RPA 及其应用简介

---新型核酸扩增技术



默瑞生物 市场部

目录

RPA---一种恒温核酸扩增技术

Recombinase Polymerase Amplification

重组酶 聚合酶 扩增技术

RPA是由多种酶和蛋白参与下，在恒温条件下实现核酸指数扩增的技术

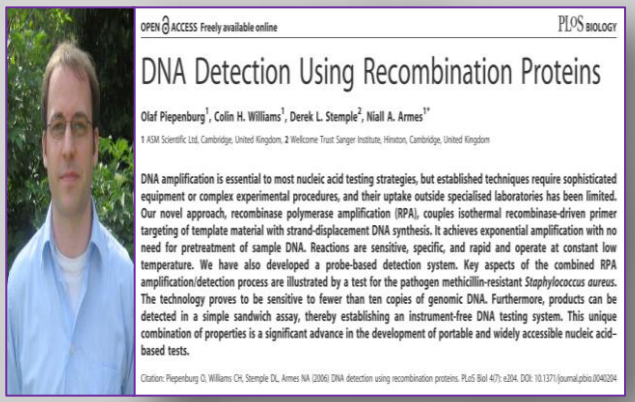
- 高灵敏度，高特异性，反应快速
- 无需温度循环仪（themocycler）
- 非常适用于POCT, 疾病诊断,流行病学监测,食品安全检测,动物病原物检测等

RPA---英国剑桥TwistDx公司专利技术

2006年

2010年

2014年



OPEN ACCESS Freely available online PLOS BIOLOGY

DNA Detection Using Recombination Proteins

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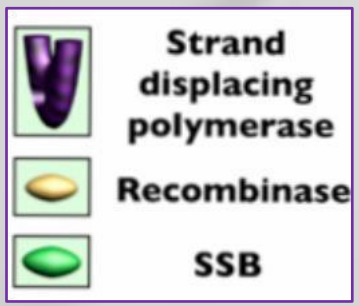
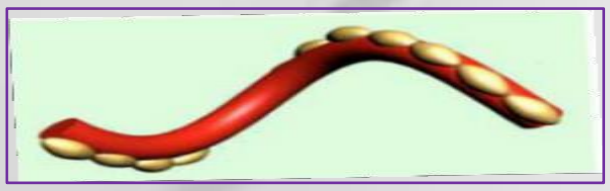
DNA amplification is essential to most nucleic acid testing strategies, but established techniques require sophisticated equipment or complex experimental procedures, and their uptake outside specialised laboratories has been limited. Our novel approach, recombinase polymerase amplification (RPA), couples isothermal recombinase-driven primer targeting of template material with strand-displacement DNA synthesis. It achieves exponential amplification with no need for pretreatment of sample DNA. Reactions are sensitive, specific, and rapid and operate at constant low temperature. We have also developed a probe-based detection system. Key aspects of the combined RPA amplification/detection process are illustrated by a test for the pathogen methicillin-resistant *Staphylococcus aureus*. The technology proves to be sensitive to fewer than ten copies of genomic DNA. Furthermore, products can be detected in a simple sandwich assay, thereby establishing an instrument-free DNA testing system. This unique combination of properties is a significant advance in the development of portable and widely accessible nucleic acid-based tests.

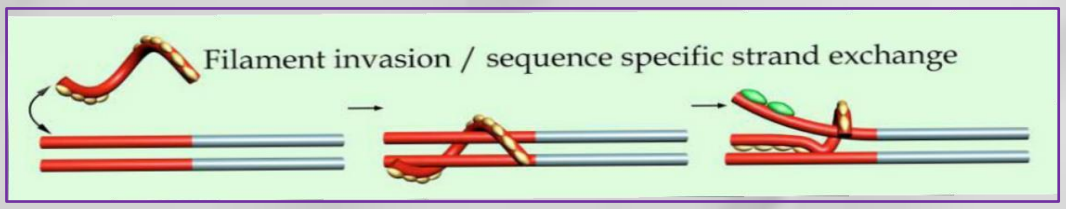
Citation: Piepenburg O, Williams CH, Stemple DL, Armes NA (2006) DNA detection using recombination proteins. PLoS Biol 4(7): e204. DOI: 10.1371/journal.pbio.0040204

