Quality Control of Poly (A)$^+$ RNA

The quality of the poly (A)$^+$ RNA is done by evaluation of the quality of the 1st strand cDNA. That is done by gel electrophoresis analysis of labeled 1st strand cDNAs.

◎*Materials and Reagents*

10X Klenow reaction buffer – Invitrogen Cat.18012-021

Klenow enzyme – Invitrogen Cat.18012-021

Cy3 dUTP or Cy5-dUTP (25 nmole) – Amersham Cat. PA53022 / PA55022

Oligo dT-V (A/C/G) (2 μg/μl)

5X Superscript II reaction buffer - Invitrogen Cat. 10864014

Superscript II reverse transcriptase (200 U/μl) - Invitrogen Cat. 10864014

50X dNTPs (25mM each except for dTTP at 10 mM) - Amersham Cat. 27-2035-01

10X dNTPs (0.25 mM each except dTTP at 0.09 mM – Amersham Cat. 27-2035-01

0.1M DTT - Invitrogen Cat. 10864014

PCR clean up kit – Qiagen Cat. 28106

TE pH 8.0

2X loading buffer (0.08% bromophenol blue, 14% (w/v) sucrose in ultra pure water)

1X TAE buffer

Lambda *Hi*nd III DNA marker

◎*Labeling 1st strand cDNA with Cy-dye*

1. Mix 2 μl RNA (80 ng mRNA) with 0.5 μl oligo dT-V (2 μg/μl)
2. Heat 10 min at 70℃ and quickly chill on ice.

3. Add

5X Superscript II buffer	1.0 μl
0.1 M DTT	0.5 μl
50X dNTPs (25 mM dNTPs except dTTP at 10 mM)	0.1 μl
Cy3- or Cy5-dUTP	0.5 μl
Superscript II reverse transcriptase (200 U/μl)	0.4 μl

4. Incubate at 42℃ for 2 hrs.
5. Clean up reaction and elute with 50 μl EB buffer from Qiagen PCR clean up kit.
6. Dry the eluted DNA down and resuspend in 3 μl TE pH 8.0.
7. Mix 3 μl of labeled 1st strand cDNA (labeled 1st strand cDNA from 80 ng mRNA) with 3 μl of 2X loading buffer.
8. Load 6 μl into each well.
9. Run a 1.2% agarose gel in 1X TAE buffer at 100mA, 30 min. A labeled DNA molecular weight marker is used for comparison. DNA molecular weight marker is labeled as follows:

◎***Labeling Lambda Hind III with Cy-dye***

1. Mix the following components and incubate at 37℃ for 1hr

Klenow 10X buffer	4 μl
10X dNTP (0.25 mM each except dTTP at 0.09 mM)	4 μl
λ/*Hind* III (0.5 μg /μl)	4 μl
Cy3-dUTP/or Cy5-dUTP	1 μl
Klenow enzyme	2 μl

Ultra pure water 25 µl

2. Clean up reaction with Qiagen PCR clean up kit and elute with 50 µl EB buffer.
3. Dry the eluted DNA down and resuspend in 10 µl TE.
4. Mix 3 µl of the labeled DNA with 3 µl of 2X loading buffer.
5. Load 6 µl into each well. Run together with your labeled sample on a 1.2% agarose gel in 1X TAE buffer at 100mA, 30 min.

◎***QC gel analysis***

Scan gel using a Fluorescence Imager (e.g., Typhoon, Variable Mode Imager, Molecular Dynamics, NJ).Generally we look for a fairly bright smear between 500-2000 bp in size.