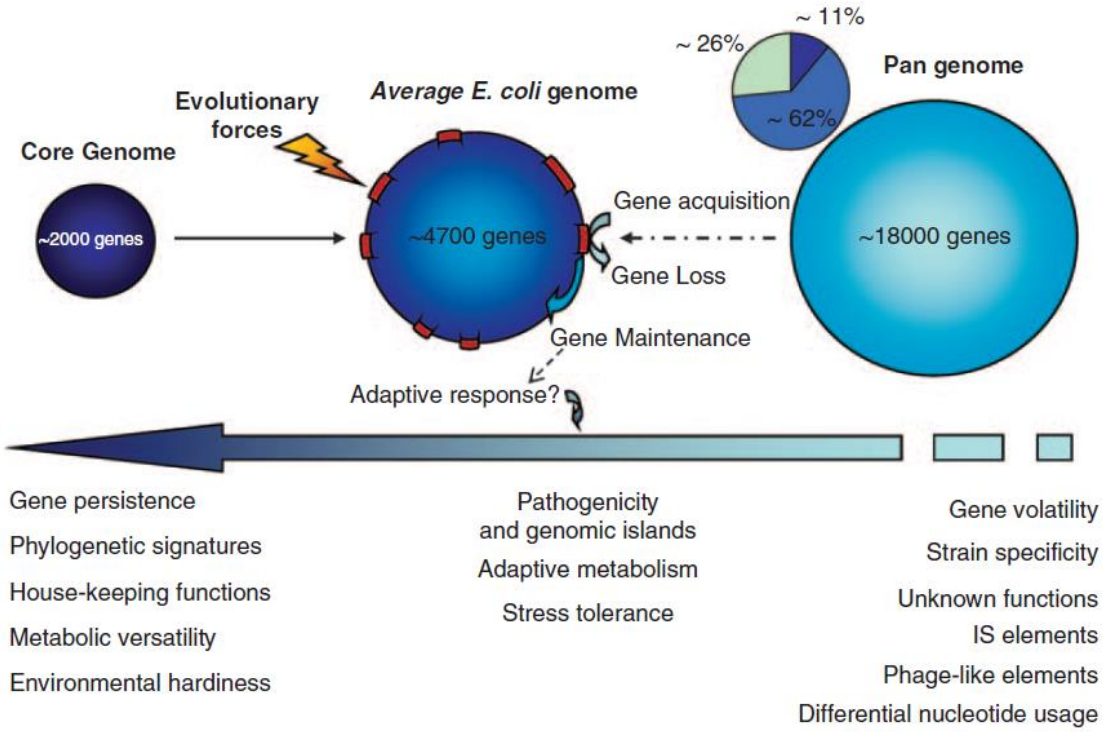
Introduction to core genome MLST (cgMLST)

Federica Gigliucci

Bioinformatics course,
11-12 July 2019

The *E. coli* pangenome

Genomic plasticity



Van Elsas J.D. et al., 2011

Pangenome **Whole genome** **Core genome** Housekeeping **Accessory genome**

Applying MLST to *E. coli*

Conventional MLST

7 housekeeping genes

Low sensitivity

High robustness

Good for phylogenetic analysis

Not good enough for outbreak investigation

MLST from WGS data

whole genome (wgMLST) – set of loci present in at least one strain

core genes (cgMLST) – set of loci present in the 95% of the strains

housekeeping genes (7-genesMLST)

Public database hosting MLST schemas

chewBBACA: assembly based allele-calling of cgMLST

Developed by INNUENDO (EFSA-funded project)

Based on cgMLST scheme developed by Enterobase

***E. coli* scheme by chewBBACA: 2360 curated loci**

https://github.com/B-UMMI/chewBBACA

MICROBIAL GENOMICS

Methods paper template

chewBBACA: A complete suite for gene-by-gene schema creation and strain identification

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chewBBACA: assembly based allele-calling of cgMLST

chewBBACA: BSR-Based Allele Calling Algorithm

Developed by INNUENDO (EFSA-funded project)

- **It works on pre-assembled contigs (.fasta)**
- **Complete coding sequence (CDS) identified by Prodigal – BLASTp search – for each genome query**
- **Blast comparison between CDS of blastp-db Vs alleles of the cgMLST scheme**
- **BLAST Score Ratio (BSR) > 0.6 to identify the allele. The 0.6 value is related to a DNA identity of 80%**

chewBBACA results – Statistics

Genome	EXC	INF	LNF	PLOT	NIPH	ALM	ASM
NC_017162.fna	892	2319	1909	0	104	5	37
NC_011586.fna	1563	1697	1809	0	116	6	75

