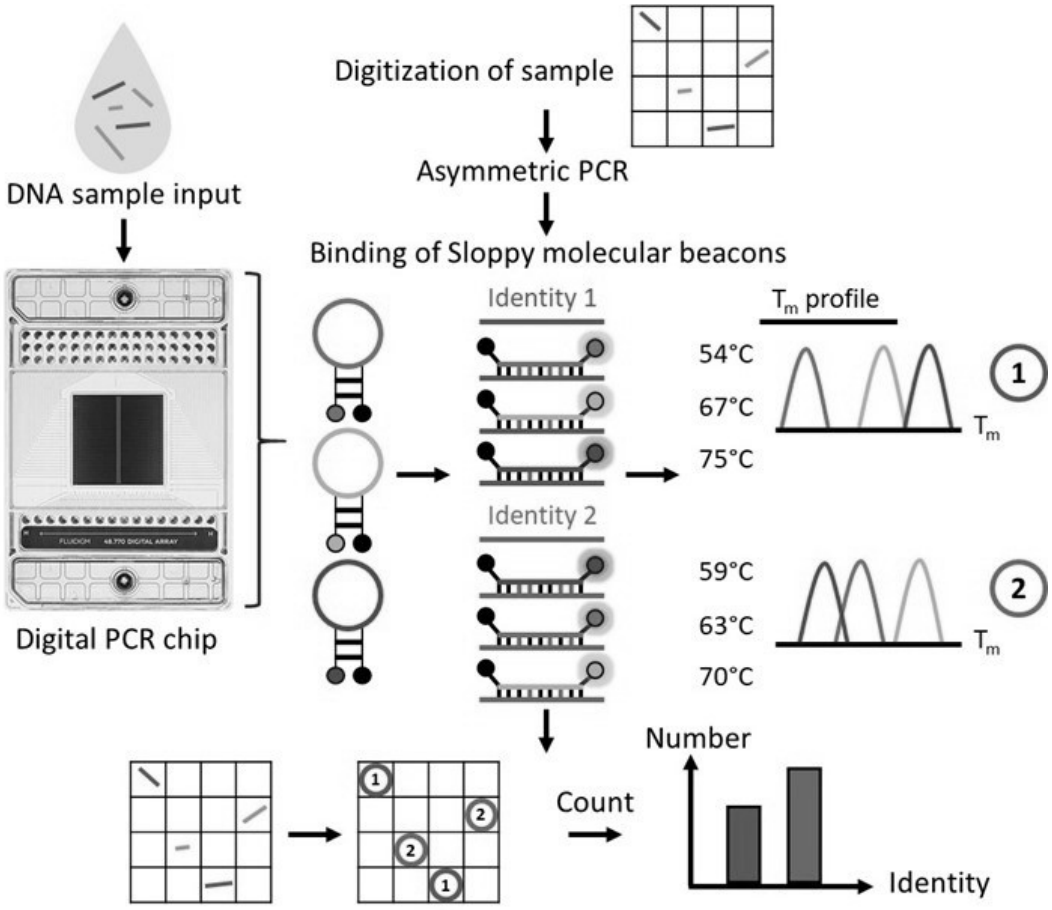

Digitization of sample
Asymmetric PCR
DNA sample input
Binding of Sloppy molecular beacons
Identity 1
T_m profile
54°C
67°C
75°C
1
T_m
Digital PCR chip
Identity 2
59°C
63°C
70°C
2
T_m
Number
Count
Identity

