体外诊断原料
首选合作伙伴

Dedication to the IVD industry?

We I.V.DO that™

Medix Biochemica
2023 技术讲座讲义

讲座时间：2023 年 5 月 29 日 10:00 - 12:00

讲座地点：南昌绿地国际博览中心一楼 A2 会议室

应您所需，量身定制
——分子诊断用工程化DNA聚合酶
Shaping DNA polymerases for your needs

RAMON KRANASTER
博士/研发总监

2023年5月29日 南昌

Medix Biochemica

目录
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Medix Biochemica

DNA聚合酶是生物行业基础技术的关键角色
DNA polymerases are key-players in fundamental techniques in Biotechnology

测序、检测、基因分型、克隆等。
Sequencing, Detection, Genotyping, Cloning, etc.

www.newsfox.com
www.telegraph.co.uk

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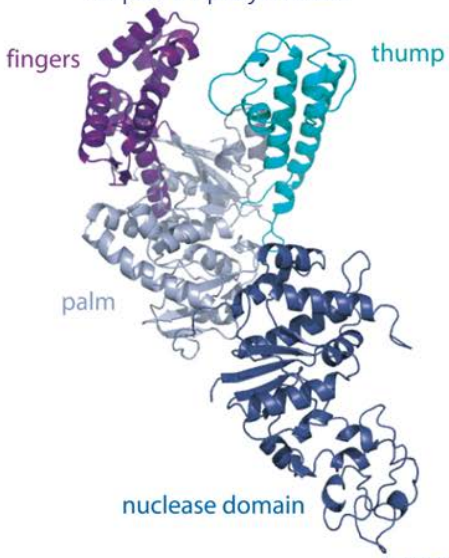
DNA聚合酶 — 天然的"分子机器"
DNA polymerases — nature's molecular machines

- 延伸速度 Velocity:
  35-150 nts/sec
- 错配率 Error rates:
  1.1 x 10^{-4} errors/bp - 8.9 x 10^{-5} errors/bp
- 碱基切除修复 (BER) 和核苷酸切除修复 (NER) 的校准和错配修复，总错配率大约是10^{-8}-10^{-10} errors/bp。
  Proofreading and mismatch repair by base and nucleotide excision repair (BER and NER) overall error-rates of 10^{-8}-10^{-10} errors/bp.
- 在真核细胞中，错配率约>10^{-10}。
  In eukaryotic cells error-rates >10^{-10} are reached.
- 人类每个细胞基因组中含有大约60亿个核苷酸!
  In living, human cells: 6 billion nucleotides!

Taq DNA **聚合酶**
Taq DNA polymerase



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DNA聚合酶 — 天然的“分子机器”
DNA polymerases — nature’s molecular machines

“大自然并未完全根据我们的需求塑造它”
“Nature didn’t shape them to our needs”

我们的贡献：
Our contribution:

- 为生物技术应用量身定制DNA聚合酶
- Tailoring DNA polymerases for biotec applications

  - 具有新特性或改良特性的DNA聚合酶突变体
  - DNA polymerase mutant with new or improved features

  - 使您的生物技术应用更简单、更快、更可靠
  - easier, faster, more reliable biotechnological application

Medix Biochemica

我们的贡献：为生物技术应用量身定制DNA聚合酶
Our contribution: Tailoring DNA polymerases for biotec applications

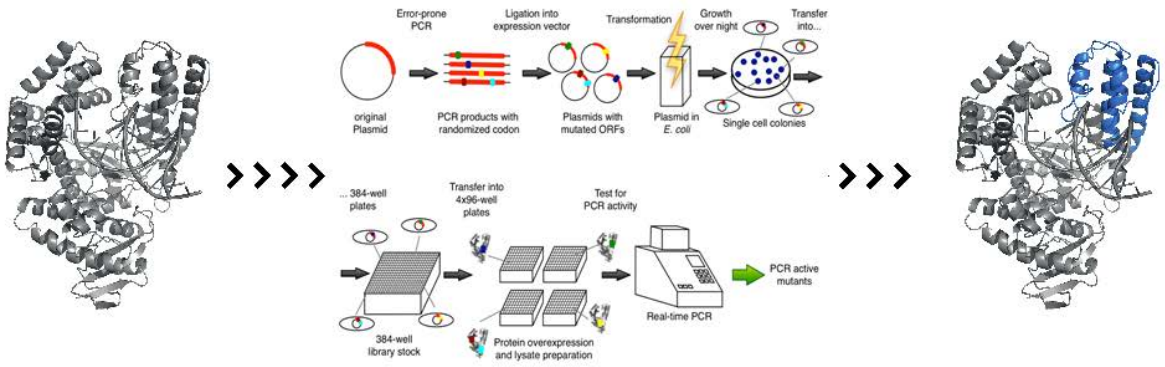
天然DNA聚合酶
natural DNA polymerase

技术平台
Technology platform

(DNA聚合酶的定向进化)
(for directed evolution of DNA polymerases)

优化后的酶突变体
Improved enzyme variant (mutated)

(用于生物技术应用)
(for biotec application)



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方法
Approach

通过诱导随机突变…
by random mutagenesis...