The column headers stand for:

- ***EXC*** - alleles which have exact matches (100% DNA identity) with previously identified alleles
- ***INF*** - inferred new alleles using Prodigal CDS predictions
- ***LNF*** - loci not found. No alleles were found for the number of loci in the schema shown. This means that, for those loci, there were no BLAST hits or they were not within the BSR threshold for allele assignment.
- ***PLOT*** - possible loci on the tip of the query genome contigs (see image below). A locus is classified as *PLOT* when the CDS of the query genome has a BLAST hit with a known larger allele that covers the CDS sequence entirely and the unaligned regions of the larger allele exceeds one of the query genome contigs ends. This could be an artifact caused by genome fragmentation resulting in a shorter CDS prediction by Prodigal. To avoid locus misclassification, loci in such situations are classified as *PLOT*.

chewBBACA results – Statistics

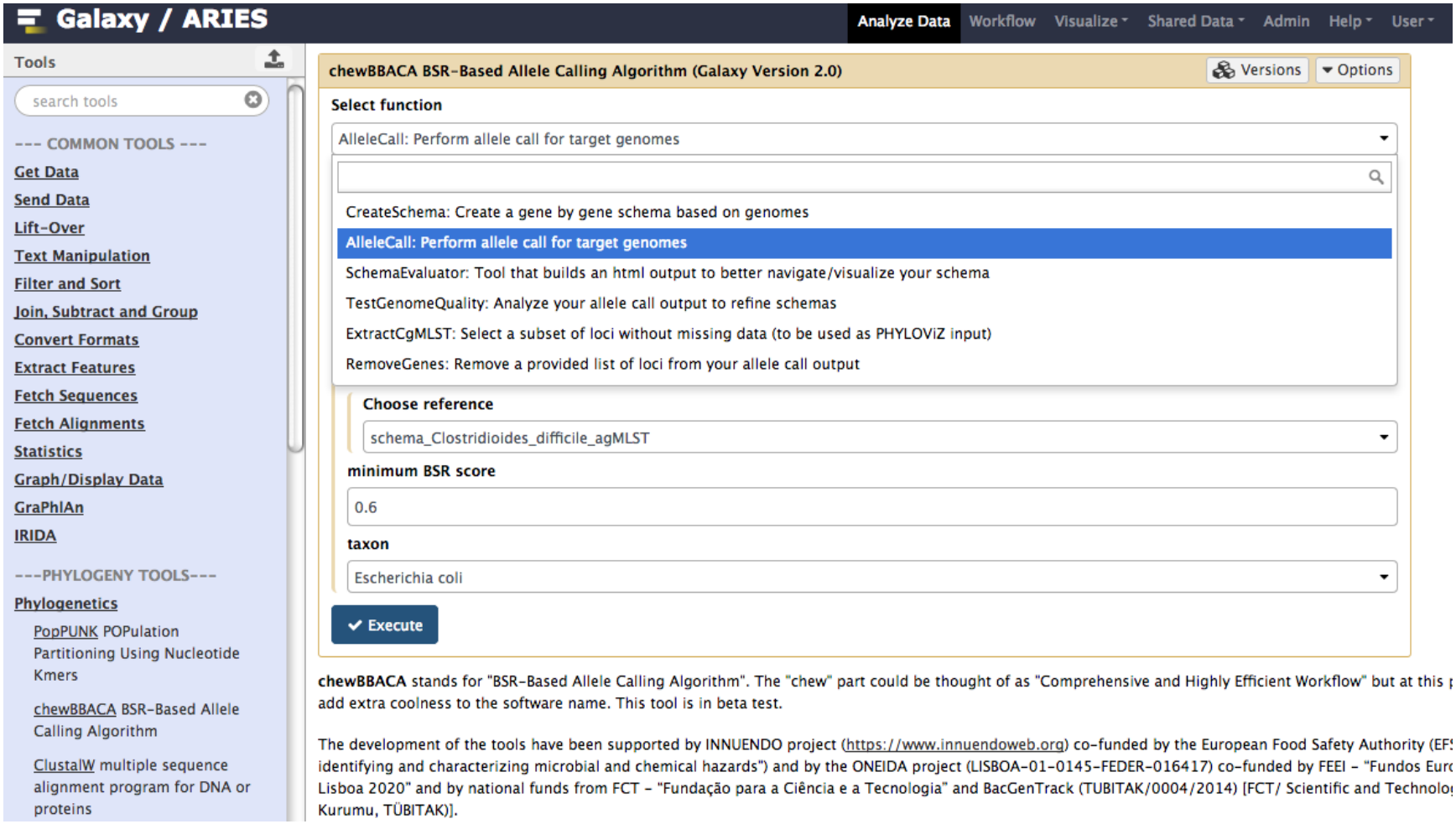
Genome	EXC	INF	LNF	PLOT	NIPH	ALM	ASM
NC_017162.fna	892	2319	1909	0	104	5	37
NC_011586.fna	1563	1697	1809	0	116	6	75

