

CLC Genomics Workbench Premiumを使用した アドホックMLST解析の方法

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MLST:

各遺伝子座のアレル番号の組み合わせに基づいて菌株の同定を行う手法

(遺伝子座1, 遺伝子座2, 遺伝子座3) =

— [1] — [1] — [1] — (1, 1, 1)

— [2] — [2] — [2] — (2, 2, 2)

— [2] — [1] — [3] — (2, 1, 3)

— [1] — [2] — [3] — (1, 2, 3)

遺伝子座1

遺伝子座2

遺伝子座3

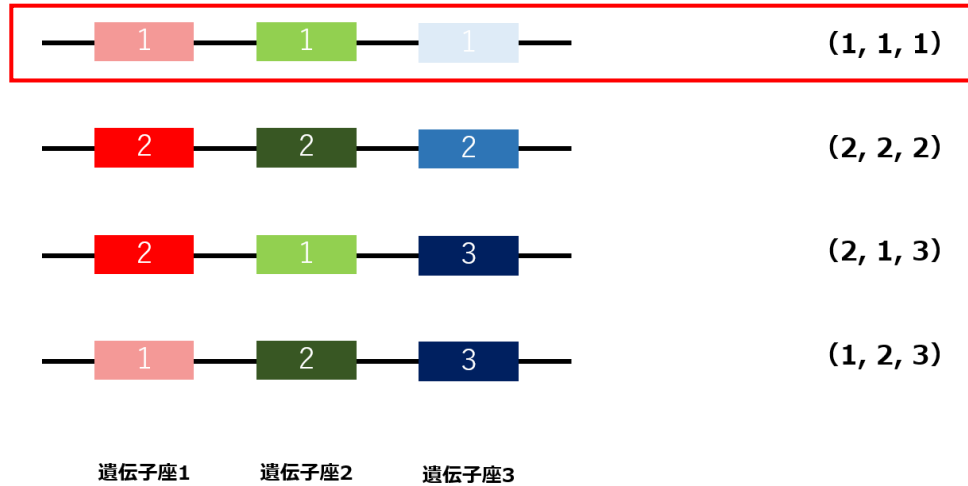
スキーム:

アレル番号の組み合わせとシーケンスタイプ（株）を対応させるデータベース

(遺伝子座1, 遺伝子座2, 遺伝子座3)	シーケンスタイプ°
(1, 1, 1)	ST1
(1, 1, 2)	ST2
(1, 1, 3)	ST3
(1, 2, 1)	ST4
(1, 2, 2)	ST5
(1, 2, 3)	ST6

●
●
●

(遺伝子座1, 遺伝子座2, 遺伝子座3) =

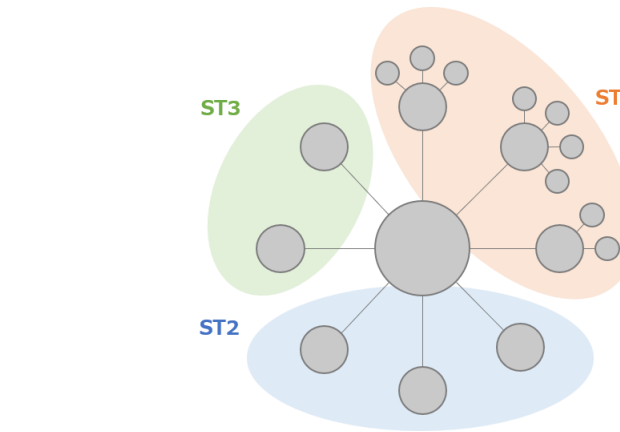
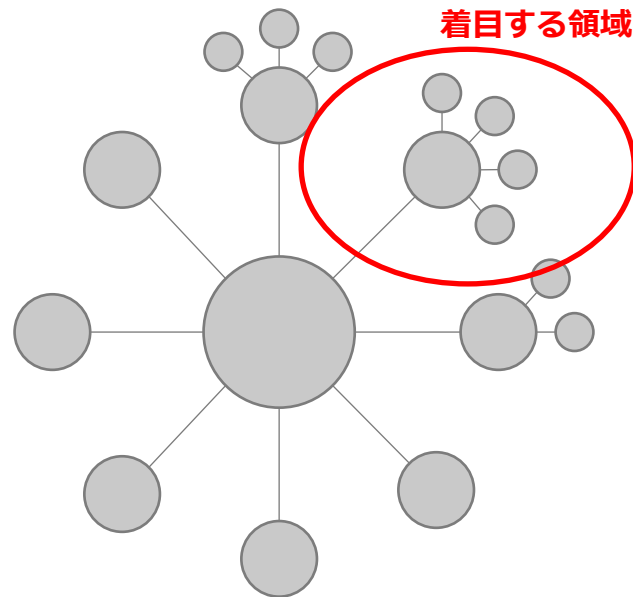


(遺伝子座1, 遺伝子座2, 遺伝子座3)	シーケンスタイプ
(1, 1, 1)	ST1
(1, 1, 2)	ST2
(1, 1, 3)	ST3
(1, 2, 1)	ST4
(1, 2, 2)	ST5
(1, 2, 3)	ST6