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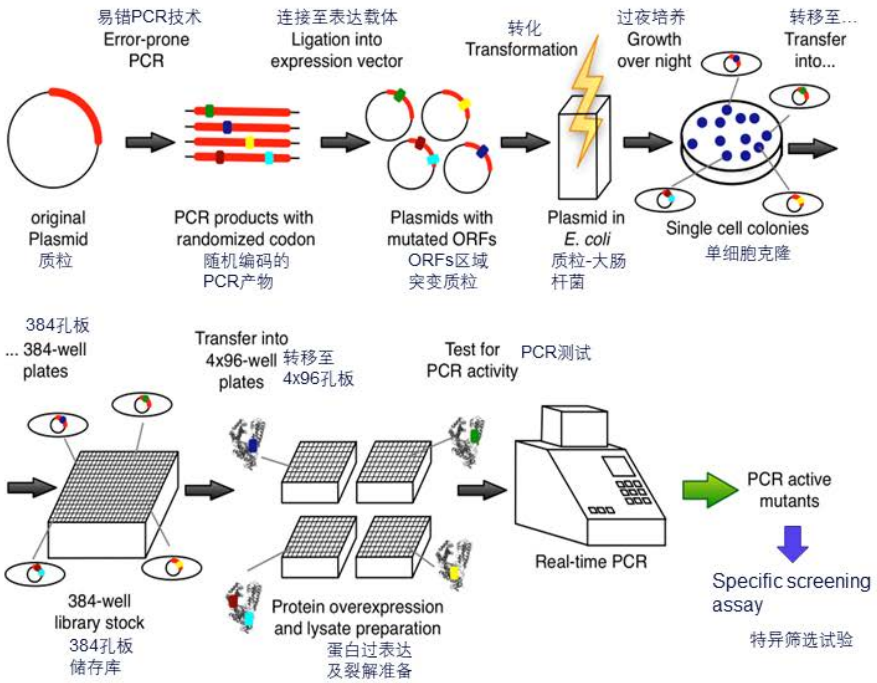
随机突变
——DNA聚合酶的工程化改造
Engineering of DNA polymerases by random mutagenesis



Kranaster, R. and Marx, A. (2010), Engineered DNA Polymerases in Biotechnology. ChemBioChem, 11: 2077–2084. doi: 10.1002/cbic.201000215

Gloeckner, C., Kranaster, R. and Marx, A. (2010), Directed Evolution of DNA Polymerases: Construction and Screening of DNA Polymerase Mutant Libraries. Current Protocols in Chemical Biology. 2:89–109.

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方法
Approach

通过人工设计…
by rational designs…

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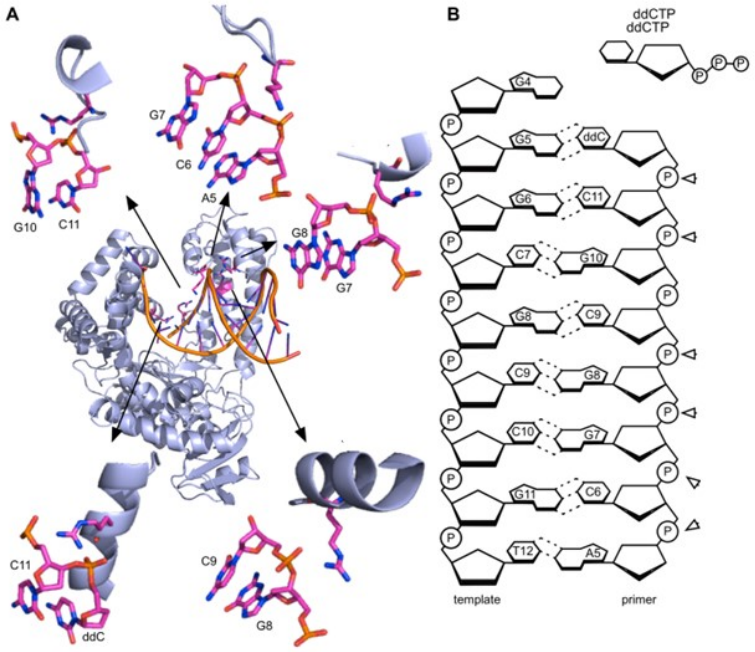
人工设计
——DNA聚合酶的工程化改造
Engineering of DNA polymerases by rational designs



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改造实例 I — HiDi® DNA polymerase
Example I — HiDi® DNA polymerase

HiDi® 意味着"High Discrimination"，即高鉴别能力。
HiDi® is standing for "High Discrimination".

Primer design

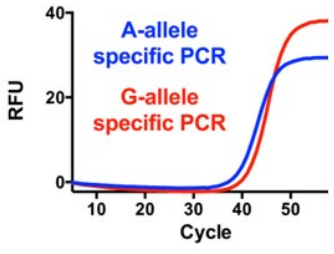
rs72921001

3' -ACAACTATCTTACCCTATCTCTTCCCTAGTTTT[C/A]GACATCC -5'

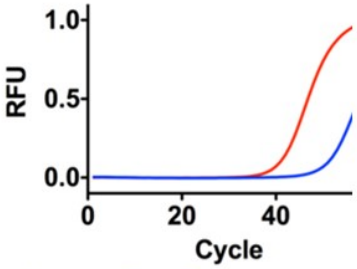
C-allele primer: 5' -GAATGGGATAGAGAAGGGATCAAAAG-3'
A-allele primer: 5' -GAATGGGATAGAGAAGGGATCAAAAT-3'

Reverse primer: 5' -CTGCTGCTTGAAAATGGATTGTG-3'

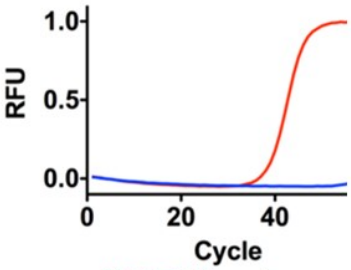
改造实例 I — 通过 HiDi® 实现高度选择性的等位基因特异性PCR
Example I — Highly selective allele-specific PCR with HiDi®



Taq DNA polymerase
(N公司)



Platinum Taq DNA polymerase
(TF公司)



HiDi® DNA polymerase
(myPOLS Biotec)

HiDi® DNA polymerase 是本实验中唯一能够准确区分引物/模板错配的酶。
HiDi® DNA polymerase is the only enzyme which separates right from wrong primer/template.

