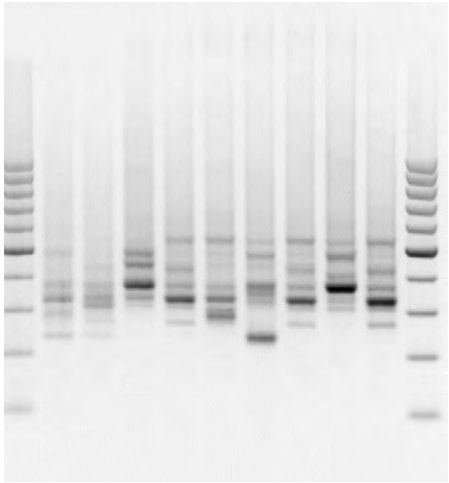
Capillary-gel-electrophoresis-based-PCR-Ribotyping

Alexander Indra

Capillary gel electrophoresis based PCR ribotyping

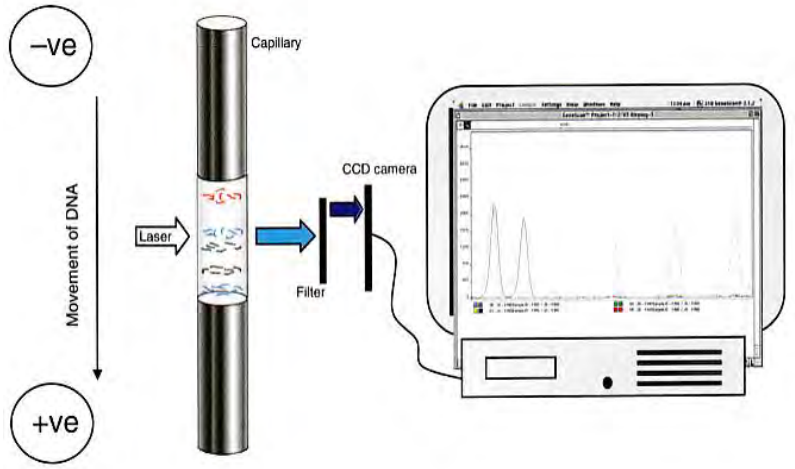
Conventional Clostridium difficile PCR-Ribotyping

- problematic inter-laboratory exchange
  - variable use of laboratory specific nomenclature
- no international database
- every sample has to be compared with controls processed under the same conditions
- inconsistent data interpretation of similar band patterns
  - e.g. PCR-ribotype 066
    - known with Toxinotype V and VI
  - untypable 001 subtypes



Capillary gel electrophoresis based PCR ribotyping

- high reproducibility by using
  - a fluorescence labeled primer
  - a uniform size-standards within every sample
  - reproducible running conditions for every sample
- high throughput
- comparability of data results
- easy-to-use data analysis
- higher discriminatory power
  - fragments with 1.5 bases difference or more can be differentiated



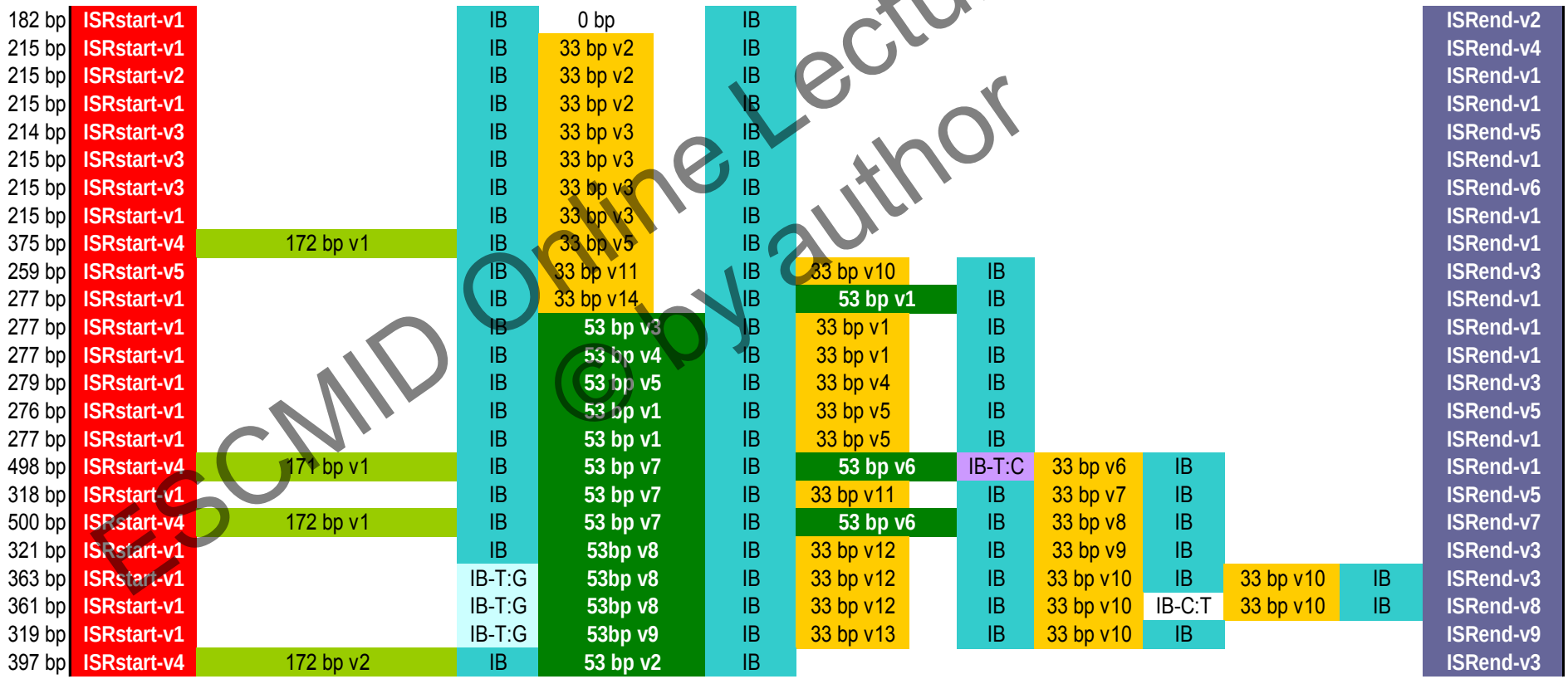
Capillary gel electrophoresis based PCR ribotyping

- 6 different 014 subtypes including 020
- Toxinotype V and VI of PCR-ribotype 066 can be differentiated

