

Smashing single cells into k -mer sketches

This manuscript ([permalink](#)) was automatically generated from [czbiohub/primate-brain-organoid-paper@90b5689](#) on November 6, 2019.

Authors

- **Olga Borisovna Botvinnik**

 [0000-0003-4412-7970](#) ·  [olgabot](#) ·  [olgabot](#)

Data Sciences Platform, Chan Zuckerberg Biohub

- **Venkata Naga Pranathi Vemuri**

 [0000-0002-5748-9594](#) ·  [pranathivemuri](#) ·  [pranuvemuri](#)

Data Sciences Platform, Chan Zuckerberg Biohub

- **Phoenix Aja Logan**

 [0000-0003-4581-0552](#) ·  [phoenixAja](#) ·  [phoenixlogan](#)

Data Sciences Platform, Chan Zuckerberg Biohub

Abstract

Single-cell RNA-sequencing is a powerful technology for identifying novel and known cell types, however its power is limited to organisms with well-annotated genomes. We demonstrate the utility of using annotation-agnostic methods which quantify cell-cell similarity using k -mer profiles. We benchmark a few methods and demonstrate the utility of converting cell types from mouse to human and back, and compare to using purely 1:1 mapped orthologous genes.

Introduction

There are a predicted 8.7 million Eukaryotic species on earth [1], yet only 14% (1,233,500) have been catalogued and 0.001% (9,449) have genomes deposited in the National Center for Biotechnology Information Genome Assembly [2]. And yet, the genome sequence is not enough. To truly understand the diversity of life on this planet, we need to determine not just the DNA blueprints of life, but understand the instantiation of the DNA, the cell types of the species. While sequencing DNA gives a quantitative measure of the nucleotide differences, it does not inform the functional strategies that change with DNA sequence. As new species can be defined by a new cell type. For example, the existence of a single cell type, the Cnidocyte [3], a stinging cell of a single-celled biological weapon, defines the phylum Cnidaria. Thus, entire clades, not only species, can be defined by the introduction of an additional cell type or state.

Novel organizations of existing cell states can also define cell types. For example, the development of genitalia in amniotes, while using similar cell types, ultimately uses a different physical organization of cell types to generate genitalia in mammals compared to reptiles [4]

Determining common gene ancestry (“orthology”) is a difficult problem. Many approaches exist [5,6].

Determining common ancestry of cell types (“orthologous cell types”) [7,8] is an additional difficult problem. Comparative transcriptomics begins with finding a common feature set for embedding molecular profiles across divergent species into a common space. Many researchers take the approach of using one-to-one orthologous genes [Cite: brawand2011, CCA, LIGER, Scanorama, basically all the single cell “alignment” packages], others use clusters of orthologous groups [9], others map reads onto a common genome derived from whole-genome alignment [cite: recent primate brain paper from Barbara Treutlein], or map onto native genomes [10] and re-annotate using a tool such as Comparative Annotation Toolkit [11].

k-mers have been proposed for comparing single cells [12] as they are a fast, simple way to create cell-cell similarities. However, the work so far has focused on using annotated organisms and not cross-species analyses.

Methods

Methods go here.

Experimental

Primate brain organoid protocols

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Single-cell capture of primate brain organoids

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Long read library prep

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Short read library prep

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Sequencing

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Computational

k-mer comparison of orthologous genes

We used ENSEMBL version 97. We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Extraction of putative coding reads from RNA-seq

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

bam2fasta conversion

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Results

To determine whether short segments of sequences could detect gene orthologues, we *k*-merized orthologous genes derived from the ENSEMBL version 97 [13] COMPARA database [14] (Fig.~). We compared human protein sequences to orthologous chimpanzee, mouse, (orangutan, bonobo, gorilla, macaque, opossum, platypus, chicken) protein sequences, as these are species used in [15]. As a background, we randomly chose 10 non-orthologous genes relative to the human gene. In addition to *k*-merizing the protein-coding sequence, we also re-encoded the protein-coding sequence into Dayhoff [16] and hydrophobic-polar encodings [17], show in Table~.

