Global Health Research Unit
Genomic Surveillance of AMR

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CGPS

WGS-Ready Checklist

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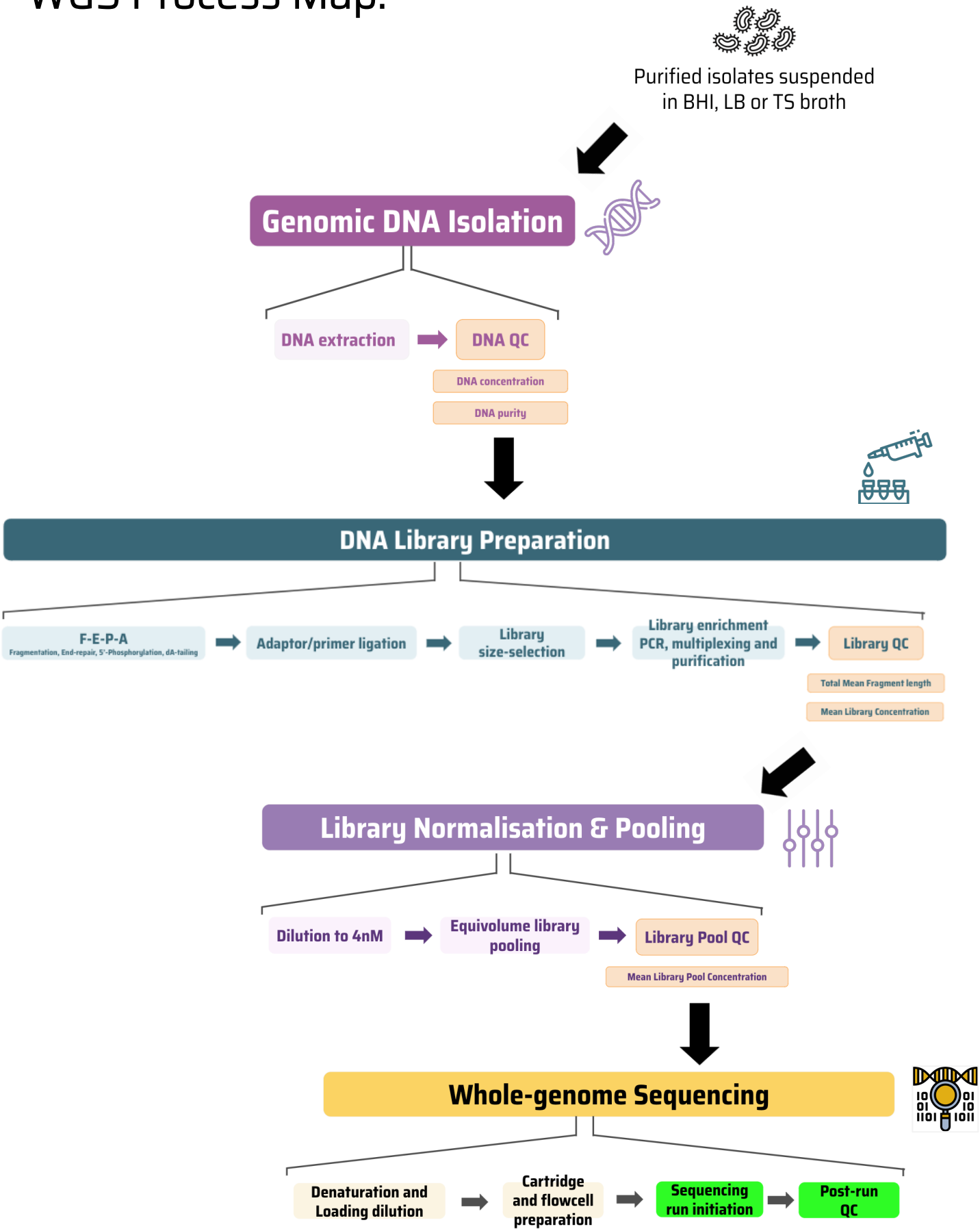

Infrastructure & Lab Design Review:

A laboratory performing routine whole-genome sequencing exhibits inner workings of an extended PCR laboratory. The purpose of considering design and designation within a WGS operation is to avoid contamination of new amplicons (generated during library preparation) with old libraries and general sample-to-sample contamination [3].