Mechanisms behind the variations in PCR-Ribotyping patterns

- differences in fragment lengths of the C. difficile 16S–23S ISR are based on the number of **9 bp direct repeats** separated by **spacer sequences**
  - spacer sequences detected show **three different lengths**
    - **20 bp**
    - **33 bp**
    - **53 bp**
- some ISR display a 172 bp spacer sequence that includes a gene for tRNA-Ala
- in contrast to the earlier findings by Sadeghifard et al. this results show a **highly structured organization** of the 16S–23S ISR of C. difficile

Mechanisms behind the variations in PCR-Ribotyping patterns



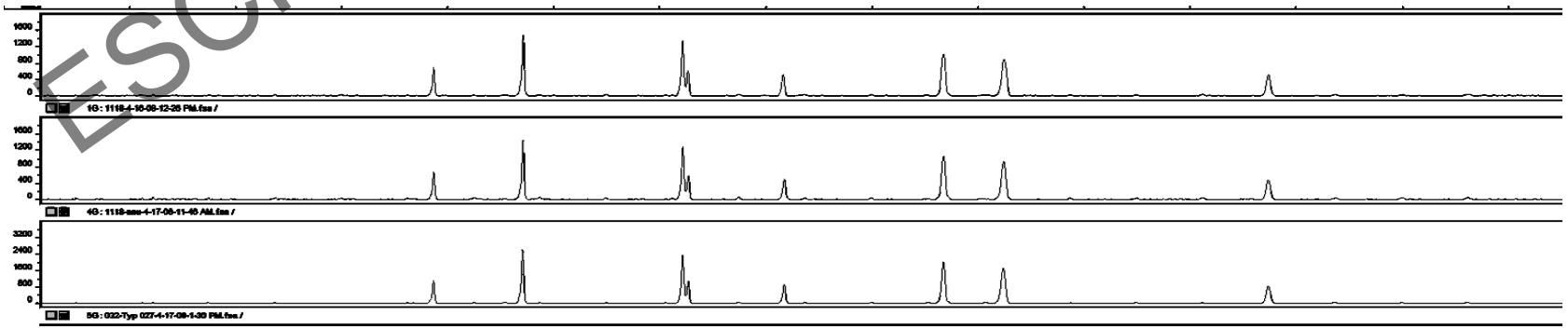
Indra & Blaschitz et al .2010

Mechanisms behind the variations in PCR-Ribotyping patterns

- this composition is responsible for the length variations of the 16S–23S ISR used in PCR ribotyping
- it has evolved as the result of several mechanisms frequently occurring during DNA replication and recombination
  - slipped-strand mispairing
  - intra-chromosomal homologous recombination
  - possible inter-chromosomal homologous recombination

Mechanisms behind the variations in PCR-Ribotyping patterns

- this highly structured and conserved organization is responsible for the comparability and reproducibility found in capillary gel electrophoresis PCR-ribotyping
  - it allows **AGES-WEBRIBO** comparison of results
    - of **different** machines
    - with **different** size-standards
    - with **different** Primer-pairs