Table 1: Dayhoff and hydrophobic-polar encodings are a reduced amino acid alphabet allowing for permissive cross-species sequence comparisons. For example, the amino acid sequence `SASHAFIERCE` would be Dayhoff-encoded to `bbbdbfecdac`, and HP-encoded to `phpphhpppp`.

Amino acid	Property	Dayhoff	Hydrophobic-polar (HP)
C	Sulfur polymerization	a	p
A, G, P, S, T	Small	b	A, G, P: h S,T: p
D, E, N, Q	Acid and amide	c	p
H, K, R	Basic	d	p
I, L, M, V	Hydrophobic	e	h
F, W, Y	Aromatic	f	h

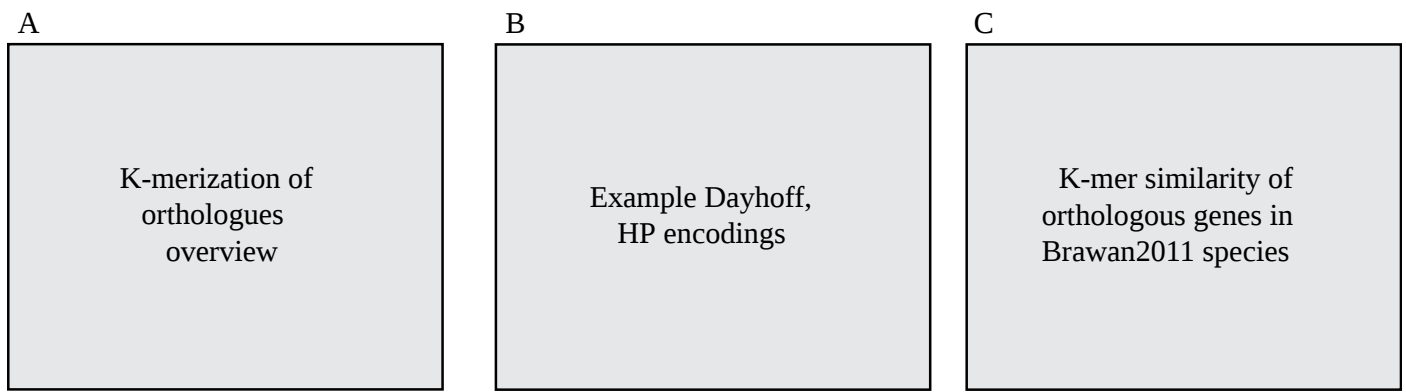


Figure 1: Figure 1.

We found that, consistent with previous knowledge, that 1:1 orthologues had higher k -mer similarities as determined by the Jaccard Index.

Additionally, more recently diverged genes had higher k -mer similarity as well.

A

Extract coding sequence using human proteins,
k-merize coding sequence and minhash, com
pare samples/cells across minhashes

B

kNN graph of
Brawand2011
MinHashes

C

kNN graph of
Brawand2011
Gene expression

D

K-mers driving
similarity in
brawand2011

E

Are the k-mers from
unmapped reads or
unannotated genes?

Figure 2: Figure 2.

Outlines

Figure 1 outline

- Kmers can approximate orthologies
 - Jaccard similarity of orthologues is higher than non-orthologues
 - Benchmarking using <https://orthology.benchmarkservice.org/cgi-bin/gateway.pl>
 - Finding orthologues
 - Gold standard
 - ENSEMBL COMPARA
 - Quest for Orthologs consortium, Altenhoff, A. M., Boeckmann, B., Capella-Gutierrez, S., Dalquen, D. A., DeLuca, T., et al. (2016). Standardized benchmarking in the quest for orthologs. *Nature Methods*, 13(5), 425–430. <http://doi.org/10.1038/nmeth.3830> [5]
 - Orthologous groups/Conserved Domain Database [18]
- Kmers can find correct reading frame of RNA-seq reads
 - Human peptides → human RNAseq
 - Human peptides → chimp RNAseq
 - Human peptides → mouse RNAseq
- Kmers can find only transcription factor reads of TFs from RNA-seq reads
 - Human TFs → human RNAseq
 - Human TFs → chimp RNAseq
 - Human TFs → mouse RNAseq
- Overview of kmermaid pipeline
 - Comparison of tissue across species
 - Partition reads to coding/noncoding bins
 - MinHash the Dayhoff-encoded coding sequences
 - Jaccard similarity on the MinHashes

