

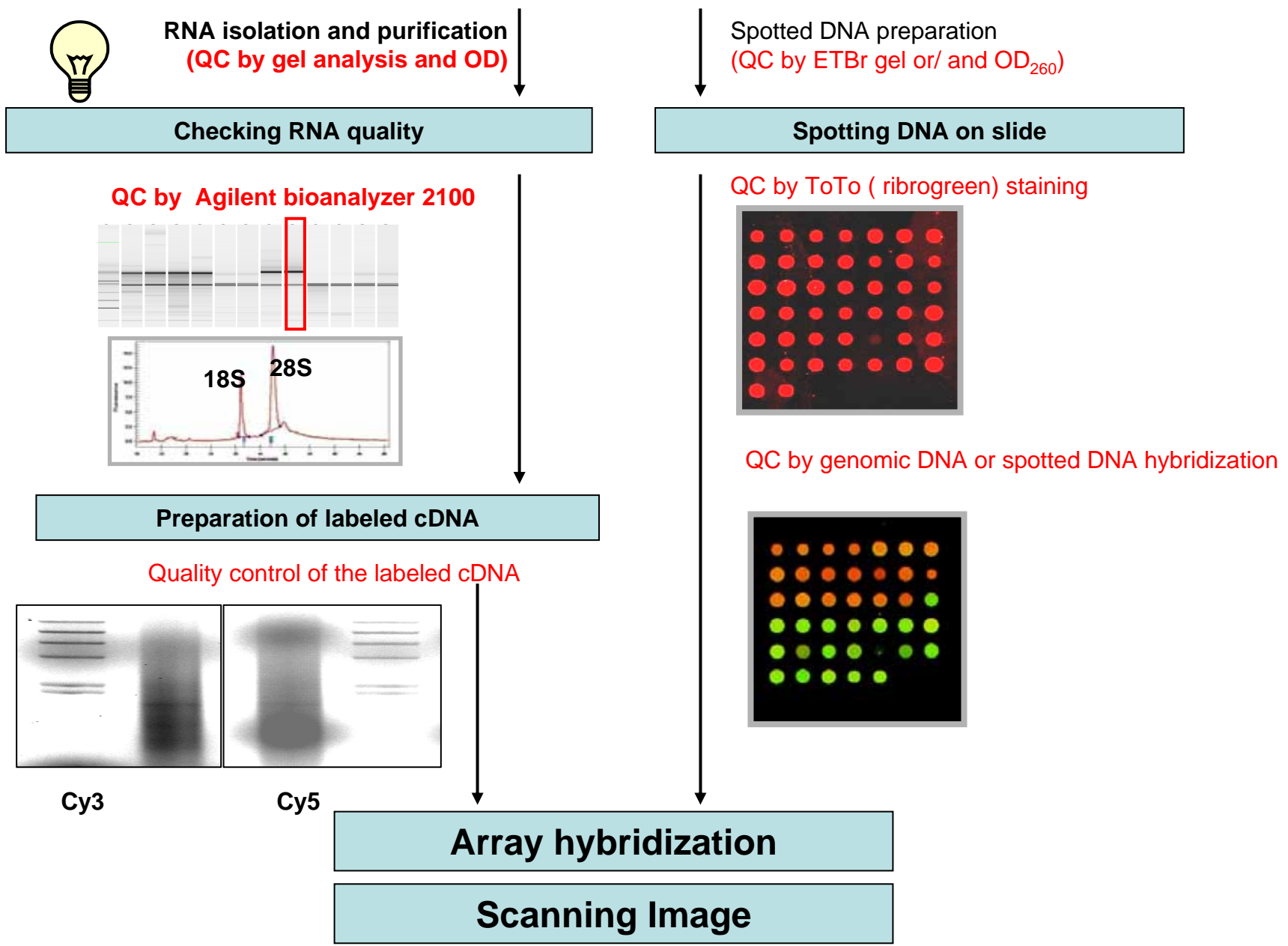
IMB Microarray Core Users Meeting (I)

RNA –Quality Control

Microarray Experiment Process

Part		Time require	Quality control test
1. Microarray experiment design	1. DNA preparation for spotting	~ months	gel OD ₂₆₀
	2. Array format design and test	1~2 day	test array
	3. Array preparation	1~3 day	genomic DNA hyb stain
2. Probe preparation	1. RNA isolation and purification		gel OD _{260/280}
	2. RNA quality control	1 hour	bioanalyzer
	3. RNA reverse transcription & labeling	1~2 day	gel
3. Hybridization and Wash	scan		
4. Data analysis	1. GeneSpring 2. SMD	~ months	

Microarray experiment design



The protocol for RNA Quality Control

Quantity OD260

Purity OD260/280 (OD 260/230)

Integrity RNA 28S/18S

1. *RNA preparation*

To obtain high quality data from your gene chip experiment, it is essential to start with high quality total RNA.

If isolating total RNA from cell culture or primary culture:

option #1: using Qiagen's RNeasy Total RNA Isolation kit.

option #2: using Gibco's Trizol reagent

If isolating total RNA from tissue:

option #1 : using Qiagen's RNeasy Total RNA Isolation kit.

option #2 : using Gibco's Trizol reagent and clean using RNeasy kit.

If isolating total RNA from plant tissue

option #1 (preferred): using pine tree methods

option #2 : using Gibco's Trizol reagent and clean using RNeasy kit.

