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Simultaneous capture of chromatin accessibility and gene expression data from single larvae and postlarvae of the marine sponge *Amphimedon queenslandica*

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We use this protocol and it's working

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Abstract

Understanding gene regulation networks is crucial to unravelling the molecular mechanisms behind a wide range of biological processes in all animals, but most existing methods are heavily tailored towards model organisms. Here we present a novel approach for simultaneous generation of ATAC-Seq and RNA-Seq (CEL-Seq2) libraries from individual larvae and postlarvae of a marine sponge, providing a valuable opportunity to capture both chromatin accessibility and gene expression profiles concurrently. Our cell separation and capture method yielded high-quality libraries from small amounts of biological material. This allowed us to directly link chromatin state changes to gene expression changes during a critical phase of the sponge life cycle, and paves the way for deeper insights into how chromatin accessibility influences transcriptional regulation in non-model animals.

Materials

Biological materials

Amphimedon queenslandica adults in reproductive state, and co-occurring articulated coralline alga *Amphiroa fragilissima*

Materials and Equipment

1. Corning Costar TC-Treated 6-well plates (#CLS3516)
2. Plastic disposable transfer pipette (Sigma-Aldrich, #Z135011)
3. Yellow (Axygen-200 µL) or white (Axygen-10 µL) pipette tips
4. 1.5 mL tubes
5. 4 °C Centrifuge (Sigma, #3-18KS)
6. Hotplate (BIBBY, #HC502)
7. Stainless steel 316 Grade woven wide mesh discs in 500/50 woven wide mesh 25 µm aperture (SEFAR <https://www.sefar.com.au/> , #1011-41.3-500)
8. Bioanalyzer 2100 (Agilent, #G2939BA)
9. Qubit 4 Fluorometer (Invitrogen, #Q33238)
10. Qubit assay tubes (Invitrogen, #Q32856)
11. Magnetic separation rack for 0.2 mL tube
12. Magnetic separation rack for 1.5 mL tube

Reagents

1. FSW: 0.22 µm filtered seawater
2. CMFSW (calcium-magnesium-free artificial seawater): 0.45 M NaCl, 9.01 mM KCl, 33 mM Na₂SO₄, 2.15 mM NaHCO₃, 10 mM Tris-HCl pH 8.0, and 2.5 mM EGTA
3. Cell lysis buffer: 10 mM Tris-HCl, pH7.4, 10 mM NaCl, 3 mM MgCl₂, and 0.1% v/v IGEPAL CA-630
4. TRIzol (Sigma, #T9424)

Reagents for ATAC-Seq library preparation

5. Illumina Tagment DNA TDE1 Enzyme and Buffer Kits (#20034197)

Reagents for RNA-Seq library preparation

6. MEGAscript™ T7 Transcription Kit (Invitrogen, #AM1334)

Note: We use the CEL-Seq2 method. A complete list of reagents can be found in (Hashimshony et al., 2016).

Reagents for library quality assessment

7. AMPure XP Reagent (Beckman coulter, #A63880)
8. RNAClean XP Reagent (Beckman coulter, #A63987)
9. Qubit dsDNA assay kit (Invitrogen, #Q32851)
10. Qubit RNA HS assay kit (Invitrogen, #Q32855)
11. Agilent High sensitivity DNA kit (Agilent, #5067-4626)
12. Agilent RNA 6000 Pico kit (Agilent, #5067-1513)



Before start

Collect the biological samples

Adult *Amphimedon queenslandica* sponges were collected from the intertidal reef flat of Shark Bay, Heron Island Reef, and transferred to outdoor aquaria at Heron Island Research Station with flow-through, unfiltered ambient seawater drawn from the adjacent reef flat (Leys et al., 2008). The coralline alga *Amphiroa fragilissima* was collected from the reef flat on the southern side of the reef and used as the settlement substratum for *Amphimedon* larvae (Degnan & Degnan, 2010; Say & Degnan, 2020). Naturally emerging larvae were collected in the early to mid-afternoon using plastic disposable transfer pipettes, and maintained in ambient conditions before transferring to 6-well plates with 10 mL of 0.22 μm filtered seawater (FSW) (10 larvae per well) for settlement on *A. fragilissima* at sunset (Leys et al., 2008; Say & Degnan, 2020). Individuals of still-swimming larvae and settled postlarvae were collected at precise timepoints designated by the experimental design and immediately dissociated as described below.

Prepare the cell dissociation tubes (Fig. 1)

- 1 Seal a disc of 25 μm aperture metal mesh to the base of a 200 μL yellow or 10 μL white pipette tip by carefully heating the edges on a hotplate set to 220 $^{\circ}\text{C}$ (Fig. 1A).

