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French agency for food, environmental and occupational health safety

EURL for *Lm*

European Union Reference Laboratory for *Listeria monocytogenes*

A molecular *Listeria monocytogenes* database: to centralize and share typing data from food, environmental and animal strains at the European level

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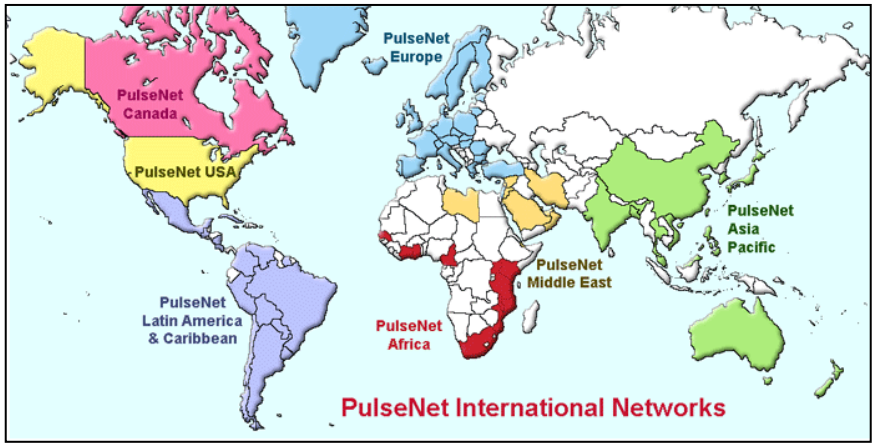
Food borne pathogens molecular surveillance networks

Success of PulseNet USA

At the International Level :



PulseNet International
The International Molecular Subtyping Network for Foodborne Disease Surveillance
USA, Canada, Europe, Asia Pacific, Latin America, Middle East
CDC

success of PulseNet International !

At the European level :

-PulseNet Europe : not active since 2006 (Lack of funding)

-European Center for Disease Prevention and Control (ECDC) : Recent launching of Tessy molecular surveillance database : strains isolated **from human cases**

-No database centralizing and sharing typing data **from food , feed and animal strains**

Anses, Maisons Alfort Laboratory for Food Safety

European Union Reference Laboratory for *Listeria monocytogenes* (*Lm*) (EURL)

since May 2006 ; Regulation EC 776/2006

Detection & Enumeration	**Typing & Epidemiosurveillance**	Risk assessment, predictive microbiology & hygiene

- Co-ordinate the network of the 35 NRLs (National Reference Laboratories)
- NRLs : responsible for typing *Lm* strains of food, feed and animal origin
- PFGE at Anses since 1998
- Other molecular methods // PFGE :
  - Semi-automated rep PCR (Roussel et al., 2010 Food Borne pathogens and Disease)
  - MLVA (Roussel et al., 2012, Gel Electrophoresis : Principles and Basics", Chapter 18)
  - FAFLP (Roussel et al., 2013, BMC Microbiology),

Introduction

For food, feed, environment and animal strains

EURL set up a database, the "EURL *Lm* DB":

- Shared within the NRL network
- Content: data submitted by the NRLs on a voluntary basis

Summary

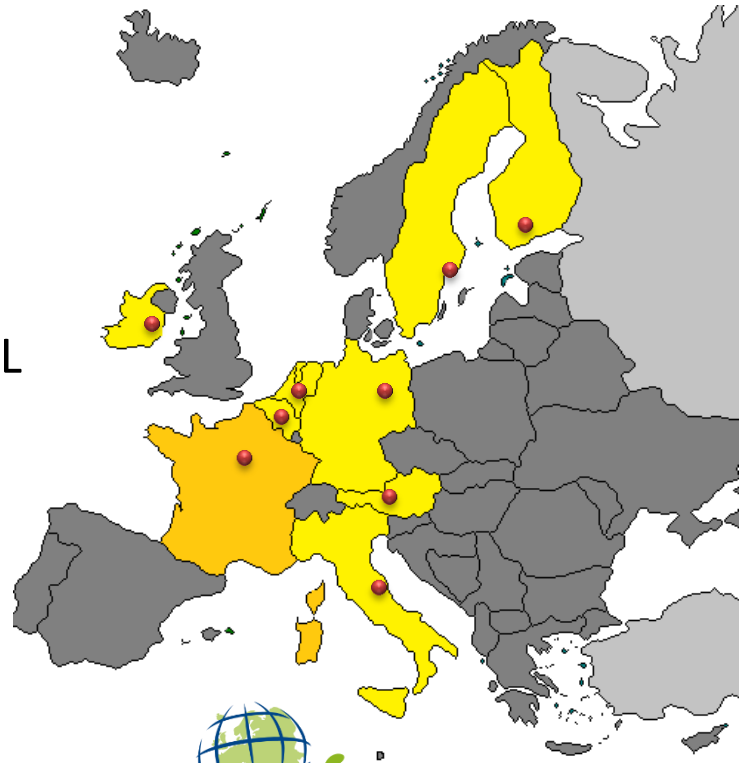
1) The steps involved in developing the EURL *Lm* DB

Technical support & organisation

Equipment:

Anses network management platform : www.moleculartyping-db.anses.fr/EUListNet
BioNumerics Server Web Edition 6.1
Oracle© database

Modified PulseNet USA communication scripts
(with the agreement with CDC)



Organization:

