.9)	0.6 (0.6-0.8)

Summary

- Nuclei extracts from LS, USP and DP can produce snRNA-seq data that sufficiently meets recommended quality control thresholds.
- Cells yielded higher median # genes than nuclei; RiboMito analysis indicates that this can mostly be attributed to the absence of cytosolic RNA in snRNA-seq data.
- ST has the potential to compensate snRNA-seq data for cytosolic RNA (albeit not at single cell resolution for the Visium platform).

Downstream bioinformatics analyses are in progress for (i) sc *vs* sn, and (ii) NL *vs* SL.

Figure 2. Ribosomal (x-axis) and mitochondrial (y-axis) RNA proportions are as expected for each transcriptomics method; both higher for scRNA-seq than snRNA-seq and ST. Representative data from one NL study participant. Spots on tissue sections coloured by unique molecular identifier (UMI) count for ST also shown (insets).



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