Electrochemical platforms for solid-phase isothermal amplification and detection of bacterial genome

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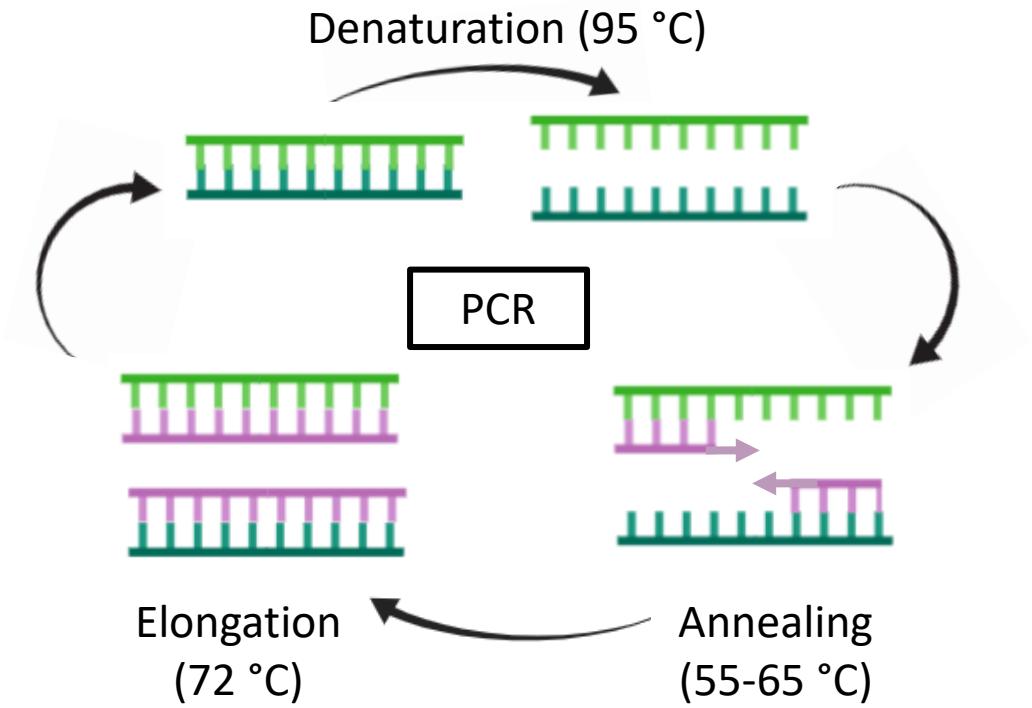
Introduction

Salmonella as many bacterial pathogens constitutes a mayor cause of foodborne disease

Monitoring and control of this human pathogenic bacterium in foodstuffs and biological fluids are necessary in order to prevent and diagnose the disease



