Supplementary Material

Table S1. GenBank accession numbers for genomes used in the study.

Species name	Genebank accession number
Merremia hederacea	MZ240749
Ipomoea biflora	MZ240739
I. dumosa	PV762244
I. quamoclit	MK086054
I. maurandioides	MK086053
I. asarifolia	MK086048
I. goyazensis	MK086051
I. cavalcantei	MK086050
I. pes-caprae	MW479982
I. alba	ON209203
I. purpurea	MG973746
I. indica	LC729553
I. nil	MG973745
I. imperati	LC729552
I. aquatica	MW250301
I. cairica	LC729551
I. tiliifolia	LC729556
I. obscura	LC729554
I. carnea	MK086049
I. marabaensis	MK086052
I. splendor-sylvae	MH173259

I. trifida	MH173261
I. batatas	MW122507
I. lacunosa	MH173257
I. cordatotriloba	MH173254
I. ramosissima	MH173258
I. triloba	MG973750
I. cynanchifolia	MH173253

Table S2. Output produced by Organelle_PBA.

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################################################################
## OrganelleRef_PBA Starts (Sun Sep 29 13:50:52 CDT 2024) ##
################################################################


================================================================
 0) Checking arguments (Sun Sep 29 13:50:52 CDT 2024)
================================================================

        Input PacBio reads file: I_dumosaREADS.HiFi.fastq
        Input type: fastq
        Output directory: cloro_out
        Organelle reference fasta file: Ipomoea_nil_chloroplast_genome_reference.fasta
                Organelle reference size: 161897 bp
                Repeat block size coverage detection: 1000
                Coverage ratio to define repeatitive region: 1.6
        Completeness fraction: 1.0
        Breaks overlap: 0
        Highest position to set up origin: 1
        Starting-End margin to check circularity: 10
        Overlap length to check circularity: 100
        Minimum BlasR alignment percentage: 5
        Passing BlasR args:
                nproc=30
        Passing Sprai ec options:
                num_threads=30

        bedtools is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-
8.3.1/bedtools2-2.27.1-7tc5po5hsox2kqmscdkr3flv2z7v5jnw/bin/bedtools)
        blasr is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-
8.3.1/blasr-5.3.1-i54qwtbzuss3feukabppjvxtuty466pq/bin/blasr)
        blastn is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-
8.3.1/blast-plus-2.9.0-svcilgox6z3a6ceq3jgzd5iqlpag2u6y/bin/blastn)
        makeblastdb is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-
ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-
ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/makeblastdb)
        runCA is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-
8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/runCA)
        pacBioToCA is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-
ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-
ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/pacBioToCA)
        PBcR is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-
8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/PBcR)
        samtools is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-
8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/samtools)
        ezez_vx1.pl is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-
8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/ezez_vx1.pl)
```

ezez4qsub_vx1.pl is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/ezez4qsub_vx1.pl)
bfmt72s is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/bfmt72s)
m52bfmt7 is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/m52bfmt7)
myrealigner is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/myrealigner)
nss2v_v3 is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/nss2v_v3)
fa2fq.pl is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/fa2fq.pl)
check_circularity.pl is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/check_circularity.pl)
dfq2fq_v2.pl is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/miniconda3-4.7.12.1-ubp7tlghaseisza4pqufq6sbehwa4pog/envs/sprai/bin/dfq2fq_v2.pl)
SSPACE-LongRead.pl is available in the PATH (/lustre/apps/spack/opt/spack/linux-centos8-ivybridge/gcc-8.3.1/sspace-longread-master-wpb2m65vosckxvilhggcd766mbftwkoo/bin/SSPACE-LongRead.pl)

```
============================================================
 1) Running BlasR (Sun Sep 29 13:50:57 CDT 2024)
============================================================

    1.1- Converting Fastq to Fasta.
         Using Seqtk to convert Fastq to Fasta.
        Input PacBio read file contains 7700636 reads

    1.2- Running BlasR mapping.

    1.3- Processing the BlasR output.
         735510 reads (9487615041 bp) will be selected
         Estimated depth (reference based): 58603 X.

    1.4- Selecting reads from the fastq file
         Using Seqtk to select reads from fastq.

============================================================
 2) Running assembly (Sprai) (Sun Oct  6 16:48:24 CDT 2024)
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    2.1- Creating the ec.spec file for Sprai.

    2.2- Creating the pbasm.spec file for Sprai.

