

Outbreaks, typing and AMR/Day 7

Outbreak detection and investigation

Section of Foodborne infections, Dep. of bacteria, parasites and fungi, Statens Serum Institute

March 2024

Intended learning outcomes

Lecture:

1. Explain the background of cgMLST
2. Explain genetic clustering and its detection in an outbreak context

Practical exercise:

1. Utilise available command-line tools for cgMLST typing
2. Detect clusters of closely related isolates using data generated by different sequencing technologies and approaches
3. Interpret genetic clusters for outbreak investigations

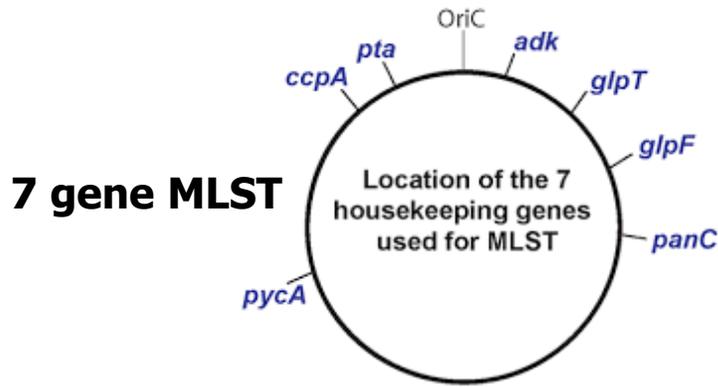
Outline

This session consists of the following elements:

<i>9.25 - 9.55</i>	Introduction to cgMLST and cluster detection
<i>9.55 - 10.55</i>	Practical exercise
<i>10.55 - 11.25</i>	Discussion

Introduction to cgMLST and cluster detection

Core genome multi-locus sequence typing (cgMLST)

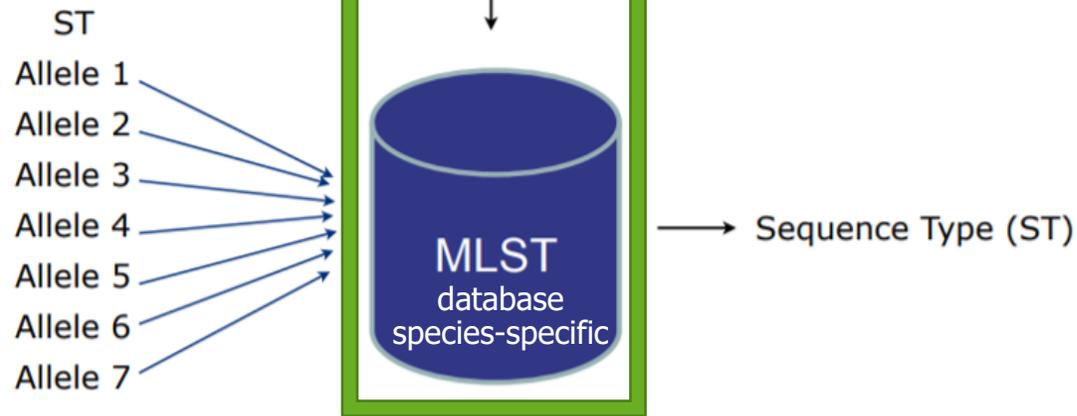


Sequence Type: 19

Locus	Identity	Coverage	Alignment Length	Allele Length	Gaps	Allele
aroC	100	100	501	501	0	aroC_10
dnaN	100	100	501	501	0	dnaN_7
hemD	100	100	432	432	0	hemD_12
hisD	100	100	501	501	0	hisD_9
purE	100	100	399	399	0	purE_5
sucA	100	100	501	501	0	sucA_9
thrA	100	100	501	501	0	thrA_2

Gene08						
Gene09						
Gene10						
Gene11						
Gene12						
Gene13						
Gene14						
Gene15						
Gene16						
Gene17						
Gene18						
Gene19						
Gene20						
Gene21						
Gene22						
Gene23						