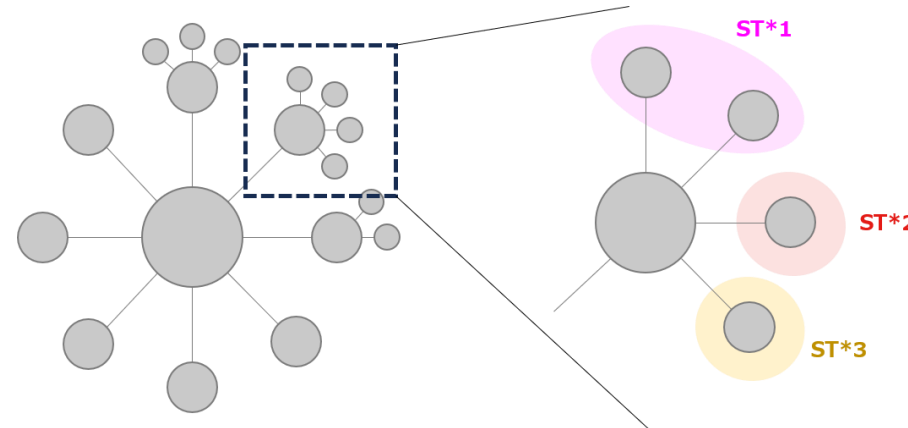
●
●
●

各配列におけるアレルの組み合わせとスキームを対応させて、株の同定を行う

キュレーション済みのスキームをPubMLSTなどからダウンロードして使用可能だが、ローカルなアウトブレイクの場合は、自分でスキームを構築してタイピングを行う方が適している場合がある。



通常のMLST



アドホックなMLST

通常のMLSTを実施し、対象と最も近い株のリファレンス配列を決定



リファレンス配列の全遺伝子座を使用してスキームを構築



リファレンス配列から構築したスキームを用いてタイピング

通常のMLSTを実施し、対象と最も近い株のリファレンス配列を決定



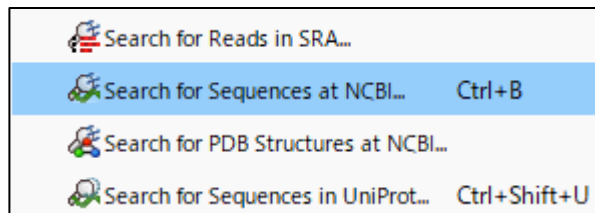
リファレンス配列の全遺伝子座を使用してスキームを構築



リファレンス配列から構築したスキームを用いてタイピング

..... SRR16508428
..... SRR16508429
..... SRR16508430
..... SRR16508431
..... SRR16508432
..... SRR16508433
..... SRR16508434
..... SRR16508435

*Acinetobacter baumannii*から得られた8つのリードデータ (fastq)



Choose database: ☒ Nucleotide ☐ Protein ☐ EST

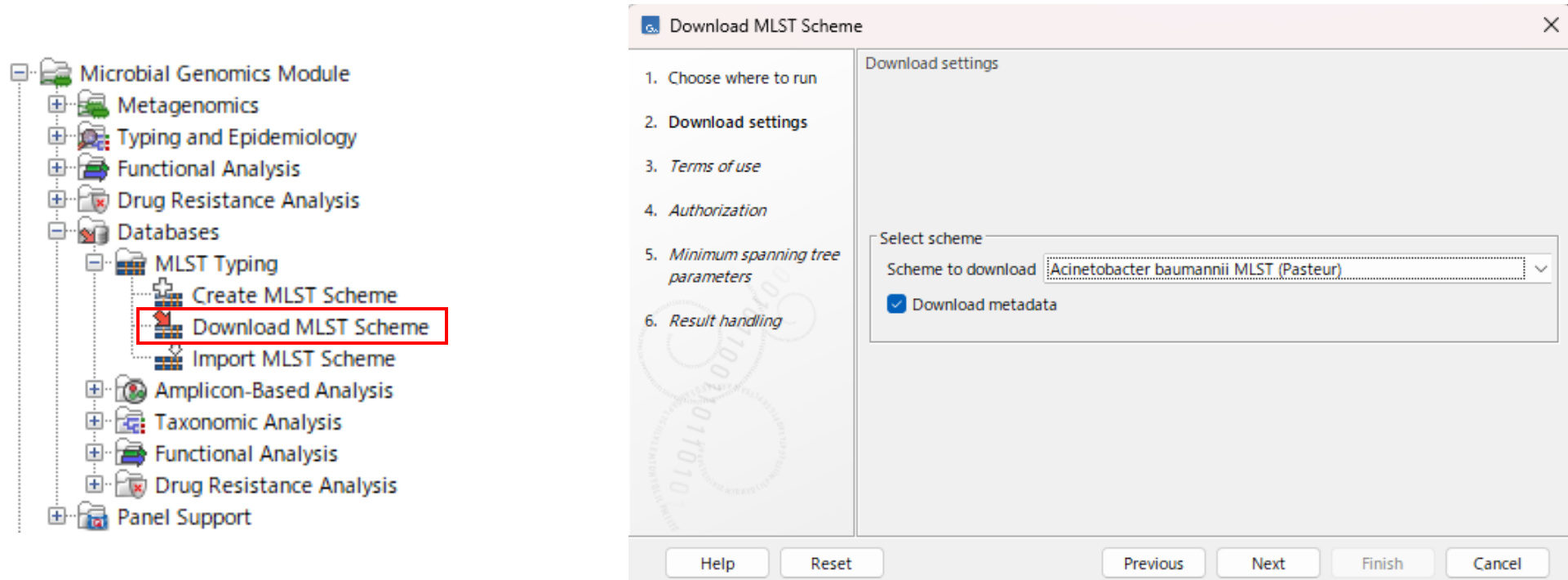
Organism:

