1. **PURPOSE:** This procedure describes a standardized laboratory protocol for whole genome sequencing of enteric bacterial organisms using the KAPA HyperPlus library preparation kit, for subsequent sequencing on an Illumina platform, thus ensuring inter-laboratory comparability of sequencing results.

2. **SCOPE:** For use by PulseNet WGS certified laboratorians when preparing libraries from DNA from enteric organisms for sequencing on Illumina platforms for submission of sequencing data to PulseNet. Laboratories may amend this procedure as necessary for use within their laboratories after validation per their laboratory's guidelines.

3. **DEFINITIONS:**
   - 3.1. **BR:** Broad Range
   - 3.2. **BSC:** Biosafety Cabinet
   - 3.3. **DNA:** Deoxyribonucleic Acid
   - 3.4. **dsDNA:** Double-Stranded DNA
   - 3.5. **GHS:** Globally Harmonized System
   - 3.6. **HS:** High Sensitivity
   - 3.7. **IEM:** Illumina Experiment Manager (Illumina Software)
   - 3.8. **LRM:** Local Run Manager
   - 3.9. **PCR:** Polymerase Chain Reaction
   - 3.10. **PHL:** Public Health Laboratory
   - 3.11. **PPE:** Personal Protective Equipment
   - 3.12. **QC:** Quality control
   - 3.13. **RNase:** Ribonuclease
   - 3.14. **SDS:** Safety Data Sheet
   - 3.15. **Tris-HCl:** Tris Hydrochloride
   - 3.16. **WGS:** Whole Genome Sequencing

4. **RESPONSIBILITIES:**
   - 4.1. **PulseNet Public Health Laboratory:**
     - 4.1.1. Prepare DNA libraries and QC, as necessary, for subsequent WGS
     - 4.1.2. Communicate with PulseNet Central, as necessary, about any complications with laboratory protocols, suspected reagent issues, or suspected instrument issues
   - 4.2. **PulseNet Central:**
     - 4.2.1. Perform additional sequence quality analysis in order to provide feedback and troubleshooting support for PHLs, as necessary
     - 4.2.2. Communicate any suspected reagent issues to PHLs, as necessary
     - 4.2.3. Maintain and review SOPs on a regular basis and post on SharePoint

5. **SAFETY:**
   - 5.1. **Biosafety Warning:** This document describes handling of DNA and associated products, and does not describe best practices for handling of biological infectious material.

5.2. **Chemical Safety Warning:** Take proper precautions, and wear appropriate PPE when handling potentially hazardous chemicals. Ensure that chemicals, spent containers, and unused contents are disposed of in accordance with governmental safety standards.

5.2.1. KAPA HyperPlus Library Prep Kit: See KAPA SDSs for additional information. Take proper precautions and wear appropriate PPE when handling reagents.

- KAPA HiFi HotStart ReadyMix (2X): GHS Category 1 for eye damage/irritant and is harmful to aquatic life and Category 3 for specific target organ toxicity single exposure.

6. REAGENTS:

6.1. Ethanol, molecular-grade, 95-100% (Fisher Cat# BP2818-500 or equivalent)

6.2. Ethanol, lab-grade, 70% or equivalent for disinfection purposes (Fisher Cat# 04-355-309 or equivalent)

6.3. KAPA Dual-Indexed Adapter Kit (96 dual-index pairs, KAPA Cat# KK8722).

- Box 1 of 3. Store at -25 to -15°C
  NOTE: *These plates have 20 µl of 15 µM indexed adapters in each well. Must be diluted with KAPA Adapter Dilution Buffer included in the kit upon use.*
  KAPA Dual-Indexed Adapter
- Box 2 of 3. Store at -25 to -15°C
  KAPA Adapter Dilution Buffer
- Box 3 of 3. Store at 2-8°C
  Sealing foils

6.4. KAPA HyperPlus Library Prep Kit (96 samples, KAPA Cat# KK8514 or 24 samples, KAPA Cat# KK8512)

- Box 1 of 1. Store at -25 to -15°C
  KAPA Frag Enzyme
  KAPA Frag Buffer (10X)
  Conditioning Solution
  End Repair & A-Tailing Buffer
  End Repair & A-Tailing Enzyme
  Ligation Buffer
  DNA Ligase
  KAPA HiFi HotStart ReadyMix (2X)
  Library Amplification Primer Mix (10X)

