**ASAP-seq protocol**

**1. Cell staining**

1. Obtain all single cell suspensions (filter if needed) and measure viability and density (if viability is <90% proceed with live cell enrichment and/or use best judgement depending on sample source / importance / rarity).
2. Resuspend ~1-2 million cells in 100 µl Staining buffer (2%BSA, 0.01%Tween in PBS).
3. Add 10 µl Fc Blocking reagent (FcX, BioLegend).
4. Incubate for 10 minutes at 4˚C.
5. While cells are incubating in Fc Block, prepare antibody pool (panel or titrated amounts).
6. Add antibody-oligo pool to cells.
7. Incubate for 30 minutes at 4˚C.
8. Wash cells 3 times with 1 mL Staining buffer spin 5 minutes 300*g* at 4˚C.
9. Resuspend cells in 450 µl PBS .

**2. Cell fixation/lysis**

1. Add 30 µl 16% formaldehyde (1% f.c) and incubate 10min at room temperature, swirl, invert occasionally
2. Quench by adding glycine to 0.125M f.c.
3. Wash with 1x ice-cold PBS by filling up the tube, invert 5 times
4. Spin 5 minutes 400*g* at 4˚C.
5. Discard supernatant and repeat wash with 1ml 1x ice-cold PBS
6. Spin 5 minutes 400*g* at 4˚C, discard supernatant.
7. Resuspended cell pellet in 100 µl chilled lysis buffer, mix by pipetting.
8. Incubate on ice for 3min (primary cells), 5min (cell lines)
9. Add 1 ml chilled wash buffer to the lysed cells, mix by pipetting
10. Spin 5 minutes 500*g* at 4˚C.

(Only if intracellular staining is desired, otherwise skip to step 15):

11. Resuspend cell pellet in 50ul block buffer, ie 40ul intracellular wash buffer + 5ul FcX + 5ul monoblock, 15min on ice
12. Add 50ul of intracellular wash bf, containing indicated amount of conjugated intracellular markers, ice 30min
13. Wash with intracellular wash bf, 3x
14. Spin 5 minutes 500*g* at 4˚C.

15. Remove supernatant, resuspend in 150 µl 1x nuclei buffer (10x Genomics)
16. Filter through 40 µm strainers (*e.g.* Flowmi cell strainer)
17. Count cells and adjust density according to 10x loading instructions.

**3. Transposition and barcoding**

18. Proceed according to 10x with the below modifications:
    1) During the barcoding reaction (step 2.1) spike in 0.5 µl of 1 µM bridge oligo (there is no dead volume in the reaction, so final volume will be 65.5 µl for v1 and 60.5 µl for v1.1)
    2) During GEM incubation (step 2.5), include a 5min incubation at 40˚C at the beginning of the protocol, ie:

| | | |
|---|---|---|
| 40˚C 5 min | | |
| 72˚C 5 min | | |
| 98˚C 30sec | | |
| 98˚C 10 sec | \| | |
| 59˚C 30sec | \| | repeat 11x (total 12 cycles) |
| 72˚C 1 min | \| | |
| 15˚C hold | | |

    (this extra step is not essential when using TSA products, but increases efficiency in TSB and especially TSC tag capture)
    3) During silane bead elution (step 3.1o) add 43.5 µl of Elution Solution I and subsequently recover ~43 µl. Keep 3ul µl aside to use as input* in the tag library PCR and with the remaining 40 µl proceed to SPRI clean up as per protocol.
    4) During SPRI cleanup (step 3.2d), save the supernatant. For the bead bound fraction proceed as per protocol. For the sup fraction, add 32 µl SPRI, let bind for 5min. Collect beads on magnet, wash 2x with 80% EtOH, remove remaining ethanol and elute beads in 42 µl EB (or more if multiple indexing reactions need to take place, ie TSA and TSB products have been used)*. This can be combined with the 3 µl left aside after the silane purification, as input in the ADT/HTO indexing reaction:

PCR:

| | |
|---|---|
| 50 µl | 2x KAPA mix |
| 2.5 µl | P5 10 µM |
| 2.5 µl | RPxx (TSA family) or D7xx (TSB family) 10 µM |
| 3-45 µl | input fragments |
| Water to 100 µl total | |

Program:

| | | | |
|---|---|---|---|
| 95˚C | 3 min | | |
| 95˚C | 20 sec | \| | |
| 60˚C | 30 sec | \| | repeat ~13-15x (total 14-16 cycles) |
| 72˚C | 20 sec | \| | |
| 72˚C | 5 min | | |

4˚C hold

Expected product: 190 bp

*note: you can use either as input in the tag indexing reaction. I find useful to combine when working with large antibody panels to increase input complexity. For simple hashing or small antibody panels you can rely on either alone.

**Buffers:**

| **LYSIS** | LLL | OMNI | Stock |
|---|---|---|---|
| Tris-HCl (pH 7.5) | 10mM | 10mM | 1M |
| NaCl | 10mM | 10mM | 5M |
| MgCl2 | 3mM | 3mM | 1M |
| NP40 (IGEPAL) | 0.1% | 0.1% | 10% |
| Digitonin | - | 0.01% | 5% |
| Tween | - | 0.1% | 10% |
| BSA | 1% | 1% | 10% |

| **WASH** | Wash buffer |
|---|---|
| Tris-HCl (pH 7.5) 1M | 10mM |
| NaCl 5M | 10mM |
| MgCl2 1M | 3mM |
| BSA 10% | 1% |

Intracellular staining buffer: Available from BioLegend. Part number 900002577

**Oligos:**

**BOA:**
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT/3InvdT/

**BOB:**
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGCTAGGACCGGCCTTAAAGC/3InvdT/

**Example of an RPxx (TruSeq Small RNA handle, present in TSA tags) :**
CAAGCAGAAGACGGCATACGAGAT*xxxxxxxx*GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA

**Example of an D7xx (TruSeq DNA handle, present in TSB tags or TSA hashing):**
CAAGCAGAAGACGGCATACGAGAT*xxxxxxxx*GTGACTGGAGTTCAGACGTGTGC

**Replace "xxxxxxxx" nucleotides with desired sample index**