Purifying Amplified Products

The C-WTA products should be purified to remove residual primers and nucleotides which may interfere with downstream processes, such as labeling reactions.

The C-WTA product should be purified on one QIAquick column as instructed in the QIAquick® PCR Purification Kit (QIAGEN, Cat # 28104 or 28106), with the following modifications to the standard protocol:

1. Add 375 µL of Buffer PB and 10 µL of 3M sodium acetate (pH 5.2) to the 75 µL product and mix by vortexing before loading the sample on a column.

2. Elute purified product in 50 µL of TE Buffer (pH 8.0), allowing column to stand for 2 minutes at room temperature after adding TE before centrifuging.

The purified, amplified product may be stored at -20℃.

Quantifying Amplified Products

UV absorbance (A260) should be used to quantify purified products, using the conversion of 1 OD = 50 µg/mL. Approximately 10-15 µg of amplified product should be generated from 10 ng of Universal Human Reference RNA (Agilent, Cat # 740000) sample.

RUBICON GENOMICS
breaking the sample barrier

Product Number: RC20050
Amount: 50 reactions
Storage: -20℃
FOR RESEARCH USE ONLY

TransPLEX® C-WTA Kit

For Whole Transcriptome Amplification of Clinical RNA Samples

Kit Components

Component Name (Part Number)	Cap Color	Volume
C-WTA Synthesis Buffer (RC20050-01)	Green	325 µL
C-WTA Stabilization Solution (RC20050-02)	Yellow	140 µL
C-WTA Synthesis Enzyme (RC20050-03)	Blue	80 µL
C-WTA Amplification Buffer (RC20050-04)	(White)	825 µL
C-WTA Amplification Enzyme (RC20050-05)	Red	65 µL
Nuclease-Free Water (RC20050-06)	(Clear)	3 x 1.8 mL
User Manual (RC20050-07, v1)		

Storage and Handling

Store the TransPLEX C-WTA Kit at -20℃. Transfer C-WTA Synthesis Enzyme and C-WTA Amplification Enzyme to ice and briefly centrifuge just before use. Thaw other components on ice and briefly vortex and centrifuge prior to use.

User-Supplied Materials

- Thermal cycler (Real-time instrument recommended)
- PCR tubes or 96-well PCR plate
- PCR plate seals
- Low-binding barrier tips
- QIAquick® PCR Purification Kit (QIAGEN, Cat # 28104 or 28106)

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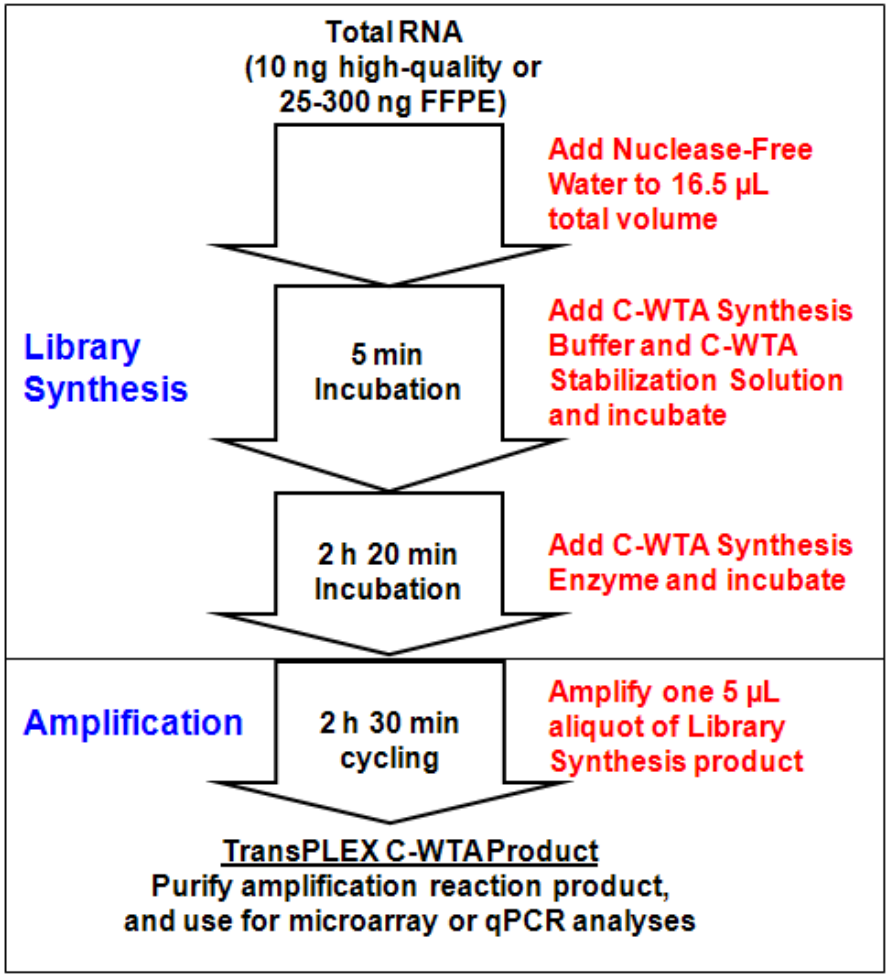
Introduction