Each gene variant has an allele number



cgMLST contains >1000 genes →

cgMLST schemes and allele calling methods

cgMLST schemes (most common)	No. of genes
<i>Salmonella</i> : Enterobase (Atchman et al., 2021)	3002
<i>Listeria monocytogenes</i> : BIGSdb (Moura et al. 2016)	1748
<i>Escherichia coli</i> : Enterobase (Zhou et al. 2020)	2513



Allele calling pipelines/methods:

Enterobase, SeqSphere, BioNumerics, chewBACCA, MentaLIST



Different cgMLST schemes and different allele calling pipelines/methods may generate different allele profiles that are not directly comparable

Cluster detection using cgMLST

In outbreak investigations **cluster is a set of isolates with nearly identical genomes**

- some allelic differences/SNPs are allowed to consider pathogen mutation rates in different hosts/environments and time

Cluster detection thresholds for cgMLST

There are no internationally agreed thresholds available

Suggested cluster thresholds/cut-offs are available (guideline):

- Comparative studies (Schürch et al. 2018)
 - ≤ 4 , *Salmonella enterica*
 - ≤ 2 , *Salmonella* Typhimurium
 - ≤ 4 , *E. coli*
 - ≤ 4 , *Listeria monocytogenes*
- FWD AMR – RefLabCap, 2022 (*Salmonella enterica*)
 - 0-3 ADs, clonal serovars
 - ≤ 5 ADs, other serovars
- EnteroBase, 2022 (*Salmonella enterica*, *E. coli*)
 - HierCCs (0, 2, 5, 10, 20, 50, 100, etc.)
- ECDC EpiPulse (*Listeria monocytogenes*)
 - ≤ 7 ADs

Cluster detection (Denmark *Salmonella*)

Cluster detection

- Cluster cut-off
 - ≤ 3 AD
 - ≤ 1 AD for clonal types (Enteritidis ST11 and monophasic Typhimurium ST34)

Inclusion criteria - not one fits all

- Type, time, place
- Typing method (SNP, cg/wgMLST)
- Clustering methods

Outbreak definitions and communication (Denmark *Salmonella*)

Outbreak definition

- Genetic cluster of ≥ 4 isolates within 3 months

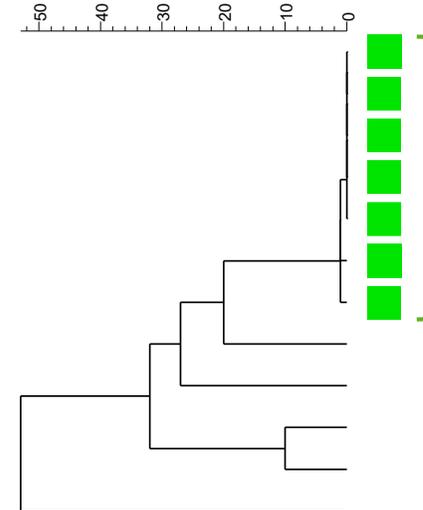
Communication

- Genetic cluster ID
- Outbreak ID

Retrospective analysis

- Cases to existing cluster – new event
- Same cluster ID but different outbreak ID

wgMLST (core (Enterobase))



ST19#65
FUD2102

EPI

- Outbreak investigation
- Patient interview
- Contact with food authorities

EpiPulse (ECDC) event – Outbreak of Enteritidis ST11



National outbreak of ST11 related to chicken kebab

Item details

ID: 2023-FWD-00045 Type: Event Title: Salmonella Enteritidis outbreak in Denmark
 Diseases: Salmonellosis Pathogens: Salmonella Enteritidis Participating domain:

Key Information

- Situation Awareness
- Comments
- Links
- Outputs

Item created on: 2023-07-21 15:25 Item last updated on: 2023-09-04 17:49
 Number of reply comments: 14 NCC: Number of confirmed cases: 86 NNC: Number of non-confirmed cases: 7 NCI