Definition/Title:

☒ Append wildcard (*) to search words

Hit	Accession	Description	Modification Date	Length
1	NZ_CP043469	Acinetobacter baumannii strain 18WIARLN0021 chromosome, complete g...	2026/08/28	3908609
2	AP051988	Acinetobacter baumannii strain KPSE34 complete genome	2026/08/22	3956519
3	AP051985	Acinetobacter baumannii strain KPSE33 complete genome	2026/08/22	3976552
4	AP051982	Acinetobacter baumannii strain KPSE32 complete genome	2026/08/22	4007363
5	AP051979	Acinetobacter baumannii strain KPSE31 complete genome	2026/08/22	4007375
6	AP051976	Acinetobacter baumannii strain KPSE30 complete genome	2026/08/22	4007379
7	AP051973	Acinetobacter baumannii strain KPSE29 complete genome	2026/08/22	4007384
8	NZ_CP107610	Acinetobacter baumannii strain 1326924-3 chromosome, complete geno...	2026/08/23	3965229
9	NZ_CP107590	Acinetobacter baumannii strain 1326569 chromosome, complete genome	2026/08/23	4062852
10	NZ_CP107601	Acinetobacter baumannii strain 1326589 chromosome, complete genome	2026/08/23	3966293
11	NZ_CP107612	Acinetobacter baumannii strain 1326927-1 chromosome, complete geno...	2026/08/23	3964660
12	NZ_CP107614	Acinetobacter baumannii strain 1326927-2 chromosome, complete geno...	2026/08/23	3901216
13	NZ_CP107581	Acinetobacter baumannii strain 1326525-2 chromosome, complete geno...	2026/08/23	3961871
14	NZ_CP107608	Acinetobacter baumannii strain 1326924-2 chromosome, complete geno...	2026/08/23	3965152
15	NZ_CP175827	Acinetobacter baumannii strain AB-40_FGL chromosome, complete geno...	2026/08/17	3984000
16	NZ_CP196959	Acinetobacter baumannii strain 17978rif chromosome, complete genome	2026/08/17	3860341
17	NZ_CP175853	Acinetobacter baumannii strain AB-25_FGL chromosome, complete geno...	2026/08/16	3958658
18	NZ_CP175876	Acinetobacter baumannii strain AB-12_FGL chromosome, complete geno...	2026/08/16	4082851
19	NZ_CP175843	Acinetobacter baumannii strain AB-34_FGL chromosome, complete geno...	2026/08/16	3925934

NCBI Search機能を利用し、対象種のゲノム配列をいくつかダウンロードしておく



Download MLST Schemeを使用し、対象種のキュレーション済みスキームをダウンロード

対象と近い株のリファレンスを取得

The image displays the Filgen software interface, specifically the 'Type with MLST Scheme' workflow. On the left, a tree view shows the 'Microbial Genomics Module' with various sub-modules. The 'Typing and Epidemiology' module is expanded, and 'Type with MLST Scheme' is highlighted with a red box. On the right, a detailed view of the 'Type with MLST Scheme' workflow is shown. It includes a 'Select sequence or sequence list' step, a 'Navigation Area' with a search bar, and a 'Selected elements (18)' list. The 'Selected elements' list contains 18 items, including SRR16508428 through SRR16508435, GCA_000695855.3, GCA_000746605.1, GCA_000746645.1, GCA_000761175.1, GCA_000794125.2, GCA_000814345.1, GCA_000828935.1, GCA_000830055.1, GCA_000963815.1, and GCA_009035845.1. The 'Batch' checkbox is unchecked. Navigation buttons (Help, Reset, Previous, Next, Finish, Cancel) are at the bottom.

Type with MLST Schemeで、対象のリードデータおよびダウンロードした配列をタイピング

対象と近い株のリファレンスを取得

Type with MLST Scheme

1. Choose where to run

2. Select sequence or sequence list

3. Typing parameters

4. Novel allele detection parameters

5. Result handling

Typing parameters

Select scheme

MLST scheme  Acinetobacter baumannii MLST (Pasteur) (2026-07-27) 

