Digestiflow: from BCL to FASTQ with ease

2

3Manuel Holtgrewe^{1,2}, Mikko Nieminen^{1,3}, Clemens Messerschmidt^{1,2},
4Dieter Beule^{1,3}
6¹ Berlin Institute of Health, Core Unit Bioinformatics, Charitéplatz 1, 10117 Berlin
7² Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin
8³ Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Straße 10, 13125 Berlin
9
10Corresponding Author:
11Dieter Beule^{1,3}
12Charitéplatz 1, 10117 Berlin
13Email address: dieter.beule@bihealth.de

15**Abstract**

16Management raw sequencing data and its preprocessing (conversion into sequences and 17demultiplexing) remains a challenging topic for groups running sequencing devices. They face 18many challenges in such efforts and solutions ranging from manual management of spreadsheets 19to very complex and customized LIMS systems handling much more than just sequencing raw 20data. In this manuscript, we describe the software package DigestiFlow that focuses on the 21management of Illumina flow cell sample sheets and raw data. It allows for automated extraction 22of information from flow cell data and management of sample sheets. Furthermore, it allows for 23the automated and reproducible conversion of Illumina base calls to sequences and the 24demultiplexing thereof using bcl2fastq and Picard Tools, followed by quality control report 25generation.

26

271 Introduction

28Laboratories operating modern sequencing facilities face a multitude of challenges. These 29include sample tracking, raw data preprocessing (conversion of raw sequencer output into 30sequences and demultiplexing of pooled experiments which is usually done in the same step), 31quality control of sequencing results, and delivery to the requesting party. While there is no clear 32consensus of what comprises a Laboratory Information Management System (LIMS), the term 33LIMS is often used to describe systems supporting these step. Simple "pure peopleware" 34implementations consist of spreadsheets on network shares while comprehensive commercial 35packages such as Illumina BaseSpace Clarity LIMS offer highly adjustable but very expensive 36solutions. A number of academic and open solutions fall in between, offering a variable number 37of features and degrees of customizability.

38The general lack of agreement of what a LIMS should cover or not cover stems from the fact that 39sequencing laboratories alone differ greatly. Areas of difference include the types of samples 40accepted (tissues/blood, DNA/RNA, final libraries/pools, or a subset thereof), and the type of 41data generated (raw base calls, sequences, aligned reads, or bioinformatics analytical reports). In 42addition, the surrounding information technology (IT) infrastructure varies greatly as does the 43degree of integration with such additional IT systems.

44In this manuscript we present our approach DigestiFlow (DF) that addresses the different needs 45of organizations by focusing on a small, well-defined subset of tasks: management of Illumina 46flow cell and sample sheet information and orchestrating the step converting base calls to 47sequences and demultiplexing pooled sequencing runs. To the best knowledge of the authors, in 48this domain DF offers unparalleled functionality. Flow cells can be filled with an arbitrary 49combination of libraries using any combination of index and molecular barcode reads. DF also 50supports the barcode being part of the template sequence. DF provides extensive features for 51sanity checking and comparison of expected indexing reads with those actually seen in the raw 52base call data.

53This is particularly important in an era where technologies such as single cell and low input 54sequencing require an ever-growing complexity of barcoding and indexing schemes and the 55amount of sequencer throughput is growing dramatically. We have encountered flow cells with 56more than 600 libraries and expect this to grow with increasing sequencer throughput. 57A fundamental link to central IT is the integration with existing authentication infrastructure via 58directory servers, e.g., Microsoft ActiveDirectory (AD). DF supports linking accounts to central 59AD instances as well as using user accounts that only exist within the system. Beyond this, the 60system provides its functionality through a REST API (representational state transfer application 61programmable interface application programming interface) such that other services can be 62easily integrated. Instead of covering all possible functionality and sample tracking schemes, DF 63avoids the complexity of a monolithic system and can be integrated as a part of a modular 64system. However, it can also just be standalone without integration with any other system. 65

