Dec 30, 2025 Version 1

# Plant nuclei extraction protocol for single nuclei RNA-seq V.1

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvmb3d6g3p/v1

Bruno Guillotin[1], Kenneth D. Birnbaum[2]

[1]CNRS - Institute of Plant Sciences of Paris - Saclay - IPS2; [2]NYU - Center for Genomics & Systems Biology

bruno guillotin
CNRS INRAE Université Paris Saclay



**DOI:** https://dx.doi.org/10.17504/protocols.io.yxmvmb3d6g3p/v1

**Protocol Citation:** Bruno Guillotin, Kenneth D. Birnbaum 2025. Plant nuclei extraction protocol for single nuclei RNA-seq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvmb3d6g3p/v1

**Manuscript citation:**
**Guillotin B**, Rahni R, Passalacqua M, Mohammed A, Xu, X, et al., Birnbaum, K. (2023). A pan-grass transcriptome reveals patterns of cellular divergence in crops. Nature. Doi:10.1038/s41586-023-06053-0

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

**Created:** August 22, 2025

**Last Modified:** December 30, 2025

**Protocol Integer ID:** 225212

**Keywords:** single nuclei RNAseq, Plant, plant nuclei extraction suitable for single nuclei rnaseq, plant nuclei extraction protocol for single nuclei rna, plant nuclei extraction protocol, single nuclei rnaseq, plant nuclei extraction, single nuclei rna, rnaseq, multiple snrnaseq platform, arabidopsi, atacseq, such as 10x genomic, 10x genomic, maize, seq this protocol, seq, multiome approach, nodule

## Abstract

This protocol describes plant nuclei extraction suitable for single nuclei RNAseq and Multiome approach (RNAseq + ATACseq).
Tested on Arabidopsis, Medicago, Maize, Sorghum, Millet, Tomato, Date palm, roots, leaves and nodules and for multiple snRNAseq platforms such as 10x genomics, Rhapsody, Scale.

## Guidelines

- If possible, extract nuclei from fresh plant tissue, this will ensure the highest quality in nuclei integrity and mRNA contents. However, studies have been published using nuclei from samples directly frozen in liquid nitrogen, or conserved in RNA later.

- If frozen in RNAlater, roots are first washed 2 times in a round petri dish of water to remove the RNA later.

-Adjusting the amount of starting material is critical to avoid too many debris. Only 15-30mg of fresh roots or leaves is usually enough to get about 50-80k nuclei.

- **All** steps should be performed on ice and centrifugations at 4°C

- The use of a **Bucket Centrifuge** is crucial to ensure that all the nuclei pellet nicely in the very bottom of the Eppendorf. We usually use 15ml falcon tube and just put the 1.5ml Eppendorf on top of it to centrifuge.

- The procedure should be carried **as fast as possible (usually about 40mins).**
We are seeing decrease in quality (#UMI, #genes per nuclei) already if the extraction takes more than 1h30. We recommend to practice ahead to get it done in 40mins (after nuclei counting it takes about 30mins more to prep the 10x chip and chromium).

- For practice and training of the protocol, DEPC and RNAse inhibitor can be avoided, as they are expensive and will only preserve RNA. So if the samples won't be sequenced no need to use.

- All nuclei extraction buffers can be made in advance, filter sterilized at 0.22um, aliquoted and frozen at -20°C for months, only add the ingredients with a * after thawing them the day of the extraction.

## Materials

**Specific material necessary for predigestion, if needed:**

- Small petri dish plastic 30mm diam
- Cell strainers 40um or 70um (example: ref VWR 732-2757)
- Vacuum chamber.
- Horizontal agitator/shaker (90 rpm)
- Tweezer
- Towel paper
- Small spatula
- Nuclei lysis buffer and ice

**a. Enzyme mix solutions:**

- Cellulases and Macerozyme from Yakult pharmaceutical (https://www.yakult.co.jp/ypi/en/product.html).
- Y23 depends on the available provider.

