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ADVANTAGES AND DISADVANTAGES OF WEB-BASED SYSTEMS TO IDENTIFY DNA PROFILES

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LAB-based database Systems: Clostridium difficile

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- one laboratory responsible for nomenclature and identification
  - Laboratory resources are limited
  - only few had/have access to typing information
- till now various „localised" nomenclature systems
  - AI, SW, …..
- limited availability of a standard strain collection
  - till 2008 !!! no international strain collection available
    - strain collection sample size is limited
    - possibility for errors
    - strain collection is always months or years behind of the users epidemiological development

INTERNET vs LAB based Database Systems

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- **INTERNET**
  - **Up to date internet-database for all users**
  - **Only** the sequencer **file is needed** to define a new type
  - **No additional costs**
    - no soft- or hardware is needed
  - **No restriction** for sequencer, gel, size-standard or primer-set
  - **ALL users have access** to new Data
  - **No shipping** costs

- **LOCAL**
  - Up to date database for **one/few local users**
  - **Strain must be send** to reference Lab
  - **Additional costs**
    - Soft- or hardware **is needed**
  - **Methods restricted to achieve minimal exchangeability**
  - **only local User** can access data
  - **shipping costs**

WEB-BASED SYSTEMS TO IDENTIFY DNA PROFILES

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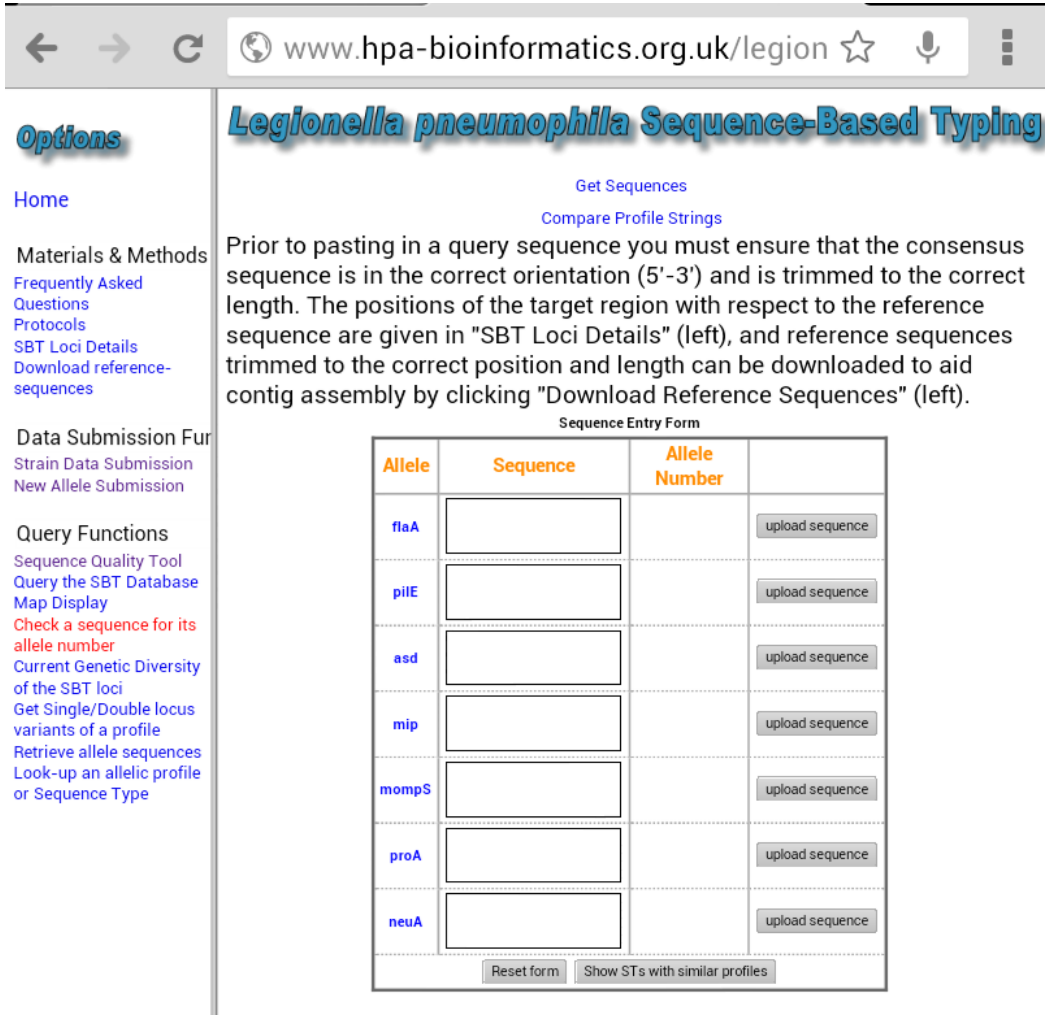
- available systems
  - **HPA-Legionella SBT Typing**