Contamination can be introduced into a microbial WGS setup from sources like: 1) residual microbiological cellular material on contaminated personal protective equipment (PPE), 2) isolated genomic nucleic acids from a different species, 3) previously amplified and aerosolized amplicons or 4) contaminated reagent stocks.

There are several methods by which contamination can be controlled, specifically with regards to whole-genome sequencing bacteria [3].

1. Physical Separation: Creating separate spaces for amplification and the original DNA generation can significantly reduce contamination. Even if separate rooms aren't feasible, these activities can be placed at opposite ends of the same room to minimise crossover. In addition, sample preparation can be performed within the confines of a HEPA and UV-enabled laminar flow cabinet that traps aerosols, cross-links active DNA and prevents contaminants landing on protective clothing. Each designated separation should contain dedicated equipment, colour-coded PPE and all of the relevant reagents stocked exclusively within each. The entrances and exits to the entire lab could be fitted with adhesive panels that trap dust and debris outside the lab space.
2. Chemical Sterilisation: Labware (like pipettes and plasticware) and reagents lacking enzymes can be sterilised using UV irradiation (or UV photolinking).
3. Experimental Control: Inclusion of relevant positive and negative controls at each sequential step within the end-to-end workflow so that assays can be validated for performance against a reference standard.

Therefore, when laid out linearly, the activities described on the WGS process map can be spread across four major enclosures inside a laboratory [4]:

1) Sample Preparation area: for DNA extraction and DNA QC
2) Pre-PCR area: for F-E-P-A, adaptor ligation and size-selection
3) Post-PCR area: for library enrichment, multiplexing, purification and library QC
4) Sequencing area: for WGS run initiation. In many cases, the sequencing area is part of the post-PCR enclosure.

These areas would normally sit downstream to the standard microbiology setup where bacterial isolates are cultured, characterised, purified and cryobanked.

Described in Figure 1 is the ideal laboratory layout for an end-to-end genome sequencing setup. In reality, due to local limitations, it is not always feasible to replicate this template. However, adjustments can be made to ensure physical separation and thereby, reduction in cross-contamination. This is not a one-size-fits-all model

but merely a guide that will help in understanding the layout of a laboratory where WGS work can be carried out.

Notably, good lab practices (GLP) ultimately define the level of success that contamination control can deliver. For example, on the template below it depends on:

1. How efficiently the unidirectional sample transfer and footfall of lab users is managed.
2. How clearly PPE is designated per room and how regularly it is cleaned.
3. How efficiently labware/reagents are stocked, sterilised and aliquoted exclusively for each separated area.

