Key features

High success rate and consistency across different bacterial and yeast strains.

Direct amplification from overnight cultures or resuspended colonies offer streamlined workflows.

Faster cycling (5 – 30 sec/kb extension time) reduces overall turnaround times.

Amplify full-length inserts instead of short internal fragments.

Step-by-step protocols suitable for KAPA2G Robust HotStart or KAPA2G Robust

PCR screening of bacterial and yeast transformants containing cloned inserts—or Colony PCR—forms an integral part of the workflow of almost all molecular biology laboratories.

Despite the obvious advantages of screening transformants directly from cultures or plates, instead of first having to isolate DNA, the utility of the technique remains limited due to the inherent limitations of Taq *DNA polymerase in crude sample PCR applications.* Taq *is easily inhibited by debris from bacterial or yeast cells and components of culture media. As a result, inconsistent results are often obtained and only short fragments of cloned inserts can be interrogated.*

Introduction

KAPA2G Robust DNA Polymerase is a highly robust and versatile second-generation enzyme derived through a process of molecular evolution. The enzyme was engineered for high performance in chemically complex reaction conditions. The result is superior tolerance to a wide range of common PCR inhibitors, which translates into unrivalled performance in Colony PCR. KAPA2G Robust and KAPA2G Robust HotStart offer the following advantages in Colony PCR:

- A high success rate with commonly used *E. coli* and *S. cerevisiae* strains.
- Fragments up to 3 kb may be reliably amplified. This offers the opportunity to interrogate full-length inserts with generic vector-specific primers, and facilitates the identification of clones carrying large deletions or insertions.
- Direct amplification from liquid overnight cultures offer improved workflows for high-throughput Colony PCR.
- Faster cycling times (5 – 30 sec/kb extension time) reduce overall turnaround times.

E. coli colony PCR

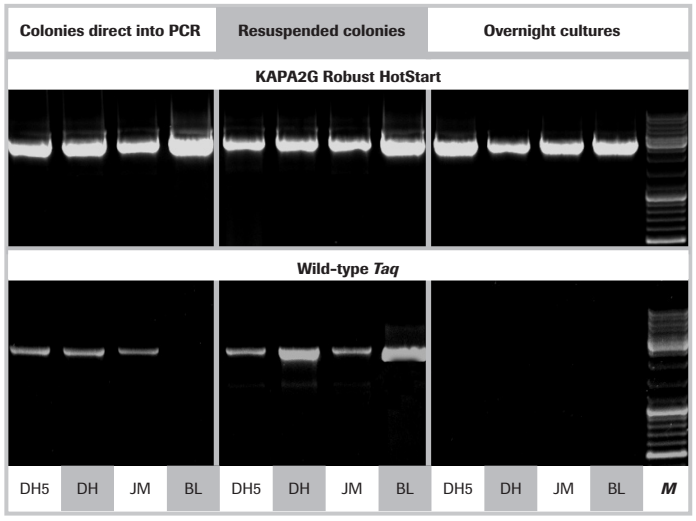


Figure 1. Amplification of a 2.7 kb insert from four commonly used *E. coli* strains (DH5a, DH10B, JM109, or BL21) using KAPA2G Robust HotStart DNA Polymerase (top) or a "robust" wild-type *Taq* (bottom). Colonies (grown on LB-agar + Amp plates) were either suspended directly in individual PCR reactions (left) or first resuspended in PCR grade water and then transferred to PCR tubes containing reaction master mixes (middle). For the right hand panel, overnight cultures (prepared in LB + Amp) were used directly in PCRs. A standard 3-step cycling profile (35 cycles), with 30 sec/kb extension time was used for KAPA2G Robust HotStart reactions. For wild-type *Taq*, the extension time was 1 min/kb.

