Programme de surveillance des variants de la COVID-19 du Québec

Michel Roger M.D., Ph. D.

Laboratoire de santé publique du Québec

GenomeQuébec

Québec
Fonds de recherche – Nature et technologies
Fonds de recherche – Santé
Fonds de recherche – Société et culture

Institut national de santé publique Québec

La surveillance des variants du SRAS CoV-2 depuis le début de la pandémie

Début des analyses du génome viral en avril 2020 et jusqu'à maintenant

- Biobanque 120 000 échantillons de cas positifs
- Échantillons analysés : 7 000/250 000 (2,8 %)

Buts

1- Suivre les trajectoires d'introduction et de transmission du virus

2- Aider à l'investigation des éclosions

3- Confirmer les cas suspects de réinfections

4- Surveillance des variants

Institut national de santé publique Québec

Qu'est-ce qu'un variant d'intérêt SRAS CoV-2?

Erreur de réplication : une mutation par 10-15 jours

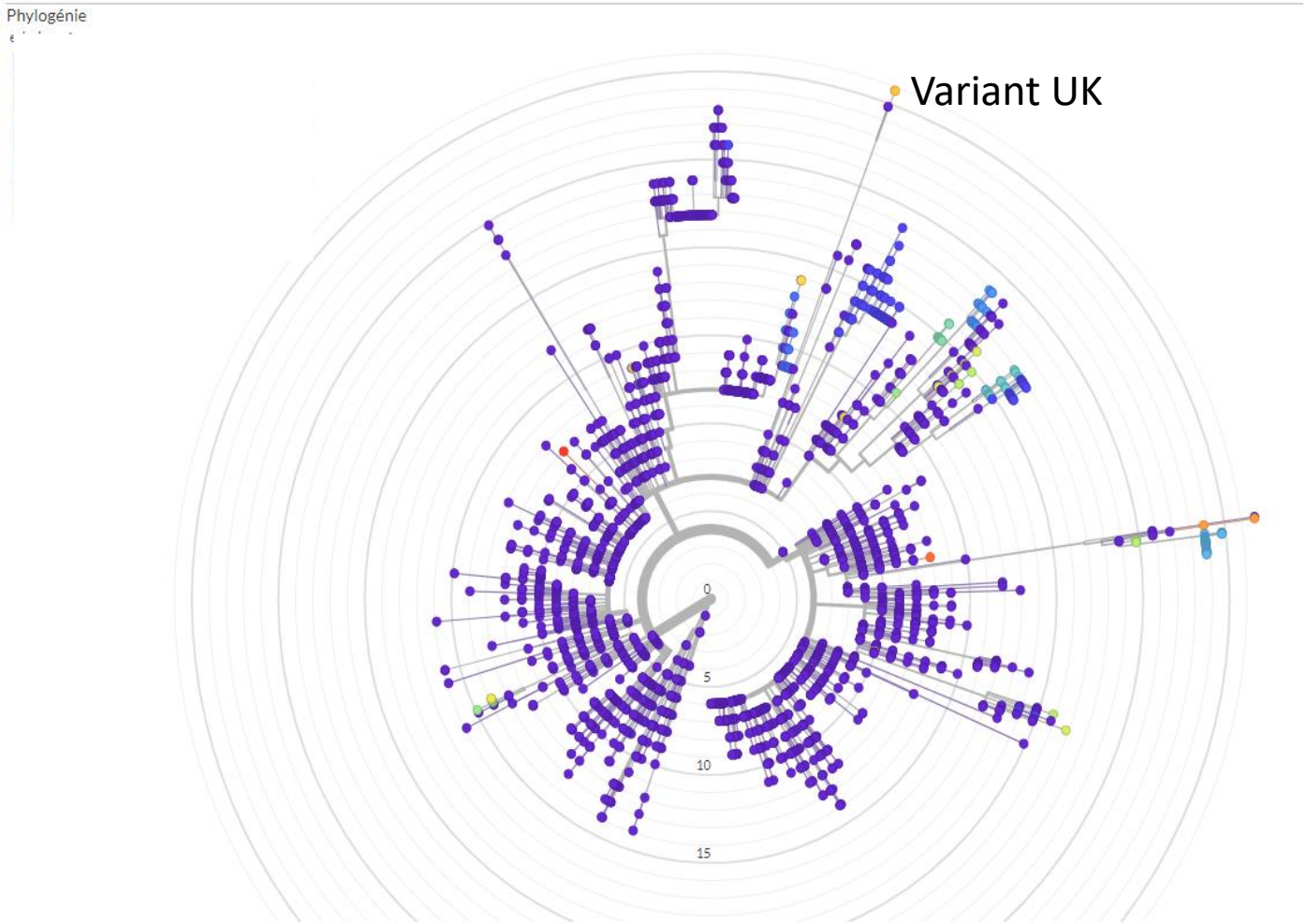
Les génomes des virus qui circulent ne sont pas tous identiques. Il y a donc plusieurs variants déjà existants au Québec

Variant d'intérêt :

- Virus qui possède un nombre de mutations anormalement élevé dans des gènes qui codent pour protéines importantes (ex. : spicule)
- Virus qui possède des mutations qui affectent la transmission, la virulence, l'efficacité d'un traitement ou d'un vaccin

Institut national de santé publique Québec

Le processus d'identification d'un variant



Institut national de santé publique Québec

Le programme de surveillance des variants du SRAS-CoV-2 au Québec en 2021

1- Augmenter la capacité de séquençage (3 % à 10 %) : 65 000 échantillons positifs en 2021

2- Séquençage aléatoire (toutes les régions) et ciblé pour augmenter les chances de trouver des variants

- Éclosions
- Tests diagnostiques discordants
- Voyageurs revenant d'une destination hors Canada
- Suspicion de réinfection
- Infection post-vaccination contre la COVID-19
- Cas de maladie grave de COVD-19

Institut national de santé publique Québec

Le programme de surveillance des variants du SRAS-CoV-2 au Québec en 2021

3- Études épidémiologiques (base de données de la Santé publique) pour identifier des variants d'intérêts

- Transmissibilité accrue ou sévérité de la maladie
- Échecs vaccinaux ou traitement
- Réinfections

4- Études fonctionnelles en collaboration avec FRQS et chercheurs pour caractériser l'impact des variants sur :

- Réplication virale, *fitness*
- Affinité pour le récepteur cellulaire et transmission modèles animaux
- Pathogenèse, échappement immunitaire, dérive antigénique
- Performance des tests diagnostics

Institut national de santé publique Québec

Les retombées potentielles de la surveillance des variants

- Recommandations de santé publique adaptées aux variants du virus en circulation pour la gestion de la pandémie (ex. : réinfections, super-transmetteurs, chaînes de transmission, éclosions, transmissibilité/morbidité accrue, etc.)
- Anticipation des demandes accrues auprès du réseau de la santé (hospitalisations, traitements, tests, par exemple) basée sur les critères de contagiosité/virulence/sévérité d'un nouveau variant circulant

Institut national de santé publique Québec

Les retombées potentielles de la surveillance des variants (suite)

- Vigie sur la performance des tests diagnostiques (tests d'amplification des acides nucléiques et sérologie); impact sur le diagnostic des cas et sur les études de séroprévalence
- Alignement fin de la stratégie de vaccination en fonction des variants en circulation si échappement de la réponse immune et dérive antigénique. Informer les compagnies pour modifier vaccins au besoin

Institut national de santé publique Québec

Institut national de santé publique Québec
Laboratoire de santé publique du Québec

Par courrier électronique

Le 22 janvier 2021

Aux responsables des laboratoires de microbiologie
Aux médecins microbiologistes infectiologues
Aux coordonnateurs techniques des laboratoires
Aux directeurs de santé publique
Aux codirecteurs OPTILAB

Objet : Priorisation des analyses de séquençage des échantillons SRAS-CoV-2 positifs

Madame, Monsieur,

Le Laboratoire de santé publique du Québec (LSPQ) coordonne le génotypage des isolats de SRAS-CoV-2 de cas de la COVID-19 confirmés en laboratoire afin de soutenir l'investigation des éclosions, de suivre la trajectoire de transmission du virus et de surveiller l'émergence de variants pouvant avoir un impact sur la transmissibilité, l'efficacité des vaccins contre la COVID-19 et la pathogénicité du virus. Un variant augmentant la contagiosité a d'abord été détecté au Royaume-Uni puis en Europe et dans le reste du monde. Au Canada, le Québec et plusieurs autres provinces ont identifié quelques cas porteurs de ce variant. À ce jour, deux autres variants d'intérêt ont été identifiés en Afrique du Sud et au Brésil.

Actuellement, le séquençage d'échantillons cliniques positifs aléatoire en routine sans critère *a priori* permet de suivre la transmission du virus au Québec et d'identifier les variants en circulation; à ce titre, une proportion de 5 % des échantillons déposés à la biobanque de Chicoutimi (CoVBanQ, Génome Québec) est sélectionnée pour séquençage systématique et sera augmentée à 10 % dans les prochaines semaines, assurant ainsi une meilleure couverture pour détecter des variants préoccupants de ce virus.

En complément de cette sélection aléatoire, une **sélection ciblée** de cas plus susceptibles de présenter des variants préoccupants est demandé aux laboratoires. Cette stratégie permettra d'investiguer plus rapidement certaines éclosions et d'identifier davantage les variants préoccupants connus, et d'autres qui pourraient émerger. À cette fin, nous demandons aux laboratoires de microbiologie effectuant les tests de dépistage COVID-19 d'**acheminer au LSPQ** plutôt qu'à la biobanque de Chicoutimi les échantillons qui répondent à l'un ou l'autre des critères ci-dessous :

1. **Éclosions de la COVID-19**

   Investigations pouvant aider à déterminer l'origine de l'infection et les liens entre les cas, à documenter un mode de propagation particulier ou à orienter les mesures de contrôle.

2. **Discordants pour le test diagnostique : cas positifs avec une des cibles amplifiables non détectée**

   Cette indication concerne les tests diagnostiques basés sur plusieurs cibles. Les échantillons demandés sont ceux dont l'une des cibles est positive franche (on exclut les faiblement positifs)

20045, chemin Sainte-Marie
Sainte-Anne-de-Bellevue (Québec) H9X 3R5
Téléphone : (514) 457-2070
Télécopieur : (514) 457-6346
www.inspq.qc.ca

et la ou les autres cibles ne sont pas détectées ou ont une Ct supérieure avec un écart inhabituel significatif. Les valeurs de cycle d'amplification dépendant de la plateforme, la valeur de l'écart significatif est laissée au jugement des laboratoires.