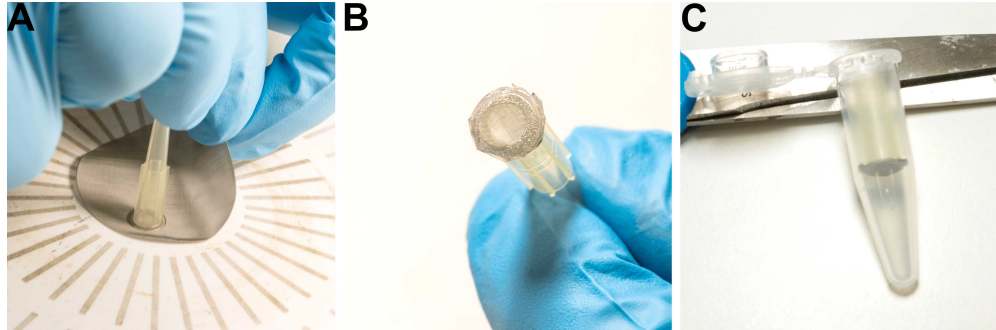


Fig. 1. Preparing the cell dissociation tube. **A.** Attach the metal mesh to the base of a yellow or white pipette tip by melting the tip into the mesh on a hotplate. **B.** Cut off the 2/3 of the tip part of the pipette tip and trim the mesh. **C.** Insert the modified pipette tip into a 1.5 mL tube with the mesh end facing downward, gently pressing to ensure proper placement.

- 2 Once the mesh is sealed, trim the excess so that it fits neatly around the base of the tip (Fig. 1B).
- 3 Cut off 2/3 (about 3.3 cm) of the tip, being consistent in the length of tip that is removed so that each sample in the experiment will receive the same treatment; removing 2/3 of the tip helps to minimise the amount of cell residue that adheres to the modified tip.
- 4 Invert the modified tip and insert it into a 1.5 mL tube (Fig. 1C).
Note: Ensure that the metal mesh is well sealed to the pipette tip. Any unsealed area may result in loss of cells during subsequent steps.

Dissociate the cells

- 5 Quickly wash individual larvae or postlarvae three times in 200 μL ice-cold FSW to remove external contaminants and maintain sample integrity.
- 6 Use a pipette to carefully transfer a cleaned larva or postlarva along with 50 μL ice-cold FSW onto the mesh of a modified tip inside a dissociation tube (prepared as above). Maintain a slow pipetting speed to minimize turbulence and maintain sample integrity during transfer.



Note: Here, FSW is used for both washing and dissociation to minimize potential stress on cells, and it is sufficient for dissociating *Amphimedon* larval and postlarval cells. If stronger dissociation is needed, CMFSW can be considered as an alternative.

Additionally, 1x trypsin solution in CMFSW can be used to wash the dissociation mesh to help collect any tissue stuck on the mesh (Cornejo-Páramo, Roper, Degnan, Degnan, & Wong, 2022).

Note: Keeping biological samples and all reagents ice cold throughout will help to minimise enzymatic activity and thus maintain the original chromatin and transcriptional states that the experiment is aiming to capture.

7 Centrifuge at 1800 g, 4 °C for 10 minutes to directly dissociate cells from a single larva or postlarva as the individual is forced down through the mesh.



8 Remove the modified tip from inside the tube, and carefully discard the supernatant.

9 On ice, resuspend the cell pellet in 50 µL of ice-cold 0.22 µm filtered CMFSW. Here the CMFSW is used to maintain the cells in their dissociated state. Then, 75% of the cells are transferred into a new 1.5 mL tube on ice for ATAC-Seq library development, leaving 25% of the cells in the original tube on ice for RNA-Seq library development (we used the CEL-Seq2 protocol).



Note: We use 75% (about 15,000) *Amphimedon* cells for the ATAC-Seq library and 20% (about 5,000) *Amphimedon* cells for the CEL-Seq2 library, which are sufficient for both library preparations. You may need to consider the cell numbers required for each library.

10 Keep the dissociated cells on ice.

- For the cells intended for ATAC-Seq library preparation, add 40 µL of ice-cold cell lysis buffer and gently pipette the mixture to ensure even mixing. Then centrifuge at 500-800 g, 4 °C for 10 minutes to pellet the nuclei. Carefully discard the supernatant and proceed with using the remaining lysate in the tube directly for library preparation.
- For the cells intended for RNA-Seq library preparation, add 50 µL of TRIzol. Then either store the samples at -80 °C freezer for later processing or proceed directly with RNA extraction.



Prepare the ATAC-Seq libraries

11 Use above nuclear pellet for ATAC-Seq library preparation as described in (Buenrostro, Giresi, Zaba, Chang, & Greenleaf, 2013).

12 Check the prepared library quality on a Bioanalyzer using a High Sensitive DNA chip. Expected fragment size range is 150-1000 bp, where nucleosomes with distinct periodicity can be observed (Fig. 2A).



