Intact Genomics®

FastAmp® qRPA SYBR Kit

Manual

Catalog #	3611	3615	3617
Package Size	25 Reactions	100 Reactions	500 Reactions

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

Intact Genomics®

FastAmp® qRPA SYBR Kit

Table of Contents

Product Description 3

RPA/qRPA Primer Design 4

Applications & Benefits 4

Components and Storage 4

qRPA Reaction Protocol 5

qRPA Optimization Experimental Design 5

RPA Technology Applications & Benefits 6

Troubleshooting 7

Challenges 8

Quality Control 8

References 8

Related Products 8

Ordering Information 8

Technical Support 9

Description:

IG® scientists engineered a novel **FastAmp® qRPA SYBR kit** that contains master mixes with ready-to-use cocktails containing all components except primers and template for the amplification, detection, and quantification of DNA in RPA. With our new technology, RPA progress can be monitored in real-time and quantified using standardized template concentrations. The FastAmp® qRPA SYBR kit is compatible with high throughput (96-well or 384-well) qPCR thermocyclers without temperature cycles for faster results, i.e. the experiment is complete in just 30 minutes at constant 37°C.

A key feature of the **FastAmp® qRPA SYBR Kit** is that it can be used to screen primer conditions for the lowest possible Non-template control (NTC) background. Most polymerases can produce erroneous byproducts with short DNA strands that result in the appearance of a non-specific NTC signal[1]. These byproducts are also capable of binding the intercalating SYBR dye, but products occur with lower probability than specific primer-target annealing and take longer to amplify. Over time the formation of byproducts eventually can lead to a background signal in both PCR and RPA. Using this knowledge to our advantage, we show that it is possible to screen for well-behaved primers that have ultra-low SYBR background relative to the specific, intended signal generated (Figure 1).

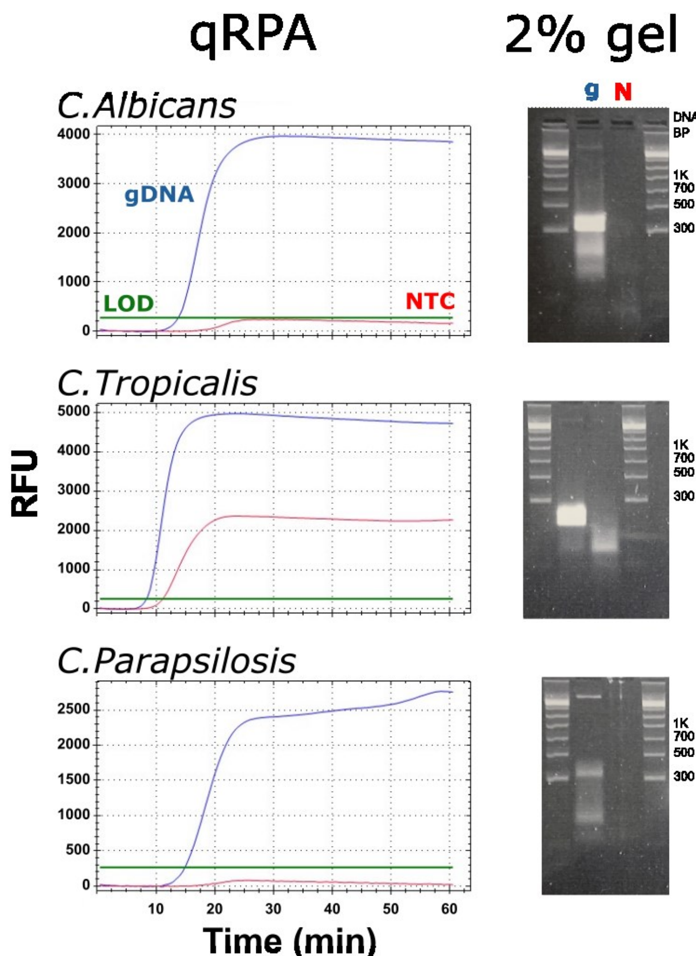


Figure 1: qRPA (Left column) using SYBR fluorescence detection was monitored for 3 fungal species with different primer sets at 37°C for over 60 minutes. After qRPA was completed, the amplified product was further analyzed on a 2% agarose gel (right column). gDNA (g) is genomic DNA ethanol precipitated from the fungal species indicated. NTC (N) is non-template control, and signal is attributed mostly to either primer-dimer formation and/or self-priming primers.

