

VTEC Network preparedness for molecular typing data collection

EU Reference Laboratory for Escherichia coli

Dept. of Veterinary Public Health and Food Safety

Istituto Superiore di Sanità, Rome, Italy

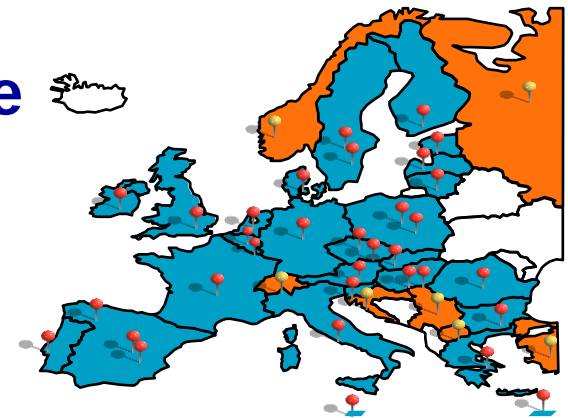


www.iss.it/vtec

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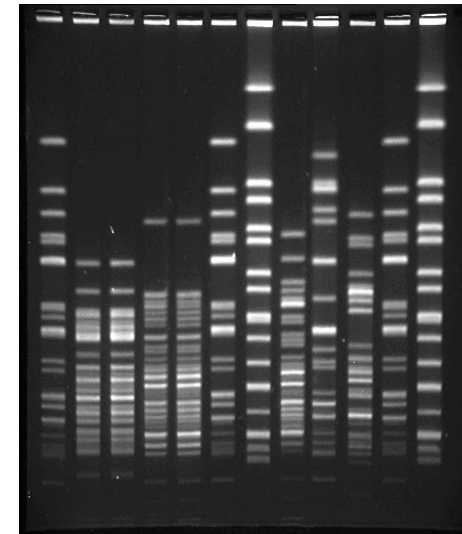
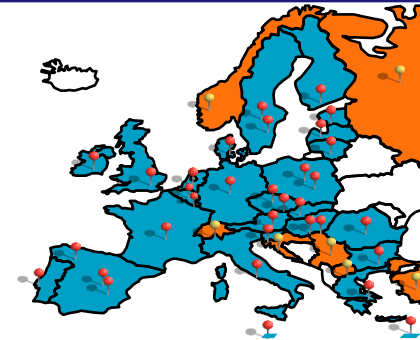
VTEC Network preparedness for molecular typing

- Proficiency tests (EQA) on PFGE (4 rounds in 2012-2015)
- Training on PFGE for NRLs staff
- Course on BioNumerics software use

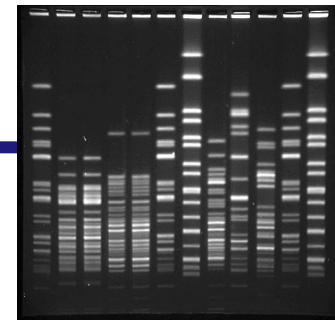


Proficiency tests on PFGE

- **PT-PFGE 1 (with PT10, January-March 2013)**
 - 16 EU NRLs + Norway and Switzerland
 - 11 *E. coli* strains
 - Conducted jointly with the ECDC-FWD network
- **PT-PFGE 2 (with PT11, June-September 2013)**
 - 23 EU NRLs + Norway and Switzerland
 - 6 *E. coli* strains
- **PT-PFGE 3 (with PT13, April-June 2014)**
 - 25 EU NRLs + Norway and Switzerland
 - 7 *E. coli* strains
- **PT-PFGE 4 (April-June 2015)**
 - 22 EU NRLs + Norway, Switzerland, Macedonia and Russia
 - 10 *E. coli* strains
 - Conducted jointly with the ECDC-FWD network