The column headers stand for:

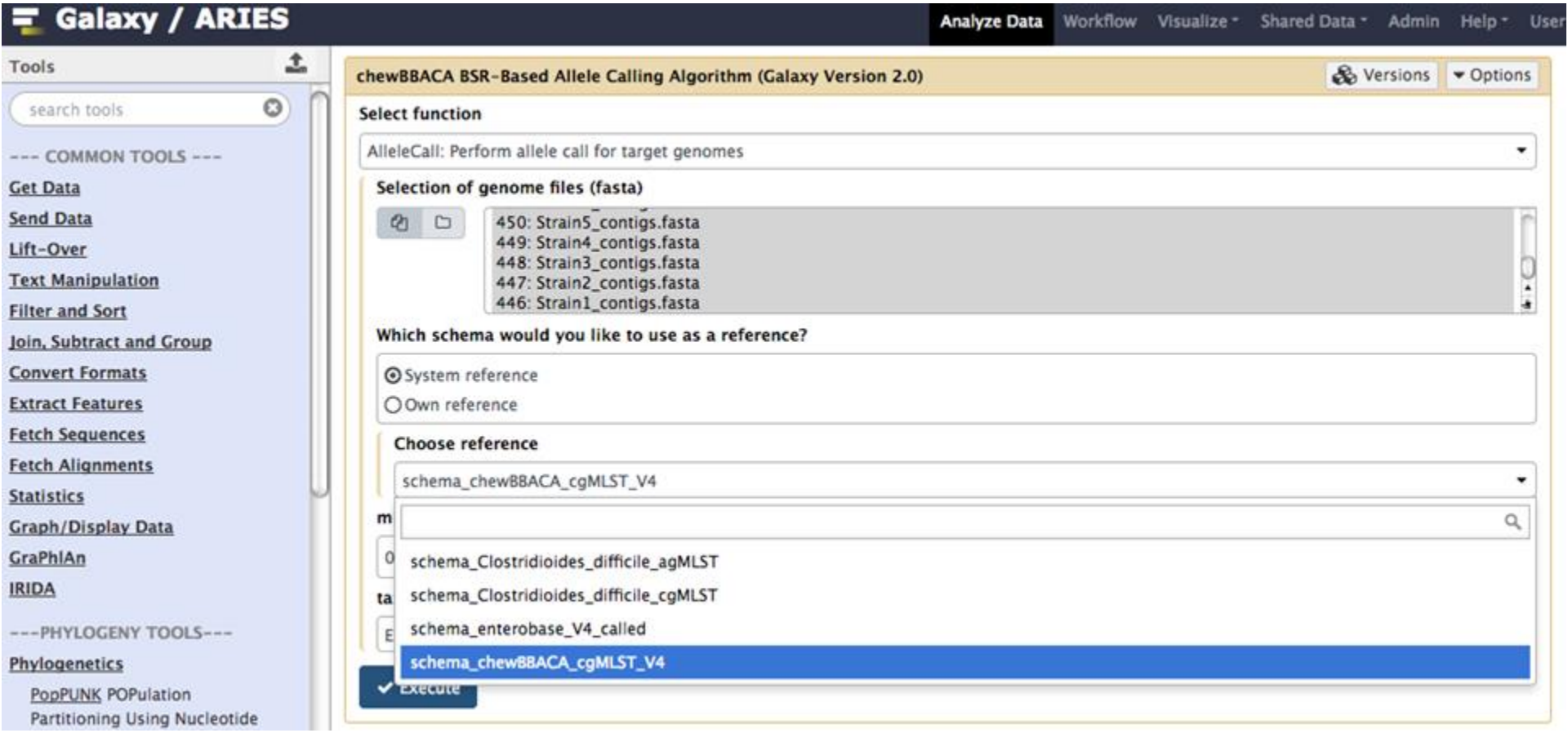
- ***NIPH*** - non-informative paralogous hit (see image below). When ≥ 2 CDSs in the query genome match one locus in the schema with a BSR > 0.6, that locus is classified as *NIPH*. This suggests that such locus can have paralogous (or orthologous) loci in the query genome and should be removed from the analysis due to the potential uncertainty in allele assignment (for example, due to the presence of multiple copies of the same mobile genetic element (MGE) or as a consequence of gene duplication followed by pseudogenization). A high number of NIPH may also indicate a poorly assembled genome due to a high number of smaller contigs which result in partial CDS predictions. These partial CDSs my contain conserved domains that match multiple loci. This classification takes precedence over *PLOT* classification.
- ***ALM*** - alleles 20% larger than length mode of the distribution of the matched loci (CDS length > (locus length mode + locus length mode * 0.2)) (see image below). This determination is based on the currently identified set of alleles for a given locus.
- ***ASM*** - similar to *ALM* but for alleles 20% smaller than length mode distribution of the matched loci (CDS length < (locus length mode - locus length mode * 0.2)).