Conclusions: In all 3 strains, robust RPA occurs in under 30 minutes, and above LOD in 15 minutes (C. tropicalis under 10 minutes). C. albicans shows high RPA amplification and low NTC background. C. tropicalis shows robust RPA, but in absence of template high NTC is present. The gel shows this NTC to be primer-dimer signal. C. parapsilosis has weak, but measurable RPA, and low NTC background.

RPA/qRPA Primer Design:

While RPA generally favors longer primers—usually more than 20 base pairs—we find that virtually any primer design software has a similar success rate between RPA and PCR if a simple tool is used to avoid strong primer dimerization and strong primer secondary structure. For RPA primer design, we suggest designing 8 to 10 primers per forward and reverse direction. For most targets, it is unnecessary to test every possible primer combination. For example, by screening all reverse primers against a single forward primer, picking the best reverse primer and then using it to screen all the forward primers, a good primer-pair can be found in 16 to 20 reactions.

Applications and Benefits:

- Primer validation and screening.
- Highly selective and sensitive isothermal amplification technique.
- Alternative to PCR. No thermocycler needed.
- Speed and sensitivity.
- No DNA pretreatment required.
- Flexible endpoint detection compatibility (e.g. lateral flow, real-time fluorescence).

Kit Includes:

- FastAmp® qRPA SYBR Reaction Master Mix
- IG® RPA Enzyme Mixture v2 (4x)
- 280 mM Magnesium Acetate (MgOAc)
- Nuclease-free Water

** The mix also includes SYBR® Green I fluorescent dye, ROX dye, $MgCl_2$, dNTPs and stabilizers. *The use of ROX dye is necessary for all Applied Biosystems instruments and is optional for the Stratagene Mx3000P Mx3005P and Mx4000 cyclers. Bio-Rad, Qiagen, Eppendorf, Illumina and Roche instruments do not require ROX dye. The concentration of ROX Dye is (5 nM) for this product*.

Storage Temperature: -20°C

qRPA SYBR Reaction Protocol:

1. Thaw FastAmp® qRPA SYBR Reaction Master Mix, IG® RPA Enzyme Mixture, Nuclease-free Water and DNA Primers on ice.
2. In a PCR tube, prepare a reaction mix according to the following table:

Component	Volume
Primer F (10 µM)	1 µL
Primer R (10 µM)	1 µL
4x IG® RPA Enzyme Mixture v2	5 µL
DNA Template (1 fg-100 ng)	1 µL
Nuclease-free Water	3 µL
FastAmp® qRPA SYBR Reaction Master Mix	9 µL

3. Due to the viscosity of the Reaction Master Mix, it is recommended to **mix the DNA primers and RPA Enzyme Mixture on ice first**. Then add the template, Nuclease-free water, and FastAmp® qRPA SYBR Reaction Master Mix and mix by pipetting 10-15 times carefully.
4. To initiate the reaction, add 1 µL of MgOAc per reaction, mix by pipetting 10-15 times carefully, and pulse spin the reaction tubes briefly. **RPA reactions start when MgOAc is added**.
5. Place the RPA samples in a qPCR machine that is set to incubate at 37°C*.
6. qRPA data can be collected by adapting real-time quantitative PCR (qPCR) using the SYBR Green method. Check the following settings on your qPCR-SYBR protocol and change for qRPA-SYBR:
   i. Temperature is held constant for RPA reaction (e.g. 37°C). No temperature cycling is required.
   ii. To track the RPA amplification of the target DNA in real-time, monitor the SYBR fluorescence signal emitted by the sample at 30-second intervals. Verify the wavelengths (λ) used for monitoring fluorescence. Typically, the excitation λ=497 nm and emission λ=520 nm.
   iv. Set the total duration to 20 minutes**.

qRPA Optimization Experimental Design:

*** Amplification Temperature**

qRPA reaction amplification temperature may vary from 24°C to 42°C.

The reaction can also be performed at ambient temperature(~24°C). However, we recommend increasing the incubation time to a minimum of 30 minutes, up to 60 minutes.

**** Amplification Time**

Optimal amplification time for an qRPA reaction is 20 minutes. For samples with high concentration/copies, amplification time can be shortened to 10–15 minutes.