662 Methods

67DigestiFlow (DF) consist of three major components. The architecture of the system is shown in68Figure 1. The figure also shows interaction with a minimal set of external systems.69

702.1 Digestiflow Server

71DF Server is a web app implemented with the SODAR-Core (Nieminen, Stolpe, Schumann, 72Holtgrewe, & Beule, n.d.) and Django frameworks in the Python programming language. It uses 73a PostgreSQL database system. It allows for the curation of flow cells and libraries together with 74arbitrarily complex index and barcoding schemes. Barcodes can be organized in barcode sets 75such that their sequence can be entered once and subsequently be referred to by name. 76Furthermore, sequencing machines can be registered with their main properties (e.g., whether the 77second barcode read needs to be reverse-complemented, depending on the paired indexing 78workflow used). DF Server allows the visualization of barcodes detected by DF Client in the 79BCL files (see Figure 2) and compares them to the libraries and barcodes entered by the users 80into the flow cell sample sheet. DF Server provides a number of sanity checks for both barcodes 81 from raw sequencing data, including a barcode frequency distribution and recognizing expected 82spike-ins such as PhiX sequence. It can also cross check between sample sheets and barcodes in 83the raw sequences, detecting barcodes present in one but missing in the other. Furthermore, users 84can add comments to flow cells and attach arbitrary files, which is useful for exchanging 85spreadsheets or concentration measurement reports from the wet lab. 86

872.2 DF Client

88DF Client is meant to be called periodically via a cron job to monitor the storage volume where 89sequencer(s) write output data. It reads the metadata files and registers any new flow cell with 90DF Server (or alternately, flow cells can be pre-registered in DF Server and their properties are 91then updated by DF Client). Once the barcodes have been sequenced completely, the DF Client 92extracts and evaluates their sequences and posts this information to DF Server. The client also 93detects when sequencing has succeeded (and various failure conditions) and updates the 94information in the server. DF Client is written in the Rust programming language. 95

962.3 Digestiflow Demux

97DF Demux is also meant to watch the storage volume where the sequencers write their output 98data. Once sequencing of a flow cell is complete and marked as ready in DF Server, it starts the 99preprocessing by first obtaining the flow cell information from DF Server. Flow cells can be 100marked for delivery as base call (BCL) files, (possibly) demultiplexed sequences, or both. If raw 101BCL files are to be delivered, DF Server simply creates a TAR (tape archive) file for each lane 102that contains all the information required for demultiplexing this one lane.

103For preprocessing, it first checks whether the flow cell can be processed by simply calling the 104Illumina vendor software bcl2fastq (version 1 or 2, depending on the needs of the raw data) and 105calls the program accordingly. Otherwise, it generates a series of calls to bcl2fastq and Picard 106Tools (http://broadinstitute.github.io/picard/) to perform the required preprocessing. An example 107for this is the Agilent XT protocol where molecular barcode sequences are stored in the second 108barcode read which bcl2fastq does not support. We refer to homogenous flow cell loads that can 109be processed with the bcl2fastq as basic preprocessing while flexible preprocessing allows 110arbitrary combination of library indexing and barcoding schemes.

111Once preprocessing is complete, FastQC

112(https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) is run on the results and the quality
113control results are be collected with MultiQC (Ewels, Magnusson, Lundin, & Käller, 2016).
114Finally, the MultiQC report is posted as a message to the flow cell in DF Server using the REST
115API together with the log files as attachment. This then allows the sequencing staff to review the
116results and react accordingly. DF Demux is implemented in the Python programming language
117with a Snakemake (Köster & Rahmann, 2012) workflow using Bioconda (Grüning et al., 2018)
118for software package management.