Per allele settings

Kmer size

Sequence type settings

Minimum locus presence

Comparing a known to a missing allele

☐ Counted as same alleles

☒ Counted as different alleles

Help Reset Previous Next Finish Cancel

Type with MLST Scheme

1. Choose where to run

2. Select sequence or sequence list

3. Typing parameters

4. Novel allele detection parameters

5. Result handling

Novel allele detection parameters

Set novel allele search

☐ Search novel alleles

Set threshold parameters

Minimum required fraction of kmers

Set acceptance parameters

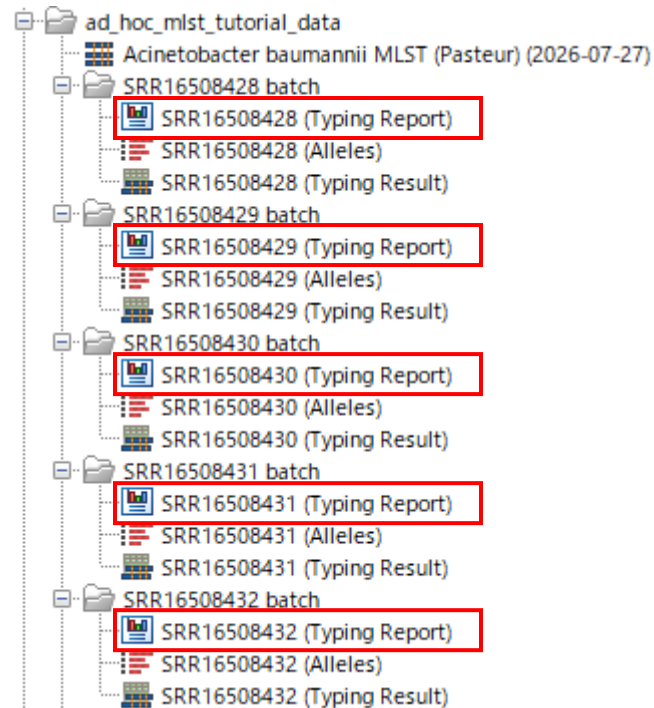
Minimum length

Minimum length fraction

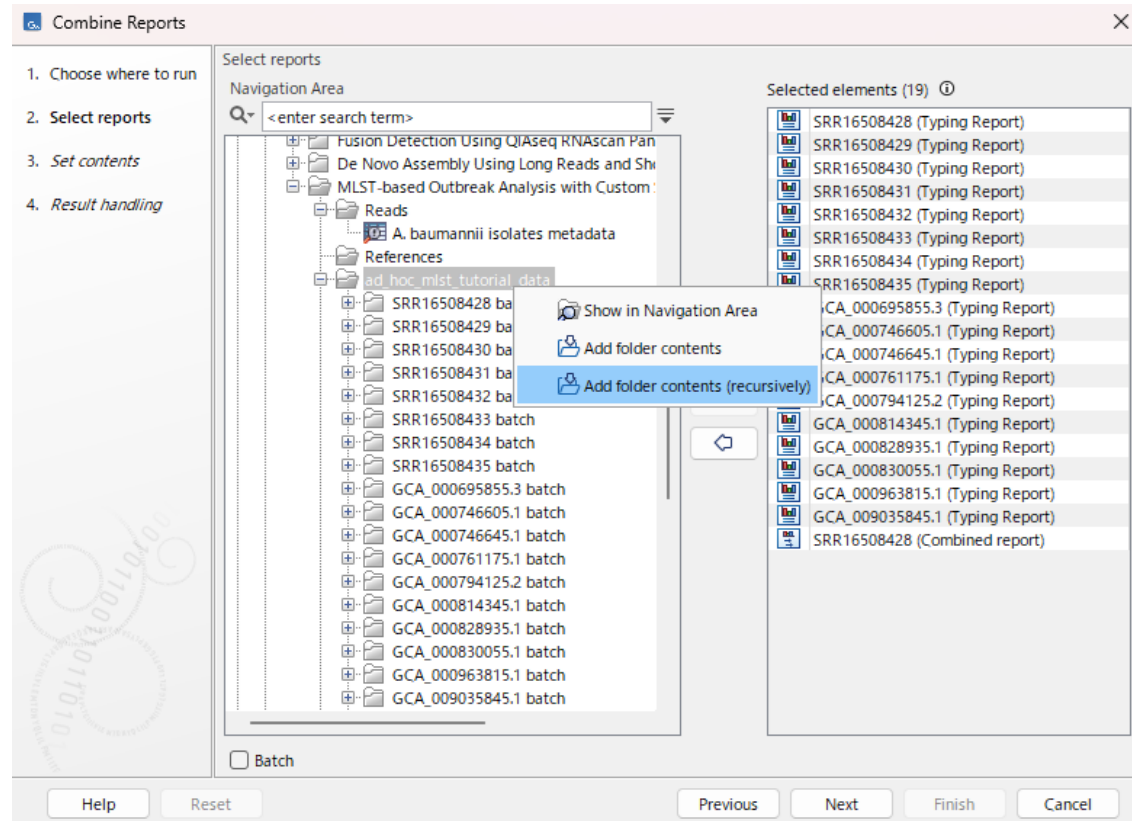
Help Reset Previous Next Finish Cancel

ダウンロードしたスキームを指定

対象と近い株のリファレンスを取得



Combine Reports



サンプルごとにタイピングレポートが作成されるので、Combine Reportsで結合する

2.1 Typing result

The table is based on 18 samples.