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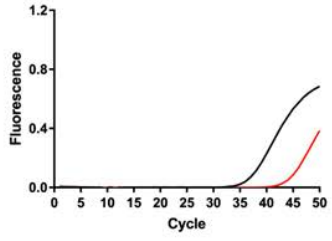
改造实例 I — HiDi® 是液体活检应用的理想选择
Example I — HiDi® is ideal for liquid biopsy approaches

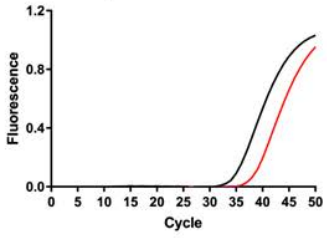
Mutation detection test: BRAF c.1799**T>A** (V600E), rs113488022, homo sapiens

target sequence: 5'...ATAGGTGATTTTGGTCTAGCTACAG**T/A**GAAATCTCGATG...
forward primer: 5'-GGTGATTTTGGTCTAGCTACAGA-3'
reverse primer: 5'-ACCATCCACAAAATGGATCCA-3'
taqman-probe: 5'-ROX-TCGATGGAGTGGGTCCCATCAGTTTG-BMNQ590-3'

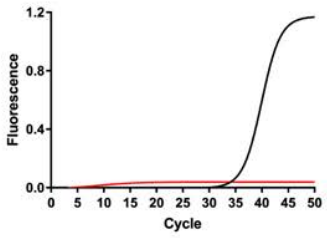
Taq DNA Polymerase
Hot Start Version (TKR公司)



GoTaq® hot start
DNA Polymerase (P公司)



HiDi Taq DNA polymerase
(myPOLS Biotec, #9201)



HiDi® Taq DNA polymerase 能够在>10,000个野生型序列中检测到单个突变拷贝。
HiDi® Taq DNA polymerase enables detection of single mutations copies in the presence of >10.000 wildtype sequences.

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Volcano® RT-PCR Master Mix

兼具逆转录酶和DNA聚合酶活性
极高耐热性
真正实现全预混RT-qPCR

改造实例 II
Example II

一种具有PCR反应活性、耐高温的逆转录酶
A PCR active, thermostable reverse transcriptase

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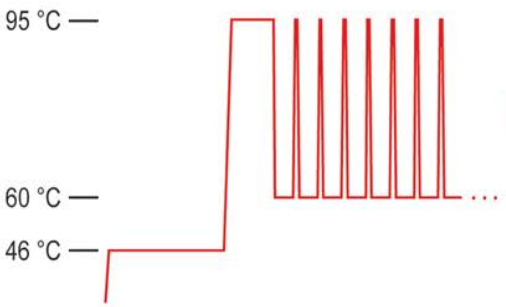
改造实例 II — 兼具DNA聚合酶活性、耐高温的逆转录酶?
Example II — a PCR active, thermostable reverse transcriptase?

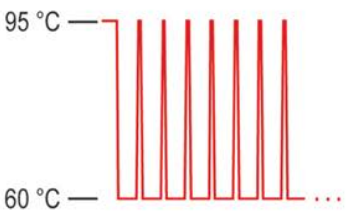
传统的
一步法 RT-qPCR

conventional
one-step RT-qPCR protocol



零步法 RT-qPCR

0-step RT-qPCR protocol



我们可以使RT-PCR更简单、更快吗?
Can we make RT-PCR easier and shorter?

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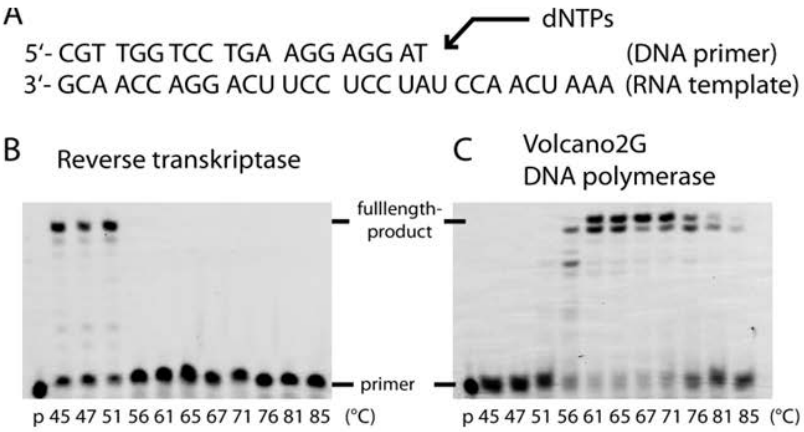
Volcano® DNA polymerase …

RNA -> DNA

… 95°C 半衰期 >40 min

… has a half-life at 95°C of >40 min

真正的热稳定性 — 兼具DNA聚合酶活性、耐高温的逆转录酶
True thermostability — a PCR-active thermostable reverse transcriptase



在更高的温度下进行逆转录有利于处理复杂样品 (如基质、RNA二级结构)，并提高特异性。
Performing reverse transcription at a higher temperature can benefit assays dealing with complex samples (e.g. matrices, RNA secondary structures) and increase specificity

Blatter, N., Bergen, K., Nolte, O., Welte, W., Diederichs, K., Mayer, J., Wieland, M. and Marx, A. (2013), Structure and Function of an RNA-Reading Thermostable DNA Polymerase. Angew. Chem. Int. Ed., 52: 11935–11939. doi: 10.1002/anie.201306655
R. Kranaster, M. Drum, N. Engel, M. Weidmann, F. T. Hufert and A. Marx, (2010), One-step RNA pathogen detection with reverse transcriptase activity of a mutated thermostable Thermus aquaticus DNA polymerase, Biotechnol. J., 5(2), 224-31.
Kranaster R, Zeller J, Kühn B, Marx A, (2016) Neues Enzym mit Reverse Transkriptase- und DNA-Polymerase-Funktion, BioSpektrum, 2, 164-165.