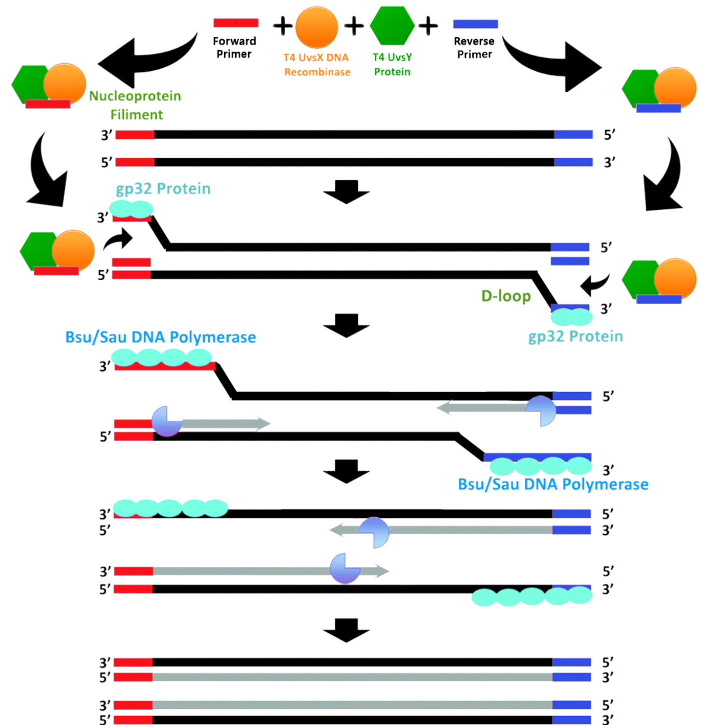
For samples with low concentration/copies or low template input, the amplification time can be increased to 30-60 minutes to increase yield and sensitivity.

Recombinase Polymerase Amplification (RPA) Technology:

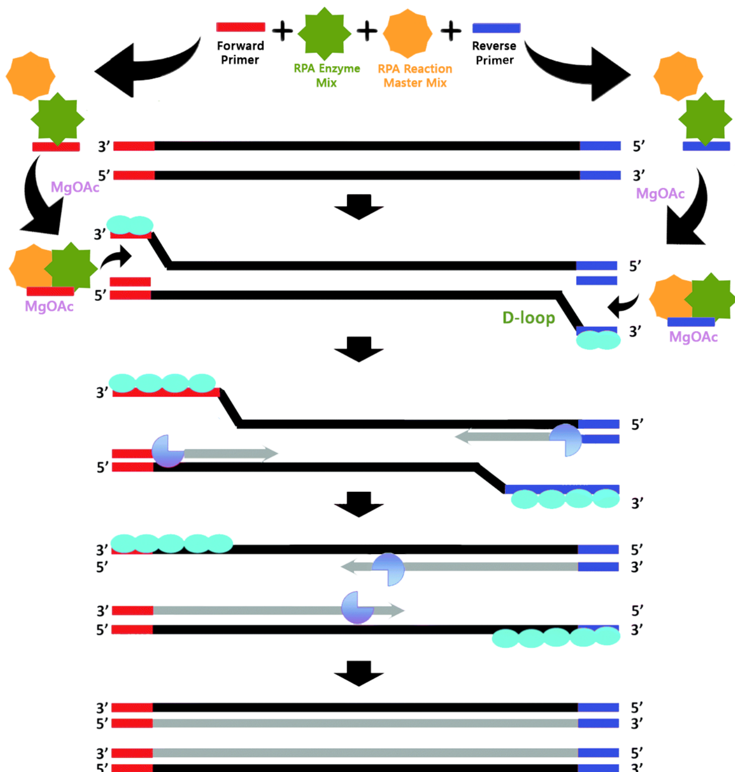
Recombinase Polymerase Amplification amplifies DNA at a 37 - 42°C using a recombinase (e.g. UvsX), primers, a single-stranded DNA binding protein (SSB), and a strand displacing DNA polymerase. T4 UvsX is used in combination with its accessory protein, UvsY. The recombinase interacts with the primers to form nucleoprotein filament. This complex can bind with homologous double-stranded DNA through a strand exchange[1-3]. After the exchange, a single-stranded binding protein, T4 gp32, stabilizes the displaced strand. Finally, Bsu/Sau DNA polymerase extends the DNA from the primers, creating a new complete copy of the template, and amplification can continue like in Polymerase Chain Reaction (PCR).

