	PACBIO®	Sequel [®] II and IIe Systems: Data Files (v10.1)
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Introduction	This document is for Customer IT or SMRT [®] Link Administrators, and describes the data files generated by Sequel II Systems and Sequel IIe Systems, and how to work with those files.
PacBio Read Files Format Description	PacBio uses unaligned BAM files as the native format to store read information.
-	hifi reads ham
	hifi_reads.bam files contains PacBio HiFi Reads (≥QV 20) and can be used directly as input for PacBio and third-party analysis tools designed to work with HiFi Reads. Additional filtering for higher read quality can be applied using the rq tag.
	The typical size for Sequel II and Sequel IIe Systems hifi_reads.bam files is <50 GB. If kinetic information is optionally included, files can be 5 times larger. More information about the PacBio BAM format can be found here.
	hifi roada faata
	IIII_reaus.iasiy
	hifi_reads.fastq files includes the same reads as hifi_reads.bam files, but contain less information about individual reads.hifi_reads.bam files can be used directly as input for PacBio and third-party analysis tools designed to work with HiFi Reads.
	The typical size for Sequel II and Sequel IIe Systems hifi reads.fastg

The typical size for Sequel II and Sequel Ile Systems hifi_reads.fastq file (gzipped) is <50 GB. More information about the PacBio FASTQ quality encoding can be found here.

reads.bam

reads.bam files contains one read per productive ZMW and consist of **both** HiFi Reads (≥QV 20) **and** non-HiFi reads (<QV 20). It is the native output file of the Sequel IIe System when running on-instrument CCS analysis. A reads.bam file is also generated when running CCS analysis in SMRT Link v10.0 or v10.1.

The typical size for Sequel II and Sequel IIe Systems reads.bam file is in the range of 50 GB. If kinetic information is optionally included, files can be 5 times larger.

subreads.bam

subreads.bam files are the native output data file of the Sequel System and the Sequel II Systems. subreads.bam files are also produced by the Sequel IIe System if users choose to skip on-instrument CCS analysis.

subreads.bam files contain the individual sequencing passes (subreads) from every productive ZMW. Subreads and HiFi Reads have different error

models, and subreads should **not** be used in HiFi Read applications or vice versa.

Notes:

- All PacBio read files are accompanied by a *.pbi index file, and a *.xml Data Set file.
- SMRT[®] Cell Data Sets transferred from Sequel II Systems also include additional files.
- The subreads.bam file size can range from 0.5 TB to 1.5 TB.

Data Flow From the Sequel IIe System to SMRT Link

Sequel lie Instrument Data Transfer/SivikT Link Server	Sint Link Gor
Generate Sequencing Data	Access using a web browser
<pre>Sequel lle System output files <your_specified_output_directory>/r64009_20200825_221039/1_A01/ m64009_200825_222052.csa_tepata_1.log m64009_200825_222052.ccs_reports.json m64009_200825_222052.ccs_reports.txt m64009_200825_222052.read.bam m64009_200825_222052.read.bam m64009_200825_222052.read.bam m64009_200825_222052.stas.wnl m64009_200825_222052.stas.ml m64009_200825_222052.stas.ml m64009_200825_22052.stas.ml</your_specified_output_directory></pre>	Automatic HiFi reads generation (Export Reads) hifi_reads.fastq.gz - FASTQ file containing HiFi Reads hifi_reads.fasta.gz - FASTA file containing HiFi Reads hifi_reads.bam - BAM file containing HiFi Reads

Sequel Ile System Output Files

The run directory output by the Sequel IIe System includes a subdirectory for each collection (SMRT Cell) associated with a sample well. In the above example figure, m64009_200825_222052 is the movie ID, including the instrument number (64009), date, and time. The collection subdirectory includes the following output files:

- baz2bam 1.log: Log file for post-primary analysis processing.
- ccs.log: Log file from CCS Analysis. This file is used internally for debugging and performance tracking by PacBio.
- ccs_reports.json, ccs_reports.txt: Contains processing metrics summarizing how many ZMWs generated HiFi Reads, and how many ZMWs failed CCS Reads generation. These files contain the same information and are used internally by PacBio Technical Support.
- consensusreadset.xml: This file is needed to import data into SMRT Link.
- sts.xml: Contains summary statistics about the collection and its
 post-processing.
- transferdone: Contains a list of files successfully transferred.
- zmw_metrics.json.gz: Contains processing information used to
 generate RunQC plots.