1203 Results

1213.1 The States of a Flow Cell

122DF tracks three components of sequencing: (a) the sequencing process itself, (b) preprocessing, 123and (c) data delivery. The possible state values differ for each of these three steps. See the 124manual in the Supplemental Material for full details, but they can be summarized as follows. 125Each step starts in the initial state. For preprocessing, an operator has to set the state of the 126preprocessing step to ready which signals DF Demux to start. Once the client detects that the 127sequencing of a flow cell has started the sequencing state changes to running. Similarly, once DF 128Demux has started, the preprocessing state changes to running. If sequencing or demultiplexing 129fails or succeeds, the corresponding state is updated accordingly (failed/success). For both, a 130human operator can set a special confirmed failure/success state manually. For example, a 131confirmed failure state will be set manually after they determine preprocessing by fixing a sample 133sheet. Or a confirmed success state may be set after a human operator determines that QC passes 134visual inspection. A special success with warning state allows users to flag situations such as 135sequencing which succeeded for all but one lane due to technical issues.

136For delivery, a human user has to set the state explicitly. This built-in system for keeping track of137the delivery state is particularly useful if more than one user is handling data delivery, especially138when used in conjunction with the message feature for leaving notes on flow cells.

1393.2 Comparison with Existing Methods

140Existing methods include the following: openBIS ELN-LIMS (Barillari et al., 2016) which 141builds on top of openBIS (Bauch et al., 2011) and has a high number of features for sample 142submission and -tracking yet also has a large number of dependencies, MendeLIMS (Grimes & 143Ji, 2014) which has basic sample tracking functionality yet is bound to a rigid data processing 144workflow, MISO (Masella et al., 2019) which offers basic sample tracking functionality yet does 145not include features for preprocessing, and Parkour LIMS (Anatskiy et al., 2019) which provides 146extensive sample tracking and advanced lab notebook features yet also does not integrate 147automated preprocessing. While being out of scope of this manuscript, we note that DF could be 148integrated with other software packages as long as they provide an API with additional code. The 149integration with Parkour LIMS appears particularly appealing as it is based on the same 150technology as DF (Python/Django) and has few other dependencies itself. Table 1 contains a 151comparison of the listed tools given some important features 152

1533.3 Features for Improving Sequencing Results

154Sample Sheet Validation. Based on practical experience, we greatly appreciate the automated 155comparison of observed adapter sequence content and sample sheet. Unexpected sequence in 156either set is an indication for possible errors. DF Server provides fine-grained control to 157acknowledge and suppress inconsistency warnings (after either fixing errors or accepting errors 158and then excluding corresponding data). Furthermore, common artifacts such as PhiX sequence 159are automatically recognized and show up as information rather than warnings or errors. Figure 2 160shows an example.

161**Reproducibility, Automation, and Quality Control.** The Digestiflow Client and Demux 162components are available from Bioconda as Conda packages and Docker images, thus allowing 163for future proof installations and creating reproducible workflows. By offering REST APIs and 164two useful client applications, DF greatly supports sequencing and demultiplexing operators in 165automating their work. Further automation can be added later as the APIs are open. Automated 166quality control using FastQC and aggregation using MultiQC also allows users to spot problems 167earlier (together with the sample sheet adapter checks described above). In our experience this 168allows for the early detection of many common issues. For example, from time to time, it occurs 169that the same adapter was used for two different libraries in the same lane. This error might be 170hard to spot on paper or in spreadsheets but applications such as DF Server can easily detect and 171report such problems similar to the example shown in Figure 2.

172

173References

175Anatskiy, E., Ryan, D. P., Grüning, B. A., Arrigoni, L., Manke, T., & Bönisch, U. (2019). Parkour LIMS: 176high-quality sample preparation in next generation sequencing. Bioinformatics.

177doi:10.1093/bioinformatics/bty820

178Barillari, C., Ottoz, D. S. M., Fuentes-Serna, J. M., Ramakrishnan, C., Rinn, B., & Rudolf, F. (2016).