6.5. KAPA Pure Beads (5 ml, KAPA Cat# KK8000 or 30 ml, KAPA Cat# KK8001). Store at 2-8°C.

6.6. Tris-HCl, 1M, pH 8.0 (Sigma-Aldrich, Cat# T3038-1L or equivalent). Store at 2-8°C.

6.7. Qubit dsDNA High Sensitivity (HS) Assay kit (100 samples, ThermoFisher Cat# Q32851 or 500 samples, ThermoFisher Cat# Q32854)

- dsDNA HS Reagent (Component A). Protect from light.
- dsDNA HS Buffer (Component B)
- dsDNA HS Standard #1 (Component C). Store at 2-8°C
- dsDNA HS Standard #2 (Component D). Store at 2-8°C

7. **SUPPLIES:**
   7.1. Conical tubes, 10 ml and/or 15 ml (Fisher Scientific Cat# 14-959A and/or Fisher Cat# 14-959-53A or equivalent)
   7.2. Ice
   7.3. Microcentrifuge tubes, 1.5 ml, sterile (ThermoFisher Cat# AM12400 or equivalent)
   7.4. Microseal A film (BioRad Cat# MSA-5001 or equivalent)
   7.5. Microseal B adhesive seal (BioRad Cat# MSB-1001 or equivalent)
   7.6. PCR plates, skirted, hard shell low profile, thin-wall, 96 well (BioRad Cat# HSP-9601 or equivalent)
   7.7. Pipette tips, sterile, filtered: 20 µl, 200 µl and 1000 µl volumes (Rainin Cat# 17001865, 17001863 & 17001864 or equivalent)
   7.8. Qubit Assay Tubes (ThermoFisher Cat# Q32856 or equivalent (clear, thin-wall 0.5-ml PCR tubes))
   7.9. Serological pipets, 1 ml to 10 ml volumes (various catalog numbers)
   7.10. Solution basins, sterile (Fisher Scientific Cat# 13-681-504 or equivalent)
   7.11. **OPTIONAL:** Deepwell storage "MIDI" plates, 96 well (Fisher Cat# AB-0859 or equivalent)

8. **EQUIPMENT:**
   8.1. Ice buckets/containers
   8.2. Magnetic Stand-96 (ThermoFisher Cat# AM10027 or equivalent)
   8.3. Microcentrifuge for quick spins
   8.4. Micropipettes, capable of volumes from 1 µl to 1000 µl. Single and multichannel (20 µl and 100µl volumes).
   NOTE: *Two sets of pipettes are suggested; one for working with pre-amplified product and reagents and one set for working with post-PCR amplified product and reagents.*
   8.5. Microplate centrifuge
   8.6. Pipet-Aid
   8.7. Qubit 2.0 or 3.0 Fluorometer, or equivalent for quantification of dsDNA
   8.8. Thermal cycler, capable of accepting a 96-well plate, with heated lid
   8.9. Vortex

9. **PROCEDURE:**
   NOTE: *Ensure that DNA going into library preparation has been assessed for quality. The 260/280 value should be between 1.75 and 2.05. See PNL33 for more information.*
   9.1. **Preparation of 10mM Tris-HCl pH 8.0**
      9.1.1. Add 10 ml of 1M Tris-HCl, pH 8.0 to 990 ml of molecular-grade water.
      9.1.2. Invert several times to mix.
      9.1.3. Store at 2-8°C for up to one year (indicate the initials of the preparer, date of preparation, and date of expiry on the bottle).
   9.2. **Preparation of Sequencing Workbook:** The steps for Sample Plate and Sample Sheet setup in IEM or LRM on the instrument can be completed at any point in this protocol, but must be performed prior to the start of the run. However, it is recommend to create the Sample Plate and Sample Sheet at the beginning of library prep.