New improved IG® Recombinase Polymerase Amplification (RPA) Kit provides a mixture of the necessary enzymes to simplify and quicken the setup for your RPA reaction at a single and constant temperature (25 - 42°C).

Schematic of Recombinase Polymerase Amplification (RPA)



Schematic of IG RPA Kits



Applications and Benefits:

- Highly selective and sensitive isothermal amplification technique.
- Alternative to PCR. No thermocycler or other heavy equipment needed.
- Speed and sensitivity. Excellent for rapid point-of-care and on-site testing.
- No DNA pretreatment required.
- Flexible endpoint detection compatibility (e.g. lateral flow, real-time fluorescence).

Troubleshooting:

Issue	Solution
No amplification	Repeat the experiment to make sure there were no pipetting errors.
	Make sure reagents are added in the correct order.
	Make sure that the magnesium acetate is added last and is mixed in well.
	Check DNA quality using gel electrophoresis or spectrophotometer; use fresh, high-quality DNA/RNA.
	Use fresh/good primers. Avoid or redesign primers that give high background.
	Increase the amount of template. Do not exceed 100 ng total DNA. In general, use 1-100 ng of genomic DNA or 1 pg—20 ng of plasmid DNA.
Low amplification	Increase incubation temperature to 42°C.
	Increase incubation time to 30, 45 or 60 minutes.
	Increase the amount of RPA enzymes/proteins added per reaction.
	Increase primer or template concentration.
False positive result	Clean pipettes and the work area before each experiment to prevent cross-over contamination
	Use sterile pipette tips and tubes only. Filtered/aerosol barrier tips are recommended.
	Prepare negative controls in an area separate from where test samples are added to the reaction.
	Replace potentially contaminated reagents with new ones. Use a new tube of nuclease-free water for each experiment. If possible, treat water with ultra-violet/germicidal light.
	Purify DNA to remove contaminants.

Challenges:

Recombinase Polymerase Amplification (RPA) is a popular isothermal amplification technique used in molecular diagnostics. However, like any new technology, it faces certain challenges. Intact Genomics has been working in the RPA field for many years and our scientists have technical expertise to support the scientists and their research with this promising technology. Contact us for tech support and collaboration opportunities. We are committed to continuous innovation and optimization and we are likely to address many of these issues in our studies.

Quality Control:

- All component proteins of the Enzyme Mixture are free from detectable nuclease activities.
- RPA activity of each lot is validated to be consistent with prior IG® lots and to be equal to or better than major competitors in the market.

References:

1. Cromie GA, Connelly JC, Leach DR (2001) Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. Mol Cell 8: 1163–1174
2. Michel B, Grompone G, Flores MJ, Bidnenko V (2004) Multiple pathways process stalled replication forks. Proc Natl Acad Sci U S A 101: 12783–12788
3. Liu J, Ehmsen KT, Heyer WD, Morrical SW (2011) Presynaptic filament dynamics in homologous recombination and DNA repair. Crit Rev Biochem Mol Biol 46: 240–270

Related Products:

1. T4 UvsX Recombinase (Cat.# 3562) , Glycerol-free T4 UvsX DNA Recombinase (Cat.# 3562GF)
2. T4 gp32 Protein (Cat.# 3515), Glycerol-free T4 gp32 Protein (Cat.# 3513GF)
3. T4 UvsY Protein (Cat.# 3572), Glycerol-free T4 UvsY Protein (Cat.# 3572GF)
4. Bsu DNA Polymerase (Cat.# 3585), Glycerol-free Bsu (Cat.# 3585GF)
5. ig® Recombinase Polymerase Amplification (RPA) Kit v2 (Cat.# 3533, 3534, 3536)
6. Exonuclease IV (Nfo) (Cat.# 3425)

Ordering Information:

- Order online within the USA. Place orders on www.intactgenomics.com using our secure Shopping Cart.
- Order by email, phone, or fax.
  Email: sales@intactgenomics.com
  Phone: (314) 942-3655 | **Toll-free :** 855-835-7172 | **Fax:** (314) 942-3656
- Order from our distributors.

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Our hours are Monday - Friday, 8AM to 5PM, U.S. central standard time.

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