3. **Voyageurs revenant d'une destination hors Canada**

   Aucun pays n'est ciblé particulièrement, car des variants préoccupants pourraient être présents dans un pays sans que cela n'ait été détecté.

4. **Suspicion de réinfection**

   On considèrera les cas avec un 2e épisode 90 jours ou plus après le 1er épisode confirmé par un TAAN ou selon les critères du guide de l'INSPQ : Prise en charge des personnes considérées rétablies et présentant ultérieurement un test positif pour le SRAS-CoV-2

5. **Infection post-vaccination contre la COVID-19**

   On considèrera les cas confirmés en laboratoire survenus 14 jours ou plus après l'administration du vaccin (1re ou 2e dose). Ces cas doivent être signalés promptement du fait du risque théorique d'émergence de variants résistants à la vaccination.

6. **Cas de maladie COVID-19 grave nécessitant la ventilation mécanique chez les individus de moins de 50 ans sans comorbidité connue.**

De manière générale, nous vous demandons d'être attentifs à tout changement inhabituel et significatif dans le profil épidémiologique des cas (par exemple, un taux d'attaque inhabituel ou un changement dans les groupes d'âge touchés) et à les communiquer avec le LSPQ. Ceci pourrait également suggérer un nouveau variant.

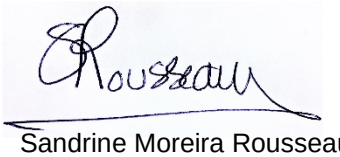
L'INSPQ met en place activement les outils pour le suivi des variants en circulation, l'analyse phylogénétique des lignées, l'identification des variants préoccupants existants et la détection de variants émergents incluant ceux localisés dans les amorces connues. Un service d'analyse génomique en contexte d'éclosion est également disponible. Un processus de communication des séquences et des variants identifiés vers les laboratoires est en cours d'élaboration et sera communiqué ultérieurement.

Pour plus d'information, veuillez consulter le guide de service du LSPQ sous la rubrique **Séquençage du génome entier (SGE) du SRAS-CoV-2**.

Il est très important de fournir avec la requête analytique, les informations cliniques requises.

Veuillez accepter, Madame, Monsieur, nos salutations les meilleures.

Michel Roger MD, Ph. D, FRCPC
Directeur médical



Sandrine Moreira Rousseau Ph. D
Conseillère scientifique spécialisée
Projet de surveillance génomique COVID-19

Séquençage du génome entier (SGE) du SRAS-CoV-2

Code MSSS : à venir

Révisé le 2021-02-12

Indications

Les situations suivantes sont jugées prioritaires pour le SGE du SRAS-CoV-2 :

1. Éclosion de la COVID-19, après entente avec le LSPQ; les critères suivants de justification sont proposés :
   - résultats de SGE pouvant aider à déterminer l'origine de l'infection et les liens entre les cas, à documenter un mode de propagation particulier ou à orienter les mesures de contrôle;
   - milieu prioritaire (notamment ceux de vie fermés et les établissements de soins);
   - cas chez des personnes vulnérables pour l'acquisition de l'infection ou d'atteinte grave (âgées de ≥65 ans ou avec comorbidités);
   - cas chez des travailleurs de la santé;
   - ampleur de l'événement (nombre élevé de cas, éclosion suprarégionale ou répercussion sur d'autres milieux);
   - éclosion liée à un ou plusieurs cas ayant acquis l'infection lors de voyage à l'extérieur du Canada;
   - éclosion dans un milieu où la vaccination contre la COVID-19 a été administrée (cas survenus >14 jours après la 1ière ou 2e dose de vaccin.

   Le formulaire : Demande de séquençage du génome entier (SGE) des souches de SRAS-Cov-2 en contexte d'éclosion de la COVID-19 doit accompagner la demande (à adresser à eclosioncovid19genomique@inspq.qc.ca).

   Une fois l'entente conclue, la liste nominale des cas inclus doit être acheminée de manière sécurisée selon un format et les modalités définis par le LSPQ. Une sélection des cas peut être suffisante pour les éclosions avec un nombre élevé de cas.

2. Réinfection suspectée de la COVID-19; les critères suivants sont proposés :
   On considèrera les cas avec un 2e épisode 90 jours ou plus après le 1er épisode confirmé par un TAAN ou selon les critères du guide l'INSPQ. https://www.inspq.qc.ca/publications/3032-personnes-retablies-nouveau-test-positif-covid19

3. Cas de la COVID-19 chez un voyageur à l'extérieur du Canada. Le pays visité devra être inscrit dans la PHAGE.

4. Résultat discordant pour un test diagnostic multiplex Les échantillons ciblés sont ceux dont l'une des cibles est positive franche (on exclut les faiblement positifs) et la ou les autres cibles

ne sont pas détectées ou ont une Ct supérieure avec un écart inhabituel significatif. Les valeurs de cycle d'amplification dépendant de la plateforme, la valeur de l'écart significatif est laissée au jugement des laboratoires. Le nom de la trousse ainsi que la cible discordante devront être inscrits dans la PHAGE.

5. Cas de la COVID-19 suite à la vaccination contre cette infection, survenu >14 jours après la 1ière ou 2e dose de vaccin.). Le nom du vaccin, le nombre de doses ainsi que la date d'administration devront être inscrits dans la PHAGE.

6. Cas de maladie COVID-19 grave nécessitant ventilation mécanique chez les individus de moins de 50 ans sans comorbidité connue.

Spécimens

- Échantillons cliniques positifs pour la détection d'acides nucléiques du SRAS-CoV-2.

Pour les réinfections et échec vaccinal seulement, veuillez acheminer (**en plus de l'échantillon clinique RT-PCR positif**) :

- Réinfections : Plasma prélevé lors de l'épisode initial (ou précédant le nouvel épisode) si disponible, à la survenue du nouvel épisode (phase aiguë), et 14 jours plus tard (phase convalescence). L'analyse d'une paire d'échantillons est fortement recommandée puisqu'il s'agit d'un test de séroconversion.
  Échec vaccinal : Plasma prélevé en phase aiguë

Critères de rejet

À **l'exception des cas suspects de réinfection**, les échantillons positifs pour lesquels la **charge virale est faible** (Ct ≥30 ou l'équivalent) **sont exclus**.

Généralement, seuls les échantillons positifs pour lesquels une proportion suffisante de la séquence du génome peut être reconstituée sont inclus dans les analyses.

Manipulation, conservation et expédition des spécimens

Ces échantillons positifs doivent être expédiés au LSPQ plutôt qu'à la bio-banque de Génome Québec (à Chicoutimi).

Échantillons respiratoires : un volume de 1 ml d'échantillon est idéal.

Les échantillons primaires peuvent être conservés au réfrigérateur et expédiés dans un colis contenant des sachets de glace (*ice-packs*) s'ils sont reçus au LSPQ < de 72 heures après le prélèvement. Sinon, ils doivent être congelés à - 80 °C et expédiés sur glace sèche.

Si une portion aliquote est envoyée, utiliser un tube conforme (tube en polypropylène, capuchon étanche, joint d'étanchéité) et l'expédier congelée sur glace sèche.

Plasma **et PBMC (réinfection et échec vaccinal seulement)** : un volume de 7 ml de sang total tube lavande, séparer le plasma et PBM.

Les échantillons de plasma et PBMC doivent être congelés à - 80 °C et expédiés au LSPQ sur glace sèche.

Conseils généraux :

Éviter les cycles de congélation-décongélation. Garder le moins longtemps possible à température pièce lors du prélèvement de la partie aliquote.

Transport : les spécimens prélevés de patients chez qui l'on suspecte une infection au SRAS-CoV-2 sont considérés comme des matières infectieuses de la catégorie B, UN 3373 pour le transport.

Le LSPQ peut recevoir des échantillons en tout temps (24/7). Il est donc souhaitable de procéder à l'envoi d'échantillons dès que possible afin de minimiser les délais.

Requête d'analyse

Chaque échantillon positif doit être accompagné d'une requête PHAGE, sélectionner

- Coronavirus (SARS-CoV-2, COVID-19) : séquençage prioritaire

Méthodes utilisées

SGE, analyse de variants par rapport à la souche de référence Wuhan-Hu-1, analyse phylogénétique et cartographie temporelle des cas (au besoin).

Sérologie pour les cas suspects de réinfection et échec vaccinal seulement, Détection des anticorps avec test CLIA RBD IgM/IgG sur une paire d'échantillons prélevés en phase aiguë et en phase de convalescence, 2 semaines après le premier échantillon.

IMPORTANT : Analyse sérologique réalisée à titre expérimental par un laboratoire de recherche au CHUM pour des fins de surveillance. Aucun rapport ne sera émis

Limites de l'épreuve

- La qualité de la séquence dépend de la qualité du spécimen prélevé et de sa bonne conservation.
- Le chauffage des spécimens en réduit la qualité.
- En général, la méthode ne permet pas de séquencer les échantillons avec faible charge virale.

Temps réponse

21 jours ouvrables après la réception des échantillons au LSPQ.

Commentaires

Les demandeurs sont invités à contacter la responsable de l'analyse ou une des personnes ressources pour les situations particulières.

Le rapport d'analyse, incluant l'interprétation des résultats, est acheminé aux demandeurs.

Personnes contacts

Personnel technique: Martine Morin, tél. : (514) 457-2070, poste 2286

Sandrine Moreira, Ph.D., Génomique et Bio-informatique, tél. : (514) 457-2070 poste 2227, courriel : sandrine.moreira@inspq.qc.ca

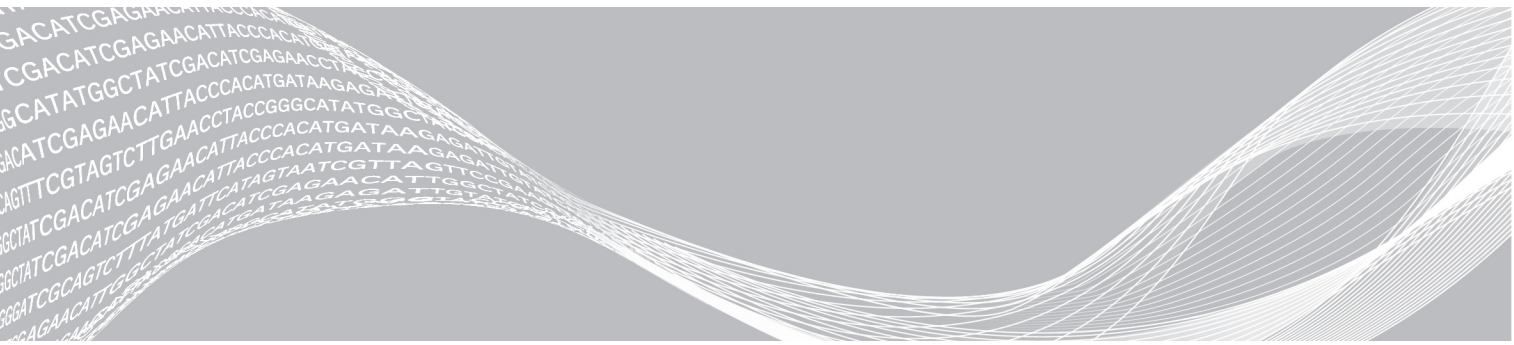
Judith Fafard, M.D., médecin microbiologiste-infectiologue-conseil, tél. : (514) 457-2070 poste 2228, courriel : judith.fafard@inspq.qc.ca

Réjean Dion, M.D., médecin-conseil en santé publique, tél. : (514) 457-2070 poste 2325, courriel : rejean.dion@inspq.qc.ca

illumina®

Illumina COVIDSeq Test

Reference Guide



Document # 1000000126053 v02

ILLUMINA PROPRIETARY

July 2020

For Research Use Only. Not for use in diagnostic procedures.