9.2.1. Prepare the "Initial Dilution" tab of the KAPA HyperPlus Library Prep Workbook as described below:

NOTE: *The workbook is designed with the following color scheme, in general:*

- *Yellow fields should be filled in*
- *Blue fields contain formulas, which will autopopulate, and should not be altered*

9.2.2. Enter the Run ID in C2, C3, and C4: labID-MXXXX-YYMMDD.

9.2.3. Enter the Library Prep date (C5), Technician (C6).

9.2.4. Enter (or select from the dropdown) Sequencing Kit Type/Chemistry (C7).

9.2.5. Enter the Sample ID in appropriate location (Column B). This is the identifier entered in the PulseNet Key field in the BioNumerics database.

9.2.6. Determine which set of indices will be used and enter (or select from the dropdown) the well position (from the index plate), into column E of the Library Prep tab of the workbook.

NOTE: *It is recommended to not use the same index pairs within 2 consecutive runs on the same sequencer to reduce the amount of carryover. See Appendix PNL37-1 for KAPA HyperPlus Indices Tracking Worksheet.*

9.2.7. Enter the 260/280 Nanodrop value into Column F.

9.2.8. Enter the Genome Size Estimate (based on Table 1 below) into Column G.

Organism	Estimated Genome Size (million bases, Mb)
E. coli spp. & Shigella spp.	**5**
Salmonella ssp.	**5**
Vibrio spp.	**5**
Listeria monocytogenes	**3**
Campylobacter spp.	**1.6**

Table 1. Estimated genome size (in Mb) by organism

9.2.9. Confirm that the number of isolates on the run is appropriate for the capacity of the instrument sequencing reagent kit to be used. The sum of the genome sizes (in Mb) for the samples on the run (from column G, found in cell G44 of the workbook) will give the estimated DNA load of the run. This cannot exceed the DNA load allowance of the reagent kit to be used. The DNA load for MiSeq cartridges can be found in Table 2 below.

MiSeq Cartridge	DNA Load (Mb)
v2 300, mixed runs	**80**
v2 300, E. coli/Shigella	**75**
v2 500	**100**
***v3 600, mixed runs**	**200**
***v3 600, E. coli/Shigella**	**175**
***Micro (300)**	**35**
***Nano (500)**	**13**

Table 2. Estimated DNA Load (in Mb) Capacity for Illumina Reagent Kits.

*DNA capacity for v3 600, Micro and Nano kits are still being evaluated. Current recommendations are based on Nextera XT and can be likely slightly increased.

9.2.10. Enter the concentration of extracted DNA into Column H.
NOTE: *PulseNet has standardized the starting mass of DNA to 105 ng.*

9.3. **Normalization and fragmentation of DNA**

9.3.1. Thaw KAPA Frag Buffer and End Repair & A-Tailing Buffer (from freezer) on ice.
NOTE: *End Repair & A-Tailing Buffer needs to be thawed and placed on ice before beginning the procedure, as it needs sufficient time to thaw and must be added quickly once fragmentation is complete.*

9.3.2. Ensure the pre-programmed thermal cycler is turned on prior to beginning fragmentation, as some steps are time sensitive.

9.3.3. Label a 96-well PCR plate, or equivalent, with Run ID.

9.3.4. Add molecular-grade water (Column J in Initial Dilution) to each sample well.

9.3.5. Add DNA (Column I in Initial Dilution) to the molecular-grade water and mix well.

9.3.6. Add 5 µl of KAPA Frag Buffer (10X) to each sample well.

9.3.7. Add 10 µl of KAPA Frag Enzyme to each well and mix well by gently pipetting 5-10 times using a multichannel pipette.
NOTE: *Fragmentation is time sensitive. It is important to perform step 9.3.7. quickly.*

9.3.8. Seal the plate with Microseal A (or equivalent) and incubate the plate at 37°C for 4 minutes, followed by a 20°C hold (volume is 50 µl) on a thermal cycler with the lid heated at ≤50°C.
NOTE1: *It is recommended to pre-program a thermal cycler for this purpose.*
NOTE2: *This is not a recommended stopping point in the procedure and End repair and A-tailing should be commenced once the samples have reached 20°C.*

9.4. **End Repair and A-Tailing of DNA**

9.4.1. Check End Repair & A-Tailing Buffer for precipitate (if present, warm at 37°C for <1 minute and vortex).

9.4.2. When the samples have reached 20°C, remove the plate from the thermal cycler.

9.4.3. Add 7 µl of End Repair & A-Tailing Buffer to each well.

9.4.4. Add 3 µl of End Repair & A-Tailing Enzyme Mix to each well and mix well by gently pipetting 5-10 times using a multichannel pipette.