2. Spectrophotometric Analysis:

A small amount of your sample will be assayed with Spectrophotometer or nana drop before you send to Core.

- Detecting your RNA in **Tris pH 7.5~8.0** with spectrometer or Nano drop including **OD260, OD280, OD260 /OD280**, and calculate the concentration ($\mu\text{g}/\mu\text{l}$) of your samples.
- The **OD 260/280** is calculated to estimate the purity of the RNA. **A ratio close to 2.00 indicates a high percentage of ribonucleotide.**

Quantity Requirement for RNA

each oligo array require	20~40 ug total RNA 1~2 ug polyA RNA
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each dsDNA array require	20 ug total RNA 0.5~1 ug polyA RNA
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For each mRNA, please send sample at a concentration of 0.5 - 2 $\mu\text{g}/\mu\text{l}$.

For each total RNA, send sample at a concentration of 1 $\mu\text{g}/\mu\text{l}$.

3. Visualize each total RNA sample

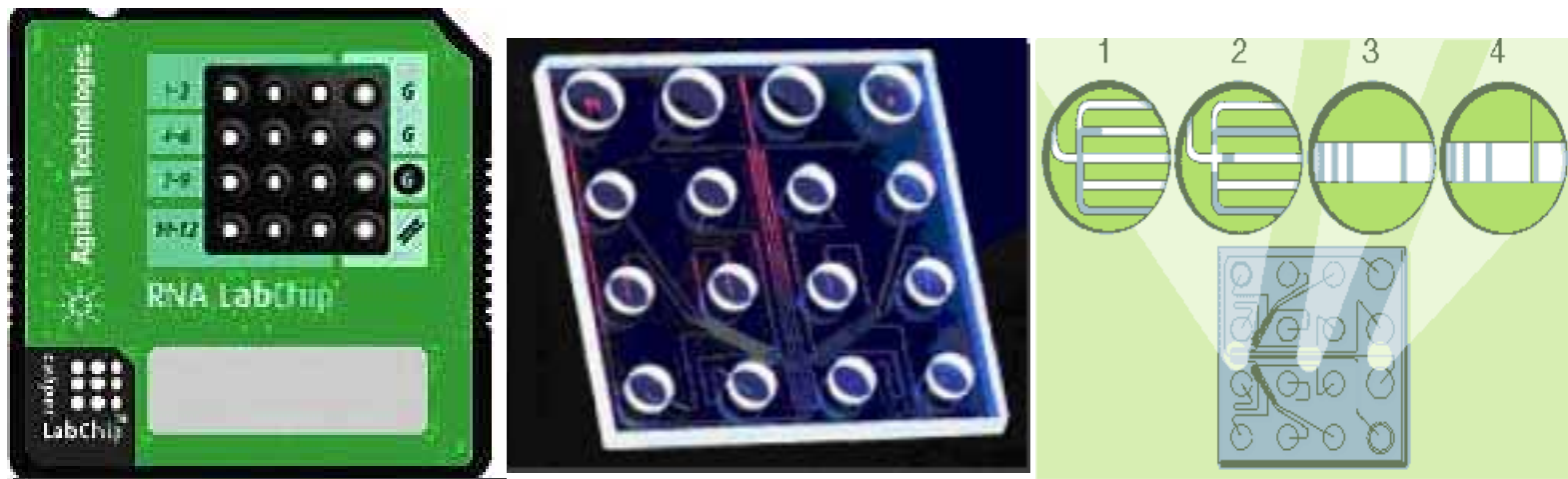
by running on an agarose gel containing 1.1% formaldehyde and photograph. **Please attach a photo of the results**

4. Bioanalyzer assay

- All of the RNA samples submitted to the Microarray core facility will be tested for RNA quality and quantity by using the **Agilent 2100 Bioanalyzer**.
- This assay is similar to gel electrophoresis in concept, but it is cleaner, more efficient, and only requires a very small amount of sample.
- Quality total RNA samples have **28S/18S ratios around 2.0**.
- Please label each tube of RNA sample prepared in RNase free water carefully and complete all the details on the [RNA submission form](#) with the volume and concentration.

Agilent's Lab-on-a-chip technology

A small amount of sample is loaded into the wells in the chip and electrodes cause the RNA to move through microchannels filled with a sieving polymer and fluorescent dye. Fluorescence signal is plotted against run-time to generate an electropherogram or translated to gel-like images.

