

NANOTOOLS V2

Long read sequence tools

Administrative User Guide

ADMINISTRATIVE USER GUIDE

Long read sequence tools

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インストール手順



本ツールは Linux で動作するプログラムを利用しています。

このプログラムを利用するため、初期設定が必要となります。

Linux で動作するツールは Windows10 WSL（Windows Subsystem for Linux）を利用して Windows 上で動作します。この WSL の設定と、利用するツール群のセットアップを行います。

※ 本ツールの起動時に以下のチェックを行っています。

Step1 : WSL (Windows Subsystem for Linux) が Windows 上で有効になっているか

Step2 : 利用するツール群を含んだ設定がされているか

このセットアップ プログラムは、本ツール起動時にチェックされ、各 Step が設定されていない場合、自動的に起動します。





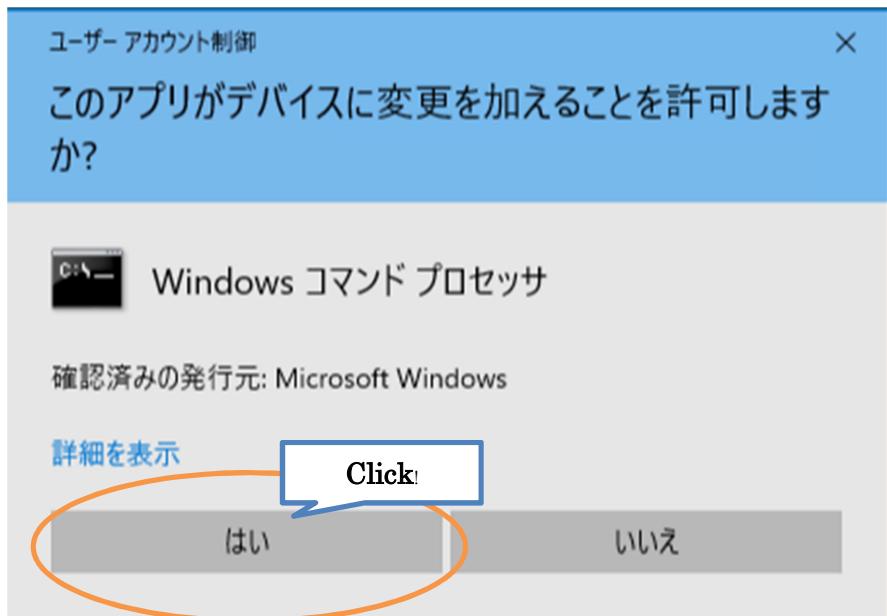
【WSL (Windows Subsystem for Linux)】の有効化

Windows10 は Linux の機能を利用するための機能があります。初期状態でこの機能は無効になっているため、ユーザが有効化する必要があります。詳しくはマイクロソフト社のページを参照してください。 <https://docs.microsoft.com/ja-jp/windows/wsl/install-win10>