9.4.5. Seal the plate with Microseal A (or equivalent) and incubate the plate at 65°C for 30 minutes, followed by a 20°C hold (volume is 60 µl) on a thermal cycler with the lid heated at 85°C.
NOTE1: *It is recommended to pre-program a thermal cycler for this purpose.*
NOTE2: *This is not a recommended stopping point in the procedure and Adapter Ligation should be commenced once the samples have reached 20°C.*
NOTE3: *Ligation master mix should be prepared while samples incubate in the thermal cycler.*

9.5. **Adapter Ligation**

9.5.1. Thaw Adapter Dilution Buffer, Ligation Buffer, DNA Ligase and Adapter Plate on ice.

9.5.2. Prepare Adapter Ligation master mix:

Reagent	Volume per Sample
KAPA Adapter Dilution Buffer	3.5 µl
PCR-grade water	5 µl
Ligation Buffer	30 µl
DNA Ligase	10 µl

Table 3. Reagent volumes per sample for adapter ligation master mix

NOTE: *It is recommended to increase the number of samples during master mix calculation by 1-2 to ensure sufficient master mix volume. See workbook (Cells J50-53) for reagent volume.*

9.5.3. Gently vortex and quick spin the Adapter Ligation master mix.

9.5.4. When samples have reached 20°C, remove the plate from the thermal cycler.

9.5.5. Add 48.5 µl of Adapter Ligation master mix to each sample well.

9.5.6. Add 1.5 µl of appropriate adapter from the adapter plate to each sample well and mix well by gently pipetting 10-15 times using a multichannel pipette.

9.5.7. Seal the plate with Microseal A (or equivalent) and incubate the plate at 20°C for 15 minutes, followed by a 20°C hold (volume is 110 µl) on a thermal cycler with no heated lid.
NOTE1: *It is recommended to pre-program a thermal cycler for this purpose.*
NOTE2: *This is not a recommended stopping point in the procedure and Post-Ligation Clean-Up should be commenced once the samples have reached 20°C.*

9.6. **Post-Ligation Clean-Up**

9.6.1. Before starting, prepare reagents:

9.6.1.1. Dilute fresh 80% ethanol sufficient for all samples:

Reagent	Volume per sample	Example: 20 samples
100% ethanol	0.4 ml	8 ml
Molecular-grade water	0.1 ml	2 ml

Table 4. Reagent volumes per sample for 80% ethanol.

9.6.1.2. Bring the KAPA Pure beads and 10mM Tris-HCl to room temperature.

9.6.2. Centrifuge the PCR plate at 800-1200 rpm (or 280 x g) for approximately 30 seconds to collect condensation.

9.6.3. Transfer 100 µl of the PCR product to a new set of wells.

NOTE: *Deepwell MIDI plate may be used for the procedure.*

9.6.4. Vortex the KAPA Pure beads until well suspended (15 – 30 seconds).

9.6.5. Add 80 µl (0.8x volume) of KAPA Pure beads to each well.
NOTE: *Avoid bead carryover by confirming no droplets are on the pipette tip. This will affect the ratio of beads to PCR reaction, which affects fragment size selection.*

9.6.6. Gently pipette up and down 10-15 times to mix.

9.6.7. Incubate at room temperature for at least 5 minutes.

9.6.8. Place the plate on the magnetic stand for a minimum of 5 minutes or until the supernatant has cleared.

9.6.9. With the plate still on the magnetic stand, carefully remove and discard the supernatant.
NOTE: *If any beads are inadvertently aspirated into the tips, dispense the beads back into the wells and let the plate rest on the magnetic stand for another 2 minutes or until the supernatant has cleared.*

