Title

Author Last and first names

Address

Abstract

Content of abstract.

Introduction

- BWA (Li and Durbin 2009)
- Minimap2 (Li 2018)
- SPAdes (Bankevich et al. 2012)
- Flye (Kolmogorov et al. 2019)
- FMLRC (Wang et al. 2018)
- GeSeq (Tillich et al. 2017)
- GetOrganelle (Jin et al. 2020)
- ptGAUL (Zhou et al. 2023)
- CLAW (Phillips et al. 2024)
- PMAT (Bi et al. 2024)
- Oatk (Zhou et al. 2024)
- TIPPo (Xian et al. 2025)
- Subsampling (Efron 1987)
- NextDenovo (Hu et al. 2024)

Materials and Methods

(Table **??**)

(Figure 1)

- `Flye` (Kolmogorov et al. 2019)
- `JellyFish` (Marçais and Kingsford 2011)
- `BLAST` (Altschul et al. 1997)
- `SeqKit` (Shen et al. 2016)
- `MAFFT` (Katoh and Standley 2013)

(Figure 1)

- `Bandage` (Wick et al. 2015)
- `Canu` (Koren et al. 2017)
- `NextDenovo` better than `Canu` (Wick and Holt 2021)

(Table **??**)

(Table S2)

Results

Comparison with other plastid assembly pipelines

(Table ??)

(Supporting Materials – Plastid genome assemblies using the six pipelines)

(Table S4)

(Table S5)

Subsampling-based plastome assemblies

(Table ??)

(Table ??)

(Table ??)

Three-stage of subsampling-based assembly

- (Table ??)

- (Table **??**)

- (Table **??**)

Discussion

Polap (Plant Organelle Long-read Assembly Pipeline v0.4.3.7), which includes the subsampling-based plastid genome assembly feature, is available under the GNU General Public License version 3.0 at http://github.com/goshng/polap.

Supplementary Material

Supplementary material, including 10 tables and three figures, is appended to the main text of this manuscript. A BASH script for executing the pipeline used to generate the results presented in the manuscript is also included.

Acknowledgements

We thank Jeffrey L. Thorne for improving the presentation of this work.

Author Contributions

S.C.C. developed the Polap pipeline and prepared the manuscript.

Conflict of Interest

The author declare no conflicts.

Data availability

`Polap` (Plant Organelle Long-read Assembly Pipeline v0.4.3.7) is available under the GNU General Public License version 3.0 at http://github.com/goshng/polap. The results presented in this manuscript are available at Figshare: https://figshare.com/s/ec1cb394870c7727a2d4.

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Tables

Table 1: Plastid genome assemblies for 23 plant species datasets using subsampled sequencing data with a maximum subsampling rate of 5% (Run Setting A). All datasets were downsampled to 10x genome coverage, and Stage 1 included 10 subsampling steps (N). Depending on the dataset, different maximum sampling rates (P) were used in Stage 1, and different replicate sizes (R) were applied in Stages 2 and 3. NA represents no assemblies in the subsampling-based method and no comparison available.

Species	P	N	R	Length (ptGAUL)	Length (Polap)	Percent identity
Anthoceros agrestis	100	10	5	160010	159938	99.93813
Eucalyptus pauciflora	100	10	5	159841	159945	99.93185

Table 2: Three stages of subsampling-based plastid genome assembly for the *Eucalyptus pauciflora* dataset with Run Setting A. The configuration includes an increasing subsample size up to a maximum subsampling rate of 5%, a step size of 10 in Stage 1, 5 replicates in Stages 2 and 3, and a maximum memory limit of 16 GB. Abbreviations are as follows: iteration in each Stage (I), subsampling rate (Rate) and read-coverage threshold (Alpha); assembly metrics including the number of segments in the assembly (N), the total length of these segments (L), and the number of circular genome paths detected (C); and the draft plastid genome assembly length (Length). Alpha at Stage 3 is the percent identity values between consecutive indices.

Stage	Index	Rate	Alpha	N	L	C	Memory	Time	Length
1	0	0.05	1.00	NA	NA	NA	8	1m	NA
1	1	0.16	0.25	1	118147	0	8	1m	NA
1	2	0.26	0.25	3	130443	4	8	1m	156251
1	3	0.37	0.25	8	253143	8	8	2m	NA
1	4	0.47	0.25	3	130676	4	9	2m	156567
1	5	0.58	0.25	4	173403	4	10	2m	156575
1	6	0.68	1.00	5	132763	4	12	2m	155609
1	7	0.79	1.75	3	130923	4	10	2m	156800
1	8	0.89	1.00	5	272624	4	16	2m	155777
2	0	0.47	0.25	6	265355	4	9	3m	158481
2	1	0.47	0.25	3	132336	4	9	2m	158528
2	2	0.47	0.25	4	180974	4	9	2m	158485
2	3	0.47	0.25	4	193091	4	9	2m	158448
2	4	0.47	0.25	5	247985	4	9	2m	158500
3	0	0.05	NA	NA	NA	NA	.05	0m	159338
3	1	0.29	99.57	NA	NA	NA	.12	.1m	159947
3	2	0.53	100.00	NA	NA	NA	.22	.2m	159944
3	3	0.76	100.00	NA	NA	NA	.31	.3m	159945
3	4	1.00	100.00	NA	NA	NA	.40	.4m	159945

Figures

Workflow of the subsampling-based plastid genome assembly. The genome assembly procedure is applied repeatedly in Stages 1 and 2.