Steering committee: 9 NRLs, EFSA, ECDC and EURL
Curator & administrator : EURL

EURL for *Lm*

European Union Reference Laboratory for *Listeria monocytogenes*

efsa European Food Safety Authority

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

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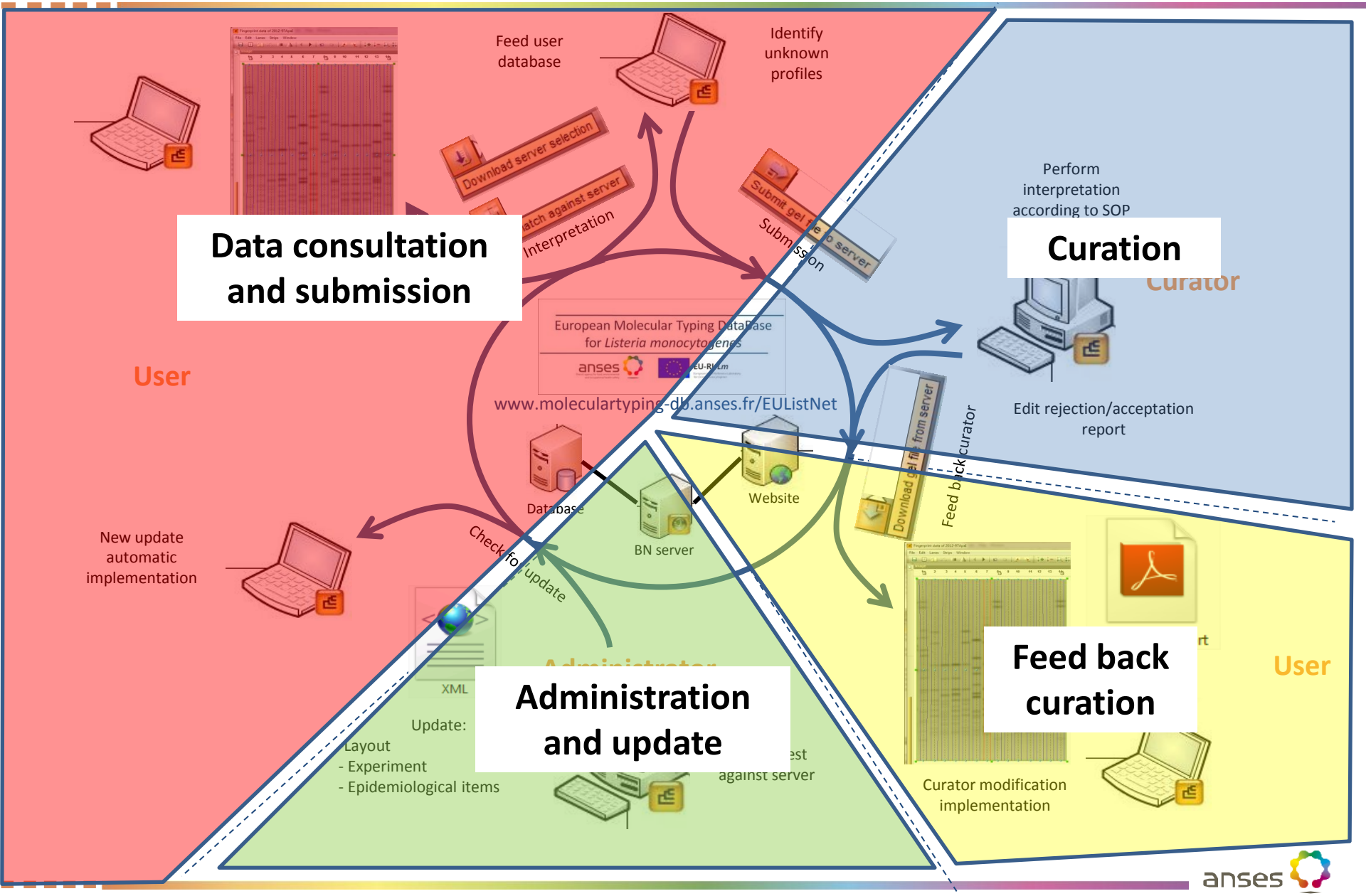
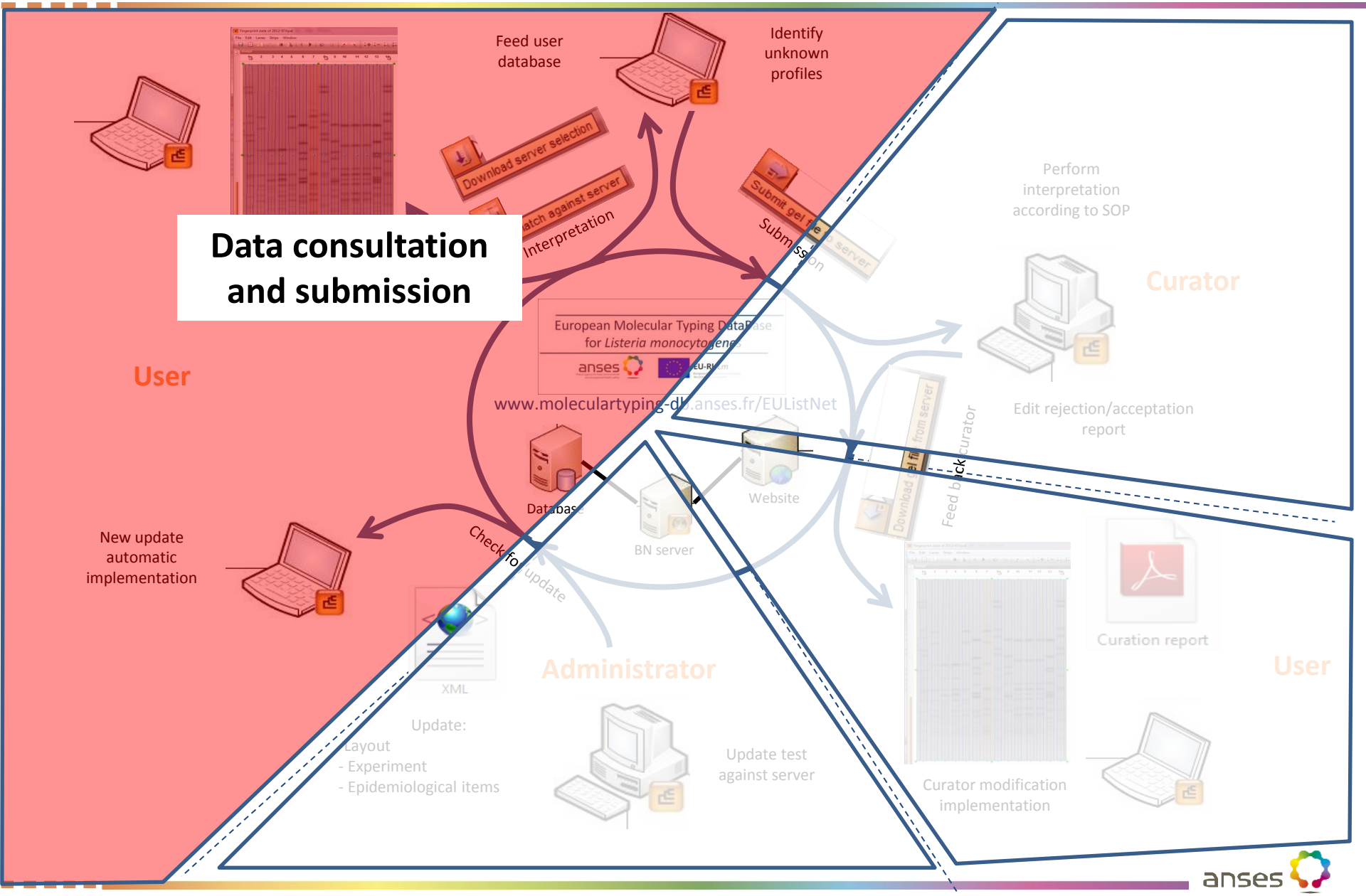
EURL Lm DB functionalites



EURL Lm DB functionalites



Data consultation and submission

Submission of data by NRLs

Two ways to use the EURL *Lm* DB

Consultation of data by NRLs (without mandatory submission)

Data submission

Participants:

(1) NRLs who successfully participated to the most recent EURL PFGE and PFGE profile interpretation PT trial

(2) Comply with the Memorandum of understanding (MoU)

Data submitted :

+ *Asc*I/*Apa*I combined PFGEprofiles
+ serotypes
+ epidemiological data
+ Do not submit duplicate strains*

Mandatory

* "Strains (1) received within the same parcel at laboratory and (2) sharing the same food product sample description. However if the typing data are different, the strains are not considered as duplicates. "

Data consultation

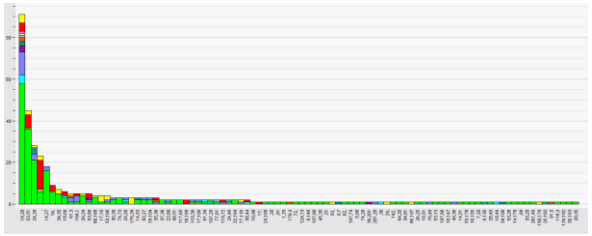
Unlimited

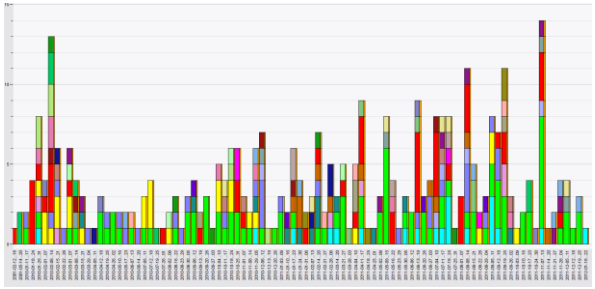
consultation of profiles & metadata

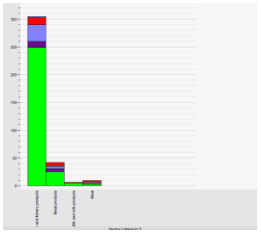
1) matching their own profile against existing entries
2) Field query (according to pulsotype, epidemiological data, etc…)

Temporary download of selected profiles from the EURL Lm DB to the user's local database

e.g.: Statistical analysis | Graphic representation | Cluster search







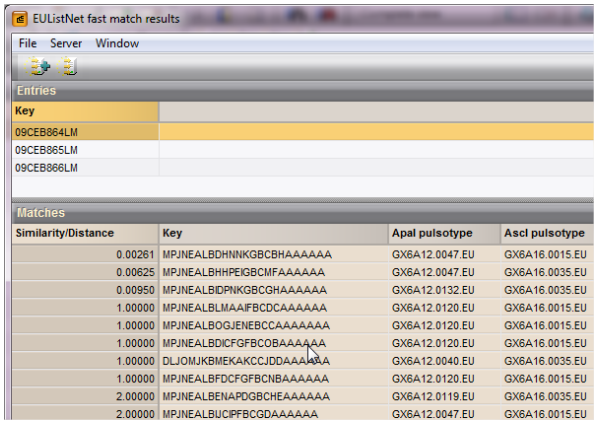
Benefits of the EURL *Lm* DB use

1) The NRLs can use the EURL *Lm* DB to make the interpretation of a PFGE profile in their own database

Utilize the profiles of the EURL *Lm* DB as “reference” for the interpretation and identification of newly analyzed profiles

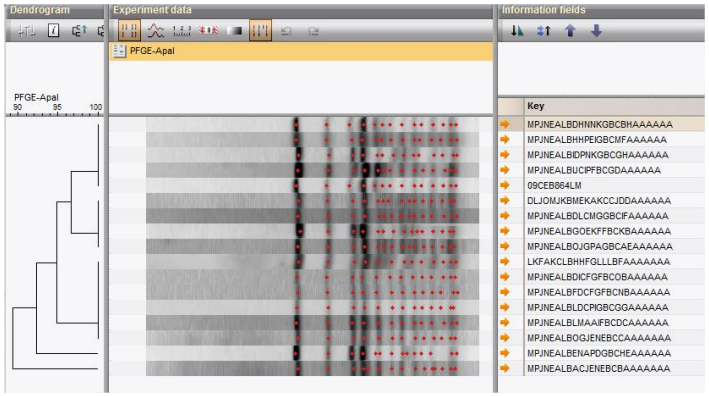
profile matching

Band of difference



Temporary profile download

Comparison and interpretation



- GX6A16.0001.EU
  (PulseNet US Pulsotype format)

