

Microarray Resource

illumina[®] TotalPrep[™] IVT to synthesize cRNA

Please do not make copies of or distribute this protocol.

A. Required reagents:

*T7 10X Reaction Buffer

*T7 Enzyme Mix

*Biotin-NTP Mix

*Reagents supplied with illumina[®] TotalPrep[™] cDNA Amplification Kit
(Ambion)

B. Equipment and supplies:

Microcentrifuge with 1.5 ml rotor

Micropipettors

Aerosol-barrier tips

Vortex mixer

Powder-free gloves

1.5 ml microcentrifuge tubes

37°C Hybridization oven or air incubator

I. illumina TotalPrep IVT to synthesize cRNA

1. At room temperature, prepare an IVT Master Mix by adding the following reagents to a nuclease-free tube in the order listed below. Be sure to include $\leq 5\%$ overage to cover pipetting error.

A master mix calculator is available at:

www.ambion.com/tools/illumina

IVT Master Mix for a single 25 ul reaction	
Amount	Component
2.5 ul	T7 10X Reaction Buffer

2.5 ul	T7 Enzyme Mix
2.5 ul	Biotin-NTP Mix

2. Mix well by gentle vortexing, spin down briefly, and place the tube on ice.
3. Transfer 7.5 ul of IVT Master Mix to each cDNA sample (volume ~17.5). Mix thoroughly by pipetting up and down 2-3 times, then flicking the tube 3-4 times. Spin briefly.
4. The recommended IVT reaction incubation time is based on the amount of input RNA used in the amplification reaction:

Input RNA	Recommended IVT Incubation
100-500 ng	4-14 hr
<100 ng	14 hr

5. Stop the reaction by adding 75 ul of Nuclease-free water to each cRNA sample to bring the final volume to 100 ul. Mix thoroughly by gentle vortexing.

*Proceed to Illumina® TotalPrep™ cRNA cleanup or store at -20°C.