Figure 1: Workflow of the subsampling-based plastid genome assembly. The genome assembly procedure is applied repeatedly in Stages 1 and 2.

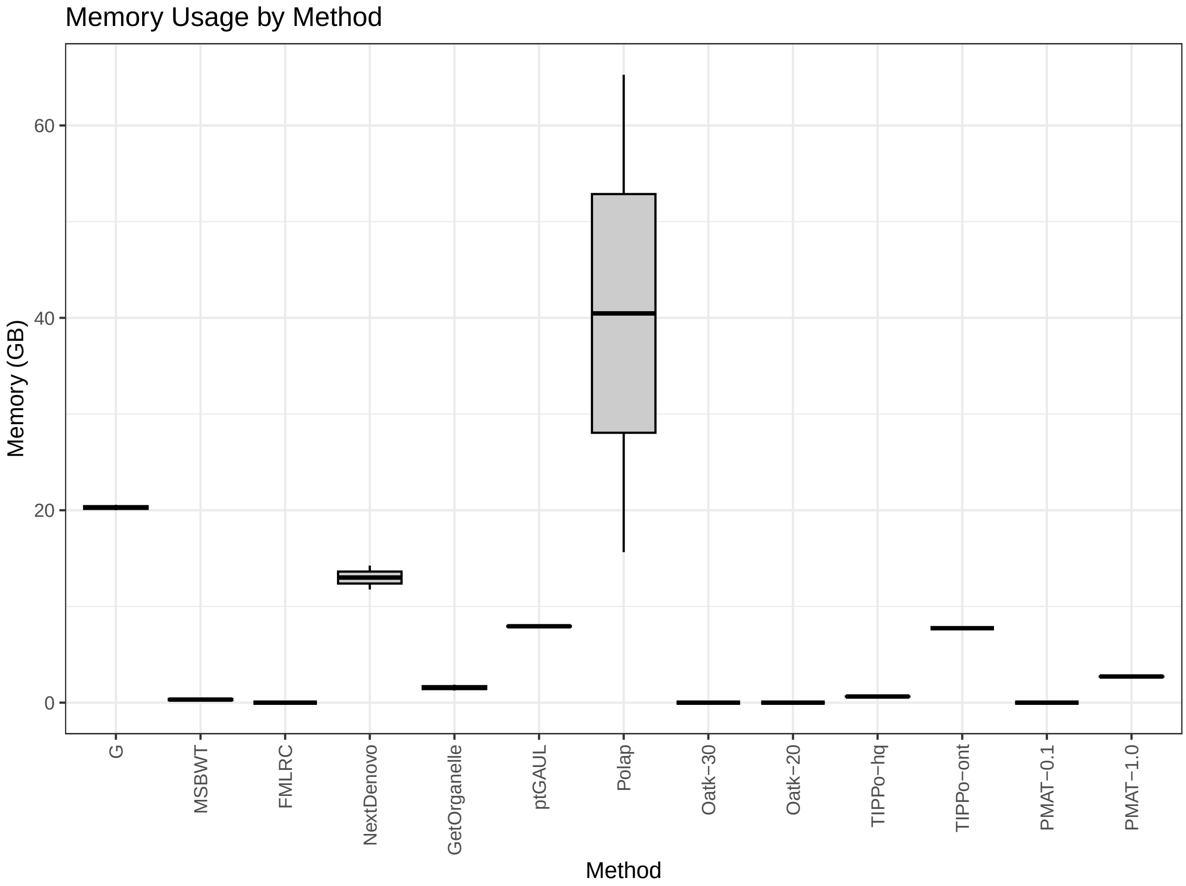


Figure 2: Workflow of the subsampling-based plastid genome assembly. The genome assembly procedure is applied repeatedly in Stages 1 and 2.

