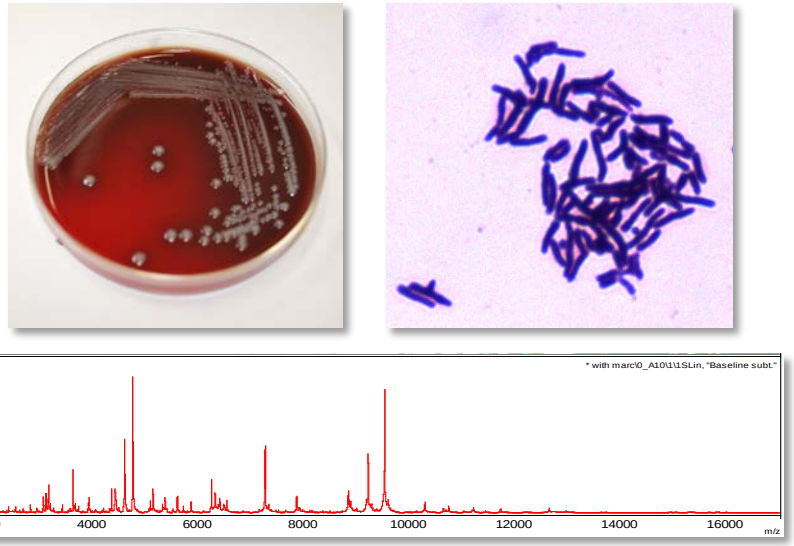
Bacterial Identification by Mass Spectrometry



Mark Fisher, Ph.D., D(ABMM)

Assistant Professor of Pathology,

University of Utah School of Medicine,

Medical Director, ARUP Bacteriology and Antimicrobials

Disclosures

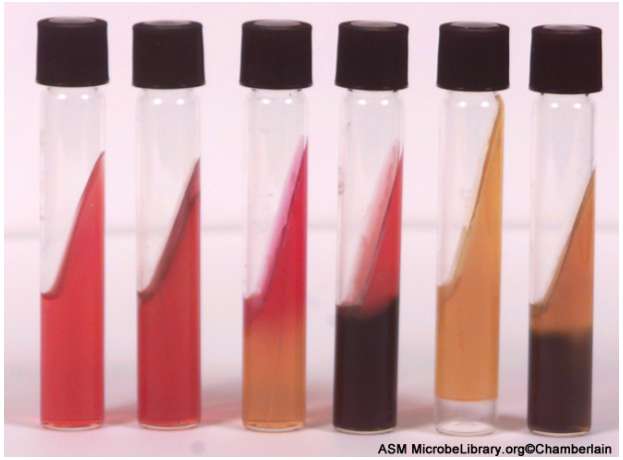
- Unrelated grant from Meridian Diagnostics
- I’m a microbiologist (not a mass spectrometrist)

Objectives

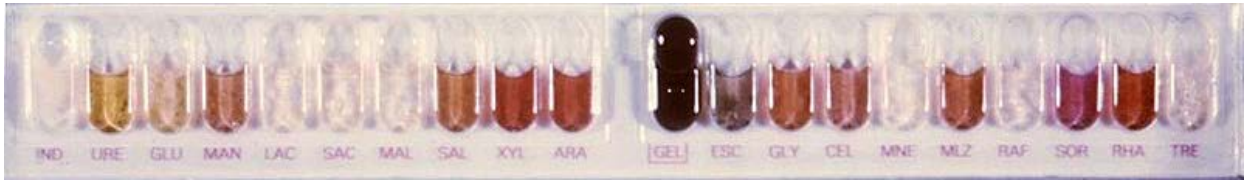
- Discuss relevant principles of mass spectrometry
- Review advantages and disadvantages of available platforms
- Discuss use of mass spectrometry in the clinical microbiology laboratory

Fermentation

- Beer, wine and bread existed among the earliest civilizations.
  - Beer = civilized
- Robert Koch – growth on solid media 1880s
  - Different substrates + indicator (pH) = fermentation-based identification system



Manual ID systems

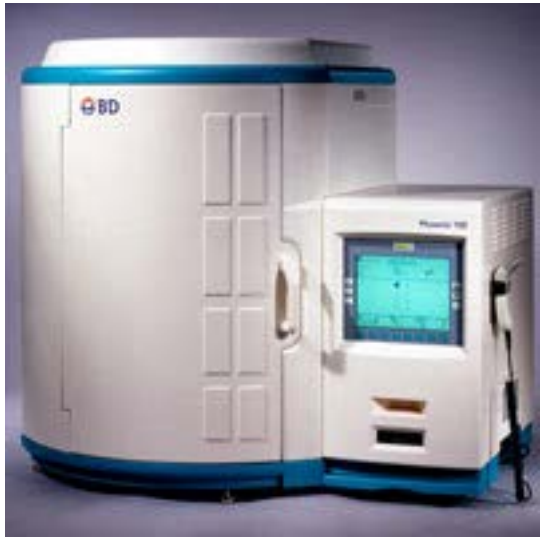


CDC/Dr. Gilda Jones

- An abnormal/weak/misread well can change ID

Automated ID systems

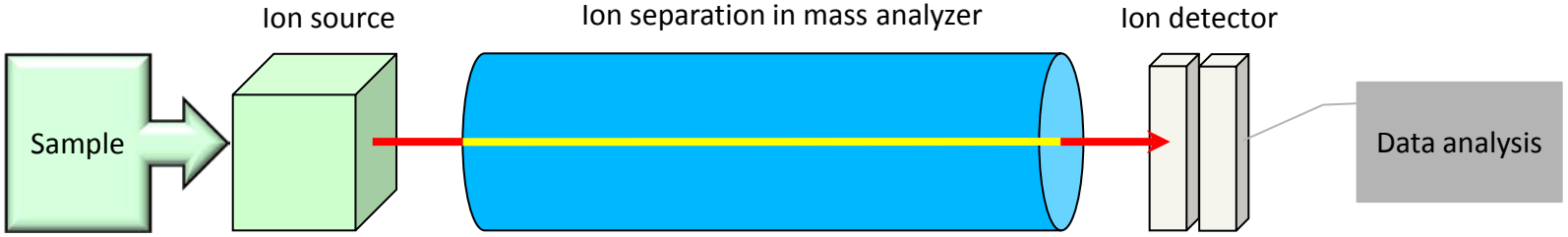
- State of the art fermentation

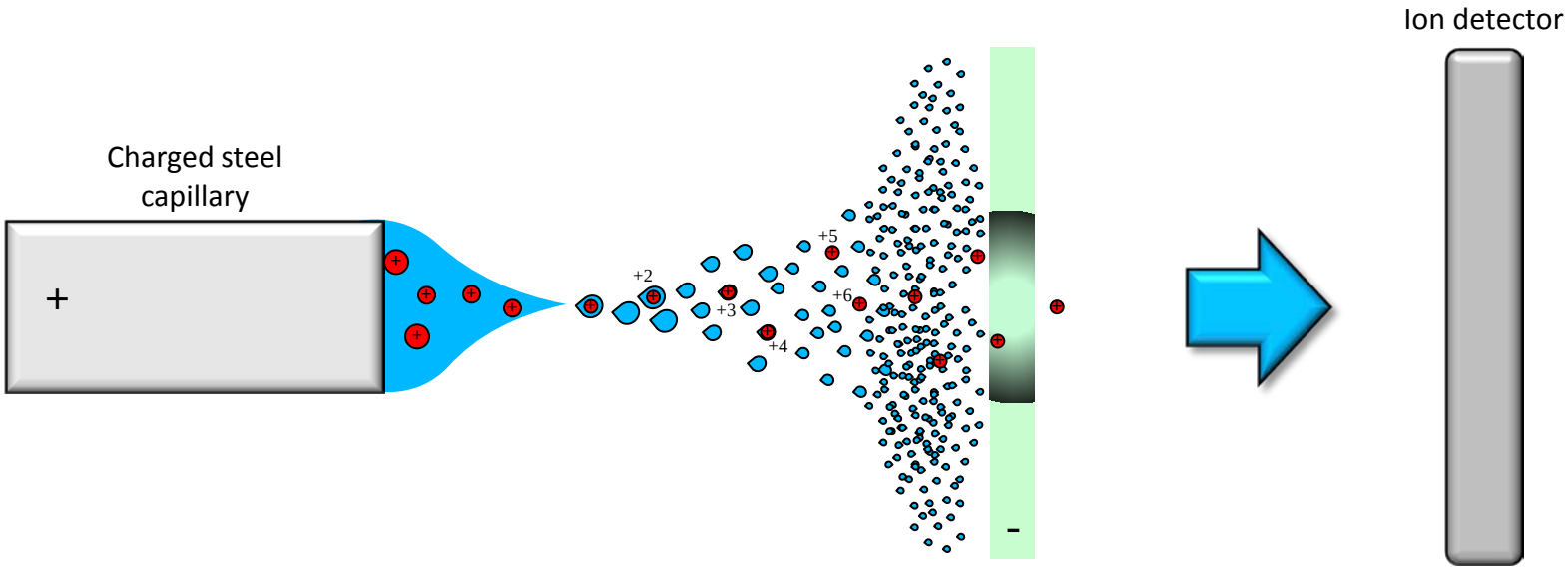


Mass spectrometry

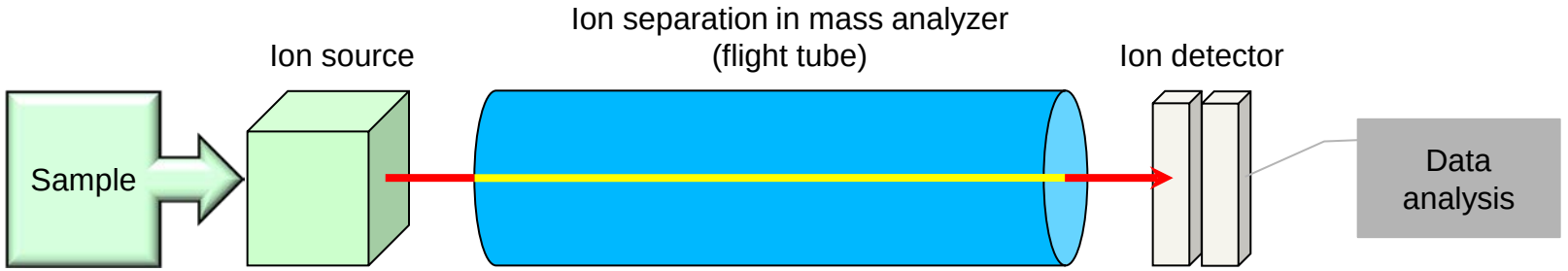
- Highly accurate method for measuring masses of ionized atoms/molecules
  - “smallest scales in the world”
- Developed around 1900
  - Often destructive ionization methods
- “soft” ionization methods → biological samples
  - Electrospray, 1968
  - MALDI, 1981
    - Matrix Assisted Laser Desorption-Ionization
- Bacterial analysis and identification, 1975/1994

Electrospray ionization

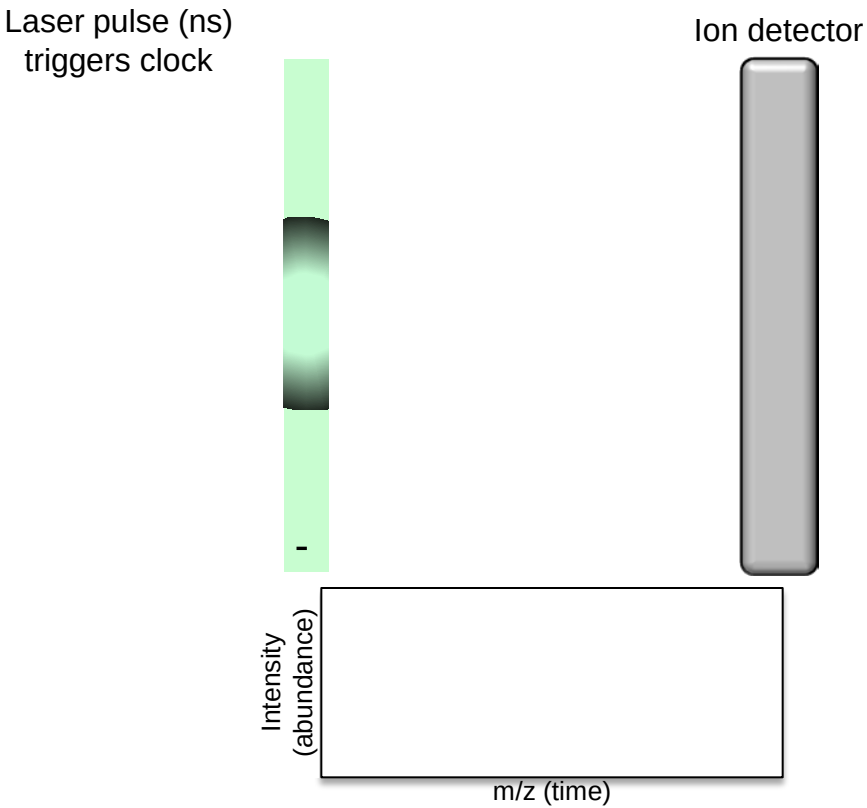




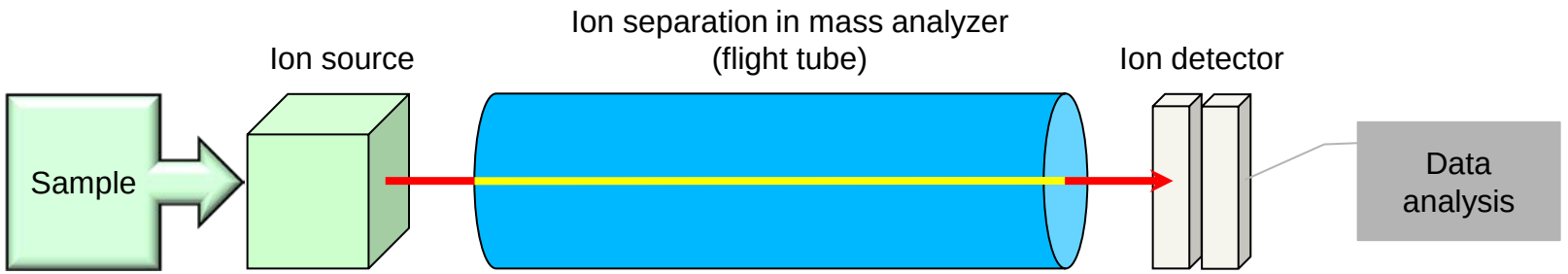
MALDI-TOF


Ion separation in mass analyzer
(flight tube)
Ion source
Ion detector
Sample
Data
analysis