Figure 2 outline

References for Primate Brain development

- Florio, M., Heide, M., Pinson, A., Brandl, H., Albert, M., Winkler, S., et al. (2018). Evolution and cell-type specificity of human-specific genes preferentially expressed in progenitors of fetal neocortex. *eLife*, 7, D635. <http://doi.org/10.7554/eLife.32332> [19]
- Mazin, P. V., Jiang, X., Fu, N., Han, D., Guo, M., Gelfand, M. S., & Khaitovich, P. (2018). Conservation, evolution, and regulation of splicing during prefrontal cortex development in humans, chimpanzees, and macaques. *Rna*, 24(4), 585–596. <http://doi.org/10.1261/rna.064931.117> [20]
- Xiong, J., Jiang, X., Ditsiou, A., Gao, Y., Sun, J., Lowenstein, E. D., et al. (2018). Predominant patterns of splicing evolution on human, chimpanzee and macaque evolutionary lineages. *Human Molecular Genetics*, 27(8), 1474–1485. <http://doi.org/10.1093/hmg/ddy058> [21]

No need for 1:1 orthology

Gene expression evolution through duplications

- Farre, D., & Alba, M. M. (2010). Heterogeneous Patterns of Gene-Expression Diversification in Mammalian Gene Duplicates. *Molecular Biology and Evolution*, 27(2), 325–335. <http://doi.org/10.1093/molbev/msp242> [22]
- Thornton, J. W., & DeSalle, R. (2000). Gene family evolution and homology: genomics meets phylogenetics. *Annual Review of Genomics and Human Genetics*, 1(1), 41–73. <http://doi.org/10.1146/annurev.genom.1.1.41> [23]
- Farre, D., & Alba, M. M. (2010). Heterogeneous Patterns of Gene-Expression Diversification in Mammalian Gene Duplicates. *Molecular Biology and Evolution*, 27(2), 325–335. <http://doi.org/10.1093/molbev/msp242> [22]

Taxa-restricted genes

- Human-specific genes in fetal neocortex Florio, M., Heide, M., Pinson, A., Brandl, H., Albert, M., Winkler, S., et al. (2018). Evolution and cell-type specificity of human-specific genes preferentially expressed in progenitors of fetal neocortex. *eLife*, 7, D635. <http://doi.org/10.7554/eLife.32332> [19]
- Insects – Santos, M. E., Le Bouquin, A., Crumière, A. J. J., & Khila, A. (2017). Taxon-restricted genes at the origin of a novel trait allowing access to a new environment. *Science*, 358(6361), 386–390. <http://doi.org/10.1126/science.aan2748> [24]

Don't need to normalize gene expression counts since we just have presence/absence

Full transcript analyses

- Brain Organoid – Droplet + PacBio data

Correlated evolution of celltypes?

- Liang, C., Musser, J. M., Cloutier, A., Prum, R. O., & Wagner, G. P. (2018). Pervasive Correlated Evolution in Gene Expression Shapes Cell and Tissue Type Transcriptomes. *Genome Biology and Evolution*, 10(2), 538–552. <http://doi.org/10.1093/gbe/evy016> [25]