- reads.bam.pbi: Provides backwards-compatibility with the APIs enabled for accessing the cmp.h5 file.
- reads.bam: The Sequel IIe System outputs one reads.bam file per collection, containing one read per productive ZMW. This is the central read data file. The file includes:
 - HiFi Reads (QV 20 or higher)
 - Lower-quality but still polished consensus reads (QV 1 QV 20)
 - Unpolished consensus reads (RQ=-1)
 - 0- or 1-pass subreads unaltered (RQ=-1)

Note: The reads.bam contains HiFi Reads and non-HiFi reads.

The BAM format is a binary, compressed, record-oriented container format for raw or aligned sequence reads. BAM files produced by all Sequel Systems are fully compatible with the BAM specification. PacBio BAM files are unaligned reads. More information about the PacBio BAM format can be found here.

Note: If CCS Analysis is run on the Sequel IIe System, the subreads.bam, scraps.bam and scraps.bam.pbi files are no longer generated or available. If CCS Analysis is run in SMRT Link, Sequel IIe System instrument output includes the subreads.bam file, and optionally, the scraps.bam and scraps.bam.pbi files.

Reads.bam Versus HiFi Reads

Once the reads.bam file is transferred from the Sequel IIe System, SMRT Link **automatically** generates files containing only HiFi Reads, using the **Export Reads** application in SMRT[®] Analysis. The following HiFi data files are **always** generated by default:

- hifi_reads.fastq.gz FASTQ file containing HiFi Reads.
- hifi reads.fasta.gz FASTA file containing HiFi Reads.
- hifi reads.bam BAM file containing HiFi Reads.

If **not** using SMRT Link for subsequent analysis, please use these three files as input with third-party analysis tools.

Input Data File Requirements for SMRT Link Analysis Applications and Third-Party Tools All SMRT Link GUI applications (as well as pbcromwell if working on the command line) accept as input the consensusreadsset.xml file that points to the reads.bam file. The analysis pipeline then filters the reads.bam file to use **only** HiFi Reads, with the exception of Iso-Seq analysis, which takes all reads at or above Q10. (See "Input for the Iso-Seq Analysis Application" on page 7 for more information about Iso-seq analysis with HiFi Reads input.) As a result, **no** manual Data Set filtering needs to be applied in SMRT Link.

This is conceptually different in SMRT Link v10.0 and later versions from previous versions. In previous SMRT Link versions, a filter for minimum QV and minimum number of passes was applied at the CCS-generation

step. These two parameters are **not** required as input in SMRT Link v10.1 and they are available as advanced parameters in SMRT Analysis.

For analysis of Data Sets with tools outside of SMRT Link, PacBio strongly recommends that you use the hifi_reads.bam or hifi reads.fastq files - not the reads.bam file.

Finding the Sequel Ile System HiFi Files

Using the SMRT Link GUI

To access the HiFi Read files generated from the reads.bam file:

- 1. Select Data Management and click on the desired Data Set Name.
- 2. On the **Dataset Details** page, click **Analyses > Completed Analyses**.
- 3. Click the name of a completed analysis to access the results of the **Export Reads** Analysis Application:

						E Copy E Ana	yze Export @ Delet
Dataset Overview	Completed Analys	es					
CCS Analysis Report	Name		State	Id	Date Created	Created By	Analysis Application
Loading Report	Auto Analyses of	20201202_64012_HG2_OICCS_Kinetics	SUCCESSFUL	32566	2020-12-01, 06:13:44 PM	jziegle	
Control Report	Export Reads of	HG002_Otter_OICCS_Kinetics_801_50pM	SUCCESSFUL	32570	2020-12-01, 06:14:45 PM	jziegle	Export Reads
Adapter Report							
Raw Data Report							

4. Click Data > File Downloads and locate and download the hifi_reads.bam, hifi_reads.fasta.gz, and hifi_reads.fastq.gz files.