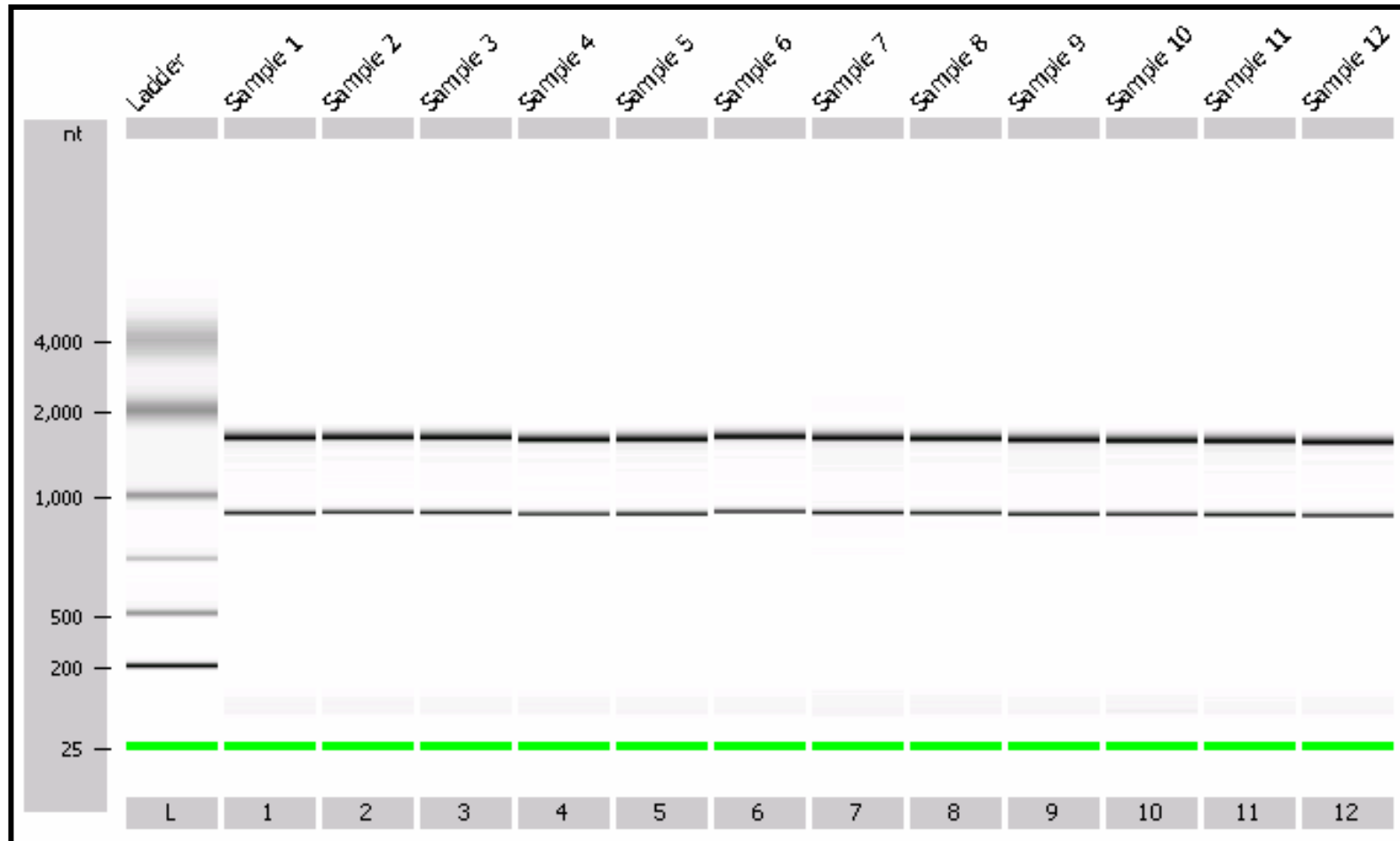


Micro-Fluidic Chip , lab-on-a-chip

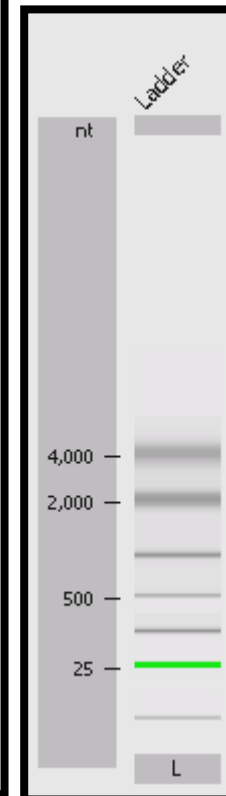
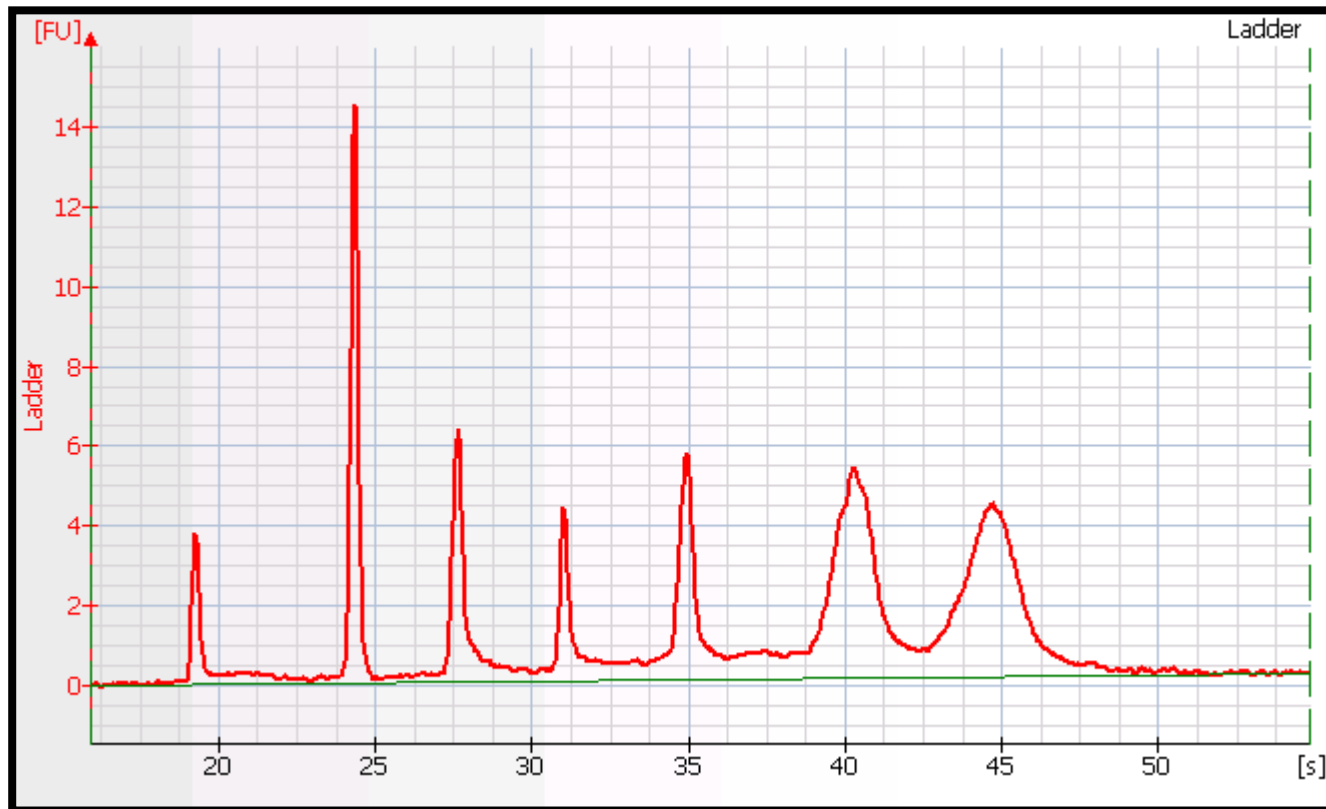
<http://www.home.agilent.com/USeng/home.html>

