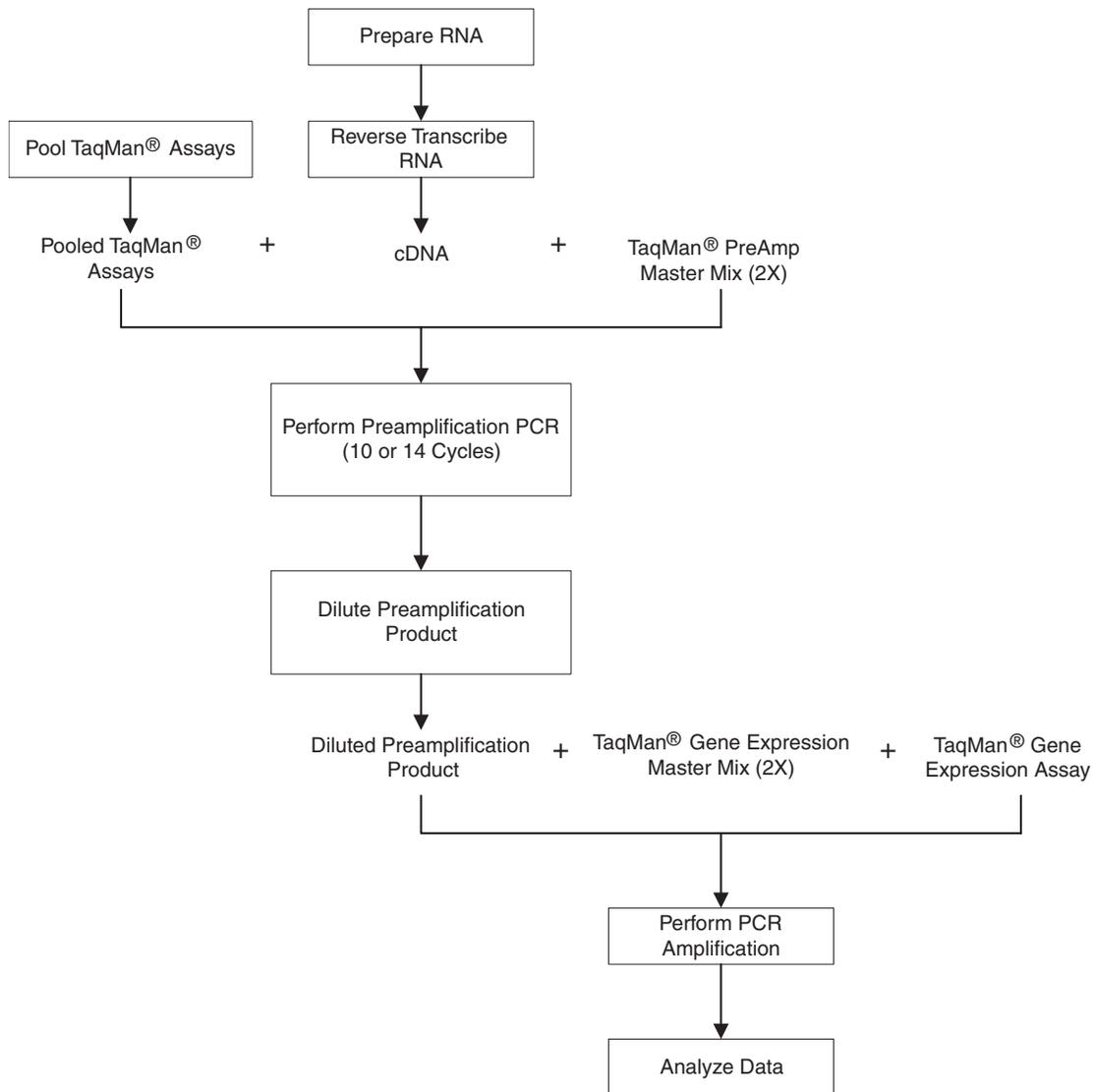


Preamplification of cDNA Quick Reference Card

For safety and biohazard guidelines, refer to the “Safety” section in the *TaqMan® PreAmp Master Mix Kit Protocol* (PN 4384557). For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To download Applied Biosystems user documents, go to <http://www.appliedbiosystems.com>, then click the link for **Support**. Click **Product & Service Literature**. On the Documents on Demand search page, select or enter your search parameters, then click **Search**.