Matrix +
analyte
+
α-cyano-hydroxy
cinnamic acid


Laser pulse (ns)
triggers clock
Ion detector
-
Intensity
(abundance)
m/z (time)

MALDI-TOF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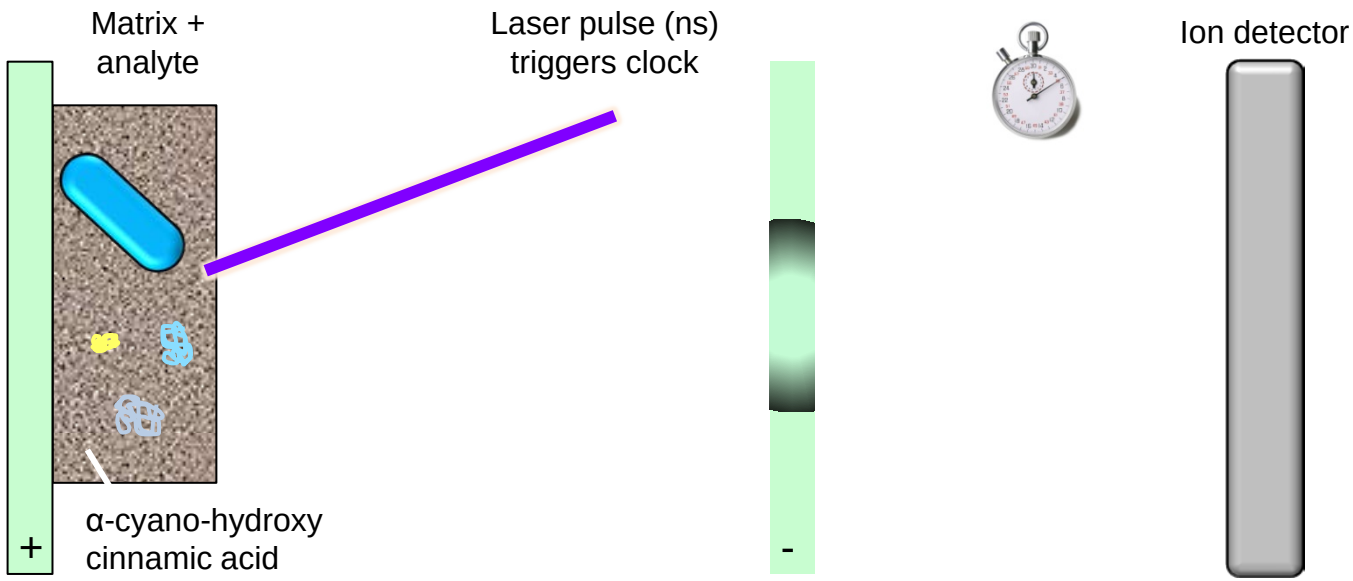
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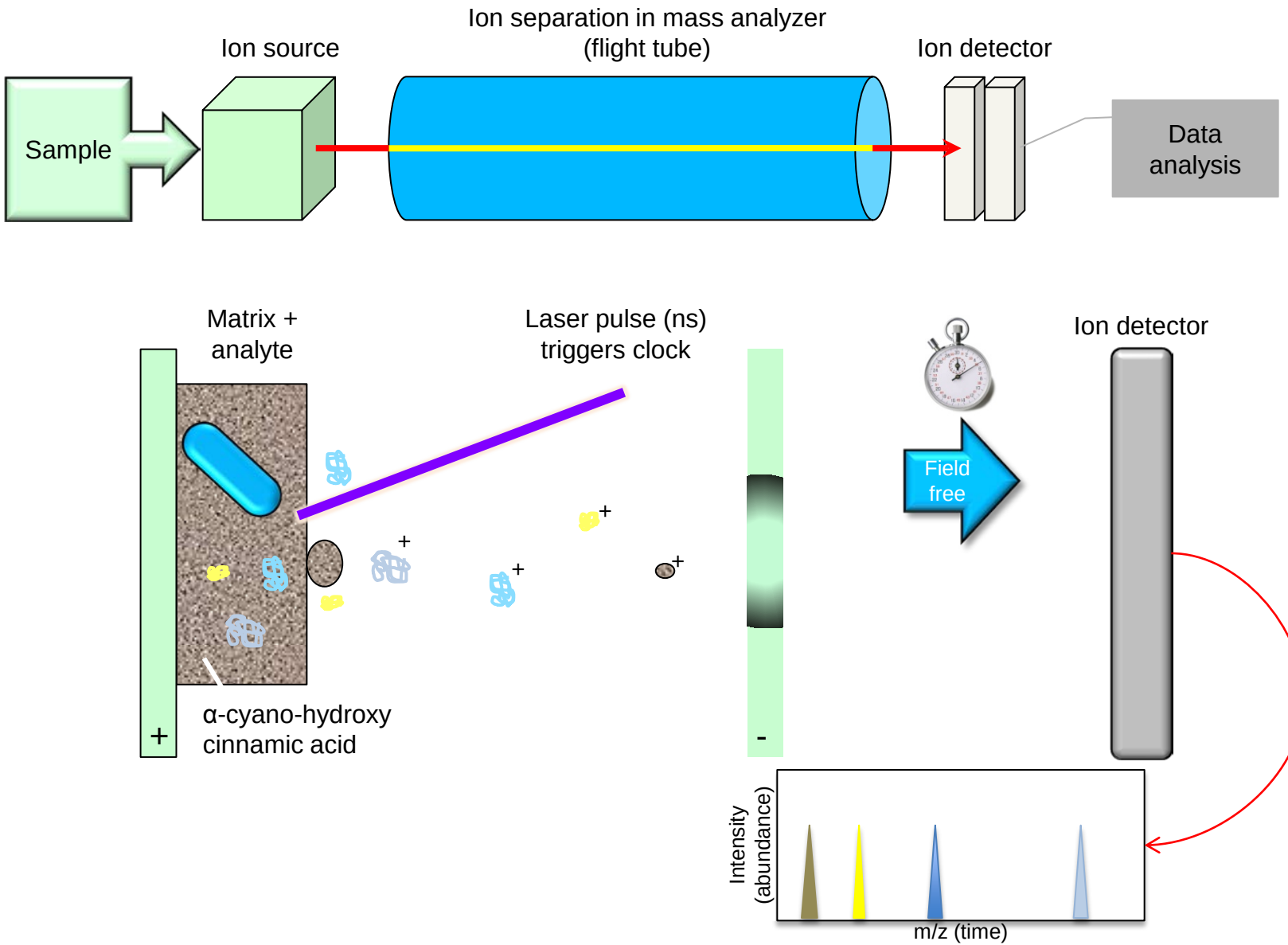
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Field
free
+
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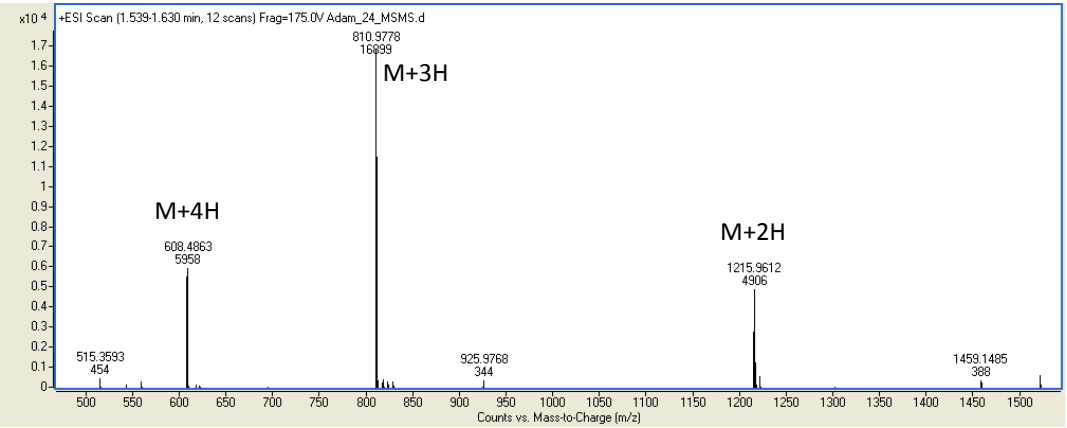
cinnamic acid
-
Intensity
(abundance)
m/z (time)

Electrospray

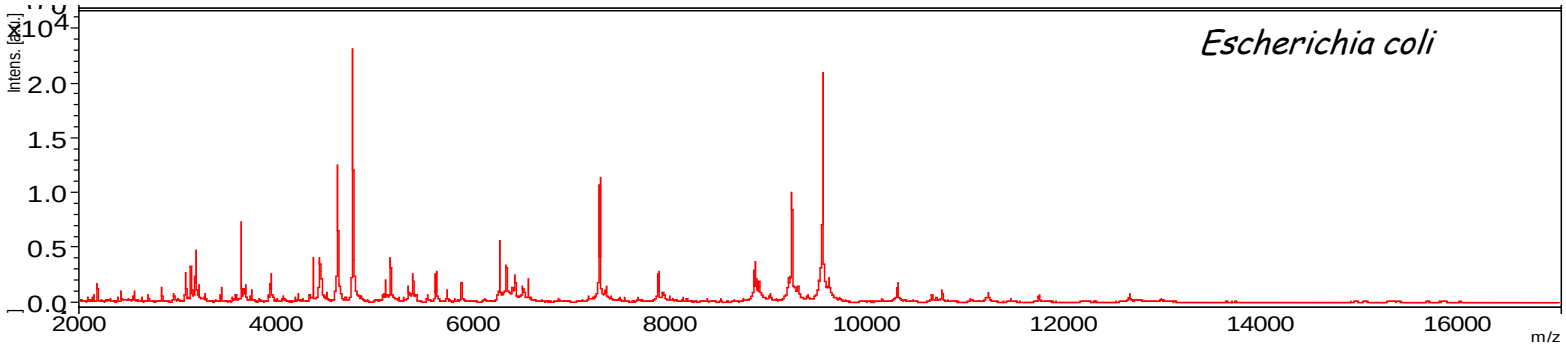
- Advantages
  - mass range up to ~70 kDa
  - femtomole to low picomole sensitivity
  - softest ionization
  - compatible with chromatography
  - multiple charges = high masses on low *m/z* instrument
  - no matrix interference → good for smaller molecules

- Disadvantages
  - salts and complex mixtures reduce sensitivity
  - multiple charges confusing in complex samples
  - requires high sample purity
  - potential run-to-run carryover



MALDI

- Advantages
  - mass range of up to ~300 kDa; ~2-20 kDa for bacterial ID
    - allows complex mixtures
  - sensitivity of femtomole to picomole; generally not a problem with bacterial ID
  - soft ionization = little fragmentation
  - tolerates salts (mM range)
- Disadvantages
  - matrix signal problematic for low MW compounds (≤~700 Da)
  - laser may degrade photosensitive molecules
  - organic acid matrix may degrade some compounds



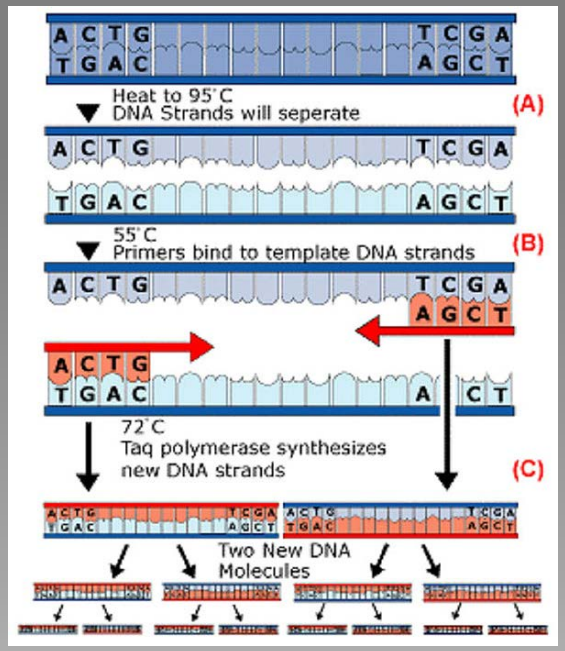
MS for Microbiology

- Assay formats:
- DNA
  - PCR/ESI – Ibis/Abbott
  - PCR/transcription/fragmentation/MALDI – Sequenom
  - Tagged PCR/APCI - Agilent
- Protein
  - Direct smear (whole cell)/extraction – Bruker, Shimadzu/Saramis (bioMerieux), Andromas

DNA – Ibis T5000/Abbott Plex-ID

Multiplex PCR → **cleanup** → **ESI-MS**

broad-range/specific | desalt, strand separation | base composition



www.noaa.gov

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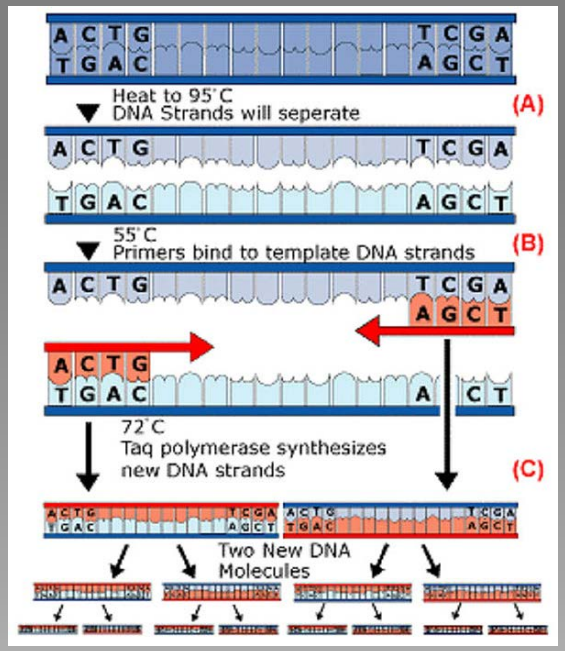