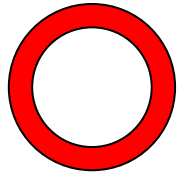
- Plant
 - Arabidopsis、Rice
- E.coli
- Insect
 - Drosophila、Sf21 Insect/virus infect
- Mouse
 - cell culture 、Tissue
- Human cell culture

Gel graph

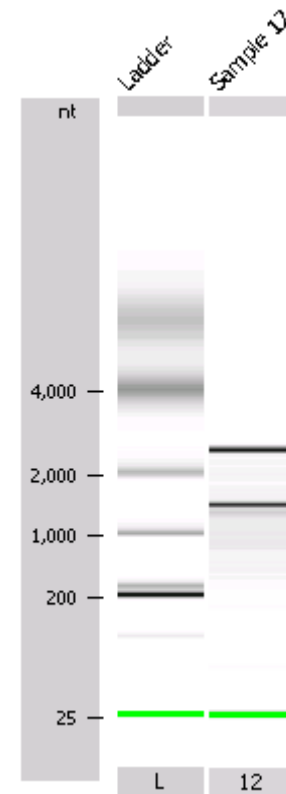
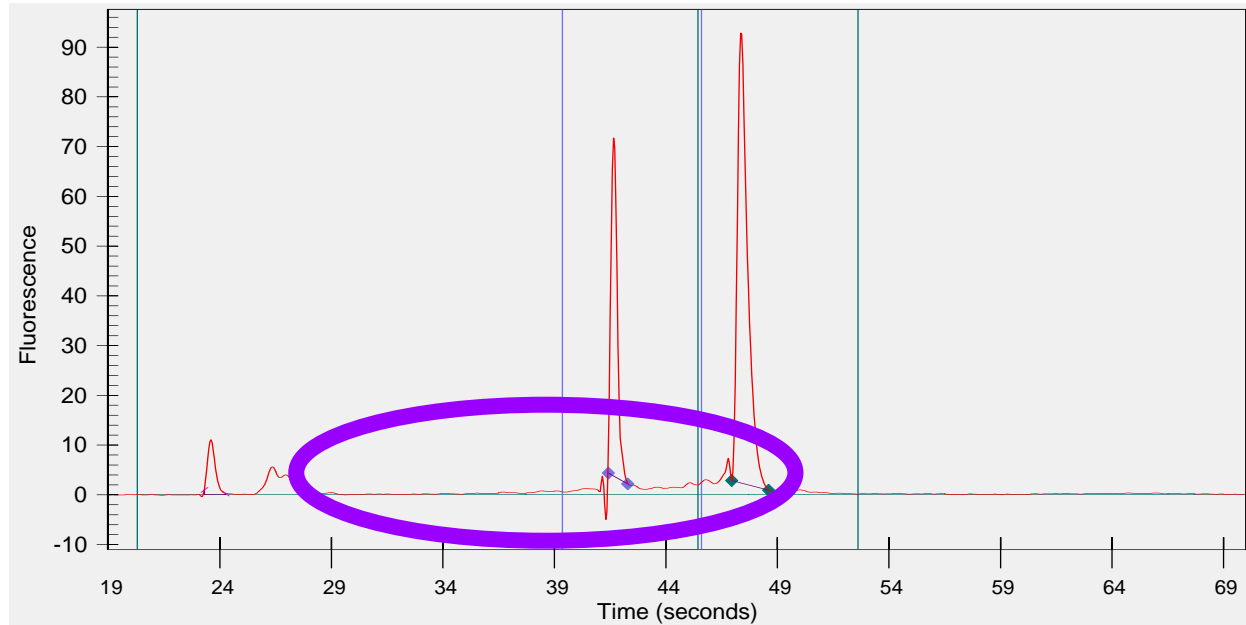