SMRT Analysis -			
xport Reads of HG002_Otter_OICCS_Kinetics_B01	_50pM		
Analysis Overview	File Downloads		
Philipsis overview	Edit Output File Name Prefix Example:analysis-HG002-32570		
◆Data			
File Downloads	File	Size	Туре
	m64012_201204_044926.hifi_reads.bam	119	Consens
SMRT Link Log	m64012_201204_044926.hifi_reads.fasta.gz	11 GB	Fasta
	m64012_201204_044926.hifi_reads.fastq.gz	29 GB	Fastq
	Analysis Log	16 KB	log

Using the File System

- 1. Select SMRT Analysis, then click an analysis.
- 2. Select Analysis Overview > Status.
- 3. The **Status** section displays the file path, which points to the location of the output data files (including HiFi Reads) for the **Export Reads** analysis within the file system directory. The files hifi_reads.bam, hifi_reads.fasta.gz, and hifi_reads.fastq.gz can be found within this output directory.

Manually Generating HiFi Reads Files from the Sequel Ile System reads.bam File If the **Export Reads** analysis application did **not** run automatically, you can run this application manually in SMRT Link. First go to **Data Management > Dataset Details** and click **Analyses > Completed Analyses** to determine if any Export Reads analysis application job has already been completed for your Data Set.

If **no** completed **Export Reads** analysis results are listed, follow the steps below to run the **Export Reads** analysis application for the Data Set of interest:

- 1. Access SMRT Link using the Chrome web browser.
- 2. Select SMRT Analysis.
- 3. Click + Create New Analysis.
- 4. Enter a name for the analysis.
- 5. Select the type of data to use for the analysis: **HiFi Reads**.
- 6. In the Data Sets table select the Data Set to export to HiFi.
- 7. Click Next.
- 8. Select the **Export Reads** analysis application from the dropdown list.
- Fill in the required parameters: Output FASTA File (ON or OFF), Output BAM file (ON on OFF), Min CCS Predicted Accuracy (Default QV 20.)
- 10. Click Start.

Extracting HiFi Reads from the Sequel Ile System reads.bam File Using the Command Line The **Export Reads** analysis application in the SMRT Link GUI has its command line-version counterpart in our developmental repository at pbbioconda/GitHub: extracthifi.

The extracthifi tool extracts HiFi Reads (≥ Q20) from the full CCS reads.bam output. For more information, see https://github.com/ PacificBiosciences/extracthifi/.

To use <code>extracthifi</code>, follow the installation instructions in the <code>pbbioconda</code> GitHub home page: https://github.com/PacificBiosciences/pbbioconda.

Usage:

extracthifi [options] <input.bam> <output.bam> input.bam STR Input CCS BAM. output.bam STR Ouput HiFi BAM.

Options:

-h,--help Show this help and exit. --version Show application version and exit.

Accessing Sequel Ile Raw Output (reads.bam) Files The SMRT Link GUI creates a report by default anytime it performs any action over data. A report is created for data transfer from the Sequel IIe instrument to SMRT Link, and another report is created once the **Export Reads** analysis application is run to generate HiFi-only data files as previously described.

To download the reads.bam file from SMRT Link, use the **Export Dataset** function:

- 1. Access SMRT Link using the Chrome web browser.
- 2. Select Run QC.
- 3. Click the name of the desired run in the table; the **Run Data** page displays.

		400)_)01	ECOII_3	BokBsut)										COMPLETE	🛓 Export
•0	verview															
	Run Start: 2018-07-27, 07:46:39	PM				Run Co 2018-07	mplete: -28, 07:3	0:06 PM						Transfer Complete: 2018-07-29, 06:57:22 AM		
	Run Id: r64003_20180728_02	1607			8	Descrip Ecoli	tion:							Instrument: Sequel		
	Instrument SN: 64003					Instrum 6.1.0.SN	APSHOT	trol SW 1 41861	Version:					Instrument Chemistry Bundle: 6.0.0.SNAPSHOT41529		
	Primary SW Version 6.1.0.SNAPSHOT4186	1														
> Co	onsumables															
xpan	d All															
-	Sample Informati >	Run Settings >	I.	l	l	Produc	tivity (%)		Reads				Control >	1	Template >	
									Polymer	rase Re	Longes	Subre			1	
														2 2 Y C C C C C C		