Fig. 1

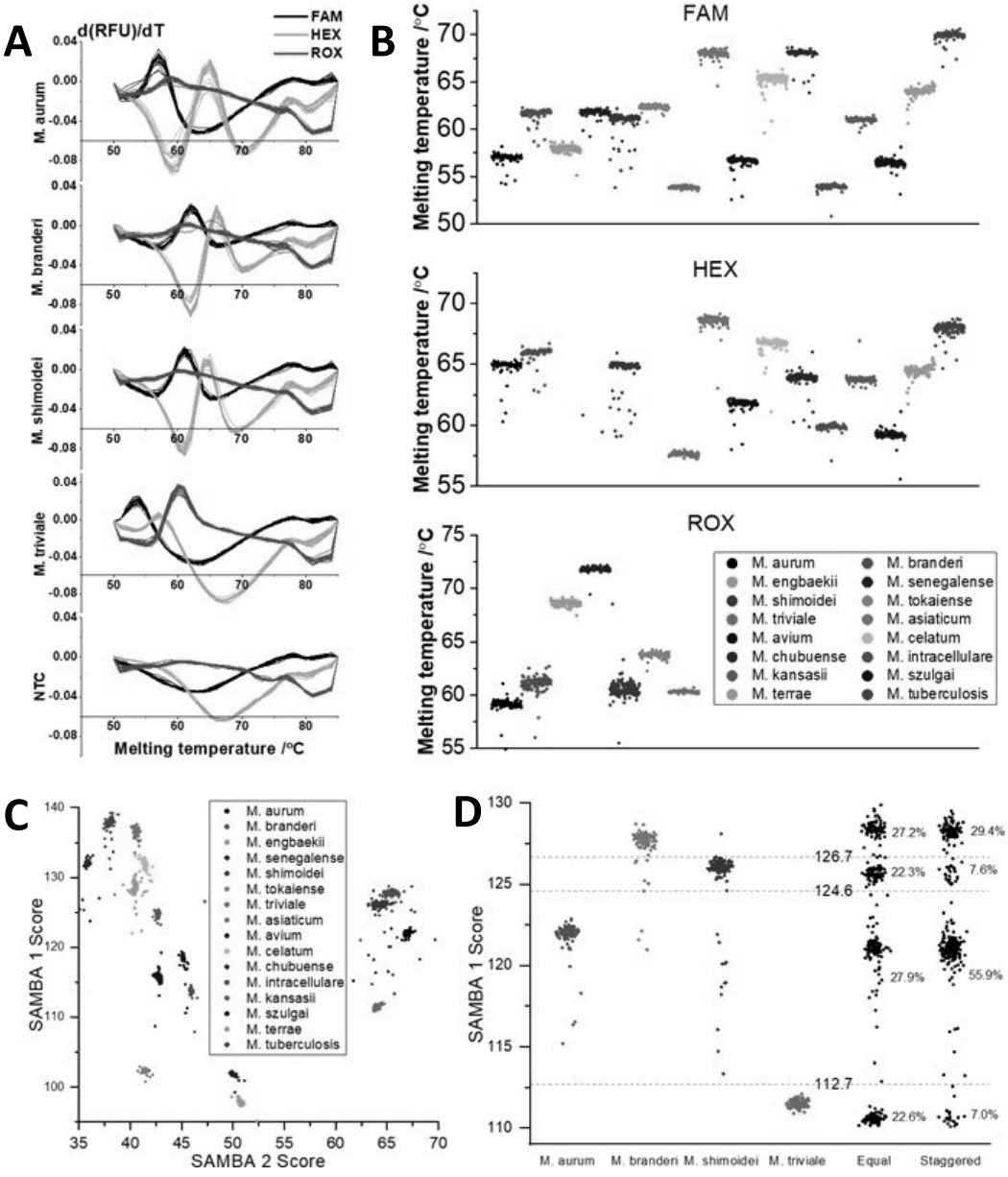

A
d(RFU)/dT
FAM
HEX
ROX
M. aurum
M. branderi
M. shimoidei
M. triviale
NTC
Melting temperature /°C
B
FAM
HEX
ROX
Melting temperature /°C
M. aurum
M. branderi
M. engbaekii
M. senegalense
M. shimoidei
M. tokaiense
M. triviale
M. asiaticum
M. avium
M. celatum
M. chubuense
M. intracellulare
M. kansasii
M. szulgai
M. terrae
M. tuberculosis
C
SAMBA 1 Score
SAMBA 2 Score
D
SAMBA 1 Score
126.7
124.6
112.7
27.2%
22.3%
27.9%
22.6%
29.4%
7.6%
55.9%
7.0%
M. aurum
M. branderi
M. shimoidei
M. triviale
Equal
Staggered