Cell type homology

- Thornton, J. W., & DeSalle, R. (2000). Gene family evolution and homology: genomics meets phylogenetics. *Annual Review of Genomics and Human Genetics*, 1(1), 41–73. <http://doi.org/10.1146/annurev.genom.1.1.41> [23]
- Tschopp, P., & Tabin, C. J. (2017). Deep homology in the age of next-generation sequencing. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1713), 20150475–8. <http://doi.org/10.1098/rstb.2015.0475> [26]
- Hejnal, A., & Lowe, C. J. (2015). Embracing the comparative approach: how robust phylogenies and broader developmental sampling impacts the understanding of nervous system evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1684), 20150045–16. <http://doi.org/10.1098/rstb.2015.0045> [27]
- Santos, M. E., Le Bouquin, A., Crumière, A. J. J., & Khila, A. (2017). Taxon-restricted genes at the origin of a novel trait allowing access to a new environment. *Science*, 358(6361), 386–390. <http://doi.org/10.1126/science.aan2748> [24]
- Mammalian decidual cell

Cell type evolution

- Erkenbrack, E. M., Maziarz, J. D., Griffith, O. W., Liang, C., Chavan, A. R., Nnamani, M. C., & Wagner, G. P. (2018). The mammalian decidual cell evolved from a cellular stress response. *PLOS Biology*, 16(8), e2005594–27. <http://doi.org/10.1371/journal.pbio.2005594> [28]

Figure 3 – long evolutionary distances with HP encoding?

Metazoan body plan formation

- Early development in Cnidarians/Hydra [29; @ 10.1016/j.cell.2018.05.019]
- sponges and others [30]
- planaria [31; doi:10.1126/science.aag1723],
- drosophila [32]
- zebrafish [33, 34, 35],
- mouse [36]

Figure 4 – What features are k-mers able to pick up that mapping doesn't?

- Which reads are found to have coding features but didn't map to the genome?
- Do these features map to novel genes or gene fusions?

Discussion

Conclusions and future directions go here.

References

1. How Many Species Are There on Earth and in the Ocean?

Camilo Mora, Derek P. Tittensor, Sina Adl, Alastair G. B. Simpson, Boris Worm
PLoS Biology (2011-08-23) <https://doi.org/fpr4z8>
DOI: [10.1371/journal.pbio.1001127](https://doi.org/10.1371/journal.pbio.1001127) · PMID: [21886479](https://pubmed.ncbi.nlm.nih.gov/21886479/) · PMCID: [PMC3160336](https://pubmed.ncbi.nlm.nih.gov/PMC3160336/)

2. Genome List - Genome - NCBI <https://www.ncbi.nlm.nih.gov/genome/browse/>

3. Cnidocyte

Wikipedia

(2019-10-20) <https://en.wikipedia.org/w/index.php?title=Cnidocyte&oldid=922123291>

4. A relative shift in cloacal location repositions external genitalia in amniote evolution

Patrick Tschopp, Emma Sherratt, Thomas J. Sanger, Anna C. Groner, Ariel C. Aspiras, Jimmy K. Hu, Olivier Pourquié, Jérôme Gros, Clifford J. Tabin
Nature (2014-11-05) <https://doi.org/f6sg74>
DOI: [10.1038/nature13819](https://doi.org/10.1038/nature13819) · PMID: [25383527](https://pubmed.ncbi.nlm.nih.gov/25383527/) · PMCID: [PMC4294627](https://pubmed.ncbi.nlm.nih.gov/PMC4294627/)

5. Standardized benchmarking in the quest for orthologs

Adrian M Altenhoff, Brigitte Boeckmann, Salvador Capella-Gutierrez, Daniel A Dalquen, Todd DeLuca, Kristoffer Forslund, Jaime Huerta-Cepas, Benjamin Linard, Cécile Pereira, ... Christophe Dessimoz
Nature Methods (2016-04-04) <https://doi.org/f3rpzx>
DOI: [10.1038/nmeth.3830](https://doi.org/10.1038/nmeth.3830) · PMID: [27043882](https://pubmed.ncbi.nlm.nih.gov/27043882/) · PMCID: [PMC4827703](https://pubmed.ncbi.nlm.nih.gov/PMC4827703/)

6. OrthoFinder: phylogenetic orthology inference for comparative genomics

David M. Emms, Steven Kelly

bioRxiv (2019-04-24) <https://www.biorxiv.org/content/10.1101/466201v2>
DOI: [10.1101/466201](https://doi.org/10.1101/466201)

