



State of Washington
Department of Health
Public Health Laboratories
1610 NE 150th Street
Shoreline, WA 98155-9701
Phone: (206) 418-5456
Fax: (206) 364-0072

Page 1 of 1

CLIA#: 50D0661453
License#: MTS-1327

Enterics Laboratory Results
Preliminary Report

Swedish Edmonds Hospital
Attn: Microbiology Lab
21601 - 76th Ave W
Edmonds WA 98026

Phone: 425-640-4129
Fax: 425 640-4491

Patient ID:	60004221429	PHL Sample #:	WA0697014
Patient Name:	Lowe, Nicholas A	Report Date:	11/29/2021
Patient Address:	6406 Augusta Crt , Mukilteo WA 98275		
Patient County:	Snohomish	Collected Date:	11/24/2021
Physician or Referring Submitter:		Received Date:	11/29/2021

Date of Birth:	07/21/1989	Date of Onset:	
Age:	32 Years	Test Reason:	Diagnostic
Sex:	Male	Specimen Source:	Stool
Submitter Lab #:	21-328-13683	Specimen Type:	Stool - Broth

Test Requested: STEC

<u>Test Description:</u>	<u>Result:</u>	<u>Result Date:</u>
Alere Shiga Toxin Quik Chek EIA	STX 1-, STX 2+	11/29/2021

Comments:



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Enterics Laboratory Results
Final Report

Swedish Edmonds Hospital

Attn: Microbiology Lab

21601 - 76th Ave W

Edmonds WA 98026

Phone: 425-640-4129

Fax: 425 640-4491

Patient ID:	60004221429	PHL Sample #:	WA0697014
Patient Name:	Lowe, Nicholas A	Report Date:	12/02/2021
Patient Address:	6406 Augusta Crt , Mukilteo WA 98275		
Patient County:	Snohomish	Collected Date:	11/24/2021
Physician or Referring Submitter:		Received Date:	11/29/2021

Date of Birth: 07/21/1989

Age: 32 Years

Sex: Male

Submitter Lab #: 21-328-13683

Date of Onset:

Test Reason: Diagnostic

Specimen Source: Stool

Specimen Type: Stool - Broth

Test Requested: STEC

<u>Test Description:</u>	<u>Result:</u>	<u>Result Date:</u>
Alere Shiga Toxin Quik Chek EIA	STX 1-, STX 2+	11/29/2021

<u>Test Description:</u>	<u>Result:</u>	<u>Result Date:</u>
E. coli Confirmation	Escherichia coli O121	12/02/2021

E. coli confirmation includes the utilization of culture methods for isolation, biochemicals for genus and species level identification and latex immunoglobulins for serotype identification. Flagellar antigen identification is performed using antisera.

Comments:

DISCLAIMER: This test was adopted and its performance characteristics determined by the WA State PHL. It has not been approved by the FDA. Results are for infection control and epidemiological purposes and should not be used as the sole means for clinical diagnosis or patient management.

CONFIDENTIAL: The confidentiality of the information in this report is protected by state law which prohibits any further disclosure without the specific written consent of the person to whom it pertains, or as otherwise permitted by state law.



State of Washington
Department of Health
PUBLIC HEALTH LABORATORY
1610 N.E. 150th Street
Shoreline, Washington 98155-97
Phone: (206) 418-5400
Fax: (206) 364-0072

MTS #1327

CLIA #50D0661453

www.doh.wa.gov/phlforms

21-328-13883
LOWE, NICHOLAS
6064221429
C ST00L
ST00L
ST00L
24NOV21
HE

FOR PHL USE ONLY

WA0697014
Lowe, Nicholas
Collected Date: 11/24/2021
Accession Date: 11/26/2021
Enterics

Please Print Clearly

MICROBIOLOGY

PATIENT	NAME (LAST) L. O. W. E.	(M)	A
	(FIRST) N. I. C. H. O. L. A. S.		
	ADDRESS 6406 AUGUSTA CRT		
	CITY MUKILTEO	STATE WA	ZIP CODE 98275
SUBMITTER	MALE ☒ FEMALE ☐	DATE OF BIRTH MO DAY YR 10/7/21	COUNTY Snohomish
	CHART OR PATIENT ID NUMBER 160004221429		
	CLINICIAN	CLINICIAN'S PHONE # () -	
	NAME OF PERSON COMPLETING THIS FORM Microbiology	PHONE # (425) 640-4129	
EPIDEMIOLOGY	MAIL RESULTS TO: Microbiology Lab Swedish/Edmonds Hospital 21601 70th Ave W. Edmonds, WA 98026 325-640-4129		
	CITY, STATE, ZIP CODE		
	COUNTY		
	AREA CODE & PHONE # () -	FAX # (425) 640-4491	
COMMENTS	SPECIMEN IS FROM ☒ SINGLE CASE ☐ CONTACT ☐ OUTBREAK ☐ CARRIER		
	SUSPECTED SOURCE OF INFECTION:		
	TRAVEL HISTORY (CONTINUE TRAVEL HISTORY IN COMMENTS, IF NECESSARY) ☐ FOREIGN ☐ USA MO DAY YR TO MO DAY YR MO DAY YR TO MO DAY YR		
	VACCINATION HISTORY		