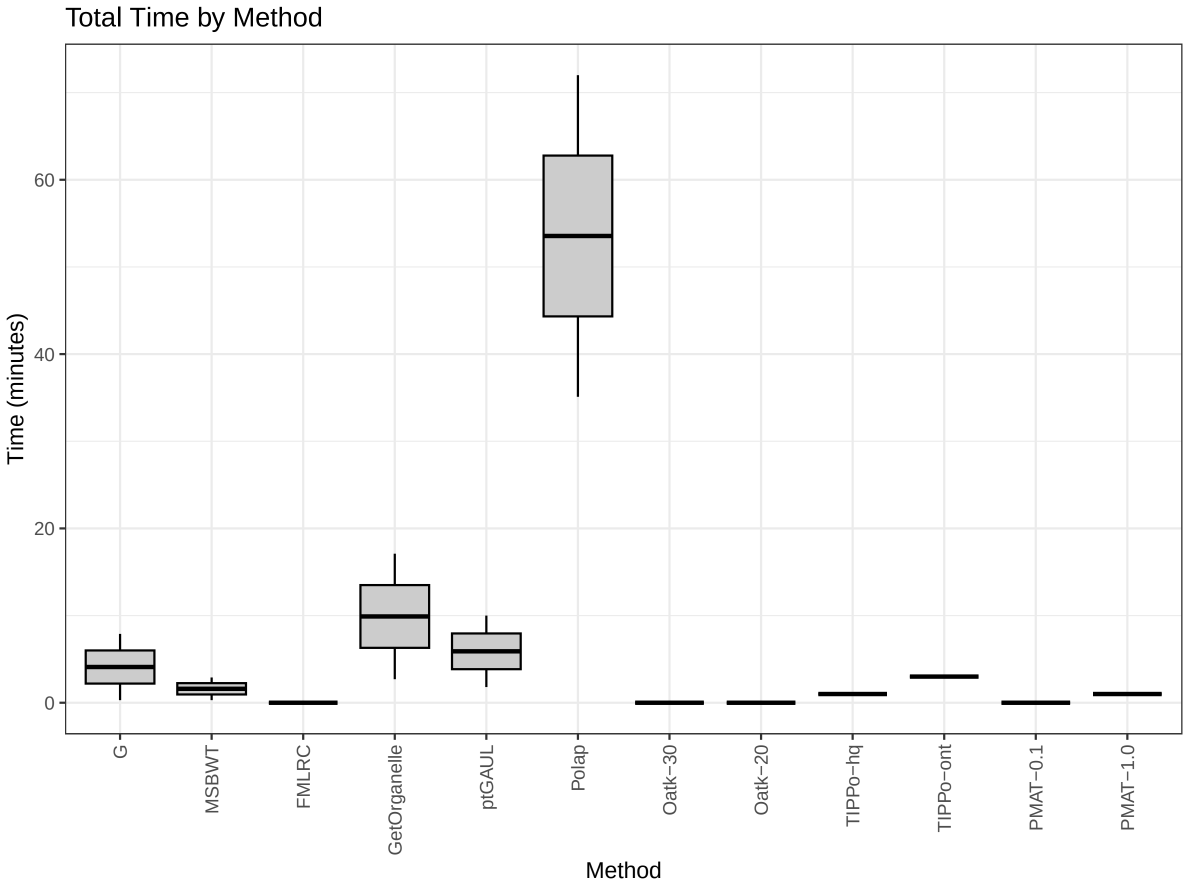


Figure 3: Workflow of the subsampling-based plastid genome assembly. The genome assembly procedure is applied repeatedly in Stages 1 and 2.

Supplementary Materials

Table S1: Sequencing data for the datasets, including species names and their corresponding taxonomic ranks studied.

Species	Order	Family	Long SRA	Long Size	Long Coverage	Short SRA	Short Size	Short Coverage
Anthoceros agrestis	Anthocerotales	Anthocerotaceae	l	191.5 Mbp	95.58	s	191.8 Mbp	95.70
Eucalyptus pauciflora	Myrtales	Myrtaceae	l	191.5 Mbp	95.58	s	191.8 Mbp	95.70

Table S2: Computer setup for the 23 datasets.

Species	CPU	Cores	Memory	Storage Type
Anthoceros agrestis	E5-2690 v4 @ 2.60GHz	56	251Gi	HDD
Eucalyptus pauciflora	E5-2690 v4 @ 2.60GHz	56	251Gi	HDD

Table S3: Replicate of plastid genome assemblies for 23 plant species datasets using subsampled sequencing data with a maximum subsampling rate of 5% (Run Setting A). All datasets were downsampled to 10x genome coverage, and Stage 1 included 10 subsampling steps (N). Depending on the dataset, different maximum sampling rates (P) were used in Stage 1, and different replicate sizes (R) were applied in Stages 2 and 3.