Revision History

Document	Date	Description of Change
Document # 1000000126053 v02	July 2020	Added instructions for extracting RNA using the Quick-DNA/RNA Viral Magbead kit. Added safe stopping point after pooling and cleaning up libraries. Updated index kit configurations to IDT for Illumina-PCR Indexes. Removed sequencing instructions. Added dilution and sequencing preparation instructions for the NovaSeq 6000 Sequencing System SP flow cell, NextSeq 500 Sequencing System, NextSeq 550 Sequencing System, and NextSeq 550Dx Instrument. Moved data analysis information to *Illumina COVIDSeq Test Pipeline Software Guide document # 1000000128122*.
Document # 1000000126053 v01	June 2020	No content changes.
Document # 1000000126053 v00	June 2020	Initial release.

Table of Contents

Chapter 1 Overview

Introduction

This guide explains how to detect the SARS-CoV-2 virus using the Illumina COVIDSeq Test.

The Illumina COVIDSeq Test offers:

- RNA extraction from decontaminated nasopharyngeal (NP), oropharyngeal (OP), and nasal swab samples, as well as mid-turbinate specimens collected from individuals who meet COVID-19 clinical or epidemological criteria, using the QIAamp Viral RNA Mini Kit or Quick-DNA/RNA Viral Magbead Kit.
- Preparation of up to 3072 samples for high-throughput sequencing using the NovaSeq 6000 Sequencing System or up to 384 samples using the NextSeq 500/550 Sequencing Systems or NextSeq 550Dx Instrument in RUO mode.
- Qualitative detection of SARS-CoV-2 RNA using the Illumina DRAGEN COVIDSeq Test Pipeline locally or on BaseSpace Sequence Hub using the Illumina DRAGEN COVIDSeq Test app.

Input Recommendations

The Illumina COVIDSeq Test supports patient samples derived from nasopharyngeal (NP), oropharyngeal (OP), and nasal swabs. Transport samples according to the governing regulations for the transport of etiologic agents applicable to your region.

Store samples according to the instructions from the manufacturer. Exceeding the storage times can negatively impact test results.

The following sample factors might affect SARS-CoV-2 detection:

- Sample collection methods, patient factors, and/or the stage of the infection.
- Viral RNA degradation during shipping and storage. RNA degradation can produce false-negative results.

CAUTION

Handle all specimens as infectious reagents.

Chapter 2 Library Prep

Introduction

This chapter describes library preparation using the Illumina COVIDSeq Test.

- Confirm kit contents and make sure that you have the required equipment and consumables. See on page 17.
- Follow the protocols in the order shown, using the specified volumes and incubation parameters.
- Make sure reagents are not expired. Using expired reagents might negatively affect performance.
- Do not allow multiple freeze-thaw cycles for CPC HT. If performing library prep multiple times, aliquot CPC HT into low-bind tubes, and then store at -85°C to -65°C.
- Do not allow more than 8 freeze-thaw cycles for all reagents, excluding CPC HT.
- Include one no template control (NTC) and one positive control per 96-well plate. The internal process control is included in the Illumina COVIDSeq Test.
- Sequence libraries as soon as possible after pooling. Pooled libraries are stable for up to 30 days at -25°C to -15°C.

Tips and Techniques

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Avoiding Contamination

- Use proper laboratory practices to prevent nuclease and PCR product contamination. Nuclease and PCR product contamination can cause inaccurate and unreliable results.
- Perform library preparation in a RNase/DNase-free environment. Thoroughly decontaminate work areas with a RNAse/DNase-inhibiting solution, such as RNAseZap and DNAzap.
- Use fresh tips and fresh consumable labware between samples and dispensing reagents.
- Use aerosol-resistant tips to reduce the risk of carry-over and sample-to-sample cross-contamination.
- Due to the potential for contamination, take extreme care to make sure that well contents remain fully in the well. Do not splash contents.
- Do not use aerosol bleach sprays when performing library preparation. Trace bleach contamination can lead to assay failure.
- Use a unidirectional workflow when moving from pre-amplification to pre-amplification environments.

Sealing and Unsealing the Plate

- Always seal the 96-well plate before the following steps in the protocol:
  - Shaking steps
  - Vortexing steps
  - Centrifuge steps
  - Thermal cycling steps
- To seal the plate, apply the adhesive cover to the plate and then seal with a wedge or rubber roller.
- Make sure the edges and wells are completely sealed to reduce the risk of cross-contamination and evaporation.
- Microseal 'B' adhesive seals are effective at -40°C to 110°C, and suitable for skirted or semiskirted PCR plates. Use Microseal 'B' for shaking, centrifuging, and long-term storage.
- Before unsealing:
  - Briefly centrifuge the 96-well plate at 1000 × g for 1 minute. For bead steps, centrifuge at 500 x g for 1 minute.
  - Place the plate on a flat surface before slowly removing the seal.

Plate Transfers

- When transferring volumes between plates, transfer the specified volume from each well of a plate to the corresponding well of the other plate.
- If beads are aspirated into the pipette tips, dispense back to the plate on the magnetic stand and wait until the liquid is clear (~2 minutes).

Centrifugation

- Centrifuge as needed at any step in the procedure to consolidate liquid or beads in the bottom of the well, and to prevent sample loss.

Handling Beads

- Pipette bead suspension slowly to prevent splashing and bubbles.
- When mixing, mix thoroughly.
- To avoid sample loss, confirm that no beads remain in pipette tips after resuspension and mixing steps.
- When washing beads:
  - Use the appropriate magnet for the plate.
  - Dispense liquid so that beads on the side of the wells are wetted.
  - Keep the plate on the magnet until the instructions specify to remove it.
  - Do not agitate the plate while on the magnetic stand. Do not disturb the bead pellet.

Extract RNA

This step extracts RNA from decontaminated viral transport medium tubes. You can extract RNA using the Quick-DNA/RNA Viral MagBead, Zymo Research, part # R2141 or the QIAamp Viral RNA Mini Kit, Qiagen, part # 5290. Follow the procedure corresponding to your extraction method.

Consumables

- ELB HT (Elution Buffer HT)
- CPC HT (COVIDSeq Positive Control HT)
- 1.7 ml LoBind tubes
- 5 ml LoBind tubes
- 15 ml tubes
- [Quick-DNA/RNA Viral MagBead] 2000 µl 96 deep well plate

About Reagents

- Aliquot CPC HT into low-bind tubes. Store at -85°C to -65°C
- Vortex before each use

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
ELB HT	2°C to 8°C	Thaw at room temperature, and then invert to mix. Keep on ice until use.
CPC HT	-85°C to -65°C	Dilute to 5 copies per µl using the following instructions. Keep diluted positive control on ice.

2 Dilute CPC HT as follows.

a Label a 1.7 ml tube Dilution 1.
b Add the following volumes to the tube *in the order listed*.
- CPC HT (5 µl)
- ELB HT (495 µl)

These volumes produce 10000 copies per µl.
c Pulse vortex to mix.

3 Dilute CPC HT a second time as follows.

a Label a 1.7 ml tube Dilution 2.
b Add the following volumes to the tube *in the order listed*.
- Dilution 1 (5 µl)
- ELB HT (495 µl)

These volumes produce 100 copies per µl.
c Pulse vortex to mix.

4 Dilute CPC HT a third time as follows.

a Label a 15 ml tube Dilution 3.
b Add the following volumes to the tube *in the order listed*.
- Dilution 2 (200 µl)
- ELB HT (3.8 ml)

These volumes produce 5 copies per µl.

c Pulse vortex to mix.

Quick-DNA/RNA Viral MagBead Procedure

1 For each sample, add 400 µl patient sample to a new deep-well plate. For every 94 samples, include one tube of dilution 3 CPC HT (positive control) and ELB HT (no template control).

2 To extract RNA, use the Quick-DNA/RNA Viral MagBead. For information, see *Quick-DNA/RNA Viral MagBead Instruction Manual* from Zymo Research.
Use the following protocol options:
- Before adding MagBinding Beads, pipette up and down ten times to mix.
- After adding 20 µl MagBinding Beads, pipette up and down ten times to mix, and then shake at 1500 rpm for 10 minutes.

QIAamp Viral RNA Mini Kit Procedure

1 For each sample, add 140 µl patient sample to new 1.7 ml microcentrifuge tube. For every 94 samples, include one tube of dilution 3 CPC HT (positive control) and ELB HT (no template control).

2 To extract RNA, use the QIAamp Viral RNA Mini Kit. For information, see *QIAmp Viral RNA Mini Handbook (document # HB-0354-006)* available on the QIAGEN website.
Use the following protocol options:
- Purify viral RNA using the spin protocol.
- Incubate elution for at least 1 minute.
- Elute in 30 µl Buffer AVE instead of 60 µl.

Anneal RNA

During this process the extracted RNA is annealed using random hexamers to prepare for cDNA synthesis.

Consumables

- EPH3 HT (Elution Prime Fragment 3HC Mix)
- 96-well PCR Plate
- Microseal 'B' adhesive seals

About Reagents

- Vortex before each use

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
EPH3 HT	-25°C to -15°C	Thaw at room temperature, and then invert to mix.

2 Save the following COVIDSeq Anneal program on the thermal cycler:
- Choose the preheat lid option
- Set the reaction volume to 17 µl
- 65°C for 3 minutes
- Hold at 4°C

Procedure

1 Label new PCR plate CDNA1.

2 Add 8.5 µl EPH3 HT to each well.

3 Add 8.5 µl eluted sample to each well.

4 Seal and shake at 1600 rpm for 1 minute.

5 Centrifuge at 1000 × g for 1 minute.

6 Place on the preprogrammed thermal cycler and run the COVIDSeq Anneal program.

Synthesize First Strand cDNA

This step reverse transcribes the RNA fragments primed with random hexamers into first strand cDNA using reverse transcriptase.