Sample name	Typing	Sequence types
SRR16508428 (Typing Report)	Conclusive	ST158
SRR16508429 (Typing Report)	Conclusive	ST2
SRR16508430 (Typing Report)	Conclusive	ST2
SRR16508431 (Typing Report)	Conclusive	ST2
SRR16508432 (Typing Report)	Conclusive	ST1
SRR16508433 (Typing Report)	Conclusive	ST1
SRR16508434 (Typing Report)	Conclusive	ST2
SRR16508435 (Typing Report)	Conclusive	ST2
GCA_000695855.3 (Typing Report)	Conclusive	ST2
GCA_000746605.1 (Typing Report)	Conclusive	ST638
GCA_000746645.1 (Typing Report)	Conclusive	ST79
GCA_000761175.1 (Typing Report)	Conclusive	ST79
GCA_000794125.2 (Typing Report)	Conclusive	ST1
GCA_000814345.1 (Typing Report)	Conclusive	ST464
GCA_000828935.1 (Typing Report)	Conclusive	ST622
GCA_000830055.1 (Typing Report)	Conclusive	ST1
GCA_000963815.1 (Typing Report)	Conclusive	ST1
GCA_009035845.1 (Typing Report)	Conclusive	ST52

 [SRR16508428 \(Combined report\)](#)

全サンプルのタイピング結果の一覧表が得られる

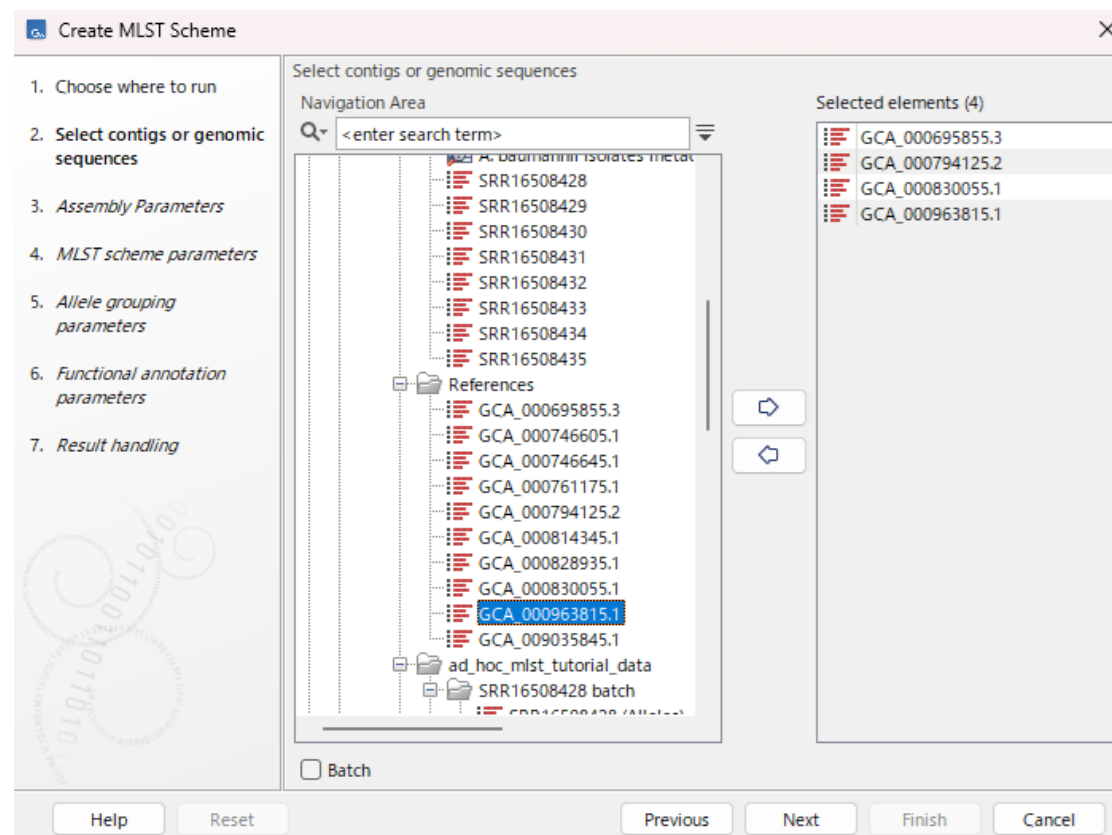
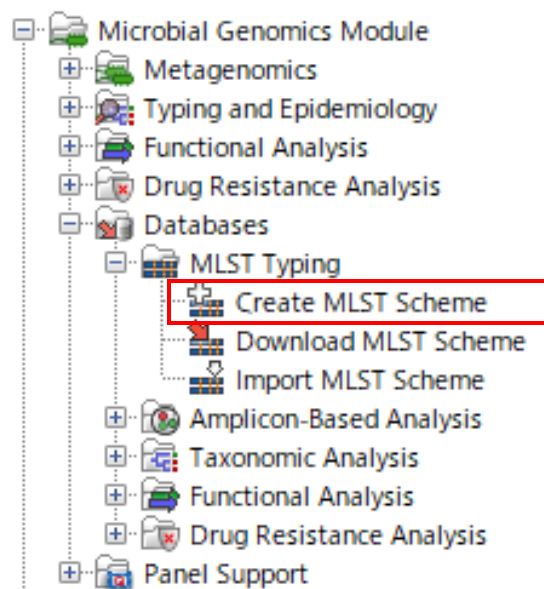
通常のMLSTを実施し、対象と最も近い株のリファレンス配列を決定