LAB-based database Systems: Clostridium difficile

- 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

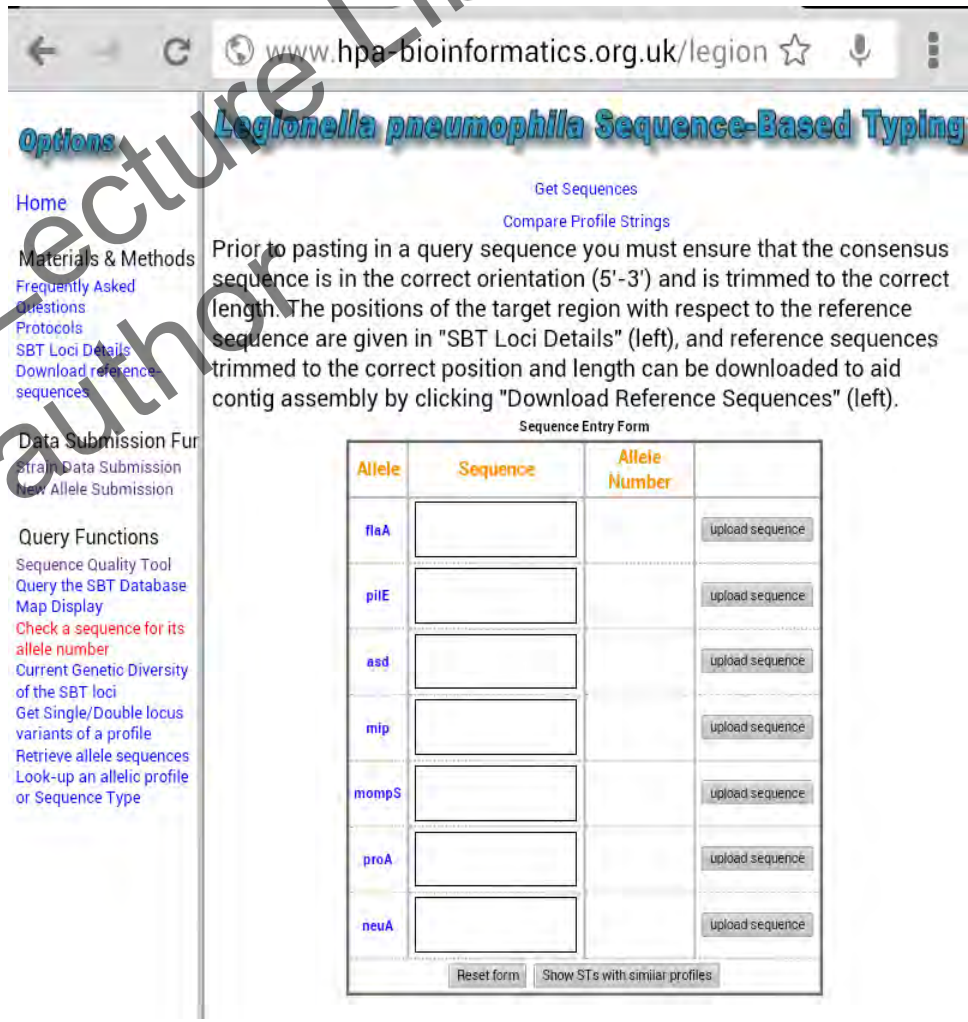
INTERNET vs LAB based Database Systems

- 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

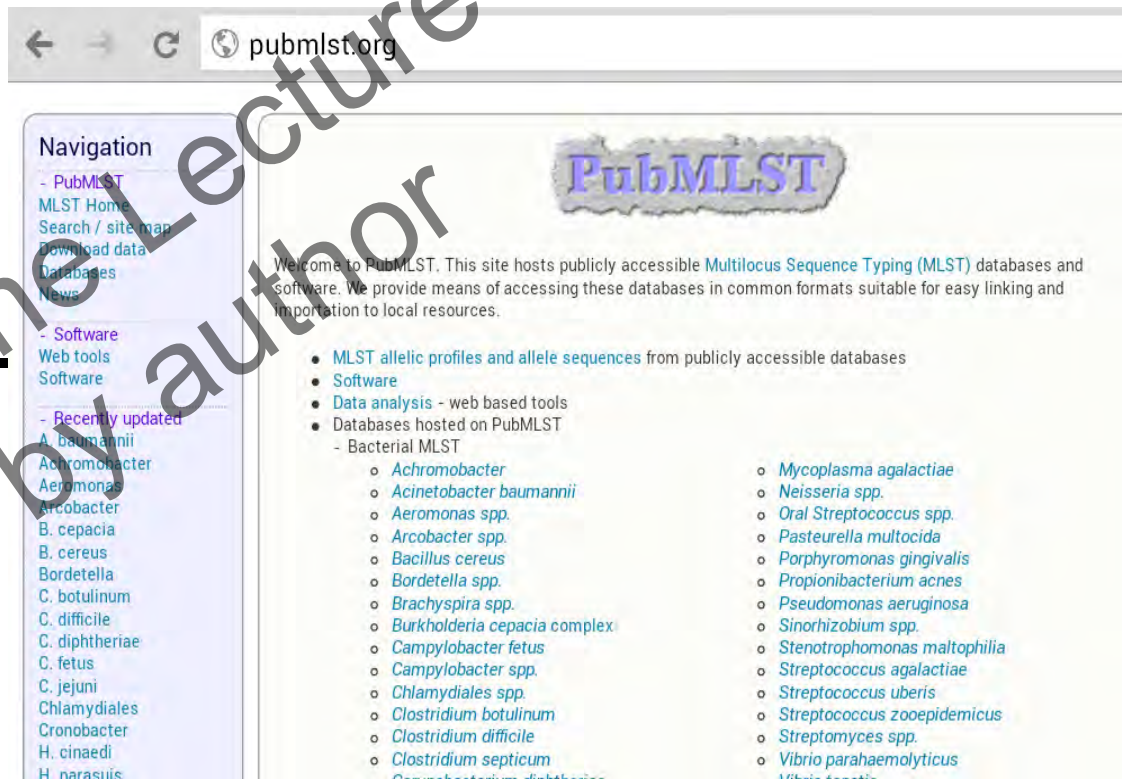
WEB-BASED SYSTEMS TO IDENTIFY DNA PROFILES

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



WEB-BASED SYSTEMS TO IDENTIFY DNA PROFILES

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



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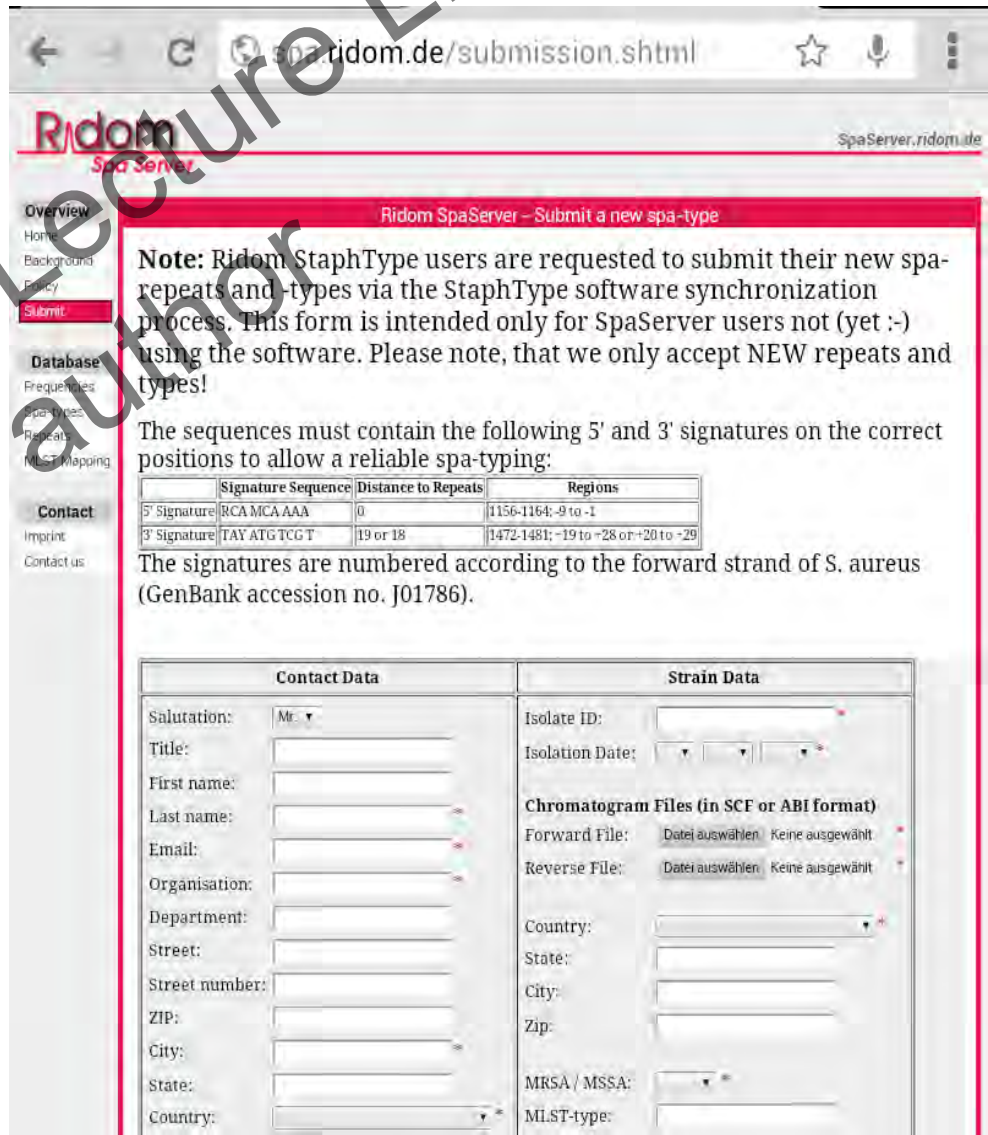