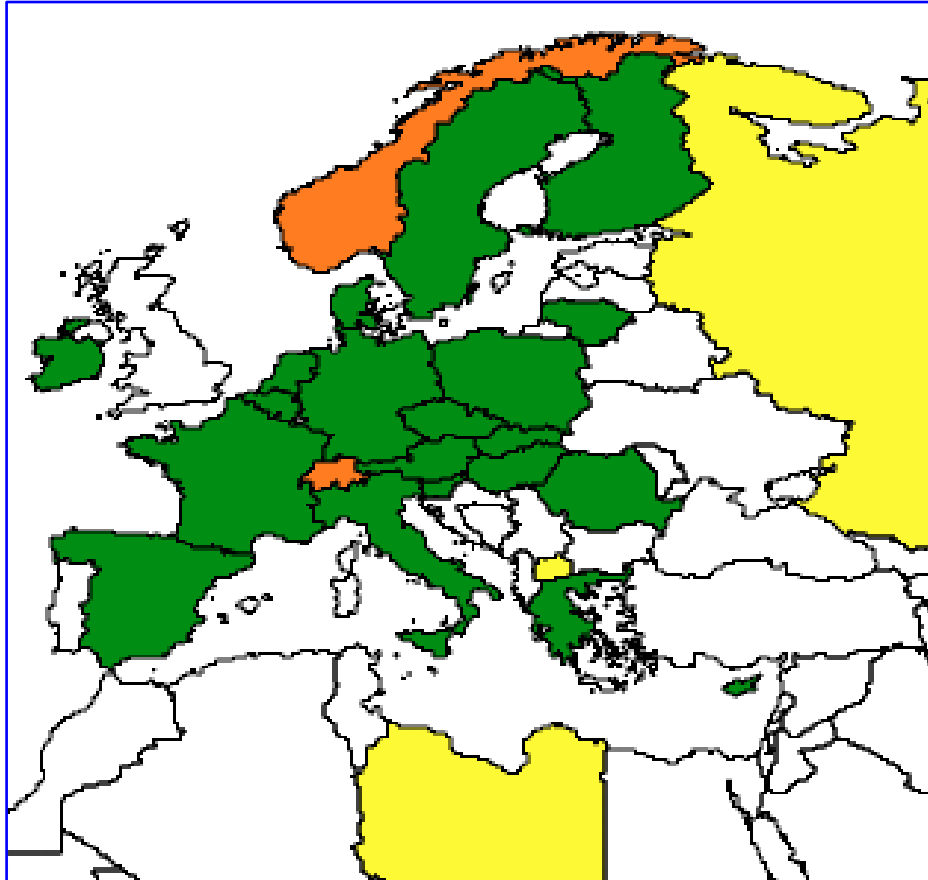


PFGE Proficiency testing (EQA)



Participation in proficiency tests on PFGE:

31 NRLs



-  26 NRLs of 21 EU MS
-  2 NRLs of 2 EEA MS
-  3 other NRLs

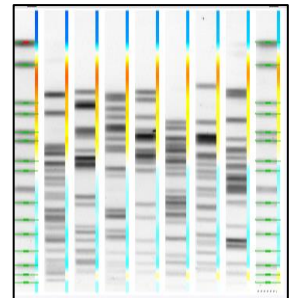
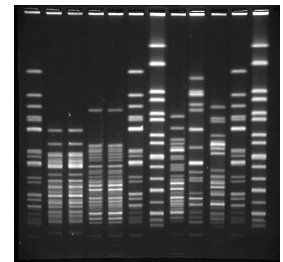
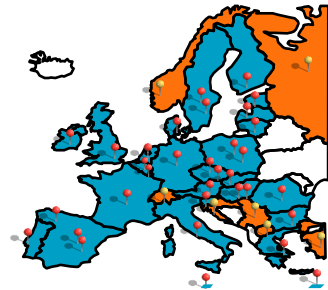
EQA on molecular typing: evaluation of the performance

➤ Evaluation NRL's proficiency:

- **PT-PFGE1 and PTPFGE2:** PulseNet
Procedure PNQ01(visual assessment)
- **PT-PFGE3 and PTPFGE4:** EFSA SOP for
PFGE profiles curation (BioNumerics
assisted)

➤ Rating:

- **Excellent, Good, Fair, Poor**

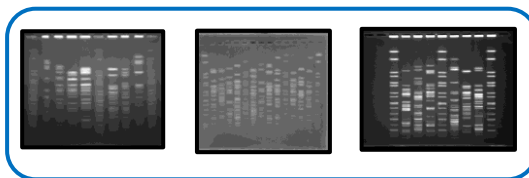


EQA on molecular typing: evaluation of NRLs' proficiency

PT-PFGE3 and PT-PFGE4: EFSA SOP for PFGE profile interpretation

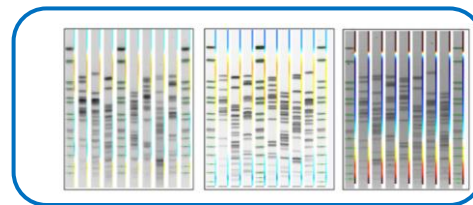
(<http://www.efsa.europa.eu/en/supporting/doc/704e.pdf>)

➤ Visual Assessment:



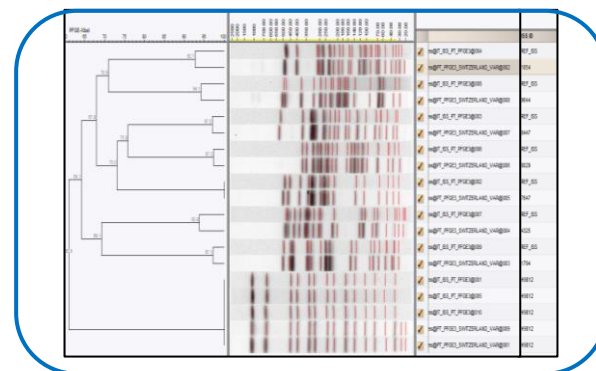
➤ BioNumerics analysis:

- Normalization and distortion analysis
- Clustering with the related reference PFGE profiles produced by the EU-RL
- Acceptable profiles: 97 % similarity with the reference

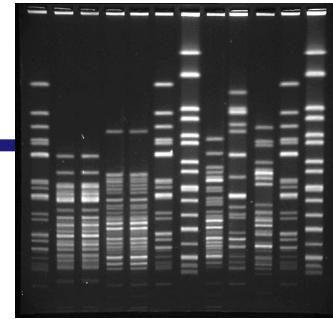


➤ Rating:

- **Excellent:** No rejected profiles
- **Good:** < 30% of rejected profiles
- **Fair:** between 30% and 60% of rejected profiles
- **Poor:** > 60% of rejected profiles