ATTENTION: (See Instructions on Reverse Side of Form)			
☒ BACTERIOLOGY ☐ MYCOBACTERIOLOGY (TB)			
☐ MOLECULAR DIAGNOSIS/PCR ☐ PARASITOLOGY			
SPECIFIC AGENT SUSPECTED: E. coli STX2			
DATE COLLECTED	MO DAY YR	TIME OF DAY	
11/24/2021	11 24 2021	7:30	AM
DATE OF ONSET	MO DAY YR	TIME OF DAY	
11/24/2021	11 24 2021	7:57	PM
DATE SENT TO STATE	MO DAY YR	FATAL?	
11/26/2021	11 26 2021	YES NO	NO
SUBMITTER'S LAB NUMBER: 21-328-13683			
☐ BLOOD ☐ CSF ☒ SPUTUM ☐ BRONCHIAL WASH			
☐ SERUM ☒ STOOL ☐ RECTAL SWAB ☐ URINE			
☐ THROAT ☐ GASTRIC ☐ URO-GENITAL ☐ NASOPHARYNGEAL			
☐ WOUND (SITE) ☐ FLUID (SPECIFY)			
☐ TISSUE (SPECIFY) ☐ OTHER (SPECIFY)			
HAVE SPECIMENS FROM THIS PATIENT BEEN SUBMITTED PREVIOUSLY? ☐ YES ☒ NO			
IS THIS REQUEST INVOLVED IN A MEDICAL-LEGAL SITUATION? ☐ YES ☒ NO			
PLEASE ATTACH YOUR TEST RESULTS: ATTACHED			
SPECIMEN SUBMITTED IS:			
☒ ORIGINAL MATERIAL ☐ PURE ISOLATE ☐ MIXED ISOLATE			
MEDIA USED FOR SUBMISSION OF SPECIMEN (SPECIFY): MAC BROTH			
LABORATORY EXAMINATION REQUESTED:			
☐ SMEAR ☐ CULTURE ☐ NAAT			
☐ MOLECULAR DIAGNOSIS/PCR ☒ IDENTIFICATION/CONFIRMATION			
☐ PFGE ☐ SEROLOGY ☐ ANTIMICROBIAL SUSCEPTIBILITY			
☐ OTHER (SPECIFY)			
TREATMENT	DATE BEGUN	DATE COMPLETED	
DRUGS USED	MO DAY YR	MO DAY YR	
FOR PHL USE ONLY			
Prelim: Stx1-, Stx2+ LT 11/24/21			
Final: E. coli O121 SK-12/2/21			
RB 12/2/2021			
Date/Time Reported: R-GW 12/2/2021 @ 1628			



LOWE, NICHOLAS ALEXANDER

Location: SED ED

MRN: 60004221429

Acct: 10391911413

Admit Date: 11/24/2021

Discharge Date: 11/24/2021

DOB: 7/21/1989

AGE: 32 years

SEX: Male

Physician: ROSS PA, KATHERINE BLISS

Patient Phone: (425)931-2515

Consulting:

Microbiology

Procedure: C Bact Stool

Collected: 11/24/2021 19:30 PST

Source: Stool

Received: 11/24/2021 19:49 PST

Body Site: Stool

Started: 11/24/2021 19:49 PST

Free Text Source:

Accession: 21-328-13683

Ordering Physician: ROSS PA, KATHERINE BLISS

PRELIMINARY REPORT

Verified: 11/26/2021 07:31 PST

No E coli O157 isolated

STAIN REPORT

Verified: 11/25/2021 16:18 PST

Shiga Toxin 2 detected.

Legend: L=Low H=High C=Critical X=Corrected A=Abnormal *Interpretive Data RC=Result Comment OC=Order Comment <=>=Performing Lab

All testing performed by Swedish Edmonds Main Lab unless otherwise specified. Alan C. Boudousquie, M.D., Director