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- available systems
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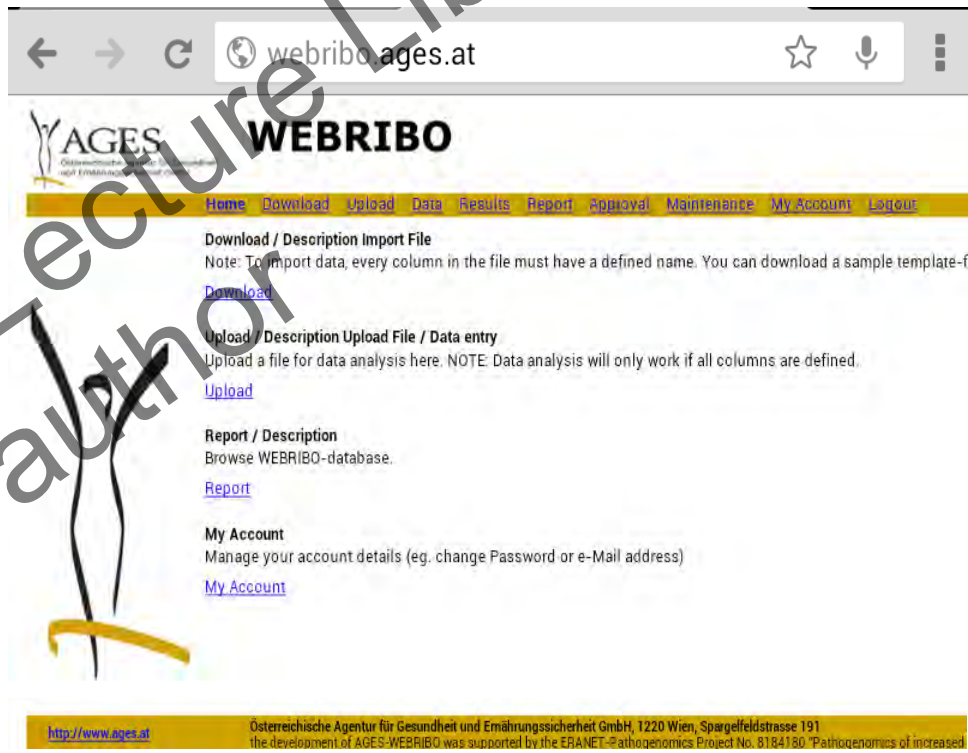
  - various MLST-Databases
  - MIRU-VNTRplus
  - RIDOM-StaphType
  - **AGES-WEBRIBO**



the only internet database doing automated fragment analysis

WEBRIBO
webribo.ages.at

- WEBRIBO allows comparison of results
  - on **different** machines
  - **different** capillaries
  - **different** POP-Gels
  - with **different** size-standards
  - with **different** Primer-pairs (Bidet, Stubbs, Janežič)