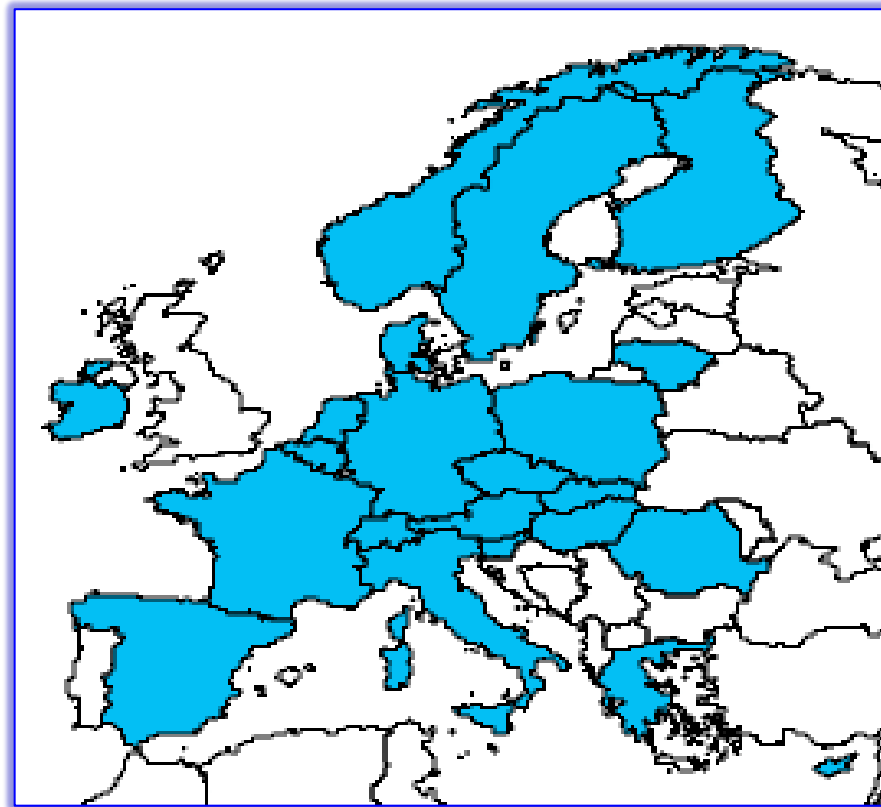


PT-PFGE4



Participation: 26 NRLs

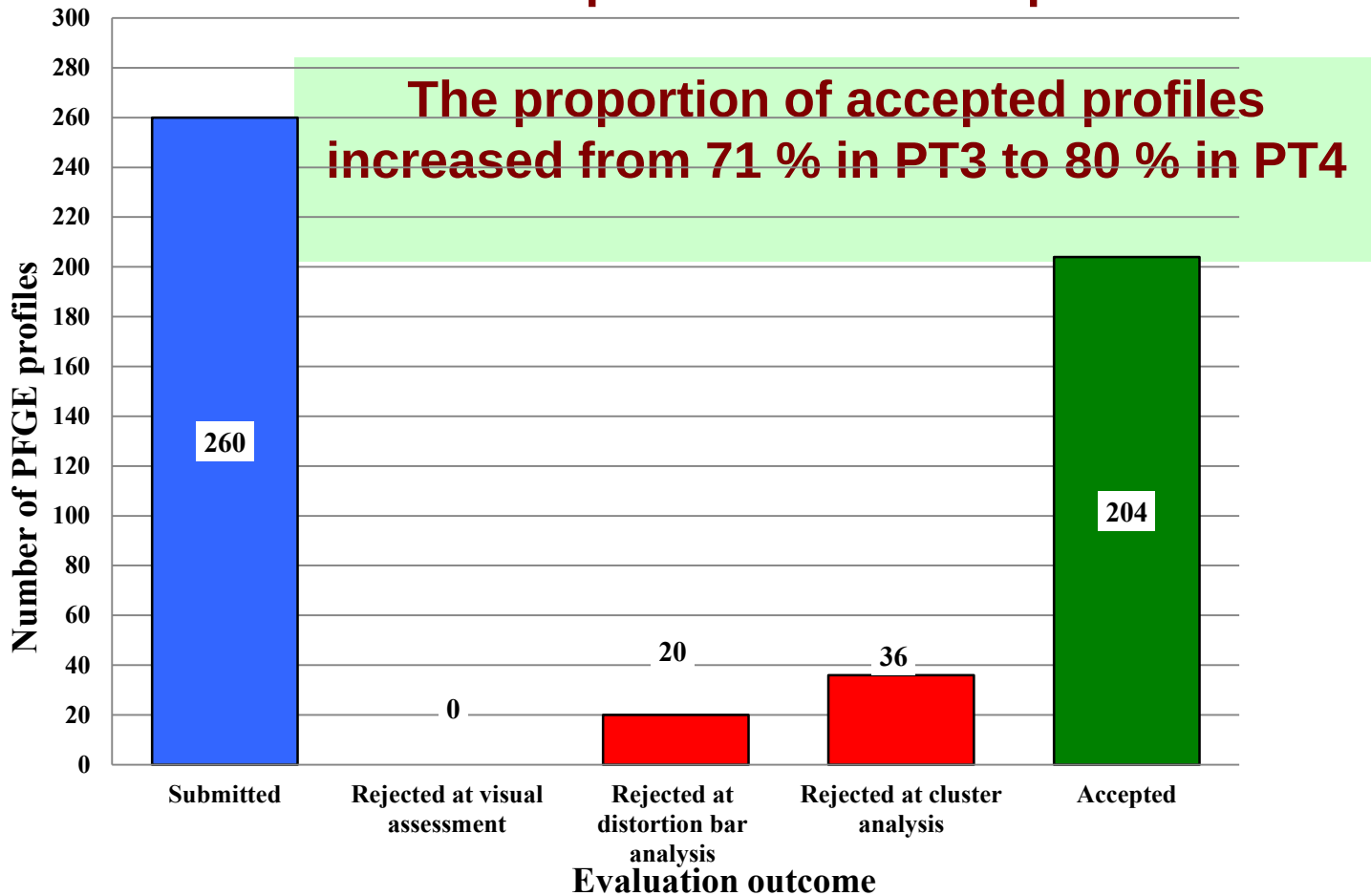
24 NRLs of 21 EU/EEA MSs