A high number of PLOT, ASM, ALM and/or NIPH usually indicates bad quality or contaminated assemblies.

chewBBACA on ARIES



chewBBACA on ARIES



cgMLST scheme for *E. coli*: Schema_chewBBACA_cgMLST_V4

chewBBACA results on ARIES

Statistics

Genome	EXC	INF	LNF	PLOT	NIPH	ALM	ASM
Genome	EXC	INF	LNF	PLOT	NIPH	ALM	ASM
ED1032_contigs	3543	16	4007	0	27	3	5
ED1088_contigs	3348	77	4120	3	16	2	35
ED1089_contigs.fasta	3105	116	4263	6	11	16	84
ED1104_contigs.fasta	3493	4	4055	1	13	5	30
ED1105_contigs.fasta	3433	12	4098	1	14	4	39

Contigs info

FILE	b0073.fasta	b0074.fasta	b0075.fasta
ED1032_contigs	scaffold_0&199417-198324&-	scaffold_0&200988-199415&-	LNF
ED1088_contigs	NODE_1_length_228150_cov_40.8159_ID_1&198543-197450&-	NODE_1_length_228150_cov_40.8159_ID_1&200114-198541&-	LNF
ED1089_contigs.fasta	NODE_1_length_227956_cov_19.4419_ID_1&197229-196136&-	NODE_1_length_227956_cov_19.4419_ID_1&198800-197227&-	LNF
ED1104_contigs.fasta	NODE_4_length_186376_cov_34.6136_ID_7&29626-30717&+	NODE_4_length_186376_cov_34.6136_ID_7&28055-29626&+	LNF
ED1105_contigs.fasta	NODE_4_length_186376_cov_40.795_ID_7&29626-30717&+	NODE_4_length_186376_cov_40.795_ID_7&28055-29626&+	LNF

Alleles

Target genome file names

Allele call data for loci present in the schema

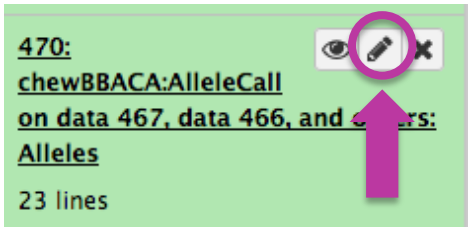
FILE	b0073.fasta	b0074.fasta	b0075.fasta	b0076.fasta	b0077.fasta	b0078.fasta
ED1032_contigs	10	11	LNF	460	13	2
ED1088_contigs	10	11	LNF	3	13	2
ED1089_contigs.fasta	10	11	LNF	3	13	2
ED1104_contigs.fasta	10	11	LNF	3	13	2
ED1105_contigs.fasta	10	11	LNF	3	13	2