harmonization of the PFGE profiles in their local database

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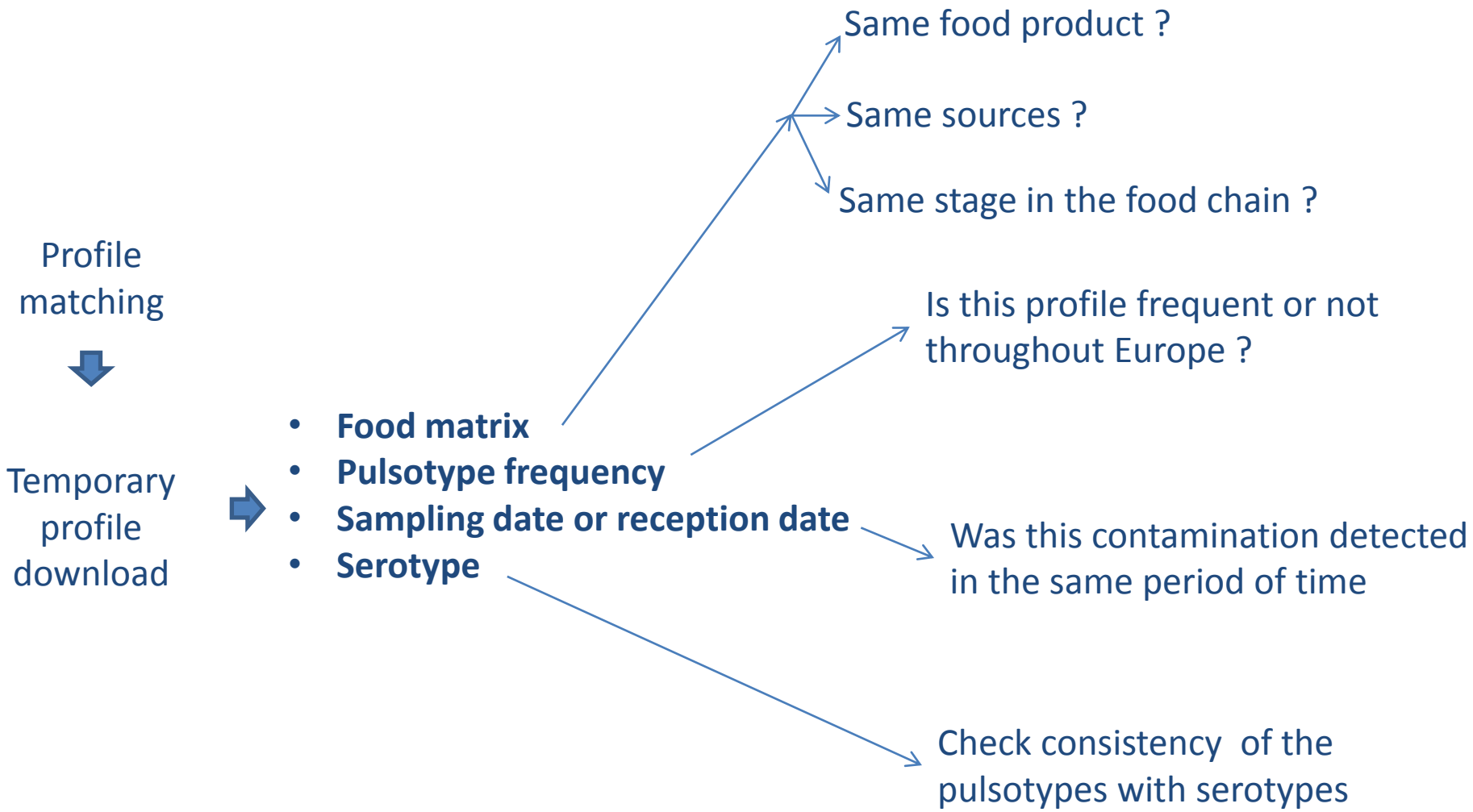
EURL *Lm* DB enables NRLS to collect information useful

for epidemiological investigations in outbreak cases



Food product description in accordance with EFSA

Four large categories

- ☐ Food
- ☐ Feed
- ☐ Animal
- ☐ Ecosystem

Business rules

1) **Adapted to Listeria**
2) **Few fields**
3) **Restricted pick lists**

Easy reporting without typo error

Same level of description for:

- Food product
- Food processing environment

→ **Food matrix**

- Meat
- Milk
- Fish
- Eggs
- Beverage
- Combining several food categories
- Other

→ **Food products**

e.g.: cheese, sausage, pate, etc…

→ **Process applied**

e.g.: cooked, raw, fermented, etc…

Epidemiological description: EU standard

Food description respect the EFSA Standard Sample Description (SSD2)

Sector | Sample type | Food matrix | Food product | Food processing

Food — Food product — Meat products — Pâté — Cooked

Lead to one
EFSA code e.g.:
A016881A

EFSA code field available

A016801A
A004241A
A016821A
A034841A
A004261A
:

The EURL *Lm* DB MoU

Memorandum of understanding (MoU)

Participation

- NRLs take part to the project on a voluntary basis

NRLs duties:

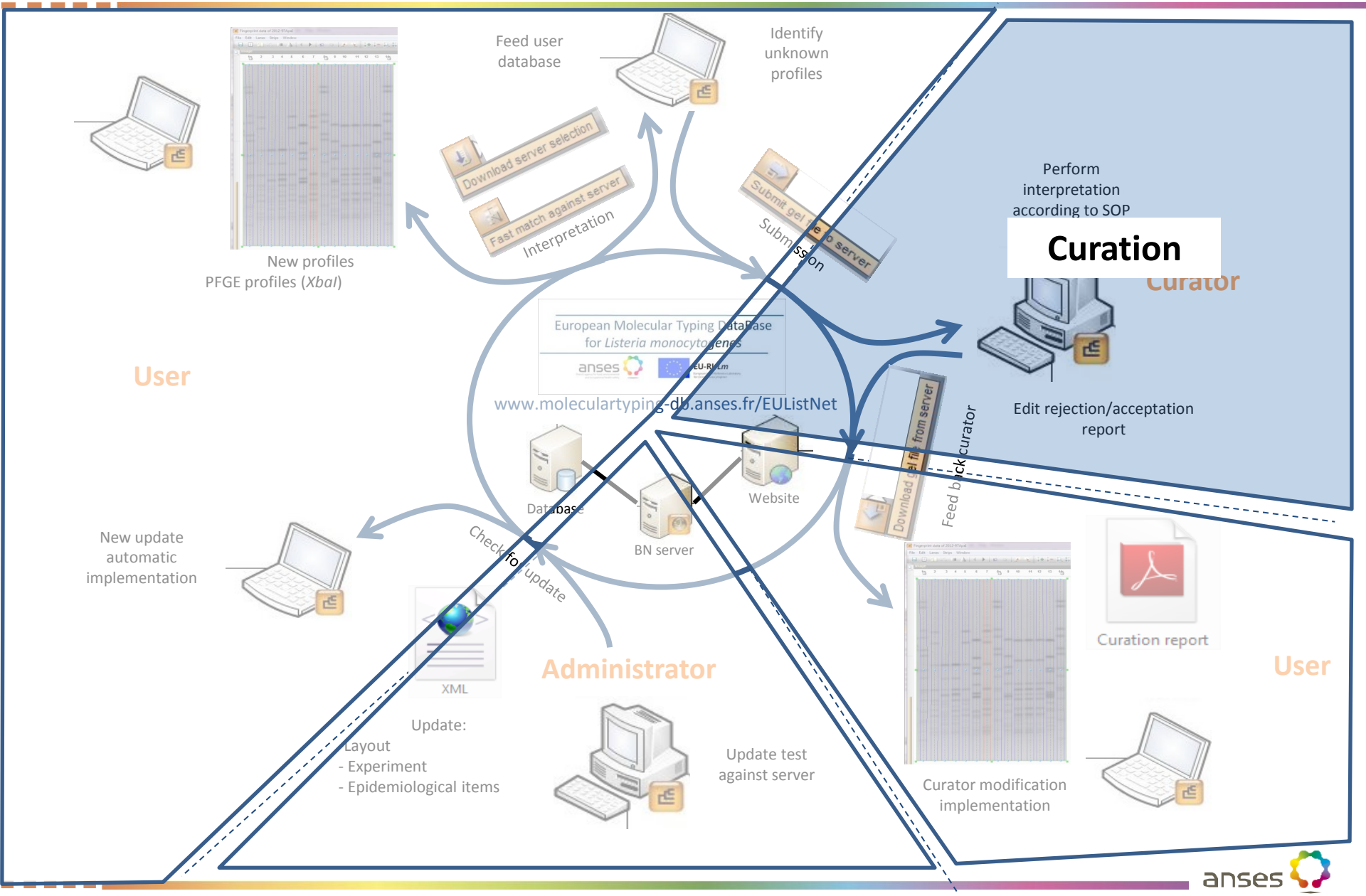
- NRLs are responsible for the data submitted
- Data disclosure imply common agreement between partners
- Citation of EU-RL *Lm* DB are possible if it discloses no specific data

NRLs right:

- NRLs pursuant the MoU can consult the EU-RL *Lm* DB
- NRLs are free to publish their own profiles even if they are submitted
- Annual report will present EU-RL *Lm* DB activity