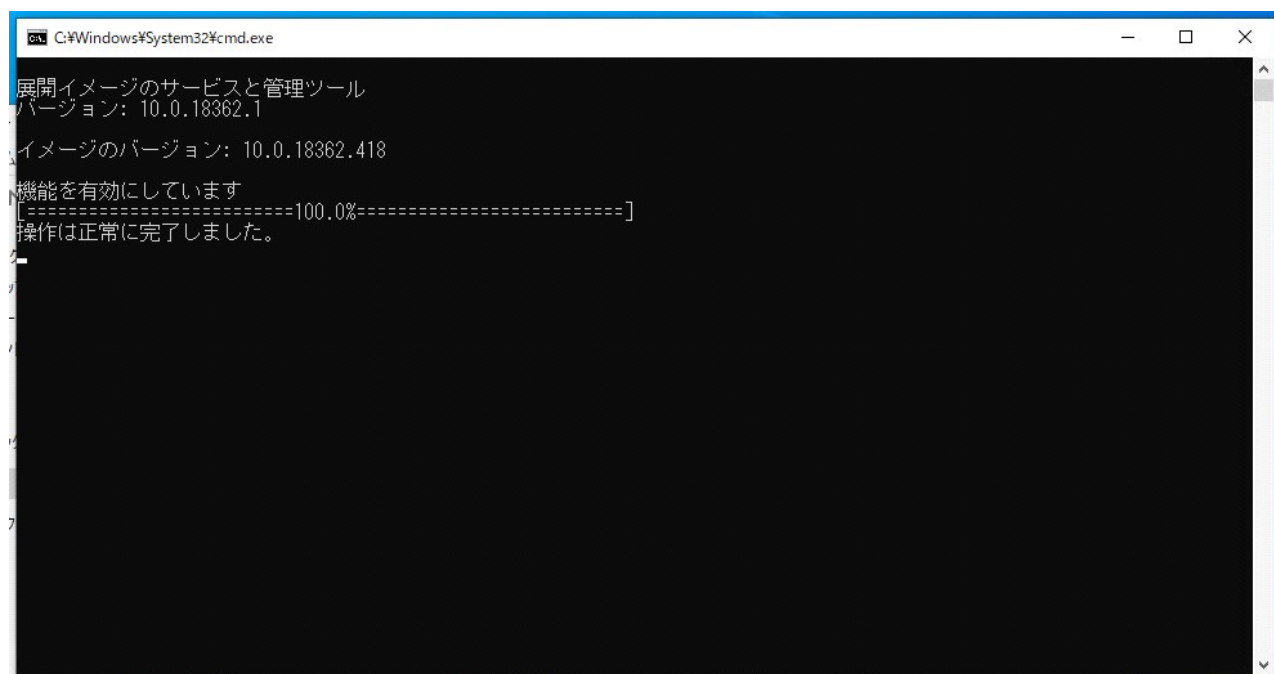
Windows 上で WSL (Windows Subsystem for Linux) が有効になって居ない場合、WSL を有効化するスクリプトを実行します。[Execute] ボタンを押下します。



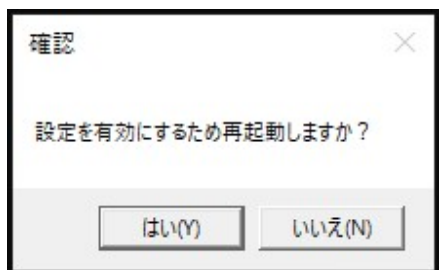
WSL を有効化するスクリプトが実行されます。実行の途中に以下の様なダイアログが表示されます。これは Windows の機能を有効化するために管理者権限が必要となるためです。



「はい」を選択して設定を有効にします。「はい」が選択されるとコマンドプロンプト（黒いコマンドが実行されているウィンドウ）が表示されます。



コマンドプロンプトの中で処理の経過が表示されます。WSLの設定を有効にするためにWindows10の再起動が必要になります。



現状態で Windows10 の再起を行う場合は「はい」を選択します。「はい」を選択すると Windows が再起動されます。「いいえ」を選択した場合は、別途ユーザが再起動を行うまで WSL（Windows Subsystem for Linux）の機能が有効になりません。

※ 設定を有効にするためには Windows の再起動が必要です!



【Ubuntu と Linux Tools】のインポート

Windows10 は Linux の機能を利用するための機能があります。本ツールは Microsoft 社が提供している Linux ディストリビューションの Ubuntu を利用しています。

詳しくは以下のマイクロソフト社のページを参照してください。 <https://docs.microsoft.com/ja-jp/windows/wsl/install-win10>

Windows 上で WSL (Windows Subsystem for Linux) が有効になっている場合、セットアップツールの Step1 は「済」のマークに変わります。



WSL が有効になり、本ツールが利用する Ubuntu とツール群がセットアップされていない場合、Step2 を実行します。以下の様に Step2 の「Execute」ボタンを押下します



実行が開始されると、Step2 が「処理中」になります。

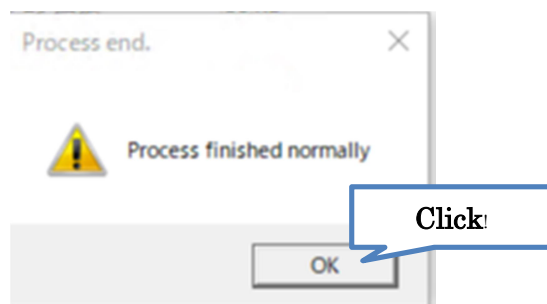
Ubuntu とツール群がインポートされるには**5～10分**程度（PC の性質によります）の時間が掛かります。変化が無いように見えますがしばらくお時間を見てください。