RPA—酶反应驱动的核酸扩增技术

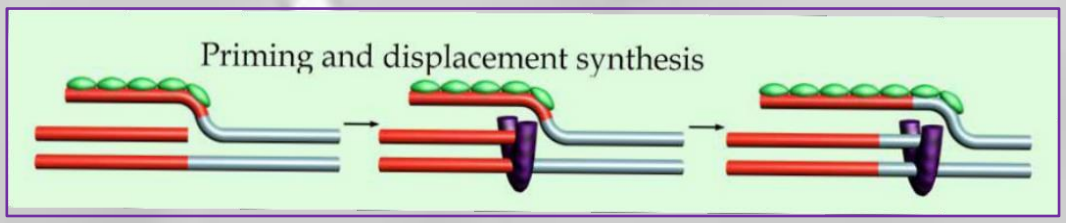




核酸蛋白复合物合成



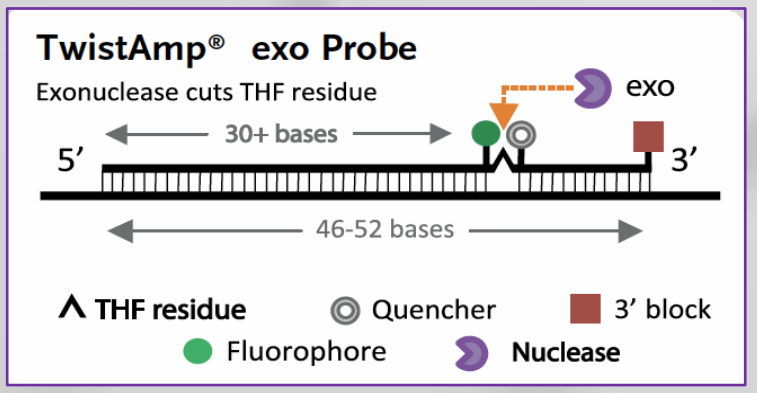
D环形成；单链固定



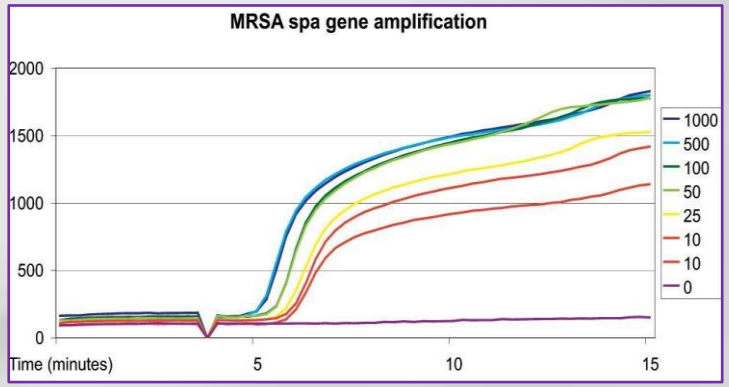
链置换；延伸

RPA---特异性高的探针检测系统

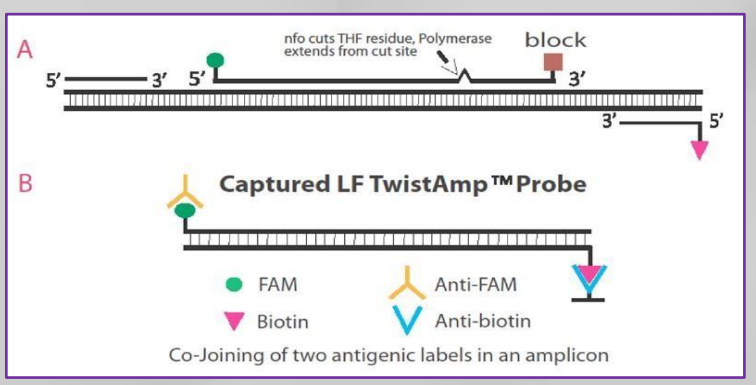
TwistAmp exo system---Real time RPA

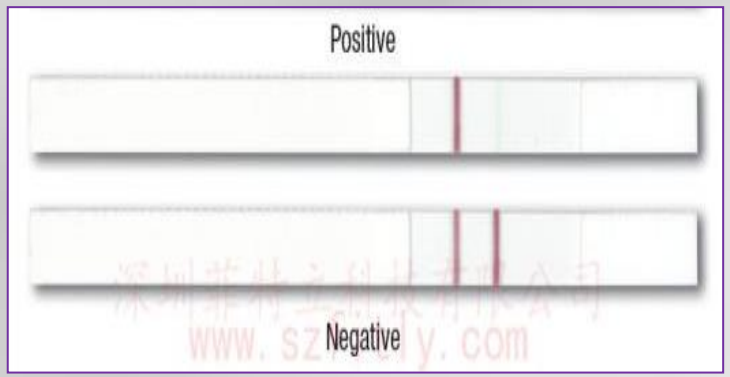


检测方法

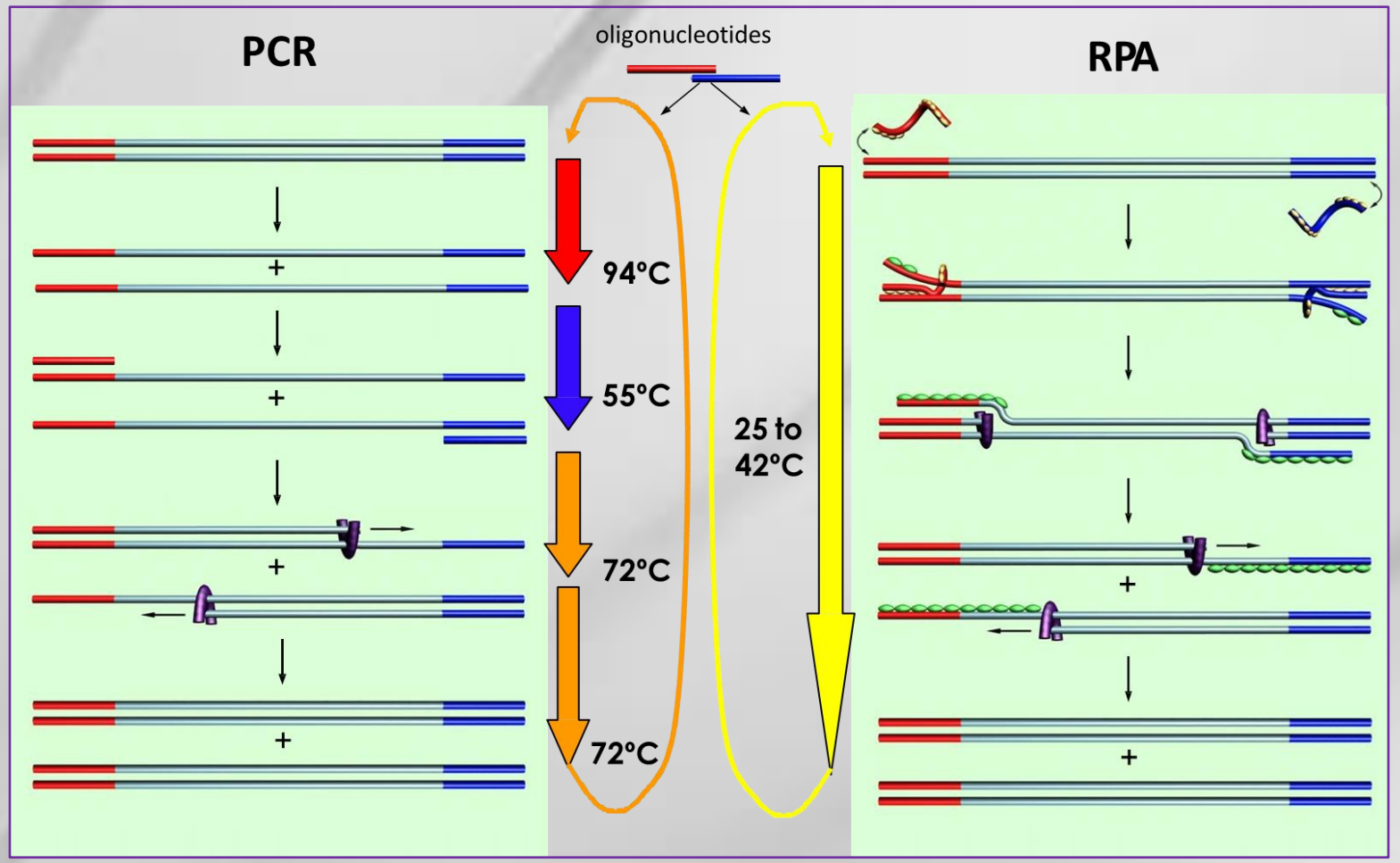


TwistAmp nfo system---LFD RPA





RPA---低能耗的核酸扩增技术



RPA---最快的恒温扩增技术

Table 1 Characteristics of RPA and other isothermal amplification techniques

Techniques	Templates	No. of Primers	No. of Enzymes	Pre-heating	Temperature (℃)	Time (min)	Products type