Capillary Gel Electrophoresis based PCR Ribotyping Analysis using WEBRIBO

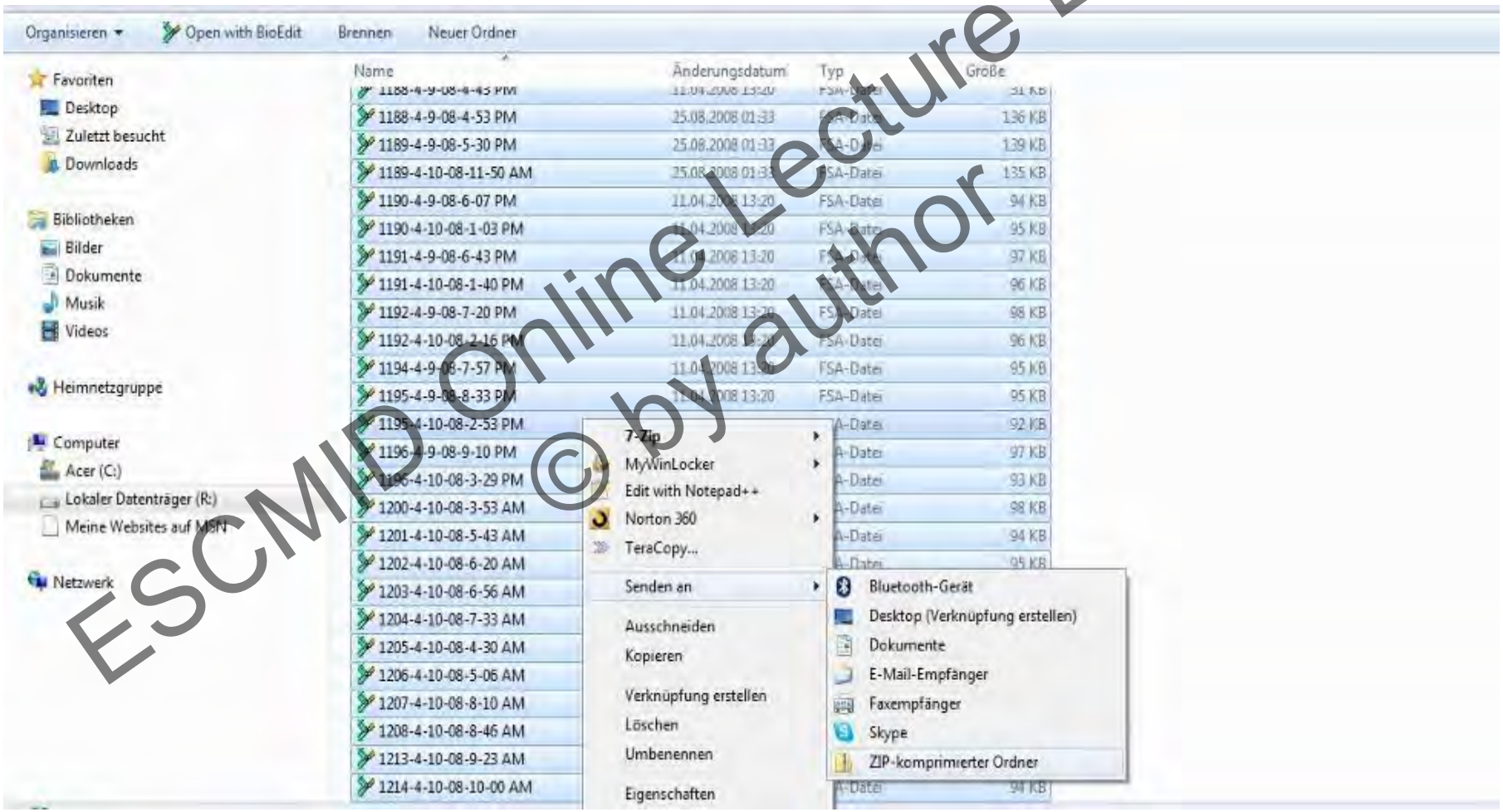
Labwork

- Culture
- DNA-Extraction
- PCR
- Capillary PCR-ribotyping

Analysis

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

Applied Biosystems fsa-File Analysis
Step 1: ZIP it

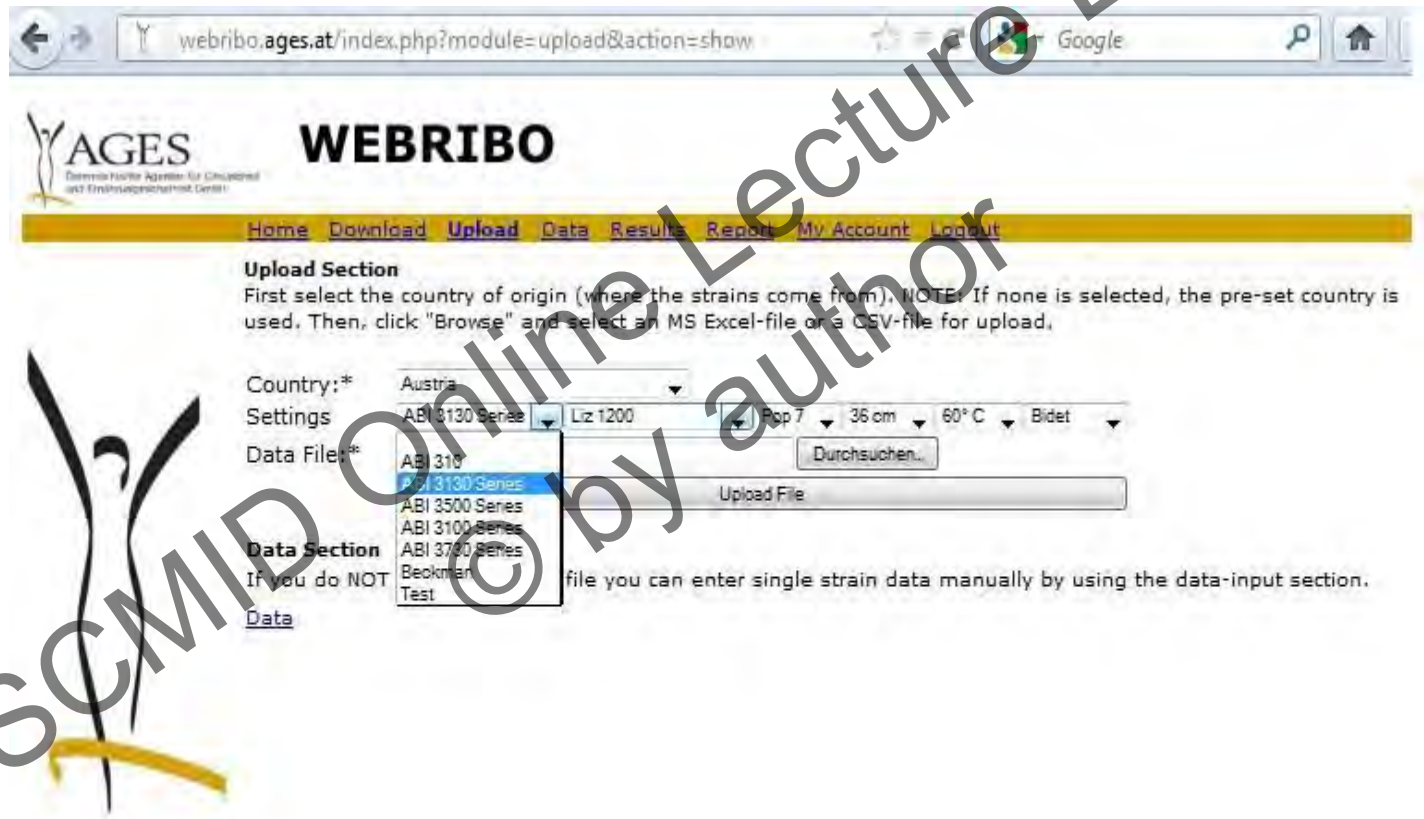


Applied Biosystems fsa-File Analysis

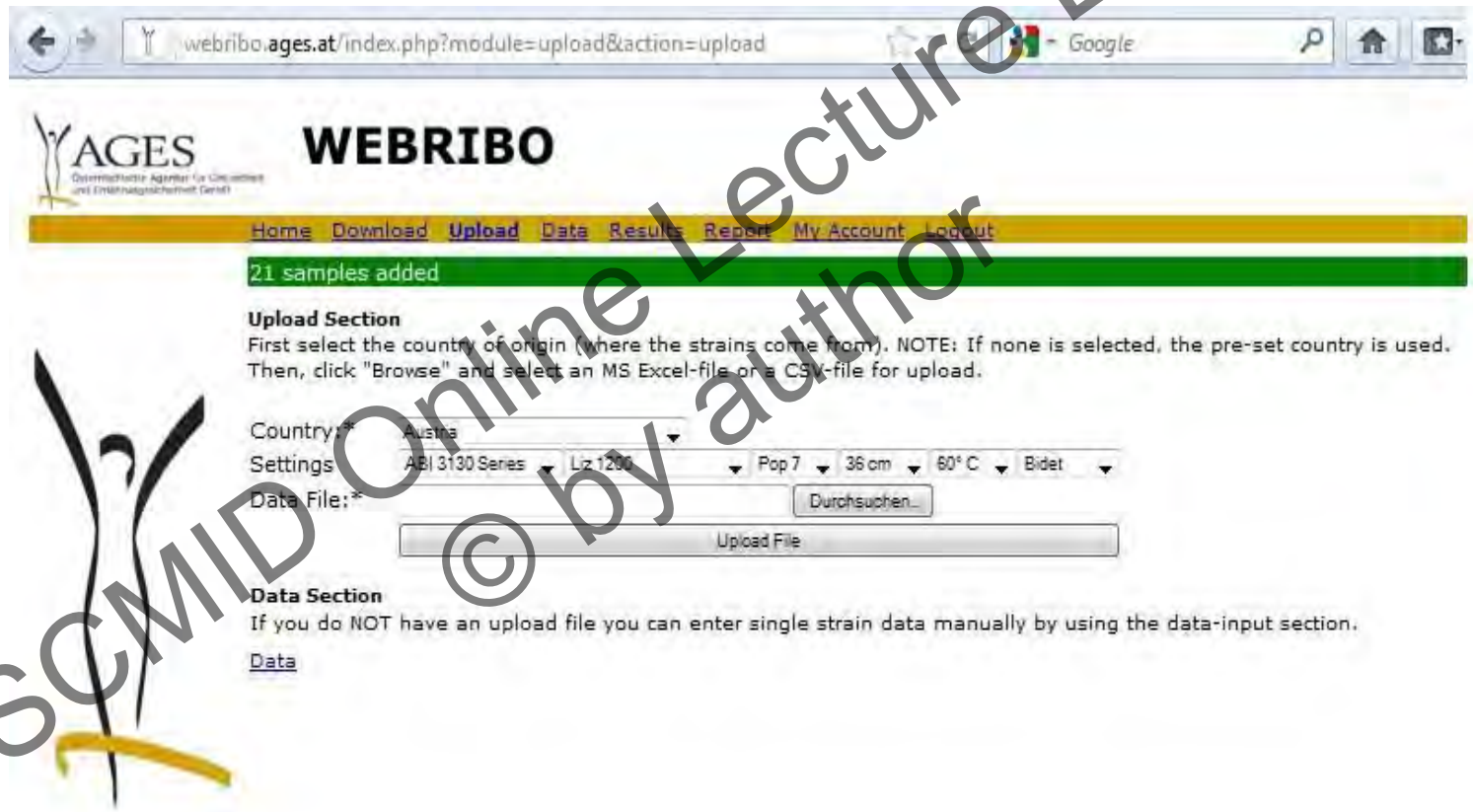
Step 2: Log into WEBRIBO