また、タスクマネージャでは以下の様なタスクが実行されています。

The screenshot shows the Windows Task Manager window with the 'タスク マネージャー' (Task Manager) title bar. The 'パフォーマンス' (Performance) tab is selected. The 'ディスク' (Disk) section shows a usage of 92%. A callout box points to the 'bsdtar' process in the list, stating 'この機能が上位に現れます!' (This function appears in the top!). Another callout box points to the '14.1 MB/秒' disk usage for 'bsdtar', stating 'ディスクの IO が高くなります' (Disk IO will increase).

名前	CPU	メモリ	ディスク	ネットワーク
bsdtar	2.4%	0.3 MB	14.1 MB/秒	0 Mbps
System	1.1%	0.1 MB	4.6 MB/秒	0 Mbps
Antimalware Service Executable	6.6%	83.2 MB	2.1 MB/秒	0 Mbps
Runtime Broker	1.4%	30.0 MB	0.1 MB/秒	0 Mbps
エクスプローラー	1.7%	17.4 MB	0.1 MB/秒	0 Mbps
タスク マネージャー	0%	4.0 MB	0 MB/秒	0 Mbps
Microsoft Windows Search Indexing				

正常に終了すると以下の様なダイアログが表示されます。[OK] ボタンを押下しセットアップツールを終了します。



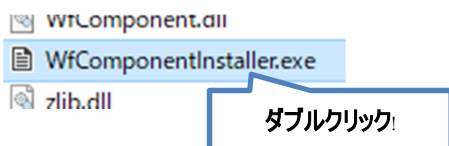


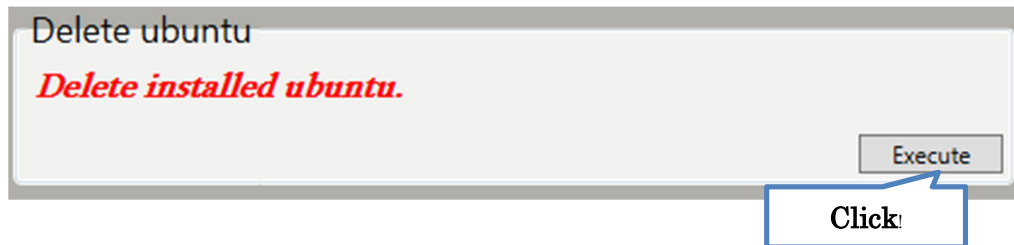
【Ubuntu と Linux Tools】の削除

本ツールが利用する Linux の機能が不要になった場合は Linux の機能を削除する事が可能です。

Ubuntu の機能とともに、Windows から削除します。

セットアップツールを起動します。本ツールを解凍したフォルダと同じ場所に [WfComponentInstaller.exe] があります。このファイルをダブルクリックして起動します。





[Execute] ボタンを押下すると、本ツールで利用する Ubuntu を削除します。

※本ツールで利用する Ubuntu は、[Ubuntu-18.04-nanopore] として登録します。

通常、ユーザが WSL (Windows Subsystem for Linux) の Ubuntu を導入する場合は [Ubuntu-18.04] として登録され、別の Ubuntu 環境として保存されています。

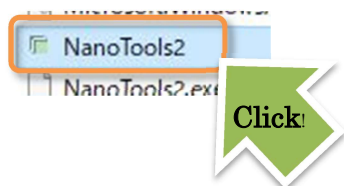
また、本ツールの [Ubuntu-18.04-nanopore] のインストール先は、ユーザごとに以下の場所へ保存されます。

