

Editorial

- Long reads decipher genomes and transcriptomes and offer novel insights into biology and diseases xi
Ana Conesa, Alexander Hoischen, and Fritz J. Sedlazeck

Mini-Reviews

- A Hitchhiker's Guide to long-read genomic analysis 545
Medhat Mahmoud, Daniel P. Agostinho, and Fritz J. Sedlazeck
- The additional diagnostic yield of long-read sequencing in undiagnosed rare diseases 559^{OA}
Giulia F. Del Gobbo and Kym M. Boycott
- Evolution of genome-wide methylation profiling technologies 572^{OA}
Carolina Montano and Winston Timp

Perspectives

- Notable challenges posed by long-read sequencing for the study of transcriptional diversity and genome annotation 583^{OA}
Carolina Monzó, Adam Frankish, and Ana Conesa
- The impact of long-read sequencing on human population-scale genomics 593
Tobias Rausch, Tobias Marschall, and Jan O. Korbel
- Unraveling the hidden complexity of cancer through long-read sequencing 599^{OA}
Qihui Li, Ayse G. Keskus, Justin Wagner, Michal B. Izydorczyk, Winston Timp, Fritz J. Sedlazeck, Alison P. Klein, Justin M. Zook, Mikhail Kolmogorov, and Michael C. Schatz

Research

- Closing the gaps, and improving somatic structural variant analysis and benchmarking using CHM13-T2T 621
Luis F. Paulin, Jeremy Fan, Kieran O'Neill, Erin Pleasance, Vanessa L. Porter, Steven J.M. Jones, and Fritz J. Sedlazeck
- Assessing DNA methylation detection for primary human tissue using Nanopore sequencing 632^{OA}
Rylee Genner, Stuart Akeson, Melissa Meredith, Pilar Alvarez Jerez, Laksh Malik, Breeana Baker, Abigail Miano-Burkhardt, CARD-long-read Team, Benedict Paten, Kimberley J. Billingsley, Cornelis Blauwendraat, and Miten Jain
- Expanded methylome and quantitative trait loci detection by long-read profiling of personal DNA 644
Cristian Groza, Bing Ge, Warren A. Cheung, Tomi Pastinen, and Guillaume Bourque

(continued)

