Preamplification of cDNA for Gene Expression with Delta Gene Assays

NOTE If using TaqMan® PreAmp Master Mix, follow the instructions on page 2 for pooling the Delta Gene™ assays, preparing the preamplification reaction solution, and thermal cycling the preamplification reaction.

Pool Delta Gene Assays

1. In a microcentrifuge tube, combine 1 µL of each 100 µM stock Delta Gene assay, up to a total of 96 assays.
2. Add DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA; TEKnova, PN T0221) to make the final volume 200 µL.
   The concentration of each assay will be 500 nM.
   Volume can be adjusted proportionally based on the number of samples to be amplified.

Prepare Preamplification Reaction Solution Using Fluidigm Preamp Master Mix

1. In a DNA-free hood, in a new, labeled 1.5-mL microcentrifuge tube, combine PreAmp Master Mix and 500 nM pooled Delta Gene assay mix as shown in Table 1.
2. Label a new 96-well plate "Sample Plate."
3. Pipet 3.75 µL of preamplification pre-mix into each well of plate.
4. In a DNA sample hood, pipet 1.25 µL of cDNA into appropriate wells of the Sample Plate, making a total volume of 5 µL.
5. Vortex the plate for 5 seconds and centrifuge at 1,000 x *g* for 1 minute.

Table 1: Preamplification reaction solution using Fluidigm Preamp Master Mix

Component	Vol. per Reaction (µL)	Vol. for 48 Reactions w/Overage* (µL)	Vol. for 96 Reactions w/Overage* (µL)
PREAMPLIFICATION PRE-MIX			
Preamp Master Mix (Fluidigm PN 100-5580 or PN 100 5581)	1.00	52.8	105.6
Pooled Delta Gene assay mix (500 nM)	0.50	26.4	52.8
DNase-free water	2.25	118.8	237.6
cDNA	1.25	—	—
Total	**5.00**	—	—

*10% for ease of pipetting.

Thermal-Cycle Preamplification Reactions Using Fluidigm Preamp Master Mix

Thermal-cycle the preamplification reactions in the 96-well plate using the conditions in Table 2.

Ten cycles are recommended as a starting point, but this number can be increased to up to 20 cycles if necessary. Determine the appropriate number of cycles empirically.

A longer time could be used if the targets were not adequately denatured, but is not necessary to activate the enzyme.

Table 2: Thermal cycling conditions for reactions using Fluidigm Preamp Master Mix

Cycles	Temperature	Time
Hold	95 °C	2 min
10	95 °C	15 sec
	60 °C	4 min
Hold	4 °C	∞

Clean Up Reactions with Exonuclease I

1. Dilute Exonuclease I (Exo I) to 4 U/µL:

Component	Per 5-µL Sample (µL)	48 Samples w/ Overage (µL)	96 Samples w/Overage (µL)
DNase-free water	1.4	84.0	168.0
Exonuclease I Reaction Buffer (New England BioLabs®, PN M0293S or PN M0293L)	0.2	12.0	24.0
Exonuclease I, 20 U/µL	0.4	24.0	48.0
Total	2.0	120.0	240.0

2. Add 2 µL of diluted Exo I at 4 U/µL to each 5-µL preamplification reaction.
3. Vortex for 5 seconds and centrifuge at 1,000 x *g* for 10 seconds.
4. Thermal-cycle the preamplification reaction and Exo I using the following conditions:

Cycles	Temperature	Time
Digest	37 °C	30 min
Inactivate	80 °C	15 min
Hold	4 °C	∞

5. Use DNA suspension buffer (TEKnova, PN T0221) to dilute the final products to an appropriate concentration for testing. The minimum amount of dilution that should be used is five-fold, but if the C_q values are consistently below 6 for some of the assays this may need to be increased to 10-fold or 20-fold.

Volume of Preamplification Reaction and Exo I Dilution (µL)	Volume of DNA Suspension Buffer to Add (µL)		
	5-Fold Dilution	10-Fold Dilution	20-Fold Dilution
7	18	43	93

You can store diluted reactions at –20 °C for at least one week or use within 60 minutes for real-time thermal cycling.

Preamplification Using TaqMan PreAmp Master Mix

NOTE If you are using **Fluidigm Preamp Master Mix**, follow the instructions on page 1.

Pool Delta Gene Assays

1. In a microcentrifuge tube, combine 1 µL of each 100 µM stock Delta Gene assay, up to a total of 100 assays when using TaqMan PreAmp Master Mix.
2. Add DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA; TEKnova, PN T0221) to make the final volume 200 µL.
   The concentration of each assay will be 500 nM.
   Volume can be adjusted proportionally based on the number of samples to be amplified.

Prepare the Preamplification Reaction Solution Using TaqMan PreAmp Master Mix

1. In a DNA-free hood, in a new, labeled 1.5-mL microcentrifuge tube, combine TaqMan PreAmp Master Mix and 500 nM pooled Delta Gene assay mix as shown in Table 3.
2. Label a new 96-well plate "Sample Plate."
3. Pipet 3.75 µL of the preamplification pre-mix into each well of the plate.
4. In a DNA sample hood, pipet 1.25 µL of cDNA into appropriate wells of the Sample Plate, making a total volume of 5 µL.
5. Vortex the plate for 5 seconds and centrifuge at 1,000 x *g* for 1 minute.

Table 3: Preamplification reaction solution using TaqMan PreAmp Master Mix

Component	Vol. per Reaction (µL)	Vol. for 48 Reactions w/Overage* (µL)	Vol. for 96 Reactions w/Overage* (µL)
PREAMPLIFICATION PRE-MIX			
2X TaqMan PreAmp Master Mix (Life Technologies PN 4391128)	2.5	132.0	264.0
Pooled Delta Gene assay mix (500 nM)	0.5	26.4	52.8
DNase-free water	0.75	39.6	79.2
cDNA	1.25	—	—
Total	**5.00**	—	—

*10% for ease of pipetting.

Thermal-Cycle Preamplification Reactions Using TaqMan PreAmp Master Mix

Thermal-cycle the preamplification reactions in the 96-well plate using the following conditions:

Table 4: Thermal cycling conditions for reactions using TaqMan PreAmp Master Mix

Cycles	Temperature	Time
Hold	95 °C	10 min
10–14	95 °C	15 sec
	60 °C	4 min
Hold	4 °C	∞

After thermal-cycling the preamplification reactions, clean up the reactions as described in "Clean Up Reactions with Exonuclease I" on page 1.

For technical support visit fluidigm.com/support

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