

MEGAscript IVT (T7 Kit: Catalog number 1334)

Transcription Reaction Assembly

This is performed to look at the quality of dsDNA prepped. We only perform this on the Jurket control; extrapolating the results to our samples tubes from that day. Needs to be done ASAP before/after sending samples to NY.

Materials:

- IVD Green Box in -20°C freezer contains:
 - dNTPs
 - 10X Reaction Buffer
- Larger Lab Bench Top Cooler contains:
 - T7 Enzyme Mix
- RNase free bench:
 - DEPC water
- 4°C Black Fridge:
 - BioRad Chromatography columns

This procedure is done at the RNase free bench and at RT.

1. Thaw frozen reagents.

Place the RNA Polymerase Enzyme Mix on ice, it is stored in glycerol which does not freeze at -20°C.

Vortex the 10X reaction buffer and the 4 ribonucleotide solutions until they are completely thawed (or flicked them with finger to vortex more gently). Once they are thawed, store the ribonucleotides ONLY on ice, but keep the 10x reaction buffer at RT while assembling the reaction.

All reagents should be microfuged briefly before opening to prevent loss and/or contamination of material that may be present around the rim of the tube.

2. Assemble transcription reaction at room temperature

The spermidine in the 10X reaction buffer can co-precipitate the template DNA if the reaction is assembled on ice.

Add the reagents in order listed. Total volume should always be 20ml.

Volume	Component
Adjust total volume to 20µl	DEPC water
2µl	ATP solution
2µl	CTP solution
2µl	GTP solution
2µl	UTP solution
2µl	10X Reaction Buffer
Amount from Table 3-1	1µg DNA sample
2µl	Enzyme Mix

Gently flick the tube or pipette the mixture up/down gently, and then microfuge tube briefly to collect the reaction

mixture at the bottom of the tube.

Table 3-1 From Affymetrix Gene Chip Expression Analysis Technical Manual:
Table 3-1: cDNA in IVT (Total RNA)

Total RNA (μg)	Volume of cDNA to use in IVT*
5.0-8.0	10 μl
8.1-16.0	5 μl
16.1-24.0	3.3 μl
24.1-32.0	2.5 μl
32.1-40.0	2 μl

* Assuming 12 μl re-suspension volume for DNA. (Note: we re-suspend our DNA in 8 μl , but follow chart volume recommendations anyways.)

3. Incubate at 37°C for 2.5 hours (up to 4 okay) using PTC.
4. Prepare chromatography column for sample about 5 minutes before sample is done at 37°C. First tap top of column upside down to collect buffer in top half. Break off bottom to allow buffer to drain through into collection tube. Spin on RNase free bench at 3K for 2 minutes; dispose of rinse in bench-top liquid waste. Follow up with (4) 0.5 ml DEPC washes. Spin on RNase free bench at 3K for 2 minutes between each new DEPC wash. Before the final spin, spin sample 20-30 seconds to collect liquid. During last DEPC spin, add 10 μl of DEPC to sample; resultant volume is 30 μl . Put 30 μl sample over column and spin for 4 minutes at 3K.
5. Read sample on UV spectrophotometer.
 - Sign in.
 - Need to warm up UV lamp for 10-15 minutes. Click on 'UV off'
 - Add 75 μl TE for blank. Tap cuvette on counter to release bubbles. Clean sides to remove lint/fingerprints. Put in Spec.
 - Hit 'Blank' on screen.
 - After blanking clean out cuvette with ddH₂O.
 - Add 75 μl of sample to cuvette. Tap cuvette on counter to release bubbles. Clean sides to remove lint/fingerprints. Put in Spec.
 - Push right mouse button anywhere in middle of screen to read. Clean out cuvette with ddH₂O.
 - Push print button on screen to send to printer. Check printer to make sure it's on line.
 - Make sure to turn off UV lamp.
 - Sign out.