21601 76th Ave West Edmonds, WA 98026

CLIA#: 50D0629863

LOWE, NICHOLAS ALEXANDER
60004221429

Page 1 of 1

Printed: 11/26/2021 10:26 PST
Request ID: 23733519



FilmArray®
GI Panel

BIO FIRE®
A BIOMÉRIEUX COMPANY

www.BioFireDx.com

Run Summary		80064221429 LONE, NICHOLAS ALEXANDER SED ED - 32 Y M 24NOV21 1931 RT/RT 21-328-13538A STOOL PATH	
Sample ID: 2132813538A			
Detected: Clostridium difficile toxin A/B Shiga-like toxin-producing E. coli (STEC) stx1/stx2			
Result Summary		90.00mL Steril C Microbiolog	
Bacteria			
Not Detected	Campylobacter		
✓ Detected	Clostridium difficile toxin A/B		
Not Detected	Plesiomonas shigelloides		
Not Detected	Salmonella		
Not Detected	Vibrio		
Not Detected	Vibrio cholerae		
Not Detected	Yersinia enterocolitica		
Diarrheagenic E. coli/Shigella			
Not Detected	Enteraggregative E. coli (EAEC)		
⊗ N/A	Enteropathogenic E. coli (EPEC)		
Not Detected	Enterotoxigenic E. coli (ETEC) lt/st		
✓ Detected	Shiga-like toxin-producing E. coli (STEC) stx1/stx2		
Not Detected	E. coli O157		
Not Detected	Shigella/Enteroinvasive E. coli (EIEC)		
Parasites			
Not Detected	Cryptosporidium		
Not Detected	Cyclospora cayetanensis		
Not Detected	Entamoeba histolytica		
Not Detected	Giardia lamblia		
Viruses			
Not Detected	Adenovirus F 40/41		
Not Detected	Astrovirus		
Not Detected	Norovirus GI/GII		
Not Detected	Rotavirus A		
Not Detected	Sapovirus		
Run Details			
Pouch:	GI Panel v2.1	Protocol:	Stool FA v3.4
Run Status:	Completed	Operator:	lab admin (labadmin)
Serial No.:	47266941	Instrument:	TM06637
Lot No.:	232821		

Sent mac
broth
instead

✓ Called RME
✓ Faxed SNO. CTY
□ Called SNO. CTY
425 339 5235
□ Sent To STYRE

Shiga-toxin producing *Escherichia coli* Work Card

Submitter: *Svedish / Edmunds Hospital*

Submitter Result: stx detected stx 1 stx 2 O157

Isolate ☐

Broth ☒

ETM ☐

WA0697014

Lowe, Nicholas

Collected Date: 11/24/2021

Accession Date: 11/26/2021

Enterics

Direct Shiga Toxin				GN Enrichment Shiga Toxin				Direct RIM O157 (from submitted isolate)			
STX 1	STX 2	Initials	Date	STX 1	STX 2	Initials	Date	RIM O157	Control	Initials	Date
	+	SLK	11/29/21								

Direct Plating				Enrichment Plating			
Colony ID	SMAC	TSI/Urea	Colony ID	TC-SMAC	TSI/Urea	Colony ID	Rainbow w/ Antibiotics
A	serb + entire serb + pink - RSTK serb + red - RSTK <i>S. dysenteriae</i>	MMG-U-		serb + serb serb - serb <i>S. dysenteriae</i>		B	gray entire <i>S. dysenteriae</i>

Colony ID	GN SMAC	TSI/Urea	Colony ID	GN TC-SMAC	TSI/Urea	Colony ID	GN Rainbow w/ Antibiotics

Shiga Toxin				Shiga Toxin			
Colony ID	STX 1	STX 2	Initial/date	Colony ID	STX 1	STX 2	Initial/date
A	-	-	SLK 12/1/21				
B	-	+					

Biochemicals			
Colony ID			
Dextrose	AG		
Lactose	A		
Maltose			
Mannitol	A		
Sorbitol			
Adonitol			
Salicin			
Mucate	+		
Indole	+		
Motility	+		
H ₂ S	-		
Citrate			
MR			
VP			
Lysine	+		
Arginine			
Ornithine	-		
Control	-		

MALDI-ToF Identification					
Colony ID	Gram Stain	MALDI ID	MALDI Score	Initials/Date	Lead Review
B	GNR	E. coli	2.50	LS 12/2/21	PL 12/2/21

Gram Stain: GNR = Gram Negative Rod, GPC = Gram Positive Cocci

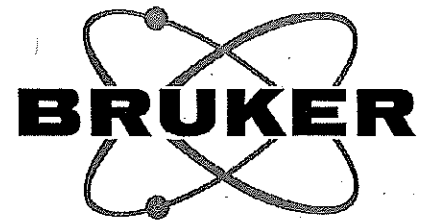
E. coli Serotyping					
Colony ID					
B					
Test	Result	Initials/Date	Test	Result	Initials/Date
ProLab Latex Agglutination			Remel RIM O157 Latex Agglutination		
O26	-		RIM O157		
O103	-		Control		
O111	-				
O145	-				
O121	+				
O45	-				
Control	-				
			H7		
			STIM #1		
			Control		
			STIM #2		
			Control		
			STIM #3		
			Control		

Mini-biochemical panel is bolded and italicized.

