biohelix®

next generation HDA nucleic acid analysis

Rapisome™ pWGA Kit

(pWGA : primase-based Whole Genome Amplification)

Catalog # H0300S
Size: 25 reactions
Store at -70°C

DESCRIPTION

Whole Genome Amplification (WGA) techniques are useful tools for cancer and genetic research, and typically used to amplify genomic DNA for archiving and down-stream analysis including genotyping, forensics, comparative genomic hybridization, and single cell analysis. The **Rapisome™ pWGA** kit non-specifically amplifies total DNA. Unlike other WGA products, the Rapisome™ pWGA kit is based upon the in vitro reconstitution of a naturally existing cellular DNA replication system, which performs fast isothermal DNA amplification without the need for thermocycling, prior heat-denaturation, or added primers.

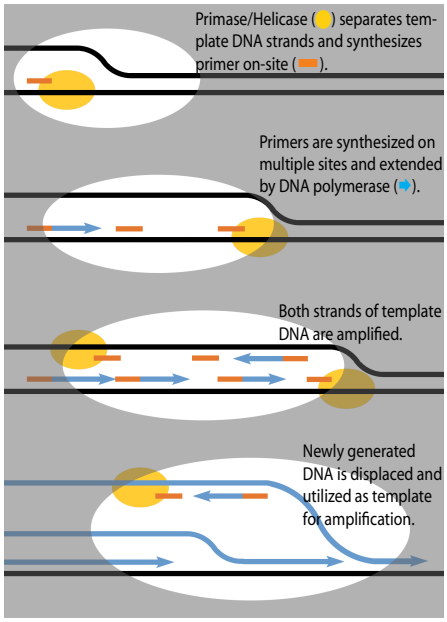

Figure 1. pWGA technology

The pWGA technology uses a **primase** to synthesize primers on-site, generating multiple initiation sites for random, whole genome amplification. Therefore, there is no need to add random primers to the reaction.

The pWGA technology also utilizes a **helicase** to denature double-stranded template DNA, eliminating the initial heating step that is required by other WGA technologies. Thus, the Rapisome™ pWGA reaction is truly isothermal.

pWGA is also faster than any other commercially available WGA techniques: the reaction time for pWGA is less than one hour. This makes **Rapisome™ pWGA** attractive technology for use in diagnostic applications more so than other conventional methods.

KIT COMPONENTS

pWGA Master Mix: 25 reactions (5 reactions/tube)
Control Template: 20 µl (Human genomic DNA, 5 ng/µl)

ADVANTAGES

No primers*: primase synthesizes primers on-site.*
No denaturation step*: helicase denatures template DNA.*
Low bias*: uses a natural replisome.*
Simple*: just add template DNA.*
Rapid*: 1hr reaction time*

PROTOCOL

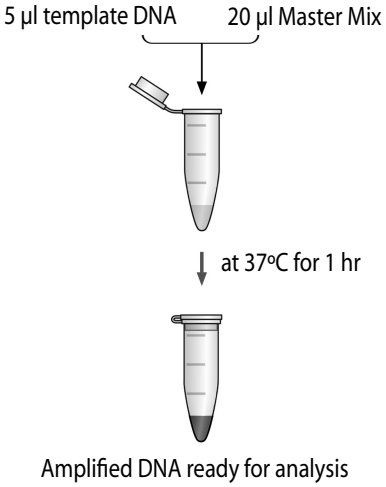

Figure 2. pWGA protocol

Preparation:

1. Prepare DNA template in a 5 µl volume with dH_2O.
   ! use 10 ng of high-quality DNA as a template.
   ! avoid introducing any contaminants into the reaction, especially Ca^{++}.

Amplification:

2. Thaw pWGA Master Mix on ice immediately before use, and add 20 µl pWGA Master Mix to the DNA template.

3. Incubate the pWGA reaction at 37°C for 1 hr.

Detection:

4. Stop the reaction by either adding EDTA to a final concentration of 5 mM or incubating at 65°C for 20 min.

5. Analyze 5 µl of the reaction by electrophoresis on 1 % agarose gel or other detection methods.

! approximately 3 - 5 µg yield.

NOTES

Storage: store the kit at -70°C. The pWGA Master Mix must be stored at -70°C; control template may be stored at -20°C; Thaw the kit components on ice immediatly before use.

Handling: the kit is sensitive to small amounts of DNA, and contamination of DNA during preparation can result in unwanted DNA amplification. Wear gloves at all time to avoid contamination.

Template DNA: Rapisome™ pWGA kit is optimized for whole genome amplification from 10 ng of high quality genomic DNA template. Use of less or low quality DNA can result in lower amplification yield.

Amplification Yield: typical yields from a Rapisome™ pWGA reaction are 3 - 5 µg per 25 µl reaction when 10 ng of high-quality genomic DNA template is used (Fig. 3). Extending the amplification time (2 hrs) may help to further increase yields.

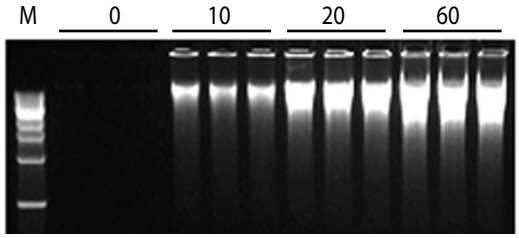

Figure 3. Yield of pWGA during 1hr incubation. The Rapisome™ pWGA reactions containing 10 ng human genomic DNA were terminated after a specified incubation time (0, 10, 20 or 60 min), and 5 µl of each 25 µl reaction was analyzed on 1 % agarose gel. The reactions are in triplicate, and lane M shows 500 ng of 1kb DNA ladder (NEB).

For technical support

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