***Chromium Total*: single-nucleus total RNA sequencing via in situ polyA-tailing**

Last Edits Made: DWM, 6/29/2021

Materials/Reagents

- Yeast PAP
- 5X Yeast PAP reaction buffer
- 1M Tris-HCl, pH 7.5
- 5M NaCl
- 1M $MgCl_2$
- 1M Sucrose
- Nuclease-Free H2O
- 10X PBS
- 10% BSA
- 20U/ul SUPERase in RNAse Inhibitor

Buffers

***Yeast PAP Enzyme Mix**:*

	Final Conc.	**Volume to add**			
		1 RXN	5 RXNs		
Yeast PAP	**0.25M**	2ul	10ul		
25mM ATP	**1%**	1ul	5ul		
SUPERase		2ul	10ul		
Total Volume	**--**	**5ul**	**25ul**		

***Yeast Reaction Buffer**:*

	Final Conc.	**Volume to add**			
5X Yeast PAP reaction buffer	**1X**	50ul	20ul		
SUPERase	**0.2U/ul**	2.5ul	20ul		
H2O	**--**	197.5ul			
Total Volume	**--**	**250ul**	**1000ul**		

***Nuclei Buffer** (from sciRNAseq3):*

	Final Conc.	**Volume to add**			
1M Tris-HCl, pH 7.5	**10mM**	500µl			
5M NaCl	**10mM**	100µl			
1M $MgCl_2$	**3mM**	150µl			
Nuclease-Free H_2O	**--**	49.25ml			
Total Volume	**--**	**50ml**			

***Nuclei Suspension Buffer** (**NSB**) (from sciRNAseq3):*

	Final Conc.	**Volume to add**			
Nuclei Buffer (NB)	**--**	4.8ml			
*20U/µl SUPERase	**1% v/v**	50 µl			
10% BSA	**1% w/v**	100µl			
Total Volume	**--**	**5ml**			

*Add just before use

Protocol

1. Set hotplate/thermomixer to 37°C and centrifuge to 4°C.
2. Prepare appropriate volumes of **Yeast PAP Enzyme Mix** and **Yeast Reaction Buffer**.
3. Thaw, resuspend, and pellet nuclei according to the methanol fixation protocol (5min, 1000 x g, 4°C).
4. Resuspend nuclei in **200ul** of **Yeast Reaction Buffer**. Pellet nuclei by spinning (5min, 1000 x g, 4°C).
5. Gently resuspend in **45ul** of **Yeast Reaction Buffer**.
6. Add **5ul** of **Yeast PAP Enzyme Mix**.
7. Incubate at 37°C for 25min without shaking (shaking causes clumping).
8. Wash nuclei in **500ul** of **NSB**.
9. Resuspend in appropriate volume for downstream applications (10x Chromium, drop-seq, etc)