RPA	DNA, RNA	2	2	No	25 ~ 42	20	DNA
LAMP	DNA	4 ~ 6	1	No	60 ~ 65	60 ~ 90	DNA
SDA	DNA	4	2	Yes	37	120	DNA
MDA	DNA	Random hexamer primers	1	No	30	480 ~ 960	DNA
RCA	Circular DNA	1	2	No	37	60	Circular DNA
HDA	DNA, RNA	2	2	No	37, 60 ~ 65	120	DNA
NASBA	DNA, RNA	2	2 ~ 3	Yes	37 ~ 42	90 ~ 120	RNA
SMART	DNA, RNA	2	2 ~ 3	Yes	41	120	DNA

RPA---产品性能卓越，应用广泛

	LAMP(环介导等温扩增)试剂盒	**RPA试剂盒**
主要组分	链置换DNA聚合酶	重组酶；单链结合蛋白；链置换DNA聚合酶
最适反应温度	60-65℃	37-40℃
推荐反应时间	40min左右	20min左右
引物数	2对	1对
保存形式	液体	冻干粉
检测方式	荧光目视；比浊法实时浊度仪	电泳；试纸条（肉眼观察）；荧光定量
扩增产物可否用于下游应用	否	是
可否Multiplex/多重反应	否	是

RPA---产品线齐全，最大限度满足客户需求

TwistAmp系列—供客户自行研发

A Basic kit：凝胶电泳检测		无需任何仪器耗材 凝胶电泳检测 适用样本：DNA,RNA
B Exo kit：荧光定量检测		需要荧光扩增仪 荧光定量检测 适用样本：DNA,RNA
C Nfo kit：侧流试纸条检测		需要胶体金试纸条 终点检测 适用样本：DNA

RPA---产品线齐全，最大限度满足客户需求

Twirla™ *mixing incubator*

Ball Dispenser MICRO

Bearing Balls

New!

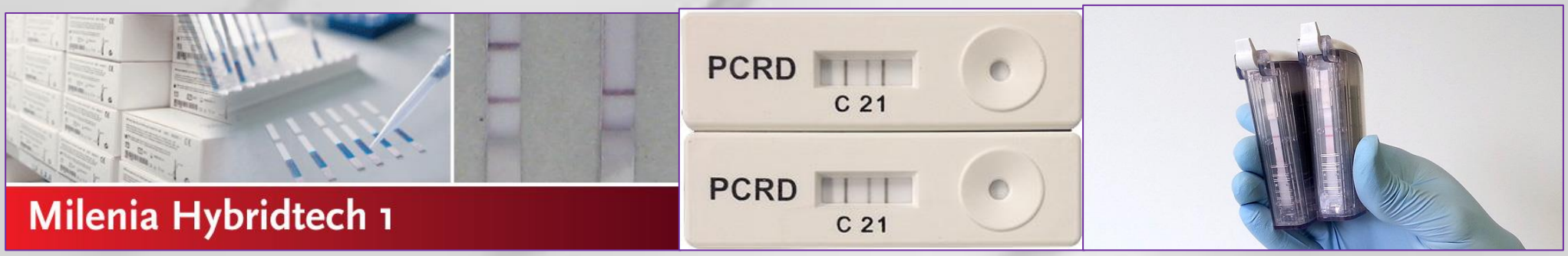
T-8 Isothermal Device

T-16 Isothermal Device

通量：8 samples 净重：1.9kg
双通道： FAM; HEX
触屏操作，含混匀功能
仪器或者PC读取

通量：16 samples 净重：1.9kg
三通道： FAM; HEX ROX
触屏操作，含混匀功能
仪器或者PC读取

RPA---产品线齐全，最大限度满足客户需求



侧流检测试纸条

Tough carry case

External battery

RPA---病原体检测神器

Real-time RPA检测结核分支杆菌（MTB)