Applied Biosystems fsa-File Analysis

Step 3: Upload your files

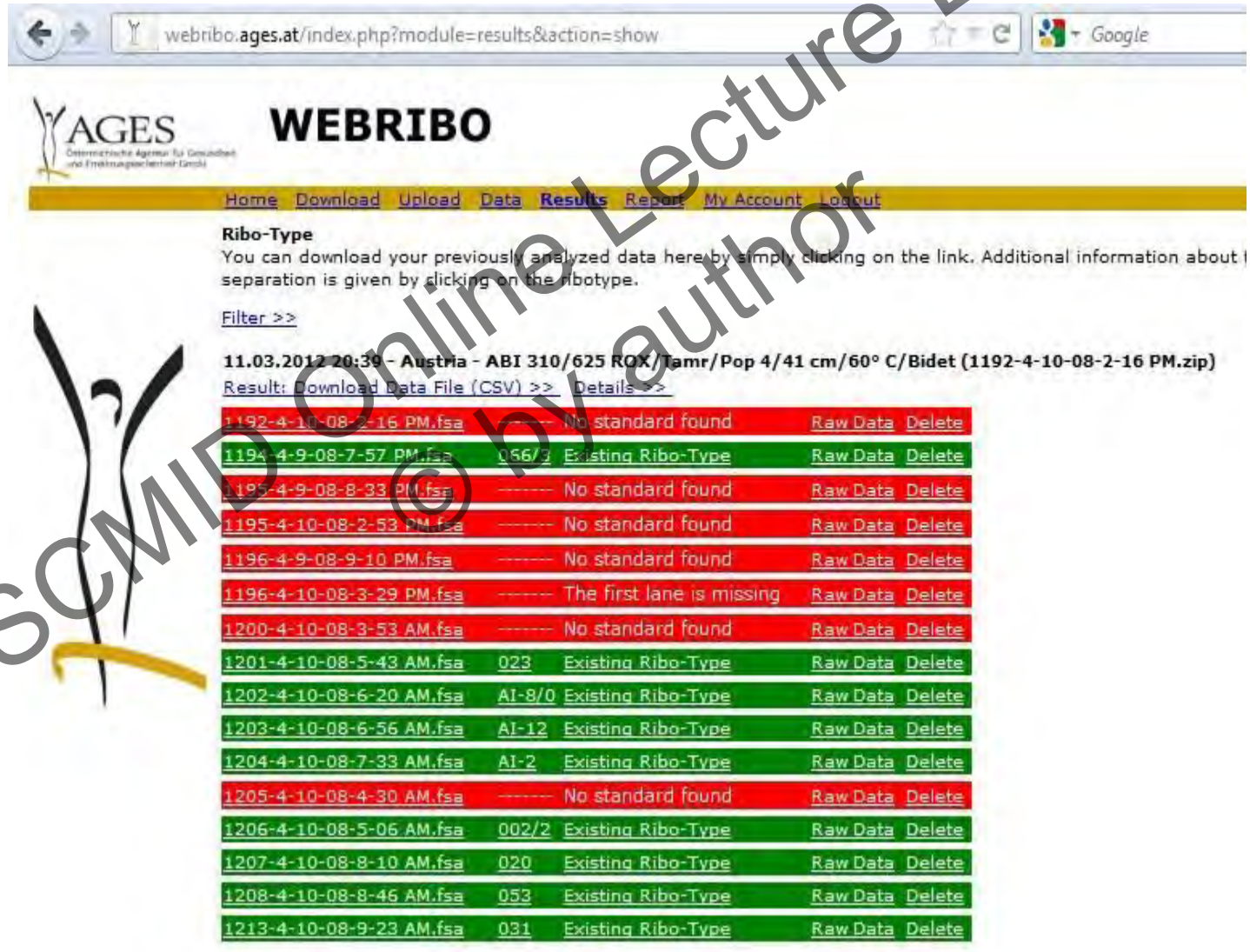


Applied Biosystems fsa-File Analysis
Step 3: Upload your files

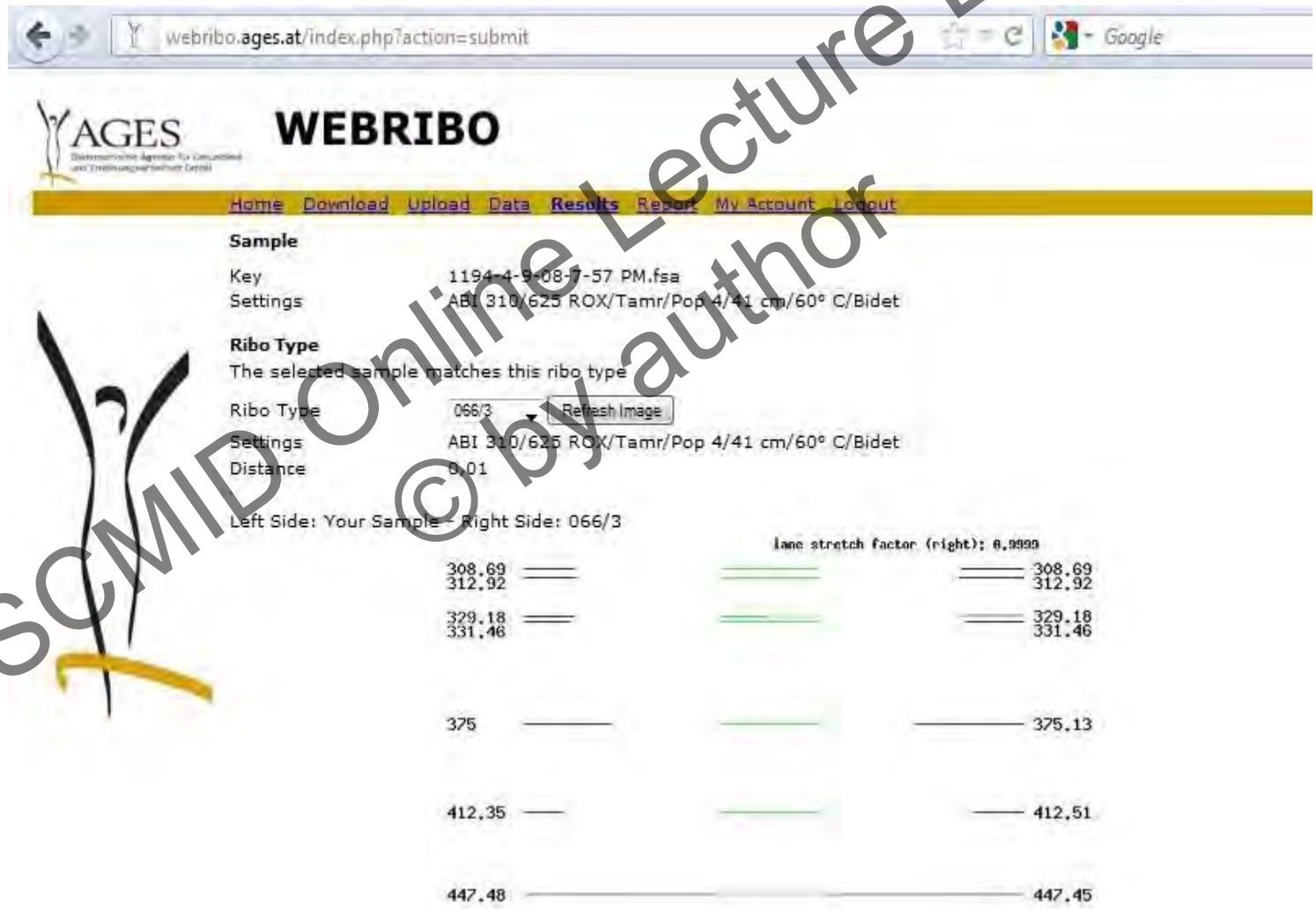


Applied Biosystems fsa-File Analysis
Step 4: Analysis

Applied Biosystems fsa-File Analysis
Step 4: Analysis

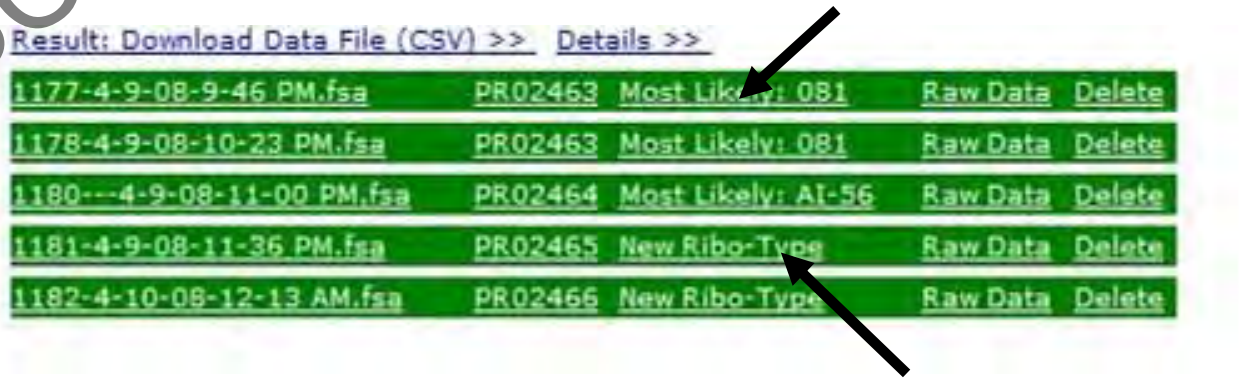


Applied Biosystems fsa-File Analysis
Compare your results