Show 10 rows

Domain	Country / Organisation	Sector	NCC	NNC	NCD	Epidemiological information	Microbiological information	
<input type="checkbox"/>	FWD	Denmark	Public Health	22	0	0	<p>In Denmark, we have a <i>Salmonella</i> Enteritidis ST11 cluster with so far 12 cases in the ages between 18-98 years. Half of the cases (n=6) are between 18-30 years old. Two cases are >90 years old. Cases live in all 5 regions of Denmark. Sample dates are between 25 May and 4 July 2023. Seven of 12 have been hospitalized. SSI has interviewed 7 cases so far. 5 of 7 have eaten kebab/pizza possibly with chicken on them.</p> <p><u>Update 3. August 2023:</u> In Denmark, we now have 15 confirmed cases, of which 8 have been admitted to hospital. In total, 12 cases have been interviewed, of which 9 out of 12 have eaten kebab/pizza possibly with chicken on them.</p> <p><u>Update as of 18 August 2023</u> The outbreak is still ongoing in Denmark with a total of 19 registered cases. The investigation still points towards imported chicken kebab, however from a different producer in Poland than the food match from Austria. Traceback investigation is ongoing.</p> <p><u>Update as of 29 August 2023</u> We now have 22 cases in Denmark. 14 of 19 interviewed cases have eaten kebab or pizza. Several of the kebab/pizza restaurants have received bags of frozen pre-roasted chicken kebab pieces from the same Polish producer. We have found an isolate of <i>Salmonella</i> in one batch of the pre-roasted chicken doner kebab which is matching (AD= 0) the human isolates. This product has been withdrawn from the market – see RASFF 2023.5788.</p>	<p>Strains are ST11, representative isolates are in Enterobase (Name=2306W37966) and another genome uploaded to SRA (SRR25381902). Also attached here is a fasta file of 2306W37966 from Enterobase. The outbreak isolates are 4-5AD to isolates from imported chicken.</p>

Epipulse - event

- Epidata
- Mikrobiological data
- Attached sequence

Turned into a international outbreak

As the source(s) have not been identified, new cases are likely to occur in this prolonged multi-country outbreak.

JOINT ECDC-EFSA RAPID OUTBREAK ASSESSMENT

Three clusters of *Salmonella* Enteritidis ST11 infections linked to chicken meat and chicken meat products

26 October 2023

https://www.ecdc.europa.eu/sites/default/files/documents/ROA_S-Enteritidis-ST11_chicken-meat_2023_amended.pdf

Three clusters of *Salmonella* Enteritidis ST11 infections linked to chicken meat and chicken meat products

Cluster 2: *Salmonella* Enteritidis ST11 (EpiPulse reference: 2023-FWD-00045/Denmark)

A confirmed outbreak case:

- A laboratory-confirmed *Salmonella* Enteritidis ST11 case with disease onset on or after 1 January 2023 (date of sampling or date of receipt by the laboratory if date of onset is not available).

AND

- Fulfilling at least one of the following laboratory criteria:
 - within **seven cg-allelic differences (AD)** from at least one of the representative Danish outbreak reference strain in the national cgMLST pipeline, OR
 - clustering within **six cg-allelic differences** in a single linkage analysis in a centralised whole genome sequencing (WGS) analysis, OR
 - belonging to the **cgMLST HC5_1358** hierarchical cluster by Enterobase scheme, OR
 - belonging to **CT2114** according to SeqSphere scheme, OR
 - clustering according to a national single-nucleotide polymorphism (SNP) pipeline within **five SNPs** of the Danish outbreak reference strain, OR
 - belonging to a **5-SNP single linkage cluster** with SNP designation 1.1.2.12.12.590.% according to the pipeline in the UK Health Security Agency (UKHSA)

AND

- No or unknown history of travel outside of the EU/EEA seven days prior to onset of symptoms.

Question for discussion

Do you use WGS for outbreak detection and investigation in your country?

- which typing method you use?