PT-PFGE4: evaluation of NRLs' proficiency

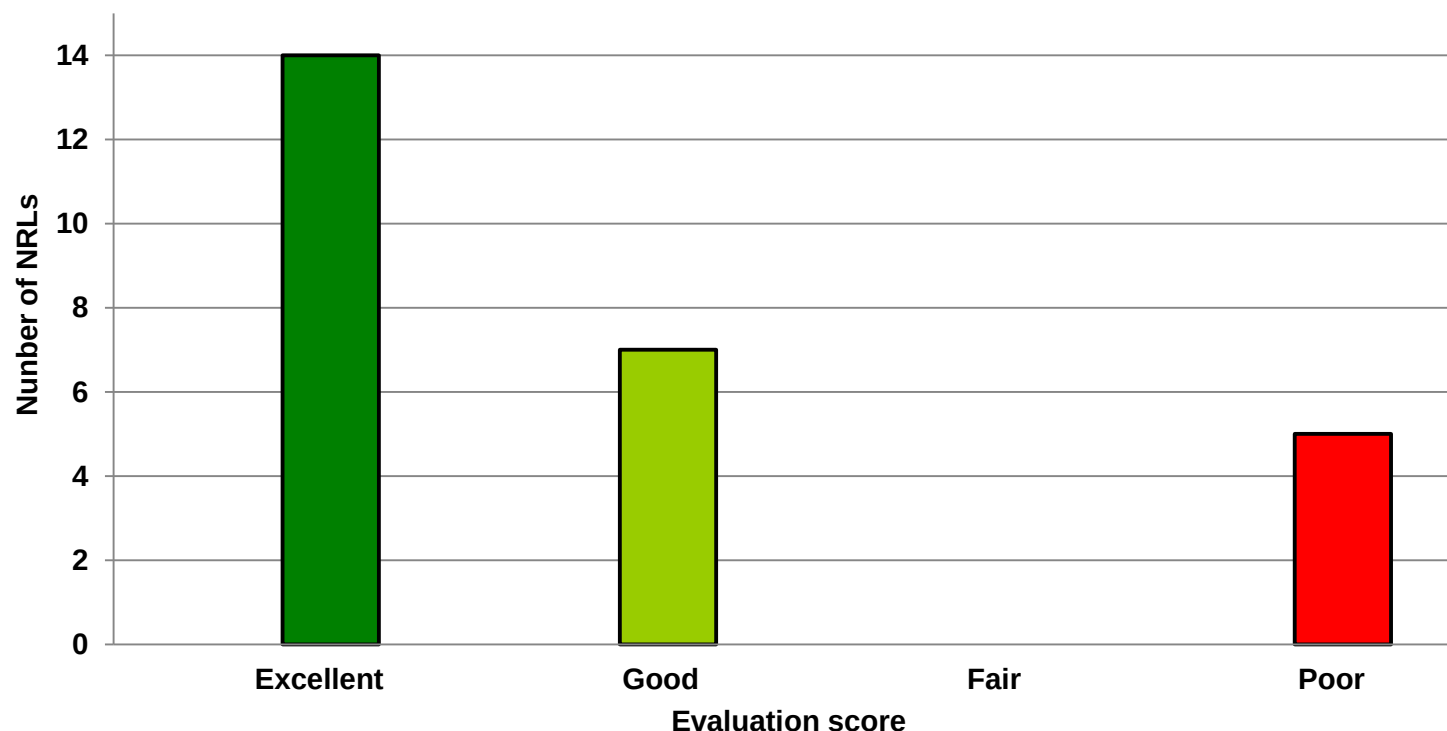
Three-step evaluation of the submitted PFGE profiles