4. Click a sample name; the **Dataset Details** page displays.

10pMchip-Cell2		🕒 Copy 🛛 🕒 Analyze	Export 🗊 Delete
Status			
Data Set	SMS_Spider_SP2.1_30kBsub_10pMchip+Cell2		
Data Set ID	34		
Data Set UUID	45741479-4808-4f8b-a277-8f030b3b82cf		
Well Sample Name	SMS_Spider_SP2.1_30kBsub_10pMchip		
Biological Sample Name	SMS_Spider_SP2.1_30kBsub_10pMchip		
5	topMchip-Cell2 Status Data Set UID Data Set UID Well Sample Name Biological Sample Name	Status SMS_Spider_SP2.1_30K8sub_10pMchip-Cell2 Data Set ID 34 Data Set UD 45751479-4808-4808-a277-8803063b82cf Well Sample Name SMS_Spider_SP2.1_3088bub_10pMchip Biological Sample Name SMS_Spider_SP2.1_3088bub_10pMchip	topMchip-Cell2 Status Sta

- 5. Click **Export** to export the data.
- 6. Another way to export data is by using **Data Management > Export Data > Export Selected**.

Export Data					
		X Cancel	🛃 Exp	ort Selected	
Data Type 🥝 HiFi Reads	\$				
Data Type HiFi Reads	*				

Downloading the reads.bam File using the Command Line

 From the Data Management > Dataset Details page, go to the Overview > Status page and look for the Data Path line:

		eguareo (Lab Teo
ata Management / Dataset Details IG002_Otter_OICCS_Kinetics_B01_50pM-Cell2	(CCS)	B Copy B Analyze. (2 Export © Delete
	Status	
✓Dataset Overview	Data Set	HG002_Otter_OICCS_Kinetics_B01_50pM-Cell2 (CCS)
Status	Data Set ID	146591
	Data Set UUID	35310d9b-5118-4b00-a17/-d56dd781a956
Thumbnails	Well Sample Name	HG002_Otter_OICCS_Kinetics_B01_50pM
	Biological Sample Name	HG002
Display All	Description	ccs dataset converted
	Number of Records	4,434,088
CCS Analysis Report	Total Length	83,083,562,169
	Status	SUCCESSFUL
>Loading Report	Date Created	2020-12-05, 07:54:40 PM
	Date Imported	2020-12-05, 08:34:44 PM
> Control Report	Date Updated	2020-12-05, 08:39:15 PM
	Job ID	32676
>Adapter Report	Data Path	[bb]collection13226/2280316/F64012_20201202_222953/2_801m64012_201204_044426.consensusreadset.xml
	Run name	20201202_64012_HG2_OICCS_Kinetics
>Raw Data Report	Cell Index	1
	Cell Io	54017
Manhurar	Instrument Name	04072
C ALLANY NEW	Well Name	DVI.

Using the **Data Path** line, find the path to the reads.bam file in the set of transferred files from the instrument. For the example data set shown above, the relevant directory path is:

\$ ls /pbi/collections/328/3280316/r64012_20201202_222953/2_B01/

```
m64012_201204_044926.baz2bam_1.log
m64012_201204_044926.ccs.log
m64012_201204_044926.ccs_reports.json
m64012_201204_044926.ccs_reports.txt
m64012_201204_044926.consensusreadset.xml
m64012_201204_044926.reads.bam
m64012_201204_044926.reads.bam.pbi
m64012_201204_044926.sts.xml
m64012_201204_044926.transferdone
m64012_201204_044926.transferdone
m64012_201204_044926.zmw_metrics.json.gz
tmp-file-4d910bcf-760a-4be3-86b0-128714ee409c.txt
```

```
Input for the
Iso-Seq
Analysis
Application
```

The Iso-Seq Analysis application does use some non-HiFi Reads as input, and will take reads at or above QV 10 as this can increase the sensitivity of the analysis. This QV 10 threshold is used as the default setting for Iso-Seq analyses and can be adjusted in the **Advanced Parameters** dialog of the Iso-Seq Analysis application. To access the dialog:

- 1. Access SMRT Link using the Chrome web browser.
- 2. Select **SMRT Analysis**.
- 3. Click + Create New Analysis.
- 4. Enter a name for the analysis.
- 5. Select the type of data to use for the analysis: HiFi Reads.
- 6. In the Data Sets table, select one or more sets of data to be analyzed.
- 7. Click Next.
- 8. Select the **Iso-Seq Analysis** application from the dropdown list.