リファレンス配列の全遺伝子座を使用してスキームを構築



リファレンス配列から構築したスキームを用いてタイピング



Create MLST Schemeを使用し、リファレンス配列からスキームを作成

Create MLST Scheme

1. Choose where to run

2. Select contigs or genomic sequences

3. **Assembly Parameters**

4. MLST scheme parameters

5. Allele grouping parameters

6. Functional annotation parameters

7. Result handling

Assembly Parameters

Assembly grouping

Assembly grouping: Each input element is one assembly

Assembly annotation type: Assembly ID

Help Reset Previous **Next** Finish Cancel

Create MLST Scheme

1. Choose where to run

2. Select contigs or genomic sequences

3. Assembly Parameters

4. **MLST scheme parameters**

5. Allele grouping parameters

6. Functional annotation parameters

7. Result handling

MLST scheme parameters

MLST options

☒ Whole genome

☐ Core genome

☐ Custom fraction

Minimum fraction: 0.9

Handle genes without CDS annotations

☐ Ignore

☒ Search alleles before clustering

☐ Search alleles after clustering

Help Reset Previous **Next** Finish Cancel

 GCA_000695855.3 (wgMLST)

Filter

Selection...

Show All

Rows: 2,249

Locus	Locus category	Number of alleles
locus0		1
locus1		1
locus2		1
locus3		3
locus4		1
locus5		1
locus6		1
locus7		1
locus8		1
locus9		1
locus10		1

Select Loci in Other Views

Allele name	Sequence length	Creation date	Gene info
ABUW_2361 (locus3 allele 1)	840	Thu Dec 18 20:19:46 JST 20...	
BL01_02650 (locus3 allele 2)	840	Thu Dec 18 20:19:44 JST 20...	
NH10_002231 (locus3 allele 3)	840	Thu Dec 18 20:19:44 JST 20...	

各遺伝子座におけるアレル数や、アレルの種類を確認できる

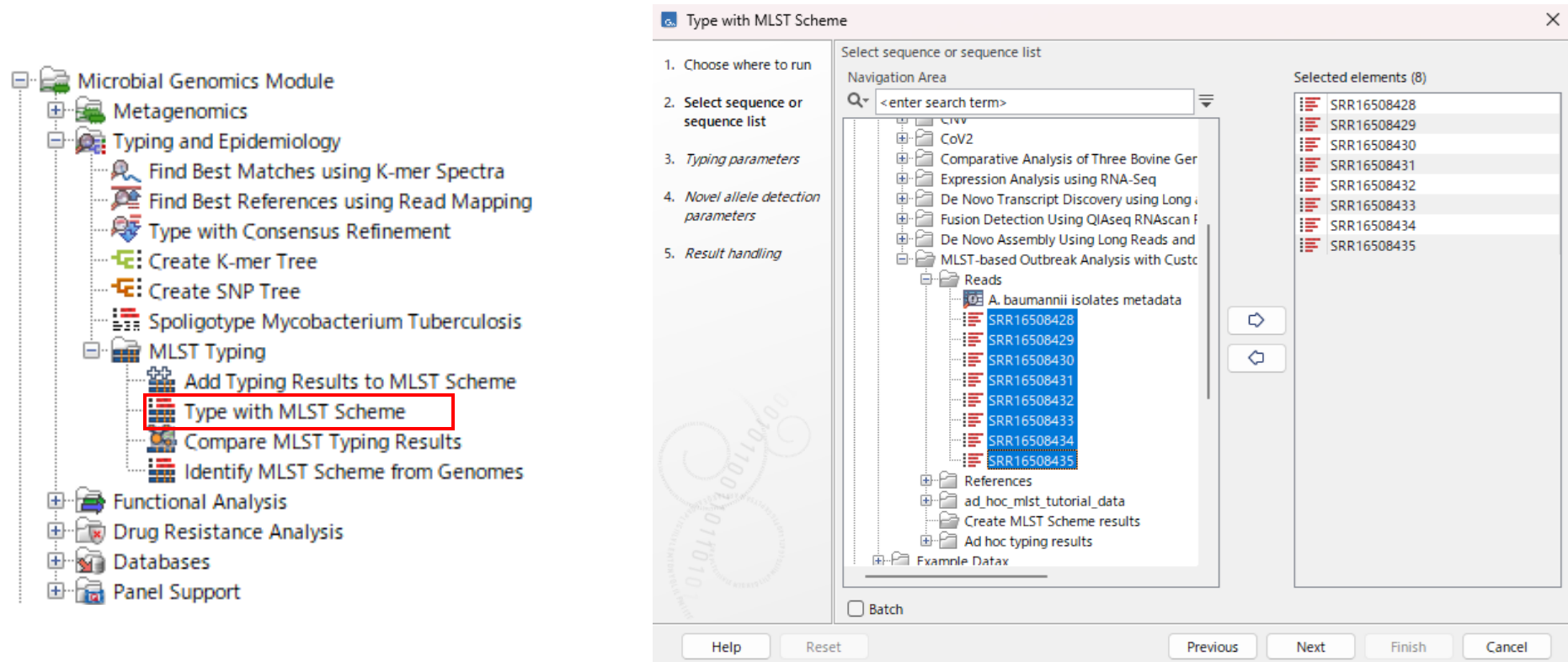
通常のMLSTを実施し、対象と最も近い株のリファレンス配列を決定



リファレンス配列の全遺伝子座を使用してスキームを構築



リファレンス配列から構築したスキームを用いてタイピング



Type with MLST Schemeで、対象のリードデータをタイピング

Type with MLST Scheme

1. Choose where to run

2. Select sequence or sequence list

3. **Typing parameters**

4. Novel allele detection parameters

5. Result handling

Typing parameters

Select scheme

MLST scheme  GCA_000695855.3 (wgMLST) 