Comments:		Results		Analyst Initial and Date	Lead Initial and Date
Ran RIM Kit off BAP for WA06970143 RIM O157, Control- LS 12.2.21		Preliminary	Stx 1-, Stx 2+	SLK 11/29/21	AP 11/29/21
		Interim			
		Final	E. coli O121	LS 12/2/21	PL 12/2/21

Bruker MBT-CA

Run Results Report



Run Info:

Run Identifier: 12022021-105405-CA-1811005486 ✓
 Comment:
 Operator: ca-user@DOHIWK150502
 Run Creation Date/Time: 2021-12-02T10:56:02.489
 Number of Tests: 18
 Type: Clinical Application
 QC: passed
 QC Position: BTS3:0

✓ PB 12/2/2021

Result Overview

'For organism identifications reported in blue, refer to "Organism Identification Hint Table" section located in the reference library package insert for further information.'

Target Position	Test Organism Label	Organism Identification	log(score) Value
BTS3 ✓	bts ✓	<u>Escherichia coli</u> ✓	2.24 ✓
BTS0 ✓	bts ✓	<u>Escherichia coli</u> ✓	2.29 ✓
BTS4 ✓	bts ✓	<u>Escherichia coli</u> ✓	2.23 ✓
A1 ✓	Blank ✓	No Identification ✓	0.00 ✓
A2 ✓	S. aureus 6358 ✓	Staphylococcus aureus ✓	2.53 ✓
A3 ✓	S. aureus 6358 ✓	Staphylococcus aureus ✓	2.53 ✓
A4 ✓	S. maltophilia 13637 ✓	<u>Stenotrophomonas maltophilia</u> ✓	2.40 ✓
A5 ✓	S. maltophilia 13637 ✓	<u>Stenotrophomonas maltophilia</u> ✓	2.39 ✓
A6 ✓	697013A ✓	<u>Escherichia coli</u> ✓	2.22 ✓

Result overview table--continued on next page

MALDI-TOF Worksheet

Initials/Date: LT / 12-2-21

BTS Lot/Exp. Date: 7030421003 / 1-31-22

Barcode: 1811005486

Matrix Lot/Exp. Date: 7020320040 / 12-7-21

A1 Blank	A2 +	A3 S. aureus 6358	A4 S. maltophilia 13837	A5	A6 697013A	A7 697407B	A8 697503A
B1 697534A	B2 697014B	B3 697351A	B4 697353B	B5 698272A	B6 698273A	B7 698275A	B8 +
C1	C2	C3	C4 +	C5	C6	C7	C8
D1	D2	D3	D4 +	D5	D6	D7	D8
E1	E2	E3	E4	E5	E6	E7	E8
F1 +	F2	F3	F4	F5	F6	F7 +	F8

Comments	
----------	--

Lead/Supervisor Initials

KG

Date:

12/2/2021

Result overview table--continued from previous page

Target Position	Test Organism Label	Organism Identification	log(score) Value
A7 ✓	697407B ✓	<u>Escherichia coli</u> ✓	2.21 ✓
A8 ✓	697503A ✓	<u>Escherichia coli</u> ✓	2.36 ✓
B1 ✓	697534A ✓	<u>Escherichia coli</u> ✓	2.38 ✓
B2 ✓	697014B ✓	<u>Escherichia coli</u> ✓	2.50 ✓
B3 ✓	697351A ✓	<u>Escherichia coli</u> ✓	2.34 ✓
B4 ✓	697353B ✓	<u>Escherichia coli</u> ✓	2.42 ✓
B5 ✓	698272A ✓	<u>Salmonella sp</u> ✓	2.19 ✓
B6 ✓	698273A ✓	<u>Salmonella sp</u> ✓	2.31 ✓
B7 ✓	698275A ✓	<u>Salmonella sp</u> ✓	2.25 ✓

Miseq Run Set-up	
Run ID:	WAPHL-M5916-211203
Library Prep Date:	12/3/2021
Library Prep Technician:	GM
Sequencing Kit Type/Chemistry	500c v2
Sequencing Date:	12/3/2021
Sequencing Technician:	GM

Verification for: LIBRARY PREP KIT
Lot #: 20567393
Rec'd Date: 2021.09.15
Exp Date: 2022.05.10
Pass Fail

Post-Sequencing Analyst QC		
	Initials	Comments
Transfer data to Z:\COMMON\WGS\CONF	DEL	
Check WGS tracker for accuracy and completeness	DEL	
BN Analyst: Update REPEAT tab for accuracy and completeness	GM	
Update Reagent Tracker	GM	
Confirm all samples meet coverage	DEL	
Cluster Density	<u>Pass</u> / Fail	DEL
Clusters Passing Filter	<u>Pass</u> / Fail	DEL
Q30	<u>Pass</u> / Fail	DEL
Analysis Software	BioNumerics 7.6.3 NCBI Uploader 2.3 PulseNet Client 0.85 PulseNet WGS Tools 1.3	GM