WEB-BASED SYSTEMS TO IDENTIFY DNA PROFILES

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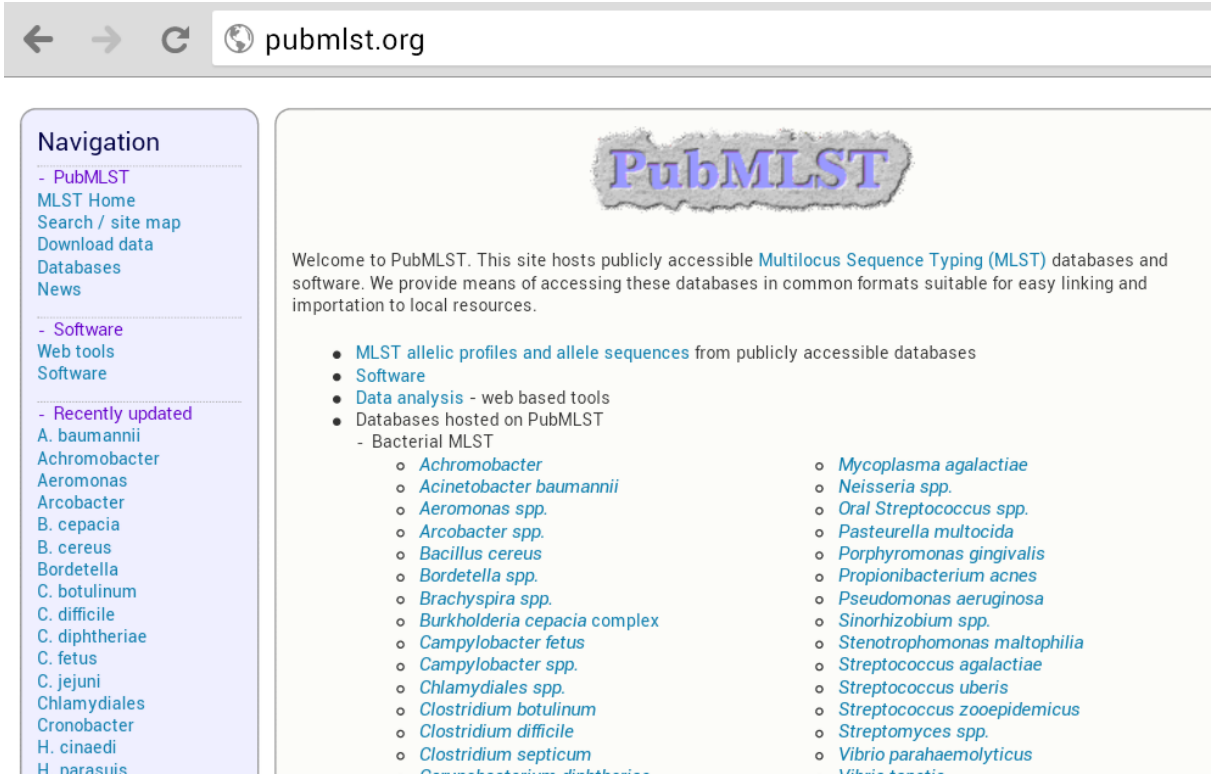
- available systems
  - HPA-Legionella SBT Typing
  - **various MLST-Databases**



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- available systems
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  - **MIRU-VNTRplus**