Highly sensitive
Reliable

Sophisticated equipment
Difficult to miniaturize
Time-consuming

Introduction

Alternative to PCR

Isothermal nucleic acid amplifications

- Constant temperature
- Short times
- Simple equipment

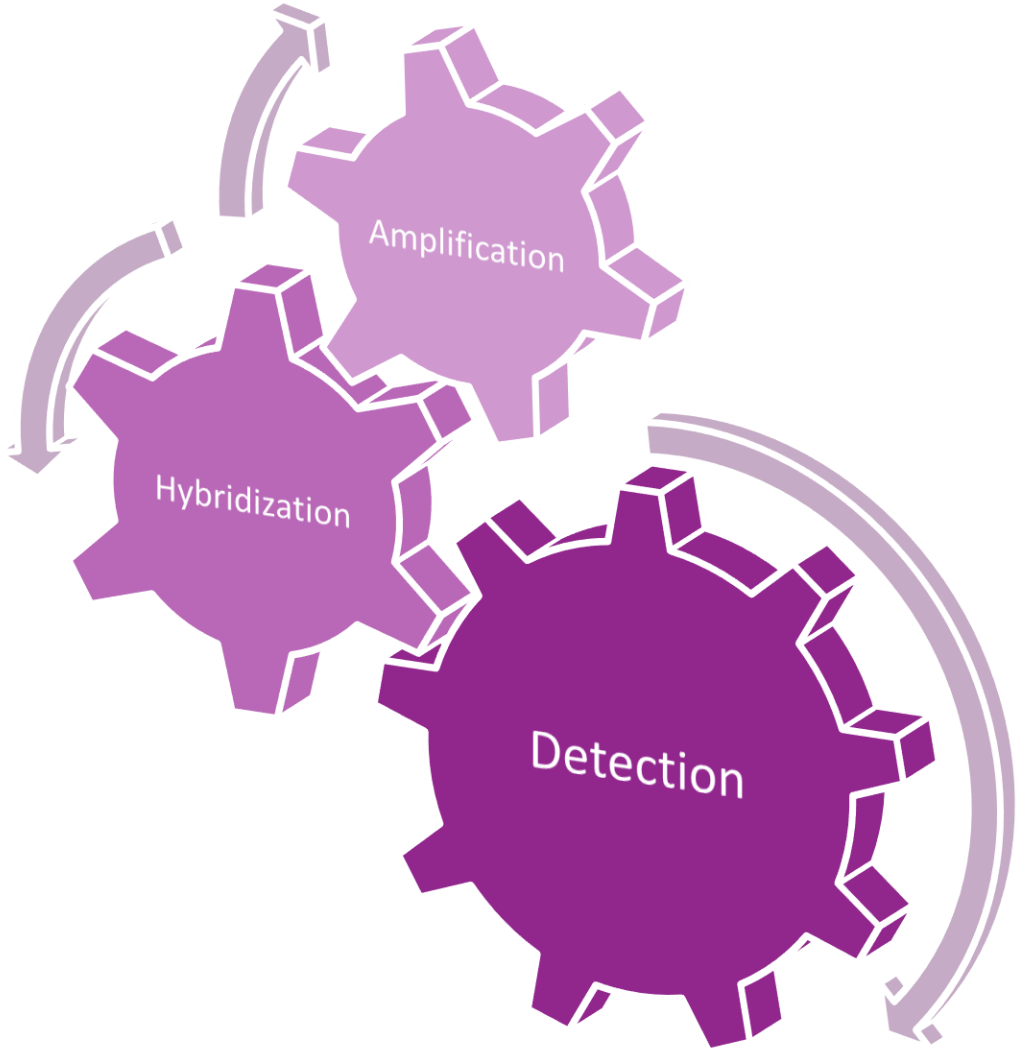
Helicase-dependent amplification (HDA)

Recombinase polymerase amplification (RPA)

On-surface isothermal amplifications

Overcome challenges

Point-of-need devices



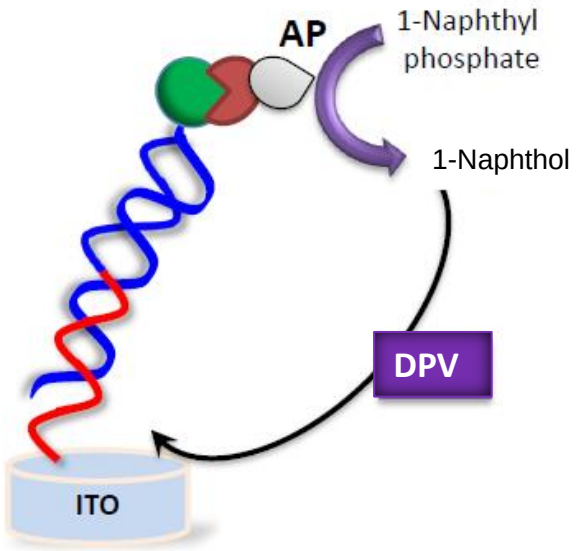
Electrochemical platform + Isothermal amplification

- Inexpensive
- Simple
- Portable
- Compatible with microfluidic technologies

Objectives

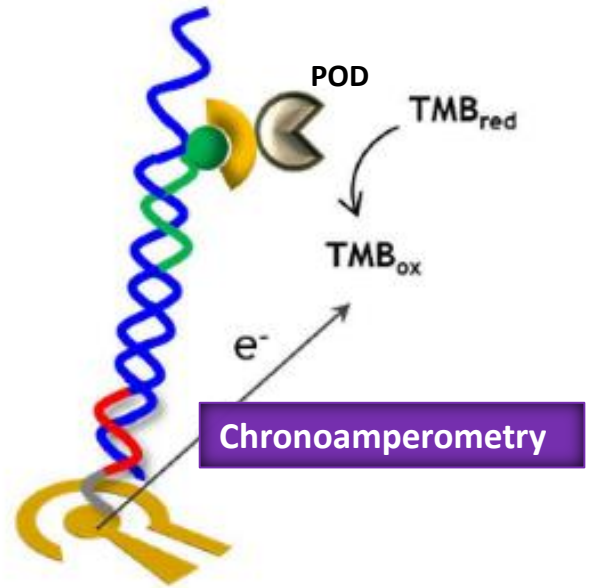
Comparison of two electrochemical platforms to detect *Salmonella* genome

