基于CRISPR的下一代分子诊断

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CRISPR：“上帝手术刀”

Science
AAAS

2012 Top 10 Breakthrough of the year

TALENs: Genomic Cruise Missiles

2013 Top 10 Breakthrough of the year

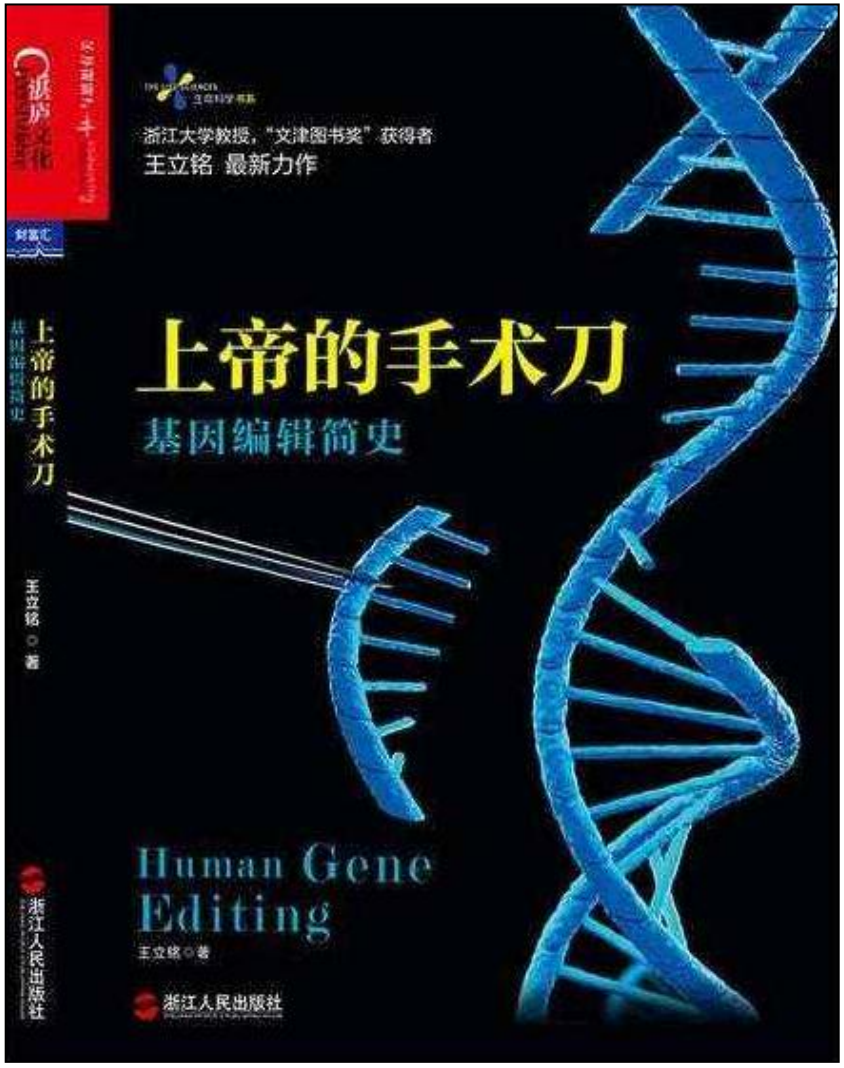
CRISPR/Cas: Genetic Microsurgery for the Masses