**Maize Protoplasting solution (»1ml per sample)**

| | A | B | C |
|---|---|---|---|
| | Components | **Final concentration** | **For 10ml** |
| | Mannitol | 400mM, 8% | 728mg |
| | MES 1M (keep frozen) | 20mM | 200ul |
| | KCL 1M | 20mM | 200ul |
| | Cellulase RS (Not for Arabidopsis) | 2% | 200mg |
| | Cellulase R-10 | 1.20% | 120mg |
| | Pectolyase Y23 (Not for Arabidopsis) | 0.40% | 40mg |
| | Macerozyme R-10 | 0.40% | 40mg |
| | CaCl2 1M | 40mM | 400ul |
| | pH 5.8 with tris 1M pH8 | | |
| | BSA 10% (keep frozen) | 100ug/ml | 10ul |

| | A | B | C |
|---|---|---|---|
| | **Filter the solution with a syringe and a 0.35-0.45um filter** | | |

**Post enzymes Washing buffer**

| | A | B | C | D |
|---|---|---|---|---|
| | **Components** | **Final [C]** | **For 10ml** | **unit** |
| | Mannitol | 400mM, 8% | 728 | mg |
| | MES 1M | 20mM | 200 | ul |
| | KCL 1M | 10mM | 100 | ul |
| | CaCl2 1M | 10mM | 100 | ul |
| | pH 5.8 with Tris 1M pH8 | | | |
| | BSA 10% (keep frozen) | 100ug/ml | 10 | ul |

**Specific material necessary (nuclei extraction):**
- Single edge Razor Blades (Gravey, 40475)
- Small petri dish plastic 60mm diam
- Blue plastic pestles
- Filter 20um (CellTrics, 04-0042-2315)
- Filter 10um (PluriSelect 43-10010-50)
- 1.5ml Eppendorf
- Bucket Centrifuge at 4°C
- Hemocytometer 0.1mm
- DAPI (for nuclei counting); mmq/Milli-Q water for dilutions
- Washing buffer; Final buffer
- Optional: Sucrose cushion buffer (washing buffer formulated with 1.7 M sucrose) for additional debris/chloroplast removal

**Additional materials for nuclei counting:**
- Fluorescence microscope with DAPI filter (40x–63x magnification)
- Normal coverslips (use instead of the thick hemocytometer coverslip for 63x imaging)

**Specific material necessary (Scale sample Fixation):**

- Bucket Centrifuge at 4°C
- Methanol 100%
- DMSO
- DEPC
- ScaleBio Sample Fixation Kit – Module A (PN 2020002) Wash buffer at −20°C (can be frozen again after use)
- ScaleBio Sample Fixation Kit – Module B (PN 2020003) Fixation Reagent at 4°C
- Your sample cells or nuclei in 50 µl of Final Buffer
- 0.2-ml PCR tubes (for counting aliquots)

All nuclei extraction buffers can be made in advance, filter sterilized at 0.22um, aliquoted and frozen at -20°C for months, only add the ingredients with a * after thawing them the day of the extraction.

For practice and training of the protocol, DEPC and RNAse inhibitor can be avoided, as they are expensive and will only preserve RNA. So if the samples won't be sequenced no need to use.

**Lysis Buffer (600ul per sample)**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| | **Lysis Buffer** | **Ref** | **Stock Solution** | **Per ml** | **What for ?** |
| | Rnase free mmq water | | | 500ul | solvent |
| | 0.3M Sucrose | fisher C12H22011 | 2.5M/solid | 120ul/102mg | Nuclei intergrity |
| | 1.25% Ficoll | Sigma F4375 | solid | 12.5mg | Nuclei intergrity |
| | 2.5% Dextran T40 | Sigma 31389 | solid | 25mg | Nuclei intergrity |
| | 15mM Tris HCl pH8 | Sigma T2694 | 1M | 15ul | Lysing cells |
| | 20mM MES | Sigma M8250 | Make 1M in Rnase free water | 20ul | Buffer pH |
| | 10mM MgCl2 | Sigma 63069 | 1M | 10ul | Lysing cells |
| | 60mM KCl | Sigma P5405 | Make 1M in Rnase free water | 60ul | Keep nuclei in the same isotonicity |
| | 15mM NaCl | Sigma S3014 | Make 5M in Rnase free water | 3ul | Keep nuclei in same isotonicity |

