Automated microbial DNA isolation from clinical samples for routine broad-range real-time PCR analysis and sequence identification

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How to make broad-range PCR more efficient

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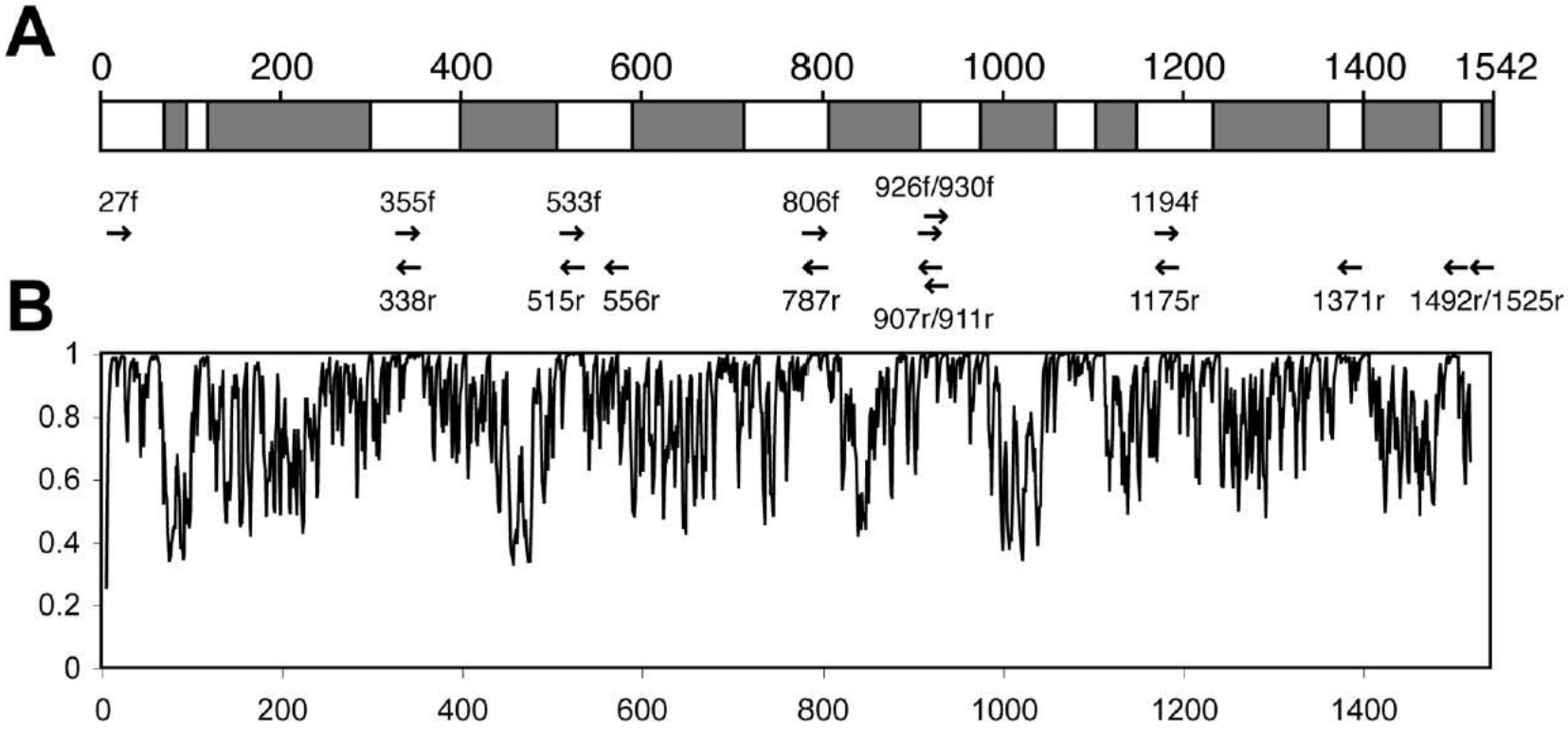
Broad-range 16S-rDNA PCR („universal“, „eubacteric“ PCR)

16S-rDNA



Maiwald et al., 2011

Issues of the broad range PCR

- Only from pure cultures / monoinfections → samples from primarily sterile body sites
- Mixed infections are difficult to detect
  - Mixed sequences
  - Selection of one of the sequences

- Sensitivity is rather low!