Species	P	N	R	Length (ptGAUL)	Length (Polap)	Percent identity
Anthoceros agrestis	100	10	5	160010	159936	99.93876
Eucalyptus pauciflora	100	10	5	159841	159936	99.93310

Table S4: Benchmark of `GetOrganelle`, `ptGAUL`, `PMAT`, `TIPPo`, `Oatk` and the method (Run Setting A) presented here in terms of data processing time. NA at the column of `NextDenovo` represents no error-corrected long-read results, resulting in no assemblies in the correction-then-assembly pipelines including `PMAT`, `TIPPo`, and `Oatk`. Abbreviations are as follows: `GetOrganelle` (GO), `ptGAUL` (pG), `NextDenovo` (ND), `PMAT` with `-fc 0.1` (P0.1), `PMAT` with `-fc 1.0` (P1.0), `TIPPo` with `-p onthq` (Thq), `TIPPo` with `-p ont` (Tont), `Oatk` with `-c 30` (O30), and `Oatk` with `-c 20` (O20).

Species	GO	ptG	MSBWT	FMLRC	ND	P0.1	P1.0	Thq	Tont	O30	O20	Polap
Anthoceros agrestis	17.1m	10.0m	2.9m	0m	4.5m	0m	1.0m	1.0m	3.0m	0m	0m	1.2h
Eucalyptus pauciflora	2.7m	1.8m	.3m	0m	.9m	0m	1.0m	1.0m	3.0m	0m	0m	35.1m

Table S5: Benchmark of `GetOrganelle`, `ptGAUL`, `PMAT`, `TIPPo`, `Oatk` and the method (Run Setting A) in terms of peak memory. NA at the column of `NextDenovo` represents no error-corrected long-read results, resulting in no assemblies in the correction-then-assembly pipelines including `PMAT`, `TIPPo`, and `Oatk`. Abbreviations are as follows: `GetOrganelle` (GO), `ptGAUL` (pG), `NextDenovo` (ND), `PMAT` with `-fc 0.1` (P0.1), `PMAT` with `-fc 1.0` (P1.0), `TIPPo` with `-p onthq` (Thq), `TIPPo` with `-p ont` (Tont), `Oatk` with `-c 30` (O30), and `Oatk` with `-c 20` (O20).

Species	GO	ptG	MSBWT	FMLRC	ND	P0.1	P1.0	Thq	Tont	O30	O20	Polap
Anthoceros agrestis	1.87	8.01	0.46	0.00	11.76	0.00	2.75	0.66	7.74	0.00	0.00	65.27
Eucalyptus pauciflora	1.26	7.87	0.18	0.00	14.25	0.00	2.69	0.62	7.74	0.00	0.00	15.65

Table S6: Plastid genome assemblies for 23 plant species datasets using subsampled sequencing data with a maximum subsampling rate of 10% (Run Setting B). All datasets were downsampled to 10x genome coverage, and Stage 1 included 10 subsampling steps (N). Depending on the dataset, different maximum sampling rates (P) were used in Stage 1, and different replicate sizes (R) were applied in Stages 2 and 3. NA represents no assemblies in the subsampling-based method and no comparison available.

Species	P	N	R	Length (ptGAUL)	Length (Polap)	Percent identity
Anthoceros agrestis	100	10	5	160010	159935	99.93689
Eucalyptus pauciflora	99	10	5	159841	159932	99.93560

Table S7: Plastid genome assemblies for 23 plant species datasets using subsampled sequencing data with a maximum subsampling rate of 1%. All datasets were downsampled to 10x genome coverage, and Stage 1 included 10 subsampling steps (N). Depending on the dataset, different maximum sampling rates (P) were used in Stage 1, and different replicate sizes (R) were applied in Stages 2 and 3. NA represents no assemblies in the subsampling-based method and no comparison available.

Species	P	N	R	Length (ptGAUL)	Length (Polap)	Percent identity
Anthoceros agrestis	100	10	5	160010	159935	99.94251
Eucalyptus pauciflora	100	10	5	159841	159935	99.93310

Table S8: Three stages of subsampling-based plastid genome assembly for the Eucalyptus pauciflora dataset with Run Setting B. The configuration includes an increasing subsample size up to a maximum subsampling rate of 10%, a step size of 10 in Stage 1, 5 replicates in Stages 2 and 3, and a maximum memory limit of 16 GB. Abbreviations are as follows: iteration in each Stage (I), subsampling rate (Rate) and read-coverage threshold (Alpha); assembly metrics including the number of segments in the assembly (N), the total length of these segments (L), and the number of circular genome paths detected (C); and the draft plastid genome assembly length (Length). Alpha at Stage 3 is the percent identity values between consecutive indices.