| | A | B | C | D | E |
|---|---|---|---|---|---|
| | Spermine 0.5mM | Sigma S3256-1G | Make 100mM in Rnase free water | 5ul | Nuclei intergrity |
| | Spermidine 0.5mM | Sigma S0266-1G | Make 1M in Rnase free water | 0.5ul | Nuclei intergrity |
| | 0.1% Triton X-100 | Sigma X100 | 10% | 10ul | Detergent |
| | Rnase free mmq water | | | to 1ml | |
| | **Add last minute *** | | | | |
| | 1%DEPC * | VWR E174 | pur | 10ul | RNAse Inhibitor |
| | 5mM DTT* | Biochemica A1101 | 1M | 5µl | antioxydant |
| | 1mM PMSF* (only stable for 30mins) | Sigma 110-5GM in ethanol | 0.1M | 10µl | Protease inhibitor |
| | 1% Plant Protease Inhibitors* 1ml | Sigma P9599 | 100% | 10µl | Protease inhibitor |
| | BSA 0.4%* If nuclei are clumping you can incease the BSA up to 1% | Sigma A3912 | 10% | 40ul | Avoid nuclei aggregation |
| | RNase inhibitor 0.4u/ul* | promega N26 | 40u/µl | 10ul | RNAse inhibitor |

**Washing Buffer (500ul per sample)**

| | A | B | C | D |
|---|---|---|---|---|
| | **Washing Buffer** | **Ref (same as lysis)** | **Stock Solution** | **Per ml** |
| | Rnase free mmq water | | | 500ul |
| | 0.3M sucrose | | 2.5M | 120ul |
| | 15mM Tris HCl pH8 | | 1M | 15ul |
| | 60mM KCl | | 1M | 60ul |
| | 15mM NaCl | | 5M | 3ul |

| | A | B | C | D |
|---|---|---|---|---|
| | 2mM EDTA | Millipore 324504 | 0.5M | 4ul |
| | Spermine 0.5mM | | 100mM | 5ul |
| | Spermidine 0.5mM | | 1M | 0.5ul |
| | 15mM MES | | 1M | 15ul |
| | 0.1% Triton | | 10% | 10ul |
| | Rnase free mmq water | | | to 1ml |
| | *** Add last minute** | | | |
| | 5mM DTT* | | 1M | 5µl |
| | 1mM PMSF* (only stable for 30mins) | | 0.1M | 10ul |
| | 1% Plant Protease Inhibitors* | | 100% | 10µl |
| | BSA 0.4%* | | 10% | 40ul |
| | RNase inhibitor 0.2u/ul* | | 40u/µl | 5ul |

**Final Buffer (100ul per sample)**

| | A | B | C | D |
|---|---|---|---|---|
| | **Final Buffer** | **Ref (same as lysis)** | **Stock Solution** | **Per ml** |
| | Rnase free mmq water | | | 500ul |
| | 0.3M sucrose | | 2.5M | 120ul |
| | 15mM Tris HCl pH8 | | 1M | 15ul |
| | 60mM KCl | | 1M | 60ul |
| | 15mM NaCl | | 5M | 3ul |
| | Spermine 0.5mM | | 100mM | 5ul |
| | Spermidine 0.5mM | | 1M | 0.5ul |
| | 15mM MES | | 1M | 15ul |

| | A | B | C | D |
|---|---|---|---|---|
| | Rnase free mmq water | | | to 1ml |
| | **∗Add last minute** | | | |
| | 5mM DTT∗ | | 1M | 5µl |
| | 0.1% Plant Protease Inhibitors* | | 100% | 1µl |
| | **BSA 1%∗** | | **10%** | **100ul** |
| | RNase inhibitor 0.2u/ul* | | 40u/µl | 5ul |

## Safety warnings

First step require DEPC in the lysis buffer to remove RNAse, DEPC is toxic so the first step should be conducted under fume hood. Washing and final buffer don't have DEPC and these steps can be conducted at the bench.

## Enzymatic pre-digestion for nuclei extraction

30m

1 *Optional: this step help soften the cell wall resulting in less debris and better nuclei quality. But it can induce transcriptional changes as samples are incubated in enzymes for 10-30mins.*

2 In a 30mm diam Petry dish, place in ≈1ml enzyme solution and put your fresh sample. Example: cut the root tips (3-4 mm) or leaves with small scissors or a surgical blade. Cut them in smaller pieces of about 1mm. Start timer

3 Place in a vacuum chamber for 1min, break vacuum and vacuum for another 1min

4 Incubate at room temperature on a horizontal agitator at 90 rpm.

5 Incubate for ≈20mins. Time should be adjusted to the different samples, Nodules 30mins, Roots 10-15mins, leaves about 20mins etc...