Consumables

- FSM HT (First Strand Mix HT)
- RVT HT (Reverse Transcriptase HT)
- 1.7 ml tubes (1 per 96-well sample plate)
- Microseal 'B' adhesive seal

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
FSM HT	-25°C to -15°C	Thaw and bring to room temperature. Invert to mix, and then keep on ice.
RVT HT	-25°C to -15°C	Invert to mix before use. Keep on ice.

2 Save the following COVIDSeq FSS program on the thermal cycler:
   - Choose the preheat lid option
   - Set the reaction volume to 25 µl
   - 25°C for 5 minutes
   - 50°C for 10 minutes
   - 80°C for 5 minutes
   - Hold at 4°C

Procedure

1 In a 1.7 ml tube, combine the following volumes to prepare First Strand cDNA Master Mix. Multiply each volume by the number of samples.
   - FSM HT (9 µl)
   - RVT HT (1 µl)

   Reagent overage is included to account for small pipetting errors.

2 Add 8 µl master mix to each well of the CDNA1 plate.

3 Seal and shake at 1600 rpm for 1 minute.

4 Centrifuge at 1000 × g for 1 minute.

5 Place on the preprogrammed thermal cycler and run the COVIDSeq FSS program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 7 days.

Amplify cDNA

This step uses two separate PCR reactions to amplify cDNA.

Consumables

- IPM HT (Illumina PCR Mix HT)
- CPP1 HT (COVIDSeq Primer Pool 1 HT)
- CPP2 HT (COVIDSeq Primer Pool 2 HT)
- Nuclease-free water
- 15 ml tube (2 for four 96-well sample plates)
- 96-well PCR plates (3)
- Microseal 'B' adhesive seal

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
CPP1 HT	-25°C to -15°C	Thaw at room temperature. Keep on ice until use.
CPP2 HT	-25°C to -15°C	Thaw at room temperature. Keep on ice until use.
IPM HT	-25°C to -15°C	Thaw at room temperature, and then invert to mix. Keep on ice until use.

2 Save the following COVIDSeq PCR program on the thermal cycler:
- Choose the preheat lid option
- Set the reaction volume to 25 µl
- 98°C for 3 minutes
- 35 cycles of:
  - 98°C for 15 seconds
  - 65°C for 5 minutes
- Hold at 4°C

Procedure

1 Label two new PCR plates COV1 and COV2.
The plates represent two separate PCR reactions on each sample and control in the CNDA1 plate.

2 In a 15 ml tube, combine the following volumes to prepare COVIDSeq PCR 1 Master Mix and COVIDSeq PCR 2 Master Mix. Multiply each volume by the number of samples.

Reagent	COVIDSeq PCR 1 Master Mix (µl)	COVIDSeq PCR 2 Master Mix (µl)
IPM HT	15	15
CPP1 HT	4.3	N/A
CPP2 HT	N/A	4.3
Nuclease-free water	4.7	4.7

Reagent overage is included to account for small pipetting errors.

3 Add 20 µl COVIDSeq PCR 1 Master Mix to each well of the COV1 plate corresponding to each well of the CDNA1 plate.

4 Add 5 µl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV1 plate.

5 Add 20 µl COVIDSeq PCR 2 Master Mix to each well of the COV2 plate corresponding to each well of the CDNA1 plate.

6 Add 5 µl first strand cDNA synthesis from each well of the CDNA1 plate to the corresponding well of the COV2 plate.

7 Seal and shake at 1600 rpm for 1 minute.

8 Centrifuge at 1000 x g for 1 minute.

9 Place in the preprogrammed thermal cycler and run the COVIDSeq PCR program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 3 days.

Tagment PCR Amplicons

This step uses EBLTS HT to tagment PCR amplicons, which is a process that fragments and tags the PCR amplicons with adapter sequences.

Consumables

- EBLTS HT (Enrichment BLT HT)
- TB1 HT (Tagmentation Buffer 1 HT)
- Nuclease-free water
- 1.7 ml tube
- 15 ml tube (1 per four 96-well sample plates)
- Microseal 'B' adhesive seal

About Reagents

- Store EBLTS HT upright at temperatures above 2°C. Make sure beads are always submerged in the buffer.
- If beads are adhered to the side or top of the 96-well plate, centrifuge at 500 × g for 1 minute, and then pipette to resuspend.

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
EBLTS HT	2°C to 8°C	Bring to room temperature. Vortex thoroughly before use.
TB1 HT	-25°C to -15°C	Bring to room temperature. Vortex thoroughly before use.

2 If COV1 and COV2 plates were stored frozen, prepare as follows.

a Thaw at room temperature.
b Check seals, and then shake at 1600 rpm for 1 minute.
c Centrifuge at 1000 x g for 1 minute.

3 Save the following COVIDSeq TAG program on the thermal cycler:
- Choose the preheat lid option
- Set the reaction volume to 50 µl
- 55°C for 5 minutes
- Hold at 10°C

Procedure

1 Label a new PCR plate TAG1.

2 Combine COV1 and COV2 as follows.

a Transfer 10 µl from each well of the COV1 plate to the corresponding well of the TAG1 plate.
b Transfer 10 µl from each well of the COV2 plate to each well of the TAG1 plate containing COV1.

3 In a 15 ml tube, combine the following volumes to prepare Tagmentation Master Mix. Multiply each volume by the number of samples.
- TB1 HT (12 µl)
- EBLTS HT (4 µl)
- Nuclease-free water (20 µl)

4 Add 30 µl master mix to each well in TAG1 plate.

5 Seal and shake at 1600 rpm for 1 minute.

6 Place on the preprogrammed thermal cycler and run the COVIDSeq TAG program.

Post Tagmentation Clean Up

This step washes the adapter-tagged amplicons before PCR amplification.

Consumables

- ST2 HT (Stop Tagment Buffer 2 HT)
- TWB HT (Tagmentation Wash Buffer HT)
- Microseal 'B' adhesive seal

About Reagents

- Dispense ST2 HT and TWB HT slowly to minimize foaming.

- Dispense TWB HT directly onto beads.

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
ST2 HT	Room temperature	Vortex before use.
TWB HT	2°C to 8°C	Vortex before use.

Procedure

1 Centrifuge the TAG1 plate at 500 x g for 1 minute.

2 Add 10 µl ST2 HT to each well of the TAG1 plate.

3 Seal and shake at 1600 rpm for 1 minute.

4 Incubate at room temperature for 5 minutes.

5 Centrifuge at 500 × g for 1 minute.

6 Place on the magnetic stand and wait until the liquid is clear (~3 minutes).

7 Inspect for bubbles on the seal. If present, centrifuge at 500 x g for 1 minute, and then place on the magnetic stand (~3 minutes).

8 Remove and discard all supernatant.

9 Wash beads as follows.

   a Remove from the magnetic stand.
   b Add 100 µl TWB HT to each well.
   c Seal and shake at 1600 rpm for 1 minute.
   d Centrifuge 500 × g for 1 minute.
   e Place on the magnetic stand and wait until the liquid is clear (~3 minutes).
   f For first wash only, remove and discard all supernatant from each well.

10 Wash beads a second time.
Leave supernatant in plate for second wash to prevent beads from overdrying.

Amplify Tagmented Amplicons

This step amplifies the tagmented amplicons using a PCR program. The PCR step adds prepaired 10 base pair Index 1 (i7) adapters, Index 2 (i5) adapters, and sequences required for sequencing cluster generation.

Consumables

- EPM HT (Enhanced PCR Mix HT)
- Index adapters (IDT for Illumina-PCR Indexes Set 1, 2, 3, 4)
- Nuclease-free water
- 15 ml tubes (1 per two 96-well sample plates)
- 96-well PCR plate

About Reagents

- Index adapter plates
  - Do not add samples to the index plate wells.
  - Index plate wells cannot be reused.

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
EPM HT	-25°C to -15°C	Invert to mix. Keep on ice until use.
Index adapters	-25°C to -15°C	Thaw at room temperature. Vortex to mix, and then centrifuge at 1000 × g for 1 minute.

2 Open each prepared index adapter plate seal as follows. Use a new PCR plate for each different index set.

a Align a new 96-well PCR plate above the index adapter plate, and then press down to puncture the foil seal.
b Discard the PCR plate.

3 Save the following COVIDSeq TAG PCR program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- Set the reaction volume to 50 µl
- 72°C for 3 minutes
- 98°C for 3 minutes
- 7 cycles of:
  - 98°C for 20 seconds
  - 60°C for 30 seconds
  - 72°C for 1 minute
- 72°C for 3 minutes
- Hold at 10°C

Procedure

1 In a 15 ml tube, combine the following volumes to prepare PCR Master Mix. Multiply each volume by the number of samples.
- EPM HT (24 µl)
- Nuclease-free water (24 µl)

2 Vortex PCR Master Mix to mix.

3 Keep the TAG1 plate on magnetic stand and remove TWB HT.

4 Use a 20 µl pipette to remove any remaining TWB HT.

5 Remove the TAG1 plate from the magnetic stand.

6 Add 40 µl PCR Master Mix to each well.

7 Add 10 µl index adapters to each well of the PCR plate.

8 Seal and shake at 1600 rpm for 1 minute.

9 If liquid is visible on the seal, centrifuge at 500 x g for 1 minute.

10 Inspect to make sure beads are resuspended. To resuspend, set your pipette to 35 µl with the plunger down, and then slowly pipette to mix.

11 Place on the preprogrammed thermal cycler and run the COVIDSeq TAG PCR program.

Pool and Clean Up Libraries

This step combines libraries from each 96-well sample plate into one 1.7 ml tube. Libraries of optimal size are then bound to magnetic beads, and fragments that are too small or large are wash away.

Consumables

- ITB (Illumina Tune Beads)
- RSB HT (Resuspension Buffer HT)
- Freshly prepared 80% ethanol (EtOH)
- 1.7 ml tube (2 per 96-well sample plate)
- PCR 8-tube strip

About Reagents

- ITB
  - Vortex before each use.
  - Vortex frequently to make sure that beads are evenly distributed.
  - Aspirate and dispense slowly due to the viscosity of the solution.

Preparation

1 Prepare the following consumables:

Reagent	Storage	Instructions
ITB	Room temperature	Vortex thoroughly to mix.
RSB HT	2°C to 8°C	Let stand for 30 minutes to bring to room temperature. Vortex and invert to mix.