OPEN ACCESS Freely available online

PLOS ONE

Rapid Detection of *Mycobacterium tuberculosis* by Recombinase Polymerase Amplification

David S. Boyle[1☯], Ruth McNerney[2☯], Hwee Teng Low[2], Brandon Troy Leader[1], Ailyn C. Pérez-Osorio[3], Jessica C. Meyer[4], Denise M. O'Sullivan[2], David G. Brooks[4], Olaf Piepenburg[4], Matthew S. Forrest[4]*

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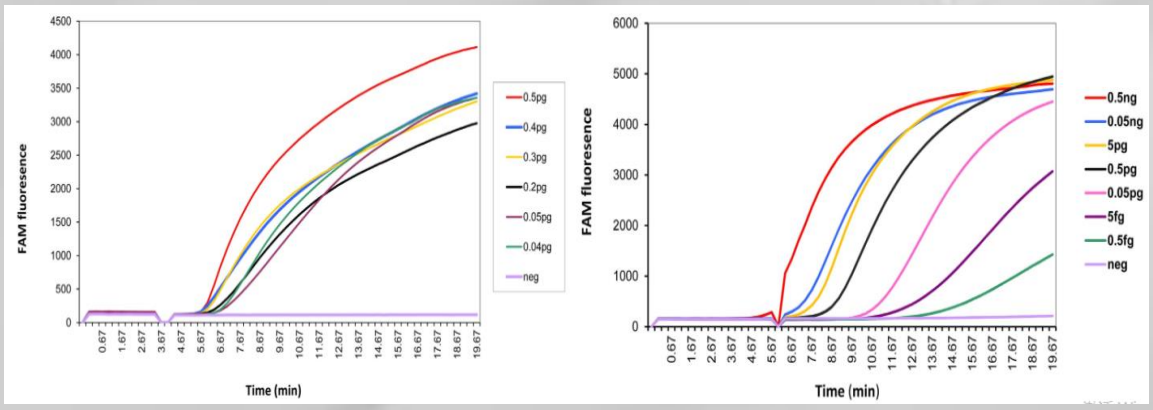

Table 4. Tuberculosis detection by indirect smear microscopy vs RPA IS*6110*.

	Culture positive	Culture negative
Indirect smear microscopy positive	34	5
Indirect smear microscopy negative	14	37
RPA *IS6110* positive	42	2
RPA *IS6110* negative	6	40

Testing pulmonary specimens (n = 90) by indirect smear microscopy and RPA IS*6110* to detect tuberculosis, with comparison to liquid culture based test data. RPA *IS6110* was more sensitive than indirect smear microscopy (87.5% (95% CI: 81.7, 93.2) vs 70.8% (95% CI: 62.91, 78.75)) and also more specific (95.4 (95% CI: 92.3, 98.1) vs 88% (95% CI: 83.6, 92.4)).

doi:10.1371/journal.pone.0103091.t004

Table 5. Tuberculosis detection by indirect smear microscopy vs RPA IS*1081*.

	Culture positive	Culture negative
Indirect smear microscopy positive	31	4
Indirect smear microscopy negative	5	31
RPA *IS1081* positive	32	0
RPA *IS1081* negative	4	35

Testing pulmonary specimens (n = 71) by indirect smear microscopy and RPA IS*1081* to detect tuberculosis, with comparison to liquid culture based test data. RPA *IS1081* was more sensitive than indirect smear microscopy (91.4% (95% CI: 85,98.9) vs 86.1% (95% CI: 78.1, 94.1)) and also more specific (100% vs 88.6% (95% CI: 80.8, 96.1)).

doi:10.1371/journal.pone.0103091.t005

RPA---病原体检测神器

RT-RPA检测禽流感病毒H7N9

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Diagnostics-in-a-Suitcase: Development of a portable and rapid assay for the detection of the emerging avian influenza A (H7N9) virus