Fig. 2

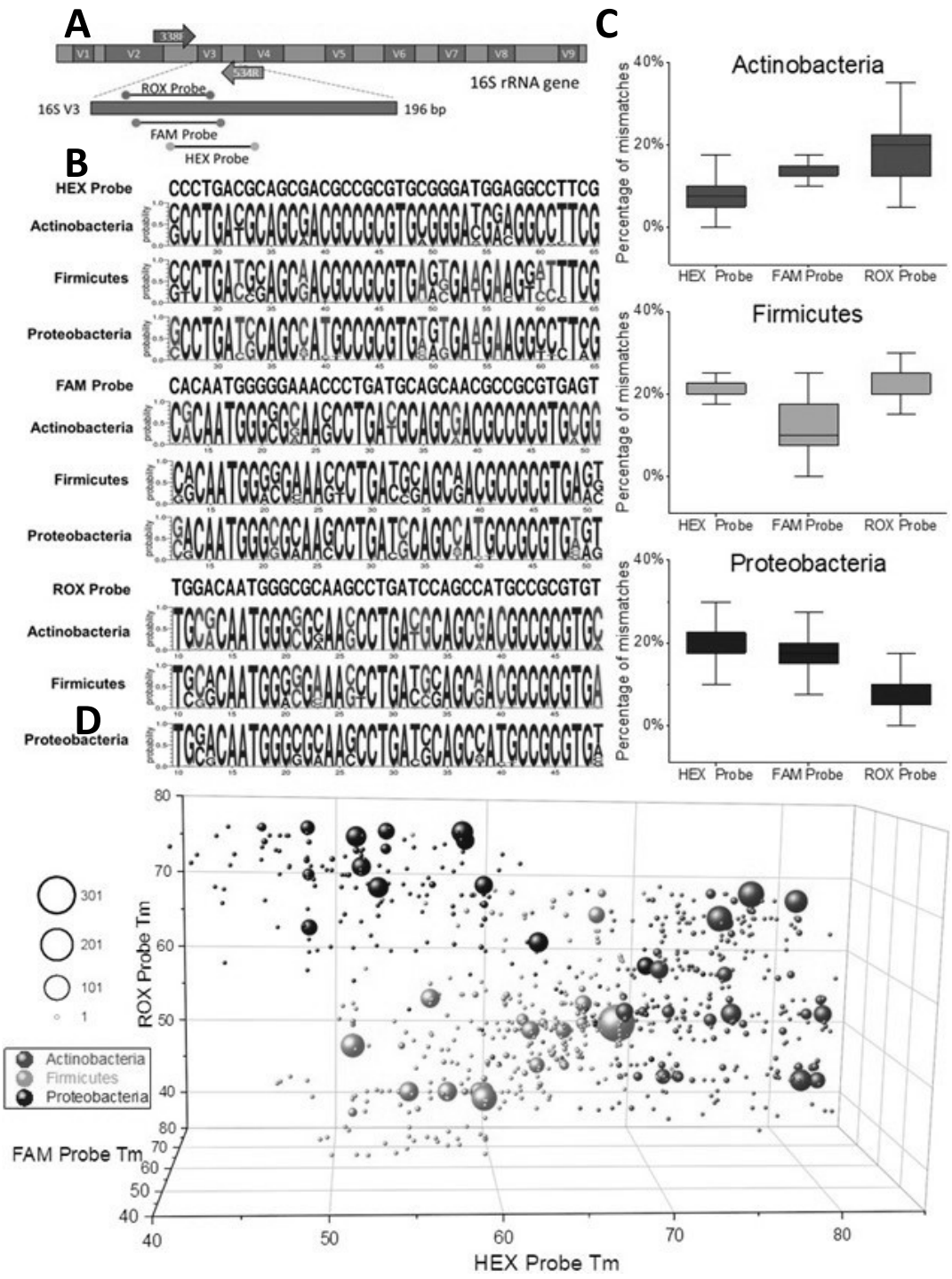

A
338F
V1
V2
V3
V4
V5
V6
V7
V8
V9
534R
16S rRNA gene
ROX Probe
16S V3
196 bp
FAM Probe
HEX Probe
B
HEX Probe
Actinobacteria
Firmicutes
Proteobacteria
FAM Probe
Actinobacteria
Firmicutes
Proteobacteria
ROX Probe
Actinobacteria
Firmicutes
Proteobacteria
C
Actinobacteria
Firmicutes
Proteobacteria
Percentage of mismatches
40%
20%
0%
HEX Probe
FAM Probe
ROX Probe
D
301
201
101
1
Actinobacteria
Firmicutes
Proteobacteria
ROX Probe Tm
FAM Probe Tm
HEX Probe Tm
80
70
60
50
40