80 % of the profiles were accepted



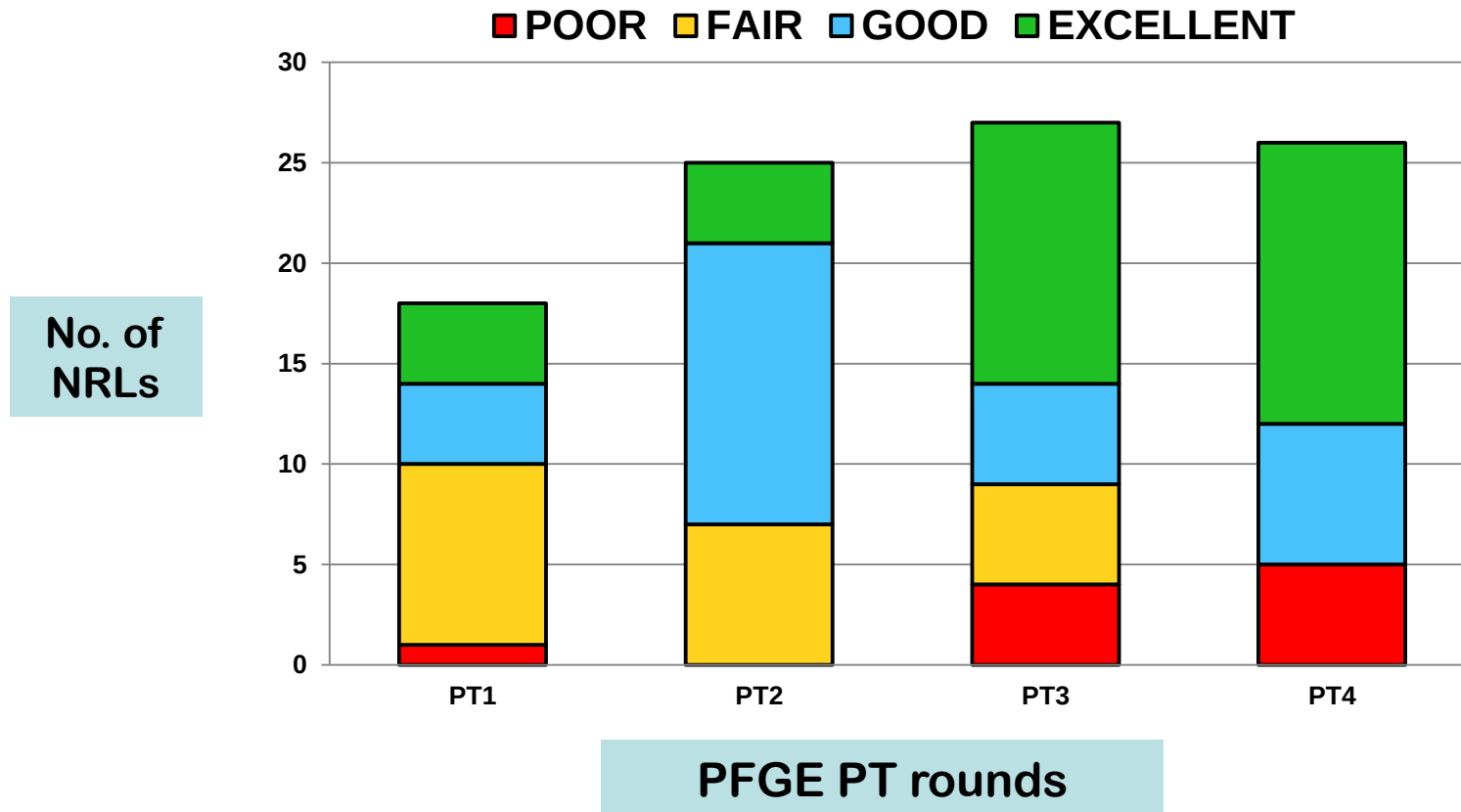
PT-PFGE4: evaluation of NRLs' proficiency