How do you define and communicate outbreaks nationally and internationally?

Concluding remarks

Not one fits all

- Interpretation of results from cluster to cluster
- Find the method that fits the data and types seen in your country
- Do validation using well defined outbreaks

Clear definitions and communication is important

- Clear information on tools and methods
- Clear information on cluster and outbreak definition (type, time, place)
- Better understanding between countries

Epi data is extremely important

Practical exercise + Discussion

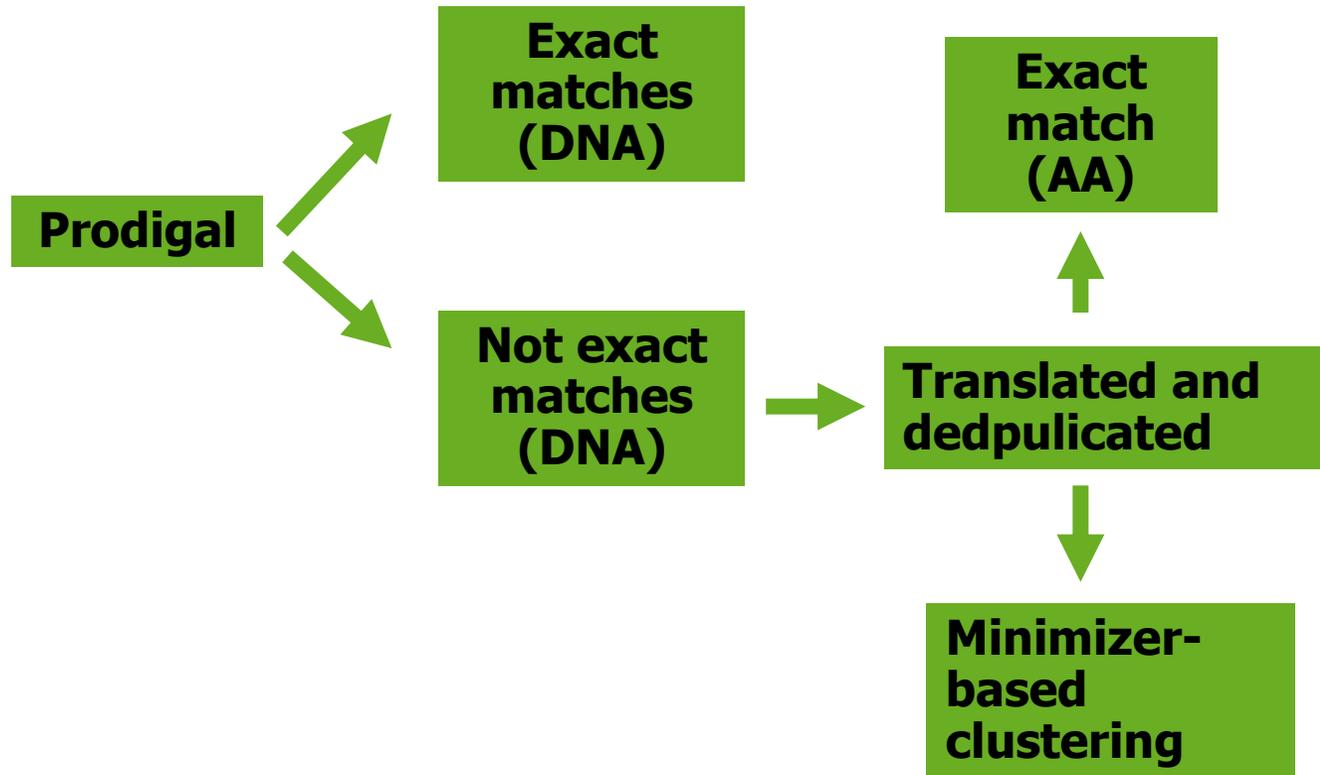
chewBBACA

- BSR-Based Allele Calling Algorithm
 - BSR: BLAST Score Ratio
 - (Comprehensive and Highly Efficient Workflow)
- Gene-by-Gene method
 - The definition of an allele is determined by the sequence similarity search method