Rearrangements of viral and human genomes at human papillomavirus integration events and their allele-specific impacts on cancer genome regulation	653^{OA}
Vanessa L. Porter, Michelle Ng, Kieran O'Neill, Signe MacLennan, Richard D. Corbett, Luka Culibrk, Zeid Hamadeh, Marissa Iden, Rachel Schmidt, Shirng-Wern Tsaih, Carolyn Nakisige, Martin Origa, Jackson Orem, Glenn Chang, Jeremy Fan, Ka Ming Nip, Vahid Akbari, Simon K. Chan, James Hopkins, Richard A. Moore, Eric Chuah, Karen L. Mungall, Andrew J. Mungall, Inanc Birol, Steven J.M. Jones, Janet S. Rader, and Marco A. Marra	
Multisite long-read sequencing reveals the early contributions of somatic structural variations to HBV-related hepatocellular carcinoma tumorigenesis	671^{OA}
Tianfu Zeng, Haotian Liao, Lin Xia, Siyao You, Yanqun Huang, Jiaxun Zhang, Yahui Liu, Xuyan Liu, and Dan Xie	
Long-read single-cell RNA sequencing enables the study of cancer subclone-specific genotypes and phenotypes in chronic lymphocytic leukemia	686
Gage S. Black, Xiaomeng Huang, Yi Qiao, Philip Moos, Deepa Sampath, Deborah M. Stephens, Jennifer A. Woyach, and Gabor T. Marth	
Heterogeneous and novel transcript expression in single cells of patient-derived clear cell renal cell carcinoma organoids	698^{OA}
Tülay Karakulak, Natalia Zajac, Hella Anna Bolck, Anna Bratus-Neuenschwander, Qin Zhang, Weihong Qi, Debleena Basu, Tamara Carrasco Oltra, Hubert Rehrauer, Christian von Mering, Holger Moch, and Abdullah Kahraman	
Genetic regulation of nascent RNA maturation revealed by direct RNA nanopore sequencing	712
Karine Choquet, Louis-Philippe Chaumont, Simon Bache, Autum R. Baxter-Koenigs, and L. Stirling Churchman	
Deciphering the largest disease-associated transcript isoforms in the human neural retina with advanced long-read sequencing approaches	725
Merel Stemerding, Tabea Riepe, Nick Zomer, Renee Salz, Michael Kwint, Jaap Oostrik, Raoul Timmermans, Barbara Ferrari, Stefano Ferrari, Alfredo Dueñas Rey, Emma Delanote, Suzanne E. de Bruijn, Hannie Kremer, Susanne Roosing, Frauke Coppieters, Alexander Hoischen, Frans P.M. Cremers, Peter A.C. 't Hoen, Erwin van Wijk, and Erik de Vrieze	
Integrating short-read and long-read single-cell RNA sequencing for comprehensive transcriptome profiling in mouse retina	740
Meng Wang, Yumei Li, Jun Wang, Soo Hwan Oh, Yexuan Cao, and Rui Chen	
Unraveling undiagnosed rare disease cases by HiFi long-read genome sequencing	755^{OA}
Wouter Steyaert, Lydia Sagath, German Demidov, Vicente A. Yépez, Anna Esteve-Codina, Julien Gagneur, Kornelia Ellwanger, Ronny Derks, Marjan Weiss, Amber den Ouden, Simone van den Heuvel, Hilde Swinkels, Nick Zomer, Marloes Steehouwer, Luke O'Gorman, Galuh Astuti, Kornelia Neveling, Rebecca Schüle, Jishu Xu, Matthias Synofzik, Danique Beijer, Holger Hengel, Ludger Schöls, Kristl G. Claeys, Jonathan Baets, Liedewei Van de Vondel, Alessandra Ferlini, Rita Selvatici, Heba Morsy, Marwa Saeed Abd Elmaksoud, Volker Straub, Juliane Müller, Veronica Pini, Luke Perry, Anna Sarkozy, Irina Zaharieva, Francesco Muntoni, Enrico Bugiardini, Kiran Polavarapu, Rita Horvath, Evan Reid, Hanns Lochmüller, Marco Spinazzi, Marco Savarese, Solve-RD DITF-ITHACA, Solve-RD DITF-Euro-NMD, Solve-RD DITF-RND, Solve-RD DITF-EpiCARE, Leslie Matalonga, Steven Laurie, Han G. Brunner, Holm Graessner, Sergi Beltran, Stephan Ossowski, Lisenka E.L.M. Vissers, Christian Gilissen, Alexander Hoischen, and on behalf of the Solve-RD consortium	

(continued)