Fig. 3

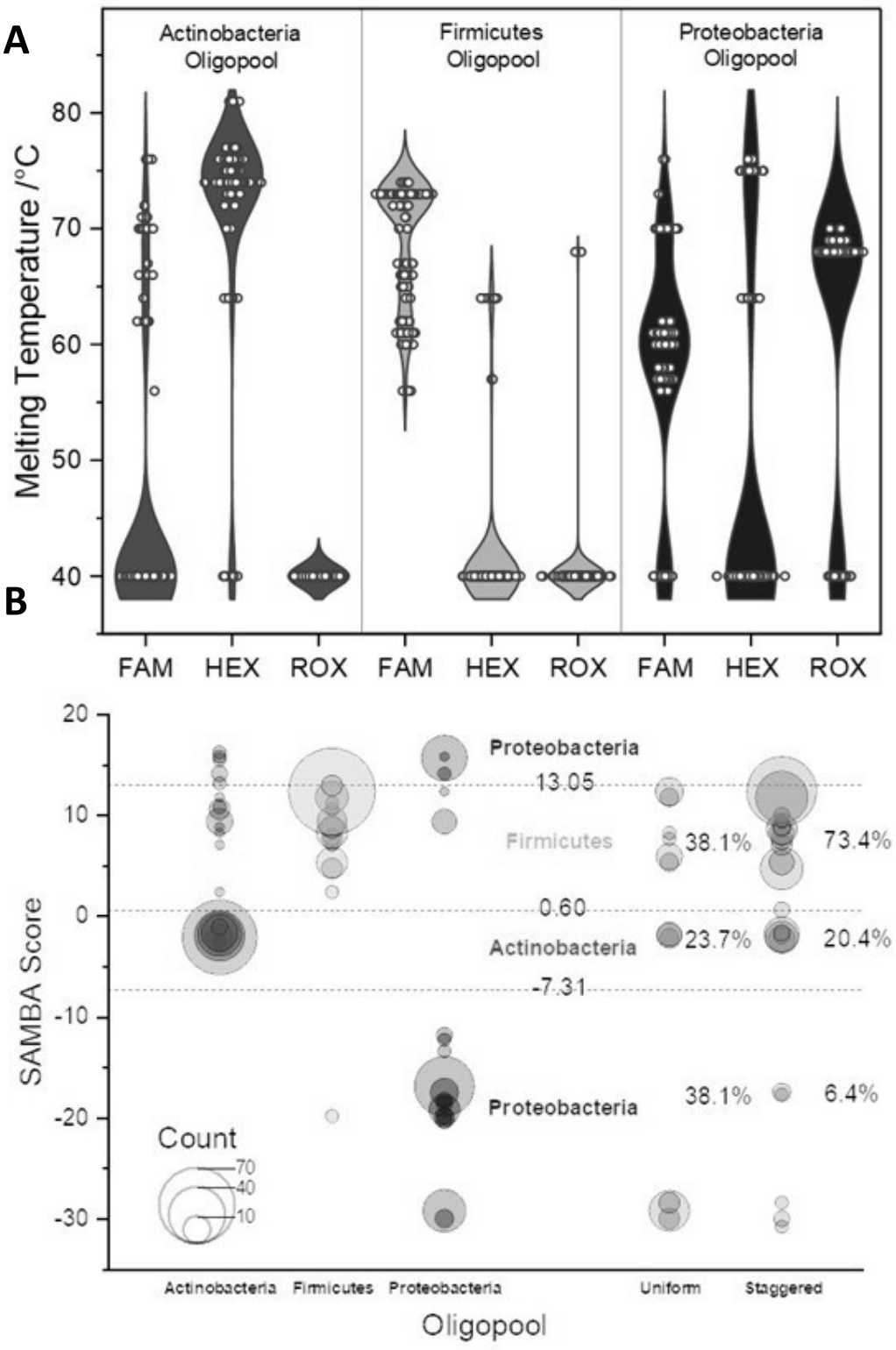

A
Actinobacteria Oligopool
Firmicutes Oligopool
Proteobacteria Oligopool
Melting Temperature /°C
80
70
60
50
40
FAM
HEX
ROX
B
SAMBA Score
20
10
0
-10
-20
-30
Proteobacteria
13.05
Firmicutes
38.1%
73.4%
0.60
Actinobacteria
23.7%
20.4%
-7.31
Proteobacteria
38.1%
6.4%
Count
70
40
10
Actinobacteria
Firmicutes
Proteobacteria
Uniform
Staggered
Oligopool