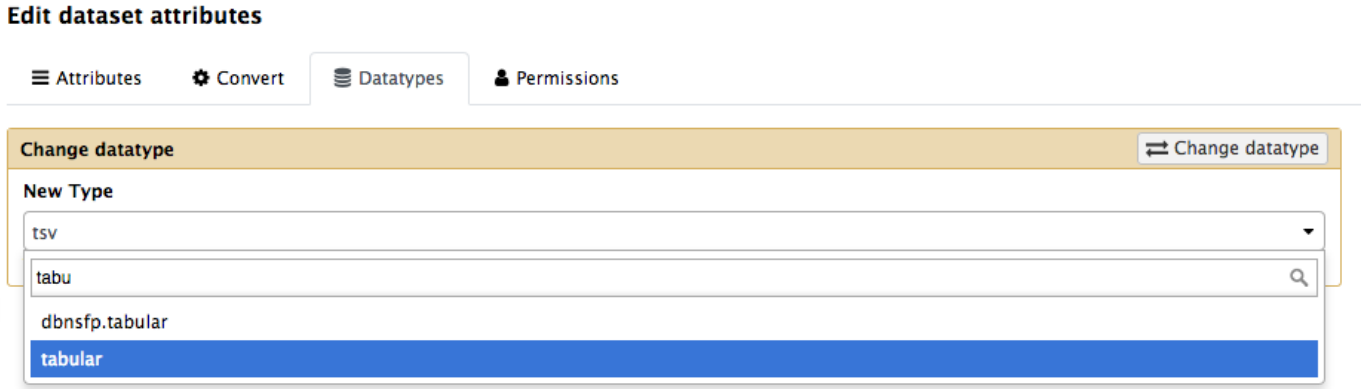
File to use for cluster analysis

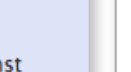
Logging info

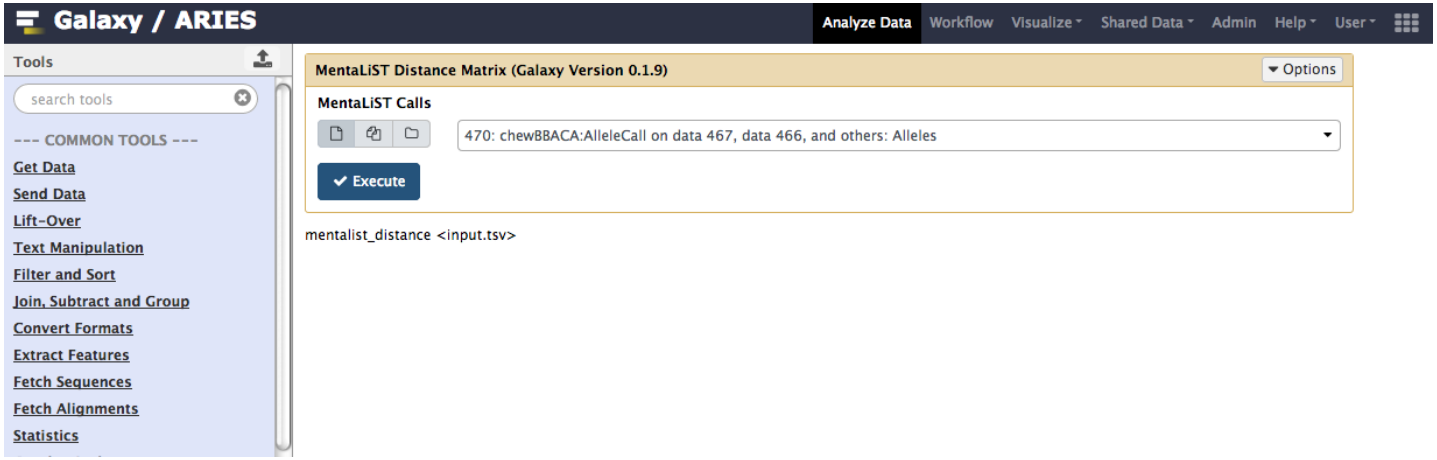
Repeated loci

Mentalist Distance Matrix tool calculates the number of allelic differences between strains









Tools

search tools

--- COMMON TOOLS ---

Get Data
Send Data
Lift-Over
Text Manipulation
Filter and Sort
Join, Subtract and Group
Convert Formats
Extract Features
Fetch Sequences
Fetch Alignments
Statistics
Graph/Display Data
GraPhlAn
IRIDA

---PHYLOGENY TOOLS---

Phylogenetics
MLST 7 Loci

MLST Scans genomes against PubMLST schemes.

MLST List Lists available schemes for the MLST tool.