Already validated by the competent authorities of the 8 SCOM members

EURL Lm DB functionalites



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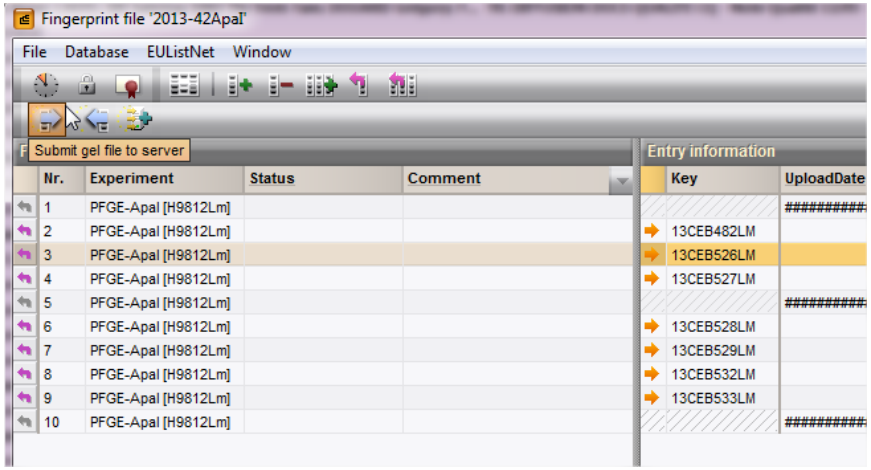
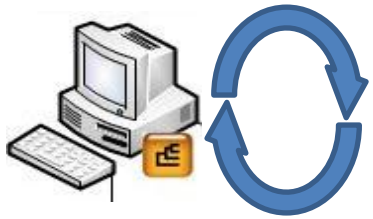
Data curation

(Félix et al. 2012 Gel Electrophoresis book Chapter 14 p.241-254)



Curator process



(1)

Quality assessment of the gel and profiles according to EURL SOP

(2)

Pulsotype assignement

GX6A16.0001.EU

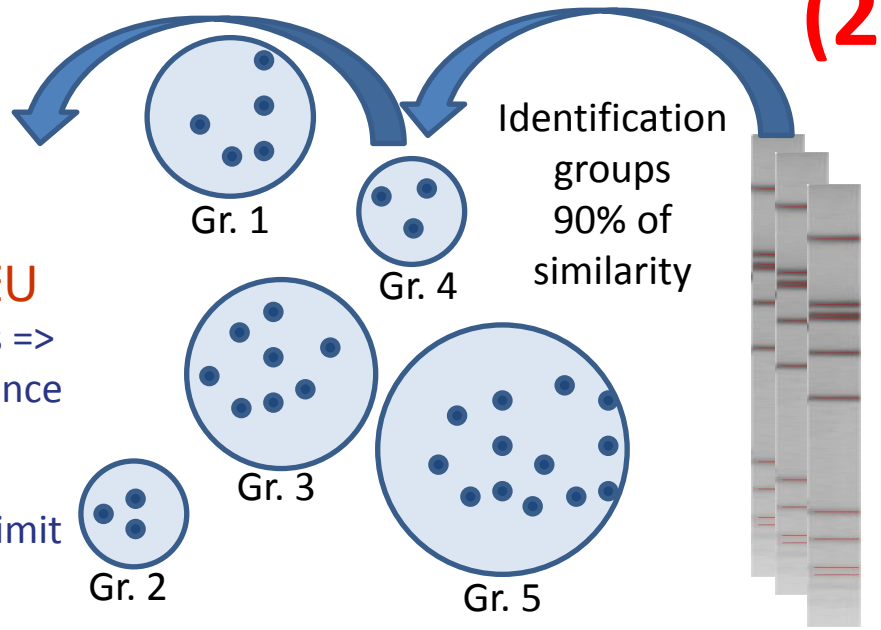
Two different profiles => at least 1 band difference

(Barrett et al. 2006)

&

Interpretation down limit 33 kbp

(Peters et al. 2006)