Evaluation of the NRL performance in producing PFGE profiles



PFGE Proficiency testing (EQA): Trends

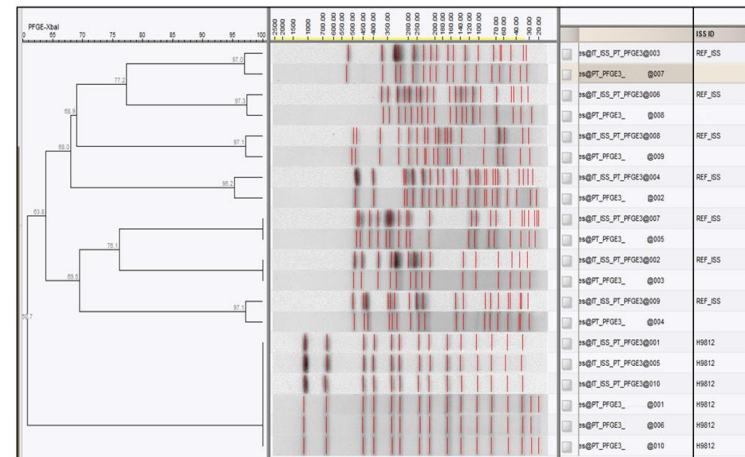
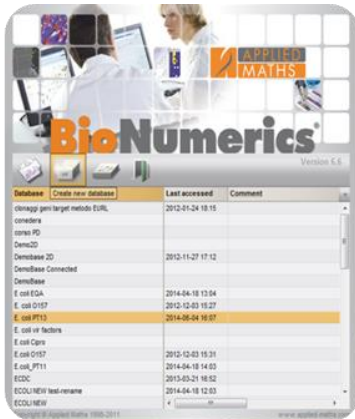
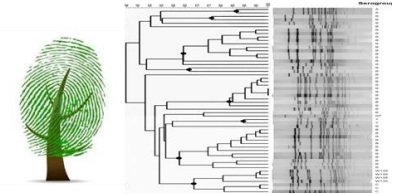
NRL performance in producing PFGE profiles



EQA on molecular typing: use of BioNumerics software

PT-PFGE4: facultative BioNumerics analysis

- Performed by 14 NRLs (50 %)
- These NRLs will receive an evaluation of their analyses in their individual report (coming soon)



Molecular typing by PFGE

Areas of improvement

- *Improvement of gel images: Background, Compressed/fuzzy bands, H9812 standard position, Extra samples/images submitted, Sample identification...*
- *For XML file production:*
 - ✓ *Band assignment in the lanes*
 - ✓ *Background subtraction*
 - ✓ *Frame, strip thickness and curve thickness*



Actions

- Individual advice through individual reports
- PFGE training modules still available
- NRLs that did not perform (or underperformed in) the **BioNumerics** analyses will receive priority in the upcoming courses on the use of the **software** (in 2016 and 2017)

