Polymerase Chain Reaction (PCR) for gDNA

Usage amount per sample:

Material ※	**Concentration**	**Addition per sample**	**Location**
gDNA †	10 ng / uL	4 µL	Usually -20°C
F-primer		0.4 µL	-20°C (2-3-4)
R-primer		0.4 µL	-20°C (2-3-4)
2X-Taq (orange lid)		5 µL	-20°C (4-3-4)
Ambion water		0.2 µL	RNA bench
Total volume:		**10 µL**	**place on ice**

※ All materials should be placed on ice.

† gDNA : The gDNA stock solution of all samples should be standardized to the same concentration, for example 10 ng/μL.

Procedure

1. Prepare the total amount of the required working solution (F-primer, R-primer, 2X-Taq);

- For example, if we had 10 samples, then me make the working solution by adding 4 µL **F-primer**, 4 µL **R-primer**, and 50 µL **2X-Taq** into a new 200 µL PCR tube.

2. Dispense the working solution into a new 200 μL PCR tubes (5.8 µL for each sample);
3. Lastly, add the sample gDNA stock solution;
4. Close the lid tightly, and swipe the tube gently with finger several time to mix the solution well;
5. Slightly centrifuge to bring down the solution;
6. Put into the PCR machine (the machine should be warmed up around 2 mins before use).

Reaction curve

Process	Temperature (°C)	Time	Cycle
Initial denaturation	95	1 min	--
Denaturation	95	15 secs	30
Annealing [†]	**56**	15 secs	30
Extension	72	1 min	30
Complete extension	72	5 min	--
PCR product storage	4	∞	--

[†] Annealing temperature depends on primers.

Done !!!

If you encounter any issues with the protocol, please report them at:
https://github.com/P10911004-NPUST/jklai/issues.