2 Prepare 80% EtOH from absolute EtOH.

Procedure

1 Centrifuge at 500 × g for 1 minute.

2 Place on the magnetic stand and wait until the liquid is clear (~3 minutes).

3 To pool libraries, do as follows. Repeat the steps for each additional sample plate.

   a Use a 20 µl eight-channel pipette to transfer 5 µl library from each well of the PCR plate to a PCR 8-tube strip. Change tips after each column.

   These volumes result in 60 µl pooled library per row.

   b Label a new 1.7 ml tube Pooled ITB.

   c Transfer 55 µl pooled library from each well of the PCR 8-tube strip into the Pooled ITB tube.

   For each sample plate, these volumes results in 440 µl pools of pooled libraries.

   If processing 3072 samples, these steps result in 32 Pooled ITB tubes.

4 Vortex the Pooled ITB tubes to mix, and then centrifuge briefly.

5 Vortex ITB to resuspend.

6 Add ITB using the resulting volume of Pooled ITB tube volume multiplied by 0.9.
For example, for 96 samples, add 396 µl ITB to each tube.

7 Vortex to mix.

8 Incubate at room temperature for 5 minutes.

9 Centrifuge briefly.

10 Place on the magnetic stand and wait until the liquid is clear (~5 minutes).

11 Remove and discard all supernatant.

12 Wash beads as follows.

   a Keep on the magnetic stand and add 1000 µl fresh 80% EtOH to each tube.
   b Wait 30 seconds.
   c Remove and discard all supernatant.

13 Wash beads a **second** time.

14 Use a 20 µl pipette to remove all residual EtOH.

15 Add 55 µl RSB HT.

16 Vortex to mix, and then centrifuge briefly.

17 Incubate at room temperature for 2 minutes.

18 Place on the magnetic stand and wait until the liquid is clear (~2 minutes).

19 Transfer 50 µl supernatant from each Pooled ITB tube to a new microcentrifuge tube.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 30 days.

Quantify and Normalize Libraries

1 Analyze 2 µl library pool using a Qubit dsDNA HS Assay kit.
If libraries are outside the standard range, dilute to 1:10 concentration, and analyze again.

2 Calculate the molarity value using the following formula.
   ▶ Use 400 bp as the average library size.

$$\frac{Library\ concentration\ ng/\mu l}{660\ \frac{g}{mol}\ x\ average\ library\ size\ (bp)} x10^6 = Molarity\ (nM)$$

3 Dilute each library pool to a minimum of 30 µl at a normalized concentration 4 nM using RSB HT.

Pool and Dilute Libraries

This step pools and dilutes libraries to the starting concentration for your sequencing system. After diluting to the starting concentration, libraries are ready to be denatured and diluted to the final loading concentration.

1 For each set of 384 samples, combine 25 µl of each normalized pool containing index adapter set 1, 2, 3, 4 in a new microcentrifuge tube. Do not combine pools with the same index adapter set.
This step produces a final pool of 384 samples diluted to a starting concentration of 4 nM. For each sequencing system, the following are the number of samples required per flow cell.
   - NextSeq 500/550 or 550Dx: 384 samples per flow cell.
   - NoveSeq 6000 system SP flow cell: 384 samples per lane and 768 total samples per flow cell.
   - NovaSeq 6000 system S4 flow cell: 384 samples per lane and 1536 total samples per flow cell.

2 Follow the denature and dilute instructions for your system to dilute to the final loading concentration.
   - For the NextSeq 500/550 Sequencing System and NextSeq 550Dx Sequencing System, see the *NextSeq System Denature and Dilute Libraries Guide (document # 15048776)*.
   - For the NovaSeq 6000 Sequencing System, see the *NovaSeq 6000 Denature and Dilute Libraries Guide (document # 1000000106351)*.

3 Use the following loading concentrations for your system.

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
NextSeq 500/550 or 550Dx	4	1.4
NovaSeq 6000 SP Flow Cell	4	100
NovaSeq 6000 S4 Flow Cell	4	100

The final loading concentrations are a starting point and general guideline. Optimize concentrations for your workflow over subsequent sequencing runs.

Prepare for Sequencing

The Illumina COVIDSeq Test is compatible with the NovaSeq 6000 Sequencing System SP and S4 flow cells, the NextSeq 500/550 Sequencing Systems, and the NextSeq 550Dx instrument.

After sequencing is complete, perform analysis on your system using the Illumina DRAGEN COVIDSeq Test Pipeline or in BaseSpace Sequence Hub using the Illumina DRAGEN COVIDSeq Test. For information on performing analysis locally, see the *Illumina DRAGEN COVIDSeq Test Pipeline Software Guide (# 1000000128122)*. For information on performing analysis on BaseSpace Sequence Hub, see *Illumina DRAGEN COVIDSeq Test App Guide (document # 1000000129548)*.

Consumables

- If using the NovaSeq 6000 Sequencing System S4 flow cell:
  - 2 NovaSeq 6000 Sequencing System S4 Reagent Kit (200 cycles), Illumina, # 20027466
  - 2 NovaSeq Xp 4-Lane Kit, Illumina, # 20021665
- If using the NovaSeq 6000 Sequencing System SP flow cell:
  - 4 NovaSeq 6000 Sequencing System SP Reagent Kit (100 cycles), Illumina, # 20027464
  - 4 NovaSeq Xp 2-Lane Kit, Illumina, # 20021664
- If using the NextSeq 500/550 System or NextSeq 550Dx Instrument:
  - 8 NextSeq 500/550 High Output Kit v2.5 (75 Cycles), Illumina, # 20024906

Sample Sheet Requirements

The Illumina DRAGEN COVIDSeq Test Pipeline requires a sample sheet for each run analysis. Use the samplesheet.csv file for your sequencing system included in the installer packager or available on the Illumina COVIDSeq Test support site as a template to create the sample sheet.

Make sure your sample sheet meets the following requirements.

1 Save the sample sheet with the name SampleSheet.csv in the sequencing run folder.

2 In Settings, enter the following value for the `AdapterRead1` parameter.

```
CTGTCTCTTATACACATCT
```

3 In the Data section, enter the following required parameters.
Make sure that there no empty rows between samples.

Field	Description	Requirements
Sample_ID	The ID used to identify the samples in the test reports and included in the output file names.	Sample IDs are not case-sensitive. Make sure Sample IDs contain the following: • Unique for the run. • ≤ 100 characters with no spaces. • Alphanumeric characters, underscores, and dashes only. An alphanumeric character must be added before and after an underscore or dash.
Index_ID	The IDT for Illumina-PCR Indexes index name associated with the sample.	See *Illumina Adapter Sequences (document # 1000000002694)* for index names and additional information. The name must be unique for each flow cell lane. If the Index_ID is not specified, the Index Set field is derived from Index and Index2. If specifying all three, the index names and associated sequences must match.
Index	IDT for Illumina-PCR Indexes i7 index sample sheet bases	See *Illumina Adapter Sequences (document # 1000000002694)* for sample sheet bases for your sequencing system and additional information. If Index_ID is specified, Index is not required.
Index2	IDT for Illumina-PCR Indexes i5 index sample sheet bases.	See *Illumina Adapter Sequences (document # 1000000002694)* for sample sheet bases for your sequencing system and additional information. If Index_ID is specified, Index2 is not required.
Lane	The flow cell lane for the sample.	If using the NovaSeq 6000 System, enter one of the following values: `1`, `2`, `3`, or `4`. If using the NextSeq 500/550 or NextSeq 500Dx, this field is not included.
Sample_Type	The sample type for each sample.	Enter one of the following case-sensitive values: `PatientSample`, `NTC`, `PositiveControl`. If using the NovaSeq 6000 System, there must be one NTC sample and one PostiveControl sample for each Index Set/Lane combination in the sample sheet. If using the NextSeq 500/550 or 550Dx, there must be one NTC sample and one PostiveControl sample for each Index Set combination in the sample sheet

4 [Optional] Enter any additional data parameters, such as `Sample_Name`.

5. Save your sample sheet.

Set Up Sequencing Run

1 If using the NovaSeq 6000 system, refer to the *NovaSeq 6000 Sequencing System Guide (document # 1000000019358)* for sequencing instructions.

- Use v1.6 of the NovaSeq Control Software (NVCS).

- If using the Illumina DRAGEN COVIDSeq Test BaseSpace Sequence Hub app, select Run Monitoring and Storage as the Configuration option.
- Use the following number of cycles and index lengths:
  - Read 1—Enter 36 as the value.
  - Index 1 and Index 2—Enter 10 as the value.
  - Read 2—Enter 0 as the value.

2 If using the NextSeq 500/550 or NexSeq 550Dx, refer to the *NextSeq 500 System Guide (document # 15046563)*, *NextSeq 550 System Guide (document # 15069765)*, or *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.
- Use v4.0 of the NextSeq Control Software (NCS).
- If using the NextSeq 550Dx, use RUO mode.
- Set up your sequencing run in manual mode.
- If using the Illumina DRAGEN COVIDSeq Test BaseSpace Sequence Hub app, select Run Monitoring and Storage as the Configuration option.
- Enter Single-Read as the Read Type.
- Use the following number of cycles and index lengths:
  - Read 1—Enter 36 as the value.
  - Index 1 and Index 2—Enter 10 as the value.

Supporting Information

Kit Contents

The Illumina COVIDSeq Test requires Illumina COVIDSeq Test (3072 Samples) and 8 IDT for Illumina-PCR Indexes.

Component	Kit	Catalog #
Library Preparation	Illumina COVIDSeq Test (3072 Samples)	20043675
Indexes	IDT for Illumina-PCR Indexes Sets 1–4 (384 Indexes)	20043137

Illumina COVIDSeq Test

Promptly store reagents at the indicated temperature to ensure proper performance.