Low sensitivity of the broad range PCR

Reasons:

- Unfavourable selection of the primers
- Contamination with human DNA

Broad-range 16S rDNA-PCR

Aims

1. To improve the results

2. To simplify the workflow

➔ Comparison study

Broad-range 16S rDNA-PCR

Comparison Study:

Inhouse PCR ⇔ SelectNA™ *plus*

→ automated DNA extraction

→ removes human DNA

SelectNA™ *plus*

Micro-Dx™
Automated sample pre-treatment, bacterial/ fungal DNA isolation and PCR Analysis
Microbe identification kit for SelectNA™*plus*

MolYsis-SelectNA™*plus*
Pathogen DNA-extraction kit for SelectNA™*plus*

numerous consumables

Workflow

	Broad-range 16S rDNA-PCR (inhouse)		SelectNA™plus (Fa. Molzym)	
	Assay time	„hands-on“ time	Assay time	„hands-on“ time
Tissue samples	60 min	5 min	15 min	5 min
Loading of machine		5 min (8 probes)		10 min (1-4 probes) 20 min (5-8 probes) 30 min (9-12 probes)
DNA-isolation	35 min		85 min (1-4 probes) 95 min (5-8 probes) 110 min (9-12 probes)	
PCR setup	10 min	10 min	10 min	10 min
PCR amplification	120 min (block cycler)		60 min (light cycler)	
Detection	70 min	10 min	melting curve analysis	5 min
Setup for sequencing	15 min	15 min	15 min	15 min
Total time	310 min	45 min	195 min	55 min


PKB
Cont. 7.5ml
LOT D02r454D049
18 to 25°C
Enzyme K
Cont. 0.08ml
LOT F16r481E020
-15 to -25°C

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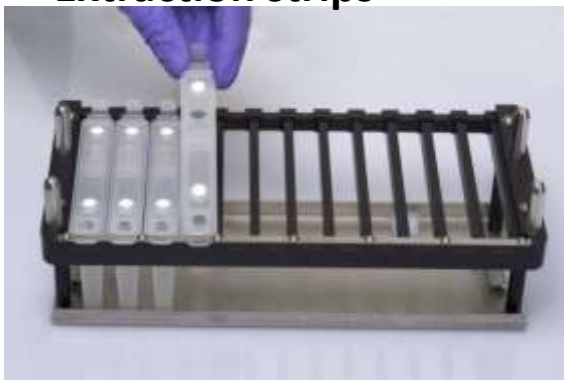
Setup of SelectNA™plus → many single components