- 13 Remove primer-dimers using 1.1:1 AMPure XP beads if the library has a visible primer-dimer peak (Fig. 2B).
- 14 Remove long fragments using 0.5:1 AMPure XP beads if the library has a visible >1000 bp peaks.
- 15 Sequence.

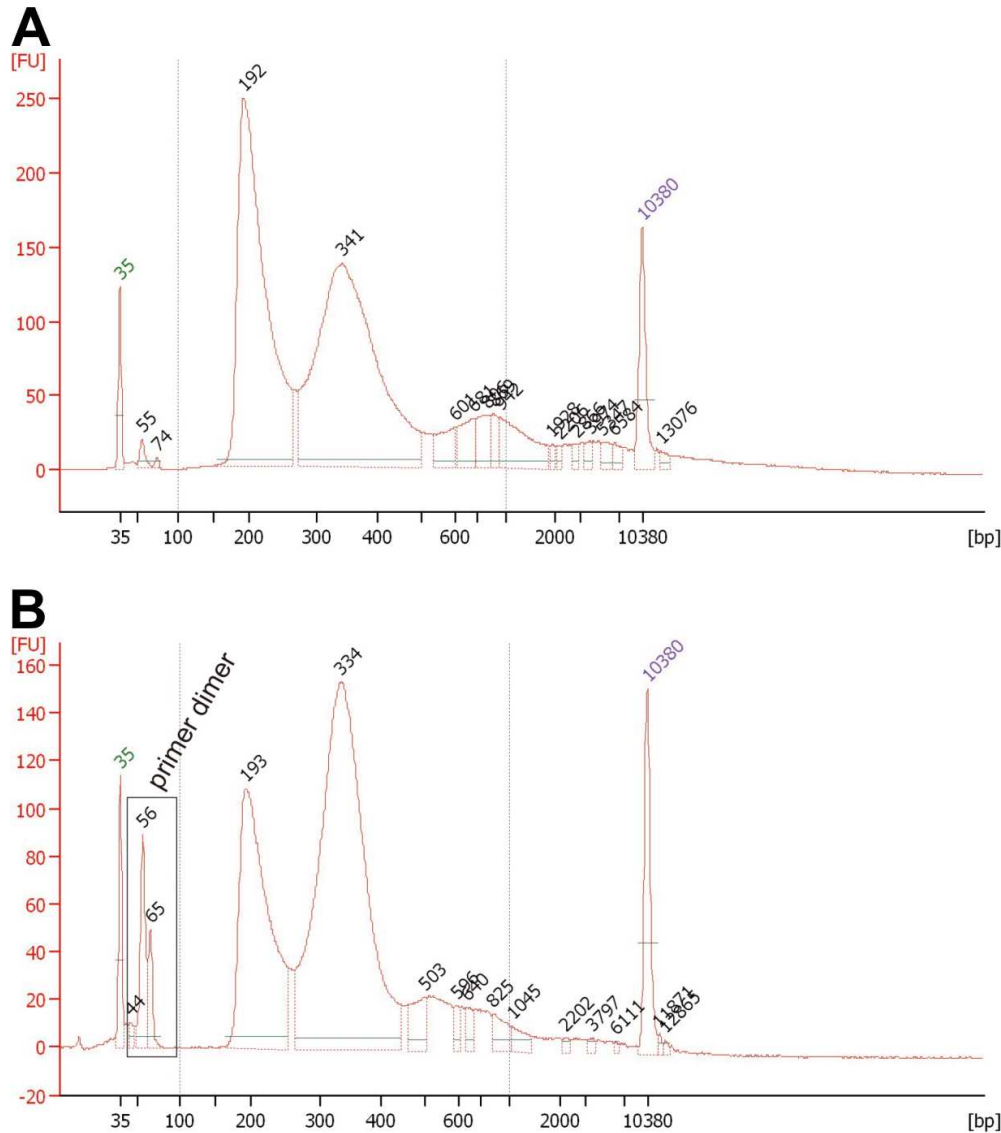


Fig. 2. Bioanalyzer analysis of example ATAC-Seq libraries. The electropherogram shows the fluorescence intensity (y-axis, relative fluorescence units) versus fragment size (x-axis, base pairs) of the ATAC-Seq library, generated using the Agilent Bioanalyzer 2100 system. The peaks at 35 and 10380 bp are high-sensitivity DNA markers. Distinct peaks correspond to mono-, di-, and tri-nucleosome fragments, indicating library suitability for downstream sequencing. **A.** An example of a high-quality library. **B.** A library showing primer dimer contamination (boxed) that needs treatment with AMPure XP beads to remove the primer dimers before sequencing.