Yeast colony PCR

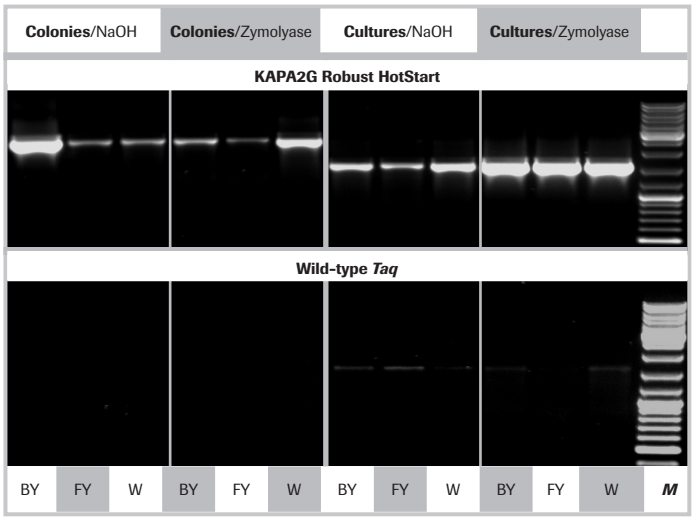


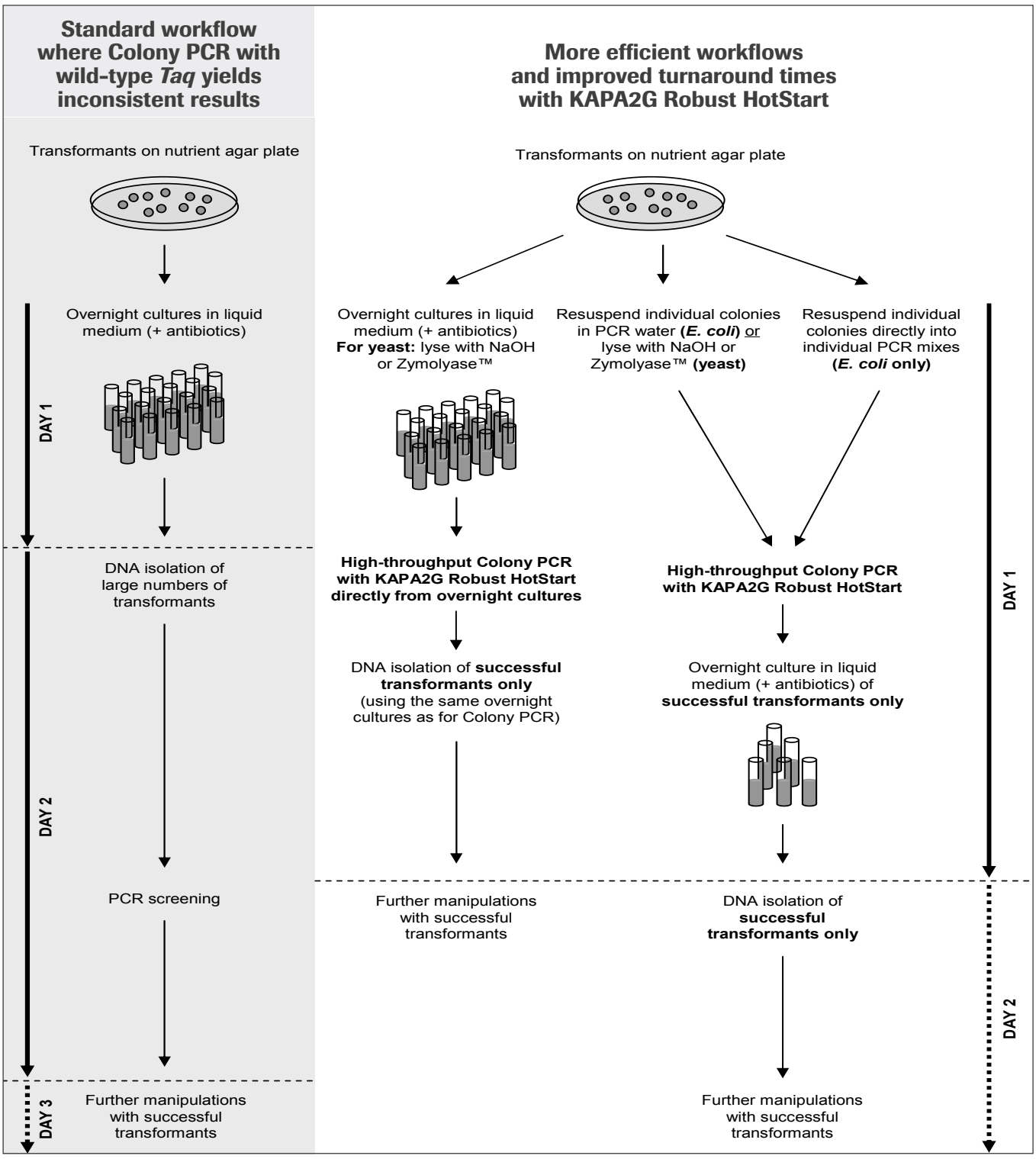
Figure 2. Amplification of a 2.5 kb (left) or 1.6 kb (right) fragment of the GSH1 gene from three commonly used *S. cerevisiae* strains (BY4742, FY23, and W303) using KAPA2G Robust HotStart DNA Polymerase (top) or a "robust" wild-type *Taq* (bottom). Colonies (from YPD-agar plates) or YPD overnight cultures were lysed in 50 µL volumes with NaOH or Zymolyase™ (as indicated). Of each lysate, 2.5 µL was used in the PCR. A standard 3-step cycling profile (35 cycles), with 30 sec/kb extension time was used for KAPA2G Robust HotStart reactions. For wild-type *Taq*, the extension time was 1 min/kb.