2015 Top 10 Breakthrough of the year

CRISPR genome-editing technology shows power

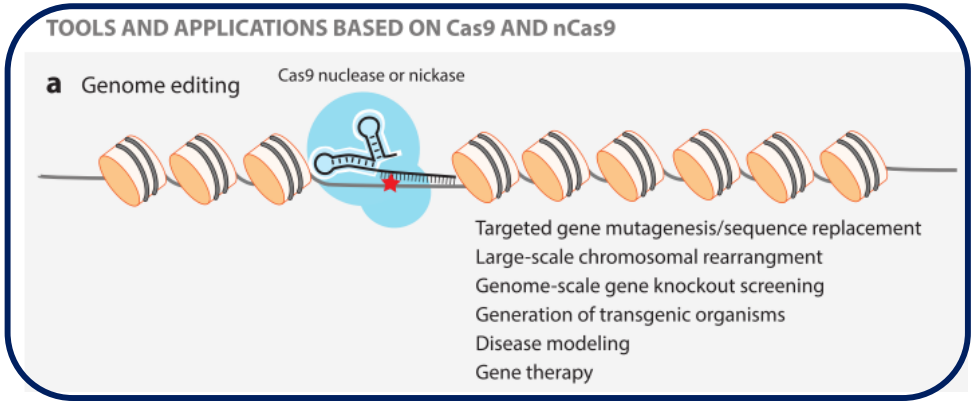
2017 Top 10 Breakthrough of the year

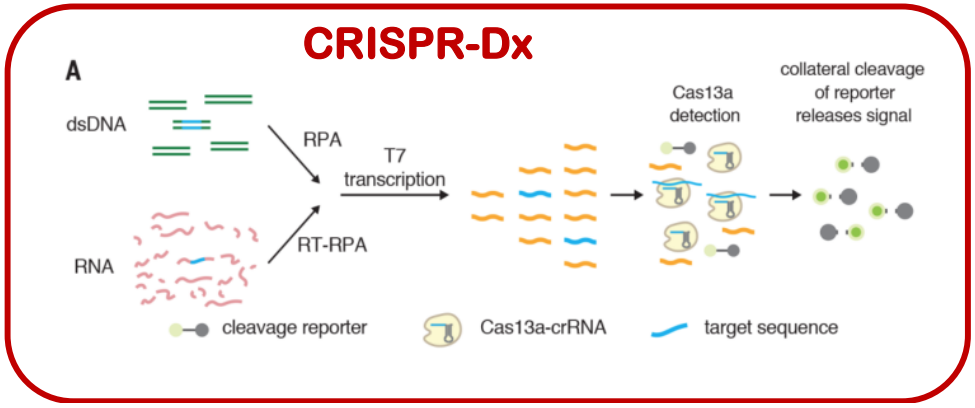
Pinpoint gene editing



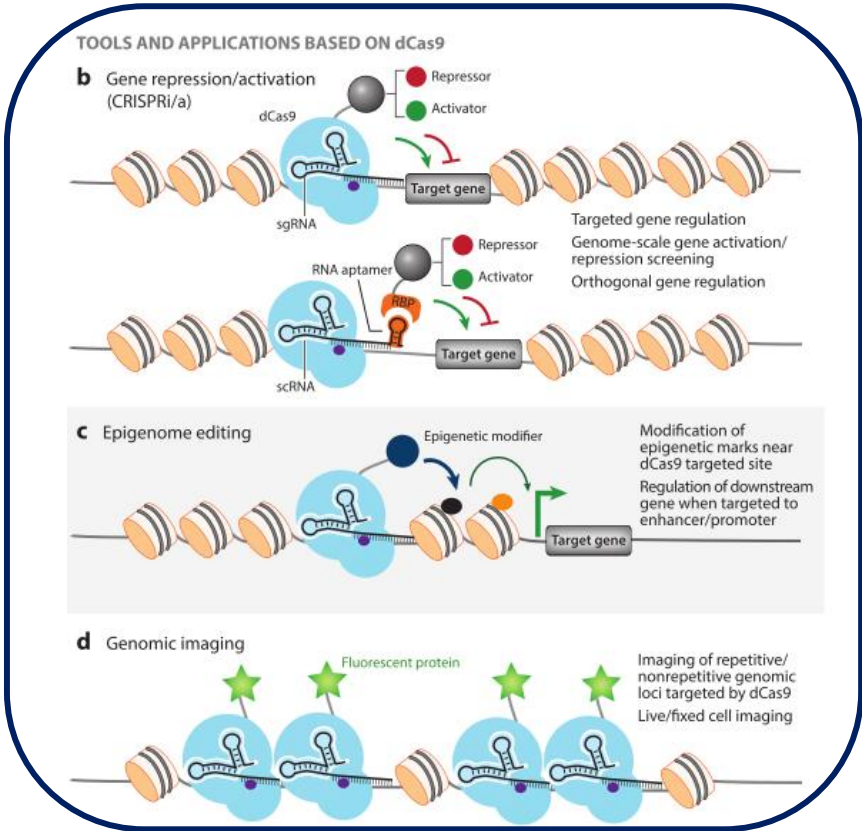
CRISPR的应用

Cas Protein



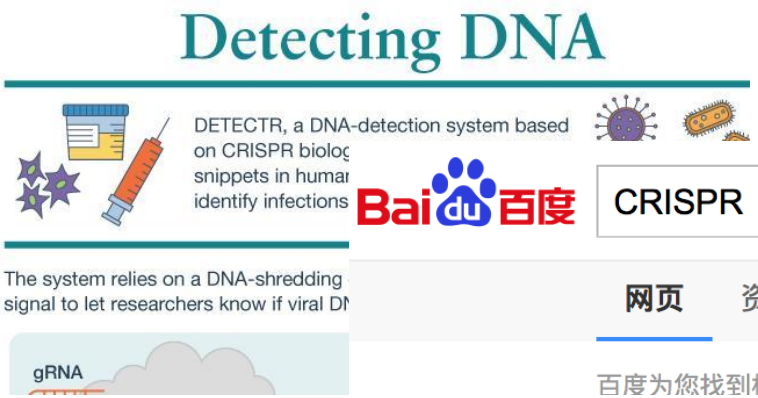


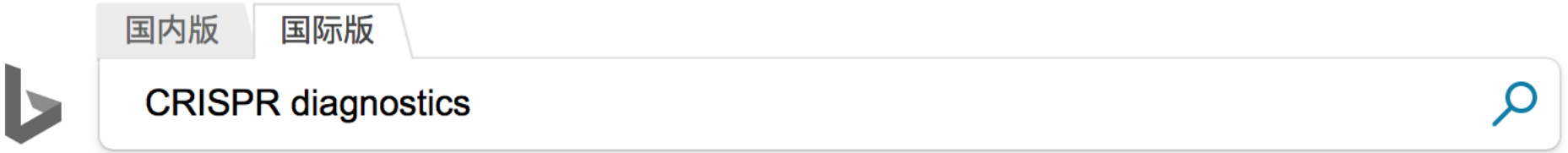
dCas Protein



Wang, *et al.* 2016; Gootenberg, *et al.* 2017

Science力推CRISPR诊断技术





百度有接近500万条关于CRISPR诊断的中文检索结果；必应则有超过1200万检索结果。

12,200,000 Results Any time

Scientists unveil CRISPR-based diagnostic platform. In their paper and patent filing, the team described a wide range of biotechnological applications for the system, including harnessing RNA cleavage and collateral activity for basic research, diagnostics, and therapeutics.

与吐露港生物联合HOLMES核酸快检技术

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(12)发

Cell Research

www.nature.com
www.cell-research.c

HOLMES（福尔摩斯）：

one-HOur Low-cost Multipurpose highly Efficient System

Shi-Yuan Li[1], Qiu-Xiang Cheng[2], Jing-Man Wang[3], Xiao-Yan Li[2], Zi-Long Zhang[4], Song Gao[5], Rui-Bing Cao[6], Guo-Ping Zhao[1,7] and Jin Wang[1]

Dear Editor,

Today, the need for time-effective and cost-effective nucleic acid detection methods is still growing in fields such as human genotyping and pathogen detection. Using synthetic biomolecular components, many methods have been developed for fast nucleic acid detection[1–3]; however, they may not be able to satisfy specificity, sensitivity, speed, cost and simplicity at the same time. Recently, a very promising CRISPR-based diagnostic (CRISPR-Dx) (namely SHERLOCK) was established, which was based on the collateral effect of an RNA-guided and RNA-targeting CRISPR effector, Cas13a[4]. SHERLOCK is of high sensitivity and specificity, and is very convenient in

trans-cleave non-targeted ssDNA reporter in the system, illuminating the HEX fluorescence (or any other fluorescence) (Fig. 1a).