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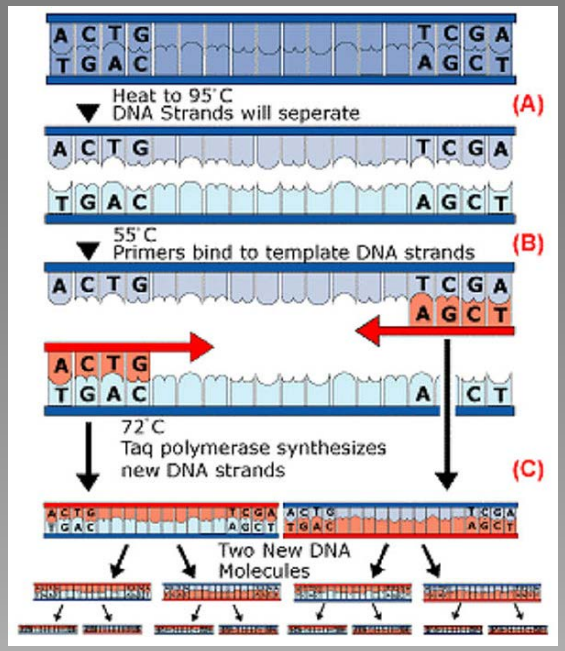

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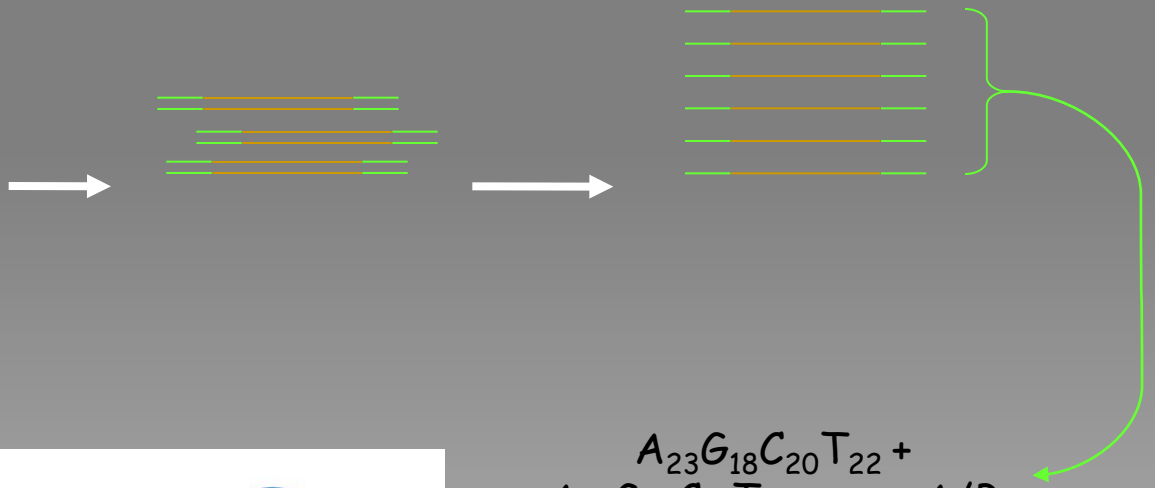

$A_{23}G_{18}C_{20}T_{22}$ +
$A_{22}G_{20}C_{18}T_{23}$ = org A/B

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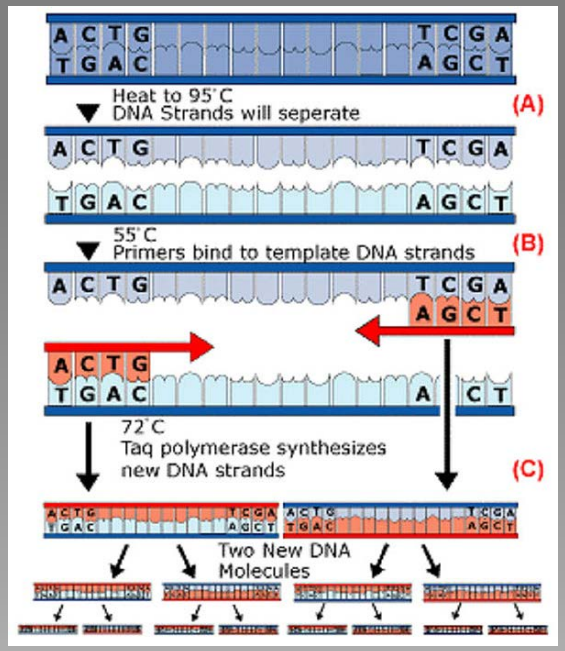

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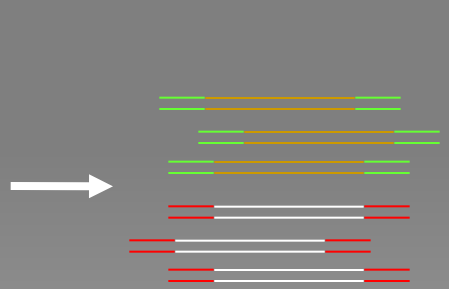

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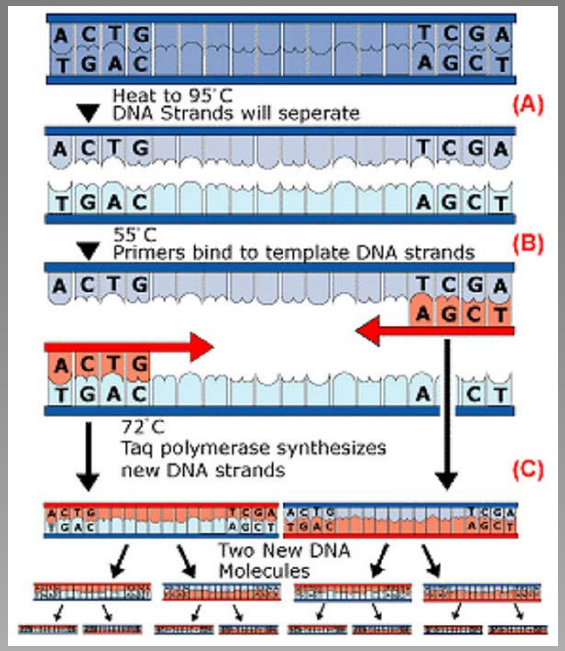

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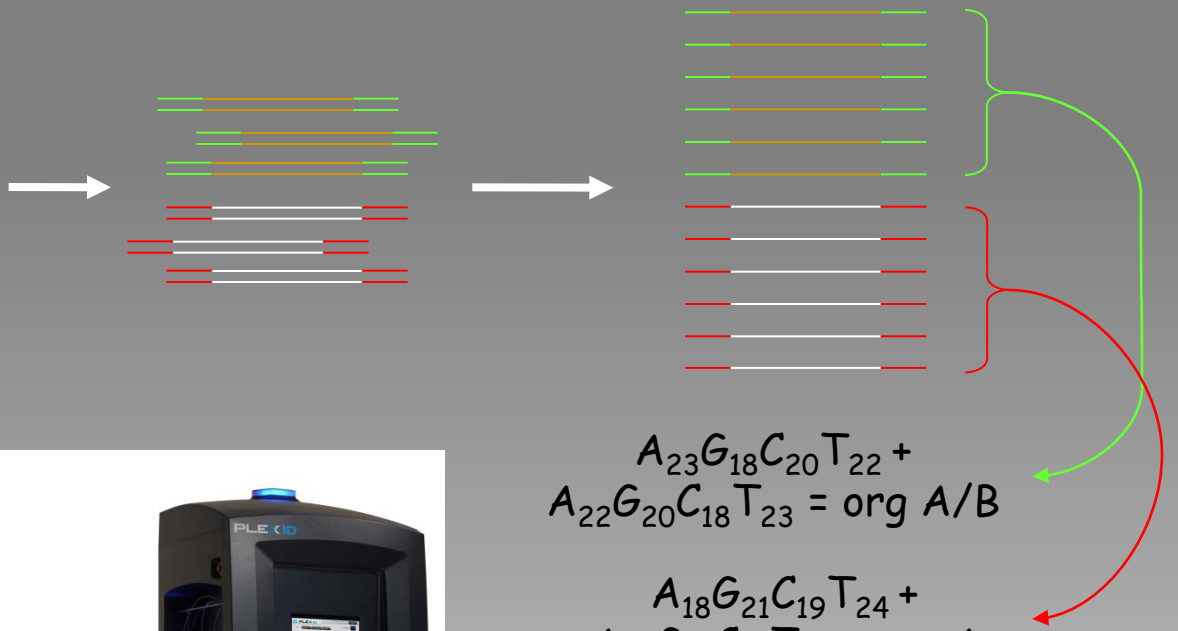

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$A_{18}G_{21}C_{19}T_{24}$ +
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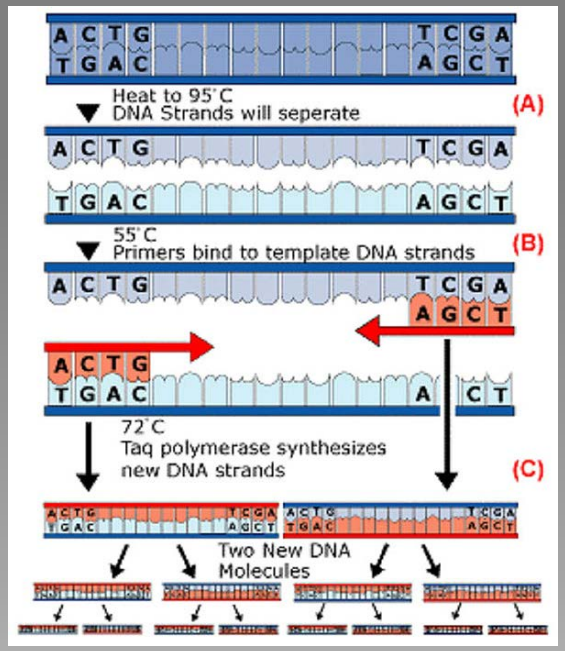

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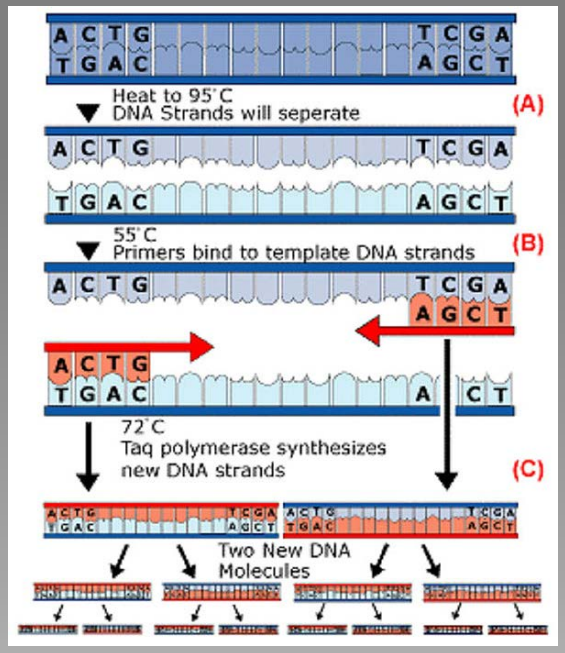
- Multiple broad-range PCRs allow ID by mass “triangulation”
- Direct from specimen
- Several hours

DNA – Sequenom iSeq

PCR → transcription → cleavage → MALDI

<800bp both orientations 4-30 base fragments base composition



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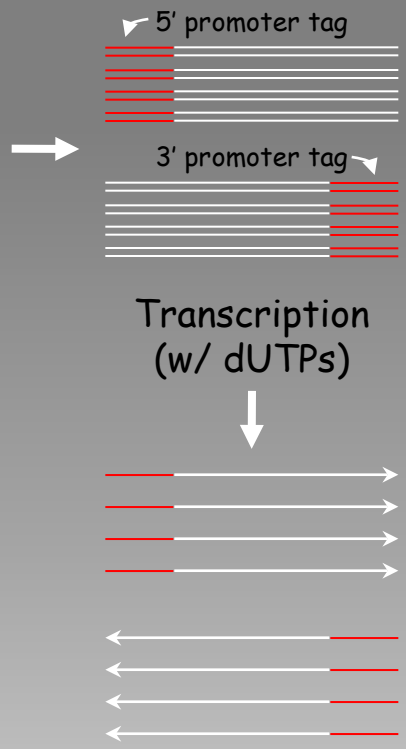

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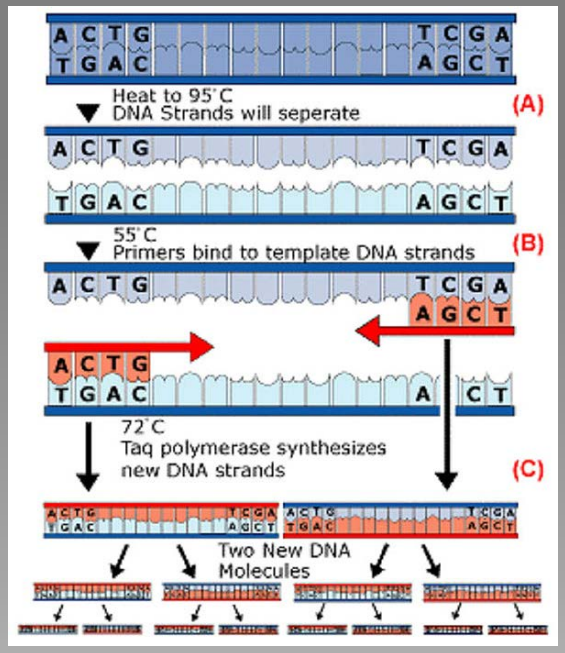

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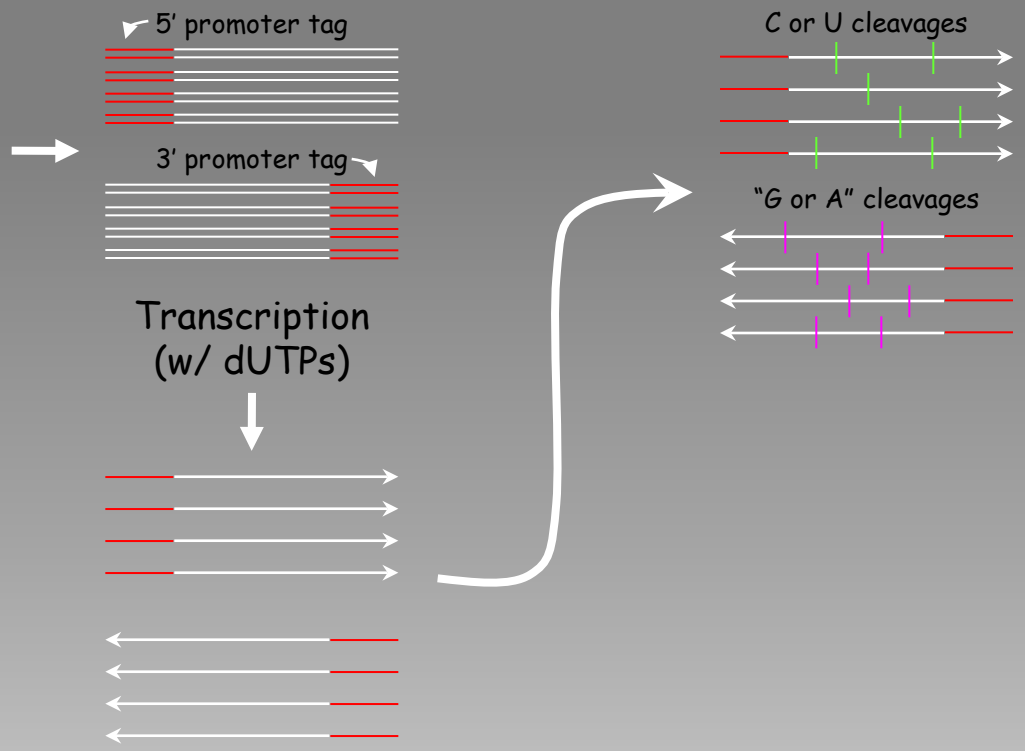