9.6.10. With the plate still on the magnetic stand, wash the beads by adding 200 µl of freshly prepared 80% ethanol to each well.
NOTE: *Do not resuspend the beads at any point during the wash steps or remove the plate from the magnetic stand.*

9.6.11. Incubate the plate on the magnetic stand for 30 seconds, then carefully remove and discard the supernatant.

9.6.12. With the plate still on the magnetic stand, perform a second 80% ethanol wash.

9.6.13. Incubate the plate on the magnetic stand for 30 seconds, then carefully remove and discard the supernatant.

9.6.14. With the plate on the magnetic stand, use a low volume pipette tip to remove any remaining ethanol droplets and allow the beads to air-dry for up to 3 minutes.
NOTE: *Exceeding the maximum drying period (3 minutes) could make resuspension of DNA fragments difficult. Over-drying is indicated by cracks in the bead pellets.*

9.6.15. Remove the plate from the magnetic stand.

9.6.16. Add 23 µl of Tris-HCl to each well.

9.6.17. Gently pipette up and down at least 10 times to mix, ensuring the beads are completely resuspended, and changing tips after each well.

9.6.18. Incubate at room temperature for 5 minutes.

9.6.19. Place the plate on the magnetic stand for at least 2 minutes or until the supernatant has cleared.

9.6.20. Carefully transfer 20 µl of the supernatant from the plate to a new set of wells for amplification.

9.7. **Amplification of DNA**

9.7.1. Thaw KAPA HiFi HotStart ReadyMix (2X) and Library Amplification Primer Mix (10X) on ice.

9.7.2. Add 25 µl of KAPA HiFi HotStart ReadyMix (2X) to each well containing 20 µl of clean adapter-ligated DNA.

9.7.3. Add 5 µl of Library Amplification Primer Mix (10X) to each well and mix well by gently pipetting 10-15 times using a multichannel pipette.

9.7.4. Seal the plate with Microseal A or equivalent, and run the following pre-programmed settings on a thermal cycler with a heated lid (100°C):

Step 1: 98°C for 45 seconds
Step 2: 8 cycles
98°C for 15 seconds
60°C for 30 seconds
72°C for 30 seconds
Step 3: 72°C for 1 minute
Step 4: Hold at 4°C
Total volume: 50 µl

9.7.5. Centrifuge plate for at 280 x g for 30 seconds.

NOTE: ***This is a safe stopping point. The plate may be sealed with Microseal B or equivalent, and stored at 2°C to 8°C for up to 3 days.***

9.8. **Post-Amplification Clean-Up**

NOTE: *The steps listed below are critical for efficient size selection, product recovery and thus cluster generation and sequencing. Always check pipette tips for correct volumes and to ensure that no beads have accidentally been aspirated. If beads have been aspirated or the bead pellet is disturbed, allow the pellet to reform (3-5 minutes on the magnet) and repeat the step.*

9.8.1. Before starting, prepare reagents:

9.8.1.1. Dilute fresh 80% ethanol sufficient for all samples:

Reagent	Volume per sample	Example: 20 samples
100% ethanol	0.4 ml	8 ml
Molecular-grade water	0.1 ml	2 ml

Table 5. Reagent volumes per sample for 80% ethanol.

9.8.1.2. Bring the KAPA Pure beads and 10mM Tris-HCl to room temperature.

9.8.2. Centrifuge the PCR plate at 800-1200 rpm (or 280 x g) for approximately 30 seconds to collect condensation.
9.8.3. Transfer 40 µl of the PCR product to a new set of wells.
NOTE: *Deepwell MIDI plate may be used for the procedure.*
9.8.4. Vortex the KAPA Pure beads until well suspended (15 – 30 seconds).
9.8.5. Add 16 µl (0.4x volume) of KAPA Pure beads to each well.
NOTE: *Avoid bead carryover by confirming no droplets are on the pipette tip. This will affect the ratio of beads to PCR reaction, which affects fragment size selection.*