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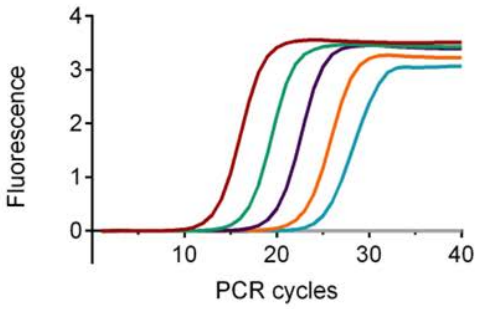
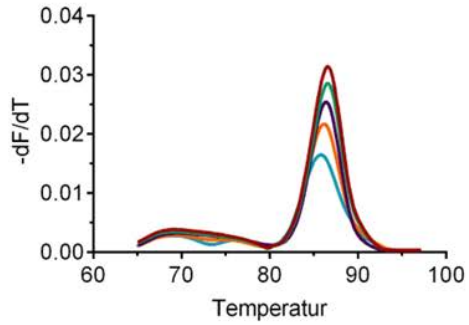
零步法RT-qPCR — 使用 Volcano® RT-PCR Master Mix 实例
0-step RT-qPCR — examples with Volcano® RT-PCR Master Mix





Volcano2G RT-PCR mastermix with GreenDye
qRT-PCR-Assay, hGAPDH mRNA
total RNA dilution series: 100-0.01 ng/reaction
Sybr Green I channel

hGAPDH-Assay

hGAPDH primer fwd	5'-GAAGGTGAAGGTCGGAGTCAAC-3'
hGAPDH primer rev	5'-GCTTCCCGTTCTCAGCCTTG-3'

RT-PCR-Protocol:

	95°C	60 sec	
	95°C	15 sec	(40 cycles)
	60°C	60 sec	
melting:			
	95°C	10 sec	
	65°C	60 sec	
	97°C 1 sec	ramp 0.2°C/sec,	5 readings/°C

-无需单独的反转录步骤
-simplified RT-PCR without isothermal RT-steps

-也适用于染料法
-dsDNA dye compatible

-定量结果更可靠
-reliable quantification

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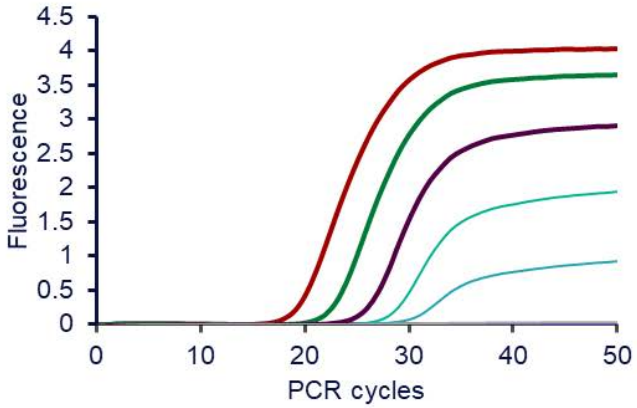
零步法RT-qPCR — 使用 Volcano® RT-PCR Master Mix 实例
0-step RT-qPCR — examples with Volcano® RT-PCR Master Mix

Volcano2G RT-PCR mastermix
IDT PrimeTime qPCR Assay Hs.PT.58.25447000 (from Integrated DNA Technologies),
NONO mRNA, FAM-Channel
total RNA dilution series: 100-0.01 ng/reaction

IDT PrimeTime qPCR Assay Hs.PT.58.25447000, NONO mRNA

NONO primer fwd	5'-TGATGCTTTCTTGGAGTATGGT-3'	
NONO primer rev	5'-AGAGGAGAAGTCGAGGTTAGAG-3'	
NONO probe	5'-/6-FAM/ACTGGGAGG/ZEN/CACTTTGCTGTCTG/IABkFQ/-3'	
RT-PCR-Protocol:	95°C	1 min
	95°C	10 sec
	60°C	1 min (50 cycles)



适用于探针法 (Taqman)
compatible with Taqman probes

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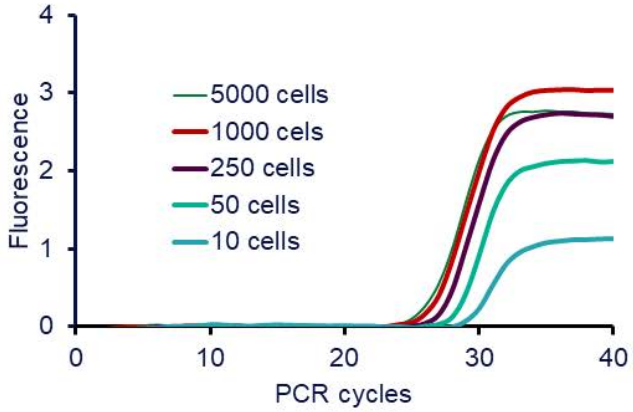
零步法RT-qPCR — 细胞样本直扩 (无需抽提!)
0-step RT-qPCR — direct from Cell samples (no clean-up!)