6 Transfer the samples into a cell strainer by just poring the solution with enzyme or helping with a tweezer.

7 Place the cell strainer on towel paper to get most of the enzyme out

8 Transfer the cell strainer in a new 30mm diam Petry dish containing 2ml of post-enzyme washing buffer. Agitate

9 Place the cell strainer on towel paper to get more of the enzyme on the filter

10 Transfer the cell strainer in a new 30mm diam Petry dish containing 2ml of post-enzyme washing buffer. Agitate.

11 Place the cell strainer on towel paper and with a small spatula transfer your sample into **300ul** of nuclei lysis buffer on ice for extraction. Proceed to nuclei extraction, step 12.

## Nuclei extraction

40m

12 Roots are transferred into a pre-chilled plastic petri dish (60mm diam) on ice with **300ul** of lysis buffer. Roots are then chopped using 2 razor blades for at least 5mins on ice, until not big pieces are visible.

13 Samples are transferred using a P1000 into a pre-chilled 1.5ml Eppendorf with pre-chilled blue plastic pestle, (If low amount of material the petri dish is washed with **100ul** of lysis buffer and transfer to the Eppendorf). 20 movement up-down with twisting are performed using the blue pestle to further grind the samples.

14 Let the samples on ice about 2–3mins on ice to finish the lysis of the cells. After 2–3mins make 10 more movements with the pestle, to release the rest of the nuclei.

15 Samples are then filtered at 20um (CellTrics, 04-0042-2315) into a prechilled 1.5ml Eppendorf. **200ul** of lysis buffer are used to wash the remains of the tissues from the filter. Shake the tube gently to get every drop of liquid in the tube.

16 Centrifuge samples at 500g (maize) or 1000g (Arabidopsis ) for 10 mins at 4°C in a **bucket** centrifuge.

17 Remove supernatant without disrupting the pellet, leaving only a few ul.

18 Add **500ul** of washing buffer and gently resuspend the nuclei with a P1000 until no clumps are visible.

19 Samples are centrifugated at 500g (maize) or 1000g (Arabidopsis ) for 5 mins at 4°C in a **bucket** centrifuge.

20 Remove supernatant without disrupting the pellet, leaving only a few ul.

21 Add **50ul** of Final buffer and flick the tube with the finger to resuspend the nuclei. Or pipette gently.

22 If using the 10X genomics multiome kit, **Do not use Final Buffer**. Resuspend the nuclei in the multiome provided nuclei buffer

23 Filter the sample on a 10um strainer (PluriSelect 43-10010-50) on a fresh pre-chilled 1.5ml Eppendorf, make pressure with you thumb on top of the filter to make the liquid go through. If low material washes the tube and the filter with additional **10ul** of Final buffer.

24 If using scale Proceed to next protocol for fixation (Step 39)

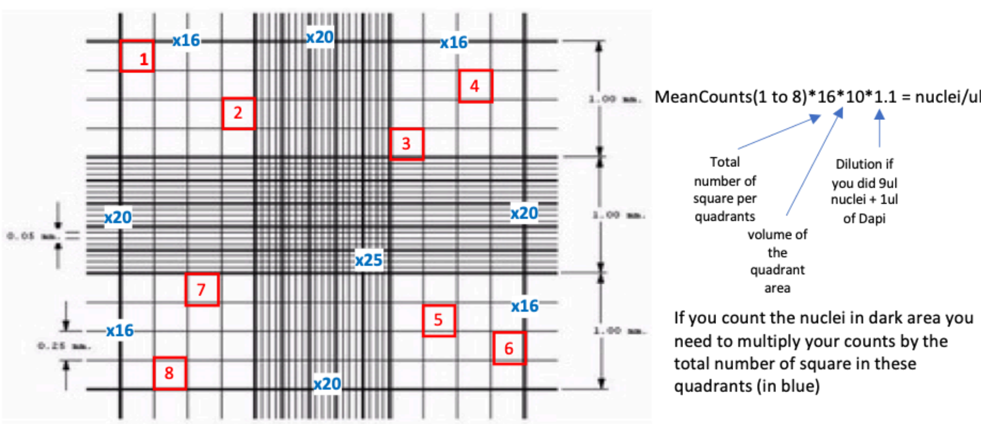
25 Use 9ul of the final volume to count nuclei on a Hemocytometer **0.1mm** with DAPI at 40x or even 63x.
**You need to use a normal coverslip not the one provided with the hemocytometer (too thick for 63x).**