9.8.6. Gently pipette up and down 10-15 times to mix.
9.8.7. Incubate at room temperature for at least 5 minutes.
9.8.8. Place the plate on the magnetic stand for a minimum of 3 minutes or until the supernatant has cleared.
9.8.9. With the plate still on the magnetic stand, carefully remove 50 µl of the supernatant and place in a new set of wells. Remove the plate from the magnetic stand for the second bead addition.
NOTE: *If any beads are inadvertently aspirated into the tips, dispense the beads back into the CAA and let the plate rest on the magnetic stand for another 2 minutes or until the supernatant has cleared.*
9.8.10. Add 5 µl (0.1x) of KAPA Pure beads to the wells containing the 50 µl of supernatant.
9.8.11. Gently pipette up and down 10-15 times to mix.
9.8.12. Incubate at room temperature for at least 5 minutes.
9.8.13. Place the plate on the magnetic stand for a minimum of 2 minutes or until the supernatant has cleared.
9.8.14. With the plate still on the magnetic stand, wash the beads by adding 200 µl of freshly prepared 80% ethanol to each well.
NOTE: *Do not resuspend the beads at any point during the wash steps or remove the plate from the magnetic stand.*
9.8.15. Incubate the plate on the magnetic stand for 30 seconds, then carefully remove and discard the supernatant.
9.8.16. With the plate still on the magnetic stand, perform a second 80% ethanol wash.
9.8.17. Incubate the plate on the magnetic stand for 30 seconds, then carefully remove and discard the supernatant.
9.8.18. With the plate on the magnetic stand, use a low volume pipette tip to remove any remaining ethanol droplets and **IMMEDIATELY** remove the plate from the magnetic stand.
NOTE: *The bead pellets are small and do not need additional drying time.*
9.8.19. Add 33 µl of Tris-HCl to each well.
9.8.20. Gently pipette up and down at least 10 times to mix, ensuring the beads are completely resuspended, and changing tips after each well.
9.8.21. Incubate at room temperature for 5 minutes.
9.8.22. Place the plate on the magnetic stand for at least 2 minutes or until the supernatant has cleared.
9.8.23. Transfer 30 µl of the supernatant to a new PCR plate – This is the final product.
NOTE1: *Qubit measurements should be performed at this point and results recorded in Column E of the Normalization and Pooling tab of the Workbook.*
NOTE2: *The DNA concentration after post-PCR clean-up can be between 5 ng/µl and 20 ng/µl.*
NOTE3: *The ideal minimum concentration required for sequencing is 2 nM. However, a sample can still be run if it is below the ideal concentration as long as DNA is detected by the Qubit. These low concentration samples should not be diluted prior to pooling libraries.*

NOTE4: *If 25% or more of the samples are below the ideal minimum concentration, consider delaying the run (depending epidemiological importance of the isolate or whether it is associated with an outbreak investigation), and repeat the library prep procedure for samples with a low concentration.*

This is a safe stopping point. The plate may be sealed with Microseal B or equivalent and stored at -20°C until use, or for long-term storage.

9.9. **Normalizing and Pooling the Libraries**

9.9.1. If libraries in the CAN plate/tubes are frozen, thaw on ice and centrifuge at 800 – 1200 rpm (or 280 x g) for 30 seconds.

9.9.2. For each library, dispense 10mM Tris-HCl (diluent) into a clean well of the PCR plate according to the volumes listed in Column H of the Normalization and Pooling tab of the Workbook.

9.9.3. Ensure that DNA is well mixed, then transfer the appropriate volume of each library (Column G of the Normalization and Pooling tab) to the corresponding well containing 10mM Tris-HCl to obtain the desired concentration (2nM, 3nM or 4nM).
NOTE1: *The recommended concentrations are 2 – 4 nM for MiSeq, 2 nM for MiniSeq, and 1 nM for iSeq. Adjust the loading concentration as needed when diluting the denatured pooled library in PNL38.*
NOTE2: *The recommended loading concentration for KAPA HyperPlus libraries is 8 – 10 pM on the MiSeq. Library loading concentration for MiniSeq and iSeq is under evaluation.*

