

UNIVERSITY OF ZAGREB
FACULTY OF ELECTRICAL ENGINEERING AND
COMPUTING

SEMINAR

MinCall — MinION end2end deep learning basecaller

Neven Miculinić

Mentor: *izv. prof. dr. sc. Mile Sikić*

Zagreb, May 2017.

CONTENTS

1. Introduction	1
2. Background	3
2.1. Sequencing overview	3
2.2. Basecalling	3
3. Method	4
3.1. Data preprocessing	4
3.2. Residual architecture	5
3.3. CTC	5
4. Results	6
4.0.1. Final model	6
4.0.2. Performance tables	6
5. Conclusion and further work	8
6. Acknowledgments	9
7. Bibliography	10

MinCall — MinION basecaller model pogonjen dubokim učenjem

Sažetak

Uređaj MinION firme Oxford Nanopore Technology prvi je prenosivi sekvencer DNA. Sposoban je pročitati nizove značajno velike duljine, preko 100 kbp, no nažalost pati od značajno više pogrešaka nego ostale metode sekvenciranja.

U ovoj studiji kreirali smo MinCall, cjelokupni model očitavanja nukleotida za MinION. Model je baziran na metodama dubokog učenja i koristi konvolucijske neuronske mreže u svojoj implementaciji. Za najbolje performanse koristi najnovije tehnike dubokog učenja i arhitekture, specifično rezidualne neuronske mreže, normalizaciju nad grupom i gubitak vezane vremenske klasifikacije.

Najuspješniji model, u koji je uključeno 270 slojeva neurona, postiže najbolje dosad zabilježene performanse od 90.5% preciznosti na skupu za učenje iz E. coli koristeći R9-kemiju nanopore i 1D-čitanja. **Ključne riječi:** Basecaller, MinION, R9, CNN, CTC, sekvenciranje sljedeće generacije

MinCall — MinION end2end deep learning basecaller

Abstract

The Oxford Nanopore Technologies's MinION is the first portable DNA sequencing device. It's capable of producing long reads, over 100 kbp were reported, however, it has significantly higher error rate than other methods.

In this study, we created MinCall, an end2end basecaller model for the MinION. The model is based on deep learning and uses convolutional neural networks (CNN) in its implementation. For extra performances it uses cutting edge deep learning techniques and architectures, notably gated residual network, batch normalization and Connectionist Temporal Classification (CTC) loss.

The best performing 270 layers deep model achieves state-of-the-art 90.5% match rate on E.Coli dataset using R9 pore chemistry and 1D reads. **Keywords:** Basecaller, MinION, R9, CNN, CTC, Next generation sequencing

1. Introduction

Oxford Nanopore Technology's MinION nanopore sequencing platform [1] is the first portable DNA sequencing device. It's small weight, of only 90 grams, low capital cost, and long read length combined with high-throughput, real-time data analysis, and decent accuracy yield promising results in various applications. From clinical application such as monitoring infectious disease outbreaks [2][3], characterizing structural variants in cancer[4] and even full human genome assembly [5].

Although MinION is able to produce long reads, they have a high sequencing error rate. This has been somewhat alleviated with new R9 pore model, replacing older R7 ones. In this paper, we show that this error rate can be reduced by replacing the default base caller provided by the manufacturer with a properly trained neural network model. In the future new R9.5 chemistry and 1D² reads supersede current models.

In the MinION device, single-stranded DNA fragments move through nanopores, which causes drops in the electric current. The electric current is measured at each pore several thousand times per second, 4000 times exactly in our dataset. The electric current depends mostly on the context of several DNA bases passing through the pore at the time of measurement. As the DNA moves through the pore, the context shifts, and the electric current changes.

A MinION device typically yields reads several thousand bases long; reads as long as 100,000 bp have been reported. 1D read has usually error rate about 10%, though it varies across different genomes, human one being lower accuracy than E.Coli one. (Check details!!, What is the error rate, how it's defined???) After parameter training, base calling can be performed by running the Viterbi algorithm, which will result in the sequence of states with the highest likelihood.

The exact error rate metric is unreliable since multiple pipeline tools could be the issue. First the sample is prepared, hopefully, uncontaminated and matching reference genome as close as possible, then sequenced using the MinION device obtaining raw data. Next, our model (or other groups ones) come along, basecall the sequence. To evaluate error rate metric basecalled read is aligned to the reference genome us-

ing aligners with their own errors/biases, mostly commonly used BWA-MEM [6] and Graphmap [7].