26 DAPI — Stain with DAPI at 300 nM final [C].
Stock Solution 1: make a 10mg/mL DAPI stock solution in mmq water.
Stock Solution 2: add 1ul of Stock Solution 1 in 499ul of mmq Water
Stock Solution 3: add 1 ul of Stock Solution 2 in 99 ul of mmq Water (Can be kept 6month at 4°C)
Working solution Add 1ul of Stock solution 3 in 9ul of Purified nuclei

## Counting the nuclei

10m

27




Hemocytometer counting of the nuclei

28 On a fluorescent microscope focus on one red square in bright field at 63x but you need to use a normal coverslip not the one provided with the hemocytometer (too thick for 63x).
Remember by eye the border of the square you focus on, switch to DAPI filter and fluorescent.

29 Count the number of well-rounded nuclei within the field of view within the approximate border of the square. **Only count the well rounded one (see pictures below) ! Broken nuclei don't have RNA inside anymore.**

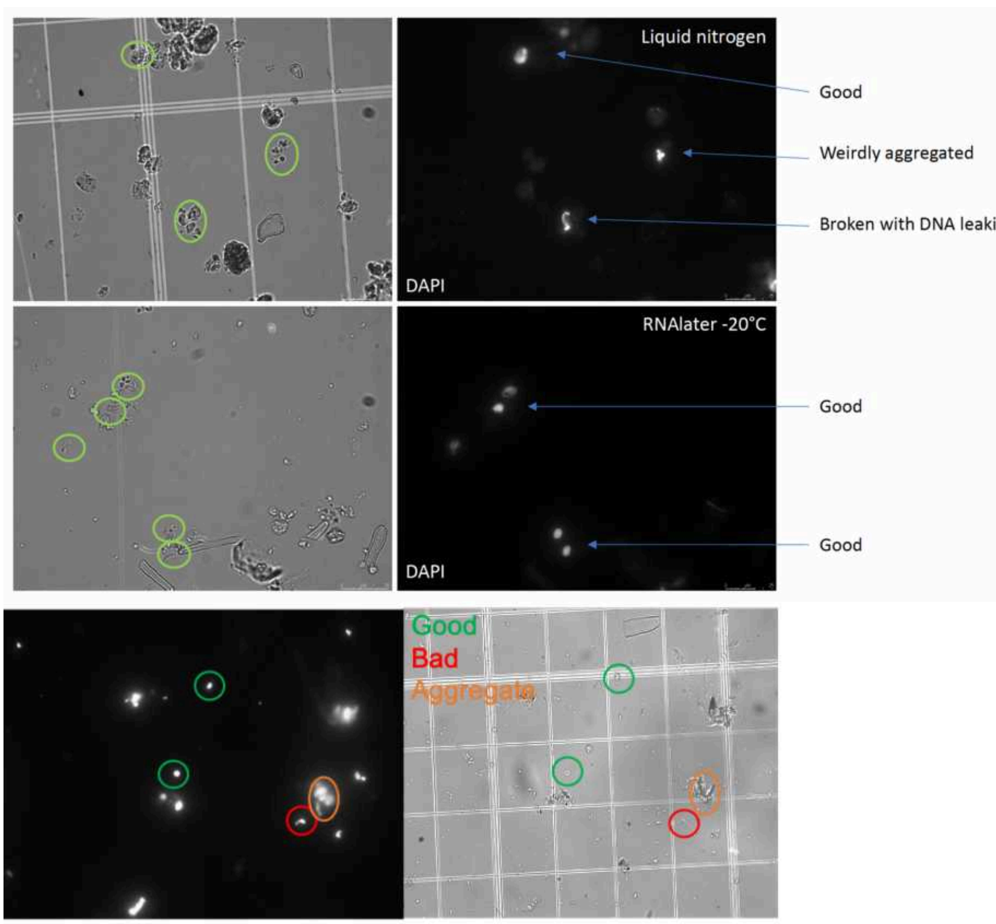
Nuclei like to stick on the coverslip and the bottom of the hemocytometer. Change the z while counting to make sure you count every nucleus.

30 Switch back to bright field and move to the next square for a new counting. Repeat from step 2. NB: depending to your microscope you can have BF on with the Dapi filter and the fluorescent lamp on. If you use very low BF light, you can see the lines of the hemocytometer and the fluorescent nuclei. So, it's easier to count within the square border.

