Introduction

This document describes the procedure for creating a Sample Sheet for your Onso sequencing run. Sample sheets from other technologies are not compatible with the Onso platform. This guide is intended for setting up a Sample Sheet for on-instrument use. For command line utilization with obc2fastq, please see **obc2fastq Reference Guide v.6.0.0**. This document is for Customer lab technicians and scientists.

- **Instrument Software Version 1.1** and **obc2fastq v.6.0.0** supports Onso SBB sequencing systems. SMRT® sequencing instruments such as Revio®, Sequel II® and Sequel IIe® systems, and Sequel® systems are **not** supported.

Note: Onso systems are for **research use only** (RUO).

Onso Sample Sheet (v.2)

Sample sheet format

The sample sheet is a comma-delimited text file (.csv) that consists of the following elements. Note: We require that you use only alphanumeric characters, dashes, and underscores for the sample sheet name.

Sections	Description
[Run Information]	Required Contains metadata about the run and can be used by downstream analysis
[Flow Cell Settings]	Required Contains the number of cycles run for Insert Read 1, Insert Read 2, Index P Read (Index), and Index A read (Index2); whether custom primers are used; and whether FASTQ output should be generated. Read1Cycles and Read2Cycles should equal the number of bases to be sequenced +2 for instrument calibration cycles (e.g., 150 base run would have a value of 152).
[Obc2fastq Settings]	Header field required but settings optional Contains the settings for obc2fastq.
[Samples]	Header fields required but settings optional Assign index sequences to individual samples when sample demultiplexing is to be performed

Onso adapters	Onso library element structure

*Bold A in SP2 comes from A-tailing in library prep not adapter oligo

	Sequence
A	CCATCTCATCCCTGCGTGTCTCCGACTCAG
SP1	TGCCGAGTACCACGGACAAGCACGAATCGAT
SP2*	**A**TCGATTCGTGCTCGATGAACCGGGCGCTTA
P1	ATCACCGACTGCCCATAGAGAGGAAAGCGGAGGCGTAGTGG

Example sample sheet

Native Onso library,

4-plex 2 x 150 bp, dual-index reads

(+obc2fastq optional settings)

Required	[Run Information]			
Required	FileFormatVersion	2		
Required	InstrumentPlatform	Onso		
Required	FlowcellBarcode	FC1000000-BCC		
Required	RunName	Demo_01		
Required	[Flow Cell Settings]			
Required	Read1Cycles	152		
Required	Read2Cycles	152		
Required	Index1Cycles	8		
Required	Index2Cycles	8		
Required	CustomPrimer	FALSE		
Required	OBC2FASTQ	TRUE		
Required	[Obc2fastq Settings]			
Optional	TrimLowScores	TRUE		
Optional	TrimQ	26		
Optional	TrimWindow	6		
Optional	R1CycleUsage	T1:Y150		
Optional	R2CycleUsage	T3:Y150		
Optional	I1CycleUsage	T2:Y8		
Optional	I2CycleUsage	T4:Y8		
Required	[Samples]			
Required	Biosample	Lane	Index	Index2
Optional	Sample1	1	TCCT TAGG	CAAC TGTA
Optional	Sample2	1	CGTC GCAC	GCGT CACT
Optional	Sample3	2	TCCT TAGG	CAAC TGTA
Optional	Sample4	2	CGTC GCAC	GCGT CACT

Example sample sheet

Converted P5/P7 library with LQC spike-in,

4-plex 2 x 100 bp, dual-index reads

(+obc2fastq optional settings)

Required	[Run Information]			
Required	FileFormatVersion	2		
Required	InstrumentPlatform	Onso		
Required	FlowcellBarcode	FC1000000-BCC		
Required	RunName	Demo_02		
Required	[Flow Cell Settings]			
Required	Read1Cycles	152		
Required	Read2Cycles	152		
Required	Index1Cycles	10		
Required	Index2Cycles	10		
Required	CustomPrimer	FALSE		
Required	OBC2FASTQ	TRUE		
Required	[Obc2fastq Settings]			
Optional	TrimLowScores	TRUE		
Optional	TrimQ	26		
Optional	TrimWindow	6		
Optional	R1CycleUsage	T1:Y150		
Optional	R2CycleUsage	T3:Y150		
Optional	I1CycleUsage	T2:Y10		
Optional	I2CycleUsage	T4:Y10		
Required	[Samples]			
Required	Biosample	Lane	Index	Index2
Optional	Sample1	1	TGTCG AACAA	CCAGA ATCTA
Optional	Sample2	1	GTGGC ACGAA	CCAAT TAGCA
Optional	Sample3	2	AAGCC TTAGA	CGTGT TATGA
Optional	Sample4	2	CGCTA AGGCT	TGTGC CGGTT