细胞mRNA直扩：
mRNA detection direct from cells:
Taqman probe-based RT-qPCR, HPRT1 mRNA, Cy5-Channel
fresh HEK-293 dilution series in water: 5000 – 10 cells/reaction,
(cell were detached, resuspended and diluted according to the manual)

HPRT1-Assay

HPRT1 primer fwd	5'-AGACTTTGCTTTCCTTGGTCAG-3'	
HPRT1 primer rev	5'-TCAAGGGCATATCCTACAACAA-3'	
HPRT1 probe	5'-Cy5-AAGCTTGCTGGTGAAAAGGA-BHQ2-3'	
RT-PCR-Protocol:	95°C	1 min
	95°C	15 sec
	60°C	1 min (40 cycles)



无需抽提——细胞样本直扩 PCR
It works also without extraction – directPCR from Cells

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Isotherm3G DNA polymerase

兼具逆转录酶和DNA聚合酶活性

高特异性

快速检测

改造实例 III
Example III

兼具逆转录活性的 LAMP DNA 聚合酶
A LAMP-capable DNA polymerase with reverse transcription activity

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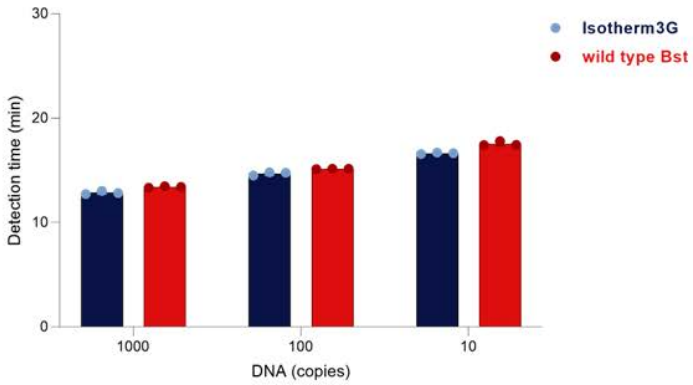
兼具反转录活性的工程化 LAMP 酶
An engineered LAMP enzyme with improved RT-activity

等温扩增
Isothermal amplification



同 Bst DNA 聚合酶一样，可检测 DNA 模板并得到可靠的检测结果
DNA targets are reliably detected as with any other Bst DNA polymerase

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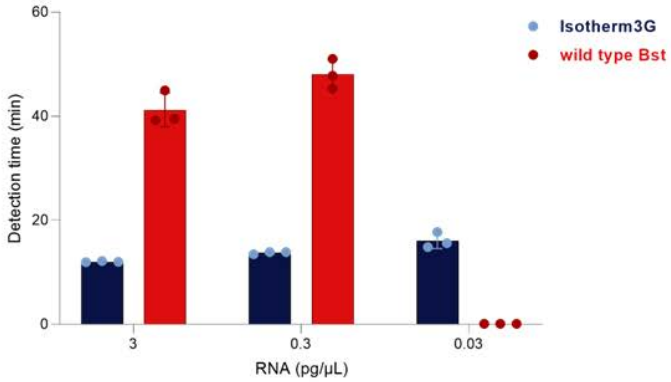
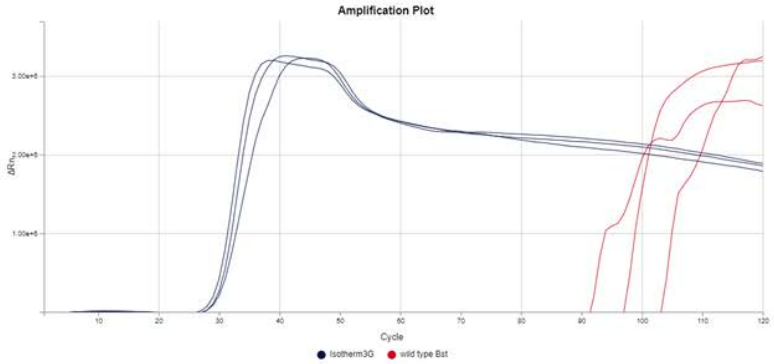
兼具反转录活性的工程化 LAMP 酶
An engineered LAMP enzyme with improved RT-activity





无需添加逆转录酶，可检测 RNA 模板、并在**更短时间内**获得可靠的检测结果。
RNA targets are reliable detected in shorter time with our engineered polymerase
No extra addition of a reverse transcriptase is needed!

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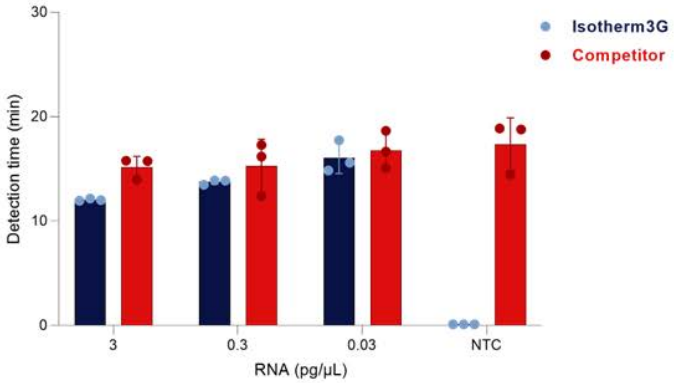
兼具反转录活性的工程化 LAMP 酶
An engineered LAMP enzyme with improved RT-activity



无需添加逆转录酶，可检测 RNA 模板并获得可靠的检测结果。
与其他供应商的酶 (聚合酶3.0版) 相比，无非特异性扩增。
RNA targets are reliable detected with our engineered polymerase
A competitor product (“Polymerase 3.0”) yields unspecific, unreliable signals

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PlexTaq®
5x qPCR Multiplex Master Mix

改造实例 IV
Example IV

粗样本直扩
高耐抑制性
多重检测

高耐抑制性、可直接用于血液样本qPCR的预混液
Inhibitor resistant polymerases a PCR mastermix for real-time DirectPCRs from blood samples

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传统的基因分型方法较单一
Standard Genotyping approach is tedious

血液样本
Blood samples

DNA **提取**
DNA extraction

基因分型
Genotyping

总耗时： > 3 - 4 h
overall time to result: > 3 - 4 h

- 耗时 Time-consuming
- 易出错 Error-prone
- 成本高 Expensive

Medix Biochemica

传统的基因分型方法较单一
Standard Genotyping approach is tedious

血液样本
Blood samples

直扩 PCR
无需 DNA 提取!
directPCR
w/o DNA extraction!

