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JAX SCBL workflow for scRNA-seq of human placenta

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ABSTRACT

Described here is the workflow used by the JAX Single Cell Biology lab to generate single cell transcriptomic libraries from human placenta samples collected at the UCSD HuBMAP Female Reproductive Tissue Mapping Center.

MATERIALS

See accompanying protocols.

OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 59523

Tissue collection

1 Tissue is collected and processed as soon as possible after Cesarean section or vaginal delivery according to the following protocol:

Human Placenta Tissue Collection and Preservation Methods - UCSD Female Reproductive TMC

Tissue is then stored in MACS storage buffer for overnight shipping.

Sample preparation

2 Upon receiving the sample, single cells are isolated through a digestion with cold active protease as described here:

Human endometrium and endometriosis tissue dissociation for single-cell RNA sequencing

2.1 The viability and cell concentration for each sample is assessed via a Countess FL II or LUNA FX7 automated cell counter.

2.2 The single cell suspension is then loaded onto one lane of the appropriate 10x Genomics gene expression chip as follows:

	A	B	C	D
	Chip	Approx Cells Loaded	Approx. Cells Targeted	Compatiable Instrument
	Chip G	12,000	6,000	Chromium Controller, Xi, X
	Chip M	40,000	20,000	Chromium X

Library Generation

3 The resulting loading, running, and library construction is performed according to the appropriate 10x Genomics protocol:

JAX - 10x Genomics - NEXTGEM v3.1 - 3' Gene Expression

Sequencing

4 We have typically targeted 100,000 read pairs/cell for human placenta sample, resulting in target library sizes of roughly 600M-2,000M read pairs. These are multiplexed on a NovaSeq 6000 S4

lane using v1.5 reagents and the reverse complement workflow in a 28/8/0/90 or 28/8/8/90 configuration.