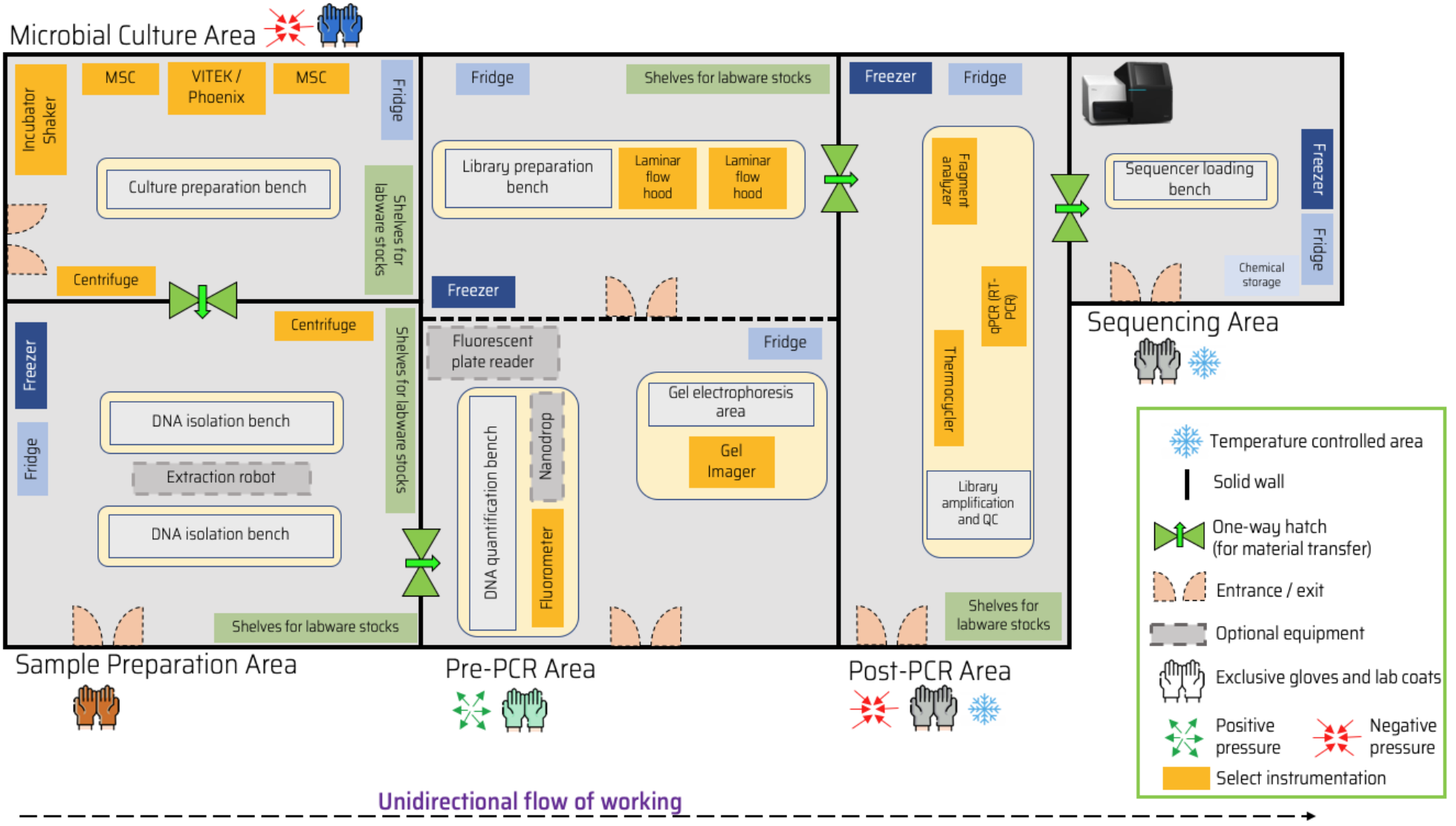

Figure 1. Ideal laboratory layout for an end-to-end bacterial genome sequencing setup.

Some of the more specific setup considerations and prioritisation of infrastructure will now be discussed within the questionnaire checklist below:

Infrastructure and Design checklist:

D.1.1. As described on Figure 1, separation needs to be maintained between the various working areas to avoid contamination. How likely is this a possibility to set up for your lab?

Note: Dimensions for individual equipment and benching can be discussed during the virtual face-to-face assessment.

- ❑ Very likely
- ❑ Very unlikely
- ❑ I need to discuss further

Details:

D.1.2. Do you have adequate area/room separation to ensure that a unidirectional workflow can be established (or at least one that doesn't involve traversing areas upstream)?

- ❑ Very likely
- ❑ Very unlikely
- ❑ I need to discuss further

Details:

D.1.3. Choose the level of feasibility for the following items considering your current laboratory arrangements.

Item	Already installed	Feasible, but not currently implemented	Not feasible, alternative needed
Cabled (network) internet connection			
Stable internet speeds			

(download and upload) of >20 Mbps			
Continuous A/C electricity supply			
Back-up generator during power outages			
At least 2 power sockets for each lab bench			
Immobilised or vibration-free lab benches			
Bench depth of at least 60 cm (23.6 in.)			
Bench strength for up to 120kg load			
Temperature and humidity control for labs (air-conditioning, heating or dehumidification)			
Dust-control for certain laboratory areas			
Refrigerators (2 to 10°C)			
Deep freezers fitted with temperature alarms (-15 to -25°C)			
Cryostorage fitted with temperature alarms (-80°C or below)			
Procurement system (for purchase orders and tenders)			
Space for room-temperature stock and inventory			
Inventory cataloguing system			
Unimpeded access to the lab for delivery and uncrating of equipment *			

*Instrument dimensions can be found on each manufacturer's website

D.1.4. Are there windows or any kind of exposure to direct heat/sunlight in your proposed post-PCR and MiSeq areas?