C:¥Users¥ [ユーザ名] ¥AppData¥Local¥Ubuntu-18.04-nanopore

初期画面構成

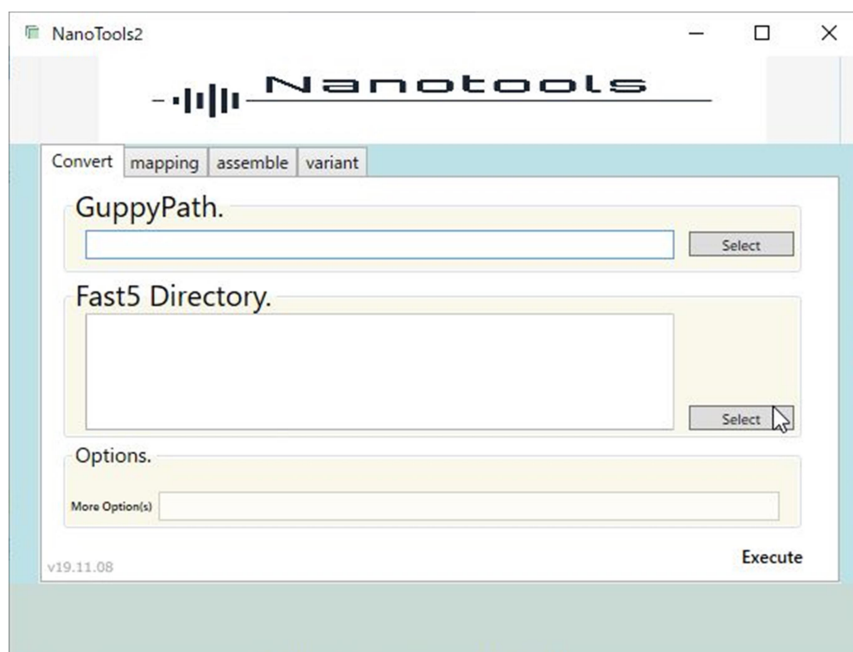


プログラムの実行は、NanoTools2.exe プログラム本体、もしくはショートカット をダブルクリックして開始します。

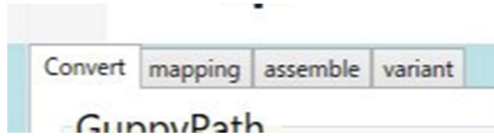


※インストールに関しては別途資料を参照してください。

起動すると以下の様なウィンドウが表示されます。



【機能タブ】の説明



それぞれのツールを切り替えます。

Convert タブ

MinION から出力された Fast5 形式から Fastq へ変換を行います。

この機能を利用するために nanopore コミュニティより取得する ont-guppy-cpu のインストールが必要です。

mapping タブ

リファレンスと Fastq 形式ファイルを指定し、マッピングを行います。対象の Fastq 形式ファイルは nanopore、PacBaio、Illumina から出力されたファイルです。

マッピングツールは minimap2 を使用します。※<https://github.com/lh3/minimap2>

assemble タブ

Fastq 形式ファイルを指定し denovo assemble を行います。内部では minimap2、miniasm、racon を利用したワークフローが実行されます。

※<https://github.com/lh3/miniasm> ※<https://github.com/isovic/racon>

variant タブ

リファレンスと Fastq 形式ファイルを指定し、構造変異検出を行います。内部では SVIM(Structural Variant Identification Method using Long Reads)を使用します。

※<https://github.com/eldariont/svim>



Fast5/Fastq 変換

Chapter

3

Nanopore MinION シーケンサから出力された Fast5 形式のファイルから Fastq への変換を行います。ツールは *guppy* を使用します。

guppy は *nanopore* のユーザサイトよりダウンロードし、インストールします。

GuppyPath.

Fast5 Directory.

Options.

More Option(s)

v19.11.08

Execute

【Guppy Path.】の指定 **必須**

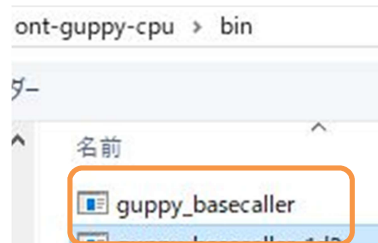
GuppyPath.

C:\Nanopore\ont-guppy-cpu_v2_3_7\ont-guppy-cpu\bin\guppy_basecaller.exe

Select

Click!