9. Fill in the relevant parameters, then click Advanced Parameters.

Min. CCS Predicted Accuracy (Phred	Require and Trim Poly(A) Tail 😢	Minimum Mapped Length (bp) 😢
Scale) 🔕	ON OFF	50
10		
Minimum Gap-Compressed Identity (%) 🔇	Minimum Mapped Coverage (%) 🔇	Maximum Fuzzy Junction Difference (bp)
95	99	0
		5
Filters to Add to the Data Set 🚯	Advanced pbmm2 Options ()	Compute Settings

To obtain/share a BAM file that contains the **same** content as the input for the Iso-Seq Analysis application, we recommend using the **Export Reads** application and manually adjusting the **Min. CCS Predicted Accuracy** (**Phred Scale**) parameter to 10 as shown below:

	PACBIO SMRT Analysis -	
	SMRT Analysis / Create New Analysis	
	1. Select Data 2. Select Analysis	
	Analysis Application Required	
	Export Reads	\$
	Output FASTA File 🕲	
	ON OFF	
	Output BAM File 🔕	
	O ON OFF	
	Min. CCS Predicted Accuracy (Phred Scale) 🔮	
	10	
	Advanced Parameters	
Data Flow From the Sequel II Systems Without On- Instrument CCS to SMRT Link	Sequel II Instrument Data Transfer/SMRT Link Server Generate Sequencing Data Sequel II System output files (your_specifiee_output_directorys/r54008_20160116_003347/1_A01 (= m54008_160116_003634.scraps.bam (= m54008_160116_003634.scraps.bam (= m54008_160116_003634.subreads.bam (= m54008_160116_003634.subreads.bam (= m54008_160116_003634.subreads.bam (= m54008_160116_003634.subreads.txml (= m54008_160116_003634.sts.xml) (= m54008_160116_003634.sts.stml) (= m54008_160116_003634.sdapters.fasta	SMRT Tink SMRT Link GUI Access using a web browser Diffi reads.fastq.gz - FASTQ file containing HiFi Reads hifi_reads.fastq.gz - FASTQ file containing HiFi Reads hifi_reads.ham - BAM file containing HiFi Reads hifi_reads.bam BAM file containing HiFi and non-HiFi Reads

Generating HiFi Reads in SMRT Link for the Sequel II System

Starting a CCS Analysis in the SMRT Link GUI to Generate HiFi Reads

When running the CCS application in SMRT Link v10.1, whether automatically set up from Run Design or manually set up from the SMRT Analysis module, the reports and output files are the **same** as those produced by Sequel IIe instruments and on-instrument CCS analysis.

- Generate HiFi Reads automatically by creating a CCS Pre Analysis Job in Run Design. (To learn more, see the SMRT Link User Guide Run Design section.) To create a CCS Pre Analysis job for a new Run Design, select Run Design > Create a New Design. The Run Design UI displays two options related to generation of HiFi Reads for Sequel II System users:
 - In SMRT Link: HiFi Reads are automatically generated after transfer to the compute cluster where SMRT Link is installed.
 - Do Not Generate: HiFi Reads are not generated automatically for this run. Only subread data are transferred to the local compute cluster where SMRT Link is installed. HiFi Reads may be manually generated later at the user's election.

Run Design / Create New New Run Design		Cancel 🗊 Delete View Summary Auto Analysi
Run Information	Sample Information	
System Type	DNA Control Complex	Sequel II DNA Internal Control 1.0
SEQUEL O SEQUEL II SEQUEL IIe	Insert Size (bp) Required	
Run Name	Recommended Concentration on Plate (pM)	30-70 pM
Run 09.09.2021 11:11	On-Plate Loading Concentration (pM) Required	0
Run Comments	Movie Time per SMRT Cell (hours)	30
	// Use Pre-Extension	YES O NO
Experiment Name	Generate HiFi Reads	O NINSTRUMENT O IN SMRT LINK O DO NOT GENERATE

Generating HiFi Reads Manually from the SMRT Analysis Module in SMRT Link

- 1. Access SMRT Link using the Chrome web browser.
- 2. Select SMRT Analysis.
- 3. Click + Create New Analysis.
- 4. Enter a name for the analysis.
- 5. Select the type of data to use: **Continuous Long Reads**. The Data Sets table displays the corresponding Data Sets available for analysis.
- 6. In the Data Sets table, select one or more sets of data to be analyzed. (For multiple selection see the SMRT Link User Guide for instructions.)
- 7. Click Next.
- 8. Select the **Circular Consensus Sequencing (CCS)** application from the dropdown list.
- 9. Click Advanced Parameters and verify that Process All Reads is set to ON. This option creates a reads.bam file containing HiFi Reads (≥QV 20) and non-HiFi reads (<QV 20) as produced by on-instrument CCS Analysis on the Sequel IIe System.

