PLANT NUCLEI DEMO DO'S AND DON'TS

DO'S

☐ **USE NUCLEI ISOLATION BUFFER OR PBS AS SHEATH**
This is the only organelle in plant biology where PBS can be used. The methods I've seen usually have an isolation buffer composed of PBS + 0.1% BSA. Nuclei isolation kits that work beautifully are made by Sysmex: Cystain ® PI Absolute PI (for ploidy analyses) and Cystain® UV Precise P (for 10x genomics).

☐ **PRIME AND CALIBRATE WITH ISOLATION BUFFER OR PBS**
This is usually PBS or something similar.

☐ **BE STRINGENT WITH GATING**
Be sure to gate on appropriate nuclei populations, removing debris and doublets from the target population. If sorting, then use scatter density plots. If analyzing ploidy, then use histograms and look for shifting of peaks. Use number of fluorescent units to determine ploidy.

☐ **KEEP SHEATH BUFFER COLD**
Nuclei need to be kept cold to maintain intactness. They will lyse otherwise.

☐ **ADJUST THRESHOLD SETTING**
Threshold off fluorescence channel (likely B620 or Y620 for PI, V450 for DAPI) and aim for a threshold of 1024 to start. Start with PMTs on all channels at 400 and adjust accordingly.

☐ **SET APPROPRIATE EXPECTATIONS AND ASK FOR NUCLEI PRESENCE PRE-SORT**
Nuclei isolation from plant tissues (leaves or callus) is not easy and varies depending on crop and ecotype. Most of the events in nuclei extractions are debris. For best results: (1) use smaller tissue quantities (~0.5 cm^2 piece of tissue), (2) don't over-chop as it will tear up everything, (3) know the age of the plant tissue and be consistent from experiment to experiment, (4) if two peaks appear of equal height, the cells were dividing. A repeat ploidy analysis is needed at a later time to determine ploidy, and (5) If the peaks are messy/heavy static, the

filtration/purification was poor. Repeat isolation.

☐ **FILTER SAMPLES**
Use a 20 µm nylon filter to remove plant debris that could clog cartridge. This is part of the nuclei isolation procedure so it must be done.

☐ **USE WIDE BORE SEROLOGICAL AND MICRO PIPET TIPS**
This is a normal method for all plant biology workflows.

nanocellect Biomedical, Inc.		Playbook for a successful plant nuclei demo
9525 Towne Centre Drive, Suite 150 San Diego, CA 92121 Tel: (858) 614-6400	Version: 1.0	**Title: Playbook for a successful plant nuclei demo**

DO NOT

☐ **VORTEX, PIPET VIGOROUSLY, OR STIR NUCLEI TO KEEP THEM SUSPENDED**
Nuclei are sensitive to manual stresses. If the nuclei start to settle during a sort, it is recommended to pause the sort, cap the tube and gently/slowly invert the tube a few times to resuspend the cells. Then continue the sort.

☐ **COMPARE RESULTS TO ANOTHER CELL SORTER DONE ON A DIFFERENT DAY**
Any comparison of the WOLF to other instrumentation or protocols should be performed concurrently with the same nuclei sample as used on WOLF.

REVISION HISTORY:

	ISSUED DATE	Description of Change	Author
(A)	(10/26/2023)	Playbook for a successful plant microspore demo – V1.0	D.Moellering