RNA 6000 ladder (ambion)





Total RNA from mouse cell culture



3517_Total-RNA-Nano_02015_2004-12-16_14-47-15 Sample 12

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_Of_Total_Area
1	18S	41.42	42.28	51.81	21.64
2	28S	46.95	48.60	103.20	43.11

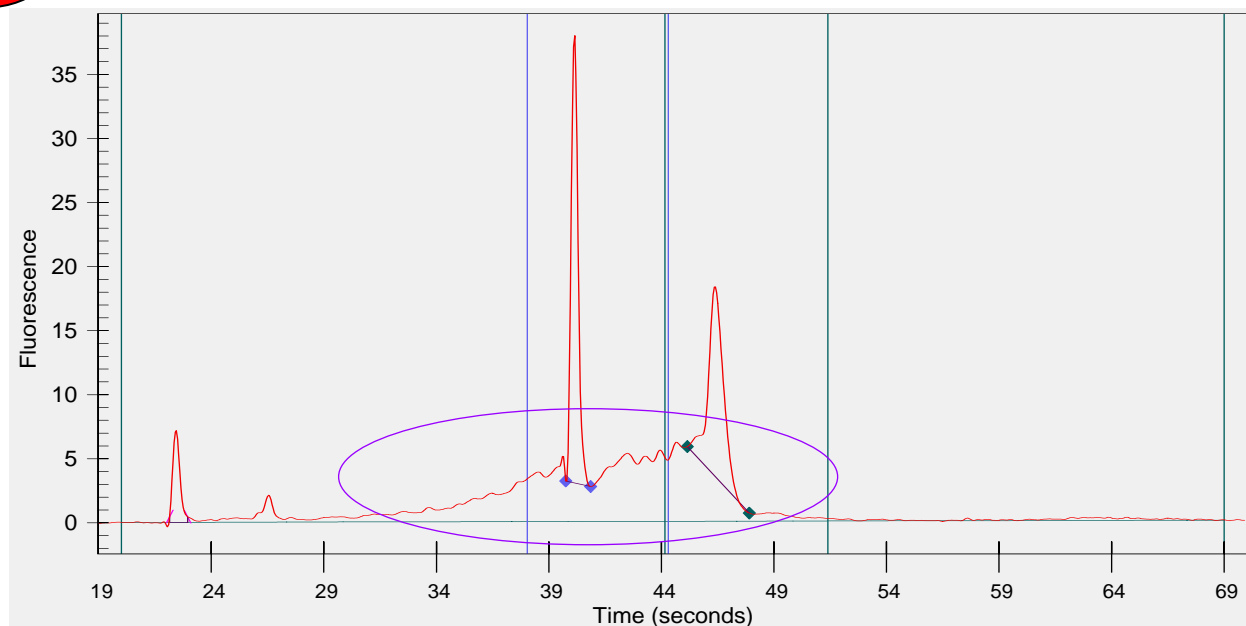
RNA Area 239.41
 RNA Concentration(ng/ul)
 rRNA Ratio [28S / 18S]

172 17
 1.99

1. 28S/18S > 1.8
2. 18S+28S > 50%
3. No degradation pattern



Total RNA from human cell culture



N214_Total-RNA-Nano_02015_2004-08-31_12-46-47 Sample 1

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area
1	18S	39.75	40.85	31.58	16.32
2	28S	45.15	47.90	25.19	13.02

RNA Area 193.47

RNA Concentration(ng/ul)

rRNA Ratio [28S / 18S]

