

TOMY DIGITAL BIOLOGY CO., LTD.





第13回 アンプリコンメタゲノミクス(その5)

メタゲノミクスは、環境サンプルから直接回収された遺伝物質の研究です。前回(<u>第 12 回 アンプリコ</u> ンメタゲノミクス(その4))から引き続き、発酵プロセスに関連する細菌群をプロファイルするため、自 然発酵したザワークラウトから PCR 増幅された 16S rRNA 遺伝子配列を解析する手法をご紹介し ます。

今回は Sequence Classifier プラグインを使用して、前回までに作成した 16S データベースを用いて、全アンプリコンデータセットを解析する手法についてです。

もし Sequence Classifier プラグインをインストールしていない場合は、Tools → Plugins で 表示されるプラグインの一覧から、Sequence Classifier を選択してインストールします。

第 9 回アンプリコンメタゲノミクス(その 1)で作成したリードファイルを選択します。デモデータでは SRR7140083\_50000 (trimmed) (merged) - length 150 to 260 というファイル名で、 トリム、マージ、length フィルタリング済みのリードセットです。OTU リストではなく、全リードのリス トに戻って使用することにご注意ください。

Tools → Classify Sequences に進み、Database Folder で、前回作成した SRR7140083 16S database を選択します。デモデータセットに含まれる 16S アンプリコン配列は、16S の V4 領域のみから得られたものであるため、種レベルに分類するために十分な解像度を有していません。 そこで、Database sequences taxonomy field を利用して、データベース配列との相同性か ら、アンプリコンリードを属レベルに分類することにします。

そのためには、以下のスクリーンショットに示すような設定を行います。まず、Sensitivity を High Sensitivity/Medium に設定し、Minimum Overlap を 100 bp に設定します。 Classificationの設定では、分類元として Database sequence taxonomy field を選択し、 minimum overlap identity for the lowest taxonomic level を 95%に、それ以降は 90% および 85%に設定します。上述のようにデモデータでは、最も低い分類レベルが種ではなく属である ことに注意してください。

Searching Database Folder:  SRR7140083 165 database Sensitivity: High Sensitivity / Medium  Minimum Overlap: 100  Hp.  Minimum Overlap Identity: 80  Minimum Overlap Identity: 80  Minimum Overlap Identity: 80  Minimum Overlap Identity to classify: 75  Minimum % identity higher than next best result to classify: 0.2  Minimum % identity higher than next best result to classify: 0.2  Minimum % identity higher than next best result to classify: 0.2  Minimum overlap identity to classify at lowest taxonomic level (e.g. species): 95  Minimum overlap identity to classify at lowest taxonomic level (e.g. species): 95  Minimum overlap identity to classify at second lowest taxonomic level (e.g. family): 85  Minimum overlap identity to classify at second lowest taxonomic level (e.g. family): 85  Minimum overlap identity to classify at third lowest taxonomic level (e.g. family): 85  Minimum overlap identity to classify at third lowest taxonomic level (e.g. family): 85  Minimum overlap identity to the first = are considered to be the same organism  Results Create table of all hit similarities for each query sequence Save all pairwise alignments. Sub-folder: Document Namel Pairwise Alignments Highlight results in green with minimum overlap identity: 95  Minimum Si exert to all hits per query Alignments Save multiple alignment of all hits per query Alignments Save tree of all hits per query Tree Options Save alignments and trees in sub-folder: (Document Name) Alignments & Trees	0 🕒	Classify Sequences
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OK をクリックすると、Sequence Classifier が実行され、完了すると結果がレポートドキュメント に保存されます。レポートは Summary、Classifications、Results の 3 つで構成されており、 Summary にはデータベースを使用して基準に従って分類された配列の数が表示されます。また、未 分類の配列の数もリストで表示されます。Classifications には、解析に用いられたすべての配列が 一覧表示され、マッチした分類の情報が表示されます。Classifications で任意の配列を選択する と、その配列に対する個々の詳細が Results に表示されます。