31 Count randomly six to eight red square (see example above) and calculate your nuclei concentration:
Average of nuclei per square * 16 *10*dilution give you the number of nuclei per ul of final buffer.

32 If you count the nuclei in the inner grid area you need to multiply your counts by the total number of square in these quadrants (in blue).

33




## After counting and adjusting concentration

34 **Don't add more than 15ul of nuclei in the 10x master mix.** Too much nuclei buffer can disturb cDNA synthesis.Add the nuclei in the 10X master mix at the very last minute, just before loading into the 10x Chromium controller. Add the appropriate amount of nuclei and mix gently by pipetting. Nuclei or plant cells don't like the 10x master mix and can become quite unstable in it.

35 Depending on the concentration, nuclei can be diluted or concentrated by centrifugation at 500g for 3mins and removing the supernatant. Adjust the concentration to about 2,000–4,000 nuclei per ul. From about 15 mg we can easily get about 50,000 nuclei. Only 16,000 nuclei are necessary for a 10x run.

## Additional purification if needed:

36 If your sample looks too dirty at the end (e.g., too many debris or chloroplast), two strategies can be tried.

37 In step 17 and step 20, centrifuge the samples for 3min at 50g to pellet the debris. Transfer the supernatant into a new tube (leaving about 50ul to make sure you don't take any debris) then perform step 17 or 20.

38 Sucrose cushion strategy (after step 19):

38.1 Add 200ul of washing buffer and pipette to resuspend the pellet. Then in another 1.5ml Eppendorf put **VERY SLOWLY** the 200ul resuspended nuclei on top of 500ul cushion buffer (same composition as the washing buffer but with 1.7M sucrose instead of 0.3M).

38.2 Centrifuge for 5min at 4°C at 1000g.

38.3 Remove the supernatant; the nuclei should have pelleted in the very bottom and the chloroplast stays in the supernatant. Remove as much buffer as possible and proceed to step 19 again or 22.

## Scale Fixation procedure for small samples

39 **Prepare Complete nuclei Fixation Solution** In a chemical fume hood, reconstitute Fixation Reagent by adding 50 µL DMSO to one Fixation Reagent tube. Fixation Reagent is lyophilized at the bottom of the tube and appears as a white pellet.Dissolve tube contents by repeatedly vortexing at high speed. Repeat until all solids are fully dissolved; this may take up to several minutes. Ensure that all solids are fully dissolved before proceeding.

After fixation, leftover reconstituted Fixation Reagent can be stored at −20°C for the next fixation.

40 **For each sample mix:**
- 222 µL of Methanol
- 5.5 µL of reconstituted Fixation Reagent
- 2.2 µL of DEPC

Vortex mix and keep on ice.
Use immediately.

41 **Fixation:**
Add rapidly 200 µL of the Complete Fixation Solution to the 1.5-mL tube containing the resuspended cell/nuclei in 50 µL of Final Buffer and pipette strongly up and down four times to mix. *This is important to avoid the nuclei/cells sticking to each other and get fixed as clumps.*

42 Incubate the tube on ice for 15 minutes. *A white precipitate should appear; this is normal. Just mix every 5 minutes by inverting the tube on ice.*

43 Add 500 µL of scale Wash Buffer and resuspend cells by flicking the tube. *The white precipitate should disappear.*

44 Centrifuge the tube at 500–1000g (Maize–Arabidopsis) for 5 min at 4°C. Note: Nuclei or small cells can be centrifuged for 8–10 minutes to maximize recovery, or increase the speed.

45 Carefully remove supernatant without disturbing the pellet, leaving ~30 µL of residual volume.

46 Add 250 µL of scale Wash Buffer and resuspend the cells by flicking the tube.

47 Centrifuge the tube at 500–1000g (Maize–Arabidopsis) for 5 minutes at 4°C.

48 Carefully remove supernatant without disturbing the pellet, leaving ~20–30 µL of residual volume; resuspend by flicking the tube.

49 Take 2–5 µL in a 0.2-ml PCR tube for counting.

50 Freeze the rest of the samples at −80°C until use.

## Protocol references

Detailed from
**Guillotin B**, Rahni R, Passalacqua M, Mohammed A, Xu, X, et al., Birnbaum, K. (2023). A pan-grass transcriptome reveals patterns of cellular divergence in crops. Nature. Doi:**10.1038/s41586-023-06053-0**