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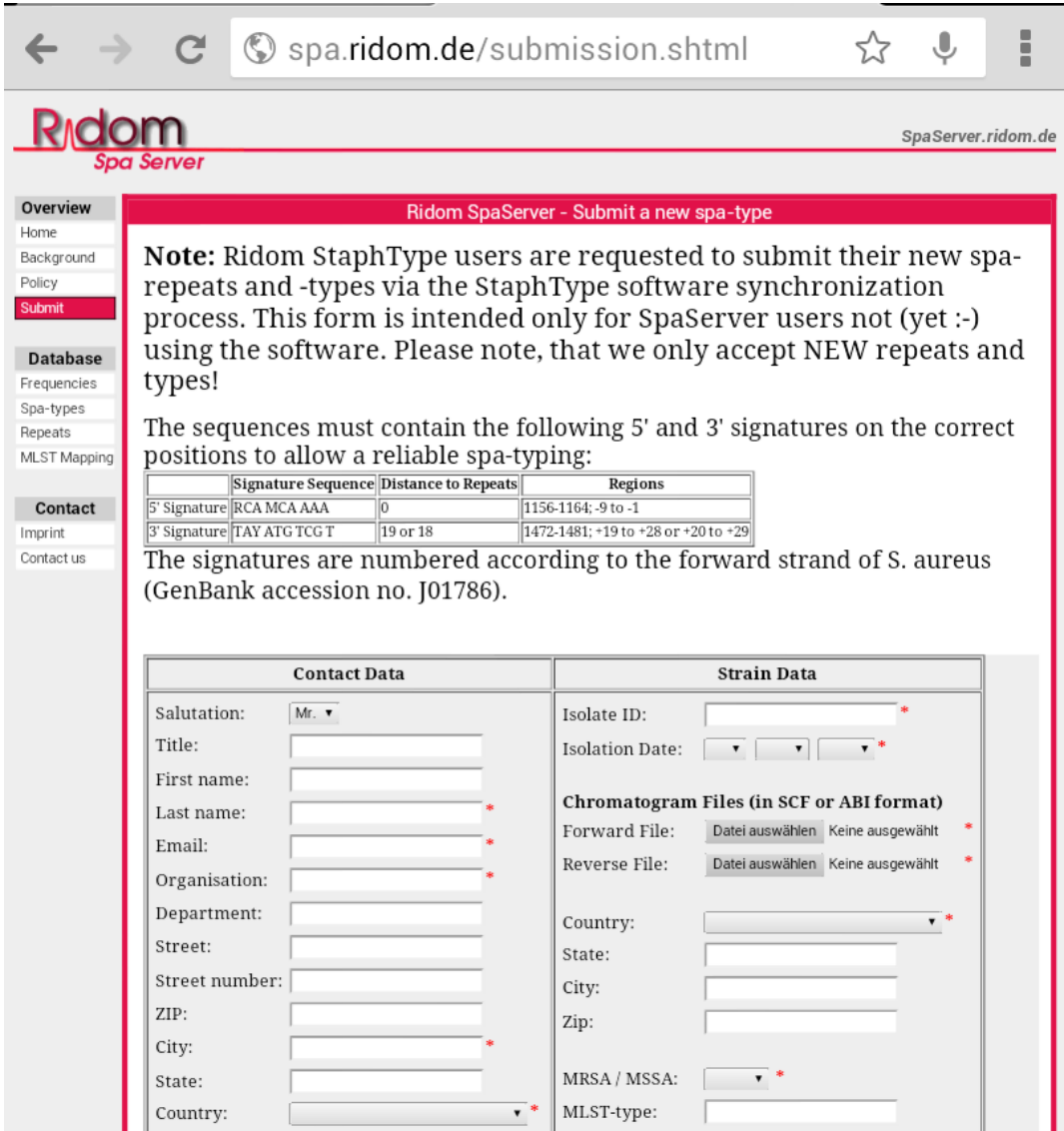
  - **RIDOM-StaphType**