	Classify S	equences l	Results Text	/iew	Lineage Info				
🛃 Export table								Ü 🛛 🖓	
	Cla	assified 12	2,458 out of 12,	465	sequences				
Summary Results for SRR7140083 21 21/1									
Classification Frequency V Frequency V					Results for SRR/140005.21 21/1				
Bacteria: Firmicutes: Bacilli: Lactobacillales: Leuconostocaceae: Leuconostoc			56.94%	n H	Database Sequence Name	Overlap identity #	Query Identity	Taxonomy	
Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae: Lactobacillus			40.26%		NK_133769 - BLAST Hit	100%	100%	Bacteria; Firmicutes; B	
Bacteria: Firmicutes: Bacilli: Lactobacillales: Leuconostocaceae			1.08%		NK_075014 - BLAST Hit	99.21%	99.21%	Bacteria; Firmicutes; B	
Bacteria: Firmicutes: Bacilli: Lactobacillales: Lactobacillaceae			0.76%		NR_074997 - BLAST Hit	99.21%	99.21%	Bacteria; Firmicutes; B	
Bacteria: Firmicutes: Bacilli: Lactobacillales			0.20%		NR_157602 - BLAST Hit	98.02%	98.02%	Bacteria; Firmicutes; B	
Bacteria: Proteobacteria: Gammaproteobacteria: Enterobacterales: Yersin.			0.18%		NR_113255 - BLAST Hit	96.84%	96.84%	Bacteria; Firmicutes; B	
Bacteria: Firmicutes: Bacilli: Lactobacillales: Streptococcaceae: Lactococcus			0.11%		NR_136428 - BLAST Hit	95.26%	95.26%	Bacteria; Firmicutes; B	
Bacteria: Cvanobacteria: Nostocales			0.11%		NR_114312 - BLAST Hit	93.28%	93.28%	Bacteria; Firmicutes; B	
Racteria: Firmicutes: Racilli: Lactobacillales: Leuconostocaceae: Weissella			0.07%		NR_136437 - BLAST Hit	92.49%	92.49%	Bacteria; Firmicutes; B	
Unclassified (no match)			0.06%		NR_029041 - BLAST Hit	92.09%	92.09%	Bacteria; Firmicutes; B	
Ractaria: Proteobacteria: Cammanroteobacteria: Pseudomonadales: Pseu			0.02%		NR_042194 - BLAST Hit	89.72%	89.72%	Bacteria; Firmicutes; B	
Bacteria: Proteobacteria: Gamman	oteobacteria: Enterobacterales: Enter	2	0.02%		NR_113289 - BLAST Hit	88.54%	88.54%	Bacteria; Firmicutes; B	
Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriales, Enter			0.02%		NR_145899 - BLAST Hit	88.14%	88.14%	Bacteria; Firmicutes; B	
Bacteria, Proteobacteria, Betaproteobacteria, Burkhouderiales, Oxabbact			0.02%		NR_125575 - BLAST Hit	88.14%	88.14%	Bacteria; Firmicutes; B	
Barteria: Cvanobarteria	connecting, whicobiares, meetyronacter.	2	0.02%		NR_125562 - BLAST Hit	88.14%	88.14%	Bacteria; Firmicutes; B	
Bacteria, Cyanobacteria			0.0276		NR_041509 - BLAST Hit	88.14%	88.14%	Bacteria; Firmicutes; B	
Classifications					NR_114339 - BLAST Hit	87.75%	87.75%	Bacteria; Firmicutes; B	
Query Sequence A Overlap Identity	Classification Clos	est Sequence	e(s)	П	NR_104573 - BLAST Hit	87.75%	87.75%	Bacteria; Firmicutes; B	