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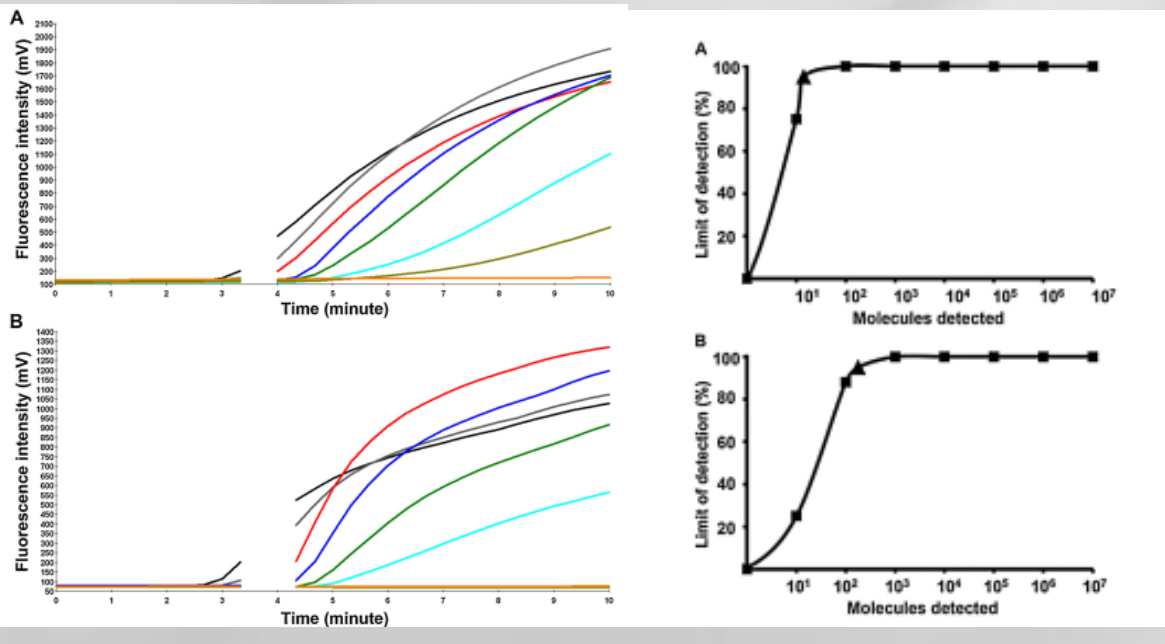
[d] Institute of Microbiology and Virology, Brandenburg Medical School Theodor-Fontane, Fehrbelliner Straße 38, 16816 Neuruppin, Brandenburg, Germany