Table 1 Illumina COVIDSeq Test Box 1 – 3072 Samples, Part # 20043645

Quantity	Label Volume	Reagent	Description	Storage
1	233 ml	ITB	Illumina Tune Beads	Room temperature

Table 2 Illumina COVIDSeq Test Box 2 – 3072 Samples, Part # 20043434

Quantity	Label Volume	Reagent	Description	Storage
1	55.6 ml	ST2 HT	Stop Tagment Buffer 2 HT	Room temperature, post-amp environment

Table 3 Illumina COVIDSeq Test Box 3 – 3072 Samples, Part # 20043646

Quantity	Label Volume (ml)	Reagent	Description	Storage
2	6.15	EBLTS HT	Enrichment BLT HT	2°C to 8°C, post-amp environment
1	10	RSB HT	Resuspension Buffer HT	2°C to 8°C, post-amp environment

Table 4 Illumina COVIDSeq Test Box 4 – 3072 Samples, Part # 20043436

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	114	ELB HT	Elution Buffer HT	2°C to 8°C, pre-amp environment
1	845	TWB HT	Tagmentation Wash Buffer HT	2°C to 8°C, post-amp environment

Table 5 Illumina COVIDSeq Test Box 5 – 3072 Samples, Part # 20043648

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	45.1	EPH3 HT	Elution Prime Fragment 3HC Mix HT	-25°C to -15°C pre-amp environment
1	100.6	IPM HT	Illumina PCR Mix HT	-25°C to -15°C, pre-amp environment
1	78.9	EPM HT	Enhanced PCR Mix HT	-25°C to -15°C, pre-amp environment

Table 6 Illumina COVIDSeq Test Box 6 – 3072 Samples, Part # 20043647

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	4.6	RVT HT	Reverse Transcriptase HT	-25°C to -15°C, pre-amp environment
1	41.1	FSM HT	First Strand Mix HT	-25°C to -15°C, pre-amp environment

Table 7 Illumina COVIDSEQ Test Box 7 – 3072 Samples, Part # 20043439

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	14.4	CPP1 HT	COVIDSeq Primer Pool 1 HT	-25°C to -15°C, pre-amp environment
1	14.4	CPP2 HT	COVIDSeq Primer Pool 2 HT	-25°C to -15°C, pre-amp environment
1	37.6	TB1 HT	Tagmentation Buffer 1 HT	-25°C to -15°C, post-amp environment

Table 8 Illumina COVIDSeq Positive Control HT, Part # 20043401

Quantity	Label Volume	Reagent	Description	Storage
1	100 µl	COVIDSeq Positive Control HT	COVIDSeq Positive Control HT	-85°C to -65°C, post-amp environment

IDT for Illumina- PCR Indexes , Store at -25°C to -15°C

The Illumina COVIDSeq Test requires 8 IDT for Illumina PCR D Indexes Sets 1–4 (384 Indexes) for a total 96 indexes, 96 sample index adapter plates.

Quantity	Description	Part Number
8	IDT for Illumina- PCR Indexes Set 1 (96 Indexes)	20043132
8	IDT for Illumina- PCR Indexes Set 2 (96 Indexes)	20043133
8	IDT for Illumina- PCR Indexes Set 3 (96 Indexes)	20043134
8	IDT for Illumina- PCR Indexes Set 4 (96 Indexes)	20043135

Consumables and Equipment

In addition to the Illumina COVIDSeq Test and IDT for Illumina-PCR Indexes, make sure that you have the required consumables and equipment before starting the protocol.

Consumables

Consumable	Supplier
10 µl pipette tips	General lab supplier
20 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
200 µl pipette tips	General lab supplier
1000 µl pipette tips	General lab supplier
Hard-Shell 96-Well PCR Plates	Bio-Rad, catalog # HSP-9601 or equivalent
96 deep-well plate, 2000 µl	Eppendorf, catalog # 951033707
1.7 ml LoBind microcentrifuge tubes	Eppendorf, catalog # 022431021
5 ml LoBind microcentrifuge tube	Eppendorf, catalog # 0030122348
15 ml tubes	General lab supplier
Lab tissue, low-lint	VWR, catalog # 21905-026, or equivalent
Lint-free alcohol wipe	General lab supplier
Microseal 'B' adhesive seals	Bio-Rad, part # MSB-1001
RNase/DNase-free Disposable Pipetting Resovoirs	VWR, part # 89094-658
One of the following, depending on the extraction method used: • 13 QIAamp Viral RNA Mini Kit • 8 Quick DNA/RNA Viral MagBead	• Qiagen, catalog # 52906 • Zymo Research, catalog # R2141
Qubit dsDNA HS Assay Kit	One of the following, depending on kit size: • ThermoFisher Scientific, part # Q32851 • ThermoFisher Scientific, part # Q32854
Qubit Assay Tubes	ThermoFisher Scientific, catalog # Q32856
If using the NovaSeq 6000 Sequencing System S4 flow cell: • 2 NovaSeq 6000 Sequencing System S4 Reagent Kit (200 cycles) • 2 NovaSeq Xp 4-lane Kit	• Illumina, catalog # 20027466 • Illumina, catalog # 20021665
If using the NovaSeq 6000 Sequencing System SP flow cell: • 4 NovaSeq 6000 Sequencing System SP Reagent Kit (100 cycles) • 4 NovaSeq Xp 2-Lane Kit	• Illumina, catalog # 20027464 • Illumina, catalog # 20021664
If using the NextSeq 500/550 System or the NextSeq 550Dx instrument: • 8 NextSeq 500/550 High Output Kit v2.5 (75 Cycles)	• Illumina, catalog # 20024906

Equipment

Equipment	Supplier
10 µl single-channel pipettes	General lab supplier
20 µl single-channel pipettes	General lab supplier
200 µl single-channel pipettes	General lab supplier
1000 µl single-channel pipettes	General lab supplier
10 µl 8-channel pipettes	General lab supplier
20 µl 8-channel pipettes	General lab supplier
200 µl 8-channel pipettes	General lab supplier
1000 µl 8-channel pipettes	General lab supplier
20 µl 12-channel pipettes	General lab supplier
200 µl 12-channel pipettes	General lab supplier
10 ml serological pipettes	General lab supplier
25 ml serological pipettes	General lab supplier
50 ml serological pipettes	General lab supplier
BioShake iQ	QInstruments, part # 1808-0506
DRAGEN Server v2 or v3	Illumina
The following, depending on the extraction method used: • QIAamp Viral RNA Mini Kit equipment • Quick-DNA/RNA Viral Magbead	 • See *QIAmp Viral RNA Mini Handbook (document # HB-0354-006)* • See *Quick-DNA/RNA Viral MagBead Instruction Manual*
Freezer, -25°C to -15°C	General lab supplier
Freezer, -85°C to -65°C	General lab supplier
Magnetic Stand-96	Thermo Fisher Scientific, catalog # AM10027
One of the following magnetic stands: • Dynabeads MPC-S (Magnetic Particle Concentrator) • MagnaRack Magnetic Separation Rack	 • Thermo Fisher Scientific, catalog #A13346 • Thermo Fisher Scientific, catalog # CS15000
Microcentrifuge	General lab supplier
Microplate Centrifuge	General lab supplier
NovaSeq Xp Flow Cell Dock	Illumina, # 20021663
Pipette Aid	General lab supplier
Quibit Fluorometer 3.0	Thermo Fisher, catalog # Q33216, Q33217, or Q33218
Refrigerator, 2°C to 8°C	General lab supplier
One of the following thermal cyclers: • C1000 Touch™ Thermal Cycler with 96–Well Fast Reaction Module • C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module	 • Bio-Rad, part # 1851196 • Bio-Rad, Part # 1851197
Sealing wedge or roller	General lab supplier
Vortexer	General lab supplier
One of the following sequencing systems: • NextSeq 500 • NextSeq 550 • NextSeq 550Dx • NovaSeq 6000	Illumina

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong, China	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
South Korea	+82 80 234 5300	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan, China	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.

Document # 1000000126053 v02

Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

For Research Use Only. Not for use in diagnostic procedures.

CanCOGeN RCanGeCO

CanCOGeN Interim Recommendations for Naming, Identifying, and Reporting SARS-CoV-2 Variants of Concern

Version: 1.0

Last Revision Date: 2021-01-15

Authors: Emma Griffiths, Jennifer Tanner, Natalie Knox, Will Hsiao, and Gary Van Domselaar on behalf of the CPHLN and CanCOGeN

Introduction

Since the publication of Public Health England's initial report describing a recently emerged variant of concern designated VOC202012/1 in December of 2020, the identification, tracking, and characterization of SARS-CoV-2 variants has become a global priority. Variants of Concern (VOCs) are subgroups of SARS-CoV-2 viruses containing combinations of mutations that have been associated with a clinically or epidemiologically significant phenotype. Naming VOCs is difficult because viral taxonomy is complex, and viral taxonomic naming schemes can evolve as the virus evolves. Terms such as strain, clade, and variant are often used interchangeably by both the public and scientific community. This taxonomic fluidity, in combination with the different nomenclature schemes implemented by different analytical platforms and a mixture of common names used in science and media communication, has created a patchwork of names and aliases for variants. Such variability poses significant challenges for public health reporting of VOCs in Canada, which in turn complicates data integration and analysis, and ultimately communication with the public. Notably, VOCs are often referred to by the location where they were first reported, such as the "UK variant" or the "South Africa variant". However, the association of pathogens and disease with geographic locations or populations has been shown to cause stigma and create xenophobia. There currently exists no international guidelines for naming VOCs, although The World Health Organization recognizes the need for one and has convened an expert working group to provide these guidelines. Canada's ability to effectively track and respond to VOCs requires a standard process for naming, identifying, and reporting VOCs. To address this pressing need, we provide here an interim variant-naming scheme along with conventions for identifying and reporting VOCs for use by the Canadian Public Health Laboratory Network and CanCOGeN. These guidelines may be revised after international guidelines become available.

Proposed Canadian SARS-CoV-2 Variant Nomenclature System

Variant Naming

To reduce stigma, the Canadian nomenclature system excludes any reference to place names and instead relies on scientifically derived designations. Two widely used genomic analysis platforms—Pangolin and Nextstrain—have had enormous influence on how variants are named and defined. Pangolin is a command-line tool and web application that assigns a lineage name to a genome using the "Pango" nomenclature scheme[1]. Nextstrain is a phylodynamic analysis platform that enables users to analyze and visualize SARS-CoV-2 genomes in a global context. Because of its utility and common usage in the scientific community and the public sphere, our proposed nomenclature system adopts the Pango nomenclature for naming VOCs. The lineage names of the three currently known VOCs are presented in Table 1, along with common aliases (synonyms), for reference and clarification.

Table 1: Pango lineage designations and common aliases for VOCs

Pango Lineage	**Alias**
B.1.1.7	clade 20I/501Y.V1, VUI 202012/01, VOC 202012/01, B1.17, B117 UK variant, Kent Variant
B.1.351	clade 501Y.V2, SA S.501Y.V2, 20H/501Y.V2, B1351, South African variant
P.1	clade 20J/501Y.V3, B.1.1.28.1, P1, Manaus variant, B.1.1.28(K417N/E484K/N501Y), B.1.1.248 (Brazil/Japan), Brazilian variant

Lineages, Variants, and Variants of Concern

A viral *lineage* is a group of viruses defined by a founding variant and its descendents. Names are assigned to SARS-CoV-2 lineages using manual and automated methods. Lineage designations are based on phylogenetic grouping followed by the identification of shared, common mutations, which are referred to as *lineage-defining mutations*. The significance of some mutations have been characterized; however, the vast majority of known mutations are uncharacterized.