分型
Genotyping

总耗时： 1.5 h
overall time to result: 1.5 h

节省成本，无需提取试剂或设备
No costs for extraction reagents and instruments

更低的出错率
Less error-prone

更高的通量
Higher throughput

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血液直扩、实时定量PCR — PlexTaq® 5x qPCR Multiplex Master Mix
Real-time DirectPCRs from Blood samples – PlexTaq® 5x qPCR Multiplex

- FAM 通道：人类凝血风险因子5-SNP基因靶点
  FAM channel: Human coagulation risk factor 5- SNP gene target
- HEX 通道： spike蛋白对照 (10^4 c/rxn)
  HEX channel: spike IC control target (10^4 c/rxn)
- Cy5 通道：RNaseP靶基因 (人血液样本中存在的) - 靶基因随着血液样本量的变化而自然变化
  Cy5 channel: RNaseP target (human gene present in human blood sample) – target will naturally vary with amount of blood sample
- 不同血液样本的稀释 (EDTA、柠檬酸盐和肝素处理) 0.1%-20% 终浓度
  Dilution of different blood samples (EDTA, citrate and Heparin treated)
  From 0.1% - 20% final concentration

PCR protocol:

Denaturation and lysis of samples)	95°C	2 min (is meant for denaturing	
Denaturation	95 °C	10 sec	50x
Annealing	60 °C	40 sec	
(Fluorescence measurement in		FAM, HEX and Cy5 channel)	
Melting curve measurement in FAM channel: 45 - 85 °C			

我们的预混液可以省略耗时的提取步骤，进行PCR直扩。
Our PCR mix allows directPCRs without time-consuming extraction steps

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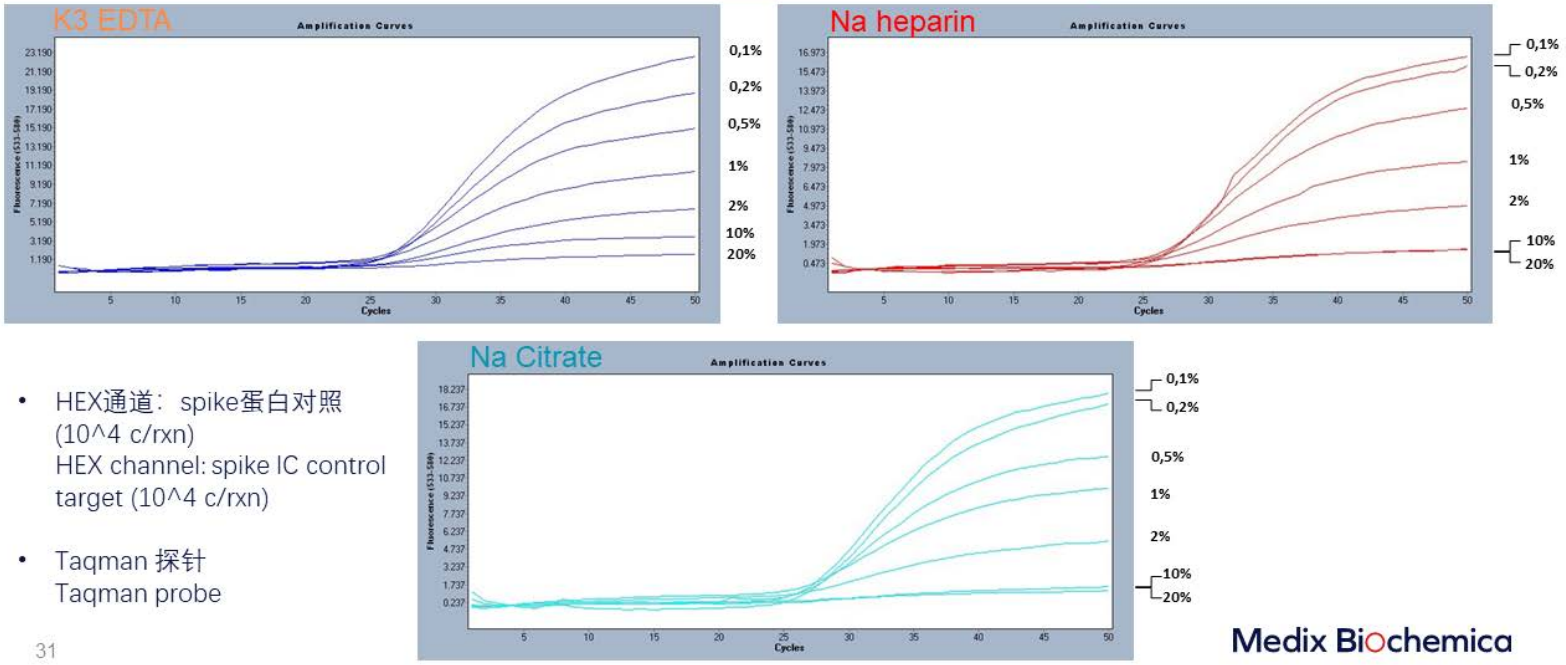
PlexTaq® 5x qPCR Multiplex Master Mix，血液样本直扩，未受任何抑制影响