Identification groups 90% of similarity

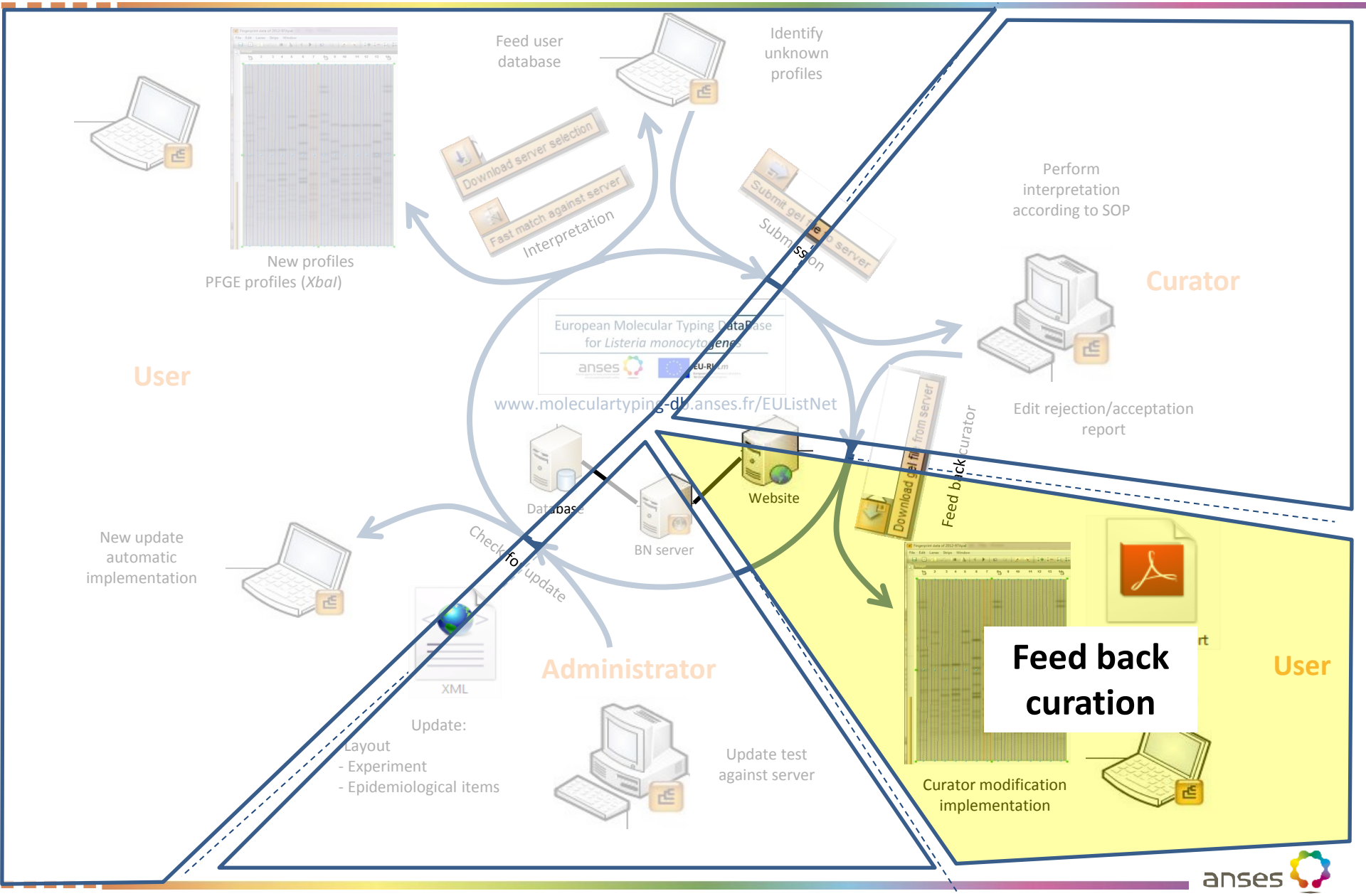
Band intensity

Migration distortion

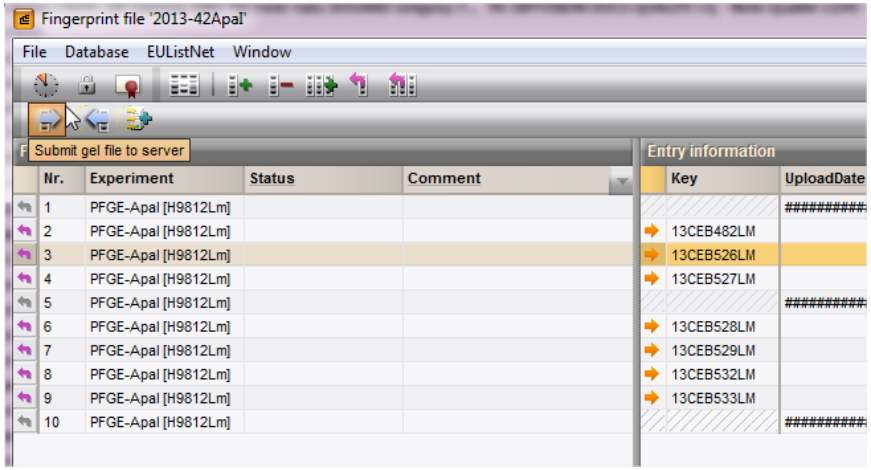
Image quality

Harmonization of the interpretation

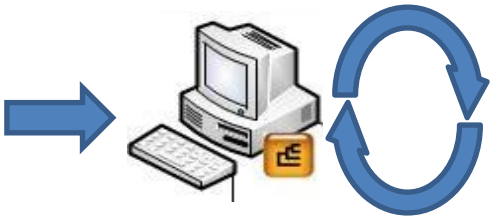
EURL Lm DB functionalites



Curator feed back content



Curator process



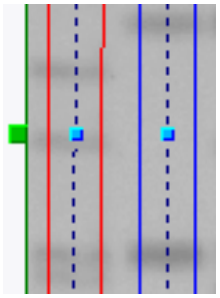
-CONFIRMED
-UNSATISFACTORY

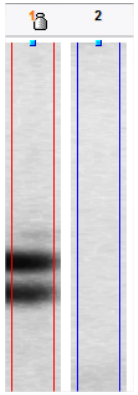
ASCI_identGr_90
APAI_IdentGr_90

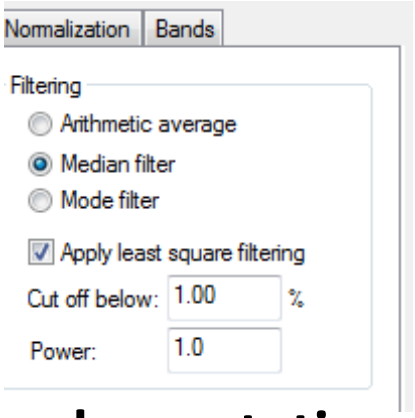
90% similarity groups

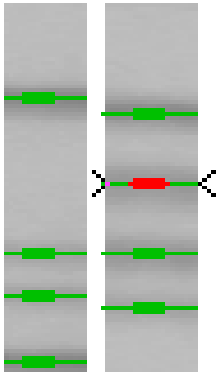
Ascl pulsotype
Apal pulsotype

Official pulsotypes









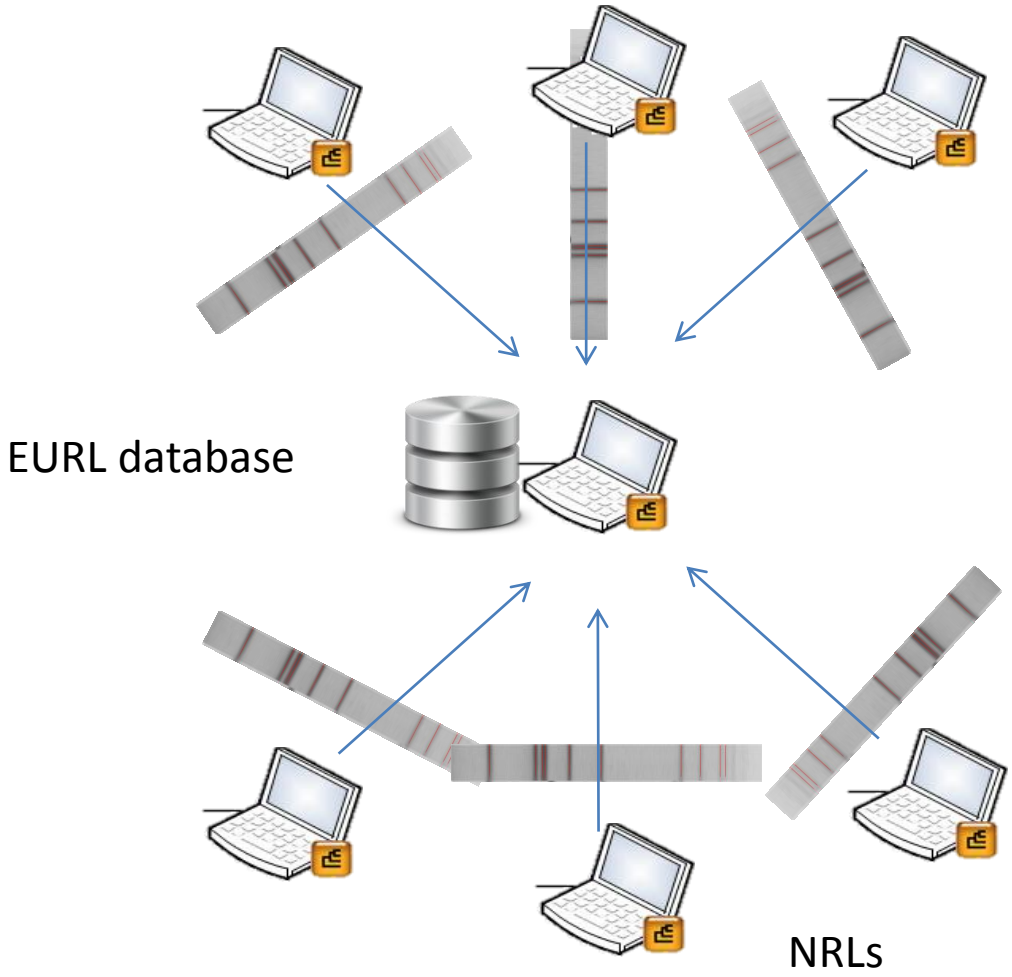
Comment
BF|good quality |all gel profiles
BF|good quality |all gel profiles

Status
Confirmed

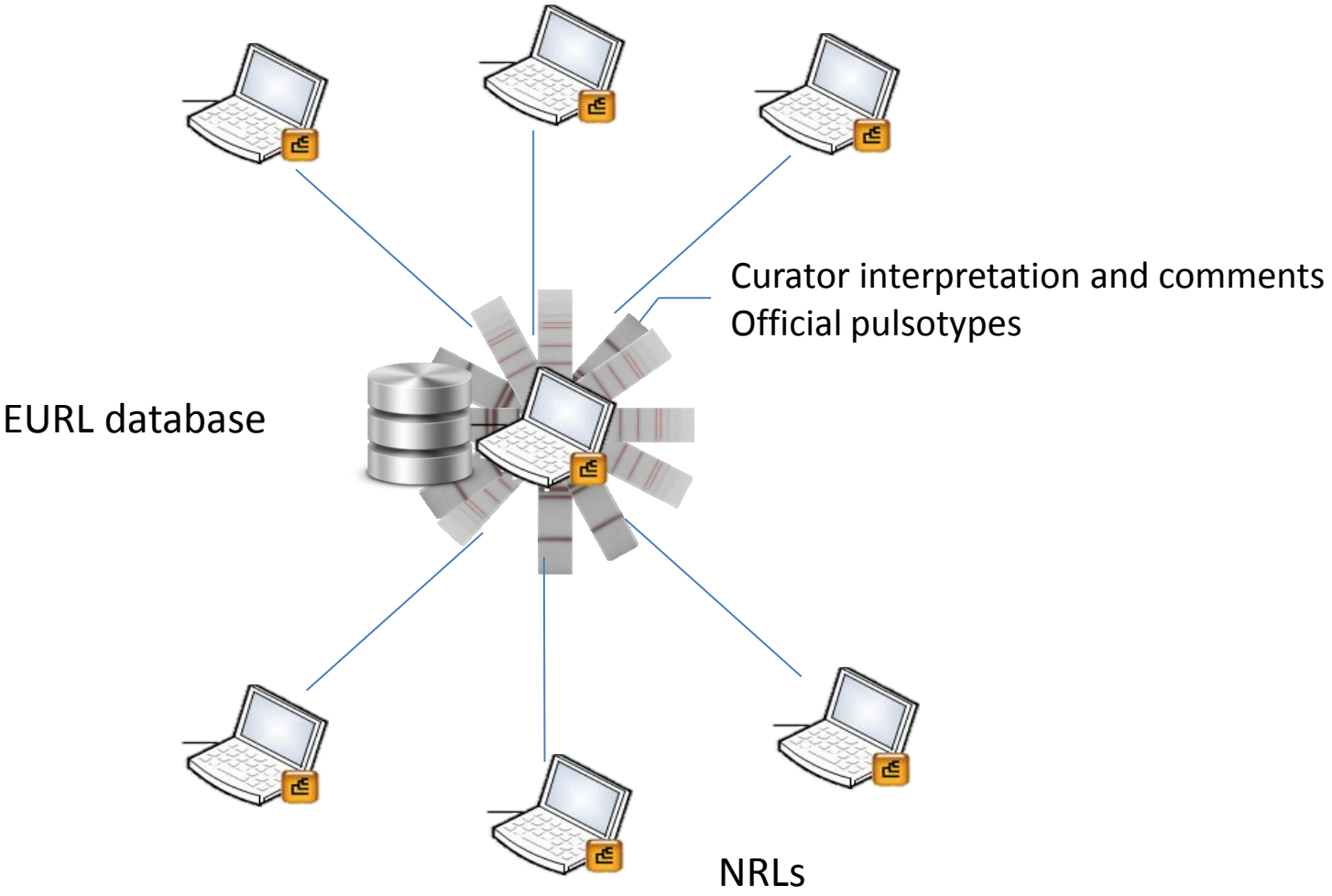
Automatic implementation of curator fingerprint modifications