Extraction columns

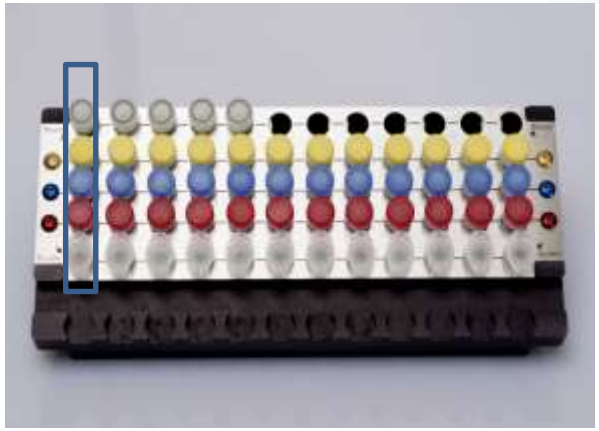
Extraction strips



Buffer strips

Inside view of machine

Buck for reagents



Samples 1ml

BugLysis plus

Proteinase K

MolDNase C

Eluate

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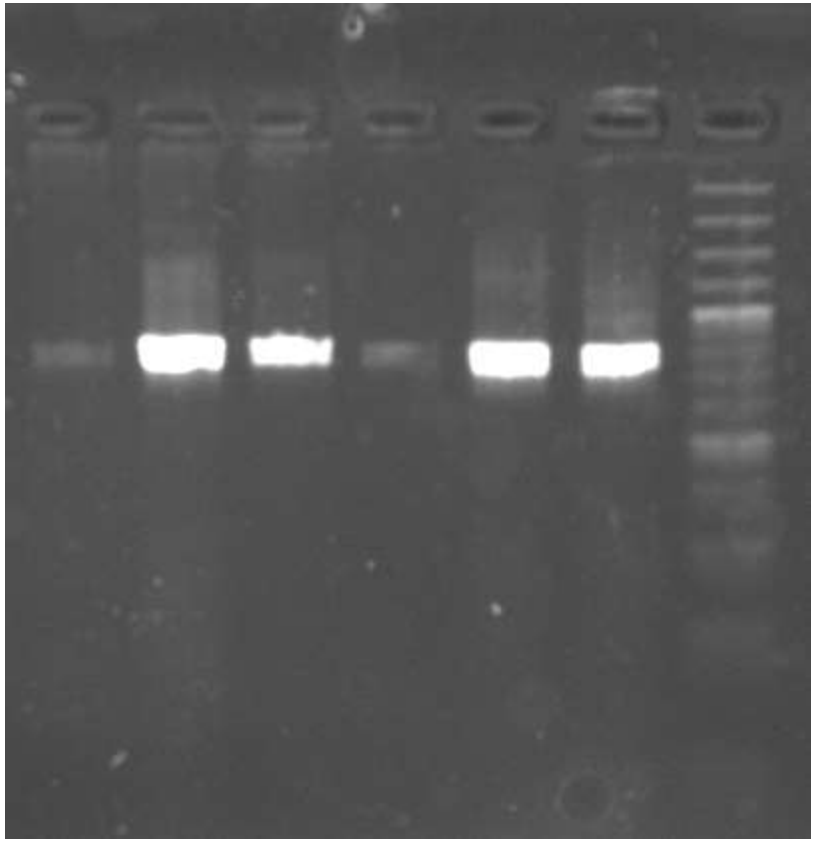
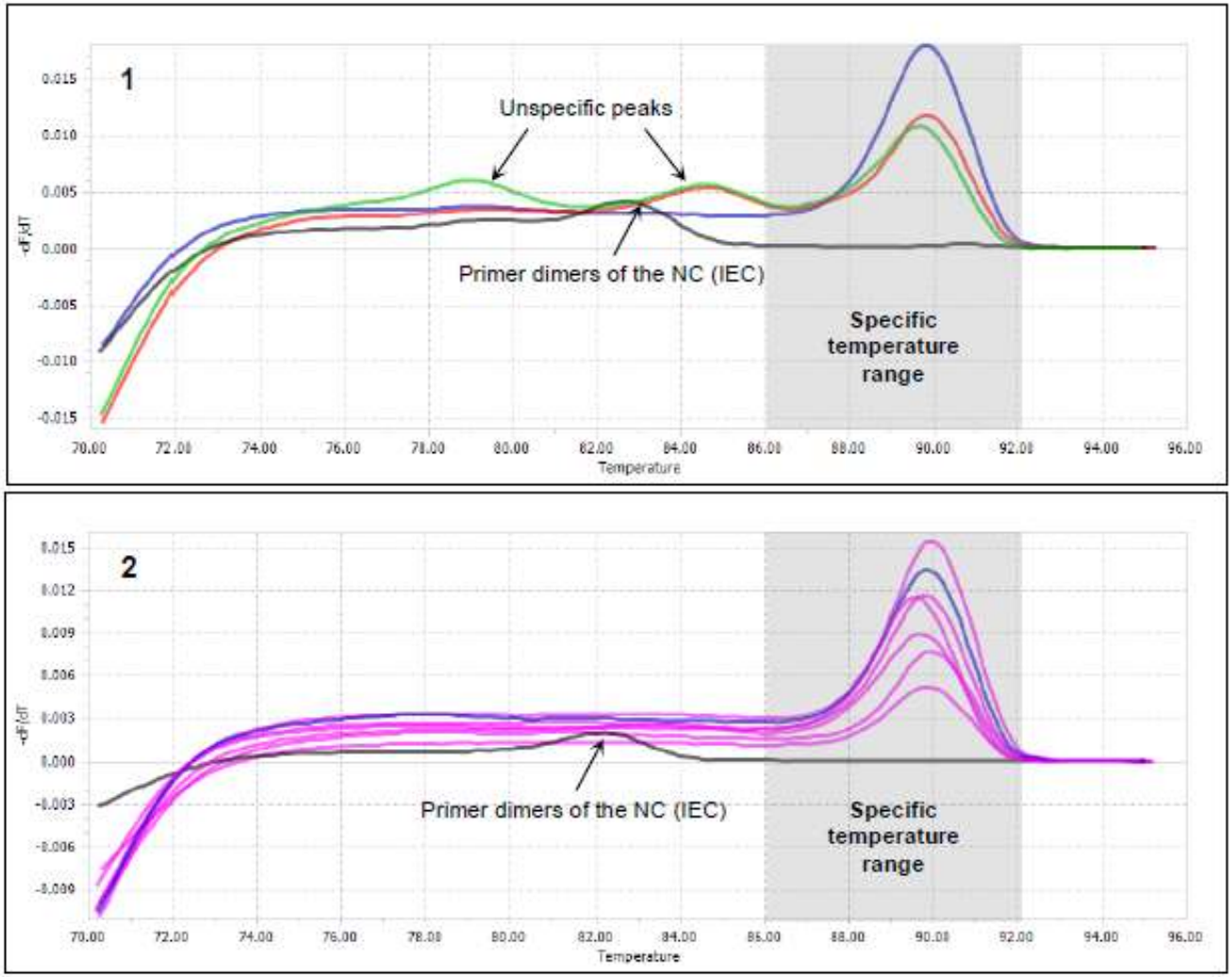
Broad range 16S rDNA-PCR (inhouse) → Agarose Gel

