***STRS*: Spatial Total RNA-Sequencing via in situ poly(A)-tailing**

Last Edits Made: DWM, 2/23/2022

Materials/Reagents

- Yeast PAP (Thermo Scientific, cat #74225Z25KU)
  - Comes with 5X Yeast PAP reaction buffer
- 40U/ul Protector RNase Inhibitor (Millipore-Sigma, cat #3335399001)
- Nuclease-free H_2O
- Visium Spatial Gene Expression Kit

Buffers

***Yeast PAP Enzyme Mix**:*

	Final Conc.	**Volume to add**		
		1 RXN	4.4 RXNs (1 slide + 10%)	8.8 RXNs (2 slides + 10%)
5X Yeast PAP reaction buffer	**1X**	15ul	66ul	132ul
Yeast PAP	**0.25M**	3ul	13.2ul	26.4ul
25mM ATP	**1%**	1.5ul	6.6ul	13.2ul
Protector	**1.6U/ul**	3ul	13.2ul	26.4ul
H_2O	**--**	52.5ul	231ul	462ul
Total Volume	**--**	**75ul**	**330ul**	**660ul**

***1X Wash Buffer**:*

	Final Conc.	**Volume to add**		
		1 RXN	4.4 RXNs (1 slide + 10%)	8.8 RXNs (2 slides + 10%)
5X Yeast PAP reaction buffer	**1X**	20ul	88ul	176ul
Protector	**0.8U/ul**	2ul	8.8ul	17.6ul
H_2O	**--**	78ul	343.2ul	686.4ul
Total Volume	**--**	**100ul**	**440ul**	**880ul**

Protocol

1. Section onto Visium Gene Expression slide according to protocols from 10x Genomics.
2. Fix, stain, and image tissue according to Visium protocol ("Step 1: Tissue Staining & Imaging").
3. Prepare appropriate volumes of **Yeast PAP Enzyme Mix** and **1X Wash Buffer**.
4. Set the thermocycler to 37°C, with the lid also set to 37°C (Step 2.1.a)
5. Place the slide into the Slide Cassette (See Visium protocol for "Tips and Best Practices").
6. Add **100ul** of **1X Wash Buffer** to each well to equilibrate tissue. Incubate at room temperature for 30sec.
7. Remove and discard buffer from each well.
8. Add **75ul** of **Yeast PAP Enzyme Mix**.
9. Place slide onto the Thermocycler Adaptor and incubate with the lid closed at 37°C for 25min.
10. Remove and discard enzyme mix from each well. Proceed directly to Step 2.1.c to permeabilize tissue, and follow the standard Visium protocol to completion.

Usage Notes

- Add the wash buffer and yPAP enzyme mix slowly and to the sides of each reaction chamber. Make sure the tissue sections are covered by each buffer. Make sure the reaction chambers are sealed during the 37°C incubation
- Keep the thermocycler set to 37oC after the polyadenylation step for the Visium protocol.