Fig. 4

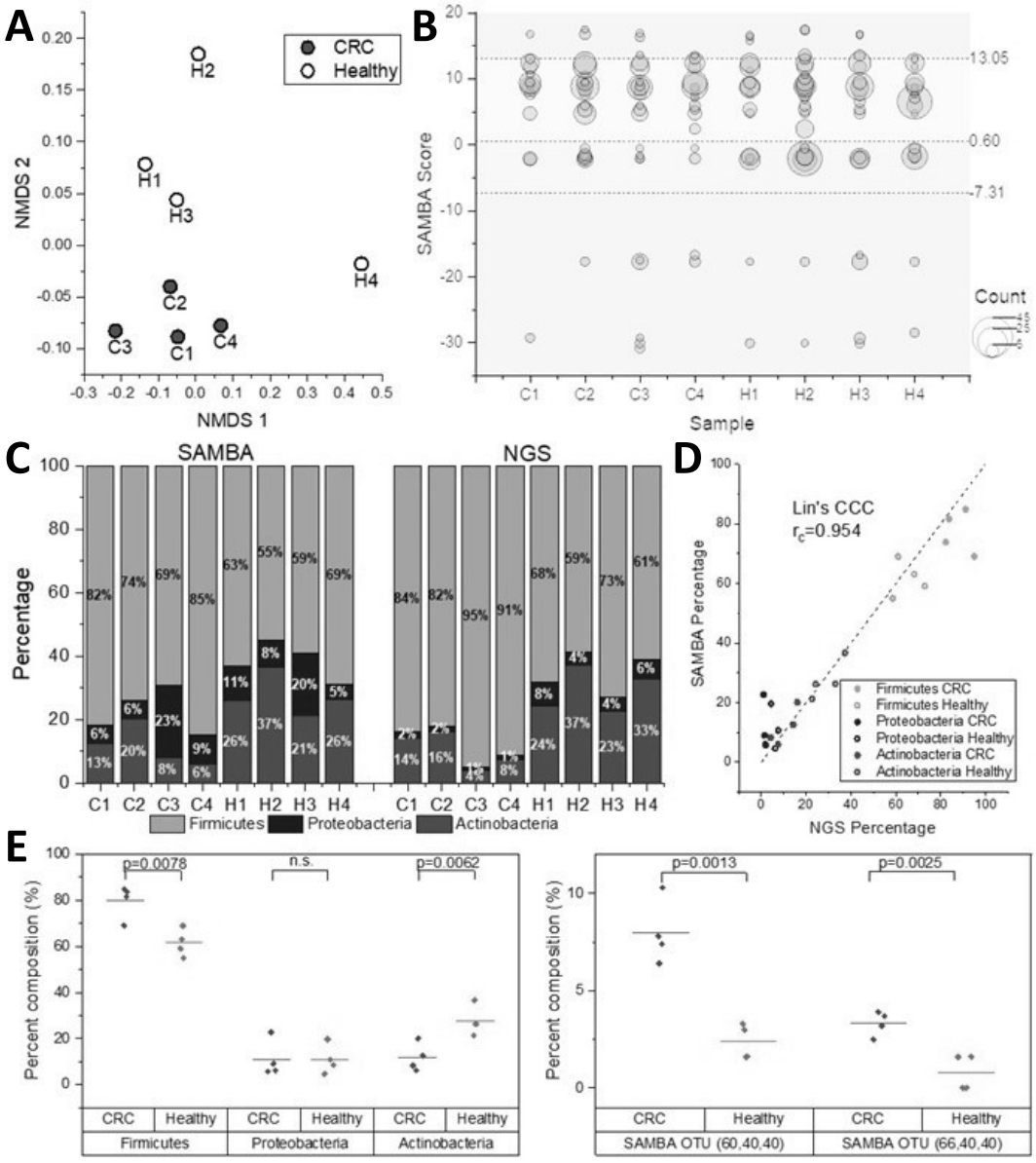

A
CRC
Healthy
NMDS 2
NMDS 1
B
SAMBA Score
13.05
0.60
-7.31
Count
Sample
C
SAMBA
NGS
Percentage
Firmicutes
Proteobacteria
Actinobacteria
D
Lin's CCC
r_c=0.954
SAMBA Percentage
NGS Percentage
Firmicutes CRC
Firmicutes Healthy
Proteobacteria CRC
Proteobacteria Healthy
Actinobacteria CRC
Actinobacteria Healthy
E
Percent composition (%)
p=0.0078
n.s.
p=0.0062
p=0.0013
p=0.0025
CRC
Healthy
Firmicutes
Proteobacteria
Actinobacteria
SAMBA OTU (60,40,40)
SAMBA OTU (66,40,40)