Solid-phase HDA onto ITO surface



Indium-tin oxide (ITO) surfaces

Solid-phase RPA onto Au surface



Gold surfaces

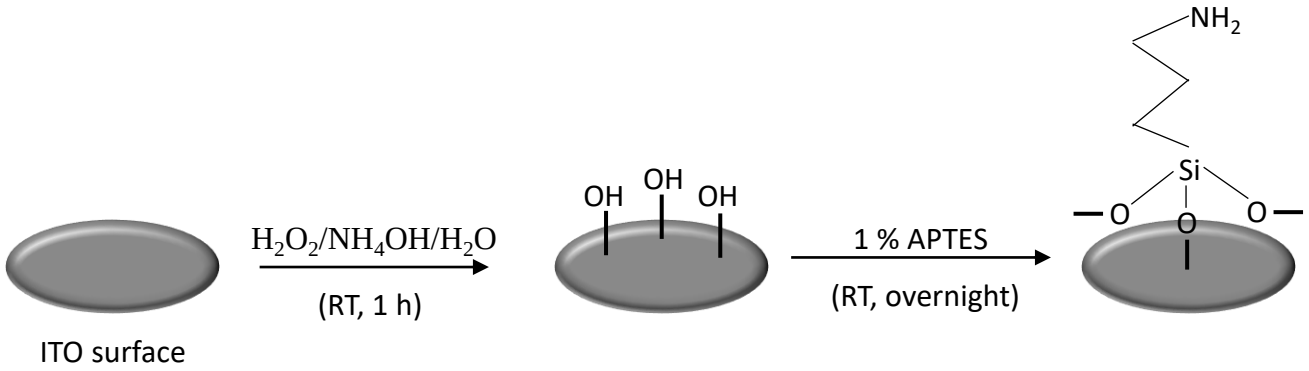
antiFITC-AP

6-FAM-Forward primer

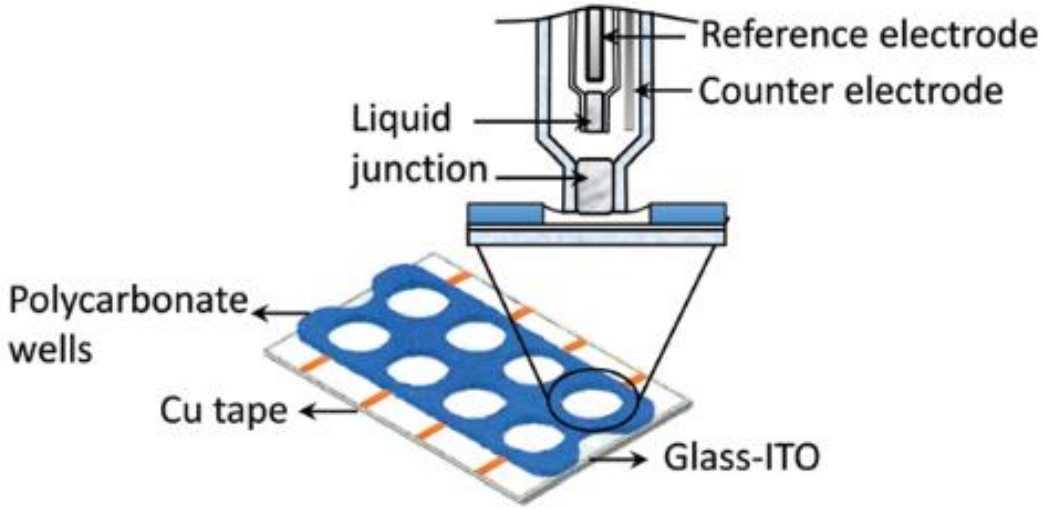
HS-T_x-Reverse primer

antiFITC-POD

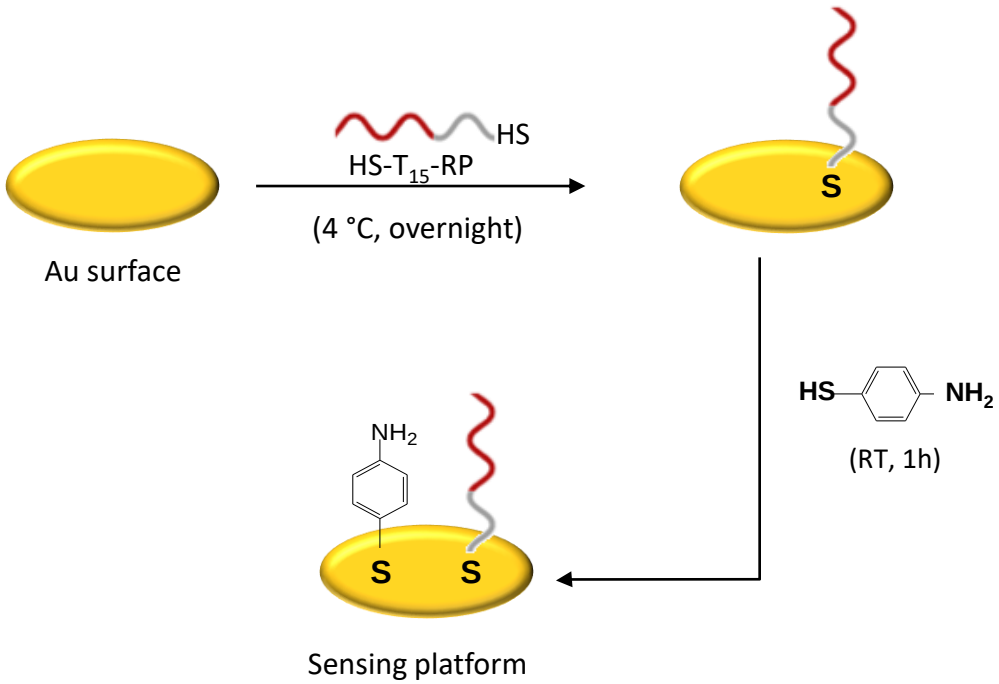
Sensing phase construction for HDA onto ITO