A prospective trial comparing programmable targeted long-read sequencing and short-read genome sequencing for genetic diagnosis of cerebellar ataxia	769
Haloom Rafehi, Liam G. Fearnley, Justin Read, Penny Snell, Kayli C. Davies, Liam Scott, Greta Gillies, Genevieve C. Thompson, Tess A. Field, Aleena Eldo, Simon Bodek, Ernest Butler, Luke Chen, John Drago, Himanshu Goel, Anna Hackett, G. Michael Halmagyi, Andrew Hannaford, Katya Kotschet, Kishore R. Kumar, Smitha Kumble, Matthew Lee-Archer, Abhishek Malhotra, Mark Paine, Michael Poon, Kate Pope, Katrina Reardon, Steven Ring, Anne Ronan, Matthew Silsby, Renee Smyth, Chloe Stutterd, Mathew Wallis, John Waterston, Thomas Wellings, Kirsty West, Christine Wools, Kathy H.C. Wu, David J. Szmulewicz, Martin B. Delatycki, Melanie Bahlo, and Paul J. Lockhart	
Multiple paralogs and recombination mechanisms contribute to the high incidence of 22q11.2 deletion syndrome	786
Lisanne Vervoort, Nicolas Dierckxsens, Marta Sousa Santos, Senne Meynants, Erika Souche, Ruben Cools, Tracy Heung, Koen Devriendt, Hilde Peeters, Donna M. McDonald-McGinn, Ann Swillen, Jeroen Breckpot, Beverly S. Emanuel, Hilde Van Esch, Anne S. Bassett, and Joris R. Vermeesch	
Optical genome mapping identifies rare structural variants in neural tube defects	798^{OA}
Nikhil S. Sahajpal, Jane Dean, Benjamin Hilton, Timothy Fee, Cindy Skinner, Alex Hastie, Barbara R. DuPont, Alka Chaubey, Michael J. Friez, and Roger E. Stevenson	
Optical genome mapping enables accurate testing of large repeat expansions	810^{OA}
Bart van der Sanden, Kornelia Neveling, Syukri Shukor, Michael D. Gallagher, Joyce Lee, Stephanie L. Burke, Maartje Pennings, Ronald van Beek, Michiel Oorsprong, Ellen Kater-Baats, Eveline Kamping, Alide A. Tieleman, Nicol C. Voermans, Ingrid E. Scheffer, Jozef Gecz, Mark A. Corbett, Lisenka E.L.M. Vissers, Andy Wing Chun Pang, Alex Hastie, Erik-Jan Kamsteeg, and Alexander Hoischen	
<i>Candida albicans</i> isolates contain frequent heterozygous structural variants and transposable elements within genes and centromeres	824
Ursula Oggenfuss, Robert T. Todd, Natthapon Soisangwan, Bailey Kemp, Alison Guyer, Annette Beach, and Anna Selmecki	
Methods	
Geometric deep learning framework for de novo genome assembly	839
Lovro Vrčec, Xavier Bresson, Thomas Laurent, Martin Schmitz, Kenji Kawaguchi, and Mile Šikić	
Multisample motif discovery and visualization for tandem repeats	850^{OA}
Yaran Zhang, Marc Hulsman, Alex Salazar, Niccolò Tesi, Lydian Knoop, Sven van der Lee, Sanduni Wijesekera, Jana Krizova, Erik-Jan Kamsteeg, and Henne Holstege	
RAMbler resolves complex repeats in human Chromosomes 8, 19, and X	863^{OA}
Sakshar Chakravarty, Glennis Logsdon, and Stefano Lonardi	
Enhancing nanopore adaptive sampling for PromethION using readfish at scale	877^{OA}
Rory Munro, Alexander Payne, Nadine Holmes, Chris Moore, Inswasti Cahyani, and Matthew Loose	

(continued)