Example sample sheet

Converted 10X 3' Single Cell gene expression P5/P7 library with LQC spike-in

4-plex asymmetric 28 x 90bp reads, 10bp dual-index reads
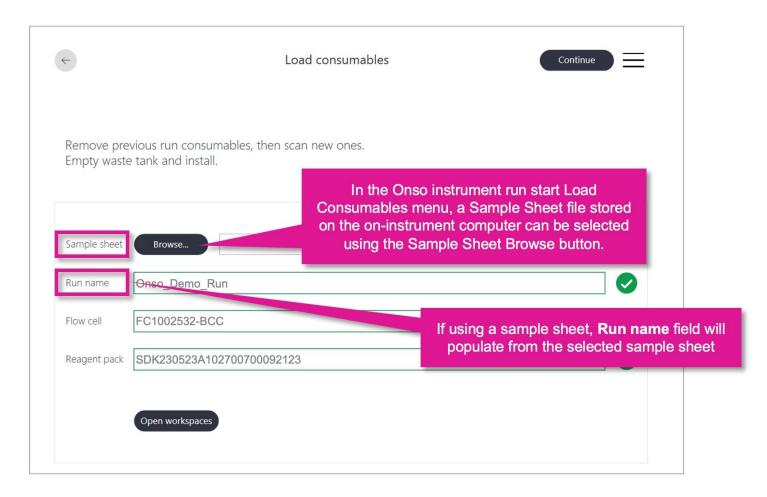
(+obc2fastq optional settings)

Required	[Run Information]			
Required	FileFormatVersion	2		
Required	InstrumentPlatform	Onso		
Required	FlowcellBarcode	FC1000000-BCC		
Required	RunName	Demo_03		
Required	[Flow Cell Settings]			
Required	Read1Cycles	92		
Required	Read2Cycles	30		
Required	Index1Cycles	10		
Required	Index2Cycles	10		
Required	CustomPrimer	FALSE		
Required	OBC2FASTQ	TRUE		
Required	[Obc2fastq Settings]			
Optional	TrimLowScores	FALSE		
Optional	R1CycleUsage	T1:Y90		
Optional	R2CycleUsage	T3:Y28		
Optional	I1CycleUsage	T2:Y10		
Optional	I2CycleUsage	T4:Y10		
Required	[Samples]			
Required	Biosample	Lane	Index	Index2
Optional	Sample1	1	GTAACATGCG	AGTGTTACCT
Optional	Sample2	1	GTGGATCAAA	GCCAACCCTG
Optional	Sample3	2	CACTACGAAA	TTAGACTGAT
Optional	Sample4	2	CTCTAGCGAG	TATCTTCATC

Example sample sheet

Converted P5/P7 library with LQC spike-in,

4-plex 1 x 75 bp, single-index reads

(+obc2fastq optional settings)

Required	[Run Information]			
Required	FileFormatVersion	2		
Required	InstrumentPlatform	Onso		
Required	FlowcellBarcode	FC1000000-BCC		
Required	RunName	Demo_04		
Required	[Flow Cell Settings]			
Required	Read1Cycles	77		
Required	Read2Cycles	0		
Required	Index1Cycles	8		
Required	Index2Cycles	0		
Required	CustomPrimer	FALSE		
Required	OBC2FASTQ	TRUE		
Required	[Obc2fastq Settings]			
Optional	TrimLowScores	TRUE		
Optional	TrimQ	26		
Optional	TrimWindow	6		
Optional	R1CycleUsage	T1:Y75		
Optional	I1CycleUsage	T2:Y8		
Required	[Samples]			
Required	Biosample	Lane	Index	Index2
Optional	Sample1	1	TCCTTAGG	
Optional	Sample2	1	CGTCGCAC	
Optional	Sample3	2	TCCTTAGG	
Optional	Sample4	2	CGTCGCAC	

How to Import the Sample Sheet

After importing the sample sheet, the Run name field will auto-populate using information provided in the selected sample sheet. Use the barcode scanner to scan in the flow cell QR code and sequencing reagent pack ID. Note: If the scanned flow cell ID differs from that specified in the imported sample sheet, a warning will be issued to notify the user. If the user chooses to proceed, the instrument control software will use the scanned flow cell ID as ground truth and overwrite the information originally specified in the imported sample sheet.

Online Resources and Getting Help

Onso Sample Sheet template, obc2fastq software installer and release documentation:
https://www.pacb.com/onso/software-downloads/

Creating a support case: E-mail techsupport@pacb.com and please attach your sample sheet. You may also use the PacBio Customer Hub to create a case and view case status.

Revision history (description)	Version	Date
Initial release	01	October 2024