Electrochemical set-up

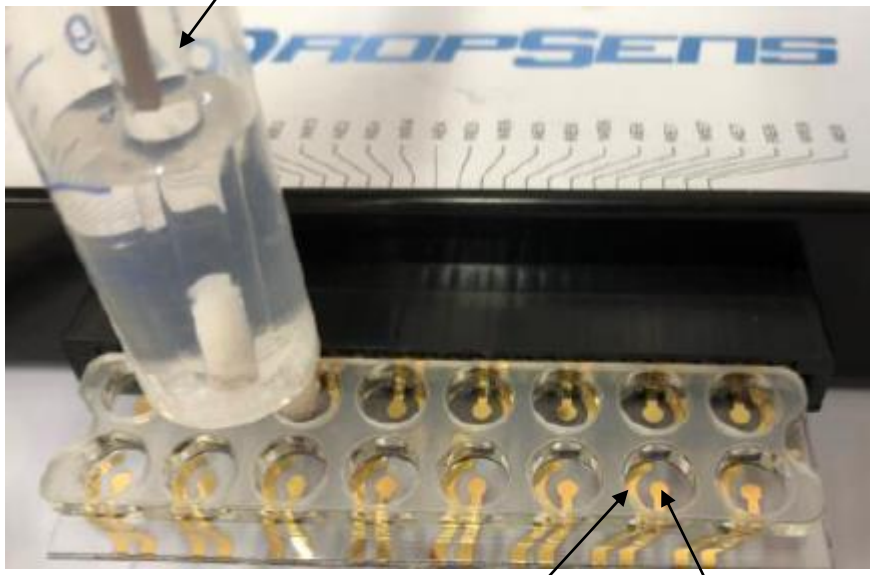


Sensing phase construction for RPA onto gold

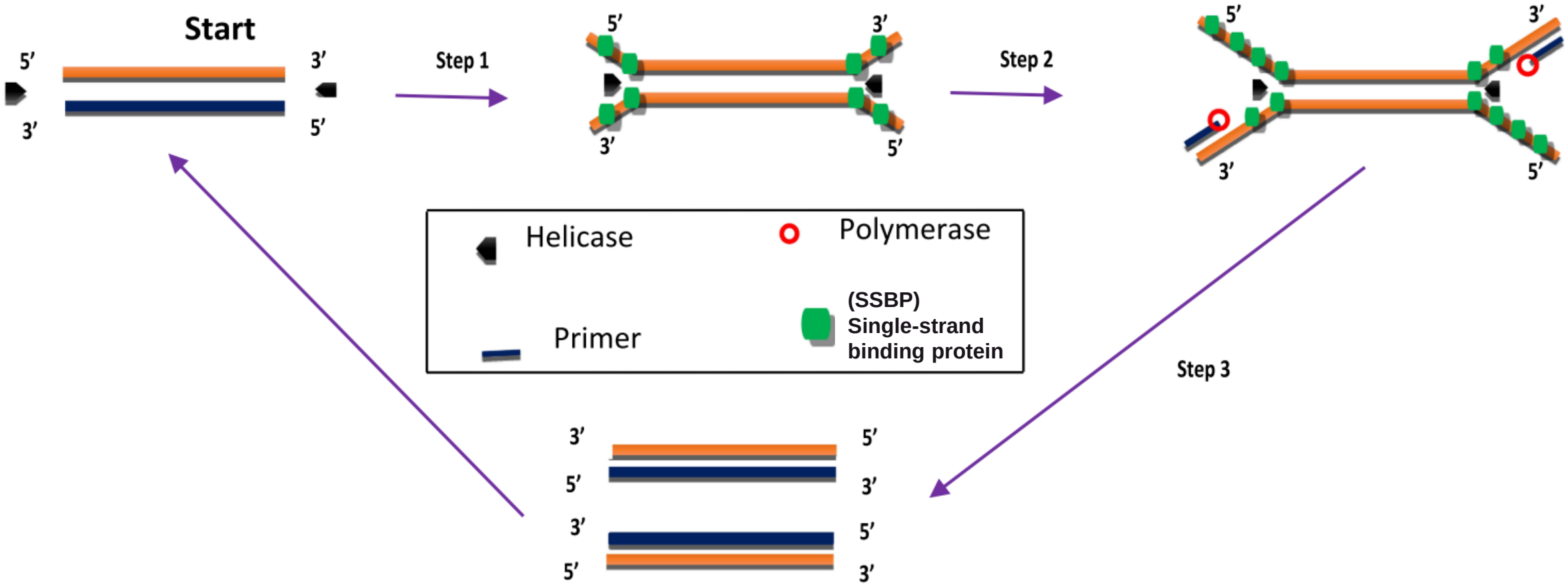


Electrochemical set-up

Reference electrode:
Ag/AgCl/KCl (3 M) electrode isolated from