Nanopore-based consensus sequencing enables accurate multimodal tumor cell-free DNA profiling	886^{OA}
Li-Ting Chen, Myrthe Jager, Dàmi Rebergen, Geertruid J. Brink, Tom van den Ende, Willem Vanderlinden, Pauline Kolbeck, Marc Pagès-Gallego, Ymke van der Pol, Nicolle Besselink, Norbert Moldovan, Nizar Hami, Wigard P. Kloosterman, Hanneke van Laarhoven, Florent Moulriere, Ronald Zweemer, Jan Lipfert, Sarah Derks, Alessio Marcozzi, and Jeroen de Ridder	
De novo detection of somatic variants in high-quality long-read single-cell RNA sequencing data	900^{OA}
Arthur Dondi, Nico Borgsmüller, Pedro F. Ferreira, Brian J. Haas, Francis Jacob, Viola Heinzelmänn-Schwarz, Tumor Profiler Consortium, and Niko Beerenwinkel	
Integration of transcriptomics and long-read genomics prioritizes structural variants in rare disease	914^{OA}
Tanner D. Jensen, Bohan Ni, Chloe M. Reuter, John E. Gorzynski, Sarah Fazal, Devon Bonner, Rachel A. Ungar, Pagé C. Goddard, Archana Raja, Euan A. Ashley, Jonathan A. Bernstein, Stephan Zuchner, Undiagnosed Diseases Network, Michael D. Greicius, Stephen B. Montgomery, Michael C. Schatz, Matthew T. Wheeler, and Alexis Battle	
De novo antibody identification in human blood from full-length single B cell transcriptomics and matching haplotype-resolved germline assemblies	929^{OA}
John Beaulaurier, Lynn Ly, J. Andrew Duty, Carly Tyer, Christian Stevens, Chuan-Tien Hung, Akash Sookdeo, Alex W. Drong, Shreyas Kowdle, Axel Guzman-Solis, Domenico Tortorella, Daniel J. Turner, Sissel Juul, Scott Hickey, and Benhur Lee	
Single-cell Rapid Capture Hybridization sequencing reliably detects isoform usage and coding mutations in targeted genes	942^{OA}
Hongke Peng, Jafar S. Jabbari, Luyi Tian, Changqing Wang, Yupei You, Chong Chyn Chua, Natasha S. Anstee, Noorul Amin, Andrew H. Wei, Nadia M. Davidson, Andrew W. Roberts, David C.S. Huang, Matthew E. Ritchie, and Rachel Thijssen	
Rapid and accurate demultiplexing of direct RNA nanopore sequencing data with SeqTagger	956^{OA}
Leszek P. Pryszz, Gregor Diensthuber, Laia Llovera, Rebeca Medina, Anna Delgado-Tejedor, Luca Cozzuto, Julia Ponomarenko, and Eva Maria Novoa	
Accurate fusion transcript identification from long- and short-read isoform sequencing at bulk or single-cell resolution	967^{OA}
Qian Qin, Victoria Popic, Kirsty Wienand, Houlin Yu, Emily White, Akanksha Khorgade, Asa Shin, Christophe Georgescu, Catarina D. Campbell, Arthur Dondi, Niko Beerenwinkel, Francisca Vazquez, Aziz M. Al'Khafaji, and Brian J. Haas	
Quality assessment of long read data in multisample lrrNA-seq experiments using SQANTI-reads	987
Netanya Keil, Carolina Monzó, Lauren McIntyre, and Ana Conesa	
Long-read RNA sequencing reveals allele-specific N⁶-methyladenosine modifications	999
Dayea Park and Can Cenik	
Biosurfer for systematic tracking of regulatory mechanisms leading to protein isoform diversity	1012
Mayank Murali, Jamie Saquing, Senbao Lu, Ziyang Gao, Emily F. Watts, Ben Jordan, Zachary Peters Wakefield, Ana Fiszbein, David R. Cooper, Peter J. Castaldi, Dmitry Korkin, and Gloria M. Sheynkman	

(continued)

- Optimizing nanopore adaptive sampling for pneumococcal serotype surveillance in complex samples using the graph-based GNASTy algorithm 1025^{OA}
Samuel T. Horsfield, Basil C.T. Fok, Yuhan Fu, Paul Turner, John A. Lees, and Nicholas J. Croucher
- Taurine pangenome uncovers a segmental duplication upstream of *KIT* associated with depigmentation in white-headed cattle 1041
Sotiria Milia, Alexander S. Leonard, Xena Marie Mapel, Sandra Milena Bernal Ulloa, Cord Drögemüller, and Hubert Pausch
- Evaluation of strategies for evidence-driven genome annotation using long-read RNA-seq 1053^{OA}
Alejandro Paniagua, Cristina Agustín-García, Francisco J. Pardo-Palacios, Thomas Brown, Maite De Maria, Nancy D. Denslow, Camila J. Mazzoni, and Ana Conesa

^{OA}Open Access paper



Cover Part 2 of the Special Issue on Long-read DNA Sequencing Applications in Biology and Medicine, Guest Edited by Drs. Ana Conesa, Alexander Hoischen, and Fritz Sedlazeck, focuses on original research offering novel biological and clinical insights gained using long-read DNA and RNA sequencing technologies and other long molecule approaches. Significant advances in long-read sequencing analysis methods and their implementation are also highlighted. On the cover, long strands representing DNA and RNA emerge from a cross section of a cell, with nucleosomes visible within. Primary colors represent different nucleotides. Floating circular motifs and the patterned background allude to characteristics of long-read sequencing technologies. The composition draws inspiration from Kandinsky, aiming to convey the inherent musicality of genetic material—the “music of life”—through the dynamic, whimsical forms of the lines and their vibrant color. This cover mirrors that of Part 1, and together they form a complete image representative of the Long-read DNA and RNA Sequencing Special Collection. (Illustration by Alex Cagan, University of Cambridge [<https://www.alexecagan.com/>].)