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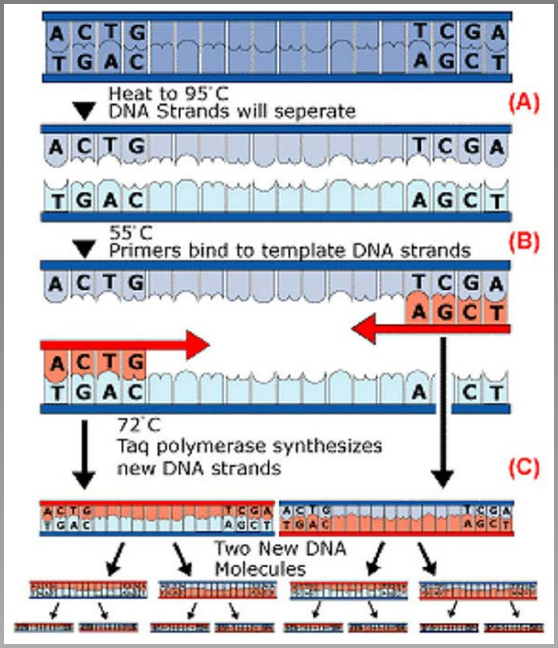

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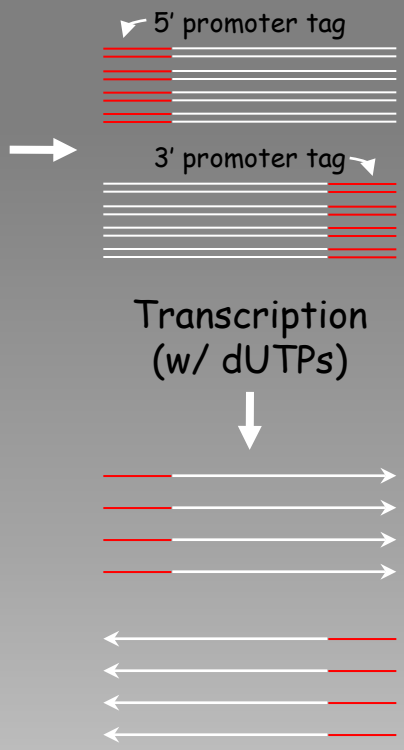
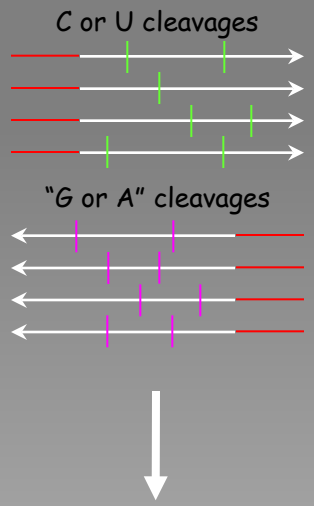




- Fragment mass/size analysis for all reactions to deduce sequence
- ~99% concordance with Sanger sequencing
- ~1 day process

DNA - MassTag PCR

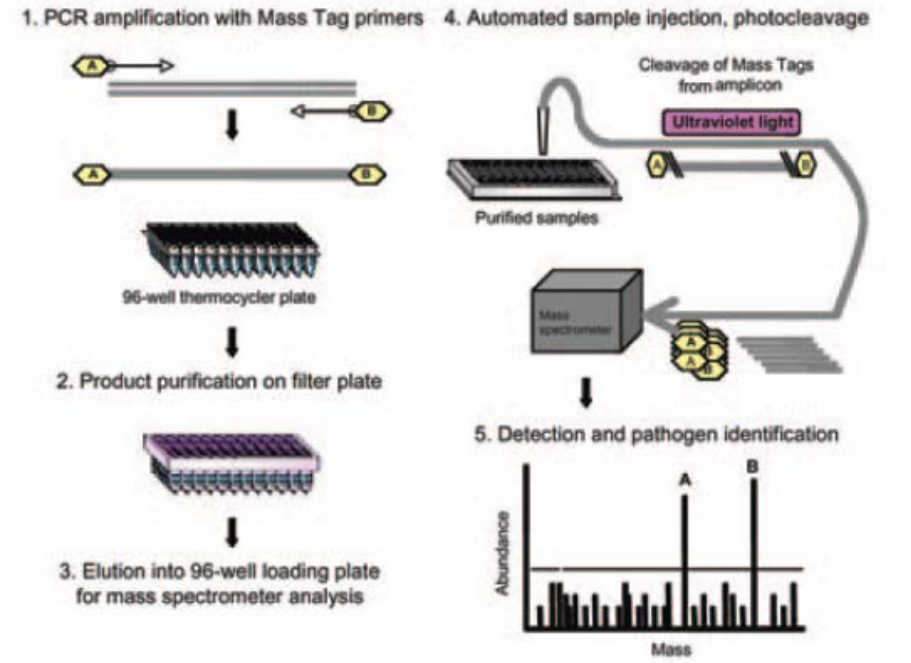


Figure 1. Schematic representation of Mass Tag polymerase chain reaction (PCR).

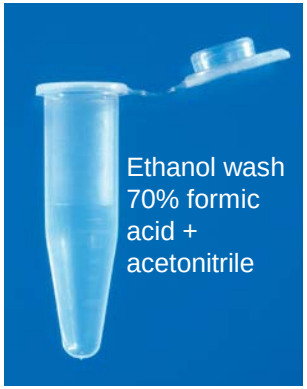
Briese, EID '05 11:310

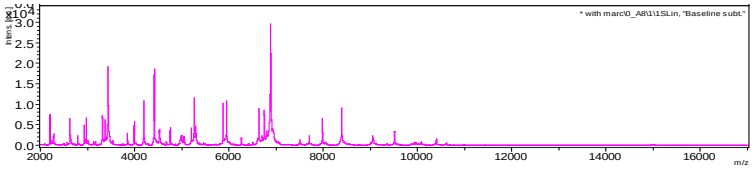
- Can be highly multiplexed (64 different tags)
- Direct from specimen
- Limited by the primers in each assay (ability to multiplex, range of organisms, sensitivity)

Protein – Bruker (or Shimadzu)

Culture → extract/smear → MALDI

actively growing | cytoplasmic proteins | pattern matching





- Analyze 2-20 kDa proteins
- Compare spectrum with database of >4600 organisms
- 1.5h / 96 isolates

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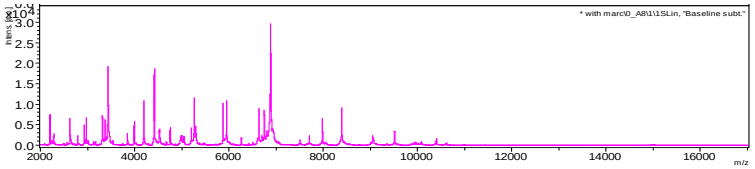

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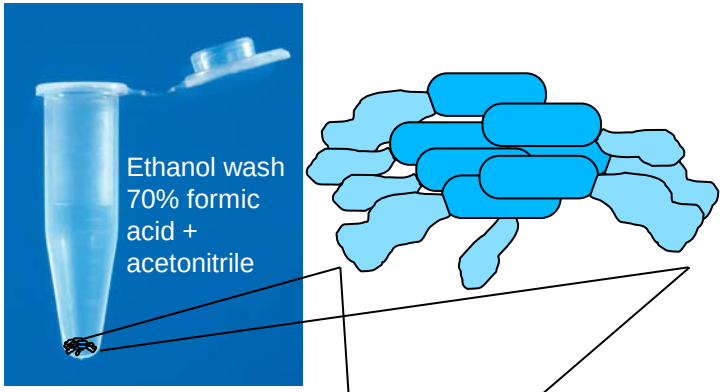

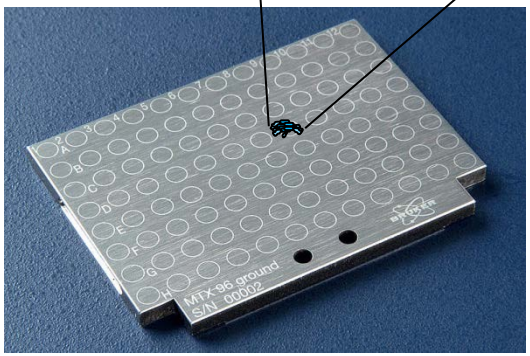

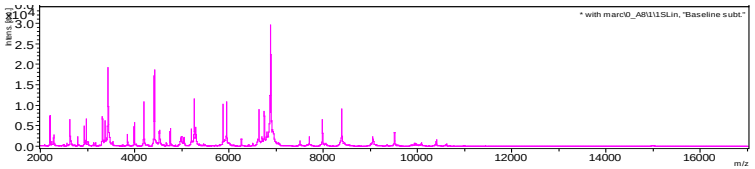

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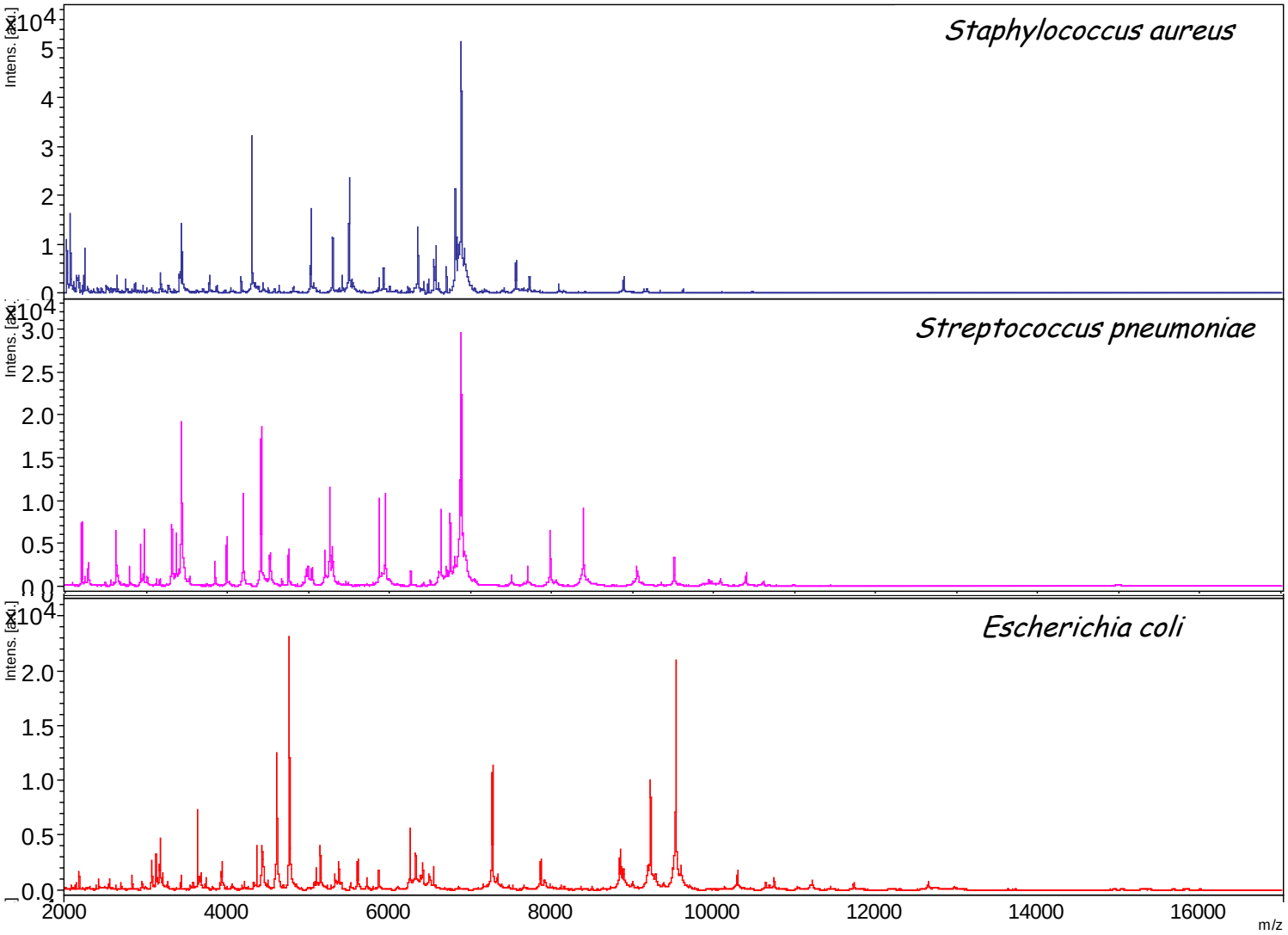