What is a PR-Type?

- PR Values are assigned
  - For new ribotypes
  - Ribotype patterns similar to ones in the database but gained with different sequencers, standards, capillaries (BM Values <3)
    - "most likely" relation to ribotype is given
      - comparison can be done by clicking on the link



What is a PR-Type?

Ribo Type

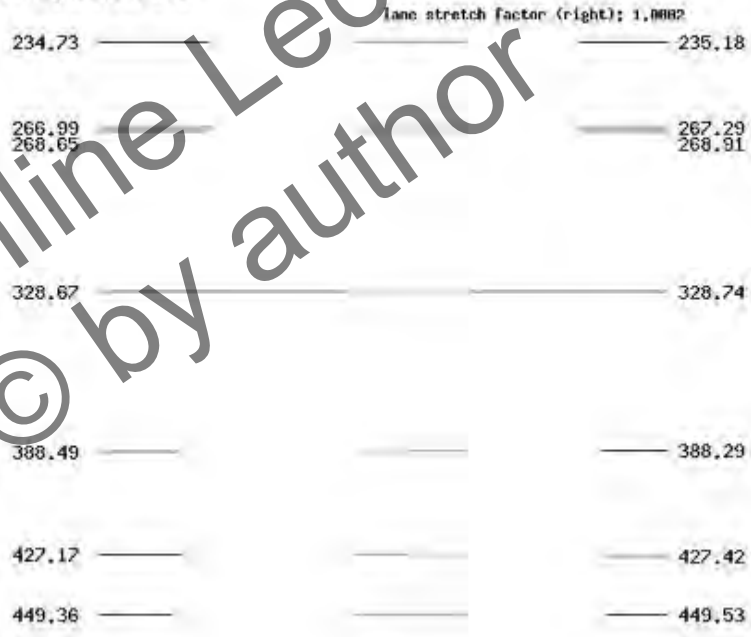
This is an automated classification that will be approved manually