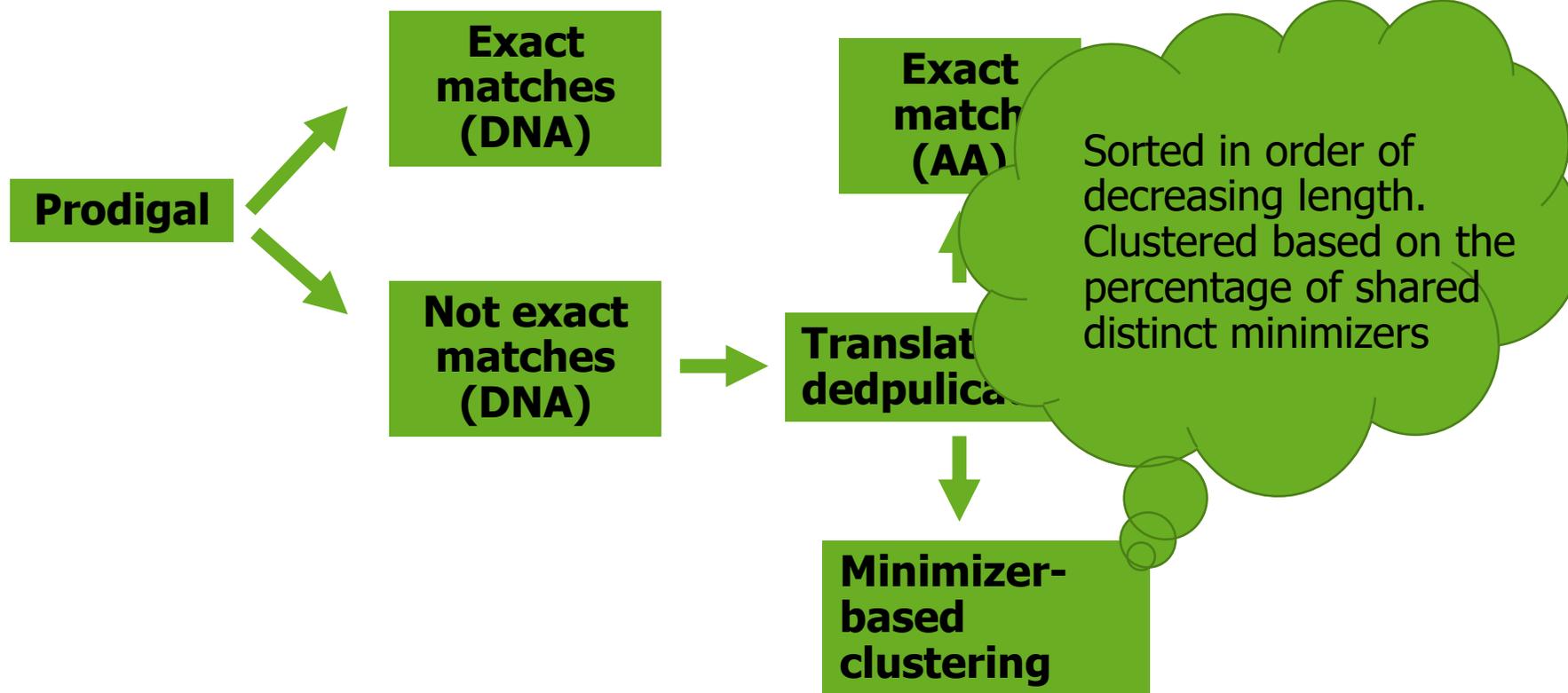
Influencing allele calling

- **Quality** of the sequence assembly
 - Sequencing method, assembler etc
- If the alleles must correspond to **coding sequences** (CDSs) and open reading frames (ORFs)
- Presence of possible homologous loci
 - Can result in an allele assignment to a possibly wrong locus – difficulty in distinguishing closely related homologs