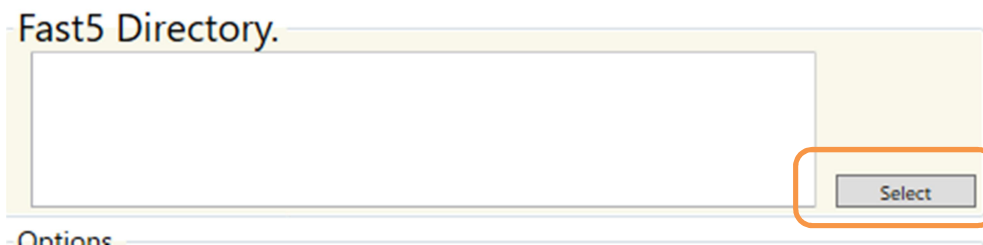
[select]ボタンを押下すると、ファイル選択ウインドウが表示されます。



nanopore ユーザサイトからダウンロードし、インストールした guppy の basecaller のパスを設定します。インストール済みの ont-guppy-cpu_(バージョン)以下にある[bin]フォルダ内の **【guppy_basecaller.exe】** を指定します。

※ 現在は guppy_barcode.exe のみ対応しています。guppy_basecaller_1d2.exe を指定すると Index ファイルが必要となり正常終了しない場合があります。

【Fast5 Directory.】の指定 **必須**



[select]ボタンを押下すると、フォルダ選択ウインドウが表示されます。

nanopore シーケンサから出力された Fast5 形式ファイルを含むフォルダを指定します。

フォルダは複数選択が可能です。

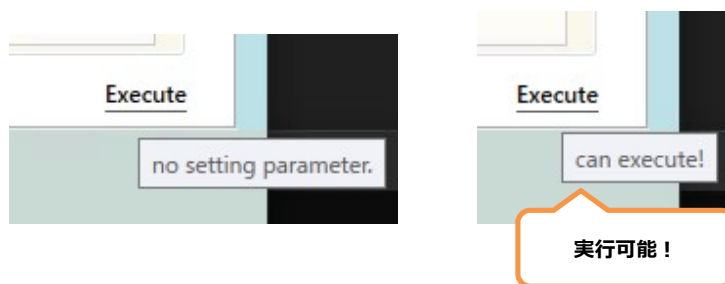
【more options】

guppy コマンドラインに追加するパラメータを記述します。

※現在は利用できません

【Execute】 ボタンを押す

必須項目が全て設定されていると、[Execute]ボタンが有効になります。ボタンを押下すると結果ファイルの出力先フォルダを選択するダイアログが表示されます。



出力先を選択すると処理が実行されます。

実行が正常に終了した場合、guppy の結果ファイルから PycoQC が実行されシーケンスの QC プロットが表示されます。 ※<https://github.com/a-slide/pycoQC>



リファレンスマッピング

Chapter

4

リファレンス配列に対して、指定した Fastq ファイルの塩基配列をマッピングします。

Fastq ファイルは、Illumina、Nanopore、Pacbio の配列が適用できます。

Illumina 由来の Fastq ファイルがペアエンドの場合、自動的にペアを認識しマッピングを行います。（※ ファイル名の「_L001_」を認識し、この文字列より前の文字列が同一の場合、ペアエンドとみなします）

Convert mapping assemble variant

Reference.

Fastq File(s).

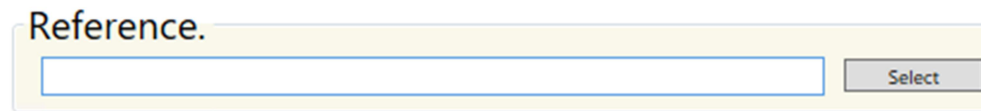
Options.