    2.3- Running Sprai.
```

2.3- Analyzing the results
Scaffold output file: cloro_out/02_1_result_20241006_164824/CA/9-terminator/asm_CA_01_20241016_100138.scf.fasta

Assembly Step 1 Results
---
* Total scaffolds: 4
* Total size (bp): 282610
* Total gaps size (Ns): 0

* Longest scaffold size (bp): 185474
* Longest scaffold gap size (Ns): 0
* Longest scaffold id: scf7180000000010

* Total assembly ref. fraction: 1.75
* Longest assembly ref. fraction: 1.15
* Different in size with ref. (bp): 23577

==========
3) Running scaffolding (SSPACE) (Wed Oct 16 10:39:01 CDT 2024)
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3.1- Skipping rescaffolding
Estimated assembly size fraction (1.15 > 1.0)

Assembly Step 2 Results
---
* Total scaffolds: 4
* Total size (bp): 282610
* Total gaps size (Ns): 0

* Longest scaffold size (bp): 185474
* Longest scaffold gap size (Ns): 0
* Longest scaffold id: scf7180000000010

* Total assembly ref. fraction: 1.75
* Longest assembly ref. fraction: 1.15
* Different in size with ref. (bp): 23577

3.2- Selecting the longest sequence (scf7180000000010)

==========
4) Evaluating repetitive regions (Wed Oct 16 10:39:01 CDT 2024)
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4.1- Searching an origin based in the reference
Origin found at 70657

4.2- Checking circularity
Circularity found: 1-24010 matches 161465-185474 (100.000 %)
Removing redundancy from circularity

Assembly Step 3 Results
---
* Total scaffolds: 1
* Total size (bp): 161464
* Total gaps size (Ns): 0

* Longest scaffold size (bp): 161464
* Longest scaffold gap size (Ns): 0
* Longest scaffold id: scf7180000000010_1_161464_NewOri_1_161464

* Total assembly ref. fraction: 1.00
* Longest assembly ref. fraction: 1.00
* Different in size with ref. (bp): 433

==========
5) Evaluating repetitive regions (Wed Oct 16 10:39:04 CDT 2024)
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TESTING cloro_out/01_1_BlasR_in.fasta should be FASTA
5.1- Running BlasR re-mapping.

5.2- Sorting bam file.

5.3- Calculating coverage.

Max. coverage: 166290
Max. coverage position: 141325
Min. coverage: 27315
Min. coverage position: 16805
Total coverage sum: 7265536748
Total length analyzed: 161464

Average coverage: 44997.8741267403
Lower limit coverage: 33566.1425395704
Upper limit coverage: 63613.7594873293

5.4- Getting the breaking points
3 breaking points have been detected

5.5.1- Breaking the assembly.
Getting the subsequence scf7180000000010_1_161464_NewOri_1_161464:1..88087

Getting the subsequence scf7180000000010_1_161464_NewOri_1_161464:88088..119118
Getting the subsequence scf7180000000010_1_161464_NewOri_1_161464:119119..131130
Getting the subsequence scf7180000000010_1_161464_NewOri_1_161464:131131..161464
cloro_out/04_2_assembly_nocirc.fasta has been broken in 4 subsequences.

5.5.2- Comparing fragments.

5.5.3- Selecting the no-redundant sequence set

5.5.4- Rescaffolding the contigs with SSPACE-Long.

No output file was found for SSPACE-OUT.
Using last assembly output.

Assembly Step 4 Results
---
* Total scaffolds: 1
* Total size (bp): 161464
* Total gaps size (Ns): 0

* Longest scaffold size (bp): 161464
* Longest scaffold gap size (Ns): 0
* Longest scaffold id: scf7180000000010_1_161464_NewOri_1_161464

* Total assembly ref. fraction: 1.00
* Longest assembly ref. fraction: 1.00
* Different in size with ref. (bp): 433

No clear improvement was produced from the repeat reassembly.
Selecting cloro_out/04_2_assembly_nocirc.fasta as final assembly.

FINAL ASSEMBLY FILE: cloro_out/Organelle_final_assembly.fasta

Final Assembly Results
---
* Total scaffolds: 1
* Total size (bp): 161464
* Total gaps size (Ns): 0

* Longest scaffold size (bp): 161464
* Longest scaffold gap size (Ns): 0
* Longest scaffold id: scf7180000000010_1_161464_NewOri_1_161464

* Total assembly ref. fraction: 1.00
* Longest assembly ref. fraction: 1.00
* Different in size with ref. (bp): 433

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#####################################################################
## OrganelleRef_PBA Ends (Tue Oct 29 20:11:00 CST 2024)   ##
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Table S3: *Ipomoea dumosa* plastome genes found (duplicated genes shown only once).

Name	Product
accD	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta
atpA	ATP synthase subunit alpha
atpB	ATP synthase subunit beta
atpE	ATP synthase subunit c
atpF	ATP synthase subunit b
atpH	ATP synthase subunit delta