Per allele settings

Kmer size

Sequence type settings

Minimum locus presence

Comparing a known to a missing allele

☐ Counted as same alleles

☒ Counted as different alleles

Help Reset Previous Next Finish Cancel

Type with MLST Scheme

1. Choose where to run

2. Select sequence or sequence list

3. Typing parameters

4. **Novel allele detection parameters**

5. Result handling

Novel allele detection parameters

Set novel allele search

☒ Search novel alleles

Set threshold parameters

Minimum required fraction of kmers

Set acceptance parameters

Minimum length

Minimum length fraction

Help Reset Previous Next Finish Cancel

作成したスキームを指定

2 Type with MLST scheme

2.1 Typing result

The table is based on 8 samples.

Sample name	Typing	Sequence types
SRR16508430 (Typing Report)	Conclusive	Novel
SRR16508428 (Typing Report)	Not possible	-
SRR16508429 (Typing Report)	Conclusive	Novel
SRR16508431 (Typing Report)	Conclusive	Novel
SRR16508432 (Typing Report)	Conclusive	Novel
SRR16508433 (Typing Report)	Conclusive	Novel
SRR16508434 (Typing Report)	Conclusive	Novel
SRR16508435 (Typing Report)	Conclusive	Novel



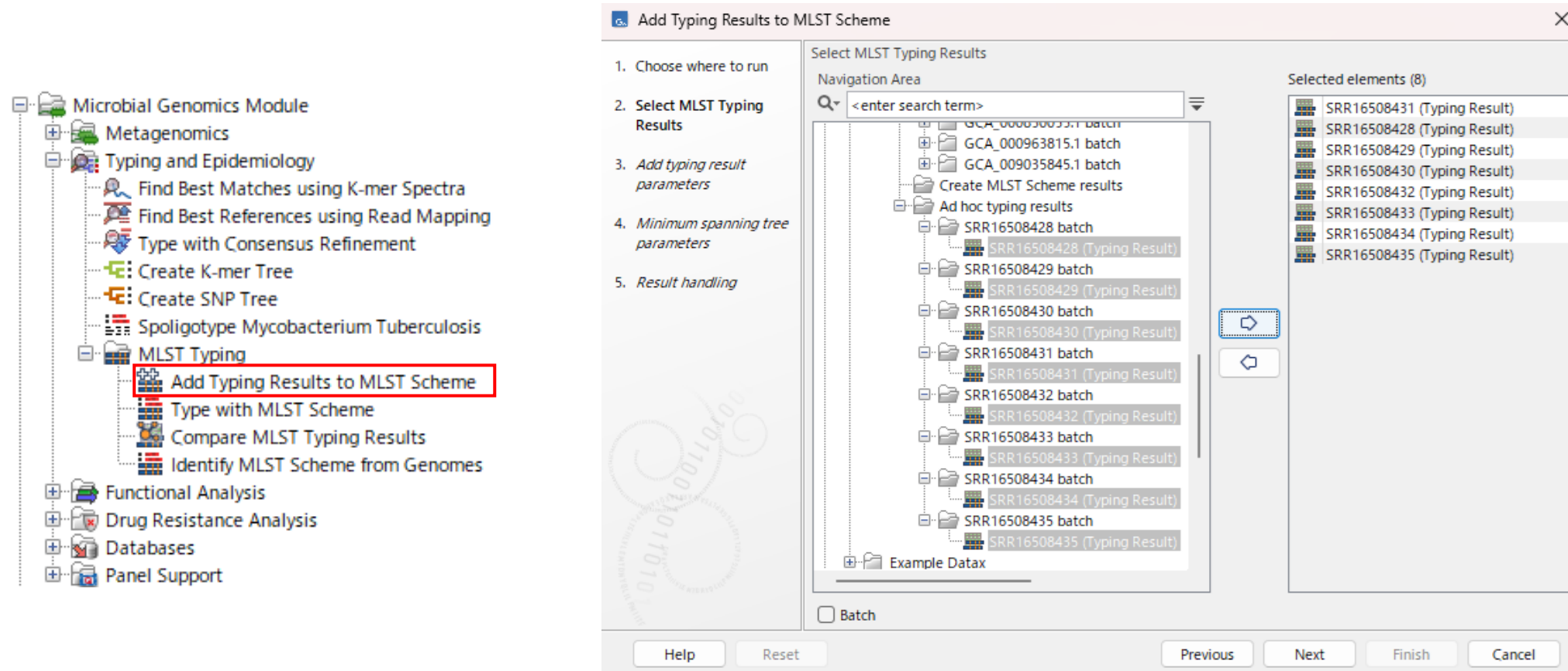
Combine Reports

2.2 Typing summary

The table is based on 8 samples.