SRR7140083.2 100%	Bacteria; Firmicutes; Bacilli; La NR_	133769 - 8	BLAST Hit	61	NR_125561 - BLAST Hit	87.35%	87.35%	Bacteria; Firmicutes; B	
SRR7140083.3 95.67%	Bacteria; Firmicutes; Bacilli; La NR_	042194 - B	BLAST Hit	1	NR_113821 - BLAST Hit	87.35%	87.35%	Bacteria; Firmicutes; B	
SRR7140083.4 100%	Bacteria; Firmicutes; Bacilli; La NR 133769 - BLAST Hit			- 11	NR_104976 - BLAST Hit	87.35%	87.35%	Bacteria; Firmicutes; B	
SRR7140083.4 100%	Bacteria: Firmicutes: Bacilli: La NR 074997 - BLAST Hit				NR_158030 - BLAST Hit	86.56%	86.56%	Bacteria; Firmicutes; B	
SRR7140083.5 97.06%	Bacteria; Firmicutes; Bacilli; La NR	133769 - B	BLAST Hit		NR_134066 - BLAST Hit	86.56%	86.56%	Bacteria; Firmicutes; B	
SRR7140083.5 99.13%	Bacteria: Firmicutes: Bacilli: La NR	157602 - B	BLAST Hit		NR_114251 - BLAST Hit	86.56%	86.56%	Bacteria; Firmicutes; B	
SRR7140083.5 98.06%	Bacteria: Firmicutes: Bacilli: La 2 be	st matches.	Names: [NR		NR_042456 - BLAST Hit	86.56%	86.56%	Bacteria; Firmicutes; B	
SRR7140083.5 98.41% Bacteria: Firmicutes: Bacilli: La NR 114844 - BLAST Hit				NR_116238 - BLAST Hit	86.17%	86.17%	Bacteria; Firmicutes; B		
RR7140083.6 95.32% Bacteria: Firmicutes: Bacilli: La NR. 114844 - BLAST Hit				NR_114844 - BLAST Hit	86.17%	86.17%	Bacteria; Firmicutes; B		
SRR7140083.6 98.02%	Bacteria: Firmicutes: Bacilli: La NR 042456 - BLAST Hit				NR_044358 - BLAST Hit	86.17%	86.17%	Bacteria; Firmicutes; B	
SRR7140083.7 99.60%	83.7 99.60% Bacteria; Firmicutes; Bacilli; La NR 113821 - BLAST Hit				NR_158060 - BLAST Hit	86.03%	77.87%	Bacteria; Firmicutes; B	
SRR7140083.7 95.65%	RR7140083.7 95.65% Bacteria; Firmicutes; Bacilli; La NR_133769 - BLAST Hit				NR 114327 - BLAST Hit	85.77%	85.77%	Bacteria; Firmicutes; B	
SRR7140083.8 99.21%	SRR7140083.8 99.21% Bacteria; Firmicutes; Bacilli; La 2 best matches. Names; [NR				NR_156080 - BLAST Hit	85.38%	85.38%	Bacteria; Firmicutes: Cl	
SRR7140083.8 93.96% Bacteria; Firmicutes; Bacilli; La 2 best matches. Names: INR				NR 044289 - BLAST Hit	83.79%	83.79%	Bacteria; Firmicutes; Cl		
SRR7140083.9 99.07%	Bacteria; Firmicutes; Bacilli; La 2 be	st matches.	Names: [NR						
				1					

デモデータセットは主に Leuconostoc と Lactobacillus という、ザワークラウト発酵で優勢であることが知られている属で構成されていることがわかります。

データをエクスポートするには、Summary、Classifications、Results の各テーブルを選択し、 左上の Export Table をクリックすることで.csv フォーマットでエクスポートすることができます。

Classify Sequences ツールの詳細なマニュアルについては<u>こちら</u>からダウンロードできます。 Sequence Classifier のチュートリアルは<u>こちら</u>から入手できます。

次回はアンプリコンメタゲノミクスのオマケとして、16S Biodiversity ツールを用いた配列の分類 についてご紹介する予定です。

Geneious 製品概要・トライアルリクエストについては<u>こちら</u>

『Geneious Prime でシークエンス解析』の過去の記事はこちらでチェック!