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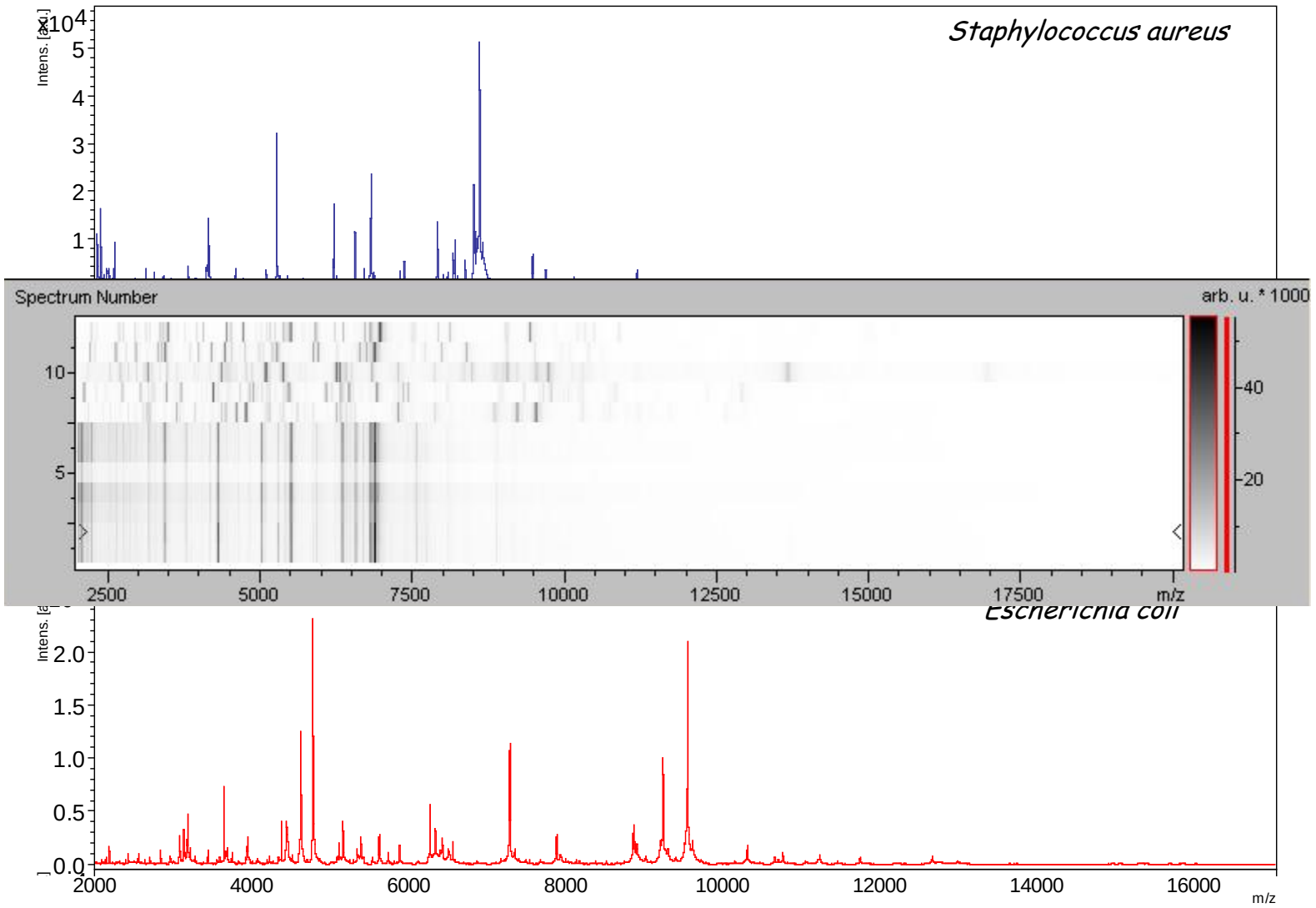
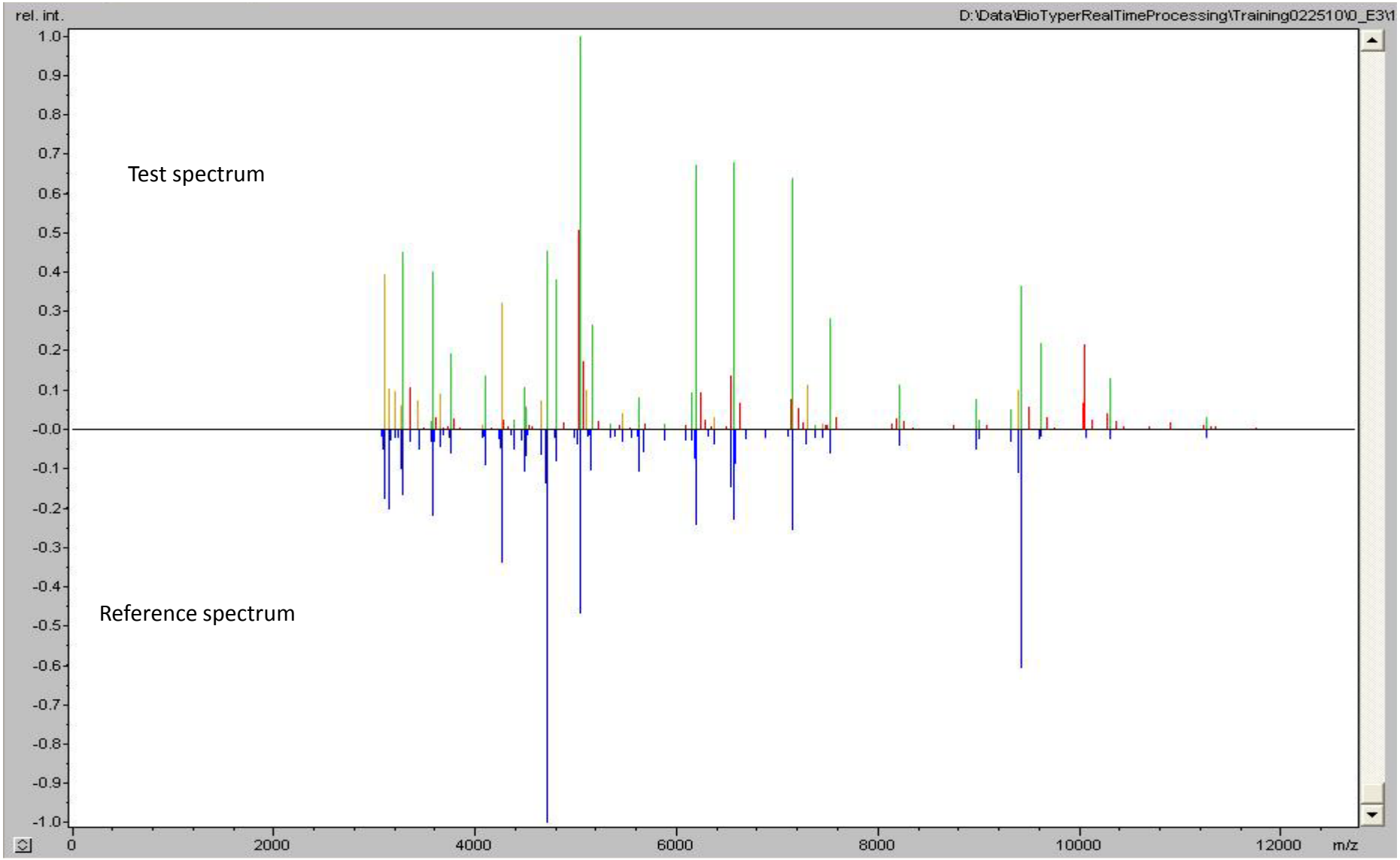


Spectrum matching



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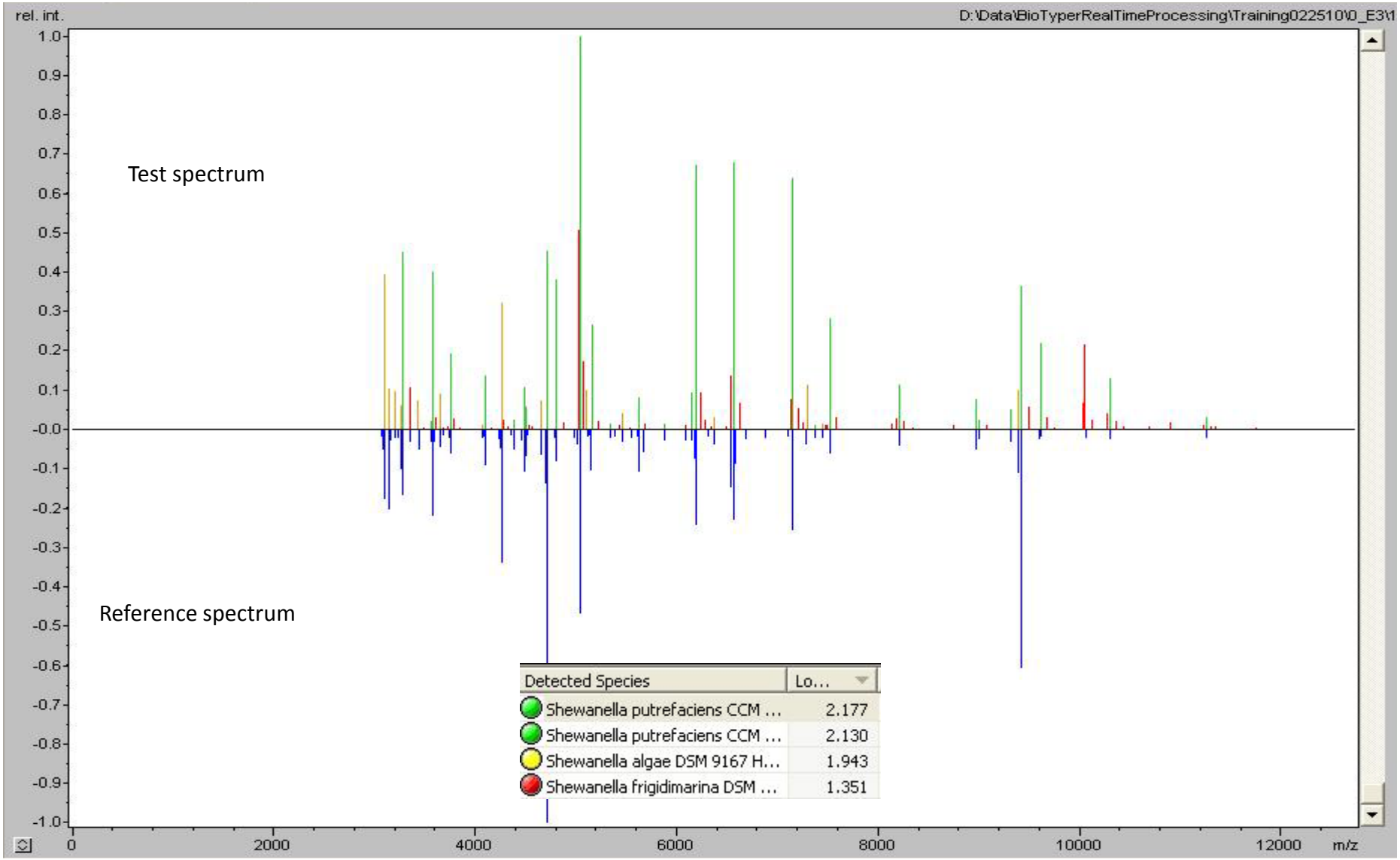

Results table

- Generated via automated analysis runs

Result Overview

AnalyteName	AnalyteID	Organism(best match)	ScoreValue	Organism(second best match)	ScoreValue
577 (++)	ID	Bordetella bronchiseptica	1.952	Bordetella bronchiseptica	1.904
444 (++)	ID	Lactobacillus rhamnosus	2.204	Lactobacillus rhamnosus	2.201
5 (+++)	ID	Providencia stuartii	2.4	Providencia stuartii	2.334
516 (-)	ID	not reliable identification	1.566	not reliable identification	1.494
59 (++)	ID	Achromobacter xylosoxidans	2.253	Achromobacter ruhlandii	2.123
BTS (++)	ID	Escherichia coli	2.249	Escherichia coli	2.227

Score	Description
1.900 ... 3.000	species identification
1.700 ... 1.899	genus identification
0.000 ... 1.699	not reliable identification

Detailed results

Rank(Quality)	Matched Pattern	ScoreValue	NCBIIdentifier
1(++)	Bordetella bronchiseptica CCM 6156 CCM	1.952	518
2(++)	Bordetella bronchiseptica CCM 6047 CCM	1.904	518
3(+)	Bordetella bronchiseptica CCM 6048 CCM	1.84	518
4(+)	Bordetella bronchiseptica CCM 6082T CCM	1.827	518
5(+)	Bordetella bronchiseptica DSM 10303 DSM	1.814	518
6(+)	Bordetella bronchiseptica CCM 6157 CCM	1.809	518
7(+)	Bordetella pertussis ATCC 8467 THL	1.735	520
8(+)	Bordetella parapertussis DSM 4922 DSM	1.72	519
9(+)	Bordetella bronchiseptica CCM 6195 CCM	1.705	518
10(-)	Bordetella pertussis DSM 4927 DSM	1.677	520

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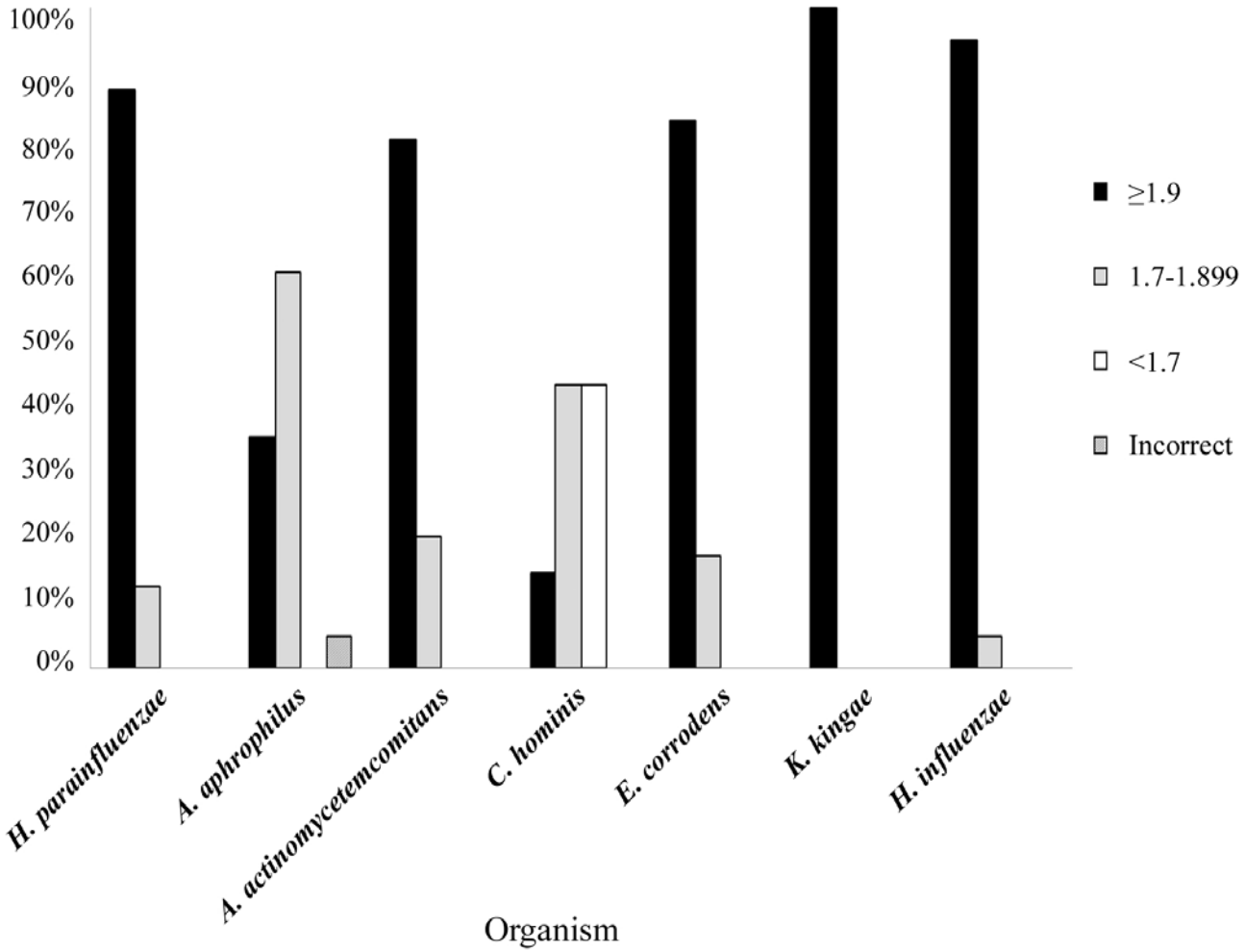
10(-)	Bordetella pertussis DSM 4927 DSM	1.677	520