179openBIS ELN-LIMS: an open-source database for academic laboratories. Bioinformatics, 32(4), 638–640. 180doi:10.1093/bioinformatics/btv606

181Bauch, A., Adamczyk, I., Buczek, P., Elmer, F.-J., Enimanev, K., Glyzewski, P., ... Rinn, B. (2011). 182openBIS: a flexible framework for managing and analyzing complex data in biology research. BMC 183Bioinformatics, 12(1), 468. doi:10.1186/1471-2105-12-468

184Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for 185multiple tools and samples in a single report. Bioinformatics, 32(19), 3047–3048.

186https://doi.org/10.1093/bioinformatics/btw354

187Grimes, S. M., & Ji, H. P. (2014). MendeLIMS: A web-based laboratory information management system 188for clinical genome sequencing. BMC Bioinformatics. doi:10.1186/1471-2105-15-290

189Grüning, B., Dale, R., Sjödin, A., Chapman, B. A., Rowe, J., Tomkins-Tinch, C. H., ... Köster, J. (2018). 190Bioconda: sustainable and comprehensive software distribution for the life sciences. Nature Methods, 19115(7), 475–476. doi:10.1038/s41592-018-0046-7

192Köster, J., & Rahmann, S. (2012). Snakemake--a scalable bioinformatics workflow engine. Bioinformatics, 19328(19), 2520–2522. doi:10.1093/bioinformatics/bts480

194Masella, A., Cooke, D., Armstrong, H., Davey, R., DeBat, T., Bian, X., ... Leipzig, J. (2019). miso-195lims/miso-lims: v0.2.183. doi:https://10.5281/ZENODO.3341739

196Nieminen, M., Stolpe, O., Holtgrewe, M., Beule, D. SODAR Core: a Django-based framework for scientific 197data management and analysis web apps. under review

199**Tables**

200

201Table 1 Comparison important properties and features in commercial and free software for the202management of Illumina flow cells information popular in the sequencing community based.203

Metric	DF	BSCL	OBLE	ML	MISO	PL
License	MIT	com.	f.f.n.	f.f.n.	GPL	GPL
Self-Hosted	✓	_	✓	✓	~	~
LDAP Auth	✓	~	✓	✓	~	_
(REST) API	✓	✓	~	_	~	~
Sample Tracking	min.	adv.	basic	basic	basic	adv.
Basic Preproc.	✓	~	✓	✓	_	~
Flexible Preproc.	✓	_	_	_	_	_
Sheet Checks	~	_	_	_	_	_
BCL Checks	~	_	_	_	_	_

204

205Abbreviations used in the table: DF (DigestiFlow), BSCL (BaseSpace Clarity LIMS), OBLE (OpenBIS LIMS206ELN), ML (MendeLIMS), PL (Parkour LIMS), com. (commercial), f.f.n. (free for non-commercial), min. (minimal),
207adv. (advanced), preproc. (preprocessing).

208Figures

209

210Figure 1 Architectural overview. Sequencing instruments write data to a specified file system
211storage. A periodically running DF Client detects new flow cells and registers them with the DF
212Server. Once sequencing is complete and sample sheet information has been approved by the
213operator, DF Demux performs the conversion to FASTQ files and creates all QC reports. Users
214can not only browse and view, but also manage and curate flow cells and their sample sheets
215through DF Server.
216



219

220Figure 2

221When adding the sample sheet (not shown), the operator made a small mistake. The adapter P37 222is given twice for the same lane in the sample sheet while the adapter sequence "AAGACCGT" 223occurs in the raw base calls but not in the sample sheet. This information can then be used for 224debugging sample sheet information. This is highlighted in the sample sheet (a) and the display 225of the adapters read from the raw base call data (b).