Fig. 5

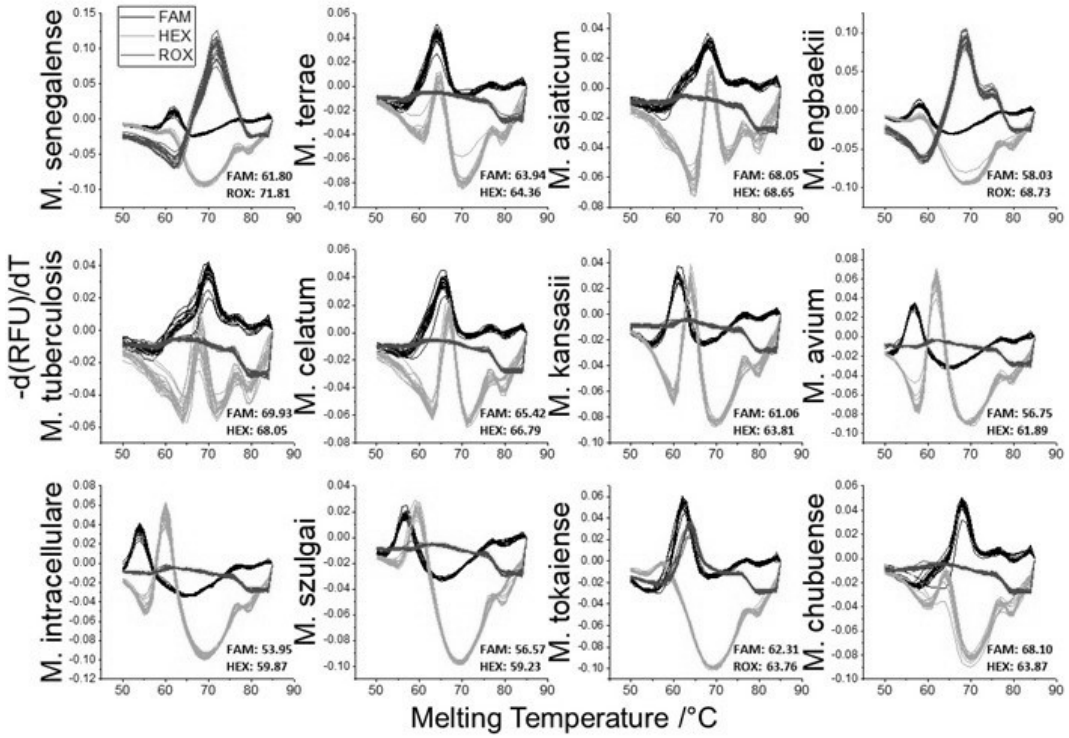

FAM
HEX
ROX
M. senegalense
FAM: 61.80
ROX: 71.81
M. terrae
FAM: 63.94
HEX: 64.36
M. asiaticum
FAM: 68.05
HEX: 68.65
M. engbaekii
FAM: 58.03
ROX: 68.73
M. tuberculosis
FAM: 69.93
HEX: 68.05
M. celatum
FAM: 65.42
HEX: 66.79
M. kansasii
FAM: 61.06
HEX: 63.81
M. avium
FAM: 56.75
HEX: 61.89
M. intracellulare
FAM: 53.95
HEX: 59.87
M. szulgai
FAM: 56.57
HEX: 59.23
M. tokaiense
FAM: 62.31
ROX: 63.76
M. chubuense
FAM: 68.10
HEX: 63.87
-d(RFU)/dT
Melting Temperature /°C

Fig. 6

Class counts for Panel ID

Class:	M. asiaticum	M. aurum	M. avium	M. branderi	M. celatum	M. chubunse	M. engbaekii	M. intracellulare	M. kansasii	M. senegalense
Count:	105	105	105	105	105	105	105	105	105	105

Class:	M. szulgai	M. terrae	M. tokaiense	M. triviale	M. tuberculosis	M.shimoidei
Count:	105	105	105	105	105	105

Total count: 1680

Threshold to used for zero probabilities: 1.0E-4

Gaussian distribution for FAM per class value

	M. asiaticum	M. aurum	M. avium	M. branderi	M. celatum	M. chubunse	M. engbaekii	M. intracellulare	M. kansasii	M. senegalense	M. szulgai	M. terrae	M. tokaiense	M. triviale	M. tuberculosis	M.shimoidei
Count:	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Mean:	67.9819	56.55718	56.64359	61.66015	65.28317	67.98118	57.96106	53.91333	61.00591	61.81155	56.4367	64.00404	62.30356	53.85765	69.87949	60.93051
Std. Deviation:	0.42613	0.5781	0.47452	0.60205	0.69555	0.56708	0.22463	0.3399	0.2082	0.27593	0.42939	0.50561	0.22673	0.10244	0.49635	1.14193
Rate:	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%

Gaussian distribution for HEX per class value

	M. asiaticum	M. aurum	M. avium	M. branderi	M. celatum	M. chubunse	M. engbaekii	M. intracellulare	M. kansasii	M. senegalense	M. szulgai	M. terrae	M. tokaiense	M. triviale	M. tuberculosis	M.shimoidei
Count:	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Mean:	68.55603	64.46068	61.76963	65.92817	66.68286	63.82838	40	59.84125	63.76459	40.38344	59.20092	64.48765	40	57.60987	67.94817	64.62477
Std. Deviation:	0.60378	0.37272	0.41529	0.5569	0.38515	0.61469	0.0001	0.29496	0.35068	2.76661	0.43827	0.46651	0.0001	0.10647	0.56293	1.13076
Rate:	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%

Gaussian distribution for ROX per class value

	M. asiaticum	M. aurum	M. avium	M. branderi	M. celatum	M. chubunse	M. engbaekii	M. intracellulare	M. kansasii	M. senegalense	M. szulgai	M. terrae	M. tokaiense	M. triviale	M. tuberculosis	M.shimoidei
Count:	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105
Mean:	40	58.87306	40	61.66216	40	40	68.67896	40	40	71.82368	40	40	63.76068	60.29808	40	60.8396
Std. Deviation:	0.0001	2.50037	0.0001	3.03675	0.0001	0.0001	0.19313	0.0001	0.0001	0.11737	0.0001	0.0001	0.22209	0.06263	0.0001	2.44218
Rate:	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%	6%

Fig. 7A

Panel ID \ Prediction (Panel ID)	M. senegal...	M. terrae	M. asiaticum	M. engbaekii	M. tubercu...	M. celatum	M. kansasii	M. avium	M. intracell...	M. szulgai	M. tokaiense	M. chubunse	M. aurum	M. branderi	M.shimoidei	M. triviale
M. senegalense	42	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0
M. terrae	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M. asiaticum	0	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0
M. engbaekii	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0	0
M. tuberculosis	0	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0
M. celatum	0	1	0	0	0	43	1	0	0	0	0	0	0	0	0	0
M. kansasii	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0
M. avium	0	0	0	0	0	0	0	44	0	1	0	0	0	0	0	0
M. intracellulare	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0
M. szulgai	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0
M. tokaiense	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0
M. chubunse	0	0	0	0	0	0	0	0	1	0	0	44	0	0	0	0
M. aurum	0	0	0	0	0	0	0	0	0	0	0	0	45	0	0	0
M. branderi	0	0	0	0	0	0	0	0	0	0	0	0	0	44	1	0
M.shimoidei	0	0	0	0	0	0	0	1	0	0	0	0	1	2	41	0
M. triviale	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45