Minimum Number of Passes (Deprecated)	Minimum Predicted Accuracy (Deprecated)	Minimum CCS Read Length 🔞		
0	0	10		
0	0			
Maximum CCS Read Length 🔞	Advanced CCS Options 🚯	Generate a Consensus for Each Strand 🔞		
50000				
Process All Reads 🚷	Include Kinetics information with CCS	Compute Settings 🚷		
Process All Reads 🚷	Include Kinetics information with CCS	Compute Settings 🚷		
O ON OFF		select		

- 10. Click **Start** to submit the analysis.
- 11. After completion, the Circular Consensus Sequencing (CCS) application creates a CCS Analysis Report in the Analysis Results page that contains information and statistics about the HiFi Read generation process. (For a full interpretation of the report see the SMRT Link User Guide.) Click Data > File Downloads (on the left side menu of the report) to download the following files:
 - Movie-name.Q20.fasta: Contains HiFi Reads in FASTA format.
 - Movie-name.ccs.bam: Contains HiFi Reads in bam format.
 - Run_name (CCS): Link to HiFi Reads report (See "Running an Analysis Using HiFi Reads from Sequel II Systems" on page 10.)
 - CCS Analysis per-Read Details: Summary of CCS Analysis performance and yield.
 - Analysis Log: CCS analysis log.
 - SMRT Link Log: pbcromwell log.
- In SMRT Analysis > Data > File Downloads, click the Run_name (CCS) link that links to the Data Management > Dataset Details page containing the HiFi Reads report for your Data Set.

RT Analysis / Analysis Results				
5002_CCS_1				SUCCESSFUL 🕒 Copy 🗊 Dele
Analysis Overview	File Downloads			
	Edit Output File Name Prefix Example:analysis-unknown-115			
CCS Analysis Report				
CCS Analysis Report	File	Size	Туре	
CCS Analysis Report Data	File ■ m84011_181218_235052.Q20.fasta	Size	Type Fasta	
CCS Analysis Report Data File Downleads	File ■ m64011_181218_235052.020.fasta ■ m64011_181218_235052.ccs.bam	Size 14 GB 11 GB	Type Fasta Consensu	
CCS Analysis Report Data File Downloads	File iii mid4011_31218_236052.020.fssta iii mid4011_31218_235052.ccs.bam H0000_CC5_1(CC5)	Size 14 GB 11 GB 80 KB	Type Fasta Consensu	
CCS Analysis Report ✓ Data File Downloads SMRT Link Log	File ■ m64011_151218_235052.020 fasta ■ m64011_151218_235052.ccs.bam H0000_CCS_1 (CCS) ■ ■ m6401_151218_235052.020 fasta	Size 14 GB 11 GB 80 KB 28 GB	Type Fasta Consensu Fastq	

 From the Data Management > Dataset Details page containing the HiFi Reads report for your Data Set, click Analyze to go the SMRT Analysis > Create New Analysis page.

Running an Analysis Using HiFi Reads from Sequel II Systems

Data Management 👻			cguarco (Admi
uta Management / Dataset Details			Le Copy La Analyze
✓Dataset Overview	Status		
	Data Set	HG002_CCS_1 (CCS)	
Status	Data Set ID	99 f3c2a06d_e665_f3a3_00d9_f246cc288d96	
Thumbnails	Well Sample Name	unknown	
	Biological Sample Name	unknown	
Display All	Description	ccs dataset converted	
	Number of Records	1,325,008	
CCS Analysis Report	Total Length	14,920,014,634	
	Status	SUCCESSFUL	
\$ Analyses	Date Created	2020-04-30 04:02:30 AM	

3. From the **SMRT Analysis > Create New Analysis** page, proceed as shown in the **SMRT Link User Guide** to create a new analysis.

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