7. The origin and evolution of cell types

Detlev Arendt, Jacob M. Musser, Clare V. H. Baker, Aviv Bergman, Connie Cepko, Douglas H. Erwin, Mihaela Pavlicev, Gerhard Schlosser, Stefanie Widder, Manfred D. Laubichler, Günter P. Wagner
Nature Reviews Genetics (2016-11-07) <https://doi.org/f9b62x>
DOI: [10.1038/nrg.2016.127](https://doi.org/10.1038/nrg.2016.127) · PMID: [27818507](https://pubmed.ncbi.nlm.nih.gov/27818507/)

8. How Single-Cell Genomics Is Changing Evolutionary and Developmental Biology

John C. Marioni, Detlev Arendt

Annual Review of Cell and Developmental Biology (2017-10-06) <https://doi.org/ggb632>
DOI: [10.1146/annurev-cellbio-100616-060818](https://doi.org/10.1146/annurev-cellbio-100616-060818) · PMID: [28813177](https://pubmed.ncbi.nlm.nih.gov/28813177/)

9. The COG database: a tool for genome-scale analysis of protein functions and evolution

R. L. Tatusov

Nucleic Acids Research (2000-01-01) <https://doi.org/fr3ggz>
DOI: [10.1093/nar/28.1.33](https://doi.org/10.1093/nar/28.1.33) · PMID: [10592175](https://pubmed.ncbi.nlm.nih.gov/10592175/) · PMCID: [PMC102395](https://pubmed.ncbi.nlm.nih.gov/PMC102395/)

10. Establishing Cerebral Organoids as Models of Human-Specific Brain Evolution

Alex A. Pollen, Aparna Bhaduri, Madeline G. Andrews, Tomasz J. Nowakowski, Olivia S. Meyerson, Mohammed A. Mostajo-Radji, Elizabeth Di Lullo, Beatriz Alvarado, Melanie Bedolli, Max L. Dougherty, ... Arnold R. Kriegstein
Cell (2019-02) <https://doi.org/gfvgzr>
DOI: [10.1016/j.cell.2019.01.017](https://doi.org/10.1016/j.cell.2019.01.017) · PMID: [30735633](https://pubmed.ncbi.nlm.nih.gov/30735633/) · PMCID: [PMC6544371](https://pubmed.ncbi.nlm.nih.gov/PMC6544371/)

11. Comparative Annotation Toolkit (CAT)—simultaneous clade and personal genome annotation

Ian T. Fiddes, Joel Armstrong, Mark Diekhans, Stefanie Nachtweide, Zev N. Kronenberg, Jason G. Underwood, David Gordon, Dent Earl, Thomas Keane, Evan E. Eichler, ... Benedict Paten
Genome Research (2018-06-08) <https://doi.org/gdpg7n>
DOI: [10.1101/gr.233460.117](https://doi.org/10.1101/gr.233460.117) · PMID: [29884752](https://pubmed.ncbi.nlm.nih.gov/29884752/) · PMCID: [PMC6028123](https://pubmed.ncbi.nlm.nih.gov/PMC6028123/)

12. K-mer counting with low memory consumption enables fast clustering of single-cell sequencing data without read alignment

Christina Huan Shi, Kevin Y. Yip
bioRxiv (2019-08-02) <https://www.biorxiv.org/content/10.1101/723833v1>
DOI: [10.1101/723833](https://doi.org/10.1101/723833)

13. Ensembl 2018

Daniel R Zerbino, Premanand Achuthan, Wasiu Akanni, M Ridwan Amode, Daniel Barrell, Jyothish Bhai, Konstantinos Billis, Carla Cummins, Astrid Gall, Carlos García Girón, ... Paul Flicek
Nucleic Acids Research (2017-11-16) <https://doi.org/gcwg6r>
DOI: [10.1093/nar/gkx1098](https://doi.org/10.1093/nar/gkx1098) · PMID: [29155950](https://pubmed.ncbi.nlm.nih.gov/29155950/) · PMCID: [PMC5753206](https://pubmed.ncbi.nlm.nih.gov/PMC5753206/)