Workflows

KAPA2G Robust HotStart offers more efficient Colony PCR workflows, which facilitate high-throughput screening of transformants and reduce turnaround times. Because of the superior inhibitor tolerance of KAPA2G Robust HotStart, Colony PCR may be performed directly from liquid overnight cultures; a workflow that is not feasible with wild-type *Taq*. This allows for large numbers of transformants to be PCR screened in 96-well plate format, directly from overnight cultures prepared in 96-well culture plates. Reduced PCR screening time with KAPA2G Robust HotStart furthermore allows for DNA isolations to be performed on the same day, using the same overnight cultures as for Colony PCR.

Alternatively, colonies may be resuspended in PCR grade water (*E. coli*) or lysed with NaOH or Zymolyase™ (yeast) in 96-well format, after which individual suspensions are transferred to PCR master mixes prepared in 96-well plates. This strategy allows for PCR screening with multiple primer sets. For *E. coli* Colony PCR, transformants may also be resuspended directly in PCR master mixes prepared in 96-well plates or PCR tubes.

NaOH or Zymolyase™ is always required for efficient lysis of yeast cells prior to Colony PCR. Please refer to **Step-by-step protocol: *E. coli* Colony PCR** and **Step-by-step protocol: Yeast Colony PCR** for specific details on sample preparation prior to Colony PCR.


Standard workflow where Colony PCR with wild-type Taq yields inconsistent results
Transformants on nutrient agar plate
Overnight cultures in liquid medium (+ antibiotics)
DAY 1
DNA isolation of large numbers of transformants
DAY 2
PCR screening
DAY 3
Further manipulations with successful transformants
More efficient workflows and improved turnaround times with KAPA2G Robust HotStart
Transformants on nutrient agar plate
Overnight cultures in liquid medium (+ antibiotics) For yeast: lyse with NaOH or Zymolyase™
Resuspend individual colonies in PCR water (E. coli) or lyse with NaOH or Zymolyase™ (yeast)
Resuspend individual colonies directly into individual PCR mixes (E. coli only)
High-throughput Colony PCR with KAPA2G Robust HotStart directly from overnight cultures
High-throughput Colony PCR with KAPA2G Robust HotStart
DAY 1
DNA isolation of successful transformants only (using the same overnight cultures as for Colony PCR)
Overnight culture in liquid medium (+ antibiotics) of successful transformants only
Further manipulations with successful transformants
DNA isolation of successful transformants only
DAY 2
Further manipulations with successful transformants

Step-by-step protocol: *E. coli* Colony PCR

1. **If resuspended colonies are to be used:** pipette 10 – 20 µL PCR grade water into each of a set of appropriately labelled PCR tubes or wells of a PCR plate. Using sterile pipette tips or toothpicks, transfer transformants to individual tubes/wells. The amount of cells resuspended must just be visible. Resuspend each colony by stirring with the tip or toothpick.
2. Prepare a PCR master mix consisting of the KAPA2G Robust HotStart PCR Kit components, as indicated below. Always prepare at least 10% more master mix than needed.

Reaction component	Final conc.	Per 25 µL rxn*
PCR grade water		15.9 µL
5X KAPA2G Buffer B with $MgCl_2$	1X	5.0 µL
$MgCl_2$**	1.5 mM included in buffer	–
dNTP mix (10 mM each dNTP)	0.2 mM each dNTP	0.50 µL
Forward primer (10 µM)	0.5 µM	1.25 µL
Reverse primer (10 µM)	0.5 µM	1.25 µL
KAPA2G Robust HotStart (5 U/µL)	0.5 unit per 25 µL rxn	0.10 µL
Total		**24.0 µL**

*If the stock concentrations of dNTP mix and/or primers are different, please adjust the volumes of these components and the PCR grade water accordingly.

**5X KAPA2G Buffer B contains $MgCl_2$ at a 1X concentration of 1.5 mM. Add additional $MgCl_2$ only if the assay has previously been optimized with a higher final $MgCl_2$ concentration. In such cases, reduce the volume of PCR grade water with the volume of $MgCl_2$ solution added.