Correct classified: 708

Accuracy: 98.333 %

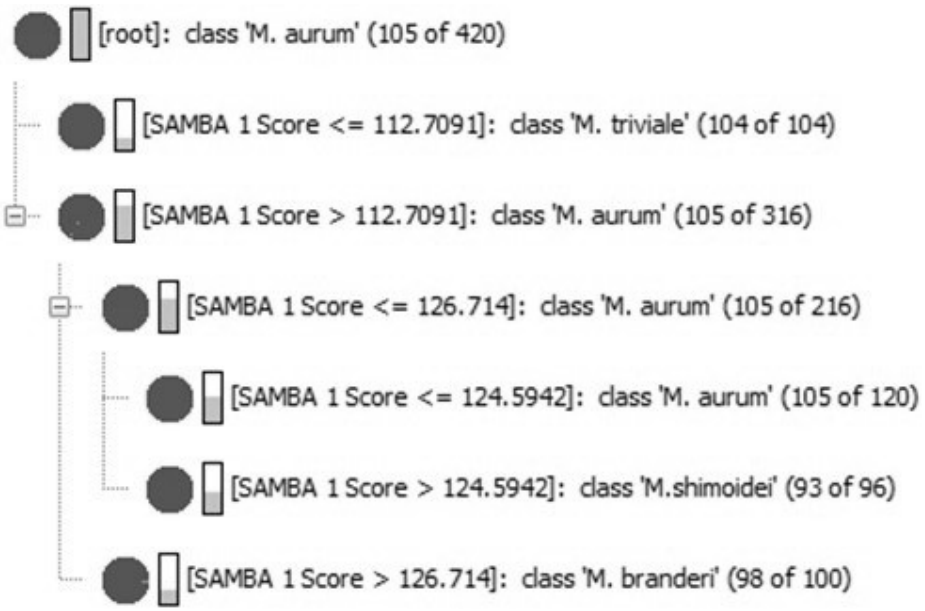
Cohen's kappa (κ) 0.982

Wrong classified: 12

Error: 1.667 %

Fig. 7B

A



B

Panel ID \ Prediction (Panel ID)	M. aurum	M. branderi	M.shimoidei	M. triviale
M. aurum	45	0	0	0
M. branderi	0	43	2	0
M.shimoidei	1	0	44	0
M. triviale	0	0	0	45

Correct classified: 177	Wrong classified: 3
Accuracy: 98.333 %	Error: 1.667 %
Cohen's kappa (κ) 0.978	

Fig. 8

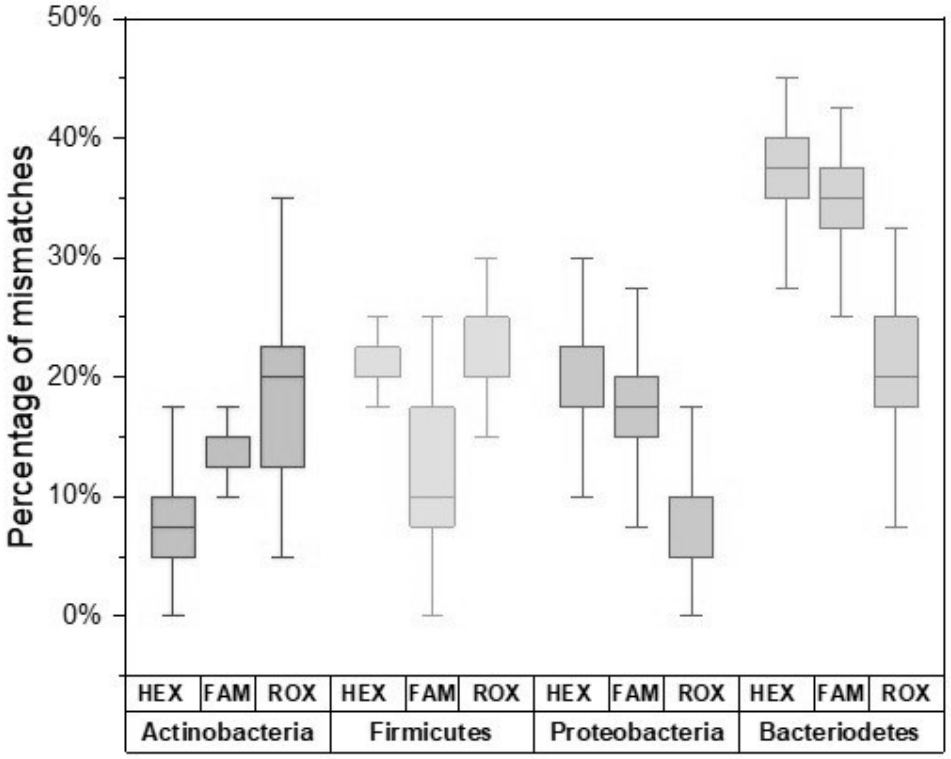

50%
40%
30%
20%
10%
0%
Percentage of mismatches
HEX
FAM
ROX
HEX
FAM
ROX
HEX
FAM
ROX
HEX
FAM
ROX
Actinobacteria
Firmicutes
Proteobacteria
Bacteriodetes