Stage	Index	Rate	Alpha	N	L	C	Memory	Time	Length
1	0	0.05	1.00	NA	NA	NA	8	1m	NA
1	1	0.16	0.25	NA	NA	NA	8	1m	NA
1	2	0.26	0.25	1	153400	2	8	1m	153400
1	3	0.36	0.25	3	129973	4	8	1m	155583
1	4	0.47	0.25	3	130068	4	9	1m	156038
1	5	0.57	0.25	3	130536	4	9	2m	156243
1	6	0.68	0.25	3	130917	4	12	2m	156930
1	7	0.78	0.25	5	170704	4	12	2m	156437
1	8	0.89	1.00	4	208162	4	13	3m	156324
1	9	0.99	1.75	3	129602	4	14	2m	155122
2	0	0.57	0.25	4	159399	4	9	2m	158516
2	1	0.57	0.25	3	132295	4	9	2m	158472
2	2	0.57	0.25	2	197574	2	9	2m	158505
2	3	0.57	0.25	4	214682	4	9	2m	158429
2	4	0.57	0.25	3	132256	4	9	2m	158422
3	0	0.05	NA	NA	NA	NA	.08	0m	159223
3	1	0.29	99.48	NA	NA	NA	.12	.1m	159932
3	2	0.52	99.99	NA	NA	NA	.21	.2m	159932
3	3	0.76	100.00	NA	NA	NA	.31	.3m	159932
3	4	0.99	100.00	NA	NA	NA	.40	.4m	159934

Table S9: Three stages of subsampling-based plastid genome assembly for the Eucalyptus pauciflora dataset with Run Setting C. The configuration includes an increasing subsample size up to a maximum subsampling rate of 10%, a step size of 50 in Stage 1, 5 replicates in Stages 2 and 3, and a maximum memory limit of 16 GB. Abbreviations are as follows: iteration in each Stage (I), subsampling rate (Rate) and read-coverage threshold (Alpha); assembly metrics including the number of segments in the assembly (N), the total length of these segments (L), and the number of circular genome paths detected (C); and the draft plastid genome assembly length (Length). Alpha at Stage 3 is the percent identity values between consecutive indices.

Stage	Index	Rate	Alpha	N	L	C	Memory	Time	Length
1	0	0.05	1.00	NA	NA	NA	8	1m	NA
1	1	0.10	0.25	4	161519	0	8	1m	NA
1	2	0.15	0.25	10	261384	8	8	1m	NA
1	3	0.20	0.25	1	155145	2	8	1m	155145
1	4	0.25	0.25	3	129647	4	8	1m	155380
1	5	0.30	0.25	3	130705	4	8	1m	156598
1	6	0.35	0.25	4	156123	4	8	1m	156499
1	7	0.40	0.25	3	130637	4	8	1m	156583
1	8	0.45	0.25	3	130116	4	9	2m	155977
1	9	0.50	0.25	4	177242	4	9	1m	156759
1	10	0.55	0.25	6	221747	4	9	2m	155102
1	11	0.60	0.25	4	154713	4	9	2m	154635
1	12	0.65	0.25	4	166791	4	9	2m	155916
1	13	0.70	0.25	6	184437	8	10	2m	NA
1	14	0.75	0.25	4	274222	4	12	3m	156546
1	15	0.80	1.00	4	209428	4	11	3m	155866
1	16	0.85	1.75	3	131009	4	10	2m	156874
1	17	0.90	1.00	5	259272	4	14	2m	156459
1	18	0.95	1.75	3	129146	4	13	3m	153495
1	19	1.00	2.50	3	130928	4	10	2m	156854
2	0	0.35	0.25	3	132312	4	8	2m	158446
2	1	0.35	0.25	4	211664	4	8	2m	158546
2	2	0.35	0.25	3	132387	4	8	2m	158585
2	3	0.35	0.25	1	158527	2	8	2m	158527
2	4	0.35	0.25	3	132021	4	8	2m	158176
3	0	0.05	NA	NA	NA	NA	.05	0m	159377
3	1	0.29	99.58	NA	NA	NA	.12	.1m	159942
3	2	0.53	100.00	NA	NA	NA	.22	.2m	159938
3	3	0.76	100.00	NA	NA	NA	.31	.3m	159938
3	4	1.00	100.00	NA	NA	NA	.40	.4m	159939