Prepare CEL-Seq2 RNA-Seq libraries

- 16 Use the RNA extracted from dissociated cells to prepare the CEL-Seq2 library as described in (Hashimshony et al., 2016; Hashimshony, Wagner, Sher, & Yanai, 2012), or an alternative library as required by your RNA-Seq method of choice.

- 17 Check concentration of cDNA by Qubit; 1 μL should be enough to measure using the high sensitivity reagent. Expected concentration is at least $\sim 1 \text{ ng}/\mu\text{L}$.
- 18 Check quality of the final prepared library on a Bioanalyzer using a High Sensitive DNA chip. Expected fragment sizes range from 200 to 1000 bp (Fig. 3).
- 19 Sequence.

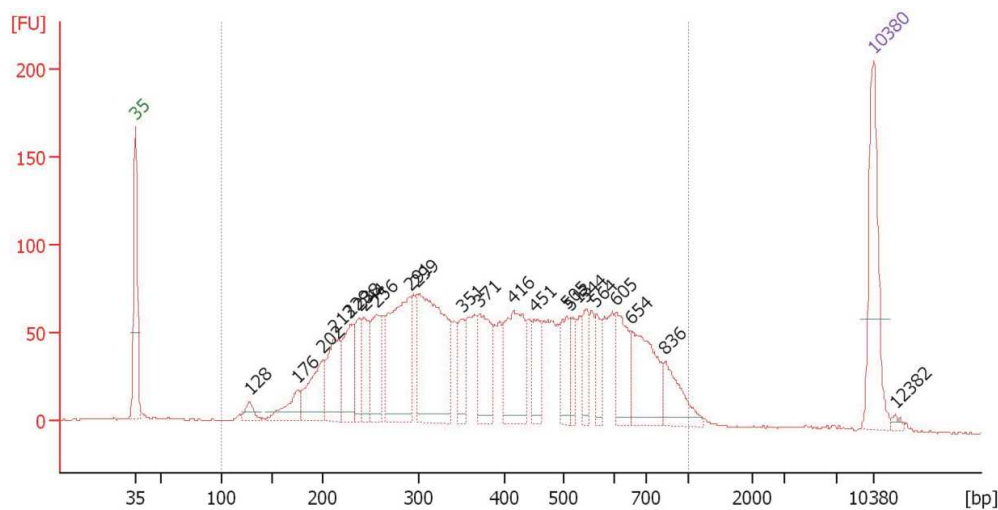


Fig. 3. Size distribution of final CEL-Seq2 library cDNA fragments. The electropherogram illustrates the fluorescence intensity (y-axis, relative fluorescence units) versus fragment size (x-axis, base pairs) of the final CEL-Seq2 library, analysed using the Agilent Bioanalyzer 2100 system. The peaks at 35 and 10380 bp are high sensitivity DNA markers. The distribution shows fragments distribution around 200–700 bp, indicating successful library preparation with the expected fragment size for sequencing.

Protocol references

- Buenrostro, J. D., Giresi, P. G., Zaba, L.C., Chang, H. Y., & Greenleaf, W. J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nature Methods*, 10(12), 1213–1218. doi:10.1038/nmeth.2688
- Cornejo-Páramo, P., Roper, K., Degnan, S.M., Degnan, B. M., & Wong, E. S. (2022). Distal regulation, silencers, and a shared combinatorial syntax are hallmarks of animal embryogenesis. *Genome Research*, 32(3), 474–487. doi:10.1101/gr.275864.121
- Degnan, S. M., & Degnan, B. M. (2010). The initiation of metamorphosis as an ancient polyphenic trait and its role in metazoan life-cycle evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1540), 641–651. doi:10.1098/rstb.2009.0248
- Hashimshony, T., Senderovich, N., Avital, G., Klochendler, A., Leeuw, Y. de, Anavy, L., ... Yanai, I. (2016). CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. *Genome Biology*, 17(1), 77. doi:10.1186/s13059-016-0938-8
- Hashimshony, T., Wagner, F., Sher, N., & Yanai, I. (2012). CEL-Seq: Single-Cell RNA-Seq by Multiplexed Linear Amplification. *Cell Reports*, 2(3), 666–673. doi:10.1016/j.celrep.2012.08.003
- Leys, S. P., Larroux, C., Gauthier, M., Adamska, M., Fahey, B., Richards, G. S., ... Degnan, B. M. (2008). Isolation of Amphimedon Developmental Material. *Cold Spring Harbor Protocols*, 2008(12), pdb.prot5095. doi:10.1101/pdb.prot5095
- Say, T. E., & Degnan, S. M. (2020). Molecular and behavioural evidence that interdependent photo - and chemosensory systems regulate larval settlement in a marine sponge. *Molecular Ecology*, 29(2), 247–261. doi:10.1111/mec.15318