	1 DigestiFlow Beta						Search term						
^	*	Home / Bit	1 Genomics MDC	Flow Cell	1 / HZWKL	BBXY (CHARITE)	i.						
4		Flow Co	ell "1901	18_ST-	K0010	07_0120_	A_H2W	KLBBXY	CHARIT	E"	Flow Cell	Operations	j.
	T.	Properties	Somple Sheet	A Index	Ştata 🛦	Messages 🔲	TXT Sheets						
	fire.		Name	Reference	Barcode Se	1#1	Barcode #1		Barcode Set #2	Ban	ode #2	Lanes	
	Contract of Contract	1 -	CH DB AL DDT	unknown	NERNING M	ulteka Olgos f.,	TTACOGAC	PD				1-8	
	111	2.0	CH DR AL 002	unknown	NERNANT M	uticies Clines (Barpode.com	bination				1-8	
	EPT2444	1.1	CH OR AL DOS		hit this set hit	and the Colorest	ACCOTCT/- Is IN	at unique for				1.0	
	les .		CHURCHOLMUNU	Science	PACIFICATION AND	output cathor i	tamital	4					
4	apress.	4 (#	DH_08_4L004	seannan	NEEIMANT M	umples Choos t.	TACGETCT	(P37) A	ž.			1-8	
	100	5 .	CH_DELAL.005	unknown	NEBNed M	ultiples Oligon f	TACOUTET	(P717) A	<u>×</u>			1-8	
	and .	6.4	CHLDB_AL006	unknown	NEBNext M	ultiplex Oligos f_	CAGGTTCA	(P61)	5			1-8.	
	eter.	7 💌	CH_08_AL_007	unknown	NEENext M	uttplice Chigos F	OGEAACTA	(19)				1-8	
	0	8 -	CH_08_AL008	unknown	NEEPiext M	uttiplex Oligos f	CACAGACT	(P21)				1-8	
	Seales	8.00	CH_08_AL009	unknown	NEBNext M	umplice Oligon f_	AGTGACCT	(P2)				1-8	
		10.00	CH 08 AL 010	unknown	MERINANT M	Michee CHaon F.	AGCETATE	94.41				1-8	
		110		T DigestiFlow Beta									
	🍸 Dige	stiFlow Beta						Search term					4
)	¶ Dige	stiFlow Beta	i Genomics MDC	/ Flow Cells	s 7 - H2WKL	BBXY (CHARITE)		-Search term	_	teenth)			
)	T Dige	stiFlow Beta	i Genomics MDC ell "1901	/ Flow Cells	коо10	BEXY (CHARITE)	A_H2W	Search term	CHARIT	E"	(1) Manual Haw Get	O Here Operation	4
)	Y Dige	StiFlow Beta Home / Bit Flow Ce Properties	i Genomics MDG ell "1901 Sample Sheet	/ Flow Cells	коотс	BEXY (CHARITE)	A_H2W	-Search term	CHARIT	E"	C) Manual	O	4 8 -
)	T Dige	How Beta Home / Bit Flow Ce Properties	I Genomics MDG ell "1901 Bample Shief	/ Flow Cells 18_ST- A Index	6 / H2WMCL K0010 Stats A	BBXY (CHARITE) 07_0120_ Messages @	A_H2W	Search term	CHARIT	E"	(1) Manual Flow Cet	O Hereit	4
)		Home / DH Flow Ce Properties O The index	t Genomics MDC ell "1901 Sample Sheet Natogram statist	/ Flow Cells 18_ST- A Index ics are comp	KOO1C	BBXY (CHARITE) D7_0120_ Mistages @ + Row cells raw b	A_H2W TXT Shorts ese calls. They	Search term	_CHARIT	E"	El Manual Flow Cet	O I I III	4
)	T Dige	etiFlow Beta Home / Dif Flow Ce Properties O The index Lane Index	I GENOMICE MDC ell "1901 Sample Sheet Natogram statist Read Frequenc	Flow Cell 18_ST-	e / H2WKAL KOO1C Starts &	BBXY (CHARITE) D7_0120_ Mestages = flow cells taw b	A_H2W	Search term	CHARIT	E"	Manual Flow Cet eets.	Operation	4