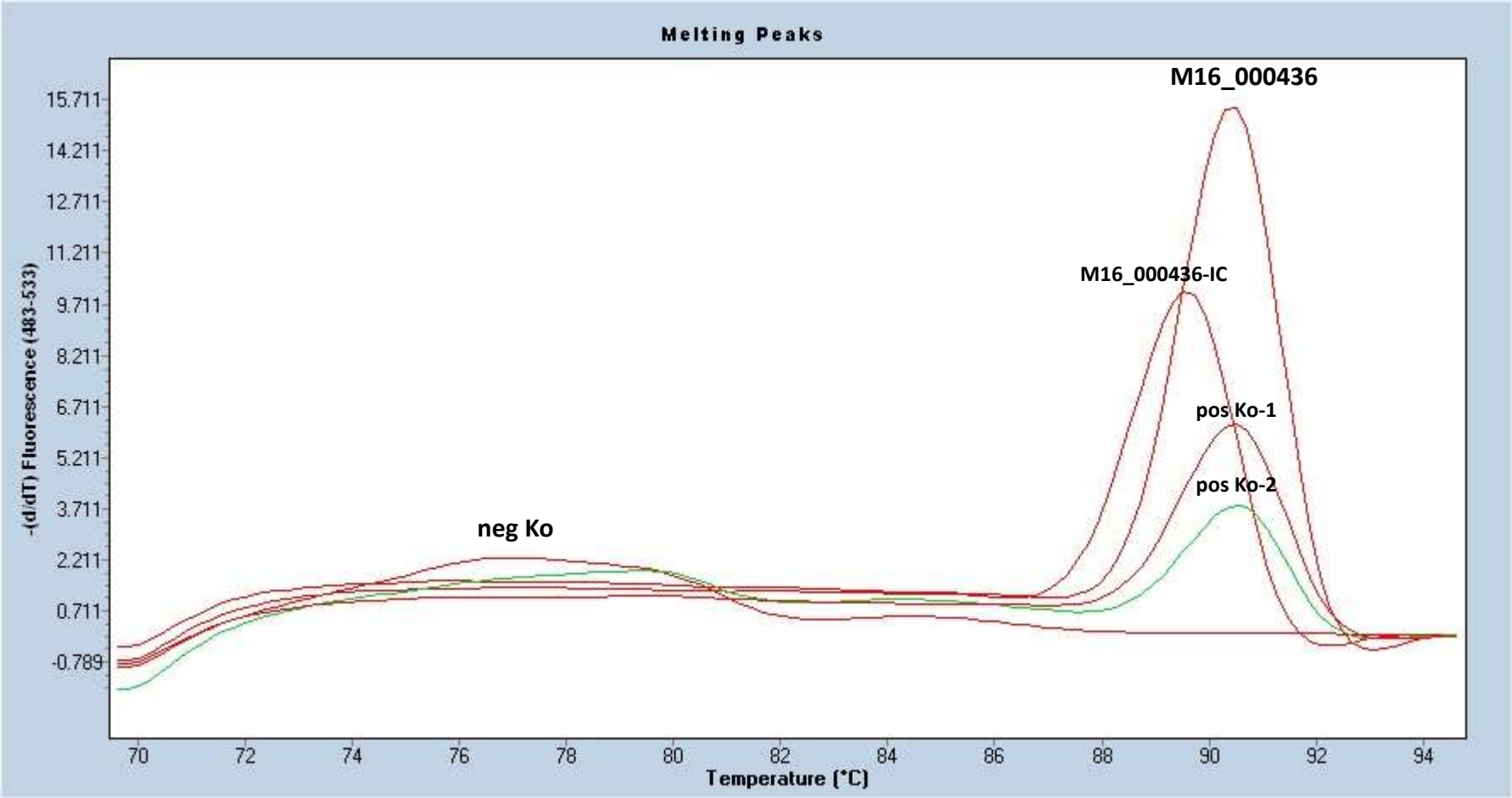




SelectNA™plus

SelectNA™plus

Sample M16_000436 → **pleura aspiration fluid / *Streptococcus pyogenes***



„inhouse PCR“ and culture → no results !!!

Broad-range 16S rDNA-PCR

Comparison Study:

Inhouse PCR ⇔ SelectNA™ *plus*

n=	Sample	„inhouse“ broad-range 16S rDNA			Molzym SelectNA		
		PCR pos	PCR neg	Sequence	PCR pos	PCR neg	Sequence

Broad-range 16S rDNA-PCR

Results

Inhouse PCR ⇔ SelectNA™ *plus*

Results:

Inhouse broad-range PCR ⇔ SelectNA™ *plus*

	positive	negative	Σ	
positive	14	2	16	**Inhouse PCR**
negative	12	36	48	
Σ	26	38	64	
	SelectNA™ *plus* Molzym			

Discrepant Results:

SelectNA™ *plus*

Sample
Aspiration fluid
Heart valve
Cerebrospinal fluid (CSF)
Heart valve (mitral)
Abscess (tissue)
Heart valve (aortic)
Pleural aspiration fluid
Tissue
Heart valve (mitral)
Heart valve (aortic)
Cerebrospinal fluid (CSF)

Discrepant Results:

- Meaningful results → clinical relevant bacteria
- Almost allways <u>no result</u> of <u>culture</u> diagnostics

Micro-Dx™ / SelectNA™ *plus*

Plus

- Easy and fast tissue preparation
- Improved sensitivity by removing human DNA
- No indication of *cross contaminations*
- Simplified DNA detection
  (LightCycler – melting curve analysis)

Minus

- Setup laborious
  - Manual screwing
  - Manual foil removing
- Costs of machine
- Costs of consumables

Conclusions:

- Simplifying of broad-range PCR with lower *hands-on*-time
- Robust System
- Improved identification of bacteria causing serious infections

Many thanks for your attention !

