Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan® RNase P Detection Reagents Kit

Publication Number MAN0007732 Revision 2.0

Overview

Purpose

RNase P is a single-copy gene encoding the RNA moiety of the RNase P enzyme. It is a useful gene for quantifying amplifiable genomic DNA when used in conjunction with a standard curve generated from a genomic DNA standard of known concentration. The TaqMan® RNase P Detection Reagents Kit contains a 20X mix of primers and probe (FAM™ dye-labeled) that will detect and quantify genomic copies of the human RNase P gene. The primers and probe are designed according to TaqMan® chemistry guidelines for quantification, and utilize universal thermal cycling parameters.

For more information and recommendations for kits to isolate genomic DNA from formalin-fixed, paraffin-embedded tissue, refer to the *Ion AmpliSeq™ Library Preparation User Guide* (Pub. Part No. MAN0006735).

Life Technologies Demonstrated Protocols

Life Technologies Demonstrated Protocols have been successfully demonstrated by Life Technologies research and development but not formally validated. There are no technical specifications for Life Technologies demonstrated protocols. Users assume all risk when using these protocols, and recognize that support for Life Technologies demonstrated protocols occurs through community discussion. All customers are encouraged to discuss and contribute via the Ion community.

Required materials and equipment

Item description	Supplier	Cat. No.
TaqMan® RNase P Detection Reagents Kit	Life Technologies	4316831
TaqMan® Universal PCR Master Mix	Life Technologies	4304437
MicroAmp® Optical 96-well reaction plates *or*	Life Technologies	N8010560
MicroAmp® Optical 384-well reaction plates	Life Technologies	4309849
MicroAmp® Optical Adhesive Film Kit	Life Technologies	4313663
Eppendorf LoBind® Microcentrifuge Tubes, 1.5 mL	Eppendorf	022431021
Nuclease-free Water	Life Technologies	AM9938
Life Technologies Real-Time PCR System†	Life Technologies	—

† See table on page 5 for list of available Life Technologies Real-Time PCR Systems.

TaqMan® RNase P assay to quantify genomic DNA

Prepare standard curve with control DNA

1. Label seven 1.5-mL Eppendorf LoBind® Tubes with numbers 1–7.
2. Add 15 µL of Nuclease-free Water to each tube.
3. Add 15 µL of control DNA from the RNase P Detection Reagents Kit to tube 1 and mix well by vortexing. Centrifuge the tube for 3–5 seconds to collect the solution at the bottom of the tube.
4. Add 15 µL of the contents of tube 1 to tube 2 and mix well by vortexing. Centrifuge the tube for 3–5 seconds to collect the solution at the bottom of the tube.
5. Repeat step 4 with the successive tubes, pipetting 15 µL from tube 2 to tube 3, etc., until the dilution series is complete, as shown in the table below:

Tube	Volume	Concentration
1	15 µL	5 ng/µL
2	15 µL	2.5 ng/µL
3	15 µL	1.25 ng/µL
4	15 µL	0.625 ng/µL
5	15 µL	0.3125 ng/µL
6	15 µL	0.15625 ng/µL
7	30 µL	0.078125 ng/µL

Dilute the samples and set up the real-time PCR reaction

1. Prepare a 1:100 dilution and a 1:500 dilution of each sample DNA in Nuclease-free Water. These can be independent or serial dilutions.
2. Calculate the total number of reactions required. Use the equation:

   Total # of Reactions = (# of Samples × 2 dilutions × 3 replicates) + 21 Standard Curve Reactions + 3 Negative Template Control (NTC) Reactions

   For example, if 5 samples are being quantified, 30 sample reactions (5 × 2 DNA dilutions/sample in triplicate) + 21 standard curve reactions (7 control DNA dilutions in triplicate) + 3 NTC reactions (where Nuclease-free Water is used in place of control of sample DNA) = 54 reactions.
3. Prepare a PCR master mix:
   - For a 384-well plate, prepare sufficient volume for 10-µL reactions using the number of reactions calculated in Step 2. Using the table below, add the calculated volume of each reagent to a new 1.5-mL Eppendorf LoBind® Tube. Vortex the tube to mix and centrifuge briefly to collect contents at the bottom of the tube.