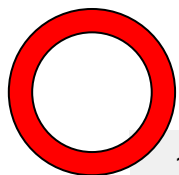
0.80

0.80

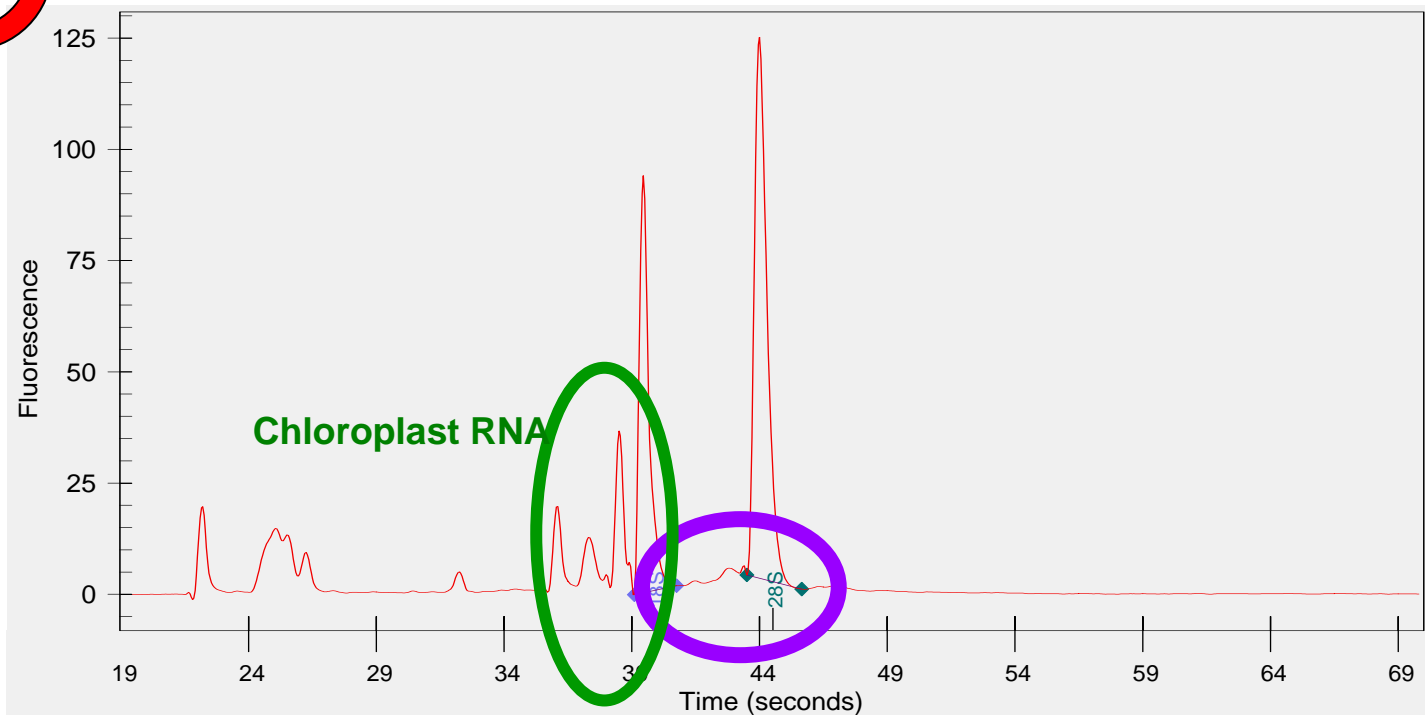
1. 28S/18S <1.8

2. 18S+28S <50%

3. Degradation pattern



Total RNA from Arabidopsis leaf



N317_Total-RNA-Nano_02015_2004-09-01_16-44-05 Sample 2

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area
1	18S	39.10	40.75	104.22	20.27
2	28S	43.50	45.65	159.10	30.95

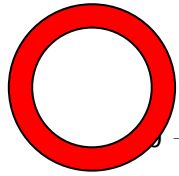
RNA Area 514.04

RNA Concentration(ng/ul)

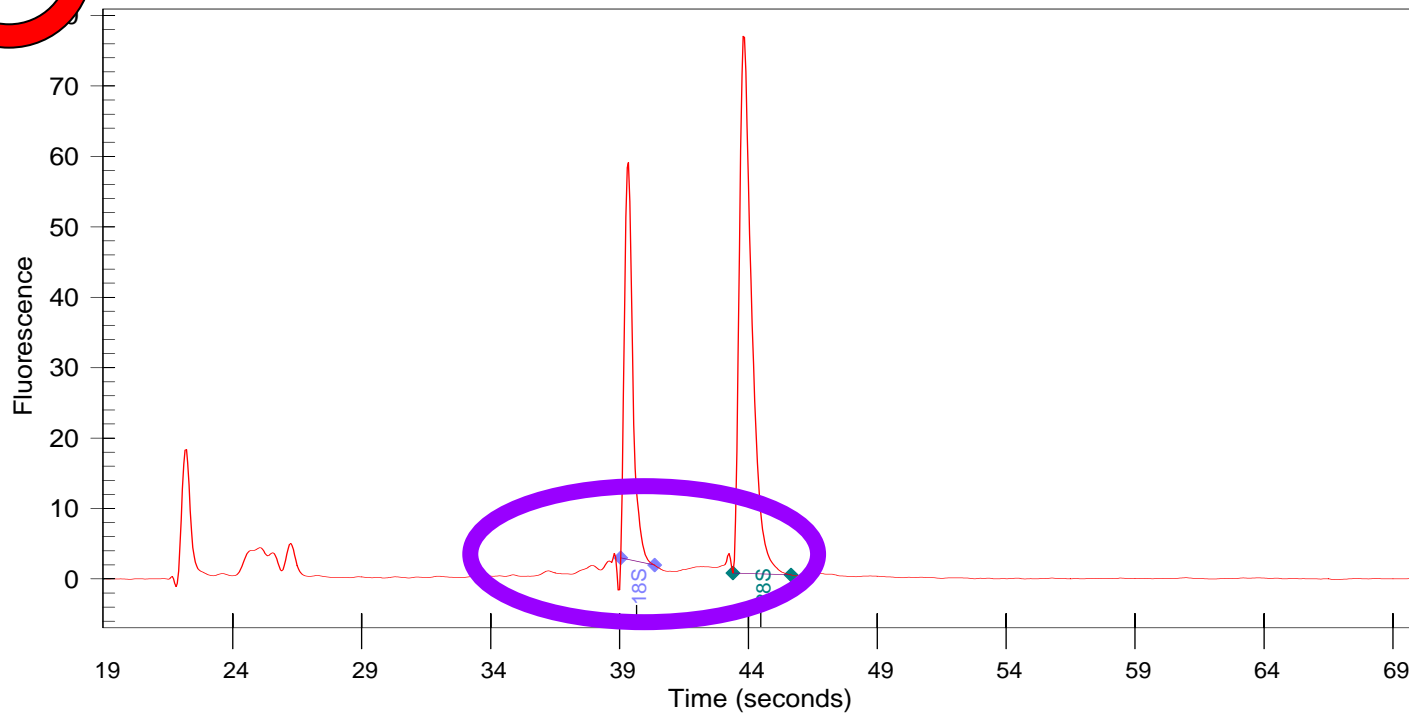
rRNA Ratio [28S / 18S]

391.21
1.53

1. 28S/18S >1.4
2. 18S+28S>40%
3. No degradation pattern
4. RT ?



