nature research

Corresponding author(s): Jeff Nivala

Last updated by author(s): Apr 27, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All experiments were performed with unmodified R9.4.1 MinION flow cells (Oxford Nanopore Technologies) by diluting analyte solution into C17 buffer for a final concentration of 0.5M KCl and 25mM HEPES (pH 8), into the flow cell priming port. Flow cells were run on the MinION at a temperature of 30°C and a run voltage of -180mV with a 10khz sampling frequency and 15 second static flip frequency. Use of a modifiable MinKNOW script (available from ONT) enabled voltage flipping cycle parameters to be set as well as collection of raw current data across the entire run.
Data analysis	The analysis pipeline for a NanoporeTER sequencing run begins with extracting the segments of the raw nanopore signal that contain capture events. A capture is defined as a region where the signal current falls below 70% of the open pore current for a duration of at least one millisecond. The fractional current values (as compared to open pore current) computed from the segmentation process, as well as the start and end times of each capture, are saved in separate data files. This information is then passed through a general filter that separates putative NanoporeTER captures from noise captures based on features of the normalized raw current (mean, standard deviation, minimum, maximum, median) as well as the duration of the capture. Captures that pass this initial filter are then fed into a classifier and classified as a specific NTER barcode or a background/noise blockade. The metadata for the captures within each NTER class are subsequently fed to a quantifier which calculates the average time elapsed between those captures and converts this time to the predicted NTER concentration using a standard curve. An alternative method of quantification is to calculate the number of reads per class per active pore per minute (reads/pore-minute or RPMs). In addition to the NTER data sets, a background/noise class data set was also used in training the models to recognize data generated from non-NTER-specific pore blockages that made it through the filtering step. This data was collected from experiments in which only running buffer, LB media, or NTER-free E. coli cultures were loaded into the flow cell.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data and code can be found at https://github.com/uwmisl/NanoporeTERs.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed.
Data exclusions	Data were not excluded from analysis except as described in Data Analysis, which includes filtering steps to remove nanopore signal noise.
Replication	Experiments were conducted in triplicate to assess reproducibility.
Randomization	Not applicable to this study; does not include subjects that require allocation into experimental groups.
Blinding	Not applicable to this study; does not include subjects that require allocation into blinded experimental groups.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK FreeStyle 293-F (Thermo catalog #R79007)
Authentication	cell lines were not authenticated
Mycoplasma contamination	cell lines were not tested for mycoplasma
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*