**Table S2: Necessary reported information to allow evaluation and repetition of a plant single cell/nucleus experiment, template from Grones et al. 2024**

|  | **Details** | **Experimental information** |
| --- | --- | --- |
| **Biological material** | Species | *Brachypodium distachyon* |
| Accession | Bd21-3 |
| Genotype | WT and *sid/bdmute-1* |
| Tissue type | Leaf developmental zones and vegetative shoot apex with leaf primordia |
| Detailed growth conditions | Greenhouse or growth chamber, 18 h light: 6 h dark (day temperature 28°C, night temperature 22°C), PPFD 200-400 μmol m−2 s–1, soil-grown (four parts Einheitserde CL ED73, 1 part Vermiculite), 2 days vernalization (in water, 4°C, dark) |
| Harvest conditions | 3-week-old plants, morning, 15 leaf developmental zones or 8-10 shoot apices |
| **Sample preparation** | Isolation protocol | Tissue was cut mechanically, incubated in water, digested using Cellulase “Onozuka RS” and Macerozyme R-10, and carefully pipetted up and down to release protoplasts |
| Tissue dissection | Young, not yet unrolled leaves were pulled out from the enveloping older leaf and lowest 3-5 mm were cut off with a scalpel to harvest developmental zones. Shoot apices were dissected and harvested using a dissecting scope, forceps and a scalpel |
| Fixation | Unfixed tissue |
| Cell enrichment | Not applicable |
| Total sample preparation time | 4 to 4.5 h (from harvest to submission for 1-2 samples at a time, time includes counting of cells at the microscope and time needed to dilute to final concentration),  2.5 to 3 h (from digestion start to submission for 1-2 samples at a time) |
| Estimated cell number loaded | 12k cells (2021 datasets), 16.5k cells (2022 datasets), 18k cells (2023 and 2024 datasets) |
| Instrument/Method/Kit | 10x Genomics Chromium Single Cell 3’ v3.1 |
| Cell viability test | Fluorescein diacetate staining |
| **Libraries** | Library construction | GEM wells were prepared to encapsule protoplasts in droplets, cDNA was extracted per protoplast and libraries were prepared according to the guidelines provided by 10x Genomics (single cell 3' version 3.1) |
| Amplification method | 12 PCR cycles to amplify cDNA |
| End bias | 3’ end |
| **Sequence results** | Instrument/method | NextSeq 550 (2021 datasets, first sequencing run of the 2022 datasets),  NextSeq 2000 (second sequencing run of the 2022 datasets, 2023 datasets),  Illumina NovaSeq 6000 (2024 datasets) |
| Library layout/paired-end | Paired-end |
| N° sequenced reads  Reads/cell | ~32-54k (2021 datasets),  ~75-84k (2022 datasets),  ~93-154k (2023 datasets),  ~24k-29k (2024 datasets) |
| **Raw data** | Reference genome | https://phytozome-next.jgi.doe.gov/info/BdistachyonBd21\_3\_v1\_2 |
| Annotation version | B. distachyon Bd21-3 v1.2 |
| Mapping method (incl. software, customized settings) | 10x Genomics Cell Ranger v7.0.1 |
| Mapping efficiency | > 91% to > 97% |
| Sequencing saturation | 38-54% (2021 datasets),  42-45% (2022 datasets),  84-90% (2023 datasets),  54-61% (2024 datasets) |
| Estimation of ambient RNA (Fraction reads in cell) | > 66% (2021 datasets),  > 71-77% (2022 datasets),  > 82-89% (2023 datasets),  > 75-85% (2024 datasets) |
| Imputation method and settings | Not applicable |
| **Processed data** | N° captured cells (before filtering) | 5’480 (WT 2021),  6’023 (sid/bdmute-1 2021),  10’023 (WT 2022),  11’438 (sid/bdmute-1 2022),  7’600 (WT 2023-05-16),  4’285 (WT 2023-05-17),  22’531 (WT 2024-1),  18’565 (WT 2024-2),  22’828 (WT 2024-3) |
| N° high quality cells | 69’686 |
| Filter criteria: % mitochondrial reads/cell | < 5% |
| Filter criteria: % chloroplast reads/cell | < 10% |
| Filter criteria: Minimum N° UMI/cell | > 1’250 and < 50’000 UMI;  > 500 and < 10’000 features |
| N° total detected transcripts | > 91% (35’584 out of 39’068 genes) |
| Doublet rate | 6% (2021 datasets), 8% (2022 datasets), 10% (2023 and 2024 datasets) |
| Replicate comparisons | Bulk RNA-seq protoplasted vs. non-protoplasted tissues |
| Batch correction method for merging (incl. reasoning for batch correction) | Not applicable |
| Additional processing | High ambient RNA filtering (SoupX), doublet removal (DoubletFinder) |
| **Validation** | Method of automatic annotation of clusters | Not applicable |
| Method of manual annotation (markers, gene function info) | Marker genes (*B. distachyon*, orthologs to genes known from other species; see Table S3) |
| Verification in planta (e.g. Number of markers used for validation) | 13 marker genes verified with Hairpin Chain Reaction (HCR) RNA-fluorescence in situ hybridization,  4 marker genes verified with transcriptional reporter lines |
| **Data availability** | Analysis scripts & codes (GitHub) | Scripts available on Github: https://github.com/raissig-lindner-lab/Berg-et-al\_2025\_ScSeq |
| Excel Tables DEG for each cluster | Supplementary information of the publication, Github: https://github.com/raissig-lindner-lab/Berg-et-al\_2025\_ScSeq |
| Objects/count matrix in repository (which one, where?) | Single-cell data on GEO: GSE307277 |
| On-line tool/browser URL | https://shiny.ips.unibe.ch/ |
| Cell-level metadata table | Available in the R Script on Github: https://github.com/raissig-lindner-lab/Berg-et-al\_2025\_ScSeq |
| **Additional** | additional comments from the authors | Intronic reads were considered in Cell Ranger 7.0.1 |