OFFICIAL CURATION REPORT
HTML format

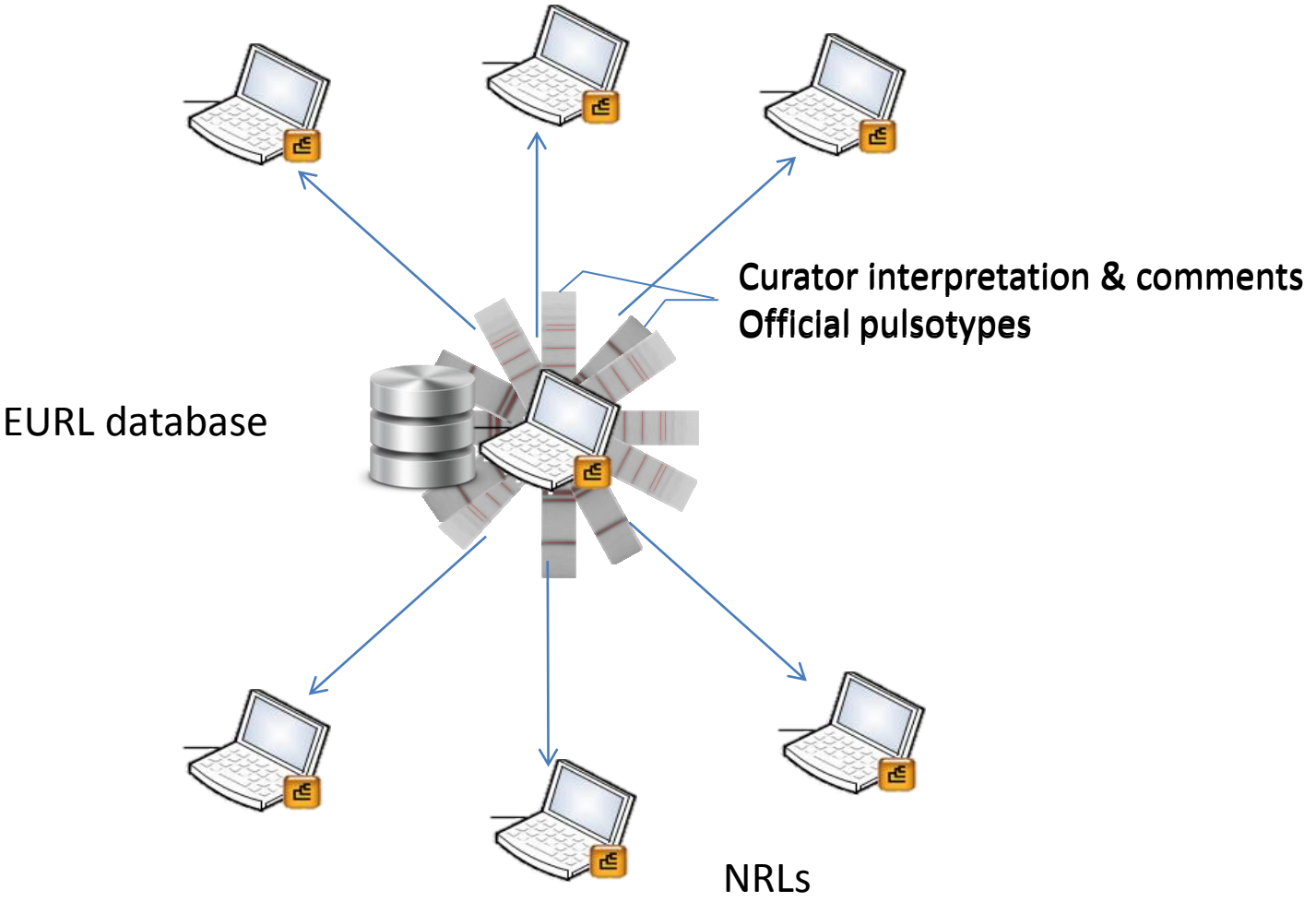
anses



Data submissions

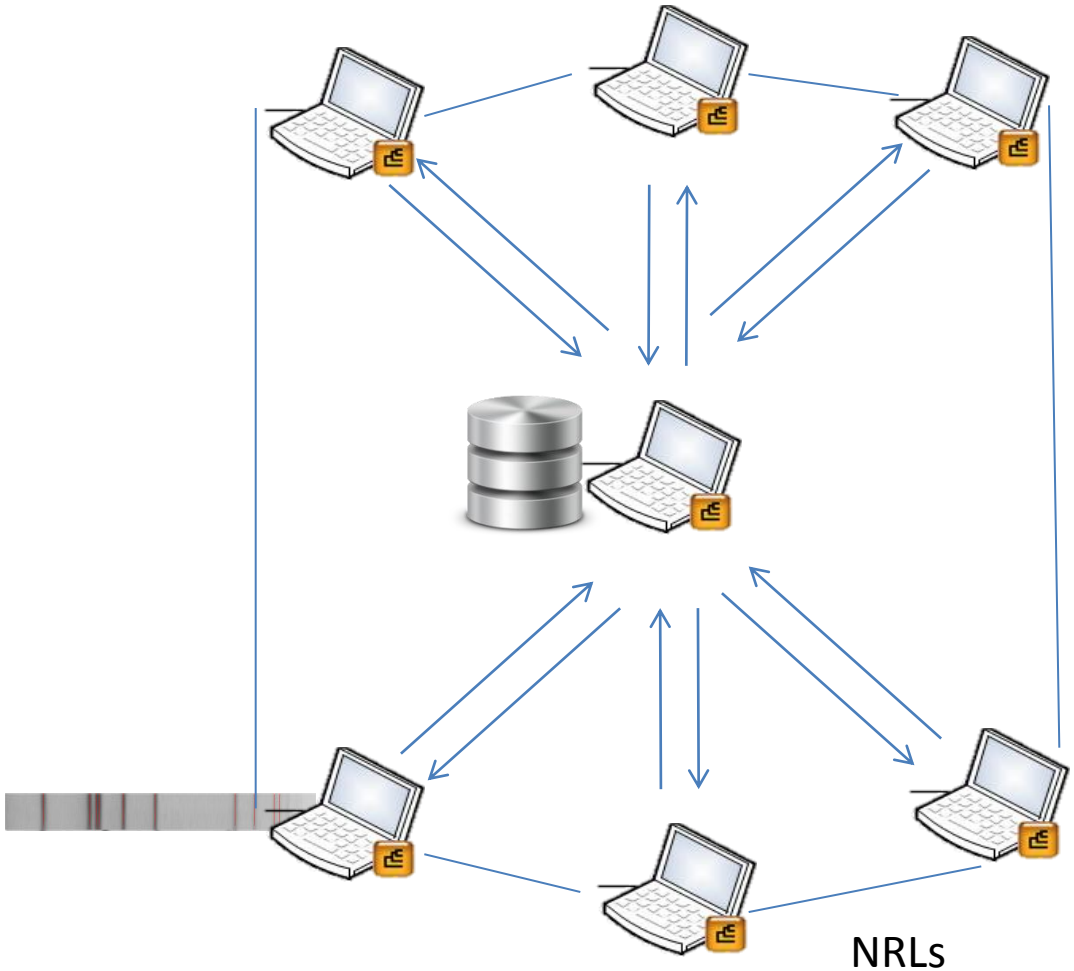


Curation process



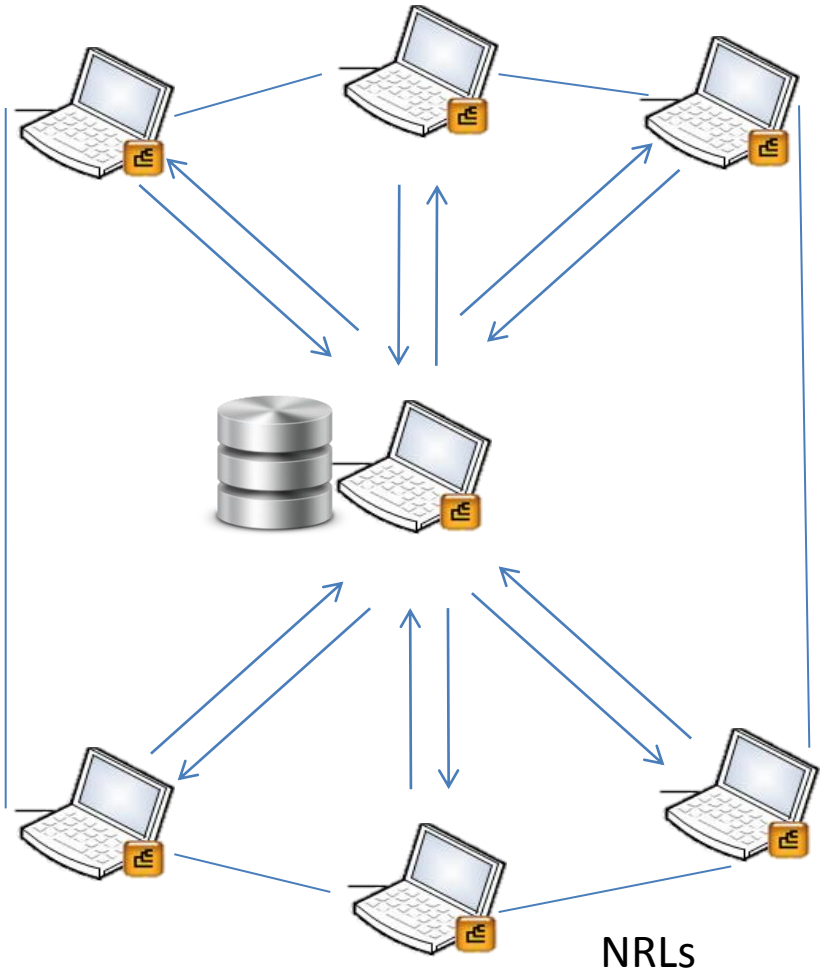
Feed back curator

Synchronization of the network



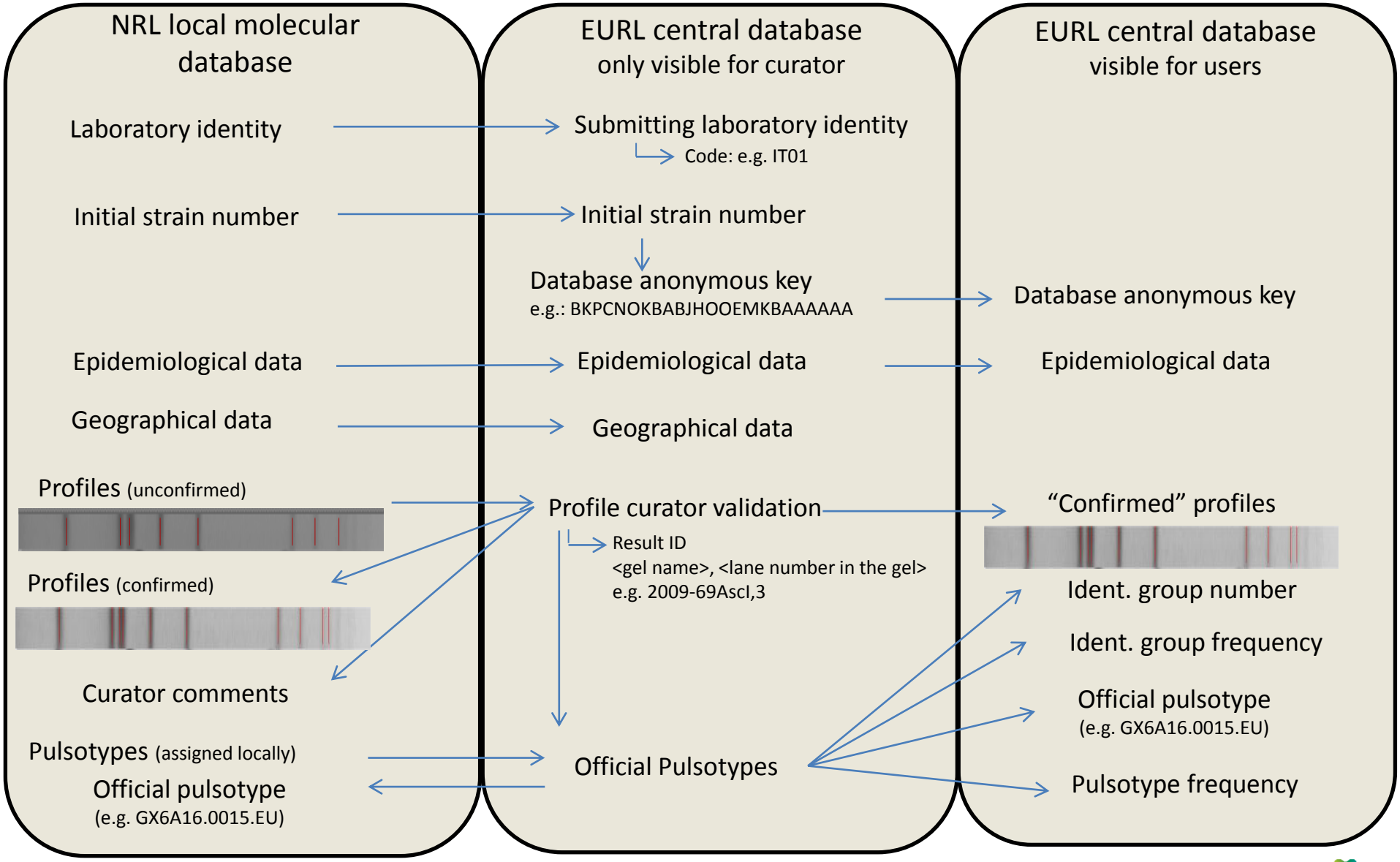
Harmonization of the data circulating within the NRLs/EURL databases network

Synchronization of the network