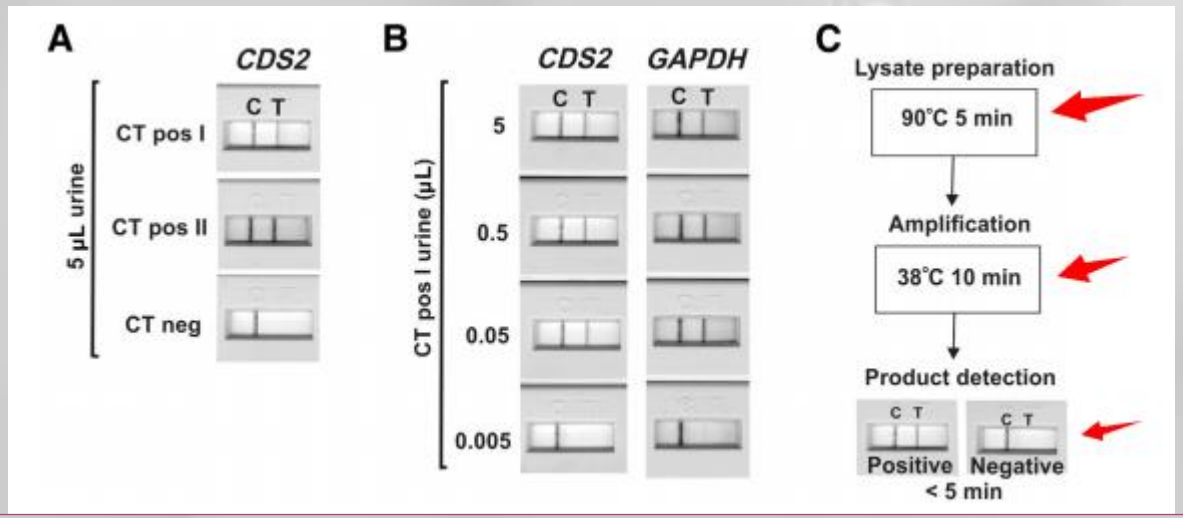


RPA---病原体检测神器

LFD-RPA检测沙眼衣原体

Sensitive and Rapid Detection of *Chlamydia trachomatis* by Recombinase Polymerase Amplification Directly from Urine Samples

Katrin Krõlov,* Jekaterina Frolova,* Oana Tudoran,*† Julia Suhorutsenko,* Taavi Lehto,* Hiljar Sibul,* Imre Mäger,* Made Laanpere,‡ Indrek Tulp,§¶ and Ülo Langel*‖



RT-RPA检测埃博拉病毒

SCIENTIFIC REPORTS

OPEN

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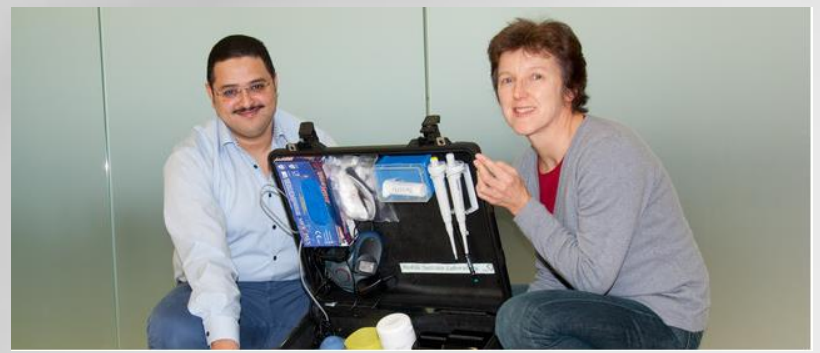
Development and Evaluation of a Rapid and Sensitive EBOV-RPA Test for Rapid Diagnosis of Ebola Virus Disease

Mingjuan Yang[1,*], Yuehua Ke[1,2,*], Xuesong Wang[1,2,*], Hang Ren[1,*], Wei Liu[1,2,*], Huijun Lu[2,3,*], Wenyi Zhang[1,2], Shiwei Liu[4], Guohui Chang[1,2], Shuguang Tian[1,2], Lihua Wang[2,5], Liuyu Huang[1], Chao Liu[1,2], Ruifu Yang[2,6,7] & Zeliang Chen[1,2,8]



RPA---与其他技术结合，应用潜力巨大

Lab on a Chip

PAPER

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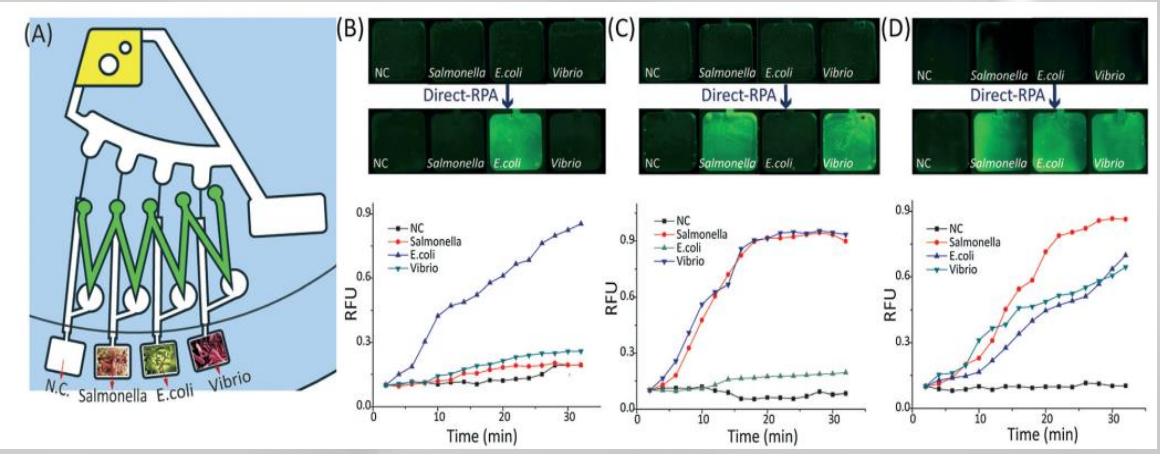
A centrifugal direct recombinase polymerase amplification (direct-RPA) microdevice for multiplex and real-time identification of food poisoning bacteria†