)	T Dige	etiFlow Beta Flow Ce Properties G The Index Lane Index	I Genomics MDG ell "1901 Bample Sheet Notogram stellst Read Frequenc	/ Flow Cells 18_ST- A Index ics are comp ies interes	KOO1C	BBXY (CHARITE) D7_0120_ Messages () e flow cell's raw b consistence () b.	A_H2W TXT Shoeta ese cally. They R35)	Search term	CHARIT	E"	E) Manual How Cell sets.	O res	4
)	T Dige	etiFlow Beta Flow Ce Properties O The Index Lane Index	I Cenomics MDG ell "1901 Bample Shert Netogram stellst Read Frequenc 1 acid	/ Flow Cells 18_ST- A Index ics are comp ics inter calls anacone (3.5)	KOO1C	BBXY (CHARITE) D7_0120_ Messages () e flow cell's raw b consector () s. consector () s.	A_H2W	Search term KLBBXY can be used for a found to ranse 1 and index of	CHARIT	E ^{II} Transfer (a. Transfer ().	Flow Cet	o	4
)	1 Dige:	etiFlow Beta Horse / Bit Flow Ce Properties © The index Lane Index	I Genomics MDC Bample Shert Nictogram stellst Read Frequence L Ass 1 Try	/ Flow Cells 18_ST- A Index ics are comp ics inter are comp ics inter class i	CODIC	BEXY (CHARITE) D7_0120_ Messages + Row cells raw b CAMETAC 5. GTEBTAC 1. STEBTAC 1.	A_H2W TXT Sheetu ese cully. They ath) 1 2993 1	Search term KLBBXY can be used for a sound barrook Adda of lard 1 and tade of CLS for 2011 is same	CHARIT	E ^{III} Transfe she Atcasca s Tracasc 1. Tracasc 1. Tracasc 1.	Flow Cet	o	
)		AtiFlow Beta Here / Bit Flow Ce Properties O The Index Lane Index	I Genomics MDC ell "1901 Bample Shert Nistogram statist Read Frequenc I of the I of	/ Flow Cells 18_ST- index ics are comp ics index ics (21.5 aaccet: (1.3 accet: (1.3 accet: (1.3) accet: (1.3	Contraction of the second seco	BDXV (CHARITE) D7_0120_ Messages + Row cells raw b conserva () 5. GTODITAC () 1. TOTATAC () 1. TOTATAC () 1. TOTATAC () 1.	A_H2W TXT Sheetu ese cults: They atto: 7993 1 2293 1 1203 1	Search term KLBBXY can be used for a	_CHARIT	E ^{III} III Sample she Attaccol 1 - Traccol	C Manual Flow Col Links 22% 12% 12% 12%	O	
)		stiFlow Beta Horse / Bit Flow Ce Properties © The index Lane Index	I Genomics MDC Ell "1901 Sample Sheet Notogram statist Read Frequenc 1 ass 1 a	/ Flow Cells 18_ST- index index icc are comp ics icc are comp	KOOTC	Advinages C Advinages C e flow celfx raw b catagoria (1, 5, ercentra (1, ercentra (1, tercentra (1,	A_H2W TXT Sheets ese calls. They atto: 7993 1 1153 1 1153 1 1153 1 1153 1 1153 1	Search term KLBBXY can be used for a Sound beroode Add in Sec. 1 and source source to source source to source source to source microwith 1 and microwith 1 and	CHARIT antly-checking yo edd Tin C edd Tin C edd Tin C edd Tin C edd Tin C control C c	E ^{nt} Transle she Artaseca (4. Transle 1) Teasaic (1. Teasaic (1.)	C Manual Fine Cel acts.	O	
)		stiFlow Beta Horse / Bit Flow Ce Properties © The index Lane Index	el Centomics MDC ell "1901 Sample Shart Alatogram statist i Ada i Territori i Control i Cont	Flow Cells IB_ST- Index I	State A	BBXY (CHARITE) D7_0120_ Mensager • flow calls raw b categorie	A_H2W TXT Shoers saw calls. They 2743 1 1 2743 1 1 2743 1 2744 1	Search term KLBBXY can be used for a foreat barrook Ard in lard 1 and the any sease(of 1 and the any sease(of 1 and the any reconstant 1 and secondart 1 a.t.	CHARIT	E ^{III} TIANGCA 4 TTANGCA 4 TTANGCC 1 TOCAGAN 1 ANGOTTA 1 NGOTTA 1 NGOTTA 1	El Manual Flow Cet 1353 2254 2354 2354 2354 2355 2354 2355 2354 2355 2354 2355 2354 2355 2354 2355 2355	O	
)		etiFlow Beta House / Bit Flow Ce Properties © The index Lane Index	Centramics MDC ell "1901: Bample Shart Natogram statist Read Frequence Read Frequence Contemportation Contempo	Flow Cells Bagst Bagst Bagst Bagst Bagst Bagst Bagst Bagst Class Class Bagst Class C	Stats A Uted from the state is a state	BBXY (CHARTE) D7_0120_ Minnager w flow calls raw by categoria () creation ()	A_H2W TXT Sheetu star calls. They 2993 1 1 2993 1 1 2995	Search term KLBBXY can be used for a Grant be used for a Grant barrouth AAX for a statistic control of the search		E ^{III} III XAITTAAGEA A ITEAGGAE A ITEAGGAE A MAGETTA L IAAGETTA L IAAGETTA L IAAGETTA L IAAGETTA L	C Manual Crow Col ects. 1153 1255 1255 1255 1255 1255 1255 1255	O	
)		stiflow Beta Here / Bit Flow Ce Properties O The Index Lane Index	I Genomics MDC ell "1901 Bample Shert Nistogram statist Read Frequenc 1 64 1 70 1 70 1 70 1 70 1 70	/ Flow Cells 18_ST- instance of the second instance of the second access of the second instance o	KOOTC State A State A state from th state sta	Advenages (CHARITE) Meterages (CHARITE) Meterages (CHARITE) e flow cell's raw b e flow ce	A_H2W TXT Sheetu asae calls. They asae c	Search term KLBBXY can be used for a contemporative second According to the se	CHARIT	Entrolo E Transfer (4. Transfer (4. Transfer (4. Nooffer (4. Nooff	C Manual Flow Cot Flow Cot 2015 2019 2019 2019 2019 2019 2019 2019 2019	O him	
)		stiFlow Beta Horse / Dif Flow Co Properties O The index Lane Index	Centromics MDC ell "1901: Sample Sheet Notogram statist Read Frequenc 1 640 1 70 1 70 1 70 1 70 1 70 1 70 1 70 1 7	Flow Cell Bank Bank Bank Bank Bank Cal.5 Bank Cal.5	CODE CODE Stats A uted from the state i state i sta	DBXY (CHARTE) D7_0120_ Advinage: @ # flow cell's raw b catacitac 5. catacitac 1. cercetaris 1. tecrcetaris 1. tecrcetaris 1. tecrcetaris 1. attracter (a. catacitac 1. cercetaris 1. cercetaris 1. cercetaris 1. cercetaris 1.	A_H2W TXT Sheen ese calls. They are calls. The	Search term KLBBXY can be used for a Sound betweed for a sound bet	CHARIT	E ^{III} UI KAITINAS ANA ATTANGA I A. TTANGA I A. HAGATTA I A. HAGATTA I A. TAGAMA I A. HAGATTA I A. TAGAMA I A. HAGATTA I A. TAGAMA I A. HAGATTA I A. TAGAMA I A.	(1) Manual Flow Cat 1353 2259 2353 2353 2353 2353 2353 2353 2	O hos	

226 Supplemental Material

227

228 • Digestiflow Server Documentation