SRST2 7 loci

MentaLiST MLST Analysis 0.2.3

MentaLiST MLST Analysis

MentaLiST Tree

MentaLiST Distance Matrix

MentaLiST MLST Analysis single-end reads

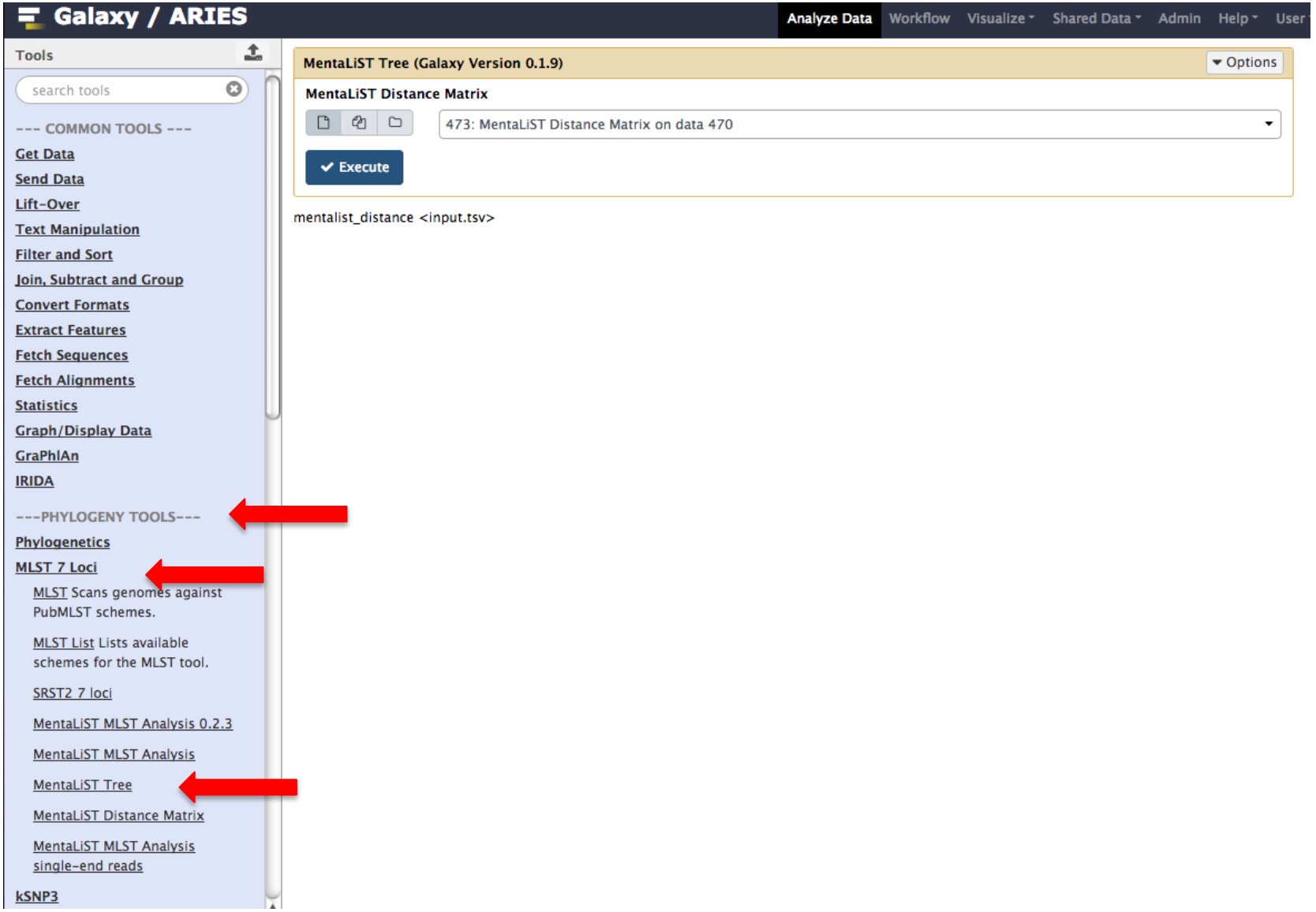
kSNP3
FDA SNP Pipeline

Distance Matrix with allelic differences

	Strain10_contigs.fasta	Strain11_contigs.fasta	Strain12_contigs.fasta	Strain13_contigs.fasta	Strain14_contigs.fasta	Strain15_contigs.fasta	Strain16_contigs.fasta	Strain17_contigs.fasta	Strain18_contigs.fasta	Strain19_contigs.fasta	Strain1_contigs.fasta	Strain20_contigs.fasta	Strain21_contigs.fasta	Strain22_contigs.fasta	Strain2_contigs.fasta	Strain3_contigs.fasta	Strain4_contigs.fasta	Strain5_contigs.fasta	Strain6_contigs.fasta	Strain7_contigs.fasta	Strain8_contigs.fasta	Strain9_contigs.fasta
Strain10_contigs.fasta	0	434	174	1647	1649	1647	1665	1650	1646	1640	536	2235	2237	2232	536	538	536	158	166	70	0	0
Strain11_contigs.fasta	434	0	531	1737	1739	1737	1752	1737	1740	1729	820	2242	2241	2234	820	821	819	514	523	444	434	434
Strain12_contigs.fasta	174	531	0	1643	1645	1643	1663	1646	1644	1636	536	2237	2239	2234	537	539	537	168	40	182	174	174
Strain13_contigs.fasta	1647	1737	1643	0	9	5	193	62	63	156	1648	2231	2232	2230	1647	1650	1648	1637	1643	1648	1647	1647
Strain14_contigs.fasta	1649	1739	1645	9	0	8	192	62	59	154	1650	2232	2233	2231	1649	1652	1650	1639	1645	1650	1649	1649
Strain15_contigs.fasta	1647	1737	1643	5	8	0	191	59	61	153	1648	2231	2232	2230	1647	1650	1648	1637	1643	1648	1647	1647
Strain16_contigs.fasta	1665	1752	1663	193	192	191	0	198	193	192	1664	2233	2236	2231	1663	1666	1664	1658	1665	1663	1665	1665
Strain17_contigs.fasta	1650	1737	1646	62	62	59	198	0	69	159	1650	2231	2233	2229	1649	1652	1650	1639	1645	1651	1650	1650
Strain18_contigs.fasta	1646	1740	1644	63	59	61	193	69	0	152	1647	2231	2233	2230	1646	1649	1647	1638	1644	1647	1646	1646