X [Signature]
Analyst Review

X [Signature] 2021.12.06
AMD Lead or Supervisor Review

X [Signature] 2021.12.06
BioNumerics Review

X [Signature] 12/6/21
Serotyping Review

Verification for: CD Index
Lot #: 20559700
Rec'd Date: 2021.07.16
Exp Date: 2022.06.14
Pass / Fail

[Signature]
12/6/21

Please include all worksheets marked with pink in the report packet

Key	FirstName	Genus	IsolatDate	LabID
WA0697014	Nicholas	Escherichia	11/24/2021	WA

Other Lab Number	Outbreak	Passed_QC	Pathotype	LastName	MLST_ST
21-328-13683/60004221429	POB2112MLEXK-1	Passed QC	STEC	Lowe	ST655

PatientAgeYears	PatientSex	PulseNet_UploadDate	ReceivedDate	SequencerRun_id
32	MALE	12/6/2021	12/2/2021	WAPHL-M5916-211203

Serotype	Serotype_wgs	SourceCity	SourceCountry	SourceCountryState	SourceCounty
E. coli O121	O121:H19	Mukilteo	USA	USA:WA	Snohomish

SourceSite	SourceState	SourceType	Species	SRR_id	Toxin_wgs
Stool	WA	Human	coli	SRR17148975	stx2a

WGS_id	Allele_code	NCBI_ACCESSION	Toxin_wgs
PNUSAE090854	EC1.0 - 18.1.14.38.16	SAMN23709422	stx2a

Run Name	WAPHL-MS916-211203
MiSeq Run Start Date	12/3/2021
MiSeq Reagent Kit	500c v2

Run Number	544
Technician	GM

Run Metrics:	Recommended Cluster Density (K/mm ²)		Recommended Q30 %			Clusters Passing Filter	Estimated Yield (Gb)
	v2, Nano, Micro	v3	v3, 600c	v2, Nano	300c v2, Micro		
	600-1200	1200 - 1400	70	75	80		
	1109		70	75	80	84.90%	89.13%
							9.54

Minimum Coverage:	Escherichia/Shigella	Salmonella	Listeria	Campylobacter	Vibrio
PulseNet/FDA	40X	30X	20X	20X	40X

- Copy values from t tex
- Ensure sampl Initial Dil
- If all cells are filled
Laboratories whose
Laboratories

Is this a repeat?	Index Number	State Key	Project	Index 1 (I7)	Index 2 (I5)	% Reads Identified (PF)	PF Reads	Total # of Reads	Coverage (if MSR/MCS v 2.6 or earlier)	Taxonomic ID/Description
		DPC25370_544	Default	TAAGGCGA	GTAAGGAG	8.1286	37121784	3017481	150.87	Salmonella_enterica
		WA0696367	Default	CGTACTAG	GCGTAAGA	6.7189	37121784	2494176	124.71	Escherichia_coli
		WA0696358	Default	AGGCAGAA	ACTGCATA	8.3268	37121784	3091057	154.55	Salmonella_enterica
		WA0696463	Default	GGACTCCT	TATCCTCT	8.1499	37121784	3025388	151.27	Salmonella_enterica
		WA0697088	Default	TAGGCATG	CCTAGAGT	5.4892	37121784	2037689	101.88	Salmonella_enterica
		WA0696363	Default	CGAGGCTG	TTATGCGA	8.543	37121784	3171314	158.57	Salmonella_enterica
		WA0696470	Default	AAGAGGCA	TCGACTAG	7.124	37121784	2644556	132.23	Salmonella_enterica
		WA0696321	Default	GCTCATGA	CGTCTAAT	7.2006	37121784	2672991	133.65	Salmonella_enterica
		WA0696365	Default	CGTACTAG	ACTGCATA	3.7044	37121784	1375139	68.76	Salmonella_enterica
		WA0695382	Default	AGGCAGAA	GTAAGGAG	4.494	37121784	1668253	83.41	Salmonella_enterica
		WA0696467	Default	TAAGGCGA	GCGTAAGA	4.1411	37121784	1537250	76.86	Salmonella_enterica
		WA0697014	Default	CTCTCTAC	TATCCTCT	4.7607	37121784	1767257	88.36	Escherichia_coli
		WA0697351	Default	GTAGAGGA	CCTAGAGT	4.4165	37121784	1639484	81.97	Escherichia_coli
		WA0697353	Default	AAGAGGCA	CGTCTAAT	4.3749	37121784	1624041	81.20	Escherichia_coli
		WA0698272	Default	GCTCATGA	TTATGCGA	3.6639	37121784	1360105	68.01	Salmonella_enterica
		WA0698273	Default	CGAGGCTG	TCGACTAG	3.7344	37121784	1386276	69.31	Salmonella_enterica
		WA0698275	Default	AGGCAGAA	GCGTAAGA	3.947	37121784	1465197	73.26	Salmonella_enterica

