TLDA: TaqMan® Array MicroRNA Cards

1. Megaplex™ RT Reactions

In this step, use the TaqMan® MicroRNA Reverse Transcription Kit and the Megaplex™ RT Primers to synthesize single-stranded cDNA from total RNA samples. The RT reaction with downstream preamplification supports 1 to 1000 ng of input total RNA. For most tissues, 30 ng of total RNA produces a comprehensive microRNA profile with pre-amplification. Applied Biosystems recommends verifying the optimal quantity of input total RNA for your sample type. The reverse transcription (RT) reaction has a final volume of 7.5 µL and contains:

- 3 µL (1 to 350 ng) total RNA*

(*If the starting material was very limited and the total RNA amounts so low that it was impossible to measure them, use a fixed, constant volume of RNA template for each reaction: 3µl)

- 4.5 µL of RT reaction mix

1. Thaw the following on ice:
   - Megaplex RT Primers
   - TaqMan® MicroRNA Reverse Transcription Kit components
   - MgCl2 (supplied with the Megaplex™ RT Primers)
2. Combine the following in a 1.5-mL microcentrifuge tube:

RT Reaction Mix Components	Volume for One Sample (µL)	Volume for Ten Samples (µL)‡
Megaplex™ RT Primers (10X)	0.80	9.00
dNTPs with dTTP (100 mM)	0.20	2.25
MultiScribe™ Reverse Transcriptase (50 U/µL)	1.50	16.88
10X RT Buffer	0.80	9.00
$MgCl_2$ (25 mM)	0.90	10.12
RNase Inhibitor (20 U/µL)	0.10	1.12
Nuclease-free water	0.20	2.25
Total	4.50	50.62

‡ Includes 12.5% excess for volume loss from pippetting.

3. Invert the tube six times to mix, then centrifuge the tubes briefly.
4. In a 96-well or 8-Tube Strip, pipette 4.5 µL of the RT reaction mix into each well or each tube, respectively.
5. Add 3 µL (1 to 350 ng) total RNA (or 3 µL of water for the No Template Control reactions) into each well or each tube containing RT reaction mix.
6. Seal the plate or tubes. Then invert the plate or tubes six times to mix. Spin briefly.
7. Incubate the plate on ice for 5 min.

Megaplex™ Reverse Transcription

Set up the run method using the following conditions:

- Ramp speed or mode: **9700** using **Std** or **Max** ramp speed. **7900HT** using **Std** ramp speed. (Alternatively, run on other thermal cycler)
- Reaction volume (µL): **7.5**
- Thermal-cycling conditions:

Stage	Temp	Time
Cycle (40 Cycles)	16 °C	2 min
	42 °C	1 min
	50 °C	1 sec
Hold	85 °C	5 min
Hold	4 °C	∞

(Optional) Stopping Point
The cDNA can be stored at −15 to −25 °C for at least one week.

2. Pre-amplification Reaction (optional)

In this step, pre-amplify specific cDNA targets to increase the quantity of desired cDNA for gene expression analysis using TaqMan® MicroRNA Arrays. (Note: For samples where the expected total RNA amounts are low this step is required)

The pre-amplification reaction has a final volume of 25 µL and contains:

- 2.5 µL RT product
- 22.5 µL PreAmp reaction mix

Prepare the PreAmp Reaction Mix

1. Thaw the Megaplex™ PreAmp Primers on ice and mix by inverting six times. Spin briefly.
2. Mix the TaqMan® PreAmp Master Mix (2✕) by swirling the bottle.
3. Combine the following in a 1.5-mL microcentrifuge tube:

PreAmp Reaction Mix Components	Volume for One Sample (µL)	Volume for Ten Samples (µL)‡
TaqMan® PreAmp Master Mix (2X)	12.5	140.62
Megaplex™ PreAmp Primers (10X)	2.5	28.13
Nuclease-free water	7.5	84.37
Total	22.5	253.12

‡ Includes 12.5% excess for volume loss from pippetting.

4. Invert the tube six times to mix, then centrifuge the tubes briefly.
5. In a 96-well plate or 8-Tube Strips, pipette 2.5 µL of each RT product into its corresponding well or tube. (Note: Using individual 0.5ml tubes allows the thawing of each sample independently of the other samples, which is useful later on at the stage of loading the array cards)
6. Dispense 22.5 µL of PreAmp reaction mix into each well of the 96-well plate or 8-Tube Strips containing the RT product.
7. Seal the plate or tubes. Then invert the plate or tubes six times to mix. Spin briefly.
8. Incubate the plate or tubes on ice for 5 min.

Perform the Pre-amplification (PreAmp)

1. Set up the run method using the following conditions:
   - Ramp speed or mode: **9700** using **Std** ramp speed. (Alternatively, run on other thermal cycler)

- Reaction volume (µL): **25**
- Thermal-cycling parameters:

Stage	Temp	Time
Hold	95 °C	10 min
Hold	55 °C	2 min
Hold	72 °C	2 min
Cycle (12 Cycles)	95 °C	15 sec
	60 °C	4 min
Hold‡	99.9 °C	10 min
Hold	4 °C	∞

‡ Required for enzyme inactivation.

2. Load, then run the plate.

Dilute the Reaction

1. Remove the 96-well plate or 8-tube strips from the thermal cycler.
2. Briefly centrifuge the tubes or plate.
3. Add 75 µL of 0.1X TE pH 8.0 to each well or tube.
4. Seal the plate or tubes, then invert six times to mix, and spin briefly.

(Optional) Stopping Point

Store the diluted preamplified product at -15 to -25°C for up to one week, or place the product on ice and proceed directly.

3. Run the Real-Time PCR Reactions

In this step, the DNA polymerase from the TaqMan® Universal PCR Master Mix amplifies the preamplified target cDNA using sequence-specific primers and probe on the TaqMan® MicroRNA

Array. The presence of the target is detected in real time through cleavage of the TaqMan probe by the polymerase 5′ - 3′ exonuclease activity.

Prepare the TaqMan MicroRNA Array Card

After the TaqMan® MicroRNA Array has reached room temperature, carefully remove it from its packaging. Refer to the *Applied Biosystems TaqMan® Array User Bulletin* (PN 4371129).

Prepare the PCR Reaction Mix

1. Thaw the diluted, stored PreAmp product on ice. Mix by inverting six times, then centrifuge the tube or plate briefly.
2. Mix the TaqMan Universal PCR Master Mix by swirling the bottle.
3. Combine the following in a 1.5-mL microcentrifuge tube:

Component	Volume for One Array‡
TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 2X	450
Diluted PreAmp product	9
Nuclease-free water	441
Total	900

‡ Includes 12.5% excess for volume loss from pippetting.

4. Invert the tube six times to mix, then centrifuge the tubes briefly.

Load and Run the MicroRNA Array (TLDA)

For detailed information on how to load, seal, and run the array, refer to the *Applied Biosystems TaqMan® Array User Bulletin* (PN 4371129).