Cite this: *Lab Chip*, 2016, 16, 2309

Multiple RPA+ 微流控



METHODOLOGY ARTICLE Open Access

Optimizing illumina next-generation sequencing library preparation for extremely at-biased genomes

Samuel O Oyola[1*], Thomas D Otto[1], Yong Gu[1], Gareth Maslen[1], Magnus Manske[1], Susana Campino[1], Daniel J Turner[2], Bronwyn MacInnis[1], Dominic P Kwiatkowski[1], Harold P Swerdlow[1] and Michael A Quail[1]

RPA+NGS

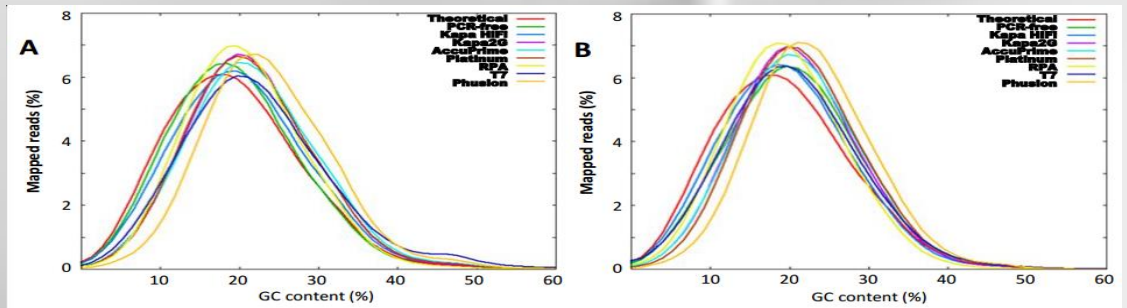
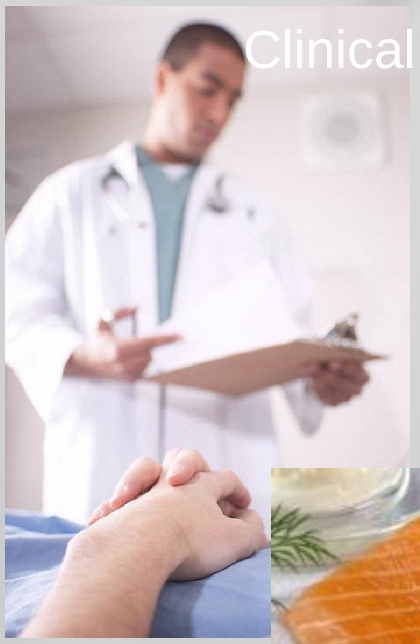


Table 1 Average GC content

Sample	Library	PCR-free	Kapa HiFi	Kapa2G	AccuPrime	Platinum	RPA	T7	Phusion
3D7	Av. %GC content	19.50	20.35	21.44	21.93	21.47	20.75	22.63	23.80
PK0076	Av. %GC content	21.92	19.79	21.07	21.37	21.91	19.66	20.95	22.87

Calculated values of average GC content corresponding to each library preparation. Deviation from the 19.4% (value of the unamplified genome) indicates the effect of amplification bias. See Figure 3 A & B for a global graphical presentation of the GC content distribution. 3D7, *P. falciparum* strain 3D7; PK0076, *P. falciparum* clinical isolate.

RPA---核酸检测技术革命



总结：RPA是一种快速，灵敏，高效的核酸扩增系统，适用于多种应用场景，搭配其他分子检测技术，，有望替代PCR的分子生物学垄断地位，掀起一场核酸检测技术革命。

Thank you for your attention

更多问题

RPA官方网站：http://www.twistdx.co.uk/

RPA中文网：http://twistdx.com.cn/

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