Evomic Science

Quick Protocol

SuperPhi DNA Amplification Kit for Bacterial or Yeast Colony Screening

SuperPhi DNA Amplification Kit has capacity to detect as low as 0.1 pictogram of template DNA under optimal condition. 0.2~0.5 µl of saturated overnight culture or 1/10 to 1/100 of the colony (approximately 10^2 ~10^4 cells) would be enough for SuperPhi DNA Amplification reaction. Please keep in mind that excess culture or colony will inhibit SuperPhi DNA Amplification reaction! Yields and kinetics may vary if crude or un-quantified samples are amplified. We recommend to consider a starting point for adapting your specific reaction.

1. Preparation of Sample Mix:

Sample Mix could be prepared, depending on material sources, as described below:

1.1. Bacterial or Yeast colonies:

Transfer 4 µl of Sample Buffer into a 0.2 ml PCR tube.
Pick 1/10 to 1/100 of the colony (approximately 10^2 ~10^4 cells) and add to the above PCR Tub.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.2. Liquid bacterial culture:

Transfer 3.5~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 0.2~0.5 µl of saturated overnight culture to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.3. Glycerol stock:

Transfer 3.5~3.8 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 0.2~0.5 µl of glycerol stock to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

1.4. Purified DNA or DNA ligation/assembly reactions:

Transfer 3 µl of Sample Buffer into a 0.2 ml PCR tube.
Add 1 µl of circular DNA (≥ 100 fg/µl) to the above PCR tube.
Heat to 95 ˚C for 3 minutes and then quickly cool to 4˚C.
Keep the samples on ice until use.

Note: *Heating at higher temperature or longer time may increase the probability of releasing host genomic DNA into cell lysis to the reaction, where the host genomic DNA will compete with the desired template DNA during amplification.*

2. DNA Amplification:

Add 5 µl of Reaction Buffer and 1 µl of SuperPhi Enzyme to the above PCR tube contains 4 µl of Sample Mix as showed below:

Component	Volume/reaction
Sample Mix	4 µl
Reaction Buffer	5 µl
SuperPhi Enzyme	1 µl

- The final volume is 10 µl. Mix well and incubate at 30 ˚C for 3~18 h.
- If multiple applications are desired, reaction volume can be appropriately scaled up to 20µl, 40µl, 60µl etc.

3. Inactivate SuperPhi: Inactivate the enzyme by incubating at 65 ˚C for 10 min, and then cool to 4 ˚C.

4. Perform Downstream Application.

- An aliquot (10µl) of the amplified DNA can be directly applied for the **cycle sequencing reaction** without further purification.
- An aliquot (10µl) of the amplified DNA can be directly applied for **DNA restriction enzyme digestion** and then examined by agarose gel.