The TransPLEX C-WTA Kit allows amplification of total RNA to over 5 micrograms of amplified DNA products suitable for both microarray and qPCR analyses in 5 hours.

TransPLEX C-WTA has two steps, library synthesis and amplification. To synthesize the library, sample RNA is incubated with a reverse transcriptase and non-self-complementary primers comprised of a quasi-random 3' end and a universal 5' end. When annealed primers are extended by polymerase, displaced single strands are generated which become new templates for primer annealing and extension. This process creates a TransPLEX library comprised of random, overlapping fragments flanked by a universal end sequence. Universal-primer PCR is then used to amplify the TransPLEX library and produce C-WTA products.

TransPLEX C-WTA Kit Sample Processing Flow



TransPLEX C-WTA Kit Protocol (Library Synthesis)

1. **Add Nuclease-Free Water to total RNA (10 ng of high quality or 25-300 ng of FFPE) to achieve a total sample volume of 16.5 µL in a PCR tube or well.**
2. **Combine the following Library Synthesis Cocktail components and mix well.**

Library Synthesis Cocktail Component	Volume Per Sample
C-WTA Synthesis Buffer	5 µL
C-WTA Stabilization Solution	2.5 µL
Total Volume	**7.5 µL**

3. **Add 7.5 µL of freshly prepared Library Synthesis Cocktail to the RNA sample and mix by pipet.**
4. **Incubate sample in a thermal cycler as follows:**

1 cycle	**70°C**	**5 min**
1 cycle	**4°C**	**Hold**

5. **Briefly centrifuge sample to collect liquid at bottom of tube/well and place sample on ice.**
6. **Add 1 µL of C-WTA Synthesis Enzyme to sample for a total of volume of 25 µL and mix by pipet.**
7. **Incubate sample in a thermal cycler as follows to produce a TransPLEX Library:**

1 cycle	**24°C**	**15 min**
1 cycle	**42°C**	**2 hour**
1 cycle	**95°C**	**5 min**
1 cycle	**4°C**	**Hold**

8. **Briefly centrifuge the Library and transfer a single 5 µL aliquot of the Library to a new tube/well to prepare for the following Amplification procedure.**

TransPLEX C-WTA Kit Protocol (Library Amplification)

9. **Combine the following Amplification Cocktail components and mix well.**

Amplification Cocktail Component	Volume Per Library Aliquot
Nuclease-Free Water	54 µL
C-WTA Amplification Buffer	15 µL
C-WTA Amplification Enzyme	1 µL
Total Volume	**70 µL**

10. **Add 70 µL of freshly prepared Amplification Cocktail to the 5 µL Library aliquot (prepared in Step 8) and mix by pipet.**
11. **Amplify sample according to thermal cycler program below:**

1 cycle	**95°C**	**2 min**
22 cycles	**95°C**	**20 sec**
	65°C	**5 min**
1 cycle	**4°C**	**Hold**

12. **Briefly centrifuge and immediately store the amplified product at -20℃ or proceed to purifying the amplified products.**