☐ Illumina ☐ Nanopore ☐ Pacbio

More Option(s)

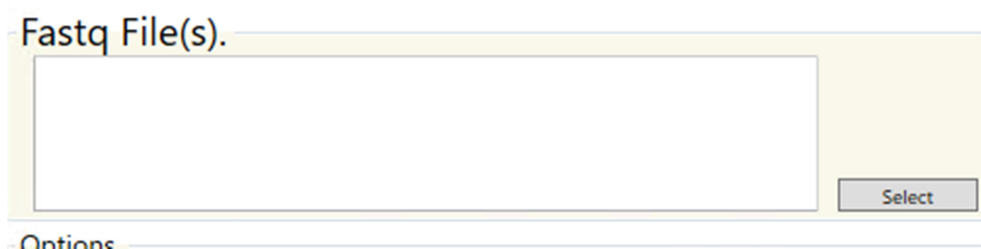
v19.11.08 Execute

【Reference.】の設定 **必須**

A form titled "Reference." with a text input field and a "Select" button.

マッピング対象のリファレンス（fasta 形式）ファイルを選択します。[Select] ボタンを押下するとファイル選択ウインドウが表示されます。リファレンスとなる FASTA ファイルを選択します。

【Fastq File(s).】の入力 **必須**

A form titled "Fastq File(s)." with a large text input field and a "Select" button.

マッピングする Fastq 形式ファイルを選択します。[Select] ボタンを押下するとファイル選択ウインドウが表示されます。複数選択された場合、選択されたファイルをマージ（結合）してから処理を行います。

【Options.】パラメータの設定

A form titled "Options." with three radio buttons labeled "Illumina", "Nanopore", and "Pacbio", and a text input field labeled "More Option(s)".

マッピング実行時にツールへ付与するオプションを指定します。

シーケンサの選択 必須

マッピングを行う Fastq ファイルのシーケンサ種類を選択します。この選択により、minimap2 実行時のオプションが自動で選択されます。

その他

その他にパラメータを指定した場合は、[More Option(s)]へ入力します。パラメータは minimap2 実行時に直接渡されます（不備なパラメータはエラー終了となります）。

オプション文字列は、minimap2[*Manual Reference Pages*]を参照してください。

※<https://lh3.github.io/minimap2/minimap2.html>

【Execute】 ボタンのクリック

必須項目が全て設定されていると、[Execute]ボタンが有効になります。ボタンを押下すると結果ファイルの出力先フォルダを選択するダイアログが表示されます。



出力先を選択すると処理が実行されます。

マッピングが正常に終了すると、結果フォルダが表示されます。有効な BAM ファイルが作成された場合、IGV が起動しマッピング状態を確認する事が出来ます。

※<http://software.broadinstitute.org/software/igv/>



De Novo assemble

Chapter

5

指定した Fastq ファイルよりコンティグを作成します。Fastq ファイルは Nanopore、Pacbio の配列が適用できます。

Convert mapping **assemble** variant

Fastq File(s).

Select

Options.

☐ Nanopore ☐ Pacbio

Target genome size

Assemble cycle: 3

Execute

【Fastq File(s).】の入力 **必須**

Fastq File(s).

Select

Options

アセンブルする Fastq 形式ファイルを選択します。[Select] ボタンを押下するとファイル選択ウィンドウが表示されます。複数選択された場合、選択されたファイルをマージ（結合）してから処理を行います。

【Options.】の設定



Options.

☐ Nanopore ☐ Pacbio

Target genome size:

Assemble cycle:

アセンブル実行時にツールへ付与するオプションを指定します。

シーケンサ選択 必須

マッピングを行う Fastq ファイルのシーケンサ種類を選択します。この選択により、minimap2 実行時のオプションが自動で選択されます。

対象のゲノムサイズ 必須

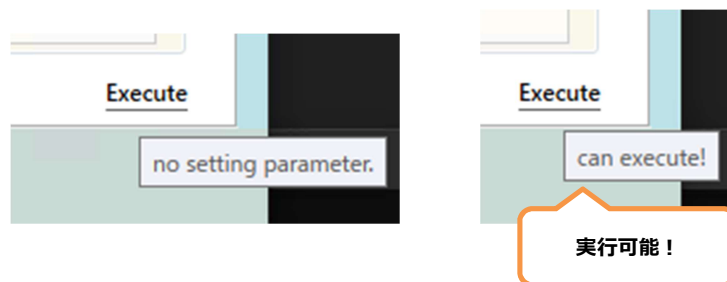
アセンブル結果の予測サイズ（数値）を記述します。小数点を含む数値と単位（K/M/G など）を含めることが可能です。