the test solution by a KNO3 (3 M) salt bridge inside a syringe



Helicase-dependent amplification (HDA) in solution



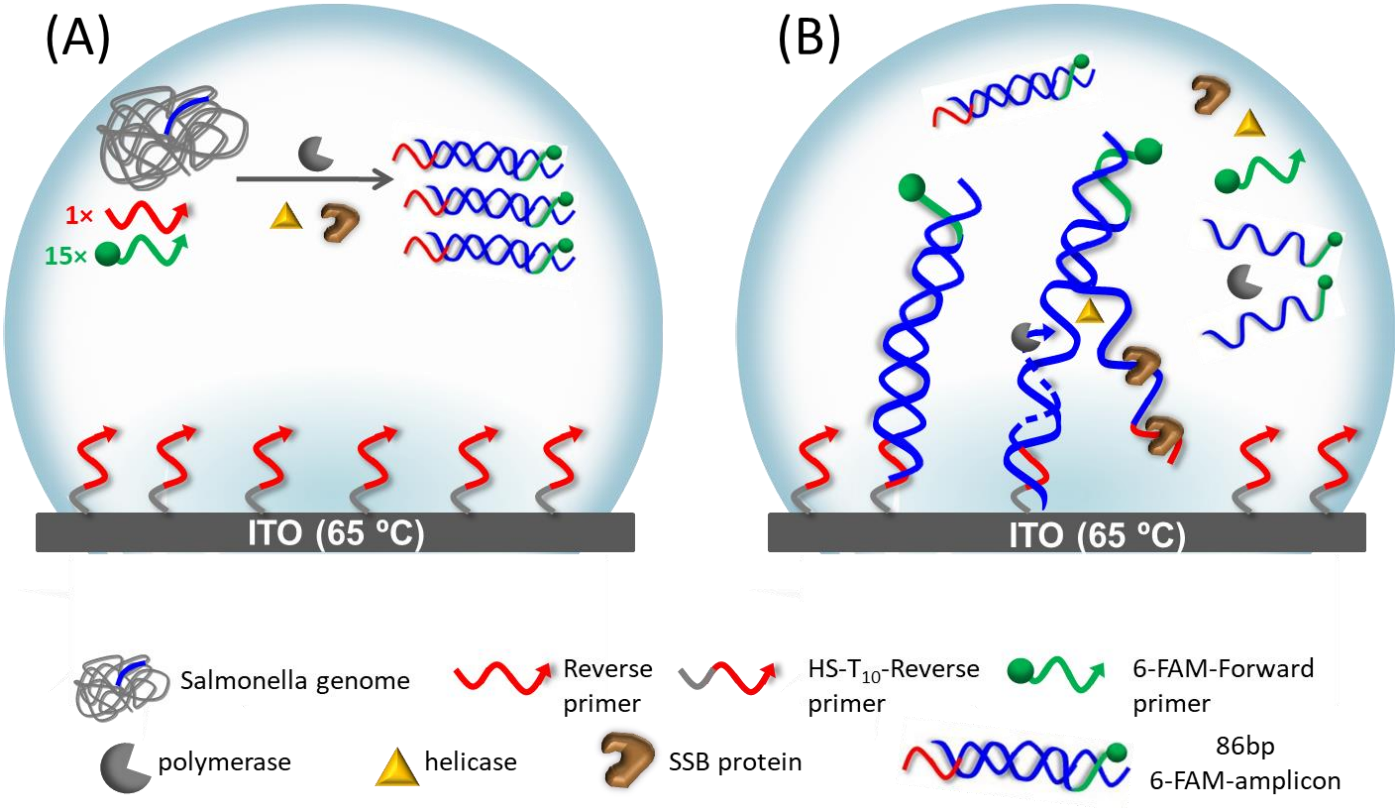
* G. A. Obande and K. K. B. Singh, *Infect. Drug Resist.*, 13 (2020) 455–483.

