Single Cell Users Group– Discussion Session 2

Sample prep: all things considered

2017-10-18

Discussion session goals:

- Topic presented briefly
- Users are encouraged to share their experiences to help users advance their knowledge of single cell experiments and analytical tools
- Moderator will help guide the conversation
- If open discussion comes to an “end”, the session can break out into smaller groups with similar interests/issues over coffee

- Feel free to email one of the organizers to submit potential topics and/or volunteer to moderate!

Mike Kelly, Jamie Diemer, Erika Kwon, Lingling Miao, Chen Yao, Luigi Alvarado, Erica Bresciani, and Ben Voisin

Check out the Users Group website for additional info and future events:
https://nih-irp-singlecell.github.io/SC-UsersGroup/

Single Cell Users Group– Discussion Session 2

- This is not meant to be a Q & A session with the moderator fielding all questions.
  - Interactive forum

- Communication and reproducibility in science are critical. So if you learn something here, trust but verify.

- **<u>Respect your peers</u>**

  - Be respectful of other people's time. If someone has knowledge of a particular area, please don't bombard them with emails and questions.

  - Give credit where credit is due. If you learn something useful here, acknowledge the source. Example: sharing scripts, protocol optimizations, etc.

“Sample prep: all things considered”

- This discussion session will be centered on common questions and issues that arise when preparing samples to perform single cell genomic experiments, mostly focused on single cell RNA-seq.

- Often, sample prep is the most critical and limiting step of the process
  - maintaining cells in an unperturbed state
  - collecting rare population of cells spanning over several days
  - Samples prepared at different times or places

- **Optimal Sample Prep Guidelines for scRNA-seq (ex. 10X Genomics platform)**

Cell suspension containing >90% viable cells at single cell suspension and free of cellular debris/aggregates

Cellular debris and aggregates removal

Accurate cell counting

Proper storage of single cell suspension

10X Genomics: https://support.10xgenomics.com/single-cell-gene-expression/sample-prep
Fluidigm: https://www.fluidigm.com/applications/single-cell-analysis#resources

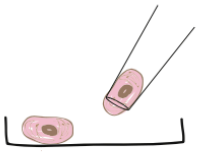
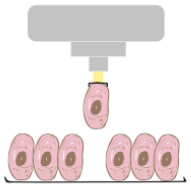
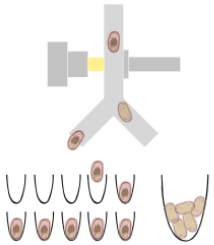
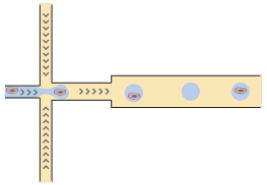
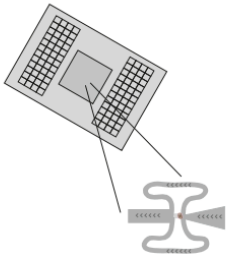
Different experimental approaches

Kolodziejczyk Mol. Cell 2015

SINGLE CELL CAPTURE

SINGLE CELL LYSIS

REVERSE TRANSCRIPTION → **PREAMPLIFICATION** → **LIBRARY PREPARATION and SEQUENCING**

MICROPIPETTING MICROMANIPULATION	LASER CAPTURE MICRODISSECTION	FACS	MICRODROPLETS	MICROFLUIDICS e.g. FLUIDIGM C1
				
low number of cells	low number of cells	hundreds of cells	large number of cells	hundreds of cells
any tissue	any tissue	dissociated cells	dissociated cells	dissociated cells
enables selection of cells based on morphology or fluorescent markers	enables selection of cells based on morphology or fluorescent markers	enables selection of cells based on size or fluorescent markers	no selection of cells (can presort with FACS)	no selection of cells (can presort with FACS)
visualisation of cells	visualisation of cells	fluorescence and light scattering measurements	fluorescence detection	visualisation of cells
time consuming	time consuming	fast	fast	fast
reaction in microliter volumes	reaction in microliter volumes	reaction in microliter volumes	reaction in nanoliter volumes	reaction in nanoliter volumes

Transcriptional changes during dissociation step

Artificially induced transcriptional perturbations during conventional whole-cell dissociation procedures (cell injury, stress, and excitotoxicity).

ARTICLE

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Nuclear RNA-seq of single neurons reveals molecular signatures of activation

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Discussion

Single-cell profiling of neural tissue has become an increasing area of interest; however, caution must be exercised when using this information to study patterns of activity-related expression. We found that, in our hands, papain dissociation of whole-cells from the DG elicited IEG expression independent of the condition of the animal. Although we have chosen to focus on

Single-nuclei RNA-seq (snRNA-seq) – more recent technique alternative to single-cell Methods

- Rapid dissociation protocol that does not require either protease digestion or heating
- Minimal contamination of ribosome genes
- Allows focused snapshot of the nuclear transcriptome
- In this paper, snRNA-seq showed consistent IEG expression pattern with the behavioral experience of the mouse

Neuron

NeuroResource

Detecting Activated Cell Populations Using Single-Cell RNA-Seq

Highlights

- Act-seq minimizes artificial transcriptional changes during tissue dissociation
- Act-seq enables unbiased characterization of cell types and their acute activation
- Application of Act-seq provides the first molecular taxonomy in the amygdala
- Application of Act-seq identifies neuronal subpopulations activated by stress

Authors

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In Brief

Wu et al. develop Act-seq, which minimizes artificially induced transcriptional changes during single-cell dissociation and thus enables faithful characterization of baseline transcriptional profiles and detection of specific cell populations activated by physiological stimuli using single-cell sequencing.

Developed “protective recovery” procedure that enhances neuronal survival during acute brain slice prep from mice by reducing cellular stress, metabolic demand and excitotoxicity.

Some Discussion Questions to Start Things Off

- What are the various sample prep protocols that people have used?
- Cell types? – frozen cells, fixed cells, solid tissues, cell lines, nuclei, etc?
- Are they any tricks to keep cells in single cell suspension? (besides working quickly, ice, vortex, or pipetting)

By show of hands..

- prepared single-cell suspensions from tissues?
- has an “easy to singularize” or “hard to singularize” sample?
  - what’s key in the process?
  - what has helped increase (or destroy!) your viabilities?
- has “hard to lyse” cells?
  - what was key in the lysis buffer or protocol? Recipe?
- has FACS sorted and ran scRNAseq?
- has fixed cells and ran scRNAseq?
- has both fixed and FACS sorted cells for scRNAseq?

- Is fresh, whole-cell best? What are some of the situations that arise the make this not feasible? What are alternative options?

Why Nuclei?

- Cell atlasing without the cells
- Expands Applications of Droplet-based Single Cell Genomics
  - Possible solution for archival (preserved), damaged or dissociation-resistant tissue samples
  - Profile complex tissues/ organs where nuclei (but not whole intact cells) can be isolated
  - Future potential for clinical samples
  - Reveal molecular genetic regulatory mechanisms specific to the nucleus
- 10x Chromium solution enables scaling of nuclei seq to >10,000 nuclei/sample
  - Turnkey solution based on existing Chromium instrument and 10x SC Reagents
  - No need to optimize DropSeq (DroNC-Seq) protocol
- Resolves biological samples equivalent (but different) to single cell
  - Similar cellular heterogeneity revealed
  - More intronic and antisense RNA species
  - Sensitive to limited nuclear polyadenylation (fewer genes detected)

10X Genomics Sample Prep Resource
https://community.10xgenomics.com/t5/10x-Blog/Single-Cell-RNA-seq-Sample-Preparation-Techniques-and-Best/ba-p/559