2. Background

2.1. Sequencing overview

Conceptually, the MinION sequencer works as follows. First, DNA is sheared into fragments of 8–20 kbp and adapters are ligated to either end of the fragments. The resulting DNA fragments pass through a protein embedded in a membrane via a nanometre-sized channel, a nanopore. A single DNA strand passes through the pore. Optionally, hairpin protein adapter can merge two DNA strands, allowing both template and complement read passing through the nanopore sequentially for more accurate reads. This technique is referred as 2D reads, while we focus on 1D reads containing only template DNA and no hairpin adapter.

Electrical current runs through the nanopore and exact nucleotides context within influences the nanopore's resistance. This resistive effect is our sensor data, that is the current fluctuations as DNA passes through the pore. The nanopore is 6 nucleotides wide, and many models use this information in their approaches, while we're opted out of this technicality.

2.2. Basecalling

The core of the decoding process is the basecalling step. Official basecaller is Metrichor, previously performed in the cloud before being open-sourced under name Nanonet. (Citation, whom?)

Earlier models were (Hidden Markov model) HMM-based where hidden state modeled DNA sequence of length 6 (6-mer) in the nanopore. Pore models were used in computing emission probabilities. [8, 9, 10, 11] and the recent open source HMM-based basecaller Nanocall [12].

Recent models opted using (Recurrent neural network) RNN, notably, DeepNano [13] and recently open sourced official Nanonet from ONT.

3. Method

Instead of opting for the traditional path using HMM or RNN we tried using CNN (Convolutional neural networks) [14], that is their residual version [15]. We opted out for gated residual network variant [16]. For loss, we used CTC (Connectionist temporal classification) [17] between basecalled and the target sequence. The implementation used is open source warp-ctc [18]. Main computation framework is tensorflow [19]

3.1. Data preprocessing

Dataset was obtained from (Ask somebody, Marko doesn't know!). In this research, models were trained on the E. Coli K-12 strand. (Should I cite someone for strand). Data was split into train and test subset, such that aligned reads map to different reference genome parts. For initial model data bootstrapping metrichorn was used, version (Ask Someone?).

The fast5 input training files were further split into smaller training blocks, consisting of fixed block size on raw signal. For each block target sequence, basecalled data is used in following way.

We're using basecalled knowledge which tells us on each raw read part which 6-mer were currently in the nanopore. Using this data, we get the intermediate target sequence for each block. To correct for model errors, we use aligned information, that is reference genome and alignment data (cigar string) to correct that information, yielding finalized target sequence. For further performance, we skip first and last training block, since most error aggregate on edges.

Adjacent matching bases are separated with surrogate one, for example, AAA -> AA'A for reasons described in the following section.

3.2. Residual architecture

We use gated residual network, that is layer function is like this: $f(x) = kg(x) + x$ Where x is input layer and k constant learnt during training. Specifically, g used is multiple, 1–3 times in our experiments, Relu-BatchNorm-CNN layers.

3.3. CTC

This section gives a brief overview over CTC, for further detail and in depth explanation we recommend original paper [17] or searching for contemporary blog posts.

In CTC we have target sequence l consisting from symbols of alphabet Σ . Our models outputs \mathbf{x} , and CTC loss maximizes $p(l|\mathbf{x})$.

\mathbf{x} is consistend of n independent discrete random variables, X_i over domain $\Sigma \cup \text{Blank}$. Path p through \mathbf{x} is assigning for each X_i single value. It's log probability is $\log P(p) = \sum_i \log P(X_i = x_i)$ where x_i s define the path.

We further have merging operator on path, $\text{merge}(x_1, x_2, \dots, x_n)$ which merges together adjacent equivalent elements. For example $\text{merge}(AAAGC) = AGC$.

Then $p(l|\mathbf{x}) = \sum_{p \in \text{paths}(\mathbf{x}, \text{merged}(p)=l)} P(p)$ or less formally, $P(l|\mathbf{x})$ is sum of all path probabilities which when merged give l

In our concrete application $\Sigma = \{A G T C A' G' T' A'\}$

For decoding, we use beam search decoder, with beam width 100.

4. Results

4.0.1. Final model

Best performing model used has 270 total layers, divided into 3 90-layer blocks. Between each 90 layers blocks, MaxPool layer is inserted with the receptive width of 2 and stride 2, to ease computation effort and add precision.