Component	Volume per reaction (384-well)	Total volume required for X number of reactions (384-well)
TaqMan® Universal PCR Master Mix (2X)	5 µL	5 µL × number of reactions × 1.2†
20X RNase P Primer-Probe mix	0.5 µL	0.5 µL × number of reactions × 1.2†
Nuclease-free Water	2 µL	2 µL × number of reactions × 1.2†

† Life Technologies recommends multiplying by a 1.2 overage factor to compensate for pipetting error.

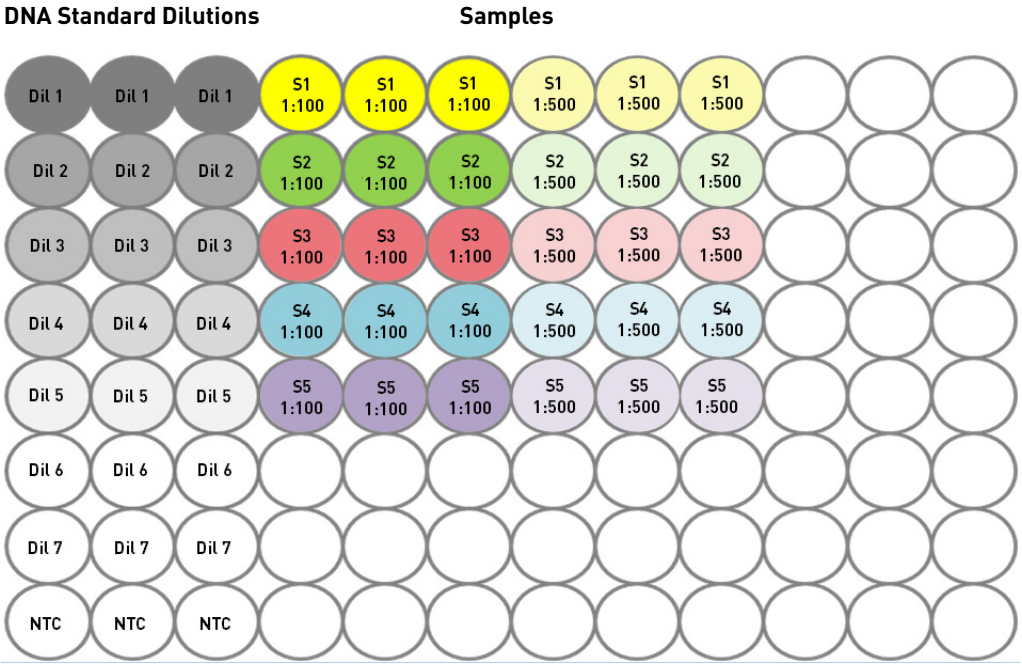
   - For a 96-well plate, prepare sufficient volume for 20-µL reactions using the number of reactions calculated in Step 2. Using the table below, add the calculated volume of each reagent to a new 1.5-mL Eppendorf LoBind® Tube. Vortex the tube to mix and centrifuge briefly to collect contents at the bottom of the tube.

Component	Volume per reaction (96-well)	Total volume required for X number of reactions (96-well)
TaqMan® Universal PCR Master Mix (2X)	10 µL	10 µL × number of reactions × 1.2†
20X RNase P Primer-Probe mix	1.0 µL	1.0 µL × number of reactions × 1.2†
Nuclease-free Water	6.5 µL	6.5 µL × number of reactions × 1.2†

† Life Technologies recommends multiplying by a 1.2 overage factor to compensate for pipetting error.

4. Add the PCR master mix to each well of the plate to be used.
   - If using a 384-well plate, add 7.5 µL per well.
   - If using a 96-well plate, add 17.5 µL per well.