14. Ensembl comparative genomics resources

Javier Herrero, Matthieu Muffato, Kathryn Beal, Stephen Fitzgerald, Leo Gordon, Miguel Pignatelli, Albert J. Vilella, Stephen M. J. Searle, Ridwan Amode, Simon Brent, ... Paul Flicek
Database (2016) <https://doi.org/ggb9tv>
DOI: [10.1093/database/bav096](https://doi.org/10.1093/database/bav096) · PMID: [26896847](https://pubmed.ncbi.nlm.nih.gov/26896847/) · PMCID: [PMC4761110](https://pubmed.ncbi.nlm.nih.gov/PMC4761110/)

15. The evolution of gene expression levels in mammalian organs

David Brawand, Magali Soumillon, Anamaria Necșulea, Philippe Julien, Gábor Csárdi, Patrick Harrigan, Manuela Weier, Angélica Liechti, Ayinuer Aximu-Petri, Martin Kircher, ... Henrik Kaessmann
Nature (2011-10) <https://doi.org/fcvk54>
DOI: [10.1038/nature10532](https://doi.org/10.1038/nature10532) · PMID: [22012392](https://pubmed.ncbi.nlm.nih.gov/22012392/)

16. Atlas of protein sequence and structure

Margaret O Dayhoff
National Biomedical Research Foundation. (1969)

17. Physical biology of the cell

Rob Phillips, Julie Theriot, Jane Kondev, Hernan Garcia
Garland Science (2012)

18. Identifiers.org < EMBL-EBI <https://www.ebi.ac.uk/miriam/main/collections/MIR:00000119>

19. Evolution and cell-type specificity of human-specific genes preferentially expressed in progenitors of fetal neocortex

Marta Florio, Michael Heide, Anneline Pinson, Holger Brandl, Mareike Albert, Sylke Winkler, Pauline Wimberger, Wieland B Huttner, Michael Hiller
eLife (2018-03-21) <https://doi.org/gc678k>
DOI: [10.7554/elife.32332](https://doi.org/10.7554/elife.32332) · PMID: [29561261](https://pubmed.ncbi.nlm.nih.gov/29561261/) · PMCID: [PMC5898914](https://pubmed.ncbi.nlm.nih.gov/PMC5898914/)

20. Conservation, evolution, and regulation of splicing during prefrontal cortex development in humans, chimpanzees, and macaques

Pavel V. Mazin, Xi Jiang, Ning Fu, Dingding Han, Meng Guo, Mikhail S. Gelfand, Philipp Khaitovich
RNA (2018-01-23) <https://doi.org/gctq4n>
DOI: [10.1261/rna.064931.117](https://doi.org/10.1261/rna.064931.117) · PMID: [29363555](https://pubmed.ncbi.nlm.nih.gov/29363555/) · PMCID: [PMC5855957](https://pubmed.ncbi.nlm.nih.gov/PMC5855957/)

21. Predominant patterns of splicing evolution on human, chimpanzee and macaque evolutionary lineages

Jieyi Xiong, Xi Jiang, Angeliki Ditsiou, Yang Gao, Jing Sun, Elijah D Lowenstein, Shuyun Huang, Philipp Khaitovich

Human Molecular Genetics (2018-02-14) <https://doi.org/gc2v79>

DOI: [10.1093/hmg/ddy058](https://doi.org/10.1093/hmg/ddy058) · PMID: [29452398](https://pubmed.ncbi.nlm.nih.gov/29452398/)

22. Heterogeneous Patterns of Gene-Expression Diversification in Mammalian Gene Duplicates

D. Farre, M. M. Alba

Molecular Biology and Evolution (2009-10-12) <https://doi.org/dxrtmd>

DOI: [10.1093/molbev/msp242](https://doi.org/10.1093/molbev/msp242) · PMID: [19822635](https://pubmed.ncbi.nlm.nih.gov/19822635/)