- ❑ Yes
- ❑ No

Details: _____

D.1.5. Based on the information above, are you able to provide clear photographs and a walk-through video of the available lab spaces for sample preparation, pre-PCR, post-PCR and sequencing?

- ❑ Yes
- ❑ No
- ❑ I need to discuss further

Details:

Skills Review:

A. Genomic DNA Isolation

The first step towards microbial WGS involves isolation of the double-stranded DNA (or circular genome) from the bacterial cell. This is achieved through disruption of the fortified cellular and nuclear wall, thereby releasing its contents into the surrounding solution. Following elution of purified DNA, samples are quantified and checked for purity. As shown above, genomic DNA isolation and its subsequent quality assessment are carried out within the sample preparation and pre-PCR areas.
A detailed list of the main labware and reagents needed to perform gDNA isolation can be found on the WGS Lab Toolkit.

Method checklist:

A.1.1. Have you performed genomic DNA extraction before?

❏ Yes
❏ No

If you answered Yes, continue to A.1.2. If not, skip to section A.2.

A.1.2. What suspension medium/broth is used to grow purified isolates prior to DNA extraction?

❏ BHI (Brain Heart Infusion) medium
❏ TSB (Tryptone Soya) medium
❏ Lysogeny broth
❏ Other Details: _____

A.1.3. Which of the following DNA extraction kits do you have prior experience with?

❏ Qiagen QIAamp DNA Mini Kit
❏ Qiagen DNeasy Kit
❏ Invitrogen PureLink Genomic DNA Purification Kit
❏ Promega Wizard Genomic DNA purification kit
❏ MPBio FastDNA Spin Kit
❏ ThermoFisher Scientific MagMax Nucleic Acid Isolation Kit
❏ Other Brand & Catalogue No: _____

A.1.4. List all of the laboratory equipment and plasticware used during your DNA extraction protocol.

Item	Manufacturer	Model / Catalogue No.	Application / Purpose	Ownership / regular access for use?
Centrifuge	Eppendorf	5801R	Flushing of spin-columns with reagents (vacuum-based filtration)	Owned by our group but is a shared resource across other institute members

A.1.5. How many bacterial samples are extracted per week using your method of choice?

_____

A.2.1. Have you performed genomic DNA QC before?

❑ Yes

❑ No

If you answered Yes, continue to A.2.2. If not, skip to section B.

A.2.2. What DNA quantification assays do you have prior experience with?

❑ Thermo Scientific Nanodrop

❑ ThermoFisher Qubit

❑ Promega QuantiFluor

❑ Other Brand & Catalogue No: _____

A.2.3. List all of the equipment and plasticware used during DNA quantification.

Item	Manufacturer	Model / Catalogue No.	Application / Purpose	Ownership / regular access for use?

A.2.4. How many bacterial samples are quantified per week using your method of choice?

_____

A.2.5. Do you record purity absorbance ratios for gDNA? (i.e. A260/280 and A260/230)

❑ Yes

❑ No

B. DNA Library Preparation

The purified DNA needs to be suitably modified to be read by the sequencer's optics and decoded into the order of nucleotides across the genome. DNA library preparation (containing an amplification step) is carried out across the pre-and post-PCR areas of the laboratory. A detailed list of the main labware and reagents needed to perform DNA Library Preparation can be found on the WGS Lab Toolkit.

Method checklist:

B.1.1. Do you have prior experience with operating a thermocycler instrument?

❑ Yes

❑ No

B.1.2. Do you have prior experience with purification or size-selection using paramagnetic beads (for eg. Ampure XP or SPRI beads)?

❑ Yes

❑ No

B.1.3. Do you have prior experience with preparing DNA libraries for Illumina-based WGS?