Estimated genome sizes by Genus (species) in bp	
Salmonella	5000000
Escherichia/Shigella	5000000
Listeria	3000000
Campylobacter	1600000
Vibrio parahaemolyticus	5000000
Vibrio vulnificus	5000000
Vibrio cholerae	4000000

[Header]
IEMFileVersion 5
Experiment Name WAPHL-M5916-211203
Date 12/3/2021
Workflow GenerateFASTQ
Application FASTQ Only
Instrument Type MiSeq
Assay Nextera DNA Flex
Index Adapters Nextera DNA CD Indexes (96 indexes plated)
Chemistry Amplicon
[Reads]
251
251
[Settings]
ReverseComplement 0
Adapter CTGTCTCTTATACACATCT
[Data]

Sample_ID	Sample_Name	Sample_Plate	Sample_Well	Index_Plate_Well	I7_Index_ID	index	I5_Index_ID	index2	Sample_Project	Description
DPC25370_544	DPC25370_544-WAPHL-M5916-211203	WAPHL-M5916-211203	A01	A01	H701	TAAGGCGA	H505	GTAAGGAG	WaitingforQC	Salmonella_enterica
WA0696367	WA0696367-WAPHL-M5916-211203	WAPHL-M5916-211203	B01	B01	H702	CGTACTAG	H517	GCGTAAGA	WaitingforQC	Escherichia_coli
WA0696358	WA0696358-WAPHL-M5916-211203	WAPHL-M5916-211203	C01	C01	H703	AGGCAGAA	H506	ACTGCATA	WaitingforQC	Salmonella_enterica
WA0696463	WA0696463-WAPHL-M5916-211203	WAPHL-M5916-211203	D01	D01	H705	GGACTCCT	H503	TATCCTCT	WaitingforQC	Salmonella_enterica
WA0697088	WA0697088-WAPHL-M5916-211203	WAPHL-M5916-211203	E01	E01	H706	TAGGCATG	H516	CCTAGAGT	WaitingforQC	Salmonella_enterica
WA0696363	WA0696363-WAPHL-M5916-211203	WAPHL-M5916-211203	F01	F01	H710	CGAGGCTG	H522	TTATGCGA	WaitingforQC	Salmonella_enterica
WA0696470	WA0696470-WAPHL-M5916-211203	WAPHL-M5916-211203	G01	G01	H711	AAGAGGCA	H513	TCGACTAG	WaitingforQC	Salmonella_enterica
WA0696321	WA0696321-WAPHL-M5916-211203	WAPHL-M5916-211203	H01	H01	H714	GCTCATGA	H510	CGTCTAAT	WaitingforQC	Salmonella_enterica
WA0696365	WA0696365-WAPHL-M5916-211203	WAPHL-M5916-211203	A02	A02	H702	CGTACTAG	H506	ACTGCATA	WaitingforQC	Salmonella_enterica
WA0695382	WA0695382-WAPHL-M5916-211203	WAPHL-M5916-211203	B02	B02	H703	AGGCAGAA	H505	GTAAGGAG	WaitingforQC	Salmonella_enterica
WA0696467	WA0696467-WAPHL-M5916-211203	WAPHL-M5916-211203	C02	C02	H701	TAAGGCGA	H517	GCGTAAGA	WaitingforQC	Salmonella_enterica
WA0697014	WA0697014-WAPHL-M5916-211203	WAPHL-M5916-211203	D02	D02	H707	CTCTCTAC	H503	TATCCTCT	WaitingforQC	Escherichia_coli
WA0697351	WA0697351-WAPHL-M5916-211203	WAPHL-M5916-211203	E02	E02	H712	GTAGAGGA	H516	CCTAGAGT	WaitingforQC	Escherichia_coli
WA0697353	WA0697353-WAPHL-M5916-211203	WAPHL-M5916-211203	F02	F02	H711	AAGAGGCA	H510	CGTCTAAT	WaitingforQC	Escherichia_coli
WA0698272	WA0698272-WAPHL-M5916-211203	WAPHL-M5916-211203	G02	G02	H714	GCTCATGA	H522	TTATGCGA	WaitingforQC	Salmonella_enterica
WA0698273	WA0698273-WAPHL-M5916-211203	WAPHL-M5916-211203	H02	H02	H710	CGAGGCTG	H513	TCGACTAG	WaitingforQC	Salmonella_enterica
WA0698275	WA0698275-WAPHL-M5916-211203	WAPHL-M5916-211203	A03	A03	H703	AGGCAGAA	H517	GCGTAAGA	WaitingforQC	Salmonella_enterica

Dilute 20 pM library pool to final loading concentration	
Desired Final Loading concentration (10 pM to 20 pM):	17
Volume of 20pM library pool (uL):	850
Volume of HT1 buffer (uL):	150