Sample name	Loci identified	Novel alleles identified	Loci not identified	Locus presence (%)	Estimated sample coverage
SRR16508430 (Typing Report)	1,816	49	433	80.75	20.30
SRR16508428 (Typing Report)	543	316	1,706	24.14	42.33
SRR16508429 (Typing Report)	1,715	90	534	76.26	32.89

サンプルごとにタイピングレポートが作成されるので、
先ほどと同様にCombine Reportsで結合する



Add Typing Results to MLST Schemeでタイピング結果をスキームに追加する

Add Typing Results to MLST Scheme

1. Choose where to run

2. Select MLST Typing Results

3. Add typing result parameters

4. Minimum spanning tree parameters

5. Result handling

Add typing result parameters

Select scheme

MLST Scheme  GCA_000695855.3 (wgMLST) 

Sequence type label

Novel allele qualification parameters

Outlier range factor

Allowed length variation fraction

☒ Allow incomplete novel alleles

Sequence type qualification parameters

Minimum average kmer fraction

Help Reset Previous **Next** Finish Cancel

Add Typing Results to MLST Scheme

1. Choose where to run

2. Select MLST Typing Results

3. Add typing result parameters

4. Minimum spanning tree parameters

5. Result handling

Minimum spanning tree parameters

Minimum spanning tree

☒ Create minimum spanning tree

Comparing a known to a missing allele

☐ Counted as same alleles

☒ Counted as different alleles

Calculate clusters

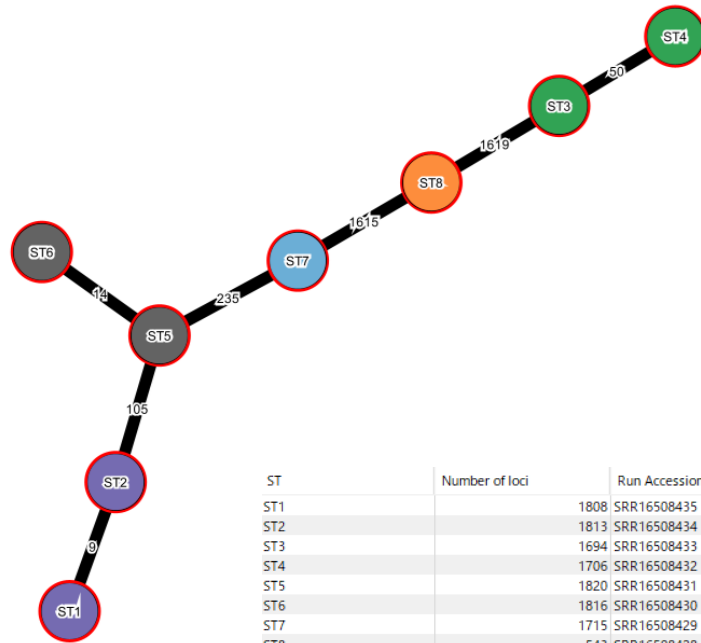
☐ Add clonal cluster metadata

Allelic distance clustering levels

Help Reset Previous **Next** Finish Cancel

作成したスキームを指定

 GCA_000695855.3 (wgMLST, updated)



ST	Number of loci	Run Accession	PFGE Apal	PFGE SmaI
ST1		1808 SRR16508435	B1	I
ST2		1813 SRR16508434	B1	I
ST3		1694 SRR16508433	A	NT
ST4		1706 SRR16508432	A	NT
ST5		1820 SRR16508431	B2	G
ST6		1816 SRR16508430	B2	G
ST7		1715 SRR16508429	C	F
ST8		543 SRR16508428	D	E

Locus	Locus category	Number of alleles	Percentage of sequence types
locus0		2	87.50
locus1		1	25.00
locus2		1	75.00
locus3		3	87.50
locus4		1	75.00
locus5		1	75.00
locus6		1	75.00
locus7		1	75.00
locus8		1	75.00
locus9		1	62.50
locus10		2	87.50
locus11		1	62.50
locus12		1	62.50
locus13		1	62.50
locus14		1	62.50
locus15		1	62.50
locus16		2	62.50
locus17		2	87.50
locus18		2	87.50
locus19		1	87.50
locus20		1	25.00
locus21		1	87.50
locus22		2	87.50
locus23		1	62.50
locus24		1	62.50
locus25		1	62.50
locus26		1	62.50
locus27		1	62.50
locus28		1	62.50

Select Loci in Other Views

Allele name	Sequence length	Creation date	Gene info	Sequence types	Percentage
ABUW_2361 (locus3 al...	840	Thu Dec 18 20:19:46 JS...		0	0.00%
BL01_02650 (locus3 all...	840	Thu Dec 18 20:19:44 JS...		5	62.50%
NH10_002231 (locus3 ...	840	Thu Dec 18 20:19:44 JS...		2	25.00%

アレル情報やMinimum Spanning Tree等を確認可能

お問い合わせ先：フィルジェン株式会社

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FAX: 052-624-4389

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