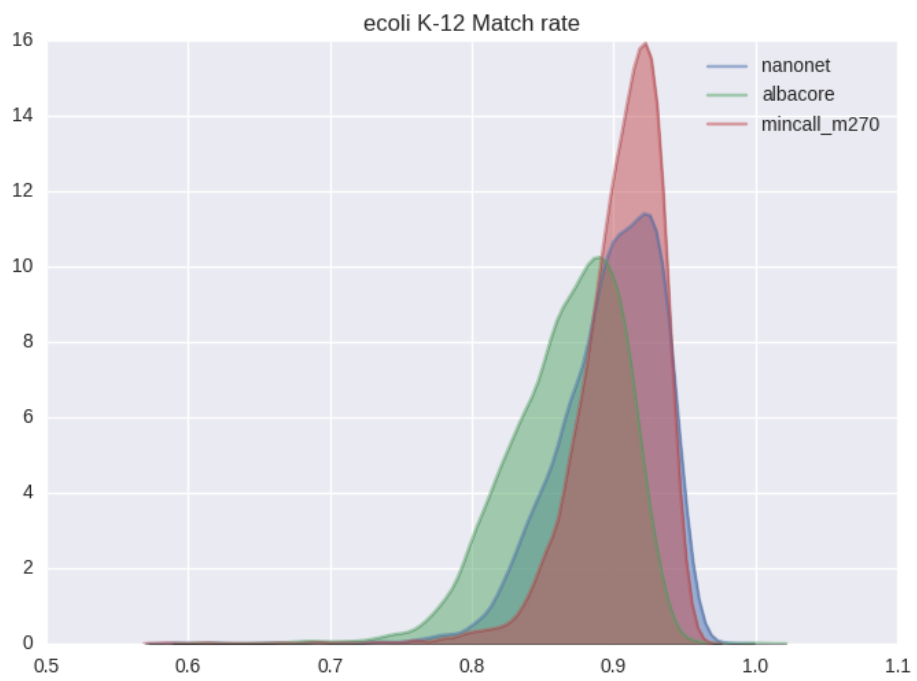
Each block consists of 30 gated residual layers, each residual layer composed of 3 sequential Relu-Batch Normalization-Conv1D layers. Each convolutional layer uses the receptive width of 3 with 64 channels as output throughout the model.

4.0.2. Performance tables

As described in the previous section, the model was tested on E.Coli test set and compared to open source Nanonet and Albacore. Albacore doesn't have specific R9 chemistry mode, thus R9.4 was used instead which explains its lower performance on this task. Mean CIGAR operation are in table4.1 and KDE Match rate plot is in figure ??.

	Deletion rate	Error rate	Insertion rate	Match rate	Mismatch rate	Read length
albacore	0.060	0.194	0.070	0.867	0.063	9843
nanonet	0.088	0.190	0.040	0.897	0.062	5029
mincall_m270	0.077	0.172	0.040	0.905	0.056	9378

Table 4.1: Mean performance metrics on E.Coli dataset, 5k sample



5. Conclusion and further work

This model used advance state-of-the-art gated residual convolutional neural network, with top models having 270 layers and over 3M parameters, yet improvements over Metrichorn baseline are marginal. As the conclusion, it might be that we've reached Bayesian error rate for R9 chemistry. Furthermore, R9.5 and 1D² reads are under development which shall yield this paper's result obsolete quite soon, yet underlying code developed could easily be adjusted and trained on new data.

Unlike Nanonet which uses custom OpenCL kernels or Albacore — a novel ONT basecaller as of May 2017 lacking GPU support, this work used world-class computational framework tensorflow with highly optimized kernels and large development community. Therefore resulting paper's effect is showcasing Residual CNN approach or pure CNN approach with CTC loss is marginally better than already established basecaller and providing code in the contemporary framework.

6. Acknowledgments

First and foremost I'd like to thank my mentor izv. prof. dr. sc. Mile Sikić for setting up the problem, guiding us through its beginnings and providing helpful advice for its completion. Next Marko Ratkovic for his not only insightful conversations, but also meaningful code contributions.

Finally, I'm thanking various other people whose code, tools and advice I've used in completing this paper: Fran Jurišić, Ana Marija Selak, Ivan Sović and Martin Šošić.

7. Bibliography

- [1] Alexander S Mikheyev i Mandy MY Tin. A first look at the oxford nanopore minion sequencer. *Molecular ecology resources*, 14(6):1097–1102, 2014.
- [2] Kim Judge, Simon R Harris, Sandra Reuter, Julian Parkhill, i Sharon J Peacock. Early insights into the potential of the oxford nanopore minion for the detection of antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy*, 70(10):2775–2778, 2015.
- [3] Joshua Quick, Nicholas J Loman, Sophie Duraffour, Jared T Simpson, Ettore Severi, Lauren Cowley, Joseph Akoï Bore, Raymond Koundouno, Gytis Dudas, Amy Mikhail, et al. Real-time, portable genome sequencing for ebola surveillance. *Nature*, 530(7589):228–232, 2016.
- [4] Alexis L Norris, Rachael E Workman, Yunfan Fan, James R Eshleman, i Winston Timp. Nanopore sequencing detects structural variants in cancer. *Cancer biology & therapy*, 17(3):246–253, 2016.
- [5] Miten Jain, Sergey Koren, Josh Quick, Arthur C Rand, Thomas A Sasani, John R Tyson, Andrew D Beggs, Alexander T Dilthey, Ian T Fiddes, Sunir Malla, et al. Nanopore sequencing and assembly of a human genome with ultra-long reads. *bioRxiv*, stranica 128835, 2017.
- [6] Heng Li. Aligning sequence reads, clone sequences and assembly contigs with bwa-mem. *arXiv preprint arXiv:1303.3997*, 2013.
- [7] Ivan Sović, Mile Šikić, Andreas Wilm, Shannon Nicole Fenlon, Swaine Chen, i Niranjana Nagarajan. Fast and sensitive mapping of nanopore sequencing reads with graphmap. *Nature communications*, 7, 2016.
- [8] Nicholas J Loman, Joshua Quick, i Jared T Simpson. A complete bacterial genome assembled de novo using only nanopore sequencing data. *Nature methods*, 12(8):733–735, 2015.