PlexTaq® 5x qPCR Multiplex doesn't show any inhibition in blood samples



- HEX通道：spike蛋白对照 (10^4 c/rxn)
  HEX channel: spike IC control target (10^4 c/rxn)
- Taqman 探针
  Taqman probe

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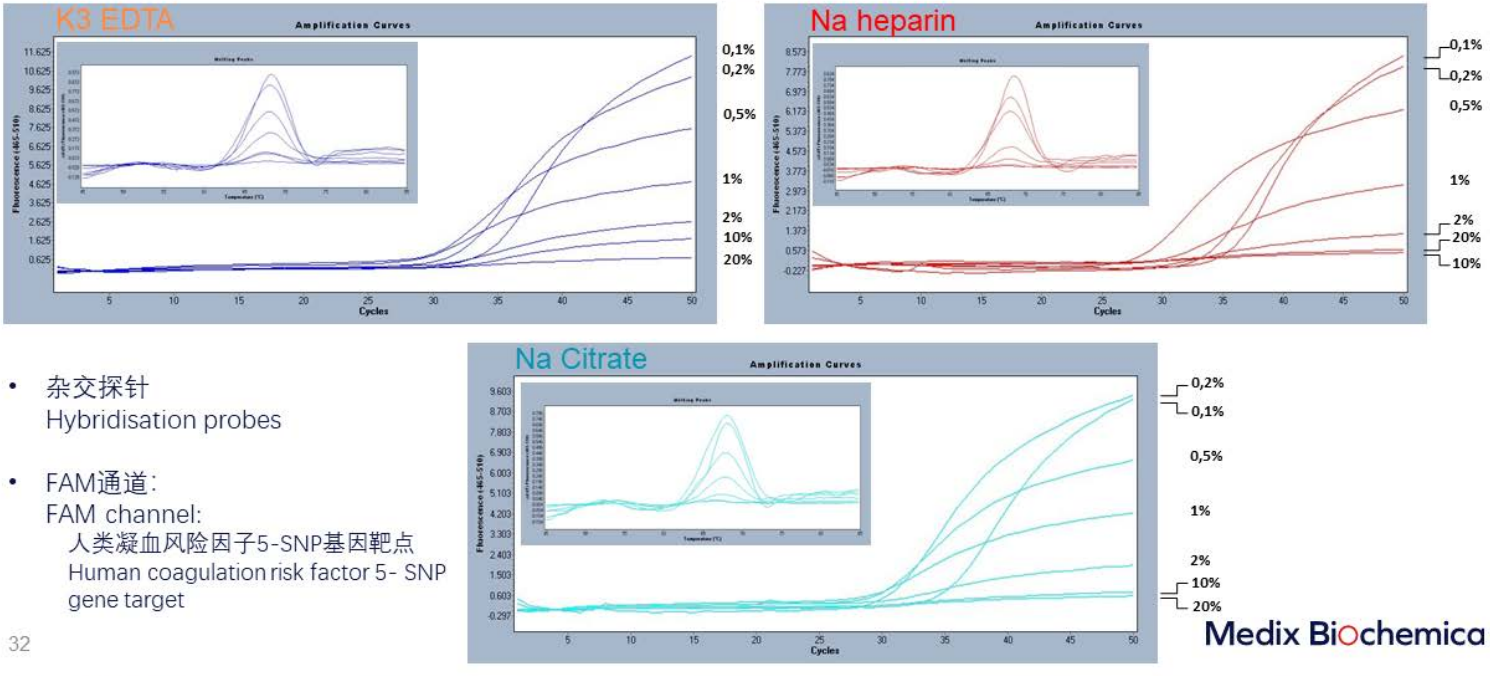
使用 PlexTaq® 进行基因分型PCR，血液样本直扩，无需抽提

Blood samples extraction steps are not needed for Genotyping PCRs with PlexTaq®

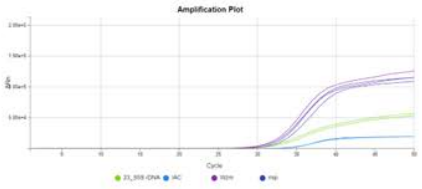


- 杂交探针
  Hybridisation probes
- FAM通道：
  FAM channel:
  人类凝血风险因子5-SNP基因靶点
  Human coagulation risk factor 5- SNP gene target

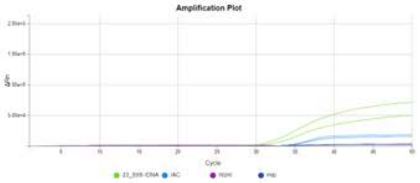
Medix Biochemica

PlexTaq® 预混液用于多重病原体检测
Multiplex pathogen detections with- PlexTaq® 5x qPCR Multiplex

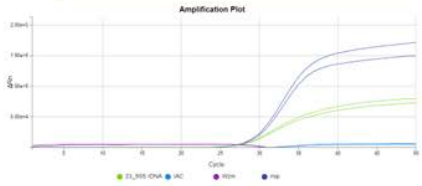
Legionella phenumophila sg1- 0,01 ng/rxn



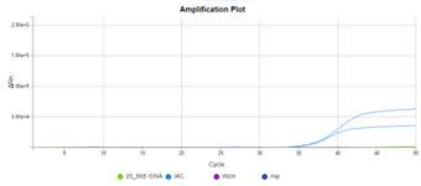
Legionella anisa - 0,01 ng/rxn



Legionella pneumophila SG4- 0,01 ng/rxn



NTC



PlexTaq® 可以支持高度多重检测应用，结果稳定均一、无明显偏差。
PlexTaq® allows high multiplexing and uniform amplification without a strong PCR bias

Medix Biochemica

Taq Hotstart DNA Polymerase

室温下高度稳定
高耐抑制性
多重检测

改造实例 V
Example V

可于室温下进行反应配置——别担心，我们已搞定!

