DNAsense

"more than just the sequence…"

Nanopore Course

17th February – 19th February 2020

February 17th

NOVI 1, Niels Jernes vej 10 (meeting room 1)

WHEN	WHAT	HOW	WHO
09.15-09.45	**Welcome** • Course introduction • Practical information • DNAsense + Participants (who is who)	**Lecture**	**RWO**
09.45-10.00	**BREAK / BUFFER**		
10.00-10.45	**General Theory** • Nanopore sensing • Applications • Error profiles • Pore types • Library options	**Lecture**	**RWO**
10.45-11.00	**BREAK / BUFFER**		
11.00-11.45	**DNA Quality Control** • Purity and Integrity • DNA extraction and cleanup • The moles vs. mass conundrum (…and its implication on the ligation based protocol)	**Lecture**	**RWO**
11.45-12.30	**LUNCH**		
12.30-14.00	**Rapid Sequencing** We will use the Rapid Sequencing protocol (SQK-RBK004) to demonstrate how fast a library can be prepared and data can be generated. You will have hands-on with library preparation, flow cell priming and loading of the flow cell.	**Hands-on**	**RWO**
14.00.14.15	**BREAK / BUFFER**		
14.15-15.00	**MinKNOW** • Configuration • Quality Control • Files and folder	**Lecture / Hands-on**	**RWO**
15.00-15.30	**Summary and Questions**		**RWO**

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February 18th

NOVI 1, Niels Jernes vej 10 (meeting room 1)

WHEN	WHAT	HOW	WHO
09.00-09.45	**Quality Control** • Run evaluation • ATP depletion and Refueling • Flow cell washing / nuclease flushing	**Lecture / Hands-on**	**RWO**
09.45-10.00	**BREAK / BUFFER**		
10.00-10.45	**Basecalling** • Introduction to basecalling and Guppy • Guppy parameters • GPU-accelerated basecalling • Demultiplexing and trimming • Quality control	**Lecture**	**RWO**
10.45-11.00	**BREAK / BUFFER**		
11.00-11.45	**Basecalling continued** During the hands-on part, you will basecall fast5 files (*E. coli* whole genome R9 and R10 data) using different algorithms, parameters and evaluate the outcome in terms of speed, quality (q-scores) and later in terms of accuracy. Prerequisites: Laptop with a SSH client (e.g. PuTTY or MobaXterm) for command line interfacing (CLI) and/or X2Go client for graphical user interfacing (GUI).	**Hands-on**	**RWO**
11.45-12.30	**Lunch**		
12.30-13.15	**Genome Assembly** • Genomics vs. metagenomics • Strategies and workflows • Assemblers and compute cost • Polishing of draft assembly • Benchmarking	**Lecture**	**RWO**
13.15-13.30	**Break / Buffer**		
13.30-15.30	**Genome Assembly continued** During the hands-on part, you will learn to filter basecalled data and how to perform whole genome *de novo* assembly (R9 and R10 data). Prerequisites: Laptop with a SSH client (e.g. PuTTY or MobaXterm) for command line interfacing (CLI) and/or X2Go client for graphical user interfacing (GUI).	**Hands-on**	**RWO**
15.30-16.00	**Summary and Questions**		**RWO**

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February 19th

NOVI 1, Niels Jernes vej 10 (meeting room 1)

WHEN	WHAT	HOW	WHO
09.00-09.45	**Genome Assembly - Quality Control** During this hand-on part you will learn how to evaluate the quality of the final polished assembly by comparing it to a reference genome. Prerequisites: Laptop with a SSH client (e.g. PuTTY or MobaXterm) for command line interfacing (CLI) and/or X2Go client for graphical user interfacing (GUI).	**Hands-on**	**RWO**
09.45-10.00	**BREAK / BUFFER**		
10.00-10.45	**Summary Overview on Basecalling and Assembly** • Basecalling Algorithms • Basecalling parameters • Assemblers • Polishing strategies • Automation (pipeline scripting)	**Lecture / Hands-on**	**RWO**
10.45-11.00	**BREAK / BUFFER**		
11.00-11.45	**Amplicon sequencing** • 16S sequencing and Classification • Illumina vs. Nanopore • Read Accuracy, error-types and Implications • UMI	**Lecture**	**RWO (SMK)**
11.45-12.30	**LUNCH**		
12.30-14.00	**Amplicon sequencing continued** We will process full length 16S Nanopore data (with and without UMI), learn how to evaluate the data and classify the data against the Silva database. During the introduction you will also learn how to perform quality control identify and identify errors such as chimeric reads. Prerequisites: Laptop with a SSH client (e.g. PuTTY or MobaXterm) for command line interfacing (CLI) and/or X2Go client for graphical user interfacing (GUI).	**Hands-on**	**SMK (RWO)**
14.00-14.15	**BREAK / BUFFER**		
14.15-15.00	**Questions and Course Evaluation**		

RWO: Rasmus Wollenberg, SMK: Søren Michael Karst

Suggested reading:

1. Deamer, D. W. *et al.* The potential and challenges of nanopore sequencing. *Genome Res.* **26**, 1146–1153 (2011).

2. Loman, N. J., Quick, J. & Simpson, J. T. A complete bacterial genome assembled de novo using only nanopore sequencing data. *Nat. Methods* **12**, 733–735 (2015).

3. Loman, N. J. & Watson, M. Successful test launch for nanopore sequencing. *Nature Methods* **12**, 303–304 (2015).

4. Loman, N. J. & Pallen, M. J. Twenty years of bacterial genome sequencing. *Nature Reviews Microbiology* **13**, 787–794 (2015).

5. Deamer, D., Akeson, M. & Branton, D. Three decades of nanopore sequencing. *Nat. Publ. Gr.* **34**, 518–524 (2016).

6. Wick, R. R., Judd, L. M., Gorrie, C. L. & Holt, K. E. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microb. Genomics* **3**, 1–7 (2017).

7. Karst, S. M. *et al.* Retrieval of a million high-quality, full-length microbial 16S and 18S rRNA gene sequences without primer bias. *Nat. Biotechnol.* (2018). doi:10.1038/nbt.4045

8. **Karst, S. M., Ziels, R. M., Kirkegaard, R. H., Sørensen, E.A., MacDonald, D., Zhu, Qiyun, Knight, R. & Albertsen, M. Enabling high-accuracy long-read amplicon sequences using unique molecular identifiers with Nanopore or PacBio sequencing. *bioRxiv* 645903 (2019). doi:10.1101/645903**

9. **Eisenstein, M. Playing a long game. *Nat. Methods* 16, 683–686 (2019).**

10. Nicholls, S. M., Quick, J. C., Tang, S. & Loman, N. J. Ultra-deep, long-read nanopore sequencing of mock microbial community standards. *Gigascience* **8**, 1–7 (2019).

11. **Schalamun, M. *et al.* Harnessing the MinION: An example of how to establish long-read sequencing in a laboratory using challenging plant tissue from Eucalyptus pauciflora. *Mol. Ecol. Resour.* 19, 77–89 (2019).**

12. **Wick, R & Holt, K.E. Benchmarking of long-read assemblers for prokaryote whole genome sequencing. F1000research (2019)**