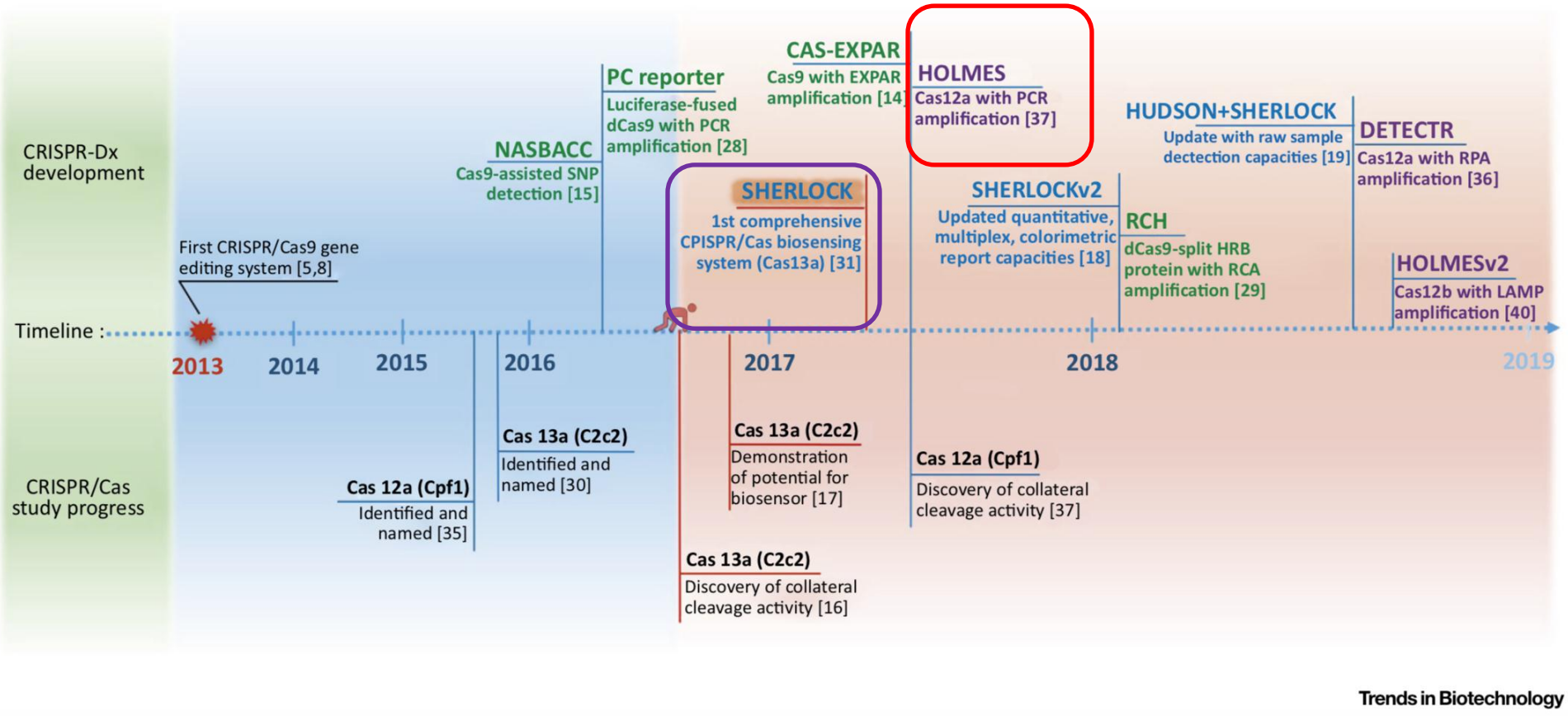
We ever purified ten Cas12a proteins (Supplementary Table S3) and found all showed the ssDNA *trans*-cleavage activity[6]. To find the most suitable Cas12a for HOLMES (i.e., with high signal-to-noise ratios), we tested all ten Cas12a proteins and found *Lachnospiraceae bacterium* ND2006 Cas12a (LbCas12a), *Oribacterium sp.* NK2B42 Cas12a (OsCas12a), *Lachnospiraceae bacterium* NC2008 Cas12a (Lb5Cas12a) and *Francisella tularensis* Cas12a (FnCas12a) showed good performance, among which LbCas12a was chosen for the following studies (Fig. 1b).

majority of the ternary complex most likely remained bound
the targeted ssDNAs after *cis*-cleavage, protecting the labelled
terminus from exposing the *trans*-activity sites of the Cas
ternary complex.

Next, we tested nine randomly selected Cas12a proteins fr
different species in addition to the above tested FnCas
(Supplementary information, Figures S5, S6a and Tables S1,
and S5), and all Cas12a proteins exhibited endonuclease acti
on plasmid dsDNA (Supplementary information, Figure S6b),
(Supplementary information, Figure S6c) and *trans*-cleava
activities on ssDNA (Supplementary information, Figure S6d).
indicates that the *cis*- and *trans*-cleavage activities on ssD
might be ubiquitous among Cas12a proteins.

When shortened targeted ssDNAs were tested, complexes w
18-nt target ssDNAs that lacked a cleavage site also showed *tra*
cleavage activity (Supplementary information, Figure S7a), indic
ing that *cis*-cleavage was not a prerequisite for *trans*-cleava

CRISPR分子诊断的发展历程



Li *et al.*, *Trends in Biotech.*, 2018

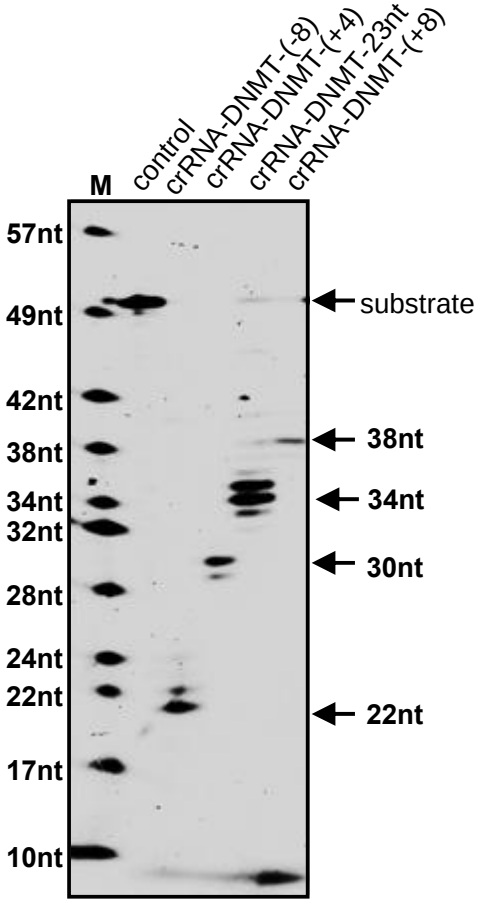
2019年9月15日，华山医院感染科与微远基因运用CRISPR技术，快速检测结核分枝杆菌的首篇研究成果被SCI杂志《Emerging Microbes & Infections》接收（2018年最新影响因子6.2），**标志着华山-微远感染精准医学转化研究中心成立以来的重大阶段性进展，两家单位联合针对结核检测开发的CRISPR-MTB技术，有望成为新一代结核诊断工具。**