   See the plate layout example below:



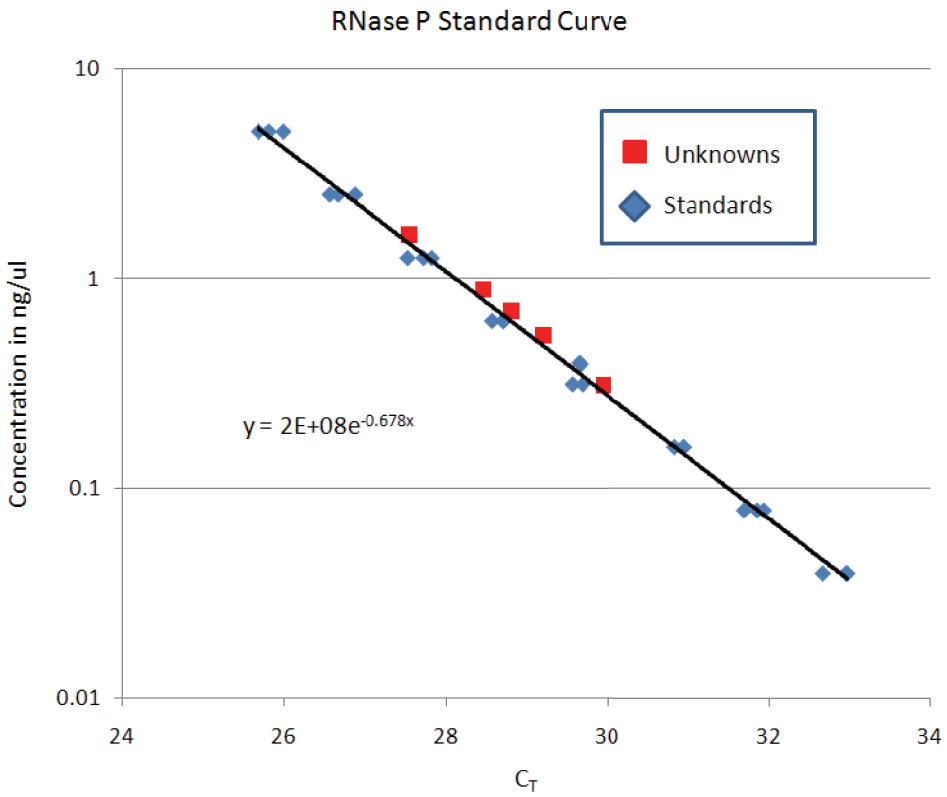
5. Add 2.5 µL of each control DNA dilution to three wells (triplicates) for a total of 21 control DNA ("Dil n") wells.
6. Add 2.5 µL of each sample DNA dilution to three wells (triplicates). Each sample with 2 dilutions will have a total of 6 ("Sn") wells.
7. Add 2.5 µL of Nuclease-free Water to the 3 NTC wells.
8. Seal the plate securely with a sheet of MicroAmp® Optical Adhesive film, vortex briefly to mix, and then spin briefly to collect the contents.

Run the real-time PCR reaction and analyze the results

1. Load the plate in your real-time PCR instrument and run the program listed in the table below. Select FAM™/TAMRA™ as reporter/quencher and ROX™ as passive internal reference dye.

Life Technologies Real-Time PCR System	Stage	Temp	Time
• 7900 HT System • 7900 HT Fast System (Fast 96-Well, Standard 96-Well, or 384-Well Block Modules) • ViiA™ 7 System • StepOne™ System • StepOnePlus™ System • 7500 Fast System • 7500 System	Hold	50°C	2 min
	Hold	95°C	10 min
	40 cycles	95°C	15 sec
		60°C	1 min

2. Generate a standard curve plotting C_T *vs.* ng/µL of input control DNA (see below) using the analysis software provided with your real-time PCR system. Alternatively, Microsoft® Excel® software loaded with the Analysis ToolPak or GraphPad Prism® software may be used for analysis. Calculate the amount of DNA present in samples from the slope and intercept of the standard curve. If sample C_T values fall outside of the standard curve, repeat the quantification after adjusting the sample dilution appropriately (see "Dilute the samples and set up the real-time PCR reaction" on page 3).



Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to: **www.iontorrent.com/support/**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Ion contact information

Website: **www.iontorrent.com**

Ion community: **http://ioncommunity.iontorrent.com**

Support email: **ionsupport@lifetech.com**

Telephone numbers
In North America: 1-87-SEQUENCE (1-877-378-3623)
Outside of North America: +1-203-458-8552

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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

The information in this guide is subject to change without notice.

DISCLAIMERS

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

TRADEMARKS

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.lifetechnologies.com/support

lifetechnologies.com

16 September 2013