atpI	ATP synthase subunit a
cemA	Potassium/proton antiporter
clpP	Chloroplastic ATP-dependent Clp protease proteolytic subunit 1
infA	Translation initiation factor IF-1
matK	Maturase K
ndhA	NAD(P)H-quinone oxidoreductase subunit 1
ndhB	NAD(P)H-quinone oxidoreductase subunit 2 A
ndhC	NAD(P)H-quinone oxidoreductase subunit 3
ndhD	NAD(P)H-quinone oxidoreductase chain 4
ndhE	NAD(P)H-quinone oxidoreductase subunit 4L
ndhF	NAD(P)H-quinone oxidoreductase subunit 5
ndhG	NAD(P)H-quinone oxidoreductase subunit 6
ndhH	NAD(P)H-quinone oxidoreductase subunit H
ndhI	NAD(P)H-quinone oxidoreductase subunit I
ndhJ	NAD(P)H-quinone oxidoreductase subunit J
ndhK	NAD(P)H-quinone oxidoreductase subunit K
petA	Cytochrome f protein
petB	Cytochrome b6
petD	Cytochrome b6-f complex subunit 4
petG	Cytochrome b6-f complex subunit 5
petL	Cytochrome b6-f complex subunit 6
petN	Cytochrome b6-f complex subunit 8
psaA	Photosystem I P700 chlorophyll a apoprotein A1
psaB	Photosystem I P700 chlorophyll a apoprotein A2
psaC	Photosystem I iron-sulfur center
psaI	Photosystem I reaction center subunit VIII
psaJ	Photosystem I reaction center subunit IX
psbA	Photosystem I P700 chlorophyll a apoprotein A2
psbB	Photosystem II CP47 reaction center protein
psbC	Photosystem II CP43 reaction center protein
psbD	Photosystem II D2 protein
psbE	PS II reaction center subunit V
psbF	PSII reaction center subunit VI
psbH	Photosystem II reaction center protein H
psbI	Photosystem II reaction center protein I
psbJ	Photosystem II reaction center protein J
psbK	Photosystem II reaction center protein K
psbL	Photosystem II reaction center protein L

psbM	Photosystem II reaction center protein M
psbN	Protein PsbN
psbT	Photosystem II reaction center protein T
psbZ	Photosystem II reaction center protein Z
rbcL	Ribulose bisphosphate carboxylase large chain
rpl2	Large ribosomal subunit protein uL 2m
rpl14	Large ribosomal subunit protein eL 14
rpl16	Large ribosomal subunit protein uL16c
rpl20	Large ribosomal subunit protein bL 20c
rpl22	Large ribosomal subunit protein uL 22c
rpl23	Large ribosomal subunit protein uL23cz/uL23cy
rpl33	Large ribosomal subunit protein bL33c
rpl36	Large ribosomal subunit protein bL36c
rpoA	DNA-directed RNA polymerase subunit alpha
rpoB	DNA-directed RNA polymerase subunit beta
rpoC1	DNA-directed RNA polymerase subunit beta
rpoC2	DNA-directed RNA polymerase subunit beta
rps2	Small ribosomal subunit protein uS2z
rps3	Small ribosomal subunit protein uS3c
rps4	Small ribosomal subunit protein uS4c
rps7	Small ribosomal subunit protein uS7cz/uS7cy
rps8	Small ribosomal subunit protein uS8c
rps11	Small ribosomal subunit protein uS11c
rps12	Small ribosomal subunit protein uS12cz/uS12cy
rps14	Small ribosomal subunit protein uS14c
rps15	Small ribosomal subunit protein uS15c
rps16	Small ribosomal subunit protein bS16c
rps18	Small ribosomal subunit protein bS18c
rps19	Small ribosomal subunit protein uS19m
rrn4.5	RNA polymerase I-specific transcription initiation factor RRN4.5
rrn5	RNA polymerase I-specific transcription initiation factor RRN5
rrn16	RNA polymerase I-specific transcription initiation factor RRN16
rrn23	RNA polymerase I-specific transcription initiation factor RRN23
trnA-UGC	Alanine transfer RNA molecule
trnC-GCA	Cysteine transfer RNA molecule
trnD-GUC	Aspartic acid transfer RNA molecule
trnE-UUC	Glutamic acid transfer RNA molecule
trnF-GAA	Phenylalanine transfer RNA molecule
trnG-GCC	Glycine transfer RNA molecule
trnG-UCC	Glycine transfer RNA molecule
trnH-GUG	Histidine transfer RNA molecule
trnI-CAU	Isoleucine transfer RNA molecule
trnI-GAU	Isoleucine transfer RNA molecule
trnK-UUU	Lysine transfer RNA molecule
trnL-CAA	Leucine transfer RNA molecule

trnL-UAA	Leucine transfer RNA molecule
trnL-UAG	Leucine transfer RNA molecule
trnM-CAU	Methionine transfer RNA molecule
trnN-GUU	Asparagine transfer RNA molecule
trnP-UGG	Proline transfer RNA molecule
trnQ-UUG	Leucine transfer RNA molecule
trnR-ACG	Arginine transfer RNA molecule
trnR-UCU	Arginine transfer RNA molecule
trnS-GCU	Serine transfer RNA molecule
trnS-GGA	Serine transfer RNA molecule
trnS-UGA	Serine transfer RNA molecule
trnT-GGU	Threonine transfer RNA molecule
trnT-UGU	Threonine transfer RNA molecule
trnV-GAC	Valine transfer RNA molecule
trnV-UAC	Valine transfer RNA molecule
trnW-CCA	Tryptophan transfer RNA molecule
trnY-GUA	Tyrosine transfer RNA molecule
ycf1	Translocon
ycf2	ATPase
ycf3	Photosystem I assembly protein
ycf4	Photosystem I assembly protein