3. Aliquot 24 µL of PCR master mix into each PCR tube/well.
4. Add the colony to be tested to each PCR tube/well in one of the following formats:
   - 1 µL of an overnight culture (e.g., LB + Amp) prepared from the specific colony or
   - 1 µL of the suspension prepared by resuspending the specific colony in PCR grade water (see 1. above) or
   - a small amount of the specific colony straight from the plate, using a sterile pipette tip or toothpick.
5. Close the tubes or seal the plate.
6. Perform the PCR using the following cycling profile:

Cycling step	Temperature and time
Initial denaturation	5 min at 95°C
Denaturation	10 – 15 sec at 95°C
Annealing	10 – 15 sec at optimal Ta
Extension	5 sec at 72°C for fragments ≤500 bp; 15 – 30 sec/kb at 72°C for fragments >500 bp
No. of cycles	30 – 35
Final extension	Not required

Notes:

- Do not reduce the final concentration of primers or dTNPs.
- Use 10 sec denaturation and annealing and 5 sec total extension time per cycle and a total of 30 cycles for short fragments (≤500 bp).
- Use 15 sec/kb extension time and 30 – 35 cycles for fragments between 0.5 and 2 kb.
- Use 30 sec/kb extension time and 35 cycles for fragments ≥2 kb.

Step-by-step protocol: Yeast Colony PCR

1. **If resuspended colonies are to be used:** pipette 50 µL of a 0.02 M NaOH solution or a Zymolyase™ solution (e.g., 2.5 mg/mL in 0.1 M sorbitol) into each of a set of appropriately labelled PCR tubes or wells of a PCR plate. Using sterile pipette tips or toothpicks, transfer transformants to individual tubes/wells. The amount of cells resuspended must just be visible. Resuspend cells by pipetting or vortexing and incubate for ≥5 min at 37°C.

   If overnight cultures are to be used: pipette 40 µL of a 0.1 M NaOH solution or Zymolyase solution into each of a set of appropriately labelled PCR tubes or wells of a PCR plate. Transfer 10 µL of each overnight culture to be tested to the appropriate tube/well and mix by pipetting up and down. Incubate for ≥5 min at 37°C.

2. Prepare a PCR master mix consisting of the KAPA2G Robust PCR Kit components, as indicated below. Always prepare at least 10% more master mix than needed.

Reaction component	Final conc.	Per 25 µL rxn*
PCR grade water		14.3 µL
5X KAPA2G Buffer B with $MgCl_2$	1X	5.0 µL
$MgCl_2$**	1.5 mM included in buffer	–
dNTP mix (10 mM each dNTP)	0.2 mM each dNTP	0.50 µL
Forward primer (10 µM)	0.5 µM	1.25 µL
Reverse primer (10 µM)	0.5 µM	1.25 µL
KAPA2G Robust HotStart (5 U/µL)	1 unit per 25 µL rxn	0.20 µL
Total		**22.5 µL**

*If the concentrations of dNTP mix and/or primers used in a specific assay are different, please adjust the volumes of these components, and PCR grade water accordingly.

**5x KAPA2G Buffer B contains $MgCl_2$ at a 1X concentration of 1.5 mM. Only add additional $MgCl_2$ if the assay has previously been optimized with a higher final $MgCl_2$ concentration. In such cases, reduce the volume of PCR grade water with the volume of $MgCl_2$ solution added.

3. Aliquot 22.5 µL of PCR master mix into each PCR tube/well.
4. Add 2.5 µL of the resuspended colony or overnight culture mixed with NaOH to the appropriate PCR tube/well.
5. Close the tubes or seal the plate.
6. Perform the PCR using the following cycling profile:

Cycling step	Temperature and time
Initial denaturation	5 min at 95°C
Denaturation	30 sec at 95°C
Annealing	10 – 15 sec at optimal Ta
Extension	15 sec at 72°C for fragments ≤500 bp; 30 sec/kb at 72°C for fragments >500 bp
No. of cycles	30 – 35
Final extension	Not required

Notes:

- Do not reduce the final concentration of primers or dNTPs.
- Use freshly plated colonies or overnight cultures as far as possible.
- Amplification of long fragments (>2 kb) is more difficult from overnight cultures than from plated colonies.
- Successful yeast Colony PCR is very dependent on the efficient lysis of yeast cells prior to the PCR. The best lysis regime (NaOH or Zymolyase; lysis time and temperature) may have to be determined/optimized for different yeast strains.

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