Early evaluation: HACEK organisms

- *Haemophilus parainfluenzae, Aggregatibacter aphrophilus, A. actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae*
  - Fastidious GNRs
  - Cause endocarditis, head and neck infections, abscesses, septic arthritis
  - Most are difficult to definitively ID
    - 16S rRNA gene sequencing in our laboratory
- 103 HACEK and 20 *H. influenzae* isolates analyzed by 16S vs. MALDI

HACEK identification



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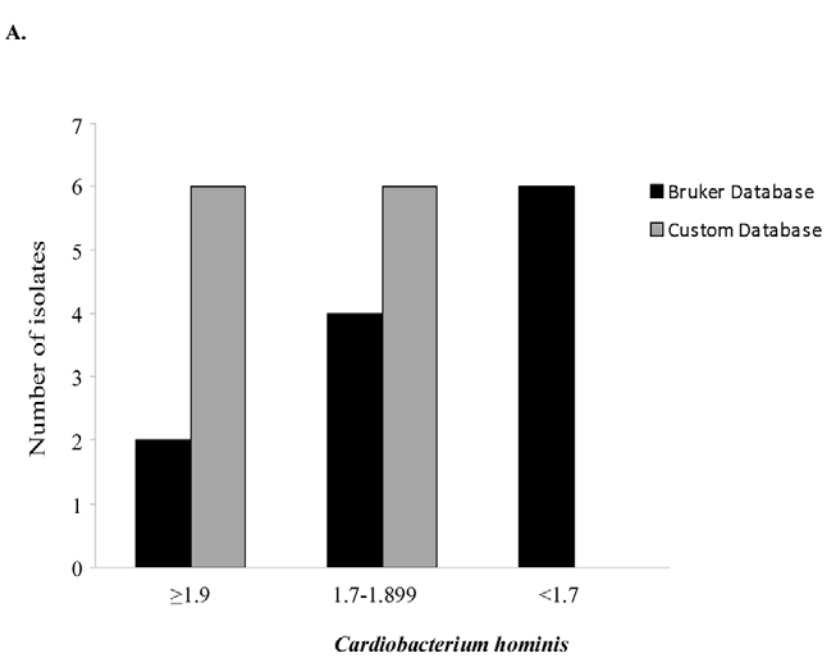
Customization of database

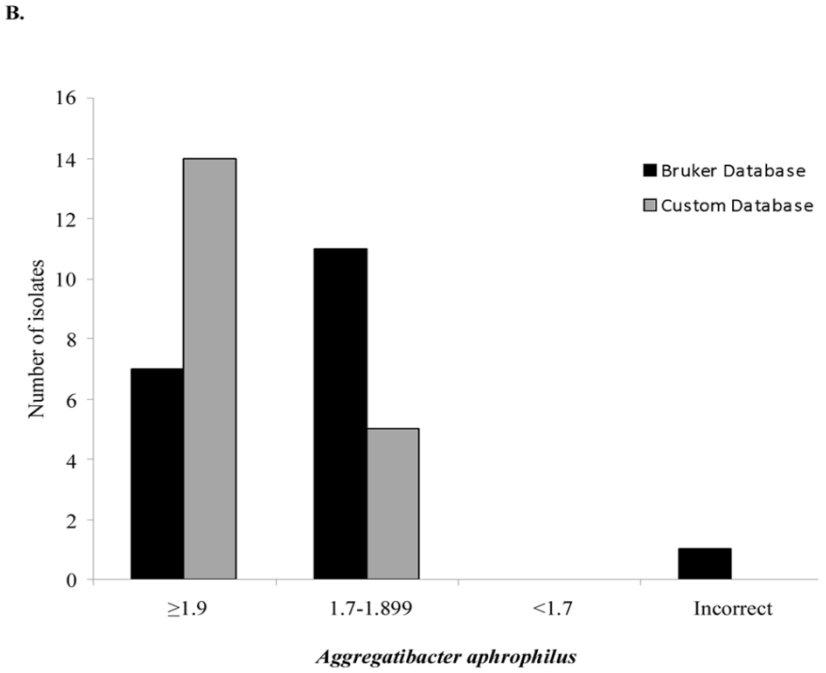
- Many isolates of *Cardiobacterium hominis* and *Aggregatibacter aphrophilus* had low scores
  - 50% *C. hominis* = no ID, 17% = species level
  - 58% *A. aphrophilus* = genus level, 37% = species level

  – Added one clinical isolate of each species to the database for each species
    - Not part of the study set
    - 3 replicate cultures, multiple spots, combine spectra
      – New MSPs (Main Spectra/Mean Spectral Pattern) added to DB

Customized database

- *C. hominis* and *A. aphrophilus* spectra reanalyzed
  - *C. hominis* : 1.759 → 1.920 (P = 0.0011), 50% to species
  - *A. aphrophilus* : 1.814 → 2.153 (P < 0.0001), 74% to species





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Score distribution

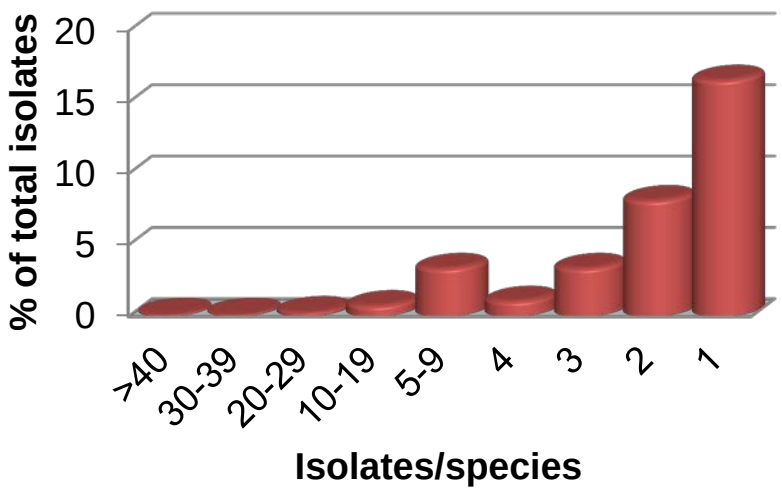
Score	Bruker DB	Customized DB
≥1.9	68 (66%)	81 (79%)
1.7-1.899	28 (27%)	22 (21%)
<1.7	6 (6%)	--
Incorrect ID	1 (1%)	--

- 103 isolates: 93% to genus with Bruker DB, 100% with customized DB

MALDI-MS Case

- *"Burkholderia cepacia"* for susceptibilities
  - Requested colistin → should be resistant
  - Isolate was susceptible
  - Client didn't want us to re-ID isolate
    - trusted their Vitek result
  - Microbiology fellow extracted cells, analyzed by MALDI-TOF → *P. aeruginosa*, high score
    - <10 min
  - Client lab director: "please re-ID for us…"

Expanded validation



- Broad analysis: 690 isolates (578 to species)
  - 225 unique species, 102 unique genera
- Compared MALDI against multiple methods
  - 16S rRNA gene sequencing (n =388)
  - automated biochemicals (BD Phoenix, n =179)
  - rapid/other biochemicals (n =123)
- Tested isolates from routine (rapid biochem, Phoenix), anaerobe (biochem, 16S seq), and difficult/reference (16S seq) benches

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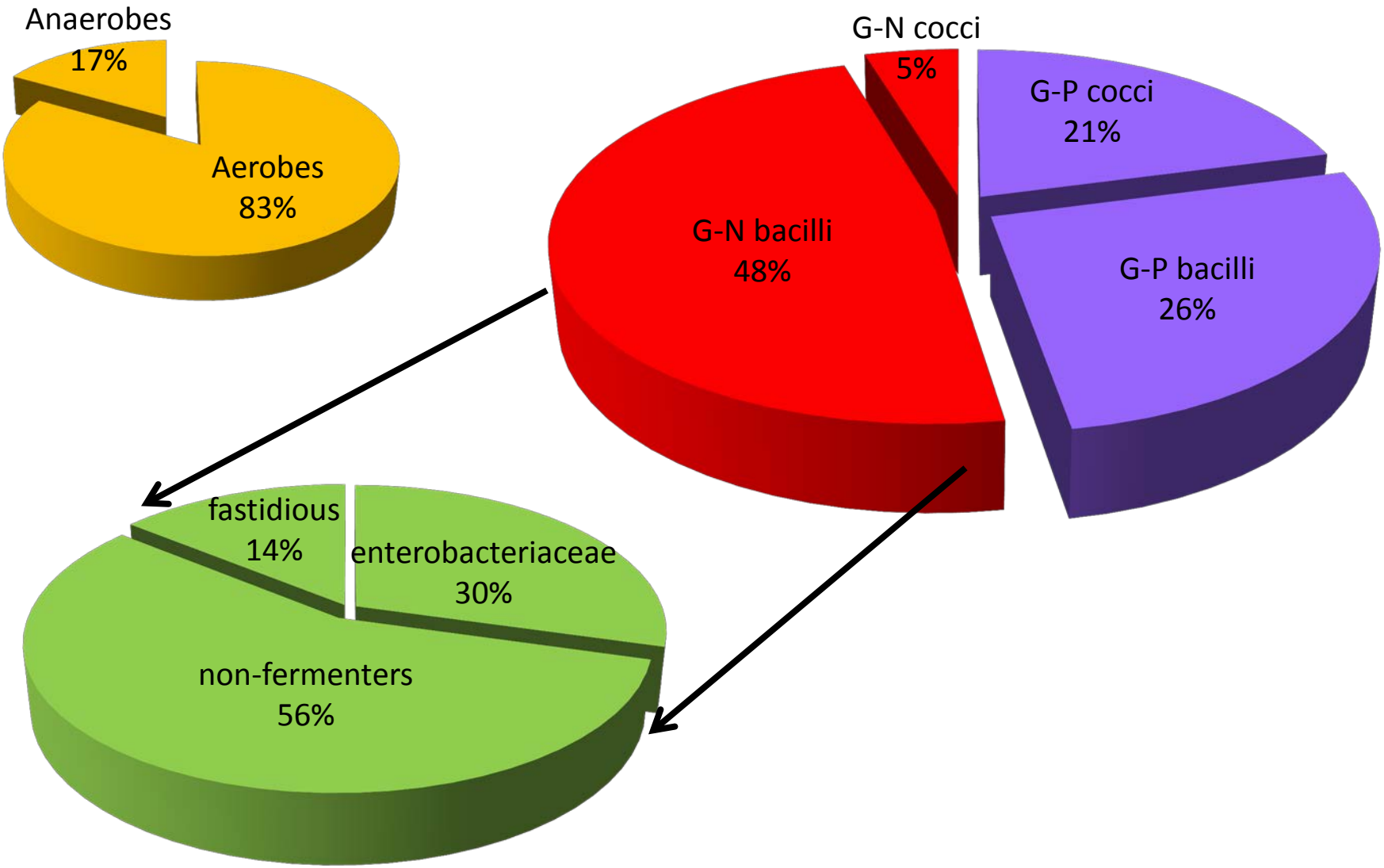
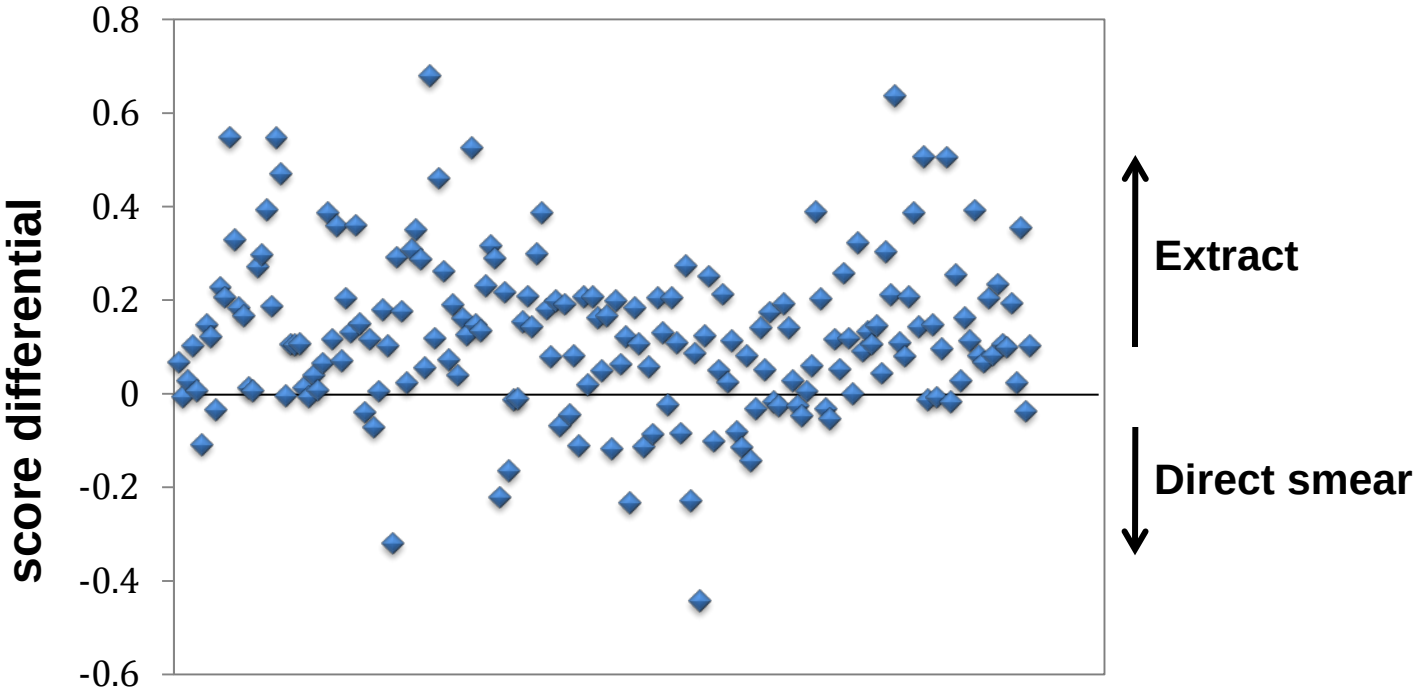
Distribution of species