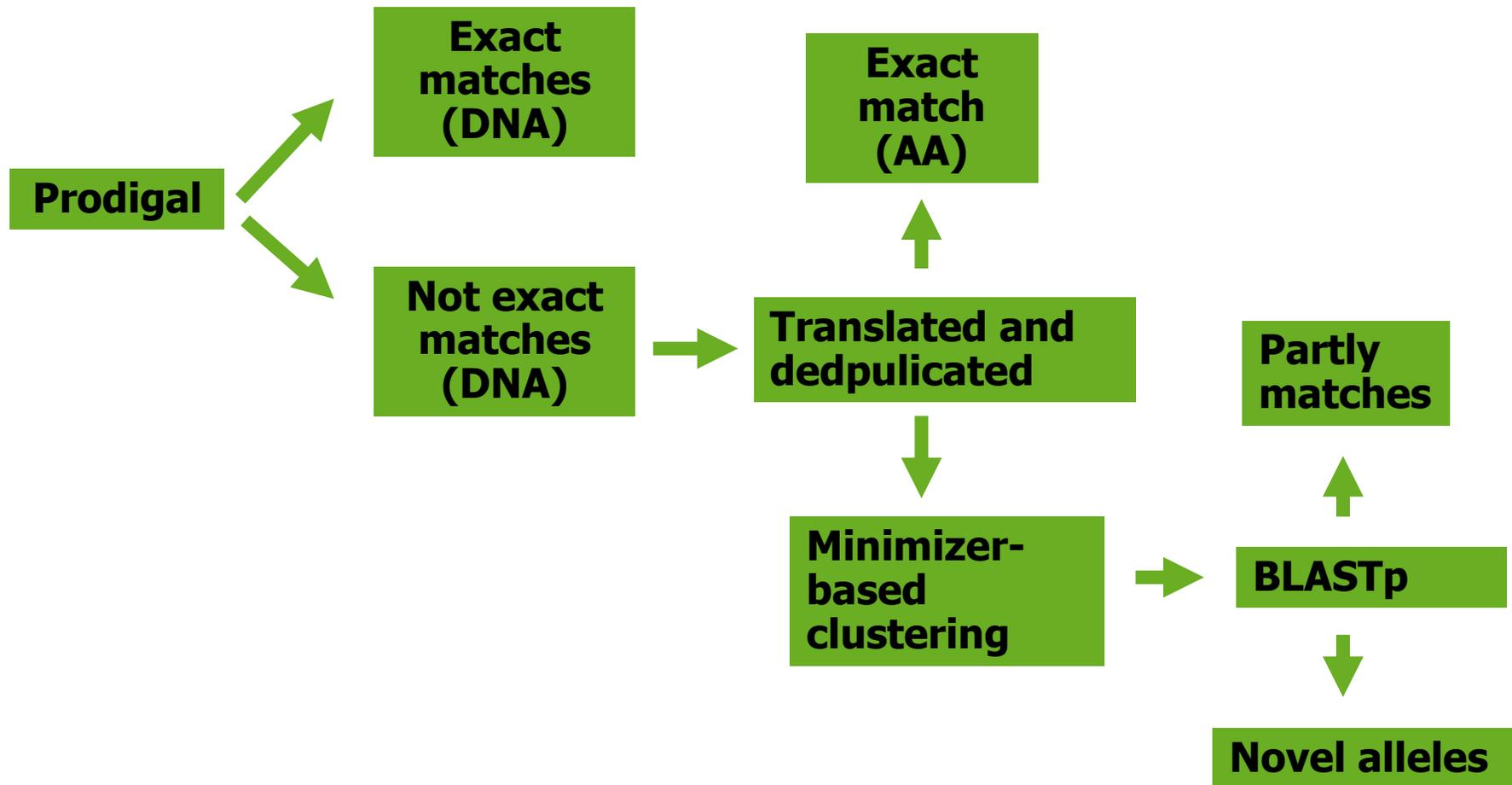
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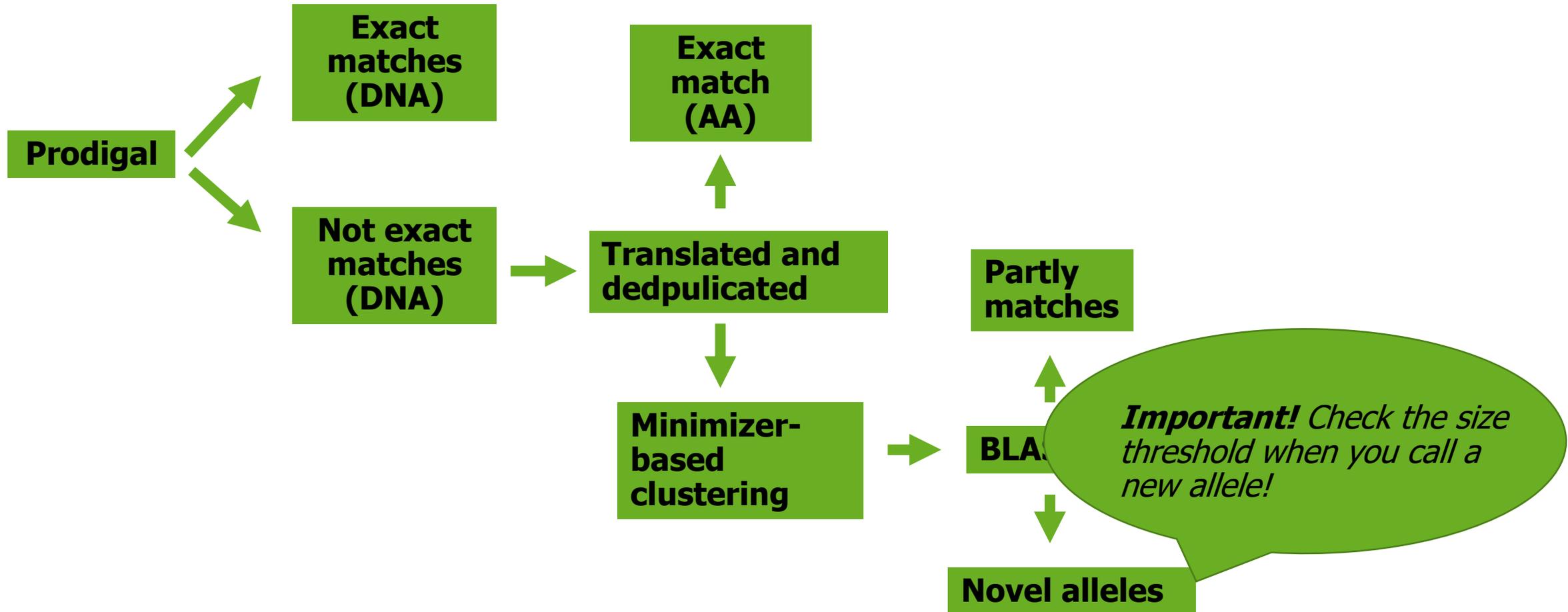
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Further reading

[chewBBACA: A complete suite for gene-by-gene schema creation and strain identification. Silva et al. 2018.](#)

[Interpretation of Whole-Genome Sequencing for Enteric Disease Surveillance and Outbreak Investigation. Besser et al. 2019](#)

[Translatability of WGS typing results can simplify data exchange for surveillance and control of *Listeria monocytogenes*. Lüth et al. 2021.](#)

[Development and evaluation of an outbreak surveillance system integrating whole genome sequencing data for non-typhoidal *Salmonella* in London and South East of England, 2016–17. Paranthaman et al. 2021.](#)

[Prolonged multi-country cluster of *Listeria monocytogenes* ST155 infections linked to ready-to-eat fish products. ECDC, 2023.](#)

Acknowledgements

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