TOROGreen® Yeast colony PCR Kit

Cat No. : DPK-100 Vol:10μL×200 Reactions

DESCRIPTION

TOROGreen® Yeast colony PCR Kit is used to amplify fragments from the yeast genome as well as transformed plasmids. The same as *E coli*.colony PCR , PCR amplification directly from a yeast colony without DNA or plasmid extraction from yeast for the robust direct PCR enzymes. The protocol of this kit is easy , rapid and low price than the traditional method. TOROGreen® 6 × DNA loading buffer contains the fluorescence dye, which is directly detected on 470nm LED transillum inator after the DNA electrophoresis.

FEATURES

- Easy-to-use: the same as *E coli*.colony PCR without DNA or plasmid extraction.
- Economical: the protocol is easy,rapid and low price than the traditional method.
- High performance: can amplify 40kb genomic DNA and ＞90% GC-rich target with 80-fold higher fidelity than Taq DNA polymerase.

APPLICATIONS

-Yeast Colony PCR

COMPONENTS

The kit includes the following reagents, which can be used for 200 reactions for a total 10ul reaction volume.

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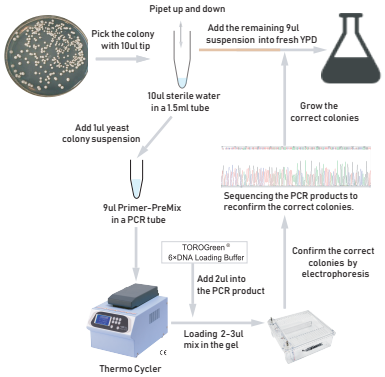
2× ASFast™ Direct PCR Mix	1ml ×1tube
TOROGreen® 6×DNA loading buffer	1ml ×1tube

BRIEF PROCEDURE



PROTOCOL

1. Pick the yeast colony.

(1)Angle the 10μL sterile pipet tip down towards a colony and just barely touch the colony.

(2) Place the tip into 10μL of sterile water in a 1.5ml clean tube and pipet up and down several times. Label the tube with a colony number and store the yeast colony in water at 4℃.

2. Standard reaction setup

(1) Prepare the 9μL Primer-PreMix on ice as the following components.

Component	Volume
PCR Grade Water	3.4μl
2× ASFast™ Direct PCR Mix	5μl
10μM Forward Primer	0.3μl
10μM Reverse Primer	0.3μl
Total Volume:	9μl

Notes:

-Primers should be 22-35 bases with Tm ≥ 65℃.

-Optimal primer concentration is 0.3uM. In the case of long targets (≥10 kb), reduced primers concentration (0.15uM) may give more effective amplification.

-When PCR yield is low, increased primers concentration (0.5uM) may give more effective amplification.

-Decreased the amount of template DNA when no PCR Product.

(2) Add 1μL yeast colony suspension into the 9μL Primer-preMix on ice, and pipet up and down several times.Gently mix the reaction solutions and spin down in microcentrifuge.

3. Set up cycling conditions.

[3-step cycle]

Predenaturation : 95°C, 5min.

Denaturation : 98°C, 10 sec.

Annealing : (Tm -5) °C, 5 sec.

Extension : 68°C, 10sec. /kb

25-45 cycles

Incubation: 4°C

Notes:

-Poor amplification may be improved by changing the denaturation step to 95°C, 15sec.

4. Confirm the correct colonies.

(1)Add 1 volume of TOROGreen® 6×DNA loading buffer to 5 volumes of PCR products.

(2) Mix well, spin down and load 5ul of the mixture and 2ul of the suitable TOROGreen® Loading Marker.

(3) Run on agarose electrophoresis to detect PCR products and marker .No additional dye is required for the PCR products and marker.

(4)Realtime observation of electrophoresis with a 470nm blue light source fitted to the electrophoresis tank and confirm the correct colonies.

5. Reconfirm the the correct colonies.

(1)Sequencing the PCR products to reconfirm the correct colonies.

(2)Add the remaining 9ul yeast colony suspension into fresh YPD in a test tube, and grow it to saturation overnight.

STORAGE

This kit should be kept at -20℃.