

## Reporting Summary

Nature Portfolio 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 Portfolio policies, see our [Editorial Policies](#) 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

- |                                     |                                     |                                                                                                                                                                                                                                                            |
|-------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | 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 | N/A                                                                                                                                                                                                                                                                                                                                                                                         |
| Data analysis   | Custom perl scripts used in this study are available at <a href="https://bitbucket.org/srouxjgi/scripts_pcr_libs_assembly_optimization/src/master/">https://bitbucket.org/srouxjgi/scripts_pcr_libs_assembly_optimization/src/master/</a> and <a href="https://bitbucket.org/bolduc/docker-read2refmapper/src/master/">https://bitbucket.org/bolduc/docker-read2refmapper/src/master/</a> . |

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Custom perl scripts used in this study are available at [https://bitbucket.org/srouxjgi/scripts\\_pcr\\_libs\\_assembly\\_optimization/src/master/](https://bitbucket.org/srouxjgi/scripts_pcr_libs_assembly_optimization/src/master/) and <https://bitbucket.org/bolduc/docker-read2refmapper/src/master/>. Reads for the different metagenomes are available on <https://genome.jgi.doe.gov/portal/> and the SRA database (<https://www.ncbi.nlm.nih.gov/sra>). Sample accession numbers are listed in Supplementary Table 2 and GenBank accession numbers for complete ssDNA vOTUs are listed in Supplementary Table 3. The HMMs generated for ssDNA viruses are available on Zenodo: 10.5281/zenodo.8353597. The 9,623 ssDNA and dsDNA vOTUs

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## 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](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Ecological, evolutionary & environmental sciences study design

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

|                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|--------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Study description        | Stordalen Mire is a long-term study site in northern Sweden that has a mosaic of habitats that span a natural permafrost thaw gradient. For more than a decade, the IsoGenie project, now the EMERGE Biology Integration Institute, has leveraged the historical data to advance a systems-level understanding of permafrost thaw with matched amplicon, meta-omic, process measurements, and biogeochemical characterization of thaw stages. We expanded these sampling efforts by collecting 20 peatland samples spanning three stages of permafrost thaw (palsa – intact permafrost, bog – partially thawed permafrost, and fen – fully thawed permafrost) across two years and multiple depths for viromic analyses. To maximize viral particle recovery, we used protocols optimized for their resuspension and DNA extraction specifically for these soils. We then sought to maximize virus sequences captured by applying two quantitative library approaches that had been previously optimized for dsDNA viruses or both ssDNA and dsDNA viruses. The 40 resulting libraries were then sequenced to create viromes to quantitatively capture ssDNA and dsDNA viruses as a foundation to study their ecology in the Mire.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Research sample          | The samples are peat soil cores from the activate layer along a natural permafrost thaw gradient. Samples from 2016 were collected at 10–14 and 30–34 cm depths and in 2017 at 1–5, 10–14, 20–24, and 30–34 cm depths. These depths were chosen based on the amount of soil that was collected and what was feasible to collect (e.g., water-logged depths of cores could not always be recovered).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Sampling strategy        | Recovery of viruses from soils is very difficult, especially in these highly organic samples. Therefore, we attempted to sample each core and have comparable DNA recovery yields. This is why some depths have additional samples.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| Data collection          | Data were collected by Moira Hough, Sky Dominguez, and Nicole Raab with support from the Abisko Naturvetenskapliga Station. Soil cores from Stordalen Mire were collected in triplicate during the third week of July 2016 and 2017, immediately frozen with liquid nitrogen, shipped to Columbus, Ohio, and stored at –80°C until processed in October 2017. Viruses were resuspended from twenty samples from depths 10–14 and 30–34 cm in 2016 samples, and from depths 1–5, 10–14, 20–24, and 30–34 cm in 2017, using a previously optimized method. Briefly, 10 ml of a 1% potassium citrate buffer amended with 10% phosphate buffered saline and 150 mM magnesium sulfate was added to 10 ± 0.5 g peat samples in triplicate. The viruses were physically desorbed from soil colloids using vortexing for 1 min and manual shaking for 30 seconds then shaking the tubes at 400 rpm for 15 min at 4°C, and finally, the tubes were centrifuged for 20 min at 4700 ×g at 4°C to pellet debris, and the supernatant was transferred to a new tube. The resuspension steps above were repeated two more times and the supernatants were combined and filtered through a 0.2 µm polyethersulfone membrane filter. The viral DNA was extracted using DNeasy PowerSoil extraction kit, using a heat lysis step: 10 min at 70°C, vortex for 5 seconds and then continue incubating at 70°C for another 5 min. The extracted DNA was quantified using a Qubit-fluorometer and the 20 samples were split into duplicates making 40 samples. Half of the samples had libraries prepared using the Accel-NGS kit and the other half using the Nextera XT kit. The 40 virome libraries were sequenced with Illumina HiSeq-1TB, run type 2 x 151. The data collection process has been standardized for the DOE IsoGenie team (now the EMERGE Biology Integration Institute) and is outlined in Bolduc B, Hodgkins SB, Varner RK, Crill PM, McCalley CK, Chanton JP, Tyson GW, Riley WJ, Palace M, Duhaime MB, Hough MA, IsoGenie Project Coordinators, IsoGenie Project Team, A2A Project Team, Saleska SR, Sullivan MB, Rich VI. 2020. The IsoGenie database: an interdisciplinary data management solution for ecosystems biology and environmental research. PeerJ 8:e9467 <a href="https://doi.org/10.7717/peerj.9467">https://doi.org/10.7717/peerj.9467</a> . |
| Timing and spatial scale | Samples were collected the third week of July 2016 and 2017, immediately frozen with liquid nitrogen, shipped to Columbus, Ohio, and stored at –80°C until processed in October 2017.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

|                                   |                                                                                                                                                                                                                                                                                                       |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions                   | N/A                                                                                                                                                                                                                                                                                                   |
| Reproducibility                   | Samples were first bioinformatically processed during G. Trubl's DOE SCGSR fellowship at JGI in 2018. Samples were re-analyzed with newer scripts, and methods in 2021. The data was analyzed a third time during the review process to add in the newer ssDNA detection methods raised by reviewers. |
| Randomization                     | Peat soil samples were homogenized over 5 cm increments.                                                                                                                                                                                                                                              |
| Blinding                          | N/A                                                                                                                                                                                                                                                                                                   |
| Did the study involve field work? | <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No                                                                                                                                                                                                                                   |

## Field work, collection and transport

|                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Field conditions       | Data available in Supplementary table 1.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Location               | Data available in Supplementary table 1.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Access & import/export | Stordalen Mire is easily accessible with built-in infrastructure that offers stable navigation of the terrain with minimal environmental disturbance, eddy covariance flux measurements, and has the Abisko field station nearby, which is well equipped to support research (e.g., laboratories, offices, sleeping quarters). The combination of these qualities has attracted researchers globally and allowed unique long-term experiments to be conducted that provide historical data spanning more than a century. Our sample permit: USDA P330-19-00010 to Virginia Rich. |
| Disturbance            | Boardwalks are available to transverse the mire and sampling sites were selected adjacent to the boardwalks. Most environmental and geochemical data are collected via non-destructive methods. The number of samples collected and the depth are reviewed each year prior to sampling and includes a survey of the mire, previous data collected, and sampling performed by other teams.                                                                                                                                                                                        |

## 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                                  |
|-------------------------------------|--------------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                        |

### Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |