Discover-sc Single Cell Kit V2

Catalog # N602

Version 6.0

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1. Introduction

Discover-sc Single Cell Kit V2 is based on multiple displacement amplification (MDA) and is designed for unbiased whole genome amplification from single cell and other micro samples. The sizes of amplification products are between 2 kb to 100 kb, with a coverage greater than 95% and an average length higher than 20 kb. The products can be widely applied to whole genome sequencing, whole exome sequencing, large scale copy number variation analysis, microsatellite analysis, qPCR analysis, or gene chip analysis.

The Phi29 DNA polymerase used in this kit is cloned from phage and has a strong strand displacement activity. It can achieve on melting and replication of the DNA chain with complex structure. Meanwhile, Phi29 has a strong chain affinity and can achieve up to 100 kb of continuous polymerization, and the amplification product can be applied to almost all of the downstream genome analysis, including large scale copy number variation (CNV) analysis. Phi29 also possesses a potent 3'→5' exonuclease (proofreading) activity which ensures high fidelity in replication, which is 1000-fold higher than that of Taq and higher than most other high fidelity polymerases.

Normally, 30-40 μg of high coverage whole genomic DNA can be obtained during one reaction with Discover-sc Single Cell V2.

2. Contents of Kits

	Components	N601-01 (24 rxn)	N601-02 (96 rxn)
Box 1	Discover-sc DNA Polymerase	48 μl	192 μl
	Discover-sc Reaction Buffer	750 μl	3 × 1 ml
	Buffer D	1 ml	2 × 1 ml
	Buffer N	1 ml	2 × 1 ml
	DTT, 1 M	1 ml	1 ml
	PBS	1 ml	2 × 1 ml
	H2O	1 ml	2 × 1 ml
	Discover-sc Primer 1	30 μl	120 μl
	Discover-sc Primer 2	30 μl	120 μl
	Discover-sc Primer 3	30 μl	120 μl
	Discover-sc Primer 4	30 μl	120 μl
	Discover-sc Primer 5	30 μl	120 μl
	Discover-sc Primer 6	30 μl	120 μl
	Discover-sc Primer 7	30 μl	120 μl
	Discover-sc Primer 8	30 μl	120 μl
	Discover-sc Primer 9	30 μl	120 μl
	Discover-sc Primer 10	30 μl	120 μl
	Discover-sc Primer 11	30 μl	120 μl
	Discover-sc Primer 12	30 μl	120 μl
	Discover-sc Primer 13	30 μl	120 μl
	Discover-sc Primer 14	30 μl	120 μl
	Discover-sc Primer 15	30 μl	120 μl
	Discover-sc Primer 16	30 μl	120 μl
Box 2	Discover-sc AceQ Master Mix	4 × 1.25 ml	16 × 1.25 ml
	ROX Dye 1	200 μl	4 × 200 μl
	ROX Dye 2	200 μl	4 × 200 μl

3. Storage

Box 1: Store at -20°C. For long time storage, please store at -70°C.
Box 2: Store at -20°C. Protect from light.

4. Applications

Discover-sc Single Cell Kit can be used for unbiased whole genome amplification from single cell and other micro samples.

Human and animal

◇ Stem cell research
◇ Research on tumor progression
◇ Cancer stem cell analysis
◇ Genetic engineering animal genotyping
◇ Pre-implantation genetic diagnosis
◇ Analysis of fetal cells in maternal circulation
◇ Study on SNP, CNVs and other biomarkers

Bacteria

◇ Pathogen analysis
◇ Metagenomics research
◇ Microbial typing

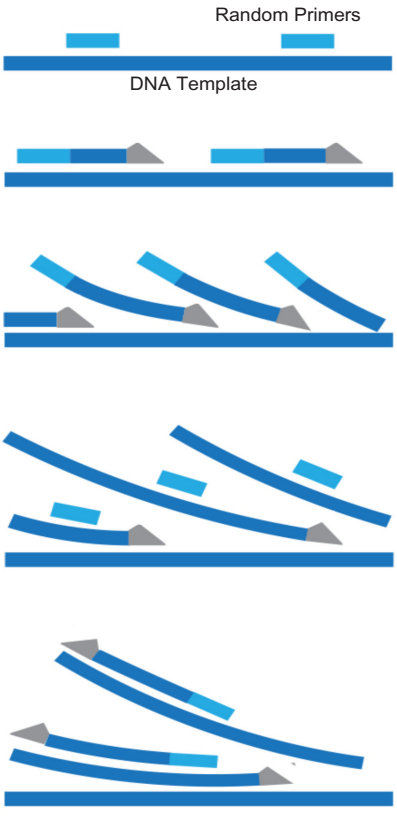
Plant

◇ Pollen analysis

5. Additional Required Equipments & Reagents

Centrifuge
Microscope
EP tube
RNase-free PCR tube
Water bath or thermal cycler
qPCR instrument

6. Schematic Diagram of Mechanism and Operating Processes



Random primers bind to multiple sites of the DNA template.

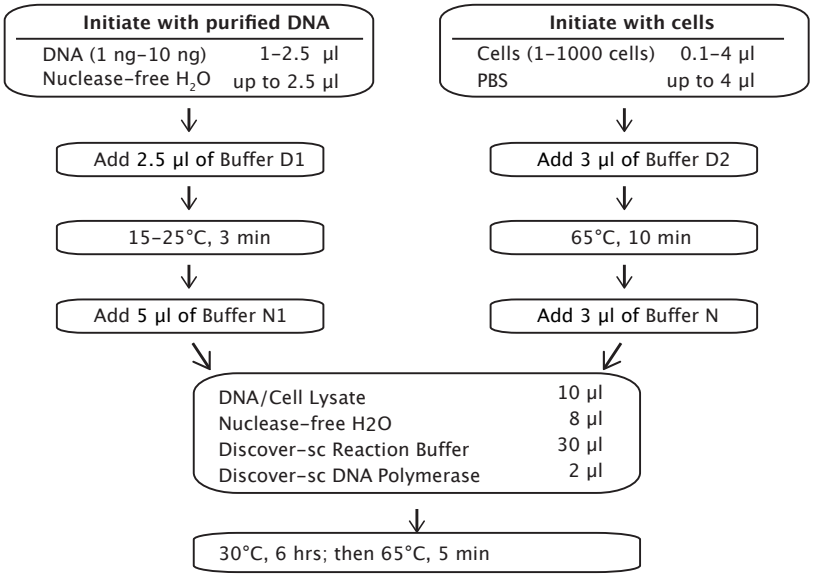
Phi29 DNA polymerase simultaneously initiates DNA replication at multiple primer binding sites.

Chain synthesis reaction in process replaces encountered DNA chain in synthesis and continue elongation, and produces replaced single strand DNA.

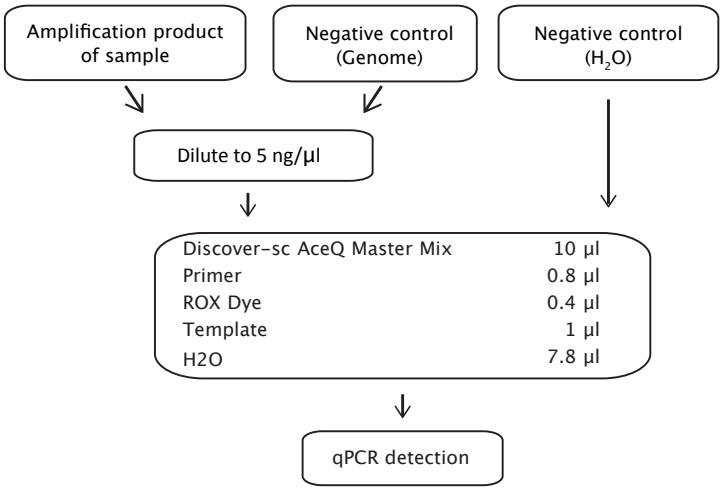
Replaced single strand DNA is bind by random primers as template.

Random primers initiate new DNA synthesis and chain replacement reaction, to synthesize double strand DNA of high molecular size.

Schematic diagram of MDA mediated with Phi29 DNA polymerase



Schematic diagram of operating processes.



Schematic diagram of amplification product integrity detection.

7. Protocol

7-1. Amplify genomic DNA from single cell.

This scheme is suitable for 1-1000 initial cells. Please use the freshly prepared cell samples to ensure the integrity of initiated genome and do not use apoptotic cells.

1. Prepare Buffer D2.

Note: The Buffer D2 volume in the following table is sufficient for 12 reactions. Store the buffer at -20°C after the experiment, and use up within 3 months.)

DTT, 1M	4 µl	
Buffer D	36 µl	
Total Volume	40 µl	

2. Add 4 µl of cell samples (resuspended in PBS) to a PCR tube. Please make up to 4 µl with PBS if the sample volume is less than 4 µl.

3. Add 3 µl of Buffer D2. Flick the tube wall to mix the cells followed with briefly centrifugation.

Note: Please make sure that the cells are not attached to the tube wall. DO NOT mix the cells with pipettors, to avoid that the cells are attached to the tips.

4. Incubate at 65°C for 10 min.

5. Add 3 µl of Buffer N, and mix by flick the tube wall followed with briefly centrifugation. Place the sample on the ice before the next reaction is prepared.

6. Prepare the mixture of reaction as follows:

H_2O	8 µl	
Discover-sc Reaction Buffer	30 µl	
Discover-sc DNA Polymerase	2 µl	
Total Volume	40 µl	

Note: Please add the above components in order. Mix with vortex and briefly centrifugation after adding H_2O and Discover-sc Reaction Buffer. Please use immediately after adding Discover-sc DNA Polymerase.

7. Immediately add 40 µl of reaction mixture to the prepared 10 µl of DNA sample (prepared in the **Step 5**), mix thoroughly and briefly centrifugate.

8. Incubate at 30°C for 6 hours.

Note: Incubation for 16 hour can obtain the maximum yield. Please prolong the incubation time if necessary.

9. Incubate at 65°C for 5 min to inactivate the Discover-sc DNA Polymerase.

10. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with water or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, CGH Array, etc.

7-2. Amplify purified genomic DNA.

This scheme is suitable for the whole genome amplification from purified genomic DNA with amount > 10 ng. Less input DNA (1-10 ng of eukaryotic genome DNA, 10-100 ng of bacterial genome DNA) can also be used if the integrity and purification of genome is high enough.

1. Prepare Buffer D1 and N1.

Note: The Buffer D1 and N1 volume in the following table is sufficient for 12 reactions. Store the buffer at -20°C after the experiment, and use up within 3 months.)

Prepare Buffer D1

Buffer D	7 µl	
H_2O	25 µl	
Total Volume	32 µl	

Prepare Buffer N1

Buffer N	9 µl	
H_2O	51 µl	
Total Volume	60 µl	

2. Add 2.5 µl of cell samples to a PCR tube. If the sample volume is lower than 2.5 µl, please make up to 2.5 µl with PBS.

3. Add 2.5 µl of Buffer D1. Flick the tube wall to mix the cells followed with briefly centrifugation.

Note: DO NOT mix the cells with pipettors, to avoid that the integrity is affected and the cells are attached to the tips.

4. Incubate at 15°C-25°C for 3 min.

5. Add 5 µl of Buffer N1. Flick the tube wall to mix the cells followed with briefly centrifugation. Place the sample on the ice before the next step.

6. Prepare the mixture of reaction.

H_2O	8 µl	
Discover-sc Reaction Buffer	30 µl	
Discover-sc DNA Polymerase	2 µl	
Total Volume	40 µl	

Note: Please add the above components in order. Mix with vortex and briefly centrifugation after adding H_2O and Discover-sc Reaction Buffer. Please use immediately after adding Discover-sc DNA Polymerase.

7. This Immediately add 40 µl of reaction mixture to 10 µl of DNA sample (prepared in **Step 5**), flick the tube wall to mix the cells followed with briefly centrifugation.

8. Incubate at 30°C for 6 hours.

Note: Incubate for 16 hours can obtain the maximum yield. Please prolong the incubation time if necessary.

9. Incubate at 65°C for 5 min to inactivate the Discover-sc DNA Polymerase.

10. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with water or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, CGH Array, etc.

7-3. Amplify genomic DNA from blood sample.

This scheme is suitable for the whole genome amplification from blood.

1. Prepare Buffer D2.

Note: The Buffer D2 volume in the following table is sufficient for 12 reactions. Store the buffer at -20°C after the experiment, and use up within 3 months.

DTT, 1M	4 µl	■
Buffer D	36 µl	■
Total Volume	40 µl	

2. Dilute the blood to more than 3-fold with PBS, and mix well followed with a briefly centrifugation to collect the sample.

3. Add 4 µl of diluted blood samples to a PCR tube. Add 3 µl of Buffer D2. Flick the tube wall to mix the cells followed with briefly centrifugation.

Note: DO NOT mix the cells with pipette, in order to avoid that the integrity is affected or the cells are attached to the tips.

4. Incubate on the ice for 10 min.

5. Add 3 µl of Buffer N after the incubation. Mix with vortex and collect with briefly centrifugation. Place the sample on ice before the next step.

6. Prepare the mixture of reaction.

H_2O	8 µl	□
Discover-sc Reaction Buffer	30 µl	■
Discover-sc DNA Polymerase	2 µl	■
Total Volume	40 µl	

Note: Please add the above components in order. Mix with vortex and briefly centrifugation after adding H_2O and Discover-sc Reaction Buffer. Please use immediately after adding Discover-sc DNA Polymerase.

7. Immediately add 40 µl of reaction mixture to the prepared 10 µl of DNA sample (prepared in the **Step 5**), flick the tube wall to mix the cells followed with briefly centrifugation.

8. Incubate at 30°C for 6 hours.

Note: Incubation for 16 hours can obtain the maximum yield. Please prolong the incubation time if necessary.

9. Incubate at 65°C for 5 min to inactivate the Discover-sc DNA Polymerase.

10. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with water or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, CGH Array, etc.

7-4. Amplify residual genomic DNA from blood on paper.

This scheme is suitable for residual genomic DNA amplification from blood on the paper. Please note that the scheme may not amplify all the genomic sequence due to the poor integrity of the sample.

1. Prepare Buffer D2.

Note: The Buffer D2 volume in the following table is sufficient for 12 reactions. Store the buffer at -20°C after the experiment, and use up within 3 months.

DTT, 1M	50 µl	■
Buffer D	450 µl	■
Total Volume	500 µl	

2. Cut the paper with blood into small pieces (3 mm × 3 mm), and place the cut paper in a 1.5 ml PCR tube.

Note: Cut multiple samples with the same device may cause cross contamination.

3. Add 40 µl of Buffer D2, flick the tube wall to mix followed with briefly centrifugation.

4. Incubate for 10 min on ice.

5. Add 40 µl of Buffer N after the incubation. Mix with vortex and collect by briefly centrifugation.

6. Take 6 µl of lysis solution to a new PCR tube and make up to 10 µl with 4 µl of water. Place on ice until next step.

7. Prepare the mixture of reaction.

H_2O	8 µl	□
Discover-sc Reaction Buffer	30 µl	■
Discover-sc DNA Polymerase	2 µl	■
Total Volume	40 µl	

Note: Please add the above components in order. Mix with vortex and briefly centrifugation after adding H_2O and Discover-sc Reaction Buffer. Please use immediately after adding Discover-sc DNA Polymerase.

8. Immediately add 40 µl of reaction mixture to the prepared 10 µl of DNA sample (prepared in the **Step 6**), flick the tube wall to mix the cells followed with briefly centrifugation.

9. Incubate at 30°C for 6 hours.

Note: Incubation for 16 hours can obtain the maximum yield. Please prolong the incubation time if necessary.

10. Incubate at 65°C for 5 min to inactivate the Discover-sc DNA Polymerase.

11. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with water or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, CGH Array, etc.

7-5. Amplify genomic DNA of oral epithelial cells from cotton swab.

This scheme is suitable for the amplification of genomic DNA from oral epithelial cells on the cotton swab. It is recommended to use fresh oral epithelial cells to ensure the genome integrity.

1. Prepare Buffer D2.

Note: The Buffer D2 volume in the following table is sufficient for 12 reactions. Store the buffer at -20°C after the experiment, and use up within 3 months.

DTT, 1M	50 µl
Buffer D	450 µl
Total Volume	500 µl

2. Place the cotton swab (containing oral epithelial cells) in a 1.5 ml EP tube.

3. Add 40 µl of Buffer D2, flick the tube wall to mix followed with briefly centrifugation.

4. Incubate for 10 min on ice.

5. Add 40 µl of Buffer N after the incubation. Mix with vortex and collect by briefly centrifugation.

6. Take 6 µl of lysis solution to a new PCR tube and make up to 10 µl with 4 µl of water.

7. Prepare the mixture of reaction.

H_2O	8 µl
Discover-sc Reaction Buffer	30 µl
Discover-sc DNA Polymerase	2 µl
Total Volume	40 µl

Note: Please add the above components in order. Mix with vortex and briefly centrifugation after adding H_2O and Discover-sc Reaction Buffer. Please use immediately after adding Discover-sc DNA Polymerase.

8. Immediately add 40 µl of reaction mixture to the prepared 10 µl of DNA sample (prepared in the **Step 6**), flick the tube wall to mix the cells followed with briefly centrifugation.

9. Incubate at 30°C for 6 hours.

Note: Incubation for 16 hours can obtain the maximum yield. Please prolong the incubation time if necessary.

10. Incubate at 65°C for 5 min to inactivate the Discover-sc DNA Polymerase.

11. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with water or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, CGH Array, etc.

7-6. Amplify genomic DNA of frozen samples or biopsy tissues.

This scheme is suitable for amplification of genomic DNA from frozen samples or biopsy tissue. Please use fresh tissue or well-preserved tissue. Improper preservation of tissue may lead to the degradation of the genome.

1. Prepare Buffer D2.

Note: The Buffer D2 volume in the following table is sufficient for 12 reactions. Store the buffer at -20°C after the experiment, and use up within 3 months.

DTT, 1M	15 µl
Buffer D	135 µl
Total Volume	150 µl

2. Place the frozen samples or biopsy tissue (< 2 mm^3) in the PCR tube. Add 10 µl of TE buffer.

3. Add 10 µl of Buffer D2, flick the tube wall to mix followed with briefly centrifugation.

4. Incubate on the ice for 30 min.

5. Add 10 µl of Buffer N after the incubation. Mix with vortex and collect by briefly centrifugation.

6. Take 10 µl of lysis solution to a new PCR tube. Place on ice until next step.

7. Prepare the mixture of reaction.

H_2O	8 µl
Discover-sc Reaction Buffer	30 µl
Discover-sc DNA Polymerase	2 µl
Total Volume	40 µl

Note: Please add the above components in order. Mix with vortex and briefly centrifugation after adding H_2O and Discover-sc Reaction Buffer. Please use immediately after adding Discover-sc DNA Polymerase.

8. Immediately add 40 µl of reaction mixture to the prepared 10 µl of DNA sample (prepared in the **Step 6**), flick the tube wall to mix the cells followed with briefly centrifugation.

9. Incubate at 30°C for 6 hours.

Note: Incubation for 16 hours can obtain the maximum yield. Please prolong the incubation time if necessary.

10. Incubate at 65°C for 5 min to inactivate the Discover-sc DNA Polymerase.

11. The amplification product is high concentration genomic DNA, please dilute the DNA to an appropriate concentration with water or TE to perform the downstream experiment. The amplification products can be widely used in downstream experiment including whole genome sequencing, whole exome sequencing, microsatellite analysis, qPCR analysis, gene chip analysis, CGH Array, etc.

8. Examples of Amplification Results using Discover-sc Single Cell Kit

8-1. Electrophoresis Analysis

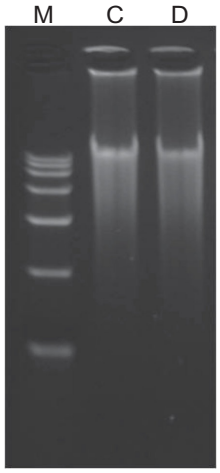


Fig.1. The electrophoresis results of amplified products using Discover-sc Single Cell Kit V2. The sizes of amplification products are between 2 kb to 100 kb, with an average length higher than 20 kb.

M: DNA marker (DL15000)

C: Amplification products of single cell

D: Amplification products of genome

8-2. qPCR Analysis

The uniformity and coverage of single cell or genome amplification product are important index to evaluate if the product is qualified. This kit employs qPCR primers targeting 16 different chromosomes for quality control.

Dilute the amplification product to 5 ng/μl for detection. Set up a negative control (no template control) to detect possible pollution in qPCR primers, qPCR reagents and the reaction system. Set up a positive control (genome) to confirm the efficiency of qPCR detection.

1. Prepare the reaction solution in a qPCR tube as follows:

Discover-sc AceQ Master Mix	10 μl	□
Discover-sc Primer	0.8 μl	■
ROX Dye 1	0.4 μl	■
Template DNA	1 μl	
ddH_2O	7.8 μl	□
Total	20 μl	

Note: The 16 pairs of primers provided in this kit are limited to detect integrity of human DNA.
Note: The amplification product is high concentration genomic DNA, and please use after dilution. Otherwise the amplification efficiency may be affected.
Note: Please use ultra-clean hood when preparing reaction mixture, and please use tips with filter to avoid contamination.