Desired final PhiX concentration (10 pM to 20 pM):	17
PhiX spiked concentration (%):	1
Available diluted PhiX volume + extra (uL):	11
Volume of 20pM PhiX (uL):	9.35
Volume of HT1 buffer (uL):	1.65

Heat Block ID:	E-95
Denaturation temperature:	96°C
Thermal Cycler ID:	E-87

	Initial	Comments
Nextera DNA Flex Library Prep Box filled out correctly		
Sample Sheet auto-populated correctly		
Indexes match between plate map and SampleSheet		
Reloaded Runs Only: prep box and sample sheet updated		
Computer has adequate drive space		
Confirm correct sample sheet uploaded to D:/ on MiSeq		
Prestart summary screen: MiSeq run is correctly named		
Prestart summary screen: Correct number of cycles assigned		

[illegible]

[illegible]

Tagmentation

bp set w:

HLA's 22559700

Pub. i. Wells 20571034

r 2021.07.16

04 2022.06.14

ex 2022-06-16 f: 2021-09-15

books + reflections 20563193

EX 2022-06-01 - 15-2021-09-15

Bundy 20567373

ex 7022, 05, 10 : 2021 09 11

Library Preparation Using Illumina DNA Prep (2 of 2)

Clean up Libraries

40	Ensure SPB and RSB are at room temperature.
41	Place spun-down PCR plate on magnet for 5 minutes (or until clear).
42	Transfer 45µL of supernatant to new MIDI plate.
43	Remove PCR plate from magnet and discard.
44	Vortex & invert SPB several times ensuring resuspension.
45	Prepare SPB master mix.

	# of samples (plus 3):	Volume (µL)
	Volume of SPB	20
	Volume of H2O	816
		884

46	Vortex SPB master mix well & add 85µL to each sample well of MIDI plate from multichannel reservoir.
47	Cover with plate seal and plate shake for 1 min @ 1600 rpm.
48	Incubate at room temperature for 5 minutes
49	Place on the magnet for 3 minutes (or until clear).
50	During the incubation, vortex the stock SPB thoroughly.
51	While on the magnet transfer 125µL of supernatant into new MIDI plate, or new wells on same MIDI plate.
52	Remove from the magnet and add 15µL of stock SPB into wells containing supernatant.
53	Plate Shake 1 min @ 1600 RPM.
54	Incubate at room temperature for 5 minutes.
55	Dilute fresh 80% EtOH (8 mL EtOH + 2 mL molecular water is enough for 28 samples.)
56	Place on magnet for 3 minutes (or until clear).
57	Remove & discard supernatant.
58	Add 170µL of 80% EtOH and incubate for 30 seconds.
59	Pipet to remove the EtOH.
60	Add 170µL of 80% EtOH and incubate for 30 seconds.
61	Pipet to remove the EtOH.
62	Remove excess EtOH with small-volume pipette if present. Examine bottom of wells to see reflection of light to determine EtOH absence.
63	Allow beads to air dry for 5 minutes.
64	Remove from magnet & add 32µL of RSB.
65	Plate Shake 1 min @ 1600 RPM.
66	Incubate at room temperature for 5 minutes.
67	Place back on the magnet for 3 minutes (or until clear).
68	Transfer 30µL of the supernatant to new wells, new plate, or new strip tubes.

May stop here, store at -25C to -15C for up to 30 days

Pooling Libraries

69	Pool 5µL of each sample column into an empty column. Then pool the pooled column into a microcentrifuge tube. Label tube as "PAL Run #".
70	Quantify the pool on Qubit - record value on Workbook.
71	Use tapestation to determine average insert size of pool -- record value on Workbook.
72	Remove a 100µL aliquot of 1M NaOH from the freezer and add 400µL of molecular grade water to dilute to 0.2N working solution. Store on ice.
73	Thaw MiSeq cartridge in DI water (1 hour). Keep an aliquot of RSB thawed.
74	Thaw HT1 on ice. Thaw 20 pM Phi-X on ice.
75	Ensure heat block is at 96°C.
76	Workbook will calculate Molarity(nM): (pool concentration x 10 ⁶)/(660 g/mol x insert size bp).
77	Workbook will calculate volume of pool required for 50µL of 4nM: ((4 nM * 50 uL)/molarity of pool).
78	Workbook will calculate volume of RSB needed (50µL minus volume above).
79	Right-click "Sample Sheet" tab and select "Move or Copy...". Copy the tab into a new workbook. Use "Save As" to save the workbook as a comma-delimited file (.CSV).
80	Name the .csv file the exact same name as the barcode of the MiSeq cartridge. "MS...-500v2" etc. Save on V:\ drive. Find sample sheet on MiSeq and copy to D:\.
81	Confirm sample sheet is present using MiSeq software. Shutdown the instrument, turn off the switch, and let sit for 5 minutes before rebooting.
82	In a new tube, dilute pool to 4nM.
83	Add 5µL of 4nM pool to microcentrifuge tube.
84	Add 5µL of diluted NaOH to the tube, vortex briefly & quick spin.
85	Incubate at room temp for 5 minutes.
86	Immediately add 990µL of chilled HT1 (now 20pM library). Store on ice.
87	Dilute 20 pM library to desired loading concentration. Dilute 20 pM Phi-X to desired loading concentration. Store both on ice.
88	Prepare MiSeq for sequencing. Take out flow cell and rinse with milli-Q water, cleaning with 80% EtOH if necessary, and dry with lens paper until glass is clear.
89	Load PR2 bottle and flow cell into MiSeq.
90	Remove 10uL from diluted library and discard. Add 10 uL of diluted Phi-X (equal to 1% spiked-in concentration) to diluted library.
91	Incubate at 96°C for 2 minutes.
92	Immediately place in ice bath for a minimum of 5 minutes, maximum 30 minutes, until ready to load.
93	Puncture foil of "Load Sample" well on reagent cartridge with clean 1000uL tip. Load 600 uL of chilled, diluted, denatured pool into well. Load on MiSeq.