アセンブルの回数

アセンブルは minimap2-minasm-racon を繰り返実行します。繰り返しの回数を指定します。0 または空白の場合はアセンブル結果が同じになるまで繰り返します。

【Execute】 ボタンのクリック

必須項目が全て設定されていると、[Execute]ボタンが有効になります。ボタンを押下すると結果ファイルの出力先フォルダを選択するダイアログが表示されます。



出力先を選択すると処理が実行されます。

実行が正常に終了するとアセンブル結果のサマリーが表示されます。

アセンブル結果 表示例

assembly info	
Contig Count	3
N50	5351083
MaxLength	5351083
MinLength	71603
Avalage	1844470.666666667
Total Nuc Count	5533412



構造変異解析

Chapter

6

指定したリファレンスと Fastq ファイルよりバリエーション検出を行います。Fastq ファイルは Nanopore、Pacbio が対応しています。

Convert mapping assemble **variant**

Reference.
 Select

Fastq File(s).
 Select

Options.
☐ Nanopore ☐ Pacbio
☐ ngmlr ☐ minimap2
 minQuality:

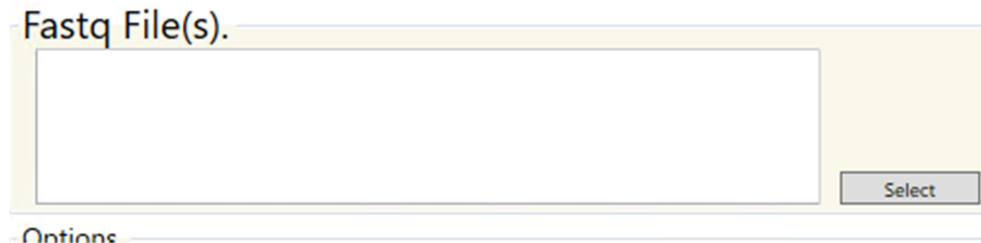
v19.11.08 Execute

【Reference.】の設定 **必須**

Reference.
 Select

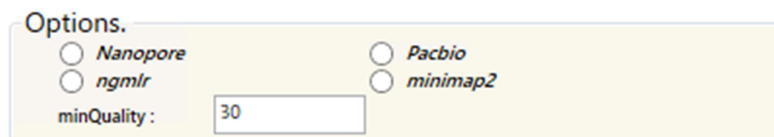
構造変異の検出対象リファレンス（fasta 形式）ファイルを選択します。[Select] ボタンを押下するとファイル選択ウィンドウが表示されます。リファレンスとなる FASTA ファイルを選択します。

【Fastq File(s).】の入力 **必須**

A screenshot of a web form section titled "Fastq File(s)." in a yellow box. Below the title is a large, empty rectangular text input field. To the right of the input field is a grey button labeled "Select". Below the yellow box is a section titled "Options." in a light blue box.

構造変異を検出する Fastq 形式ファイルを選択します。[Select] ボタンを押下するとファイル選択ウインドウが表示されます。複数選択された場合、選択されたファイルをマージ（結合）してから処理を行います。

【Options.】の設定

A screenshot of the "Options." section in a light blue box. It contains four radio buttons arranged in two columns. The left column has "Nanopore" and "ngmlr". The right column has "Pacbio" and "minimap2". Below the radio buttons is a label "minQuality:" followed by a text input field containing the number "30".

構造変異検出の実行時にツールへ付与するオプションを指定します。

シーケンサの選択 **必須**

構造変異検出を行う Fastq ファイルのシーケンサ種類を選択します。この選択により、minimap2 実行時のオプションが自動で選択されます。

マッピングツールの選択 **必須**

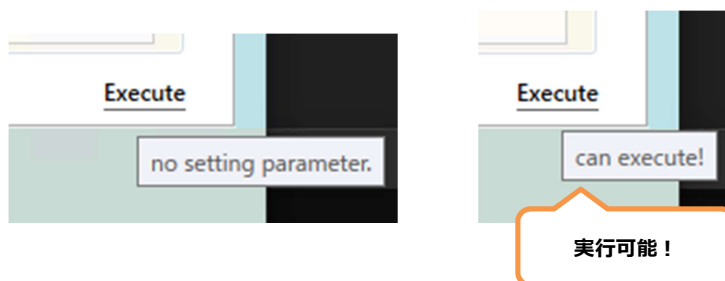
マッピングに使用するアライメントツールを選択します。構造変異を含むシーケンスリードのアライメントには NGMLR を推奨しています。