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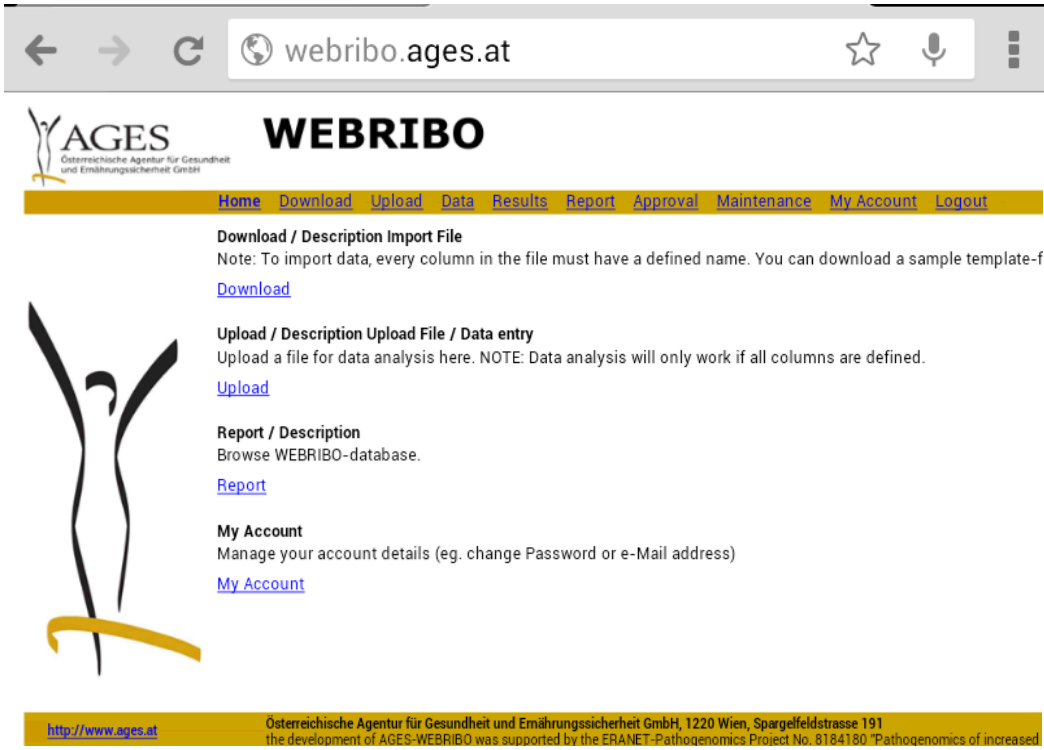
- available systems
  - HPA-Legionella SBT Typing
  - various MLST-Databases
  - MIRU-VNTRplus
  - RIDOM-StaphType
  - **AGES-WEBRIBO**



the only internet database doing automated fragment analysis

WEB-BASED SYSTEMS TO IDENTIFY DNA PROFILES: **Cons**

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- analysis algorithm **quite sensitive**
  - HPA-Legionella
- **RAW-data** import for **Beckman or Licor-Systems** not always available
  - most Databases missing RAW-Data Import
- **nomenclature can be divergent**
  - overdiscrimination
  - Kreiswirth vs. RIDOM, AGES-WEBRIBO - Cardiff
- **Analysis can take some time**

WEB-BASED SYSTEMS TO IDENTIFY DNA PROFILES: **Wishlist**

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- **Freely accessible constantly updated internet-database**
- **ABI gained files** are **analyzed** automatically
- **Reliable results** regardless of any primer, gel, size-standard or sequencing machine used
- **No commercial software is needed** (Bionumerics, Genemapper, …)
- **no** manual **data manipulation** needed

Capillary Gel Electrophoresis based PCR Ribotyping Analysis using WEBRIBO

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Labwork

- Direct or culture samples
- DNA-extraction
- PCR
- Capillary sequencer PCR-ribotyping

Analysis

- results in 4 steps using **WEBRIBO** (webribo.ages.at)

WEBRIBO:
Equipment requirements

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- **PCR-Cycler**
- **Primers**
  - any labelled PCR-Ribotyping primer available (e.g. Bidet, Stubbs or Janežič)
- **Sequencer**
  - Applied Biosystems (ABI) Systems tested (310, 3100, 3130(xl), 3730)
    - any POP-Gel
    - any capillary length
  - Beckman Coulter Sequencer
- **Size-Standard**
  - any Size-Standard like
    - Geneflo 625 ROX/Tamra
    - LIZ 600/LIZ 120
    - Genemapper 1000
    - Beckman GenomeLab 600