9.9.4. Mix the dilutions by pipetting up and down 5-7 times with a multichannel pipet.

9.9.5. For each sample library, transfer the volume listed in the "Pooling Volume" column (Column J) into a single well on the PCR plate or in a new 0.2 ml tube for a total pooled volume of 50 µl.
NOTE: *The pooling factor is based on the genome size. When genomes of different sizes are run together, the amount of each single library is added proportionately to genome size to reduce the over-representation of small genomes in the pooled library.*

9.9.6. Mix the pooled library by pipetting up and down 10 times.

NOTE: The libraries are now ready for sequencing. Please proceed to the appropriate instrument sequencing SOP for instructions to denature and dilute the pool to the proper loading concentration.

10. **FLOW CHART:** N/A

11. **RELATED DOCUMENTS:**

Document Number	Title
PNL33	**DNA Extraction and QC SOP**
PNL38	**Sequencing on the MiSeq SOP**
PNQ07	**Illumina MiSeq Data QC**
PNL37.W1	**KAPA HyperPlus Workbook**

12. **REFERENCES:**

12.1. KAPA Biosystems, KAPA Dual-Indexed Adapter Kit TDS. Version 1.17. May 2017. https://www.kapabiosystems.com/document/kapa-dual-indexed-adapter-kit/?dl=1

12.2. KAPA Biosystems, KAPA HyperPlus Kit TDS. Version 4.17. September 2017. https://www.kapabiosystems.com/document/kapa-hyperplus-library-preparation-kit-tds/?dl=1

13. **CONTACTS:**

13.1. PulseNet NGS Lab troubleshooting account: PulseNetNGSLab@cdc.gov

14. **APPENDICES:**

14.1. PNL37-1: KAPA HyperPlus Indices Tracking Worksheet

15. **AMENDMENTS:**

15.1. 5/29/19 New Document

16. **APPROVAL SIGNATURES:**

Approved By: Yoo Jin Joung -S (Affiliate) Digitally signed by Yoo Jin Joung -S (Affiliate) Date: 2019.06.07 11:37:16 -04'00' Date: 06/07/2019
Author

Approved By: Patricia C. Lafon -S (Affiliate) Digitally signed by Patricia C. Lafon -S (Affiliate) Date: 2019.06.07 14:15:09 -04'00' Date: 06/07/2019
PulseNet QA/QC Personnel

Approved By: N/A Date: N/A
PulseNet Outbreak Detection and Surveillance Unit Chief

Approved By: N/A Date: N/A
PulseNet PFGE Reference Unit Chief

Approved By: Eija Trees Digitally signed by Eija Trees Date: 2019.06.07 14:06:15 -04'00' Date: 06/07/2019
PulseNet Next Generation Subtyping Methods Unit Chief

Approved By: Efrain M. Ribot -S Digitally signed by Efrain M. Ribot -S Date: 2019.06.07 13:45:00 -04'00' Date: 06/07/2019
PulseNet Reference Outbreak Surveillance Team Lead

Appendix PNL37-1:
Example of KAPA HyperPlus Indices Tracking Worksheet

KAPA HyperPlus Indices Tracking Worksheet

	1	2	3	4	5	6	7	8	9	10	11	12
A	D501	D501	D501	D501	D501	D501	D501	D501	D501	D501	D501	D501
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
B	D502	D502	D502	D502	D502	D502	D502	D502	D502	D502	D502	D502
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
C	D503	D503	D503	D503	D503	D503	D503	D503	D503	D503	D503	D503
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
D	D504	D504	D504	D504	D504	D504	D504	D504	D504	D504	D504	D504
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
E	D505	D505	D505	D505	D505	D505	D505	D505	D505	D505	D505	D505
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
F	D506	D506	D506	D506	D506	D506	D506	D506	D506	D506	D506	D506
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
G	D507	D507	D507	D507	D507	D507	D507	D507	D507	D507	D507	D507
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712
H	D508	D508	D508	D508	D508	D508	D508	D508	D508	D508	D508	D508
	D701	D702	D703	D704	D705	D706	D707	D708	D709	D710	D711	D712

Lot Number	
Expiration Date	
Rec'd Date	
Open Date	

Runs