Results & discussion

HDA onto ITO surfaces

A) First stage:

Amplification starts in solution, giving rise to an 86 bp product

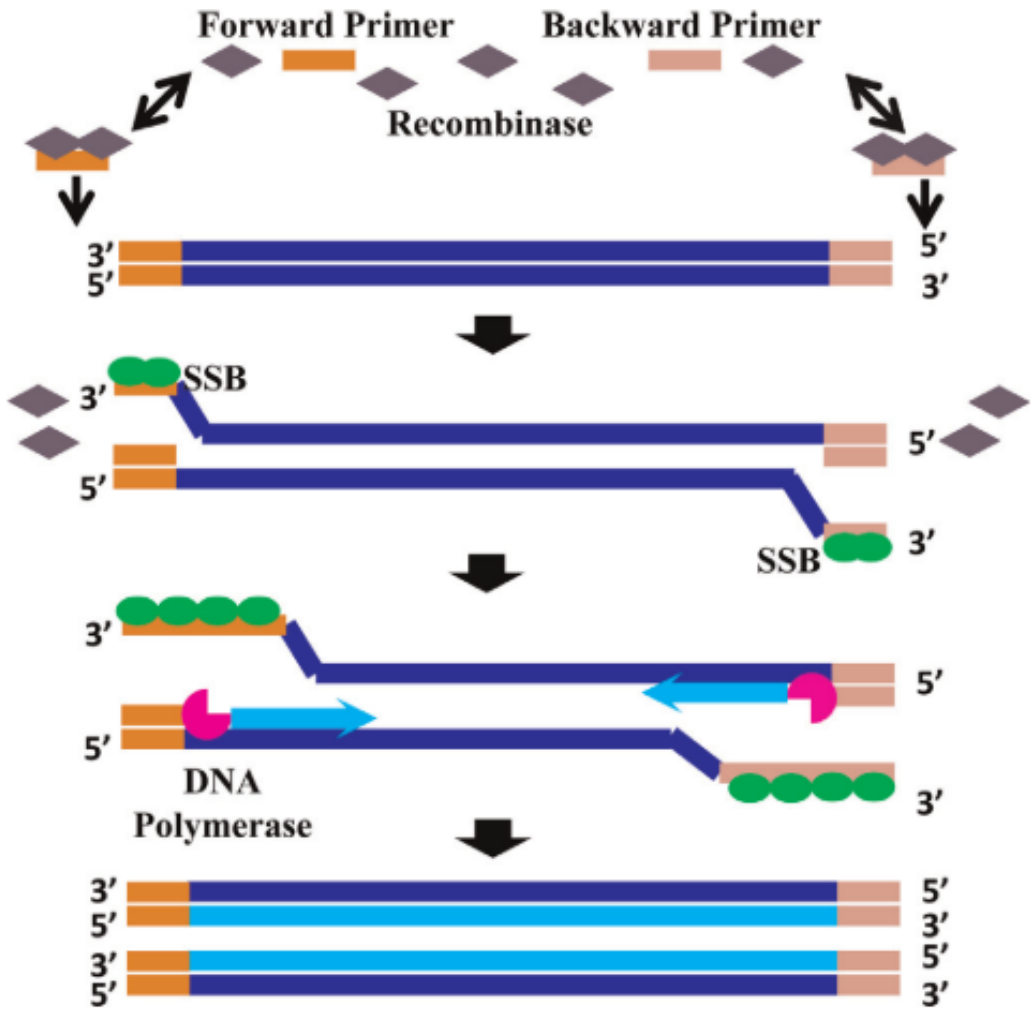


B) Second stage:

Amplification takes place on surface boosted by the immobilized primer

*Barreda-García, S.; Miranda-Castro, R.; de-los-Santos-Álvarez, N.; Miranda-Ordieres, A.J.; Lobo-Castañón, M.J., *Chem. Commun.