Total RNA from Arabidopsis root



N317_Total-RNA-Nano_02015_2004-09-01_16-44-05 Sample 8

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	% of total_Area
1	18S	39.05	40.37	48.56	20.72
2	28S	43.40	45.65	97.76	41.71

RNA Area 234.38

RNA Concentration(ng/ul)

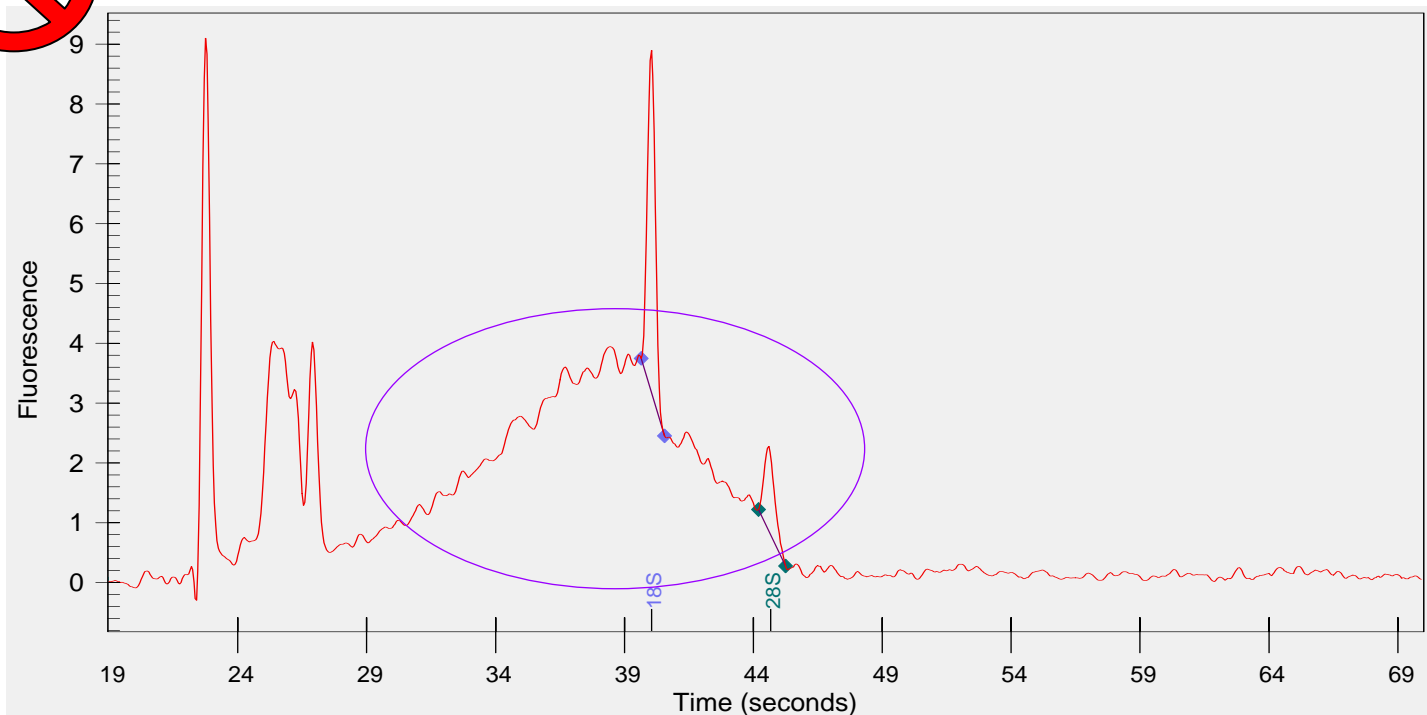
rRNA Ratio [28S / 18S]

178.37
2.01

1. 28S/18S >1.8
2. 18S+28S>50%
3. No degradation pattern
4. RT ?



Total RNA from Arabidopsis root



N317_Total-RNA-Nano_02015_2004-08-31_12-02-11 Sample 12

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area
1	18S	39.65	40.55	5.15	3.73
2	28S	44.20	45.25	1.52	1.10

RNA Area 138.05

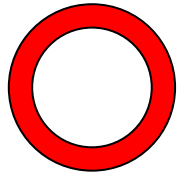
RNA Concentration(ng/ul)

rRNA Ratio [28S / 18S]