MS4 #1 MS3102048-SD062
20581643 r: 21109 ex: 220802

MS4 #2
20585514
r: 21109 ex: 220830

Nextera DNA Flex Library Prep	
Run ID:	WAPHL-M5916-211203
Library Prep Date:	12/3/2021
Library Prep Technician:	GM
Sequencing Kit Type/Chemistry	500c v2
Sequencing Date:	12/3/2021
Sequencing Technician:	GM
Total number of samples:	17

ROWS SHOULD NOT BE DELETED!! If less than
96 Isolates are being prepped & sequenced, the
additional, unused rows may be hidden. **DO NOT
DELETE ROWS.**

Key
White cells: Fillable
Blue cells: Formulas
Dark gray cells: Optional

Total number of samples:		17		DPC naming format: DPC12345_Run#		Optional					
Sample Well	State Key	Taxonomic ID/Description	Project ID	Genome Size (Mb)	Input DNA Qubit Reading (ng/ul)	Input DNA Nanodrop (260/280)	Volume of input DNA (ul)	Volume of input H2O (ul)	Quantity of Input DNA (ng)	Index Plate Well	Qubit Reading (ng/ul) of library
A01	DPC25370_544	Salmonella enterica	WaitingforQC	5	29.089		6	24	174.534	A01	11.873
B01	WA0696367	Escherichia coli	WaitingforQC	5	48.474		6	24	290.844	B01	14.708
C01	WA0696358	Salmonella enterica	WaitingforQC	5	73.595		6	24	441.57	C01	15.55
D01	WA0696463	Salmonella enterica	WaitingforQC	5	49.494		6	24	296.964	D01	15.139
E01	WA0697088	Salmonella enterica	WaitingforQC	5	61.51		6	24	369.06	E01	16.963
F01	WA0696363	Salmonella enterica	WaitingforQC	5	64.038		6	24	384.228	F01	15.138
G01	WA0696470	Salmonella enterica	WaitingforQC	5	46.018		6	24	276.108	G01	16.427
H01	WA0696321	Salmonella enterica	WaitingforQC	5	16.867		6	24	101.202	H01	10.55
A02	WA0696365	Salmonella enterica	WaitingforQC	5	32.64		6	24	195.84	A02	2.433
B02	WA0695382	Salmonella enterica	WaitingforQC	5	41.486		6	24	248.916	B02	2.589
C02	WA0696457	Salmonella enterica	WaitingforQC	5	44.964		6	24	269.784	C02	2.438
D02	WA0697014	Escherichia coli	WaitingforQC	5	32.228		6	24	193.368	D02	2.247
E02	WA0697351	Escherichia coli	WaitingforQC	5	46.588		6	24	279.528	E02	2.553
F02	WA0697353	Escherichia coli	WaitingforQC	5	37.242		6	24	223.452	F02	2.978
G02	WA0698272	Salmonella enterica	WaitingforQC	5	30.821		6	24	184.926	G02	2.61
H02	WA0698273	Salmonella enterica	WaitingforQC	5	36.206		6	24	217.236	H02	2.447
A03	WA0698275	Salmonella enterica	WaitingforQC	5	30.229		6	24	181.374	A03	2.493
Total MB:				85							

Illumina Sequencing Kit	DNA Load (Mb)
HiSeq v2, 300c	60
HiSeq v2, 500c	100
HiSeq v3, 600c* (mixed runs = no ECI)	200
HiSeq v3, 600c* (<i>E. coli/Shigella</i> runs)	175
HiSeq Micro, 300c	25
HiSeq Nano, 500c	13
MiniSeq Mid Output	60
MiniSeq High Output	100