23. GENEFAMILYEVOLUTION ANDHOMOLOGY: Genomics Meets Phylogenetics

Joseph W. Thornton, Rob DeSalle

Annual Review of Genomics and Human Genetics (2000-09) <https://doi.org/bjp5pm>

DOI: [10.1146/annurev.genom.1.1.41](https://doi.org/10.1146/annurev.genom.1.1.41) · PMID: [11701624](https://pubmed.ncbi.nlm.nih.gov/11701624/)

24. Taxon-restricted genes at the origin of a novel trait allowing access to a new environment

M. Emília Santos, Augustin Le Bouquin, Antonin J. J. Crumière, Abderrahman Khila

Science (2017-10-19) <https://doi.org/gcgjbs>

DOI: [10.1126/science.aan2748](https://doi.org/10.1126/science.aan2748) · PMID: [29051384](https://pubmed.ncbi.nlm.nih.gov/29051384/)

25. Pervasive Correlated Evolution in Gene Expression Shapes Cell and Tissue Type Transcriptomes

Cong Liang, Jacob M Musser, Alison Cloutier, Richard O Prum, Günter P Wagner

Genome Biology and Evolution (2018-01-23) <https://doi.org/gc69v9>

DOI: [10.1093/gbe/evy016](https://doi.org/10.1093/gbe/evy016) · PMID: [29373668](https://pubmed.ncbi.nlm.nih.gov/29373668/) · PMCID: [PMC5800078](https://pubmed.ncbi.nlm.nih.gov/PMC5800078/)

26. Deep homology in the age of next-generation sequencing

Patrick Tschopp, Clifford J. Tabin

Philosophical Transactions of the Royal Society B: Biological Sciences (2017-02-05)

<https://doi.org/gfzpbj>

DOI: [10.1098/rstb.2015.0475](https://doi.org/10.1098/rstb.2015.0475) · PMID: [27994118](https://pubmed.ncbi.nlm.nih.gov/27994118/) · PMCID: [PMC5182409](https://pubmed.ncbi.nlm.nih.gov/PMC5182409/)

27. Embracing the comparative approach: how robust phylogenies and broader developmental sampling impacts the understanding of nervous system evolution

Andreas Hejnol, Christopher J. Lowe

Philosophical Transactions of the Royal Society B: Biological Sciences (2015-12-19)

<https://doi.org/ggcd2m>

DOI: [10.1098/rstb.2015.0045](https://doi.org/10.1098/rstb.2015.0045) · PMID: [26554039](https://pubmed.ncbi.nlm.nih.gov/26554039/) · PMCID: [PMC4650123](https://pubmed.ncbi.nlm.nih.gov/PMC4650123/)

28. The mammalian decidual cell evolved from a cellular stress response

Eric M. Erkenbrack, Jamie D. Maziarz, Oliver W. Griffith, Cong Liang, Arun R. Chavan, Mauris C. Nnamani, Günter P. Wagner

PLOS Biology (2018-08-24) <https://doi.org/gd5b9s>

DOI: [10.1371/journal.pbio.2005594](https://doi.org/10.1371/journal.pbio.2005594) · PMID: [30142145](https://pubmed.ncbi.nlm.nih.gov/30142145/) · PMCID: [PMC6108454](https://pubmed.ncbi.nlm.nih.gov/PMC6108454/)

29. Stem cell differentiation trajectories in Hydra resolved at single-cell resolution

Stefan Siebert, Jeffrey A. Farrell, Jack F. Cazet, Yashodara Abeykoon, Abby S. Primack, Christine E. Schnitzler, Celina E. Juliano

Science (2019-07-25) <https://doi.org/ggcd2p>

DOI: [10.1126/science.aav9314](https://doi.org/10.1126/science.aav9314) · PMID: [31346039](https://pubmed.ncbi.nlm.nih.gov/31346039/)

30. **Cnidarian Cell Type Diversity and Regulation Revealed by Whole-Organism Single-Cell RNA-Seq**