Ribo Type 081 Refresh Image

Settings ABI 310/625 ROX/Tamr/Pop 4/41 cm/60° C/Bidet

Distance 0,04

Left Side: Your Sample - Right Side: 081



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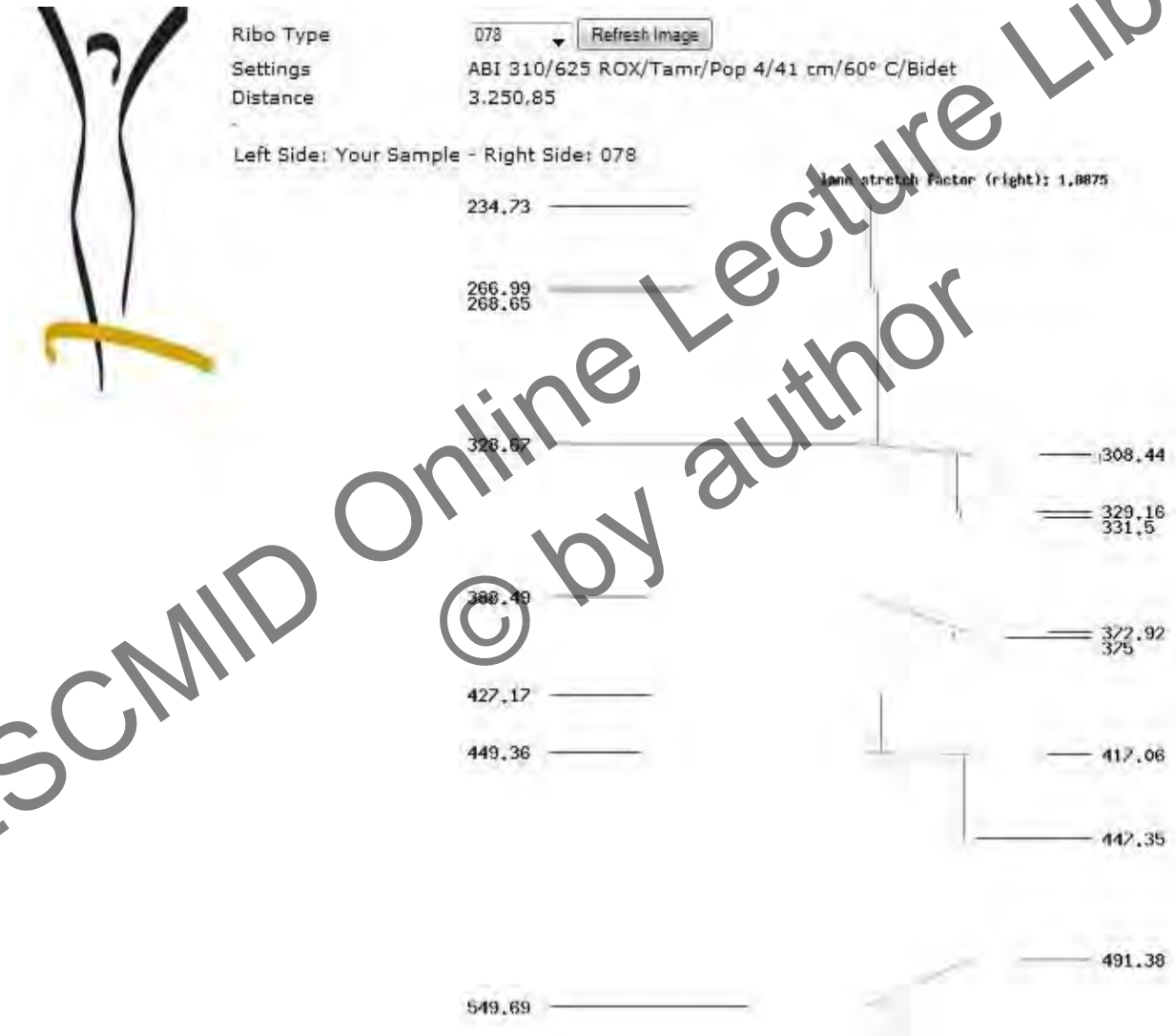

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    - "most likely" relation to ribotype is given
      - comparison can be done by clicking on the link
    - the final assignment is currently done manually
      - automatic assignment in next update (April-May 2012)

WEBRIBO:
Equipment requirements

- Sequencer
  - Applied Biosystems (ABI) Systems tested (310, 3100, 3130(xl), 3730)
    - any POP-Gel
    - any capillary length
  - Beckman Coulter Sequencer
- Primers
  - any PCR-Ribotyping Primer available (e.g. Bidet, Stubbs or Janežič)
- Size-Standard
  - any Size-Standard like
    - Geneflo 625 ROX/Tamra
    - LIZ 600
    - LIZ 1200
    - Genemapper 1000
    - Beckman GenomeLab 600

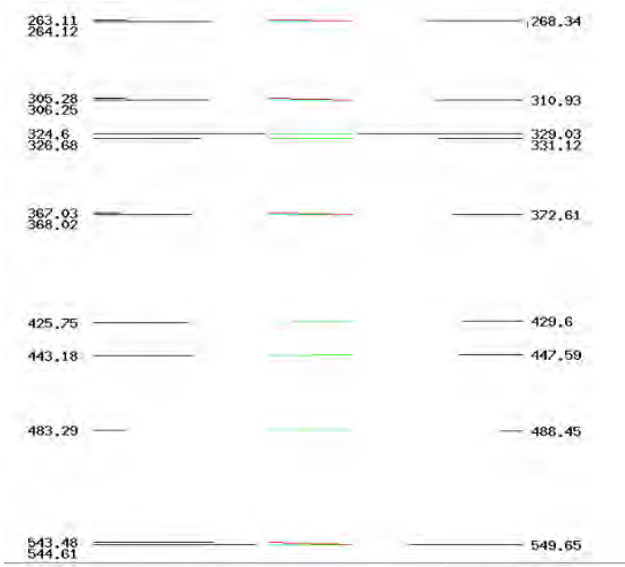
WEBRIBO:
Software requirements

- Computer
  - PC, Mac, Linux,...
  - iPhone, Android, Amazon Kindle....
- Webbrowser
  - IE, Safari, Chrome, Opera,....
- Raw-Data Analysis Software
  - ABI-Peakscanner-Pro (free Software)
  - Beckman
- Spreadsheet Software
  - LibreOffice, Excel, OpenOffice, KDE-Office, ...

WEBRIBO
webribo.ages.at

- 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



WEBRIBO
webribo.ages.at

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

WEBRIBO: Pro/Cons

Pros

- Database is **always up to date**
  - **follow epidemiological trends**
- **Access via internet**
- **No training** needed
- **Only** the .fsa **file is needed** to define a new ribotype
- **No additional costs**
  - no soft- or hardware is needed
- **No restriction** for sequencer, gel, size-standard or primer-set

Cons

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

WEBRIBO
webribo.ages.at

- Freely accessible internet-database for *Clostridium difficile* PCR-ribotyping
- No commercial software is needed (Bionumerics, Genemapper, ...)
- Real-time update
- ABI gained .fsa files can be analyzed automatically
- Reliable results regardless of any size-standard or sequencing machine used

THANK YOU FOR YOUR QUESTIONS