Fig. 9

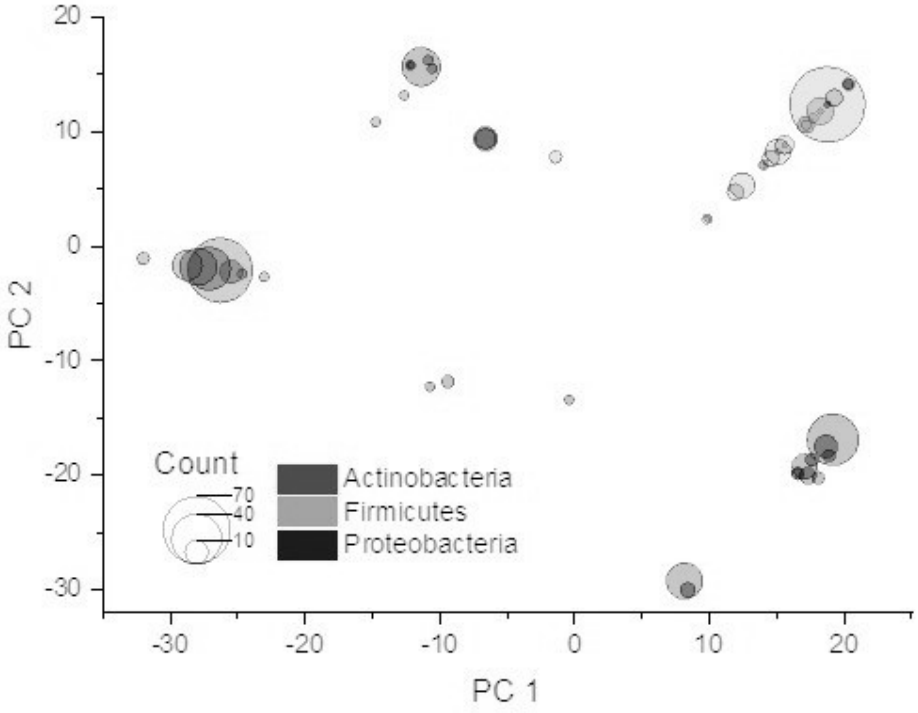
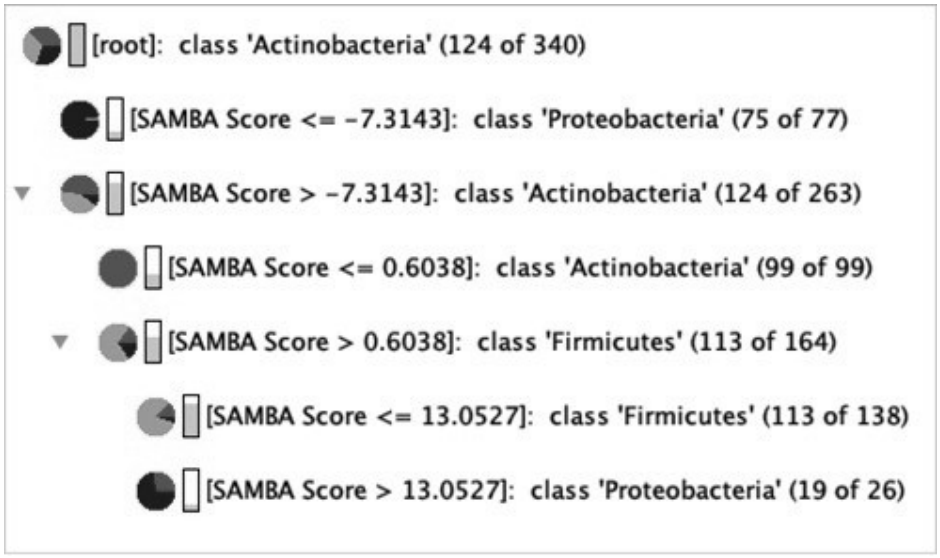

20
10
0
-10
-20
-30
PC 2
-30
-20
-10
0
10
20
PC 1
Count
70
40
10
Actinobacteria
Firmicutes
Proteobacteria

Fig. 10

A



B

Phylum \ Prediction (Phylum)	Actinobacteria	Firmicutes	Proteobacteria
Actinobacteria	47	2	4
Firmicutes	0	49	0
Proteobacteria	0	1	43

Correct classified: 139 Wrong classified: 7

Accuracy: 95.205 % Error: 4.795 %

Cohen's kappa (κ) 0.928

Fig. 11