Extract vs. direct smear



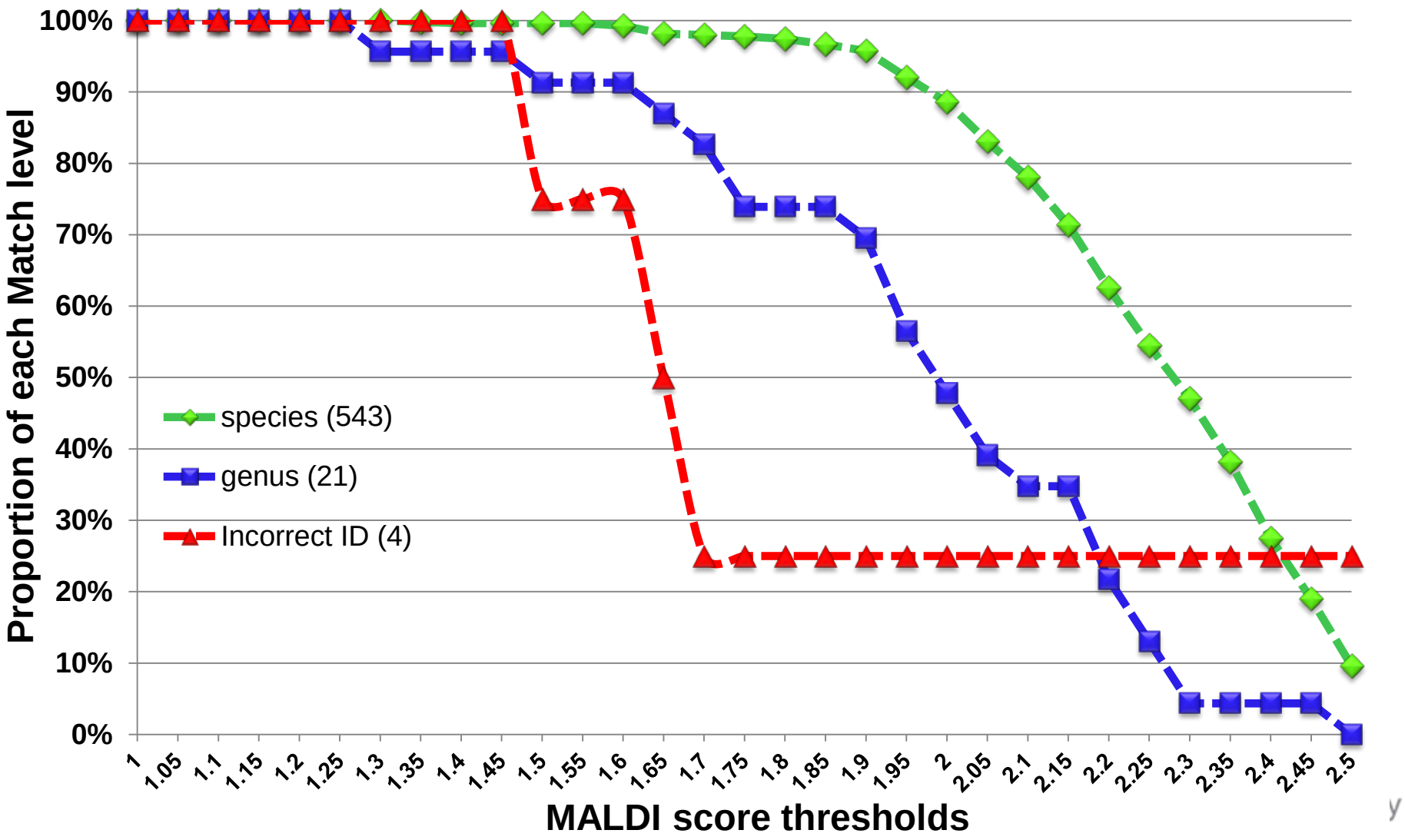
Average MALDI scores (n=184)

Extract	DS
2.187	2.061

p<.0001

146 unique species, 72 unique genera

Score threshold analysis



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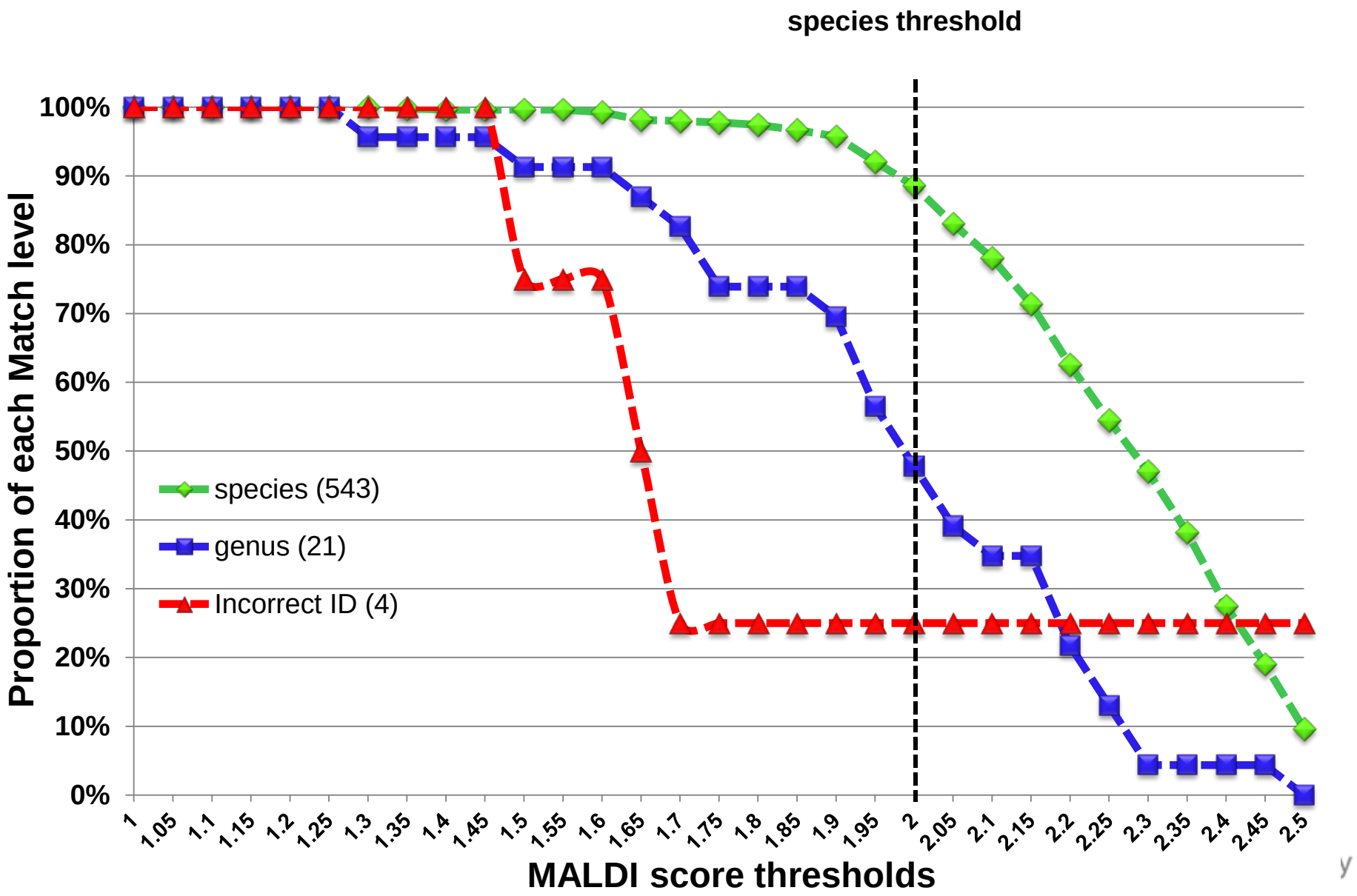
Score threshold analysis



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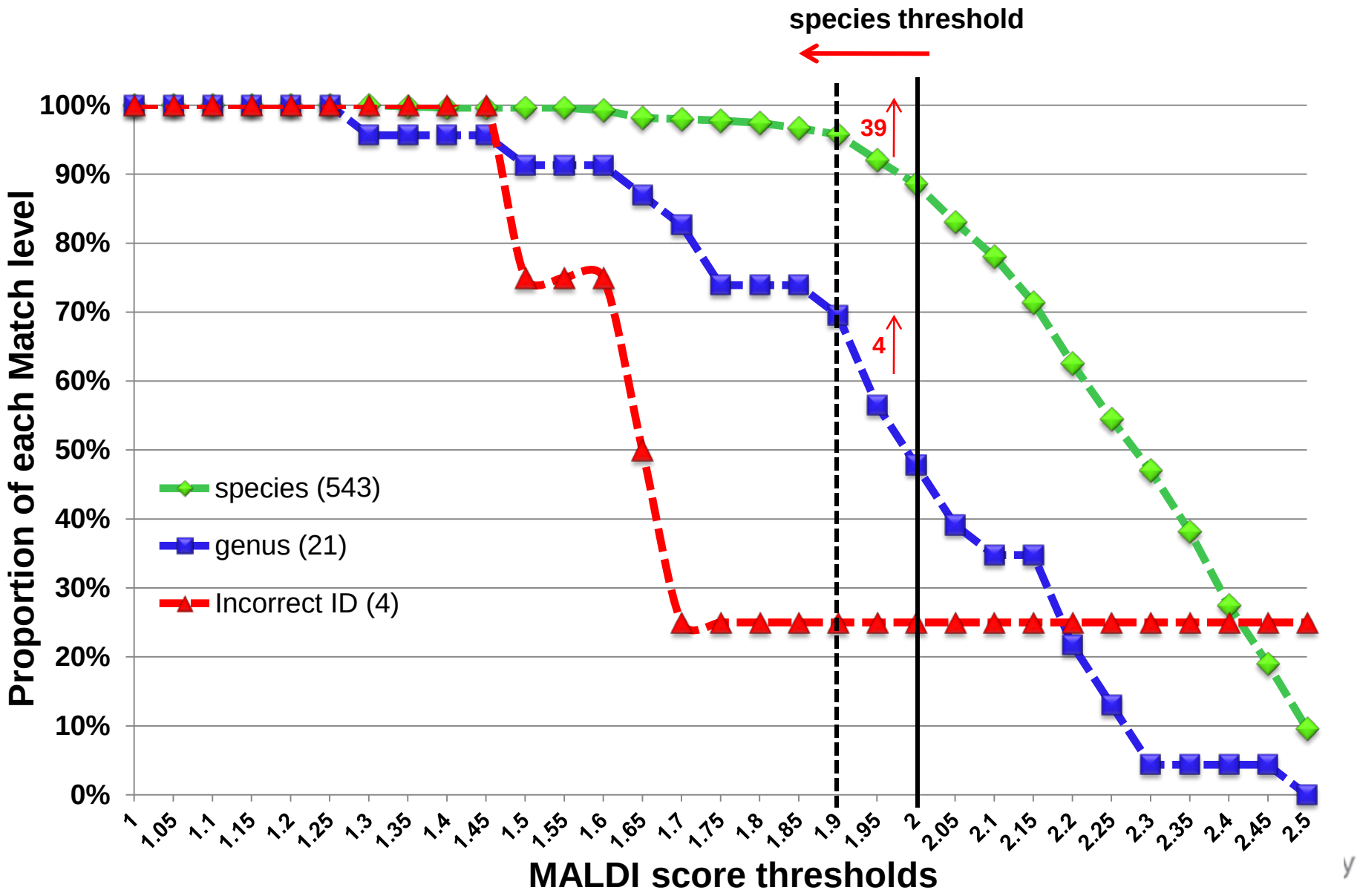
Score threshold analysis