Preamplification Workflow



Pooling the TaqMan Assays

- Combine equal volumes of each **20X TaqMan® Gene Expression Assay**, up to 100 assays.
For example, to pool 50 TaqMan assays, combine 10 µL of each assay in a microcentrifuge tube.
- Dilute the pooled TaqMan assays using 1X TE buffer so that each assay is at a final concentration of 0.2X.
For the above example, add 500 µL of 1X TE buffer to the pooled TaqMan assays for a total final volume of 1 mL.

Preparing cDNA from RNA

Applied Biosystems recommends the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (PN 4368814).

Performing Preamplification PCR

Determine the preamplification conditions, based on the number of preamplification cycles:

Number of Preamplification Cycles	Dilution Factor of Preamplification Products	Final Volume of Diluted Preamplification Product
10	1:5	250 µL
14	1:20	1 mL

- Prepare the preamplification reactions, then transfer to a reaction plate:

Component	Volume (µL)/Reaction
TaqMan® PreAmp Master Mix (2X)	25.0
Pooled assay mix (0.2X, each assay)	12.5
1–250 ng cDNA sample + nuclease-free water	12.5
Total	50.0

- Seal the plate with a MicroAmp™ Clear Adhesive Film, then gently invert the plate. Centrifuge the plate briefly, then place a MicroAmp™ Optical Film Compression Pad on top of it.
- Run the preamplification reactions (standard mode):

	HOLD	CYCLE (10 or 14 cycles)	
Temp	95 °C	95 °C	60 °C
Time	10 min	15 sec	4 min

- Upon completion, *immediately* remove the plate from the thermal cycler and place it on ice.
- Dilute the preamplification product (step 1 below), or you may store aliquots of the preamplification product at –20 °C.

Performing PCR Amplification

- Dilute the preamplification product according to the number of preamplification cycles you performed:
 - 10 preamplification cycles: Dilute 1:5 with 1X TE Buffer
 - 14 preamplification cycles: Dilute 1:20 with 1X TE Buffer

- Prepare the PCR reactions, then transfer to an optical plate:

Component	Volume (µL) /Reaction	
	20-µL Reactions	50-µL Reactions
TaqMan® Gene Expression Assay (20X)	1.0	2.5
Preamplified cDNA products (diluted 1:5 or 1:20)	5.0	12.5
TaqMan® Gene Expression Master Mix (2X)	10.0	25.0
Nuclease-free water	4.0	10.0
Total Volume	20.0	50.0

- Seal the plate with a MicroAmp™ Optical Adhesive Film or with optical flat caps, then centrifuge briefly.
- Run the plate in a Real-Time PCR instrument (standard mode):

	HOLD	HOLD	CYCLE (40 cycles)	
Temp	50 °C	95 °C	95 °C	60 °C
Time	2 min	10 min	15 sec	1 min

- Analyze the results. Refer to your instrument user guide or appropriate getting started guides for instructions.

Applied Biosystems Products

See the *TaqMan PreAmp Master Mix Protocol* for a complete list.

Product	Part Number
Applied Biosystems High Capacity cDNA Reverse Transcription Kit	4368814
<i>Applied Biosystems High-Capacity cDNA Reverse Transcription Kit Protocol</i>	4375575
MicroAmp™ Clear Adhesive Films	4306311
MicroAmp™ Optical 384-Well Reaction Plates	4309849
MicroAmp™ Optical 96-Well Reaction Plates	4306737
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical Film Compression Pads	4312639
TaqMan® Gene Expression Assays	4331182, 4351372
TaqMan® Gene Expression Master Mix (5 mL)	4369016
<i>TaqMan® Gene Expression Master Mix Protocol</i>	4371135
TaqMan® PreAmp Master Mix Kit	4384267

For Research Use Only. Not for use in diagnostic procedures.

NOTICE TO PURCHASER: PLEASE REFER TO THE *TaqMan® PreAmp Master Mix Kit Protocol* (PN 4384557) FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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TaqMan is a registered trademark of Roche Molecular Systems, Inc.

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