A *Variant* is a distinct virus defined by the unique constellation of mutations contained in its genome. Most mutations are unremarkable; however, some variants contain mutations that may alter viral transmissibility, disease severity, or propensity for immune system and/or vaccine escape. Variants that contain these *Mutations of Concern* are candidates for national surveillance, but they have not yet been classified as a Varian of Concern, and thus are not considered reportable.

A *Variant of Concern (VOC)* is a variant associated with an experimentally verified functional change in the virus affecting transmissibility, disease severity, immune escape, vaccine escape, or any other important clinical or epidemiological trait. Because of their increased risk to public health, VOCs have been identified as a priority for surveillance and response. The process for raising the surveillance priority of any variant to that of a VOC has yet to be developed by the broader scientific community, although in general, the process proceeds through a formal investigation, during which the variant is considered a Variant Under Investigation (VUI). A VUI can be designated a VOC depending on the outcome of the investigation[2]. Efforts are underway by international health authorities to establish these processes, and Canada is defining similar standards and processes for defining VOCs, which will be addressed in a subsequent guidance document.

The three currently known VOCs are B.1.1.7, B.1.351, and P.1. The canonical lineage-defining mutations of these VOCs are presented in Table 2.

Table 2: Canonical lineage-defining mutations for known variants of concern

Protein Name	**B.1.1.7**	**B.1.351**	**P.1**
ORF1ab	T1001I, A1708D, I2230T, 3675-3677SGFdel,	K1655N	S1188L, K1795Q, 3675-3677SGFdel, E5662D (synT733C, synC2749T, syn C12778T, synC13860T)
Spike (S)	69-70HVdel, Y144del, **N501Y**, A570D, P681H, T716I, S982A, D1118H	K417N, **E484K**, **N501Y**, **D614G**, A701V	**L18F**, T20N, P26S, D138Y, R190S, K417T, **E484K**, **N501Y**, H655Y, T1027I
ORF8	Q27stop, R52I, Y73C		E92K (ins28269-28273)
Nucleocapsid (N)	D3L, S235F	T205I	P80R
Envelope (E)		P71L	

syn = synonymous genetic mutation
ins = genetic sequence insert
del = amino acid deletion
stop = mutation resulting in a stop codon
bold = mutations common to more than one VOC

Data Standard for Naming Variants

To capture and structure information pertaining to the results and methods used to determine the presence of VOCs in patient samples, a data standard prescribing a set of standardized fields and terms is recommended for recording and communicating results. Encoding this information in a standardized way facilitates and streamlines database queries, data analysis, and reporting. The fields and suggested values, along with their definitions and guidance for usage, are provided in Table 3.

Table 3: Standardized fields and terms for naming variants

Field Name	Definition	Values	Guidance
Lineage name	The lineage name of the virus	B.1.1.7 B.1.351 P.1 Undetermined	Determine the lineage using Pangolin, by assessing lineage-defining mutations, or by VOC-specific RT-qPCR assay. If the technique used cannot distinguish the lineage, use “Undetermined” or leave blank.
Variant designation	The designation used to classify lineages as Variants or Variants of Concern	Variant of Concern Variant	Track whether the lineage is a variant or variant of concern. If the lineage assigned is neither, leave this blank.
Variant evidence	The evidence used to determine the lineage of a Variant of Concern	Free text	List the assay/technique used for testing, and any mutations of concern/interest used as criteria for the lineage/variant designation. If the lineage is not a Variant/VOC, leave blank.

Laboratory Confirmation of Variants

Different techniques offer different thresholds of evidence for characterizing a variant of concern, with WGS offering the highest level of evidence. VOCs were first identified and characterized by WGS, and there is growing evidence that lineages continue to adapt under evolutionary pressure. As such, it is therefore recommended that a VOC be sequenced, when possible, in order to determine its spectrum of mutations. However, laboratories may confirm the presence of a viral variant from complete genome sequence, partial genomic sequence, and from RT-qPCR assays that target one or more variant-defining mutations, each with diminishing

confidence. Genome sequencing and analysis can take days to weeks to complete, while RT-qPCR-based assays can provide results in a much shorter time frame—a saving which can be critical for effective public health response. The definitions, testing criteria, and naming conventions of confirmed VOCs are provided in Table 4.

Confirming Variants

A flowchart depicting the process for confirming variants is provided in Figures 1 and 2.

Confirming Variants From Whole Genome Sequence Data

Whole-genome sequencing provides the strongest evidence for confirming a VOC. The most straightforward method to confirm a variant from WGS data is from its Pango lineage assignment; therefore, **we recommend that VOCs be confirmed from whole genome sequence data from its Pango lineage assignment.**

Confirming Variants From Partial Genome Sequence Data

Pango lineage assignment from a high quality, complete genome is the preferred method for confirming a variant; however, some specimens (e.g., specimens with a high Ct value) may yield an incomplete genome of insufficient quality for Pangolin to assign a lineage. Other partial genome sequencing approaches, such as metagenomic sequencing of wastewater samples, can also be unsuitable for lineage assignment by Pangolin. In these cases, it may be necessary to manually identify a VOC from the mutation data. The minimum set of mutations required to confirm a VOC depends on the power of the mutations in a given VOC to discriminate it from other circulating variants. For example, Pangolin assigns lineages from 5 of the 17 defining B.1.1.7 mutations[3], 5 of the 9 defining B.1.351 mutations[4], and 10 of the 17 defining P.1 mutations[5]. **We recommend that the same number of minimal lineage-defining mutations as those used by Pangolin be identified for each specific variant in order to confirm a VOC.** We also urge caution when using this approach, since variants that are not considered VOCs may share common mutations with VOCs. A good example is the recently emerged B.1.525 variant (see for example EPI_ISL_961609|2021-01-13), which shares the S:69/70del, S:Y144del, and Orf1ab:3675-3677SGFdel in common with the B.1.1.7 variant.

Confirming Variants From From RT-qPCR Assay

Some RT-qPCR Assays are currently being used as a proxy to detect VOCs, the most familiar example being the Thermo Fisher TaqPath 3-gene assay, which exhibits a reliable S-gene target failure due to the presence of the 69-70del mutation in the B.1.1.7 VOC. Other RT-qPCR assays are in development, such as the single-target N501Y SNP assay and multiplex RT-qPCR assays that can detect and discriminate the B.1.1.7, B.1.351, and P.1 VOCs[6].

There is some debate as to whether RT-qPCR assays are confirmatory for a VOC. Some feel that RT-qPCR assays based on the detection of a single target is insufficient to confirm a VOC since it is not definitive for a given variant[7]. Multiplex RT-qPCR assays that detect two or more

mutations in a given VOC can substantially increase its confirmatory power; however, they are not robust to viral evolution. For example, the E484K mutation, which is present in B1.351 and P.1 and is thought to be responsible for immune escape, has recently been detected in a subset of B.1.1.7 VOCs[8] and as of February 10, 2020, is considered a VOC by NERVTAG. Existing RT-qPCR assays for the B.1.1.7 VOC do not discriminate between B.1.1.7 and this evolved variant.

One option for addressing the problems associated with molecular diagnostic detection of VOCs is to label them as “presumptive” if they are detected by RT-qPCR assay, and “confirmed” by follow up genome sequencing. This approach is problematic, however, since the large number of VOCs detected by screening, and the delay in confirming by whole genome sequencing, would result in the initial reporting of many VOCs detected by RT-qPCR assay as “presumptive,” which can create confusion with reporting to public health authorities and the public. To avoid these problems, we recommend not to use the terms “presumptive" and “confirmed” when reporting VOCs. Instead, the evidence used to report the detection of a VOC should be provided in the Variant Evidence field. The evidence can be used to judge the confirmatory power of the method used to to detect the VOC. For this reason, we do not include a field to capture the classification status of VOCs in our interim naming standard.

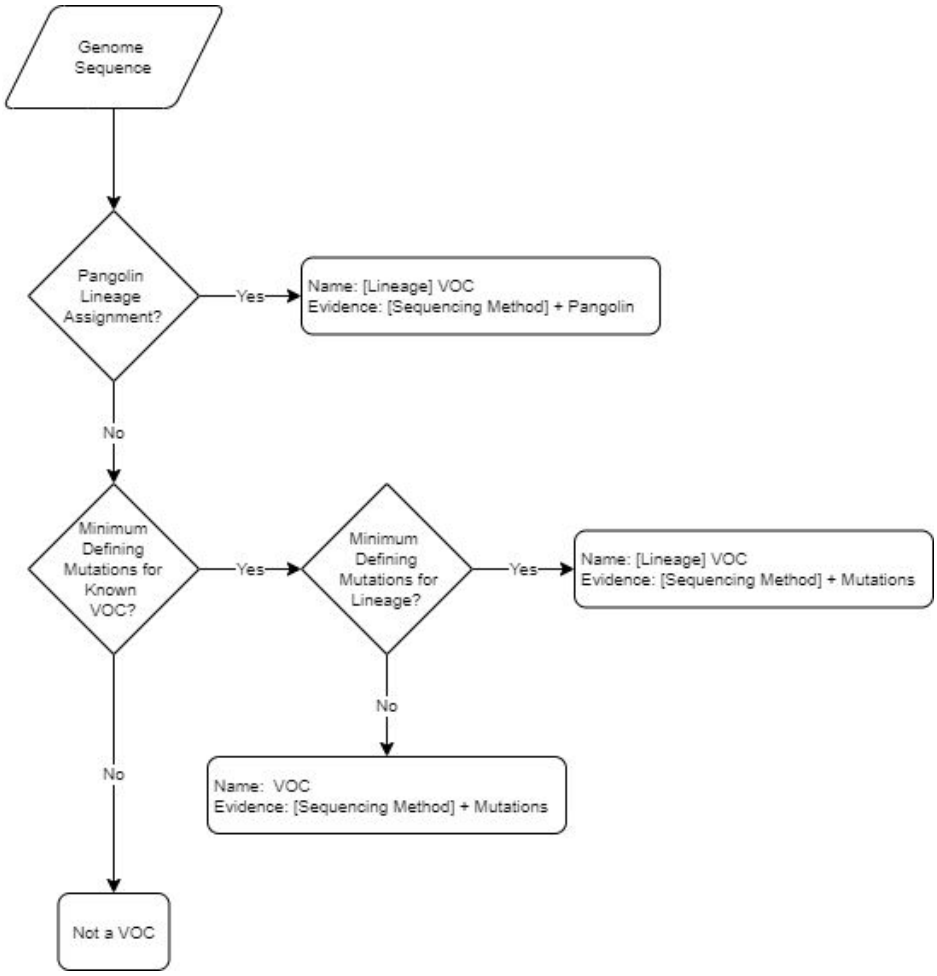


Figure 1. Flowchart for naming VOCs from sequence data.