配列クオリティの閾値（数値）

マッピング結果からバリエーション検出を行う際に、Fastq クオリティ(Phread Score)の閾値を指定します。

【Execute】 ボタンのクリック

必須項目が全て設定されていると、[Execute]ボタンが有効になります。ボタンを押下すると結果ファイルの出力先フォルダを選択するダイアログが表示されます。

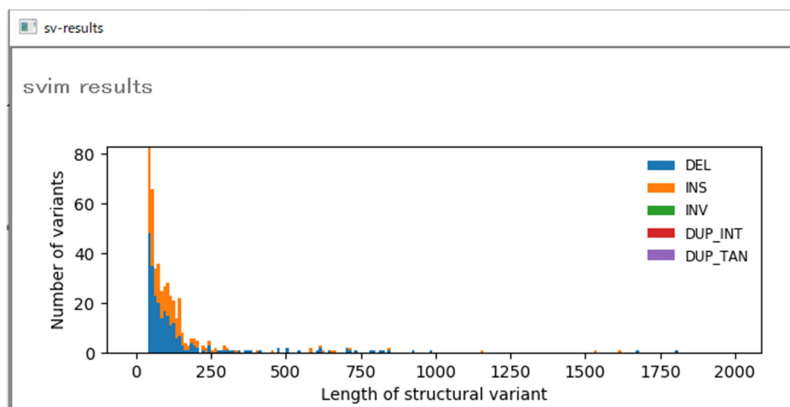


出力先を選択すると処理が実行されます。

処理が正常に終了すると、結果フォルダが表示されます。有効な BAM ファイルが作成された場合、IGV が起動しマッピング状態を確認する事が出来ます。

※<http://software.broadinstitute.org/software/igv/>

また、検出結果が有効な場合に SVIM が出力するレポートを表示します。



※ 初めてマッピングおよび構造変異解析の結果を表示する際に、以下の様なダイアログが表示されることがあります。

この場合、[Microsoft HTML アプリケーション ホスト]（通常は表示の際に選択されています）を選択し、[OK]ボタンを押下してください。

結果表示に HTA(HTML Application)を利用しているために表示されます。



IGVによる構造変異部位の マッピング状況とゲノム位置の観察方法

1. IGVゲノムブラウザの起動

Nano Toolsに付属のIGVを
NanoTools2/bin/IGV_Win
にあるigv.batをダブルクリックしてIGVゲノムブラウザを立ち上げます。

2. ゲノム情報の取り込み

IGVのメニューから
Genomes->Load Genome From Serverをクリックして生物種を選択し、ゲノム配
列とアノテーションを取得します。

3. BAMファイルの表示

IGVのメニューから
File->Load from Fileをクリックして.bamファイルをIGVに取り込みます。
.bamファイルはNanoToolのVariant解析結果出力先フォルダに作成されています。

4. 構造変異検出部位のゲノムブラウザでの表示

IGVのメニューから
File->Load from Fileをクリックして.bedファイルをIGVに取り込みます。
.bedファイルはNanoToolのVariant解析結果出力先フォルダに作成されており、イベント
の種類ごとに作成されています(e.g del.bed, dup_tan_dest.bed)。

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<https://github.com/WorldFusion/WfComponent>

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If the program does terminal interaction, make it output a short notice like this when it starts in an interactive mode:

```
{project}Copyright (C) {year}{fullname}
```

This program comes with ABSOLUTELY NO WARRANTY; for details type show w.

This is free software, and you are welcome to redistribute it under certain conditions; type show c' for details.

The hypothetical commands show w' and show c' should show the appropriate parts of the General Public License. Of course, your program's commands might be different; for a GUI interface, you would use an "about box".

You should also get your employer (if you work as a programmer) or school, if any, to sign a "copyright disclaimer" for the program, if necessary.

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