* **2017**, *53*, 9721–9724,

Recombinase polymerase amplification (RPA) in solution

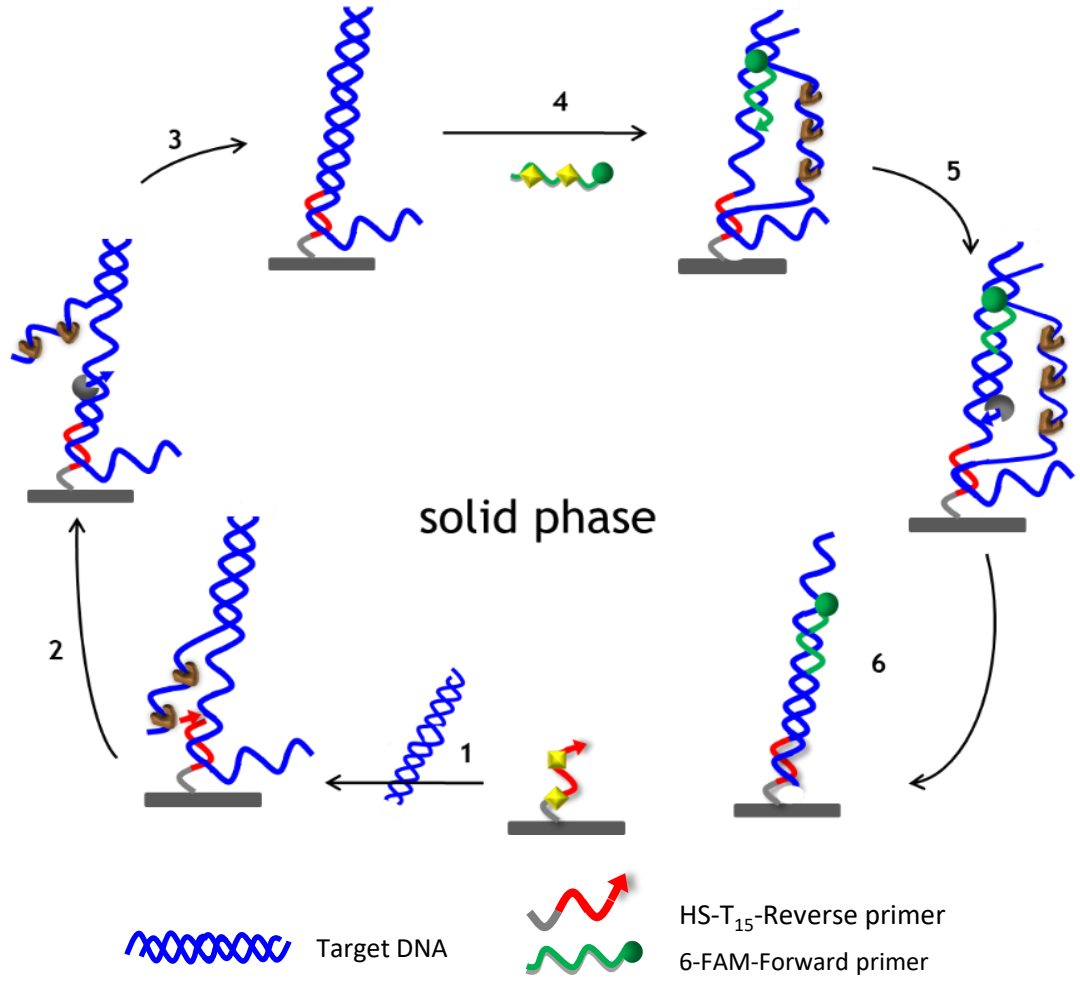


1. Formation of recombinase-primer complex
2. Homologous sequence recognition
3. Stabilization by single-strand DNA binding proteins (SSBs)
4. DNA polymerase elongation