Arnau Seb -Pedr s, Baptiste Saudemont, Elad Chomsky, Flora Plessier, Marie-Pierre Mailh , Justine Renno, Yann Loe-Mie, Aviezer Lifshitz, Zohar Mukamel, Sandrine Schmutz, ... Heather Marlow
Cell (2018-05) <https://doi.org/gdkxf5>
DOI: [10.1016/j.cell.2018.05.019](https://doi.org/10.1016/j.cell.2018.05.019) · PMID: [29856957](https://pubmed.ncbi.nlm.nih.gov/29856957/)

31. **Cell type transcriptome atlas for the planarian *Schmidtea mediterranea***

Christopher T. Fincher, Omri Wurtzel, Thom de Hoog, Kellie M. Kravarik, Peter W. Reddien
Science (2018-04-19) <https://doi.org/ggcd2n>
DOI: [10.1126/science.aag1736](https://doi.org/10.1126/science.aag1736) · PMID: [29674431](https://pubmed.ncbi.nlm.nih.gov/29674431/) · PMCID: [PMC6563842](https://pubmed.ncbi.nlm.nih.gov/PMC6563842/)

32. **The *Drosophila* embryo at single-cell transcriptome resolution**

Nikos Karaikos, Philipp Wahle, Jonathan Alles, Anastasiya Boltengagen, Salah Ayoub, Claudia Kipar, Christine Kocks, Nikolaus Rajewsky, Robert P. Zinzen
Science (2017-08-31) <https://doi.org/gbvp2z>
DOI: [10.1126/science.aan3235](https://doi.org/10.1126/science.aan3235) · PMID: [28860209](https://pubmed.ncbi.nlm.nih.gov/28860209/)

33. **Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis**

Jeffrey A. Farrell, Yiqun Wang, Samantha J. Riesenfeld, Karthik Shekhar, Aviv Regev, Alexander F. Schier
Science (2018-04-26) <https://doi.org/gdgtf>
DOI: [10.1126/science.aar3131](https://doi.org/10.1126/science.aar3131) · PMID: [29700225](https://pubmed.ncbi.nlm.nih.gov/29700225/) · PMCID: [PMC6247916](https://pubmed.ncbi.nlm.nih.gov/PMC6247916/)

34. **The dynamics of gene expression in vertebrate embryogenesis at single-cell resolution**

James A. Briggs, Caleb Weinreb, Daniel E. Wagner, Sean Megason, Leonid Peshkin, Marc W. Kirschner, Allon M. Klein
Science (2018-04-26) <https://doi.org/gdgg4d>
DOI: [10.1126/science.aar5780](https://doi.org/10.1126/science.aar5780) · PMID: [29700227](https://pubmed.ncbi.nlm.nih.gov/29700227/) · PMCID: [PMC6038144](https://pubmed.ncbi.nlm.nih.gov/PMC6038144/)

35. **Single-cell mapping of gene expression landscapes and lineage in the zebrafish embryo**

Daniel E. Wagner, Caleb Weinreb, Zach M. Collins, James A. Briggs, Sean G. Megason, Allon M. Klein
Science (2018-04-26) <https://doi.org/gdgg3>
DOI: [10.1126/science.aar4362](https://doi.org/10.1126/science.aar4362) · PMID: [29700229](https://pubmed.ncbi.nlm.nih.gov/29700229/) · PMCID: [PMC6083445](https://pubmed.ncbi.nlm.nih.gov/PMC6083445/)

36. **The single-cell transcriptional landscape of mammalian organogenesis**

Junyue Cao, Malte Spielmann, Xiaojie Qiu, Xingfan Huang, Daniel M. Ibrahim, Andrew J. Hill, Fan Zhang, Stefan Mundlos, Lena Christiansen, Frank J. Steemers, ... Jay Shendure
Nature (2019-02) <https://doi.org/gfvqww>
DOI: [10.1038/s41586-019-0969-x](https://doi.org/10.1038/s41586-019-0969-x) · PMID: [30787437](https://pubmed.ncbi.nlm.nih.gov/30787437/) · PMCID: [PMC6434952](https://pubmed.ncbi.nlm.nih.gov/PMC6434952/)