2. Run the following program for qPCR:

Stage 1	Pre-denaturation	Reps: 1	95℃	5 min
Stage 2	Denaturation	Reps: 40	95℃	10 sec
	Annealing + Extension		60℃	30 sec
Stage 3	Melting Curve	Reps: 1	95℃	15 sec
			60℃	15 sec
			95℃	60 sec

3. Data analysis.

After reaction, CT values (**Fig. 2**) and melting curves (**Fig. 3**) are confirmed. The specificity of amplification product can also be confirmed by agarose electrophoresis. Negative control shows no obvious signal, otherwise it indicates contamination in qPCR process. CT values between 22 and 28 are accurate; if CT value is higher than 32, then qPCR detection is not effective.

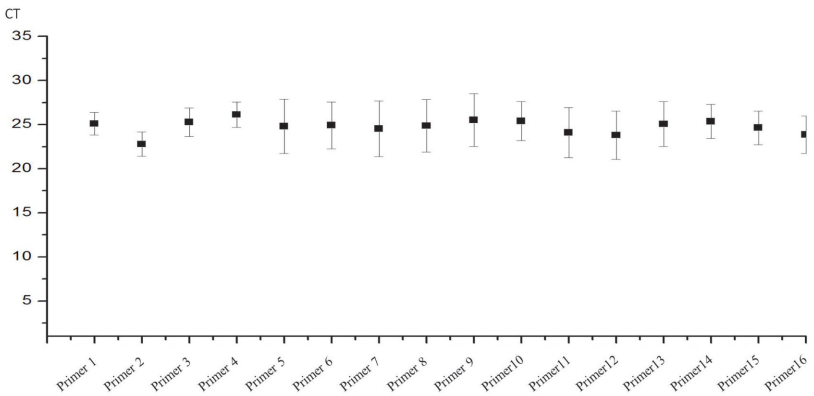


Fig.2. qPCR analysis results.

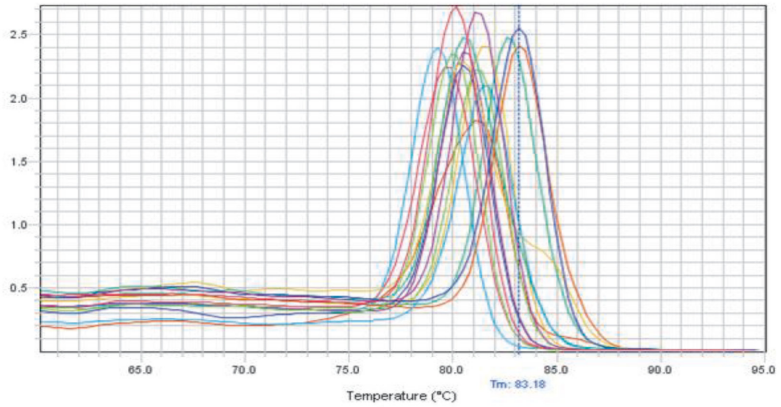


Fig.3. Melting curves.

9. Troubleshooting

1. No amplified products

A. Sample loss during collection

Redo the sample collection.

B. Genome DNA samples contains components that inhibit the reaction.

Purify or dilute DNA sample. If there was ethanol precipitation or rinsing process during the purification of samples, the residual alcohol can inhibit the reaction and purification process should make full volatile of the alcohol.

C. High reaction temperature

The reaction temperature should be controlled under 30°C. Over high temperature can inactivate the enzyme. If use PCR instrument with hot-lid, please set the temperature of hot-lid at 70°C.

2. 10-40 μg of DNA is amplified but loss some loci or alleles.

A. For reaction of using genomic DNA as initial template:

The genomic DNA may be degraded, please use complete DNA or more amount of DNA as template.

B. For reaction of using cell as initial template:

Cell apoptosis or DNA degraded during the fixed step. Or there is cell wall (i.e. plant cells) which is not suitable for direct initial material.

3. 10-40 μg of DNA was amplified in the negative control but the downstream detected results were negative (i.e. qPCR).

The high molecular weight DNA products were amplified by the random annealing of primers in the negative control reactions, but the products do not affect downstream analysis of the target product.

4. 10-40 μg of DNA was amplified in negative control and the downstream detected results were positive (i.e. qPCR).

The reaction is contaminated with exogenous DNA. Since the reaction is very sensitive to micro DNA, please replace all the reagents and supplies that may be contaminated with exogenous DNA.