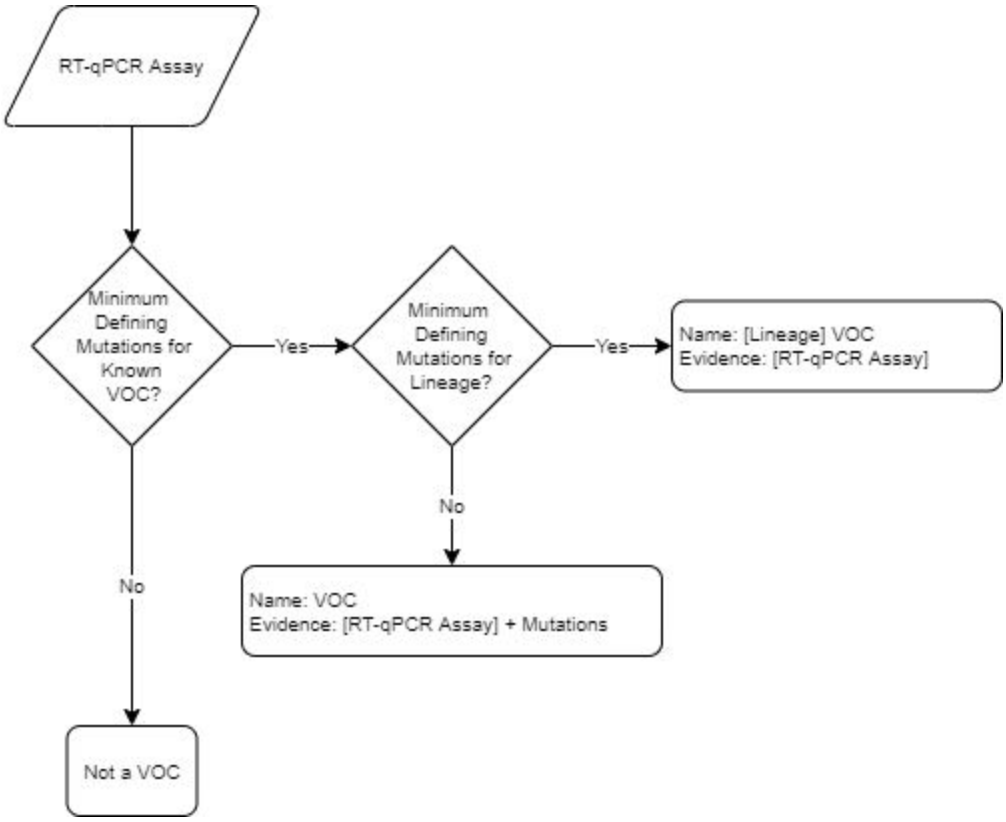


Figure 2. Flowchart for naming VOCs from RT-qPCR assay

Table 4: Definitions, testing criteria and naming conventions of SARS-CoV-2 variants

Nomenclature	Definition	Testing Criteria
B.1.1.7 VOC	A B.1.1.7 VOC confirmed by RT-qPCR assay or sequencing	RT-qPCR screening assay targeting B.1.1.7-specific mutations (e.g., TaqPath RT-qPCR assay) AND/OR Whole or partial genome sequencing; Pango lineage assignment or minimal set of VOC-defining mutations
B.1.351 VOC	A B.1.351 VOC confirmed by RT-qPCR assay or sequencing	RT-qPCR screening assay targeting B.1.351-specific mutations (e.g., A701V) AND/OR Whole or partial genome sequencing;

		Pango lineage assignment or minimal set of VOC-defining mutations
P.1 VOC	A P.1 VOC confirmed by RT-qPCR assay or sequencing	RT-qPCR screening assay targeting a P.1-specific mutation (e.g., P26S) AND/OR Whole or partial genome sequencing; Pango lineage assignment or minimal set of VOC-defining mutations
VOC	A test result containing a single VOC-defining mutation	RT-qPCR screening assay targeting non-discriminatory mutations (e.g., N501Y RT-qPCR assay)
[Lineage] Variant	Any variant of interest containing variant-defining mutations	Whole or partial genome sequencing Pango lineage assignment or minimal set of variant-defining mutations

Worked Examples for Data Standard

We provide here a number of worked examples to illustrate how the data standard should be implemented. A number of hypothetical scenarios are presented below, and the corresponding values for the fields defined by the data standard are shown in Table 5.

Sample Descriptions

Sample 1: The genome was fully sequenced and a lineage of B.1.1.7 assigned using Pangolin. Upon analysis, the genome was found to contain all canonical mutations.
Sample 2: The sample had a Ct value >34 and did not meet the quality threshold for genome sequencing. Targeted sequencing was used to analyze regions of the genome. Five B.1.351 lineage-defining mutations were found to be present.
Sample 3: The genome was fully sequenced and a lineage of B.1.1.7 assigned using Pangolin. The minimal set of variant-defining mutations were identified; however, a few non-lineage defining mutations were also present.
Sample 4: A TaqPath qPCR screen was carried out and all 3 targets indicate the sample is a B.1.1.7 VOC.
Sample 5: A N501Y qPCR screen was carried out and the sample gave a positive result. The sample is presumed to be a VOC but the lineage could not be determined (N501Y is present in all three lineages).
Sample 6: The genome was fully sequenced and a lineage of B.1.429 was assigned. The lineage contains a L452R (Spike) mutation of concern and is thought to be “the California variant,” which is not currently considered a VOC.

Sample 7: The genome was fully sequenced and a lineage of B.1.1.1 was assigned by Pangolin.

Table 5: Worked examples for naming variants

Sample	Lineage name	Variant designation	Variant evidence
Sample 1	B.1.1.7	Variant of Concern	Genome sequencing; Pango lineage assignment
Sample 2	B.1.351	Variant of Concern	Partial genome sequencing; minimal lineage-defining mutations (m_1,m_2...m_n)
Sample 3	B.1.1.7	Variant of Concern	Genome sequencing; Pangol lineage assignment
Sample 4	B.1.1.7	Variant of Concern	TaqPath; SGTF, N, ORF1ab mutations
Sample 5	Undetermined	Variant of Concern	N501Y RT-qPCR screen
Sample 6	B.1.429	Variant	Genome sequencing; Pango lineage assignment; L452R
Sample 7	B.1.1.1	Variant	Genome sequencing; Pango lineage assignment

Reporting Variants

Characterizing the genomic content of a lineage can provide information that can help tease out factors influencing increased viral spread such as types of human behaviour (phylogenetic analysis resolving transmission due close social interactions, travel, transport between hospitals and care facilities, etc.) and viral evolution (mutations resulting in increased transmissibility etc). However, even in the absence of genomic characterization, knowing where and when VOCs are identified is actionable information. As such, federal health authorities recommend that all VOCs be reported. Reporting should be carried out weekly through the submission of the COVID Variants of Concern Report form. Since not all variants of interest are variants of concern, **we recommend that only VOCs be officially reported to jurisdictional and federal health authorities.**

At present, there are no changes to treatment regimens based on VOC determinations. While there is thought to be an elevated risk for transmission and immune escape, there is little evidence to date that suggests an increase in clinical severity for those infected with VOCs. These findings should be kept in mind when communicating VOCs with the public.

Glossary of Terms

Mutation: a change of a nucleotide in the viral RNA genome, or an insertion or deletion event. Some mutations result in an amino acid substitution. Substitutions are denoted by the wildtype amino acid followed by the site in the amino acid sequence and the replacement amino acid (e.g., N501Y denotes and asparagine-to-tyrosine substitution at amino acid site 501). The mutation is sometimes presented with the gene name prepended (e.g., S:N501Y).

Mutation of Interest: a mutation *that may cause a functional change in the virus* affecting transmissibility, disease severity, immune escape, vaccine escape, or any other biological, clinical, or epidemiological trait.

Mutation of Concern: a mutation *associated with a known functional change in the virus* affecting transmissibility, disease severity, immune escape, vaccine escape, or any other biological, clinical, or epidemiological trait.

Variant: a distinct virus defined by the collection of mutations it harbours (the “variant-defining mutations”). Variants adopt the name of the lineage in which they reside (e.g., “a B.1.1.7 variant”).

Variant-Defining Mutation: a non-synonymous substitution or indel found in a variant in addition to the lineage-defining mutations that characterize a given variant.

Variant of Interest: a variant that warrants ongoing surveillance but is otherwise not a variant of concern (e.g., the P.2 variant).

Variant of Concern: a variant *associated with a known functional change in the virus* affecting transmissibility, disease severity, immune escape, vaccine escape, or any other important biological, clinical, or epidemiological trait. Variants of concern are classified as a national and global surveillance priority.

Lineage: a founding variant and its descendants. Lineages are assigned using genomic data and epidemiological data (e.g., “the B.1.1.7 lineage”). We have adopted the Pango lineage nomenclature for naming lineages and variants.

Lineage-Defining Mutation: a shared, common mutation found in at least five genomes of epidemiological significance (“genotypes” with epidemiological significance (geographical prevalence, implicated in outbreaks, etc.). Canonical lineage-defining mutations are assigned using manual and machine-learning approaches.

References

1. Rambaut, A. *et al.* A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nature Microbiology* 1–5 (2020).
2. Coronavirus: The Global COVID-19 Observatory - Science Museum Group. https://www.sciencemuseumgroup.org.uk/blog/coronavirus-the-global-covid-19-observatory

3. B.1.1.7 report. https://cov-lineages.org/global_report_B.1.1.7.html.
4. B.1.351 report. https://cov-lineages.org/global_report_B.1.351.html.
5. P.1 report. https://cov-lineages.org/global_report_P.1.html.
6. Vogels, C. Multiplexed RT-qPCR to screen for SARS-COV-2 B.1.1.7 variants. (2021) doi:10.17504/protocols.io.brcrm2v6.
7. Vogels, C. B. F. *et al.* PCR assay to enhance global surveillance for SARS-CoV-2 variants of concern. *bioRxiv* (2021) doi:10.1101/2021.01.28.21250486.
8. Public Health England. Investigation of novel SARS-CoV-2 variant - Variant of Concern 202012/01. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/957504/Variant_of_Concern_VOC_202012_01_Technical_Briefing_5_England.pdf.