Strain19_contigs.fasta	1640	1729	1636	156	154	153	192	159	152	0	1639	2229	2232	2230	1638	1641	1639	1630	1637	1639	1640	1640
Strain1_contigs.fasta	536	820	536	1648	1650	1648	1664	1650	1647	1639	0	2239	2241	2235	2	4	3	501	530	539	536	536
Strain20_contigs.fasta	2235	2242	2237	2231	2232	2231	2233	2231	2231	2229	2239	0	226	302	2238	2238	2238	2235	2237	2235	2235	2235
Strain21_contigs.fasta	2237	2241	2239	2232	2233	2232	2236	2233	2233	2232	2241	226	0	237	2240	2240	2240	2237	2239	2237	2237	2237
Strain22_contigs.fasta	2232	2234	2234	2230	2231	2230	2231	2229	2230	2230	2235	302	237	0	2234	2234	2234	2232	2234	2232	2232	2232
Strain2_contigs.fasta	536	820	537	1647	1649	1647	1663	1649	1646	1638	2	2238	2240	2234	0	4	3	501	531	539	536	536
Strain3_contigs.fasta	538	821	539	1650	1652	1650	1666	1652	1649	1641	4	2238	2240	2234	4	0	5	503	533	541	538	538
Strain4_contigs.fasta	536	819	537	1648	1650	1648	1664	1650	1647	1639	3	2238	2240	2234	3	5	0	501	531	539	536	536
Strain5_contigs.fasta	158	514	168	1637	1639	1637	1658	1639	1638	1630	501	2235	2237	2232	501	503	501	0	160	166	158	158
Strain6_contigs.fasta	166	523	40	1643	1645	1643	1665	1645	1644	1637	530	2237	2239	2234	531	533	531	160	0	175	166	166
Strain7_contigs.fasta	70	444	182	1648	1650	1648	1663	1651	1647	1639	539	2235	2237	2232	539	541	539	166	175	0	70	70
Strain8_contigs.fasta	0	434	174	1647	1649	1647	1665	1650	1646	1640	536	2235	2237	2232	536	538	536	158	166	70	0	0
Strain9_contigs.fasta	0	434	174	1647	1649	1647	1665	1650	1646	1640	536	2235	2237	2232	536	538	536	158	166	70	0	0

Threshold of maximum 10 - 15 allelic differences to consider *E. coli* strains related

Mentalist Tree tool uses the matrix with allelic differences to build a phylogenetic tree



Change format from “txt” to “tre” to the Mentalist Tree output