很高兴看到CRISPR检测技术的第一篇临床论文发表！

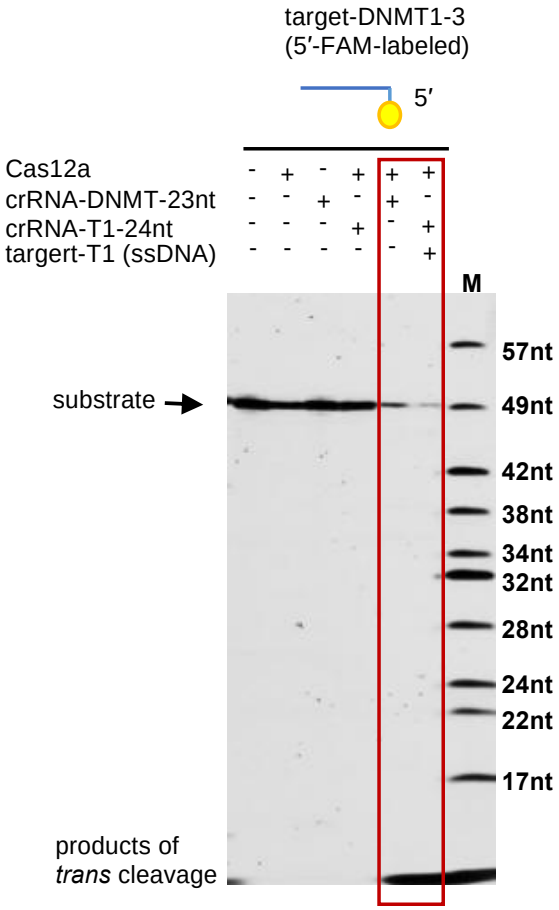
ABSTRACT

Rapid and simple-to-use diagnostic methods for tuberculosis are urgently needed. Recent development has unveiled the diagnostic power of the CRISPR system in the detection of viral infections. However, its potential use in detecting the *Mycobacterium tuberculosis* complex (MTB) remained unexplored. We developed a rapid CRISPR-based assay for TB detection and conducted a retrospective cohort study of 179 patients to evaluate the CRISPR-MTB test for identifying MTB in various forms of direct clinical samples. Its diagnostic performance was compared, in parallel with culture and the GeneXpert MTB/RIF assay (Xpert). The CRISPR-MTB test is highly sensitive with a near single-copy sensitivity, demands less sample input and offers shorter turnaround time than Xpert. When evaluated in the clinical cohort of both pulmonary and extra-pulmonary tuberculosis, the CRISPR-MTB test exhibited an overall improved sensitivity over both culture (79% vs 33%) and Xpert (79% vs 66%), without comprise in specificity (62/63, 98%). The CRISPR-MTB test exhibits an improved overall diagnostic performance over culture and Xpert across a variety of sample types, and offers great potential as a new diagnostic technique for both pulmonary and extra-pulmonary tuberculosis.