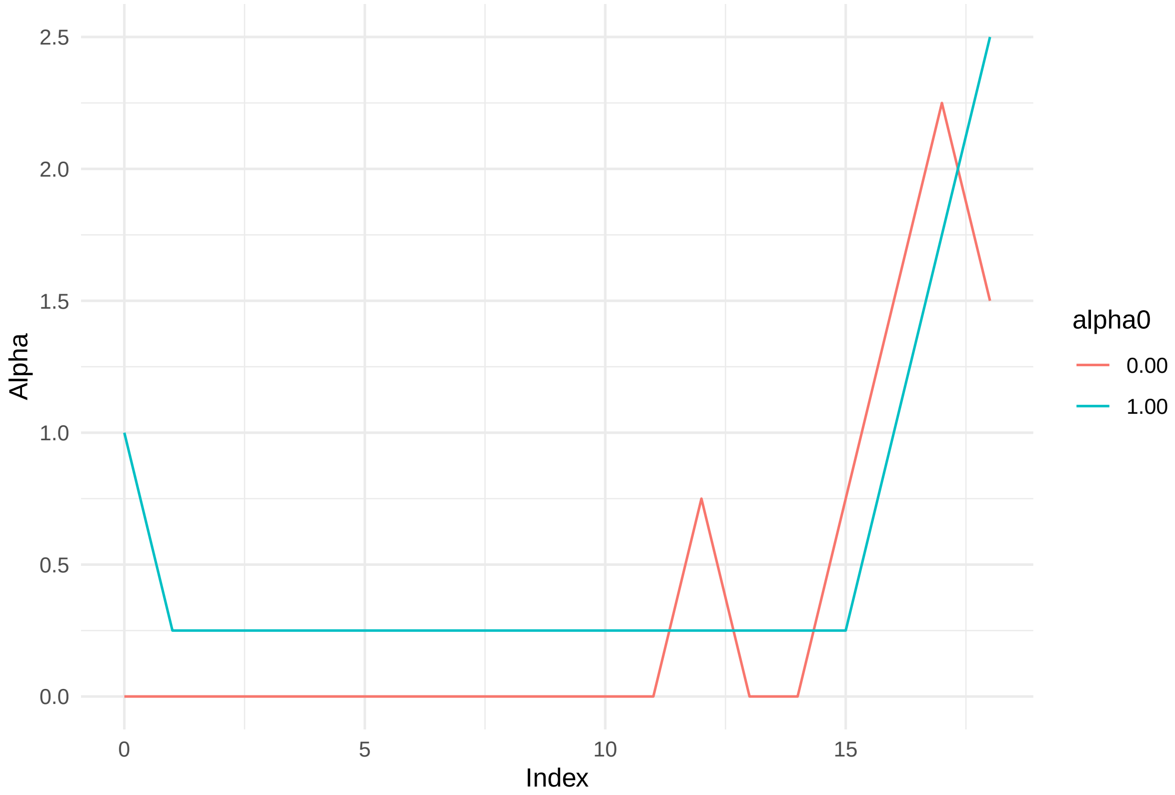


Figure S1: Line plot of read-coverage thresholds versus subsample size index in Stage 1 of the subsampling-based assemblies for *Eucalyptus pauciflora*.