Max von Pettenkofer-Institut:

Thanks to the diagnostic team of the Großhadern lab

Birgit Groß

Agenda

1. Universelle PCRs
2. Vergleichsstudie:

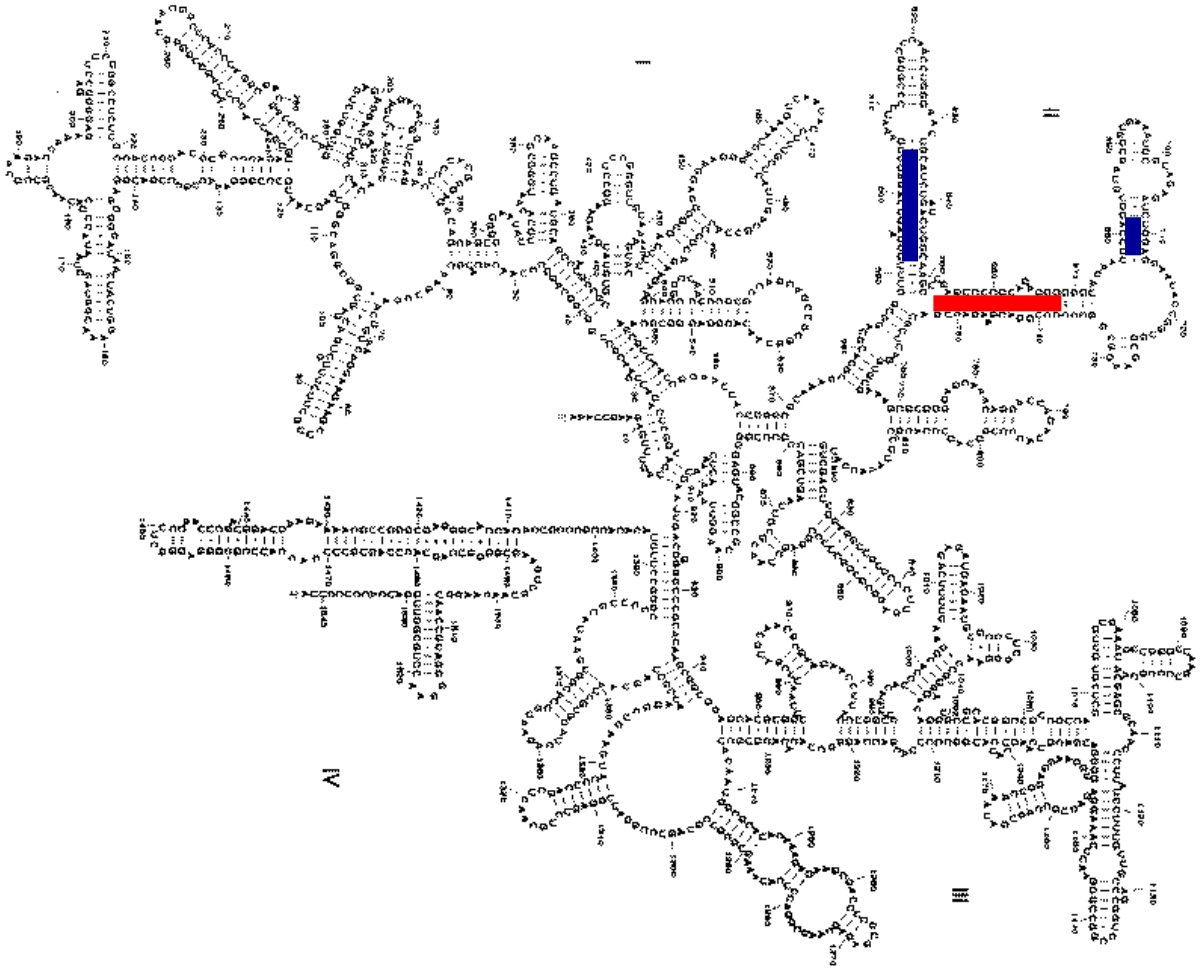
   Mirco-Dx™ / Select NA™ plus ⇔ inhouse UNI-PCR

   – Methodik
   – Ergebnisse
3. Mirco-Dx™ / Select NA™ plus

   – Vorteile und Nachteile
   – Zusammenfassung

PCR der 16S-rDNA („universelle“, „eubakterielle“ PCR)

Ribosomale RNA (16S-rRNA)