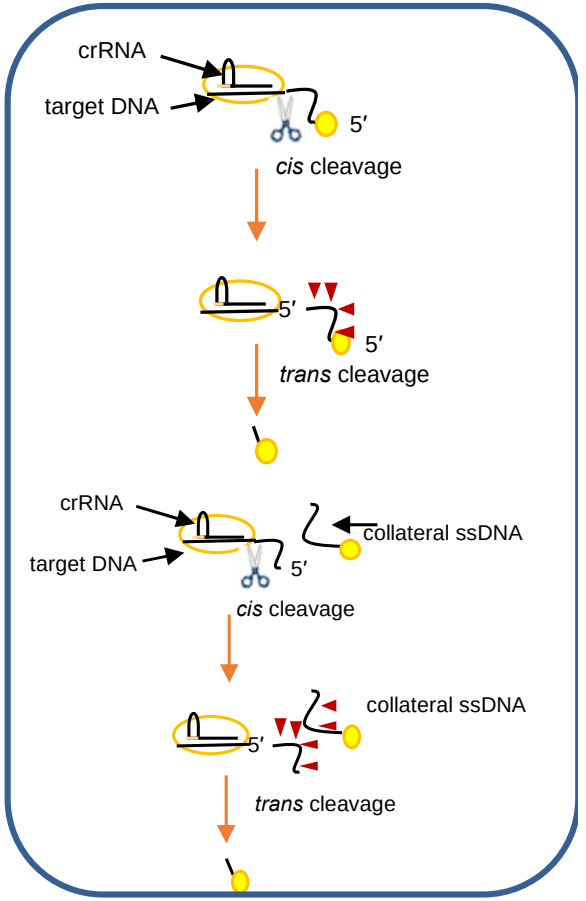
Cas12a非理性切割ssDNA



（理性切割）

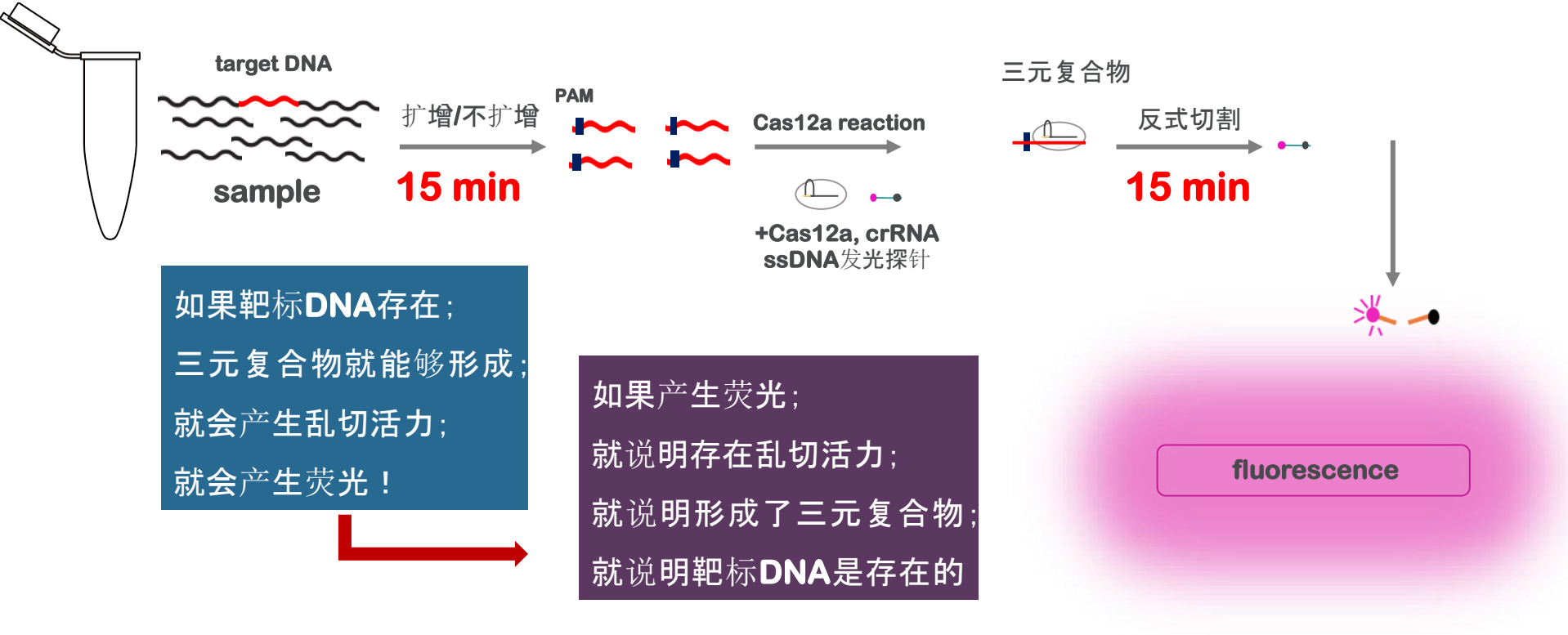


（非理性切割）



Li *et al., Cell Res,* 2018

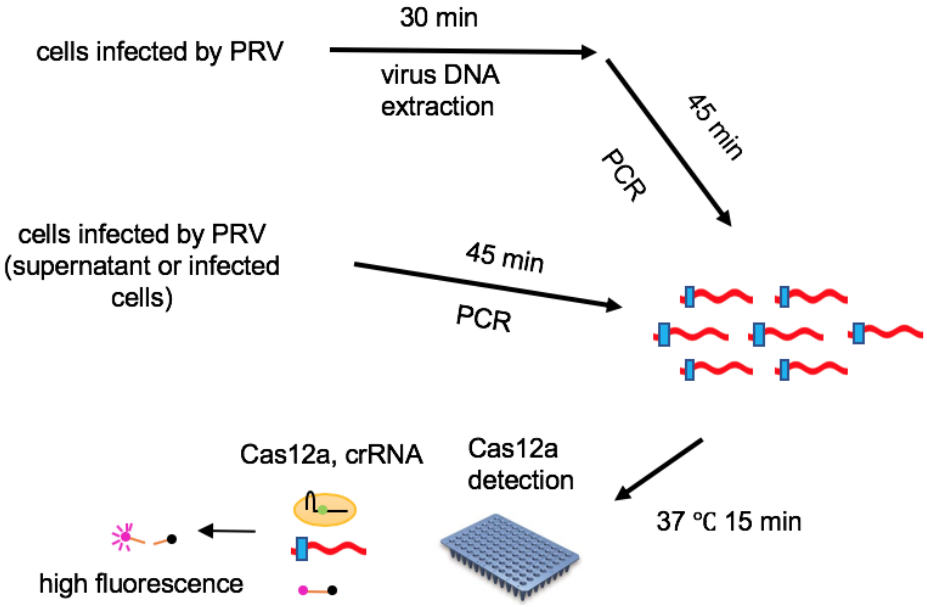
基于非理性切割特点开发的HOLMES

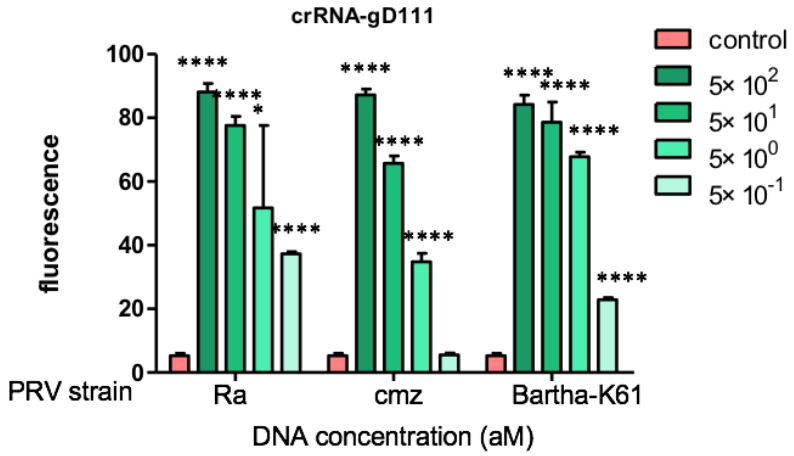


特点：灵敏度高；快速；特异性强；傻瓜式操作；全封闭；成本低。

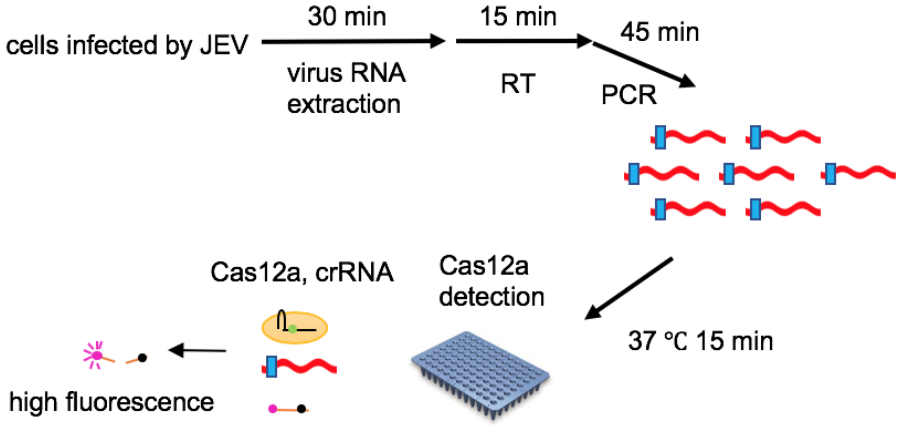
Li *et al., Cell Dis,* 2018

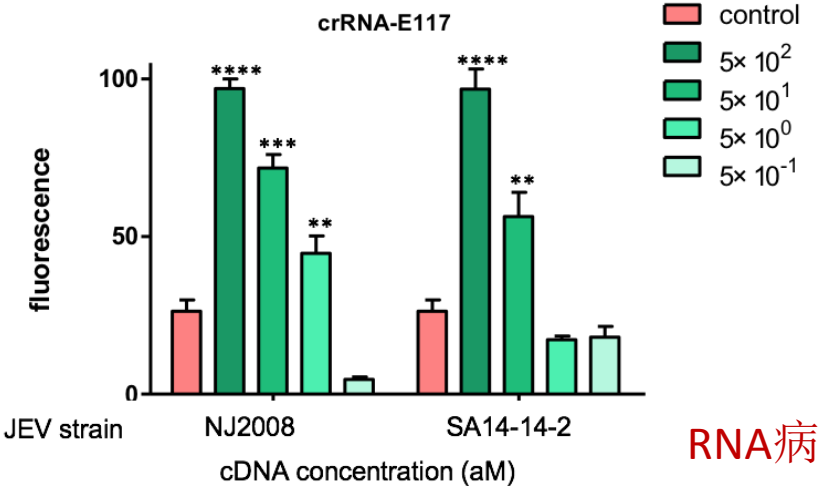