Improvement of the NRL network reproducibility and internal communication

Global database scheme



2) Further developments

Quality assessment tools for curators

Automatic QA tools:

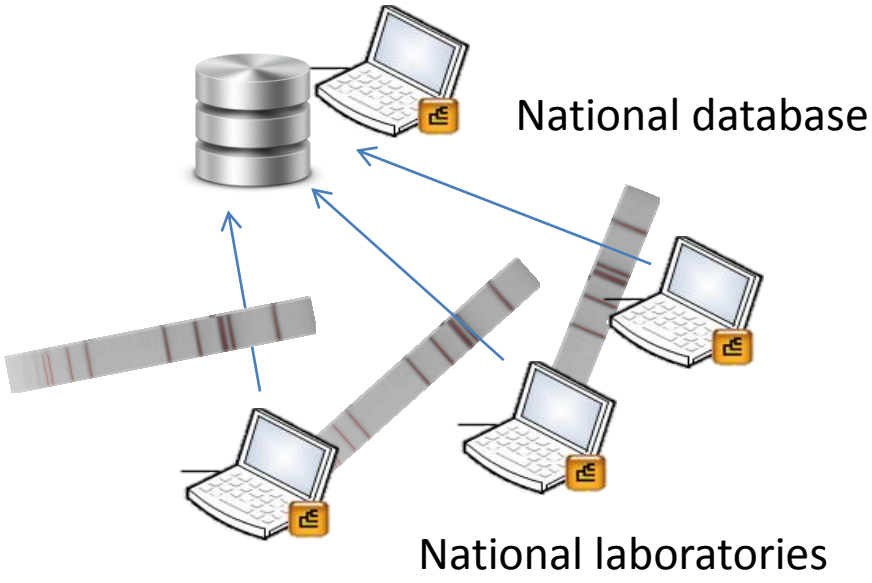
- Automatic reference system validation
- Assessment of higher bands intensity (variable parameters: metrics, height limit)
- Maximum – minimum band count
- Band marking limit