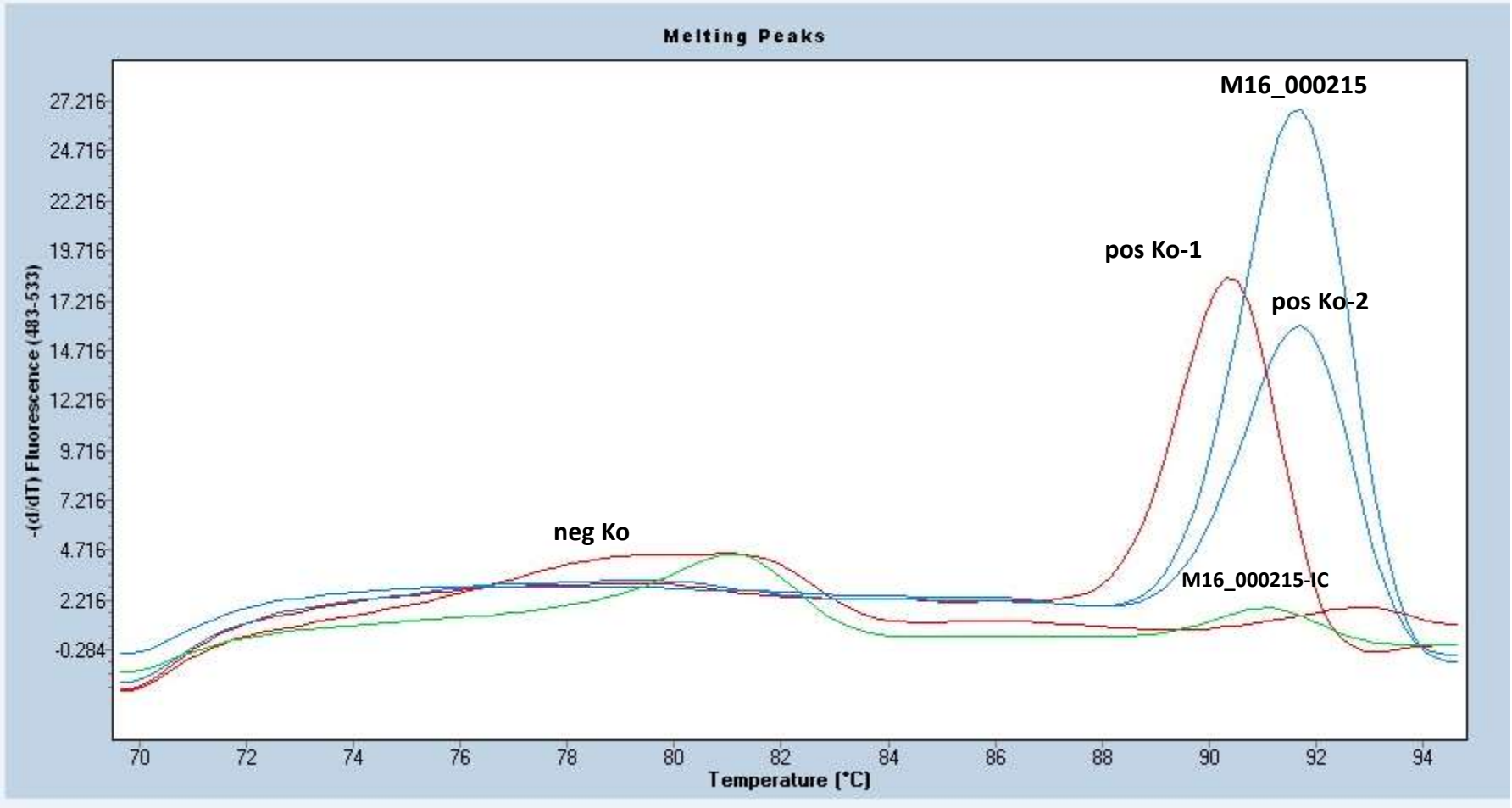


: = konstant

: = variabel

SelectNA™plus

Sample M16_000215 → **heart valve** / ***Staphylococcus aureus***



„inhouse PCR“ → ***Staphylococcus aureus***

2016

	Inhouse PCR	Molzym SelectNA *plus*
Tissue sample preparation	**60 min**	**15 min**
Setup / Loading	**5 min**	**10 - 30 min**
DNA Extraction (bacterial)	**35 min**	**110 min**
PCR	**200 min**	**70 min**
∑	**240 - 300 min = 4 - 5 h**	**205 - 225 min ≈ 3,5 h**