HOLMES高灵敏检测DNA/RNA病毒





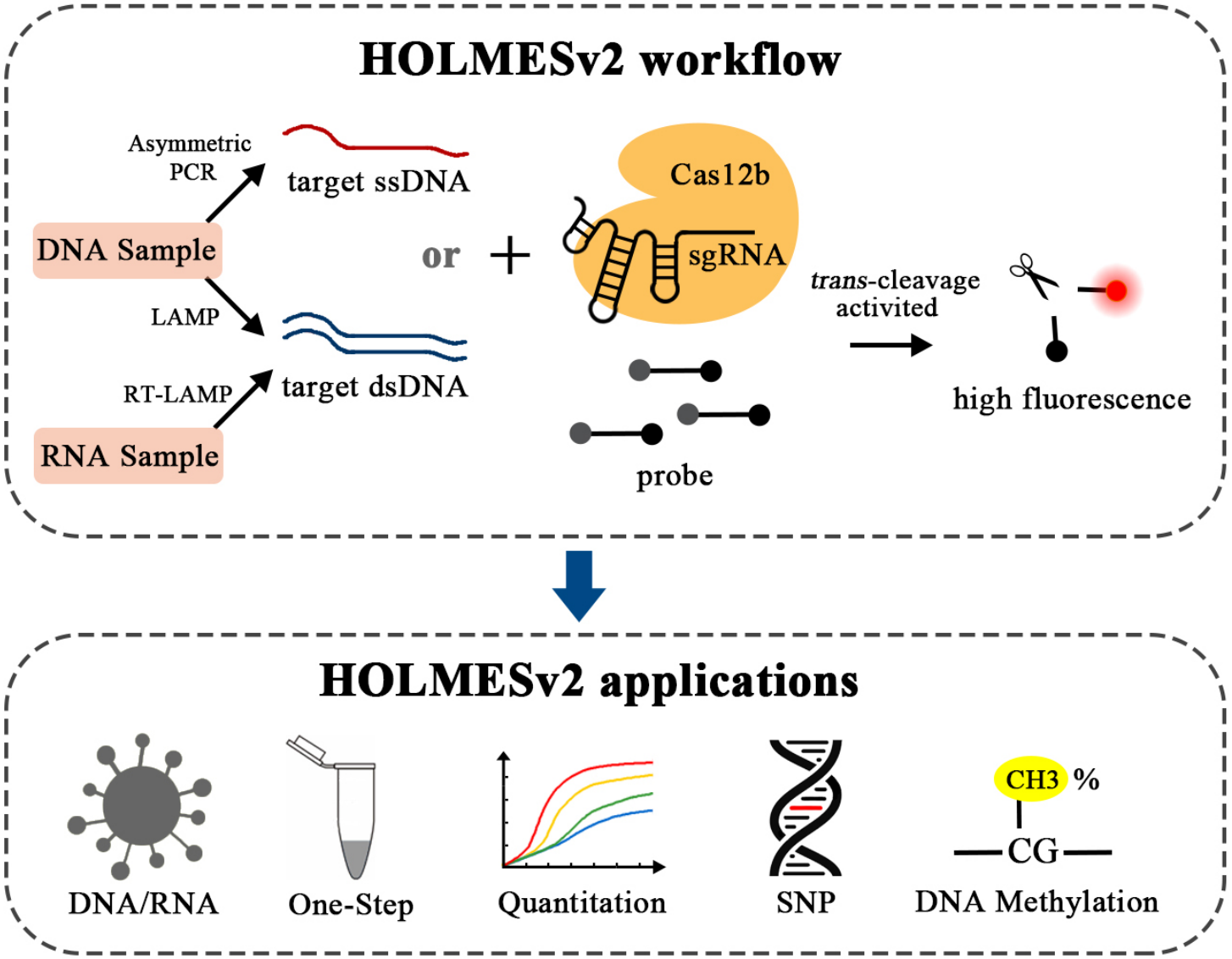
DNA病毒





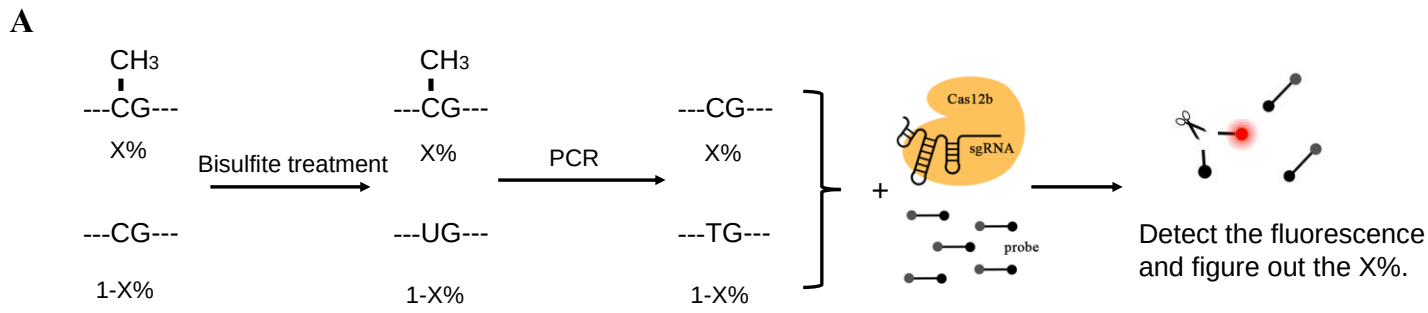
RNA病毒

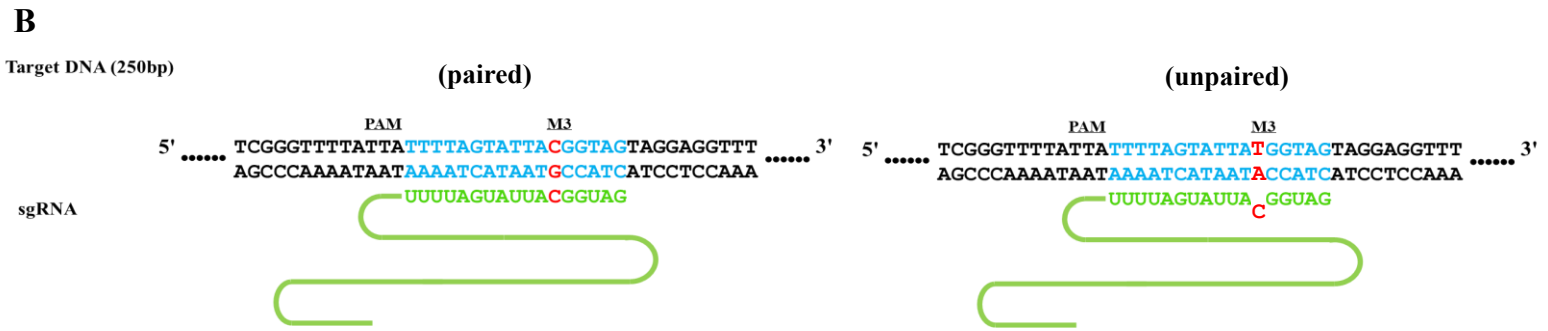
HOLMESv2可实现“一锅法”快检

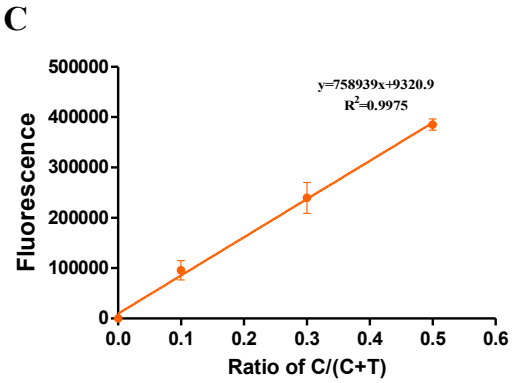


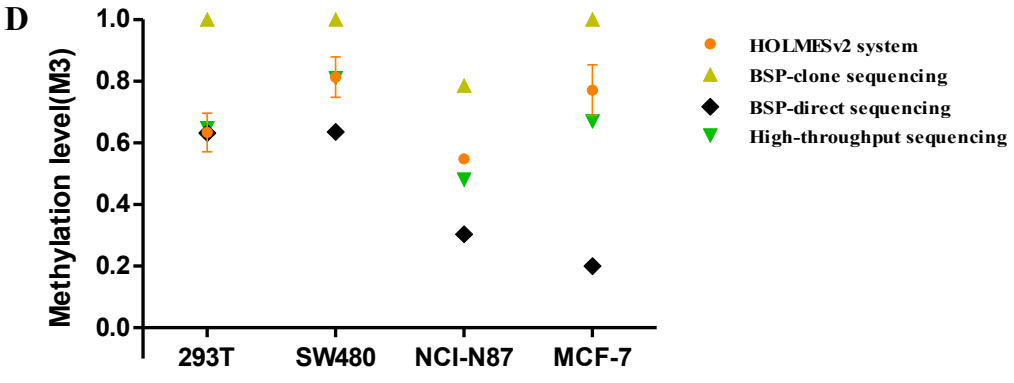
Li *et al., ACS Syn Bio,* 2019

HOLMESv2定量检测靶标的甲基化水平





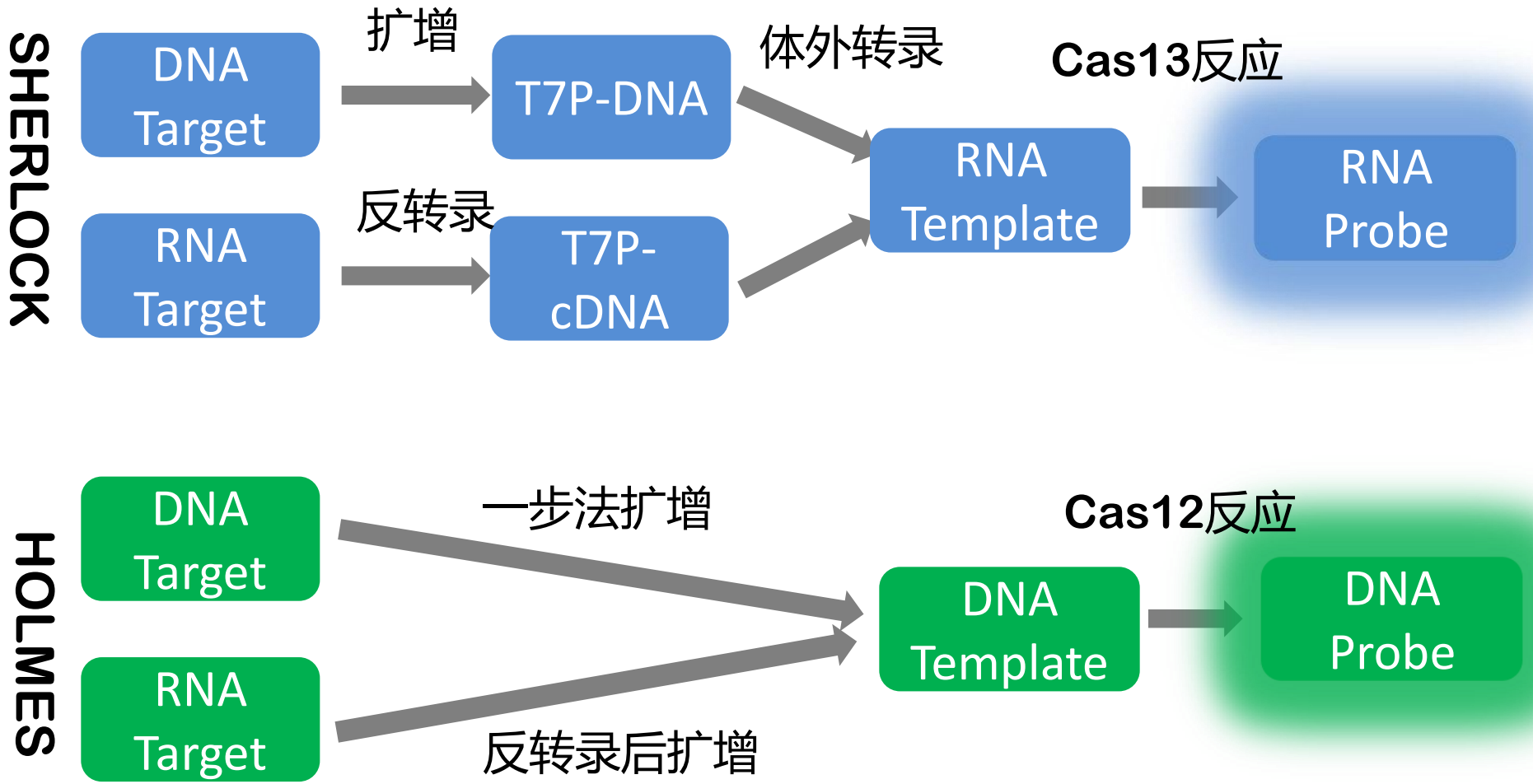




CRISPR分子诊断核心IP列表

	申请号	保护内容	发明团队	说明