WEBRIBO
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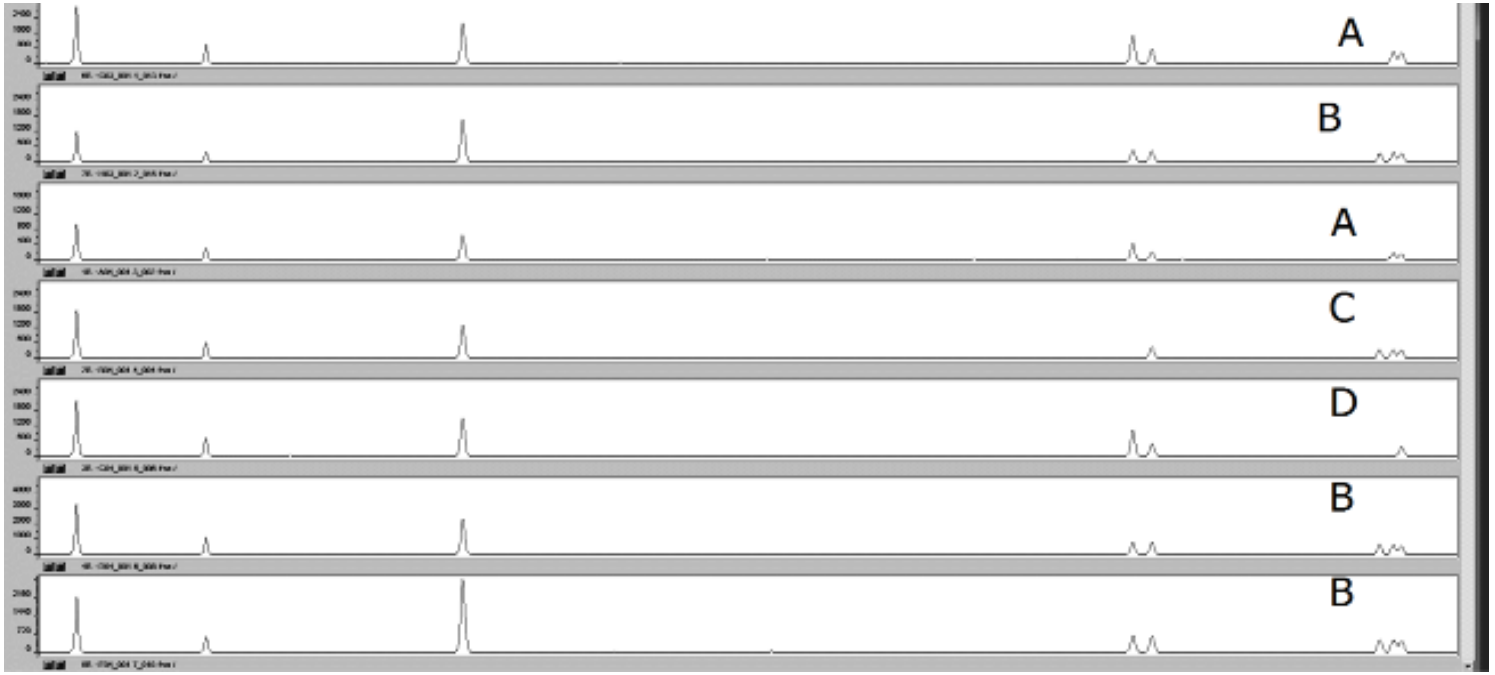
- **Capable of** differentiating **ribotypes 014 and 020**
  - additional 014 subtypes can be separated
- **Capable of** differentiating **various 001 subtypes** first described by Northey et al.



WEBRIBO: Cons

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- .fsa data analysis algorithm **quite sensitive**
- **No RAW-data** import for **Beckman Systems**
- PCR-Ribotye **nomenclature not in total accordance**
  - **Subtype** nomenclature is sometimes **confusing**
- **Assignment algorithm has to be improved**
- **Analysis can take some time**

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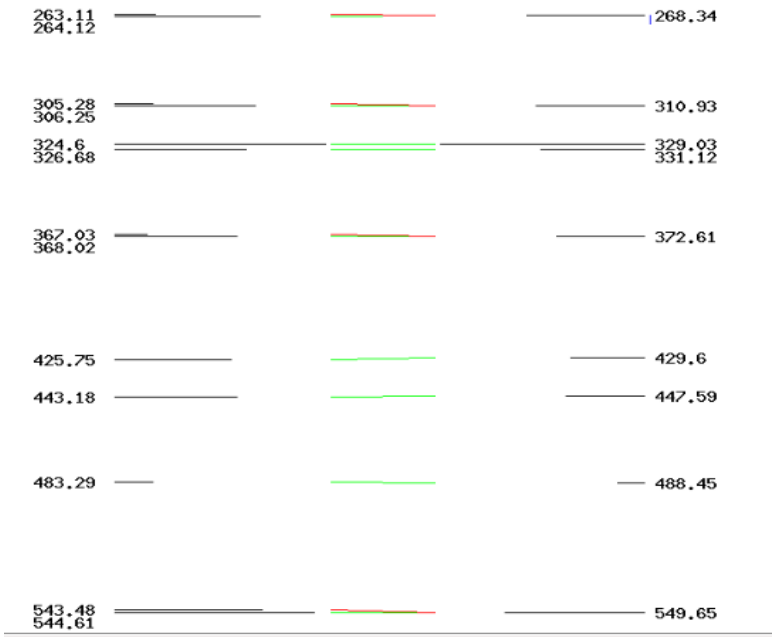
- Up to 99% correct **identification of samples analysed with**
  - **different** size-standards
  - **different** sequencing machines
  - **different** primers
    - Stubbs, Bidet, Janežič
  - a **supervised learning algorithm** copes with double-peaks due to PCR-artefacts



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QUESTIONS?

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