❑ Yes

❑ No

If you answered Yes, continue to B.1.4. If not, skip to section B.2.

B.1.4. Which of the following DNA library preparation kits do you have prior experience with?

❑ New England Biolabs NEBNext Ultra II DNA Library Prep Kit for Illumina®

❑ New England Biolabs NEBNext Ultra II FS DNA Library Prep Kit for Illumina®

❑ Illumina DNA Prep (previously Nextera XT or Nextera Flex)

❑ ThermoFisher Collibri DNA Library Prep kits for Illumina® systems
❑ Roche KAPA DNA Library Preparation kits for Illumina®
❑ Other Brand & Catalogue No: _____

B.2.1. Do you have prior experience with operating a fragment analyzer or electrophoresis?
❑ Yes
❑ No
If you answered Yes, continue to B.2.2. If not, skip to section B.3.

B.2.2. Which of the following electrophoresis systems do you have experience with?
❑ Agarose Gel Electrophoresis
❑ Agilent Bioanalyzer
❑ Agilent TapeStation
❑ Agilent Fragment Analyzer
❑ Agilent Femto Pulse
❑ Agilent ZAG DNA Analyzer
❑ Other Brand & Catalogue No: _____

B.2.3. Do you own and/or have regular access to this electrophoresis system?
Ownership:
❑ Yes
❑ No

Regular access:
❑ Yes
❑ No

B.3.1. Do you have prior experience with operating a real-time quantitative PCR (qPCR) instrument?
❑ Yes
❑ No
If you answered Yes, continue to B.3.2. If not, skip to section C.

B.3.2. Provide the brand and catalogue number of qPCR instrument used. Do you own it and/or have regular access to it?

_____

B.3.4. How many samples (excluding any assay controls) can be quantified on a single qPCR run in your lab using your preferred choice of kit?

_____

C. Whole-genome Sequencing

Short-read whole-genome sequencing using Illumina platforms works on the principle of sequencing-by-synthesis (SBS). The sequencing reaction involves amplification of each library read to form clonal clusters upon which modified deoxy-ribonucleotides (dNTP) are 3'-ligated simultaneously across millions of template molecules[1,2]. A terminator, bonded to the modified dNTPs, contains a unique fluorescent label that is detected by the sequencer optics to confirm when the correct base is incorporated[1,2].
For optimal operation, the sequencer should be housed either within its own enclosure or on a separate vibration-free benchtop, with the ambient temperature of the room between 18-25°C (and non-condensing relative humidity between 30-75%)[5]. A detailed list of the main labware and reagents needed to perform bacterial whole-genome sequencing using an Illumina MiSeq instrument can be found on the WGS Lab Toolkit.

Method checklist:

C.1.1. Do you have prior experience with initiating and operating an Illumina MiSeq instrument?

❑ Yes

❑ No

If you answered Yes, continue to C.1.2. If not, skip to the next chapter (Internal Process Review).

C.1.2. What MiSeq sequencing reagents have you had experience with?

- ❏ MiSeq v2 (50-cycle)
- ❏ MiSeq v2 (300-cycle)
- ❏ MiSeq v2 (500-cycle)
- ❏ MiSeq v3 (150-cycle)
- ❏ MiSeq v3 (600-cycle)

Internal Process Review (IPR):

Based on responses to the above questions and checklists, a customised IPR exercise will be designed to test your laboratory proficiency for process gaps that are essential and accessory to generating quality-assured genomes from bacterial isolates.

References:

1. Illumina Sequencing by Synthesis - YouTube. https://www.youtube.com/watch?v=fCd6B5HRaZ8.
2. Illumina Sequencing Technology - YouTube. https://www.youtube.com/watch?v=womKfikWlxM.
3. Mifflin, Theodore E. "Setting up a PCR Laboratory." CSH Protocols, vol. 2007, July 2007
4. Dieffenbach, C. W., and G. S. Dveksler. "Setting up a PCR Laboratory." PCR Methods and Applications, vol. 3, no. 2, Oct. 1993, pp. S2-7.
5. MiSeq System Site Prep Guide. https://emea.support.illumina.com/downloads/miseq_site_prep_guide_15027615.html.