1	WO2016/205764	Cas13体外核酸检测	美国/张锋	基于CRISPR-Cas13的反式切割活性，即利用Cas13蛋白识别RNA靶标**并切割RNA发光探针**。专利包括13a和13b等蛋白。
2	WO2017/218573	Cas13体外核酸检测	美国/Doudna	
3	WO2018/107129	Cas13体外核酸多重检测	美国/张锋	
4	WO/2019/011022	Cas12体外核酸检测	**中国/王金 2017.7.14**	基于Cas12反式切割活性，即利用Cas12识别靶标DNA并**切割DNA发光探针**。专利包括12a和12b等蛋白。
5	WO2019/104058	Cas12体外核酸检测	美国/Doudna 2017.11.22	

HOLMES与SHERLOCK的比较



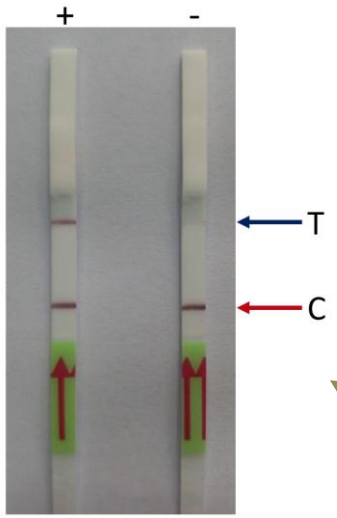