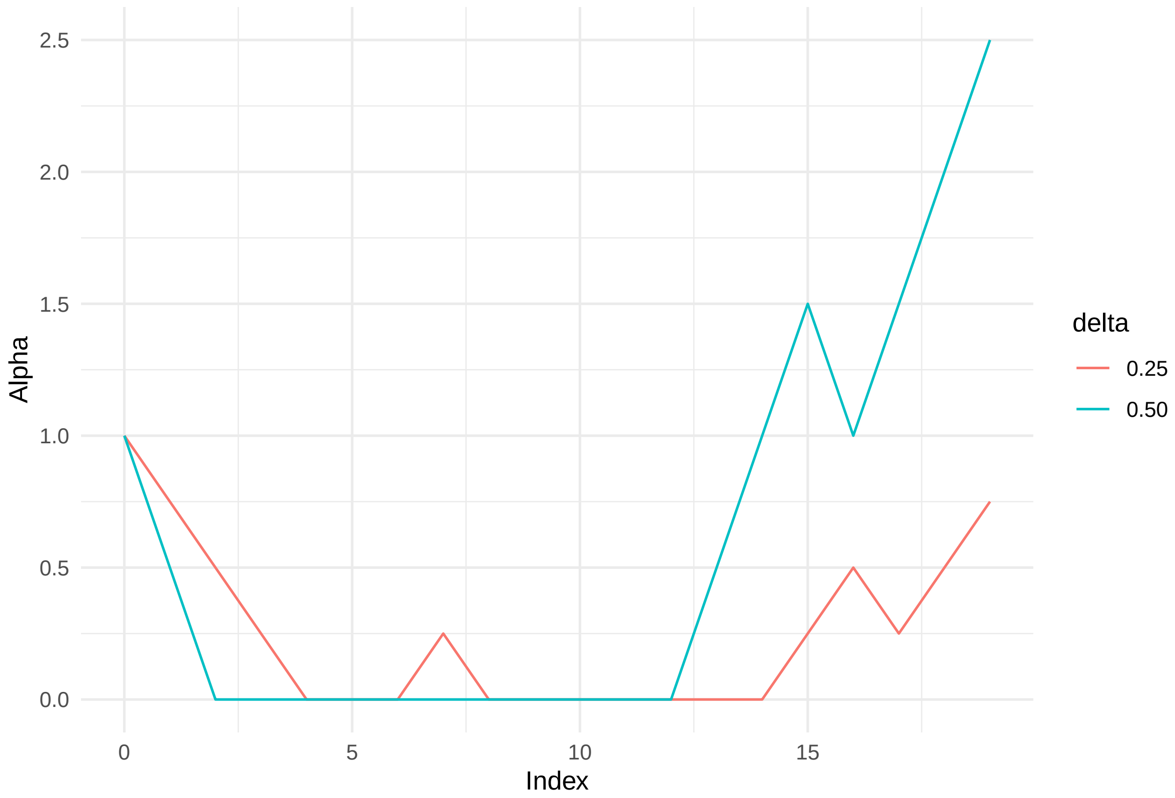


Figure S2: Line plot of increment size versus subsample size index in Stage 1 of the subsampling-based assemblies for *Eucalyptus pauciflora*.

Code

Polap (Plant Organelle Long-read Assembly Pipeline v0.4.3.7) is available at http://github.com/goshng/polap. A quick start guide of the subsampling-based plastome assembly is provided for use on a Linux system with an Internet connection. A detailed guide is also available, offering a step-by-step explanation of test of the procedures outlined in the quick start.

Requirements

- **Operating System**: Linux (not compatible with macOS or Windows)
- **Dependencies**: Requires Bash (>= 5.0) and **Miniconda**

Quick Start

To replicate the results presented in this manuscript on a Linux computer with `git` installed and an Internet connection, follow the steps below. Most steps complete in a relatively short time, except for the final step, which includes both data downloading and full analysis:

```
mkdir -p all/polap/cflye1
cd all/polap/cflye1
```

```
git clone https://github.com/goshng/polap.git
bash polap/src/polap-data-cflye -y install conda
```

Log out and back in to the terminal.

```
cd all/polap/cflye1
source ~/miniconda3/bin/activate
bash polap/src/polap-data-cflye setup conda
bash polap/src/polap-data-cflye -y install minimal
bash polap/src/polap-data-cflye setup polap
```

Log out and back in to the terminal.

```
conda activate polap
polap-data-cflye delete-polap-github
polap-data-cflye sample-csv polap-data-v2.csv test
polap-data-cflye -y download-test-data
# run time: about 1 hour
polap-data-cflye local-batch Taxon_genus t off
polap-data-cflye -y install-getorganelle
# polap-data-cflye -y download-pmat
# polap-data-cflye -y install-pmat
```

```
polap-data-cflye sample-csv polap-data-v2.csv all on
# edit the CSV file if necessary
polap-data-cflye local-batch each
```

Now, go to step 10 of the next subsection to create tables and figures.

Detailed Guide

1. Open a new terminal: Open a new terminal in a Linux computer, such as one with Ubuntu.

2. Install Miniconda: Download and install **Miniconda** using the instructions. The following is a script that works at the time of writing this manuscript. Otherwise, one could easily find a resource for the installation.

```
mkdir -p ~/miniconda3
wget https://repo.anaconda.com/miniconda/Miniconda3-latest-Linux-x86_64.sh \
  -O ~/miniconda3/miniconda.sh
bash ~/miniconda3/miniconda.sh -b -u -p ~/miniconda3
rm ~/miniconda3/miniconda.sh
```

After installing, close and reopen your terminal application.

3. Setup the conda channels: If you did not close and reopen a new terminal, please do so. Then, execute the followings to setup the conda channels for `polap`.

```
source ~/miniconda3/bin/activate
conda config --add channels bioconda
conda config --add channels conda-forge
conda config --set channel_priority strict
```

4. Install the Bioconda Polap package: You setup `polap` and `polap-fmlrc` conda environments using Polap conda package.

```
conda create -y --name polap polap=0.4.3.7.6
```

5. Installation of Flye for disjointig filtering: Note that `Flye` with disjointig filtering feature is a slightly modidified version of the original `Flye`. You activate the `polap` conda environment and setup `polap-fmlrc` environment

```
conda activate polap
conda install -y goshng::cflye
base_dir=$(dirname "$(command -v polap)") && \
  conda env create -f $base_dir/polap-conda-environment-fmlrc.yaml
```

6. Polap assemble run with a test dataset: This tests the basic execution of the `polap` commmand.

```
wget -q https://github.com/goshng/polap/archive/refs/tags/0.4.3.7.6.zip
unzip -o -q 0.4.3.7.6.zip
cd polap-0.4.3.7.6/test
polap assemble --test
```

7. Plastid genome assembly with *Eucalyptus pauciflora* dataset: Your assembled plastid genome sequence will be `o/ptdna.0.fa`.

```
polap x-ncbi-fetch-sra --sra SRR7153095
polap x-ncbi-fetch-sra --sra SRR7161123
polap disassemble -l SRR7153095.fastq \
  -a SRR7161123_1.fastq \
  -b SRR7161123_2.fastq
```