*J. Li and J. Macdonald *Biosensors and Bioelectronics, 69* (2015) 196-211

On-gold RPA

1. Hybridization between genome and the attached primer
2. Elongation of the primer
3. Label-free amplicons
4. Second surface amplification
5. Incorporation of the tag
6. Labeled inmobilized amplicon

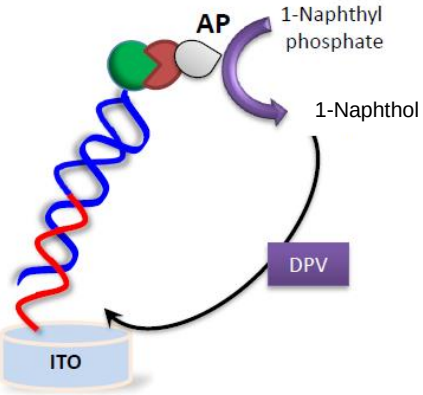


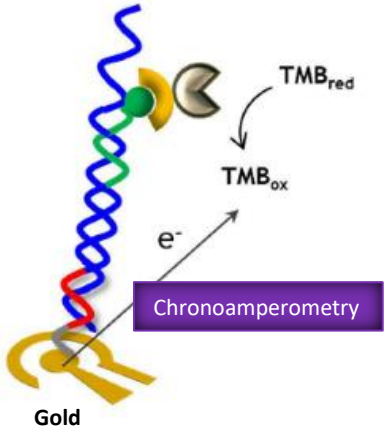
*Sánchez-Salcedo, R.; Miranda-Castro, R.; de-los-Santos-Álvarez, N.; Lobo-Castañón, M.J., *ChemElectroChem* **2019**, *6*, 793-800.

Evaluation of the analytical performance of both platforms

Features related to the surface

	HDA	RPA
Temperature (°C)	65	37
Surface	ITO	Gold
Enzyme conjugate	AntiFab-AP	AntiFab-POD
Enzyme substrate	1-naphthyl phosphate	TMB
Detection technique	DPV	Chronoamperometry
Storage stability	9 months	1 month

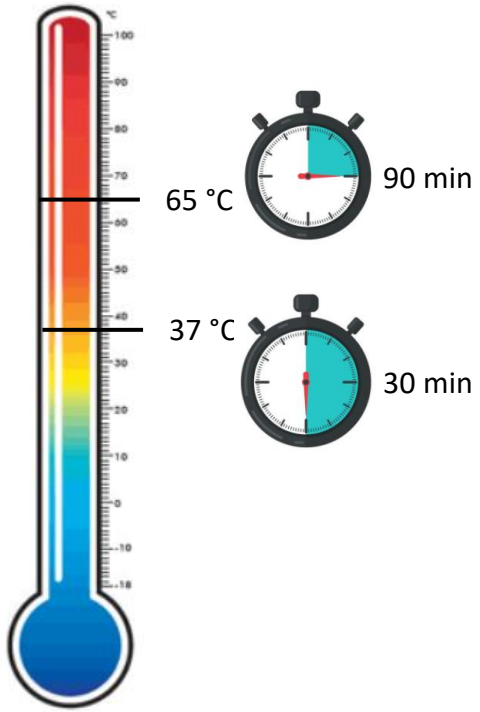




Evaluation of the analytical performance of both platforms

Features related to the amplification

	HDA	RPA
Temperature (°C)	65	37
Available format	Kit	Kit
Time (min)	90	30
LOD (genomes)	10	10^5
Reproducibility (%)	20	30



Conclusions

1. The devices compared in this work successfully integrate isothermal amplification in electrochemical platforms

2. Achieving efficient detection of small amounts of the *Salmonella* genome without thermal cycling while using simple equipment is accomplished in both cases.

3. The results of this study may be of general utility in the design of sensors for detecting other bacteria.

Acknowledgement

Electroanalysis group

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