基于Cas12的HOLMES扩增简便、探针稳定、成本便宜、定量准确！

HOLMES技术的应用领域

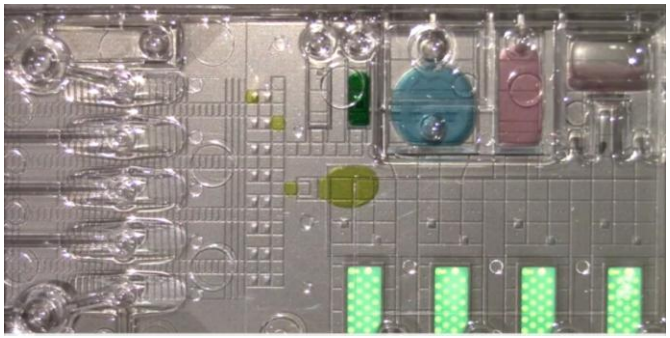
检测领域	应用场景	HOLMES技术优点
人传染病	医院，海关，CDC，家庭	快、准、简单、便宜
肿瘤筛查和精准用药	医院，家庭	快、准、通量高、便宜
动物疫病	大规模养殖场、海关	快、准、简单、便宜
农业转基因	农场、食品公司、家庭	快、简单、便宜
生物反恐	政府安全部门	快、准、通量高、便宜

针对任何DNA或RNA的快速检测

HOLMES是平台型的技术：特异、灵敏度高



LFA



microfluid

ddPCR

HOLMES

DNA/RNA of known sequences

(IVD/农业/海关/……)

Joii

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