8. Check the accuracy of the plastid genome assembly: We use the Polap disassemble command with *Eucalyptus pauciflora* dataset and check its similarity with its known plastid genome sequence Your assembled plastid genome sequence will be `o/ptdna.ref.0.fa`. The text file named `o/0/mafft/pident.txt` has the percent identity between the assembled ptDNA and the knomn reference.

```
polap get-mtdna --plastid --species "Eucalyptus pauciflora"
cp o/00-bioproject/2-mtdna.fasta o/ptdna-reference.fa
polap disassemble \
  --disassemble-i 1 \
  --stages-include 3 \
  -l SRR7153095.fastq \
  -a SRR7161123_1.fastq \
  -b SRR7161123_2.fastq \
  --disassemble-align-reference \
  --disassemble-c o/ptdna-reference.fa


mkdir -p o/0/mafft
polap mafft-mtdna \
  -a o/ptdna-reference.fa \
  -b o/0/disassemble/2/pt.subsample-polishing.reference.aligned.1.fa \
  -o o/0/mafft
cat o/0/mafft/pident.txt
```

9. Batch script that creates the results in the manuscript:

```
polap-data-cflye -y install-getorganelle
# polap-data-cflye -y download-pmat
# polap-data-cflye -y install-pmat

polap-data-cflye example-data polap-data-v2.csv all on

polap-data-cflye local-batch each
```

10. Tables in the manuscript: Tables in Markdown format will be generated and saved in the `man` directory after executing the following command. You should download a precompiled binary version 0.8.1 of `Bandage` genome assembly graph visualization tool from the official Bandage GitHub.

```
polap-data-cflye -y install-bandage

# Install xelatex if necessary ...

# sudo apt-get install texlive texlive-latex-recommended texlive-xetex

# sudo apt-get install texlive-fonts-recommended texlive-fonts-extra texlive-lang-all

polap-data-cflye -y install-man

polap-data-cflye -y download-man

polap-batch-v2.sh

polap-data-cflye -y make-man
```

Supporting Material - Plastid genome assemblies using the six pipelines

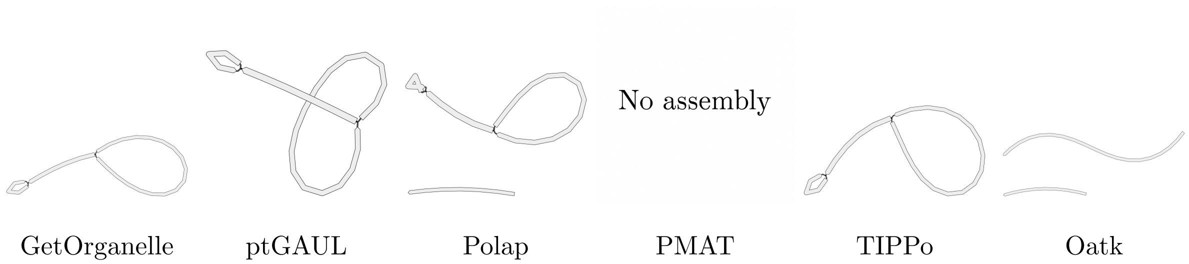
On the plastid genome assembly by subsampling low-quality Oxford Nanopore long-read data

Sang Chul Choi

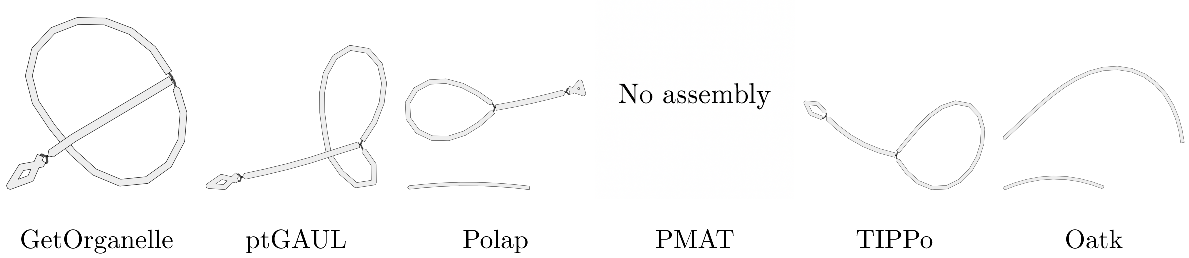
Figure description

We used `GetOrganelle`, `ptGAUL`, `PMAT`, `TIPPo`, and `Oatk` to assemble plastid genomes using low-quality Oxford Nanopore long-read data. All of the figures were created using Bandage software (Wick et al. 2015).

Anthoceros agrestis



Eucalyptus pauciflora


No assembly
GetOrganelle
ptGAUL
Polap
PMAT
TIPPo
Oatk