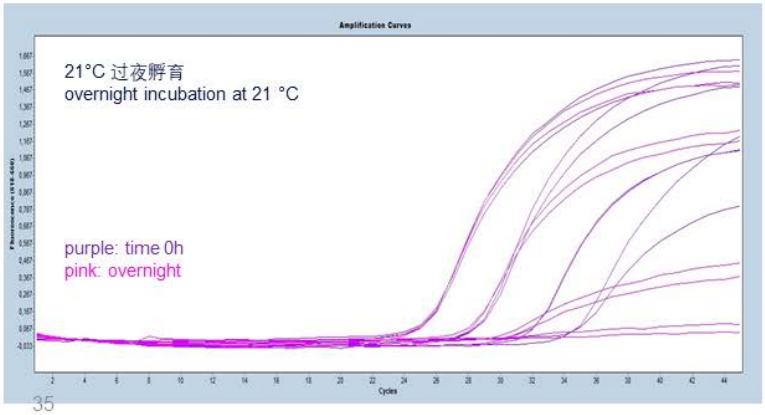
Engineered DNA polymerase for room-temperature reaction setups – don't worry we have it covered!

Medix Biochemica

将包含引物/探针的酶预混液，于室温下孵育
Incubation of a full mastermix including primers and probes at room-temperature

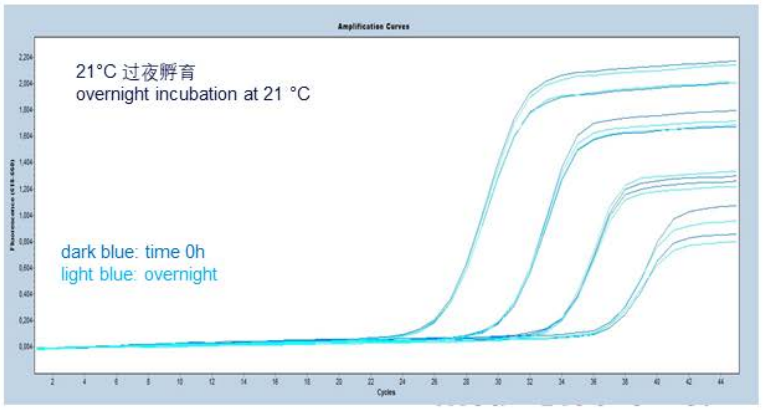
其他供应商Taq DNA 聚合酶经21℃过夜孵育：信号强度减弱、灵敏度降低

Competitors, standard Taq DNA polymerase:
Loss of signal intensities and reduced limit of detections



工程化热启动 Taq DNA 聚合酶经21℃过夜孵育：信号强度及灵敏度未受任何影响

Our engineered, Taq Hotstart DNA Polymerase:
Don't worry anymore about room-temperature setups!

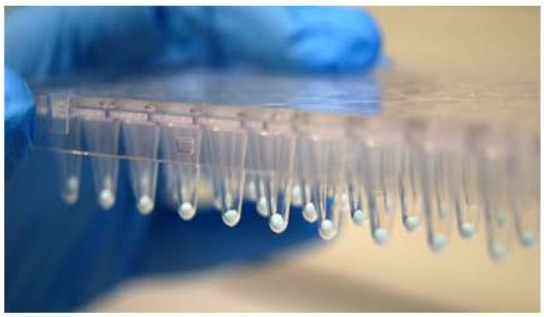


运输不畅时，你会担心产品的质量吗？
Your shipment is stuck, delayed or returned?

每个冻干微球可包含多对引物/探针
Every bead can contain multiple primers and probes

室温运输或储存
Shipping and storage at room-temperature

仅需添加样品即可
Only sample needs to be added



LyoBeads 冻干微球
(1 Bead = 1 rxn) (1冻干微球=1个反应)

Medix Biochemica

让我们的冻干技术为您赋能！
Make use of our freeze-drying capabilities!

冻干粉原料用于多次反应:
1份冻干粉=25次反应
Multiple reactions LyoCakes:
For example 1 Cake = 25 rxns

联系我们获得订制化服务!

Establishement and production of customized products possible.
Get in touch!

Medix Biochemica

总结
Summary

为生物技术应用量身定制DNA聚合酶
Tailoring DNA polymerases for biotec applications.

- 随机突变——DNA聚合酶的工程化改造
  Engineering of DNA polymerases by random mutagenesis
- 人工设计——DNA聚合酶的工程化改造
  Engineering of DNA polymerases by rational designs

专业，应您所需——赋能分子诊断
Your needs are our expertise - make molecular diagnosis capable.

- 突变检测 (HiDi®)
  Mutation detections (HiDi®)
- 耐高温RT-PCR (Volcano®)
  Hot RT-PCR (Volcano®)
- 适用于DNA和RNA模板的通用LAMP酶 (Isotherm3G)
  One LAMP for all (Isotherm3G)
- 血液样本直扩、无需抽提 (PlexTaq® & 血液直扩PCR)
  Skip Blood extraction steps (PlexTaq® & DirectBlood)

让我们的冻干技术为您赋能
Make use of our freeze-drying capabilities

客户定制服务
Other customized service

Medix Biochemica

Thank you

Meet us at booth A6-2609

Medix Biochemica

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