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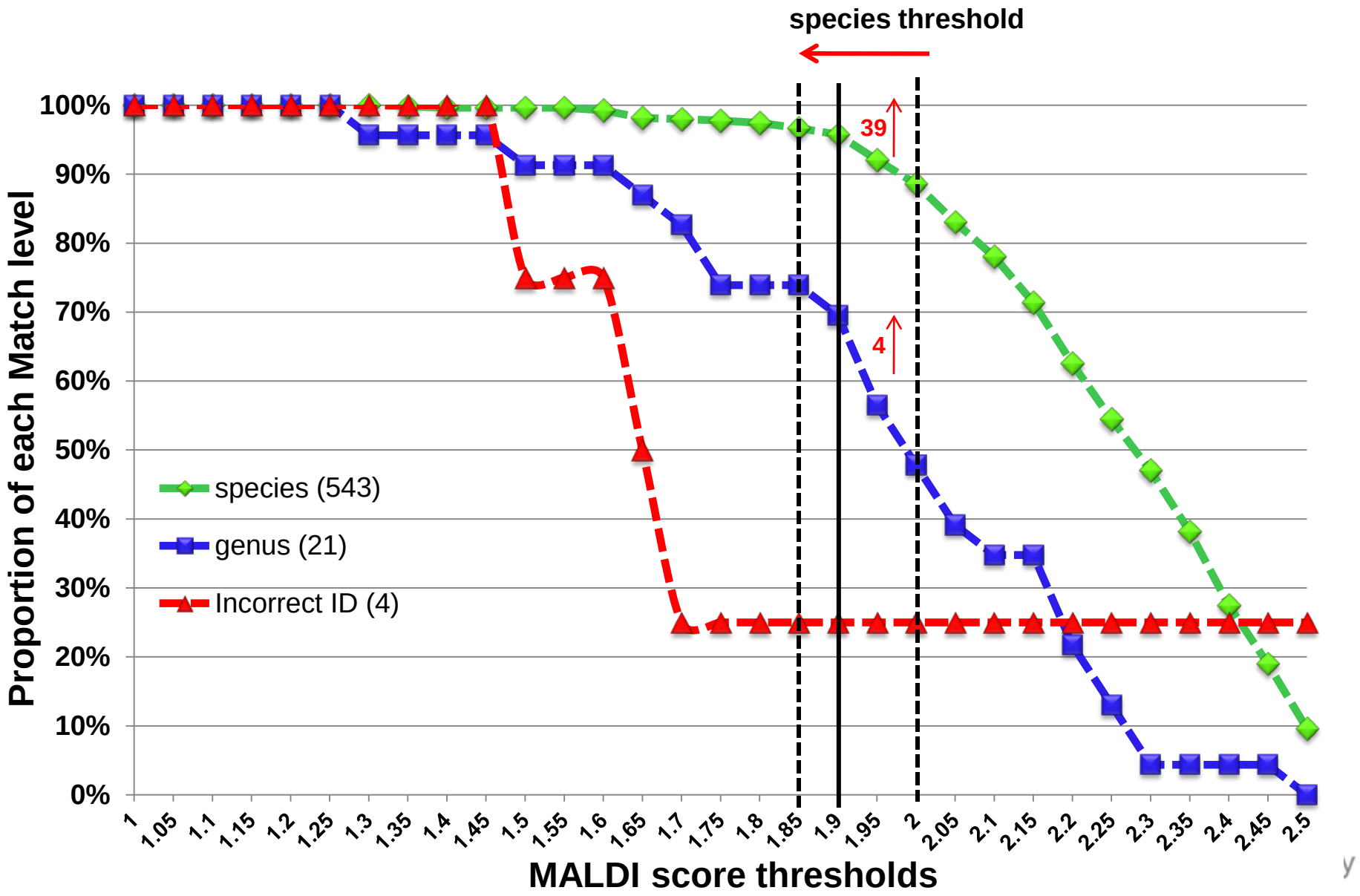

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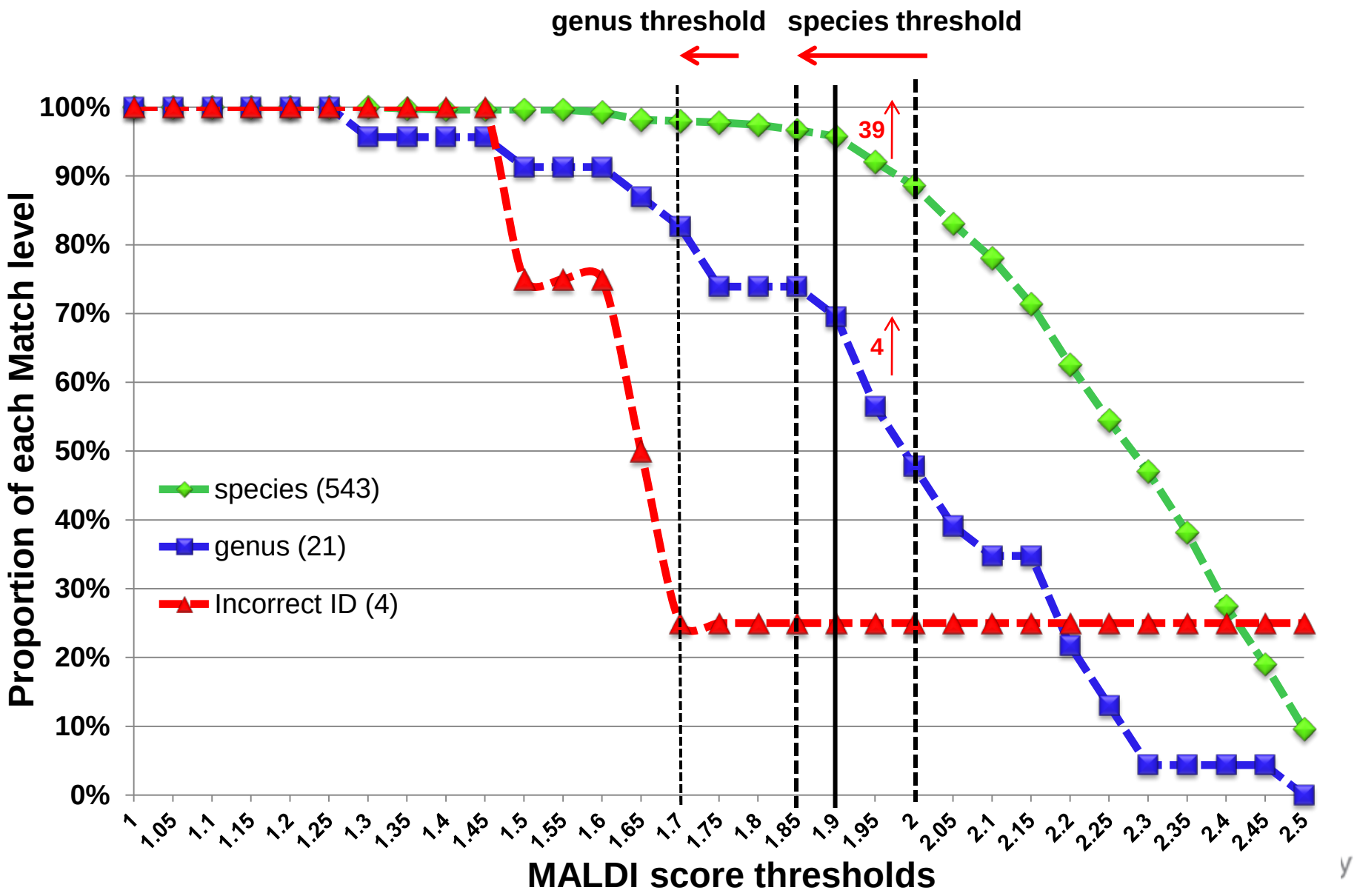

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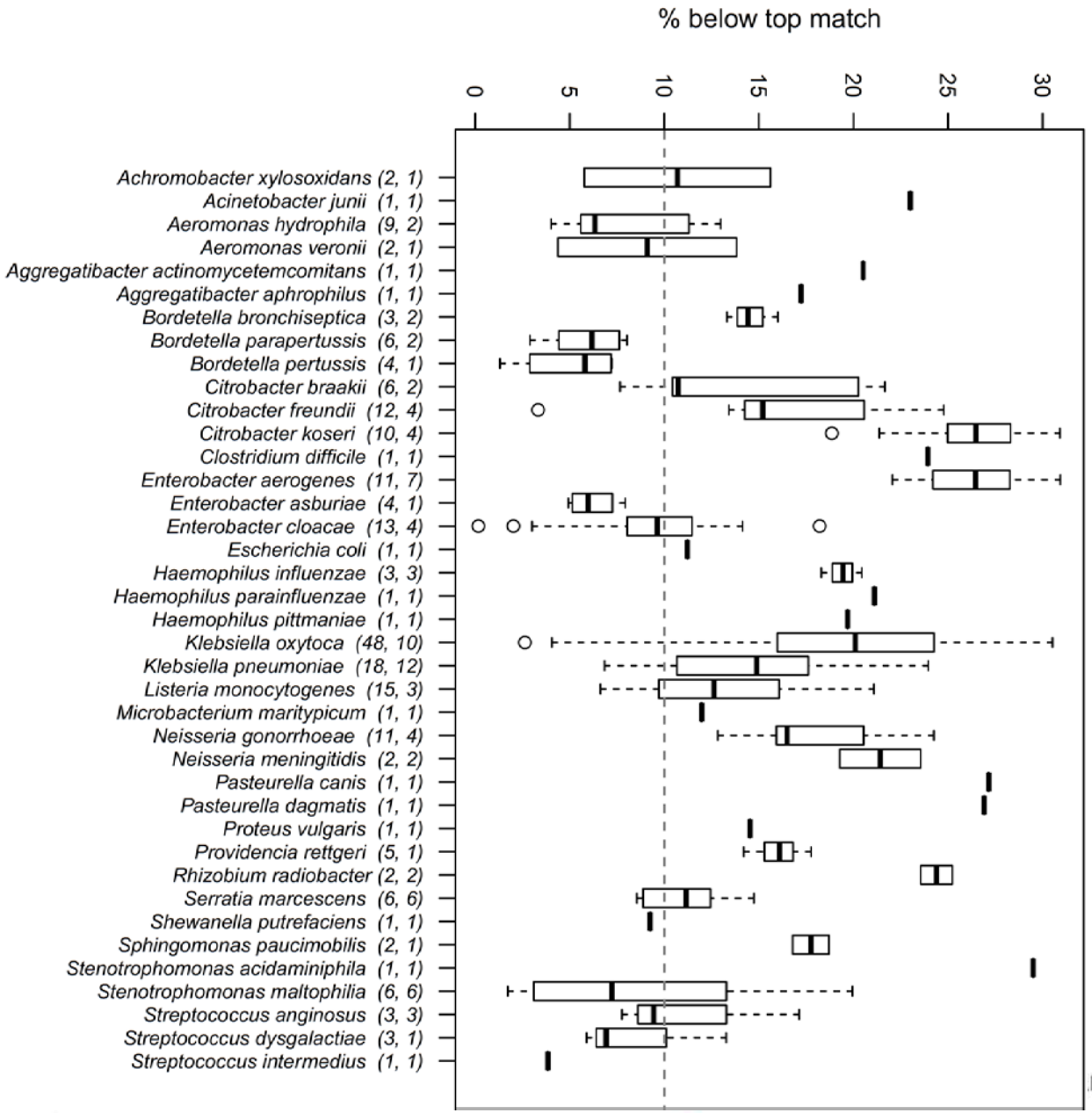
Validation summary

Category	% w/ scoring	% w/o scoring
species	90%	95%
genus	5%	4%
incorrect	1%	1%
no ID	4%	

- Very good considering the scope of organisms evaluated
  - 620 isolates ID'd to species, gp or cplx by std methods
    - 9 isolates not in Biotyper DB
  - Difficulties with actinomycetes, non-fermenters, anaerobes
  - Known issues with *S. pneumoniae/mitis* gp., *E. coli-Shigella, S. maltophilia*
- Since going live (~1yr), validated ~200 new species (~50 new genera)

Tough bugs…



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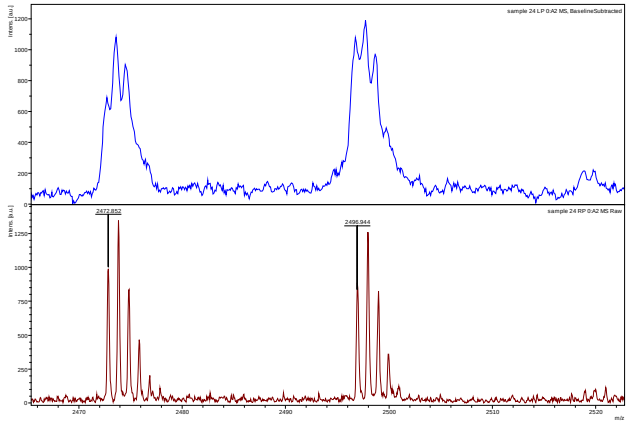
MS advantages/disadvantages

- Ibis/Abbott - DNA
  - Direct from sample, triangulation
  - Cost, design of new assays, not sequence data
- Sequenom - DNA
  - Good quality sequence, direct from sample
  - Complex sample prep, no kit for bacterial ID
- MassTag – DNA
  - Ability to multiplex, direct from sample, less expensive MS
  - Little data on commercial system, optimization, sensitivity
- Bruker/Shimadzu - Protein
  - No/rapid sample prep, some direct applications, cost
  - Typically requires culture, closely related organisms can cause problems (*S. mitis/S. pneumoniae*)

Microbiology mass spec summary

- High sensitivity and accuracy, low reagent cost
- Electrospray (Ibis/Abbott) vs. MALDI (Sequenom, Bruker, Shimadzu)
  - No salt vs. salt
  - Lower mass vs. higher mass
    - Minor differences for complex mixtures
- DNA (Ibis/Abbott, Sequenom) vs. Protein (Bruker)
  - $$$ vs. $
  - PCR + cleanup/processing vs. culture
  - Hours vs. minutes from starting material
  - Gene present vs. gene expressed



MALDI-MS Case #2

- Positive blood culture
  - GPC-clusters by Gram stain
  - Yellow catalase + bacitracin-sensitive GPC-clusters
    - Next day: wet/mucoid colonies in bacitracin zone
    - GN coccoid cells, weak catalase +, oxidase +
      - Sent for 16S sequencing
      - MALDI on rounds → direct smear, add matrix, fire

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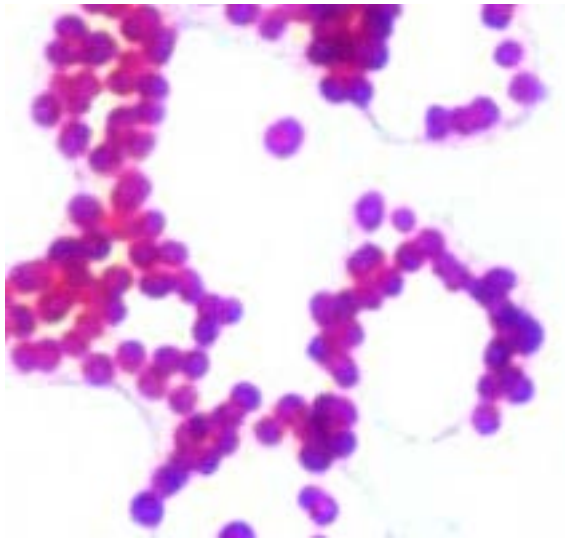
    - Next day: wet/mucoid colonies in bacitracin zone
    - GN coccoid cells, weak catalase +, oxidase +
      - Sent for 16S sequencing
      - MALDI on rounds → direct smear, add matrix, fire
    - Good score for *Paracoccus yeei*
      - "donuts" on Gram stain
      - Confirmed by sequencing (24h later)



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