- [9] Jacob Schreiber i Kevin Karplus. Analysis of nanopore data using hidden markov models. *Bioinformatics*, stranica btv046, 2015.
- [10] Tamas Szalay i Jene A Golovchenko. De novo sequencing and variant calling with nanopores using poreseq. *Nature biotechnology*, 33(10):1087–1091, 2015.
- [11] Winston Timp, Jeffrey Comer, i Aleksei Aksimentiev. Dna base-calling from a nanopore using a viterbi algorithm. *Biophysical journal*, 102(10):L37–L39, 2012.
- [12] Matei David, Lewis Jonathan Dursi, Delia Yao, Paul C Boutros, i Jared T Simpson. Nanocall: an open source basecaller for oxford nanopore sequencing data. *Bioinformatics*, stranica btw569, 2016.
- [13] V. Boža, B. Brejová, i T. Vinař. DeepNano: Deep Recurrent Neural Networks for Base Calling in MinION Nanopore Reads. *ArXiv e-prints*, Ožujak 2016.
- [14] Y. LeCun, L. Bottou, Y. Bengio, i P. Haffner. Gradient-based learning applied to document recognition. *Proceedings of the IEEE*, 86(11):2278–2324, November 1998.
- [15] Kaiming He, Xiangyu Zhang, Shaoqing Ren, i Jian Sun. Deep residual learning for image recognition. U *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, stranice 770–778, 2016.
- [16] Pedro HP Savarese. Learning identity mappings with residual gates. *arXiv preprint arXiv:1611.01260*, 2016.
- [17] Alex Graves, Santiago Fernández, Faustino Gomez, i Jürgen Schmidhuber. Connectionist temporal classification: labelling unsegmented sequence data with recurrent neural networks. U *Proceedings of the 23rd international conference on Machine learning*, stranice 369–376. ACM, 2006.
- [18] Dario Amodei, Rishita Anubhai, Eric Battenberg, Carl Case, Jared Casper, Bryan Catanzaro, Jingdong Chen, Mike Chrzanowski, Adam Coates, Greg Diamos, Erich Elsen, Jesse Engel, Linxi Fan, Christopher Fougner, Tony Han, Awni Y. Hannun, Billy Jun, Patrick LeGresley, Libby Lin, Sharan Narang, Andrew Y. Ng, Sherjil Ozair, Ryan Prenger, Jonathan Raiman, Sanjeev Satheesh, David Seetapun, Shubho Sengupta, Yi Wang, Zhiqian Wang, Chong Wang, Bo Xiao, Dani Yogatama, Jun Zhan, i Zhenyao Zhu. Deep speech 2: End-to-end speech

recognition in english and mandarin. *CoRR*, abs/1512.02595, 2015. URL <http://arxiv.org/abs/1512.02595>.

- [19] Martín Abadi, Ashish Agarwal, Paul Barham, Eugene Brevdo, Zhifeng Chen, Craig Citro, Greg S. Corrado, Andy Davis, Jeffrey Dean, Matthieu Devin, Sanjay Ghemawat, Ian Goodfellow, Andrew Harp, Geoffrey Irving, Michael Isard, Yangqing Jia, Rafal Jozefowicz, Lukasz Kaiser, Manjunath Kudlur, Josh Levenberg, Dan Mané, Rajat Monga, Sherry Moore, Derek Murray, Chris Olah, Mike Schuster, Jonathon Shlens, Benoit Steiner, Ilya Sutskever, Kunal Talwar, Paul Tucker, Vincent Vanhoucke, Vijay Vasudevan, Fernanda Viégas, Oriol Vinyals, Pete Warden, Martin Wattenberg, Martin Wicke, Yuan Yu, i Xiaoqiang Zheng. TensorFlow: Large-scale machine learning on heterogeneous systems, 2015. URL <http://tensorflow.org/>. Software available from tensorflow.org.