Curation assistance tool

- Pick list for standard comments
- Editable pick list

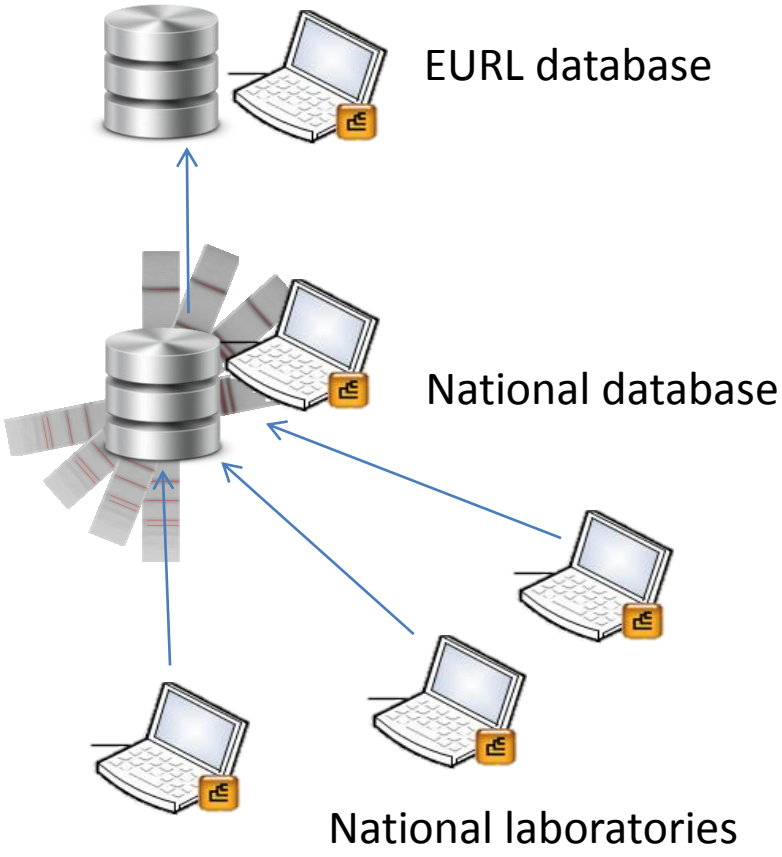
Synchronization with national network

Submissions to national central database



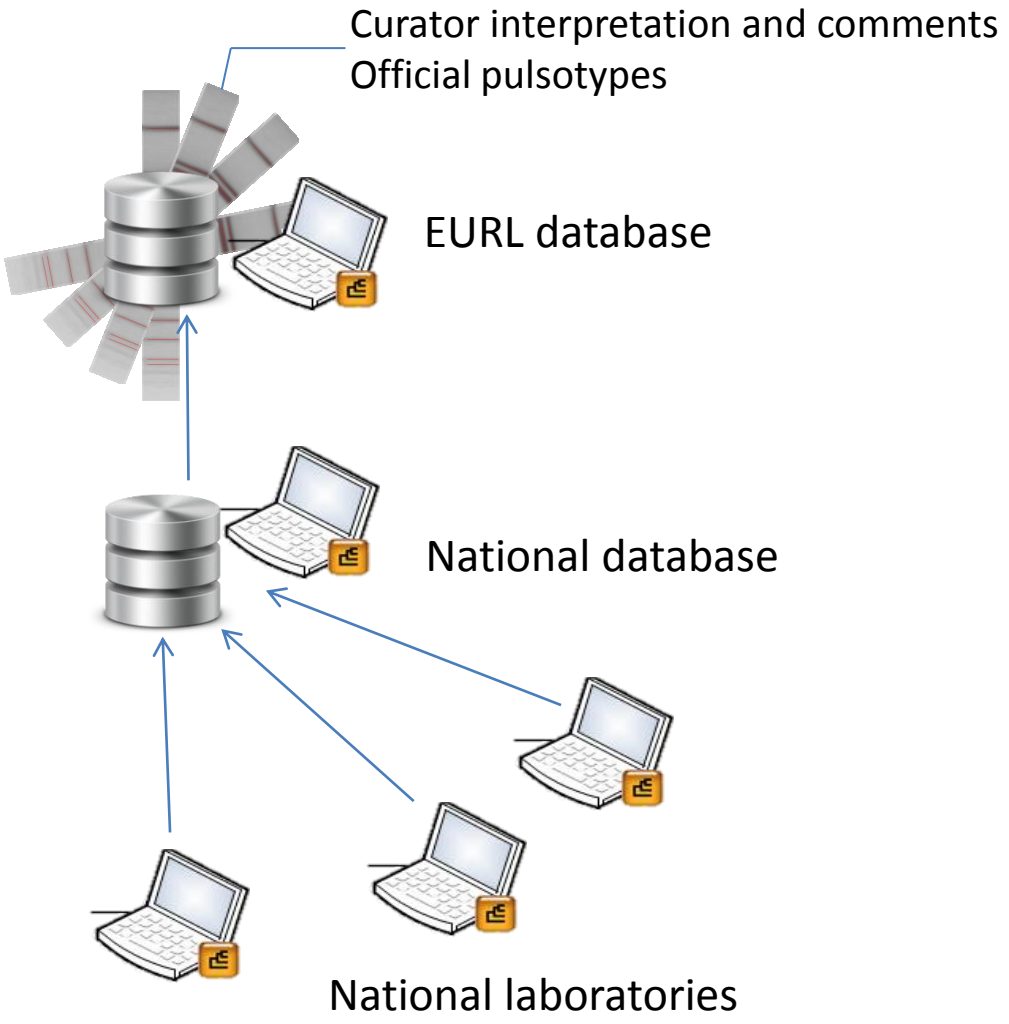
Synchronization with national network

Direct transmission to EURL database, without curation at national level



Synchronization with national network

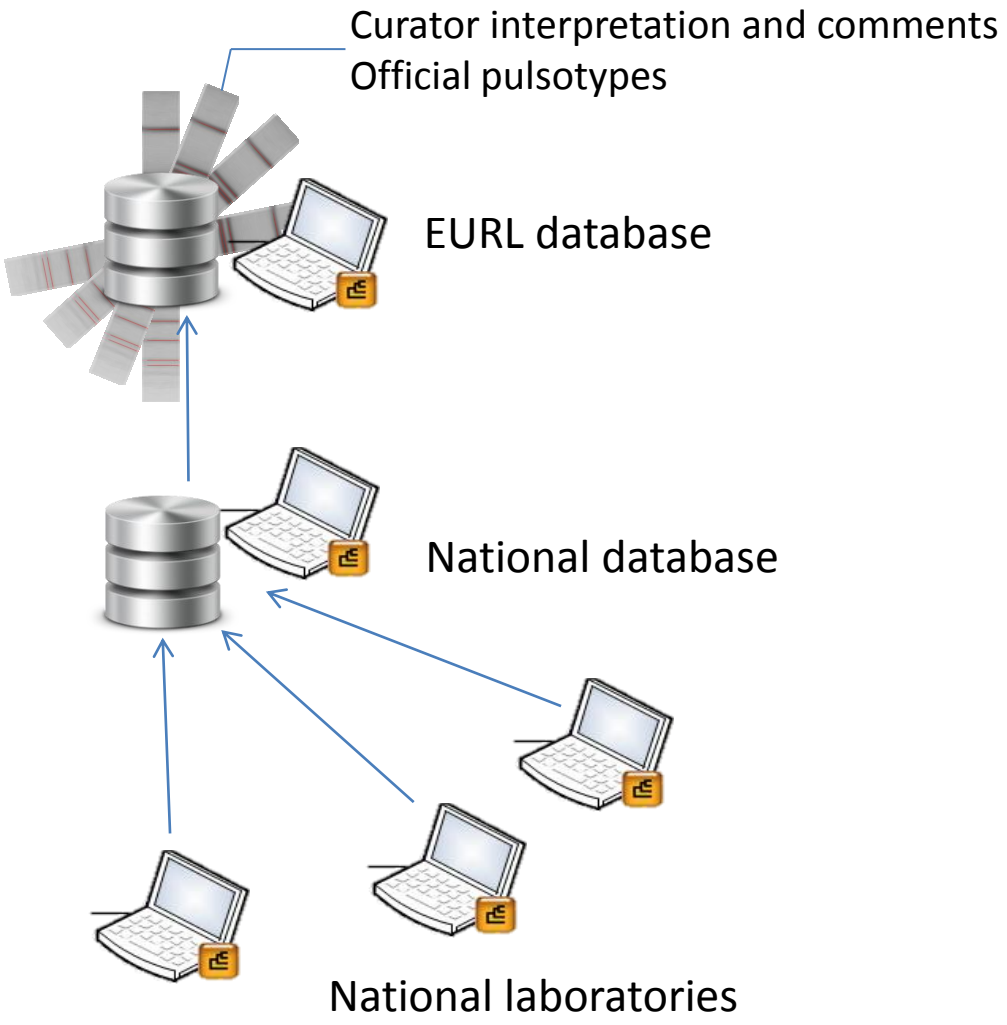
Curation process



Synchronization with national network

Feed back curator

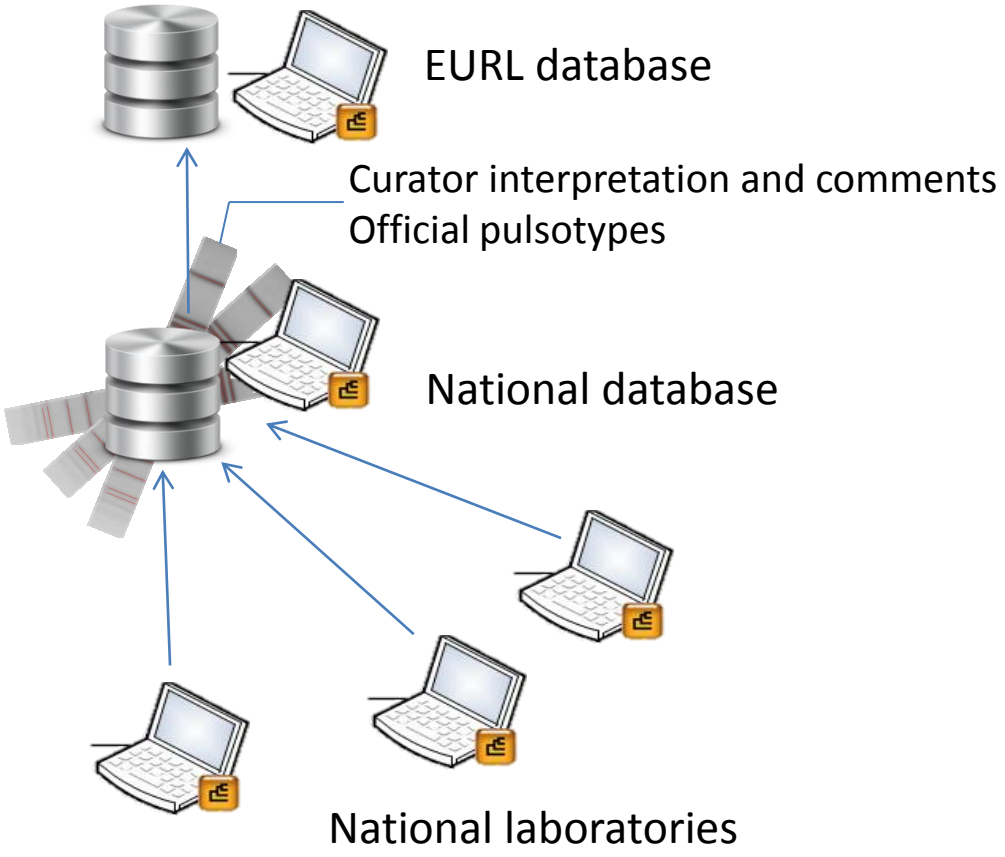
1) To national database



Synchronization with national network

Feed back curator

1) To national database
2) To local laboratory databases



EURL *Lm* Database-Conclusions

In combination with epidemiological investigations and databases on human strains :

(1) Strengthen the national surveillance in each European country

(2) better allowing the detection of European contamination clusters,

(3) optimizing the detection of emerging *Lm* strains

(4) suggesting links with potential sources of contamination.

(5) facilitating communication between NRLs, EURL, EFSA and ECDC

The EURL *Lm* DB : improve the surveillance of *Lm* in the food chain

Acknowledgement

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