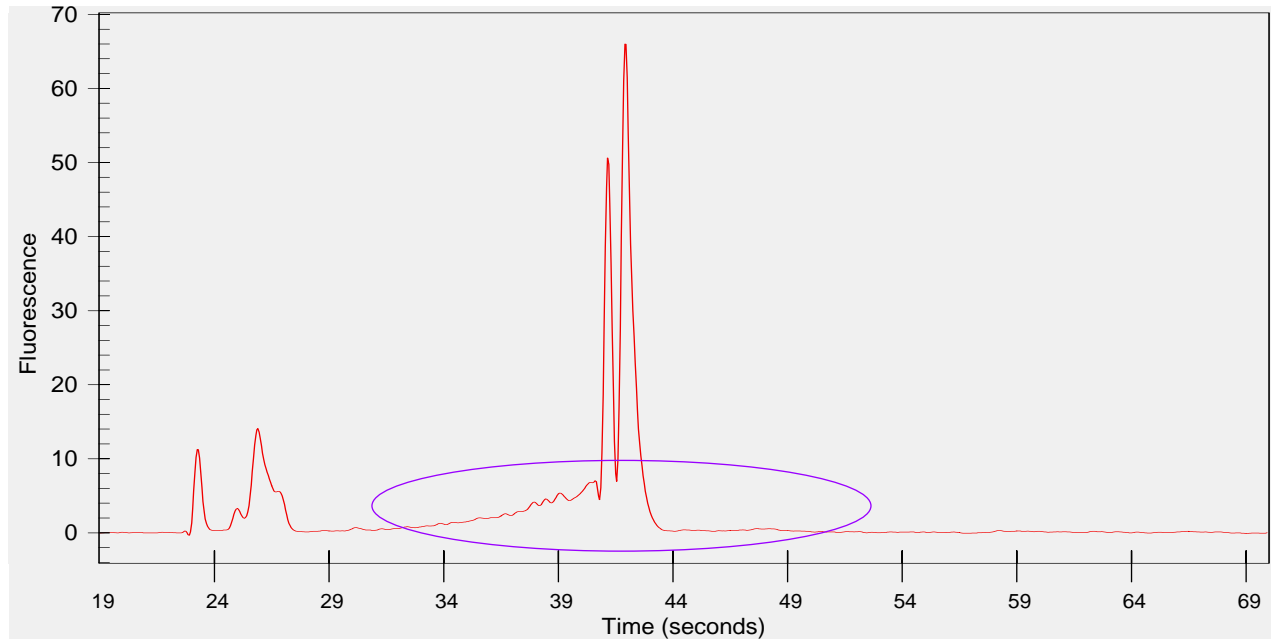
150.13

0.29

1. 28S/18S <1.8
2. 18S+28S <50%
3. Degradation pattern
4. RT ?



Total RNA from Drosophila



N523_Total-RNA-Nano_02015_2004-12-29_14-21-58 Sample 7

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	% of total_Area
1	18S	40.76	41.51	38.69	13.88
2	28S	41.53	43.10	76.72	27.53

RNA Area 278.64

RNA Concentration(ng/ul)

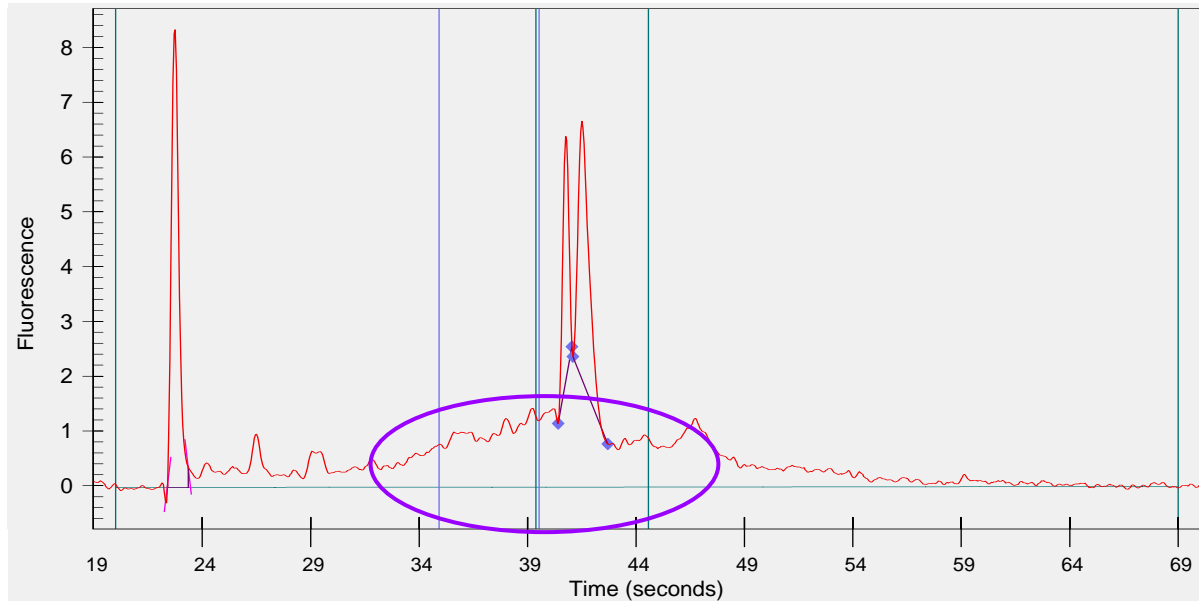
rRNA Ratio [28S / 18S]

199.18
1.98

- 28S/18S >1.8
- 18S+28S>50%
- No degradation pattern
- RT ?



Total RNA from Drosophila



N523_Total-RNA-Nano_02015_2003-10-16_11-39-59 Sample 7

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	% of_total_Area
1	18S	40.41	41.05	3.53	5.34
2	28S	41.09	42.69	6.45	9.77

RNA Area 66.02

RNA Concentration(ng/ul)

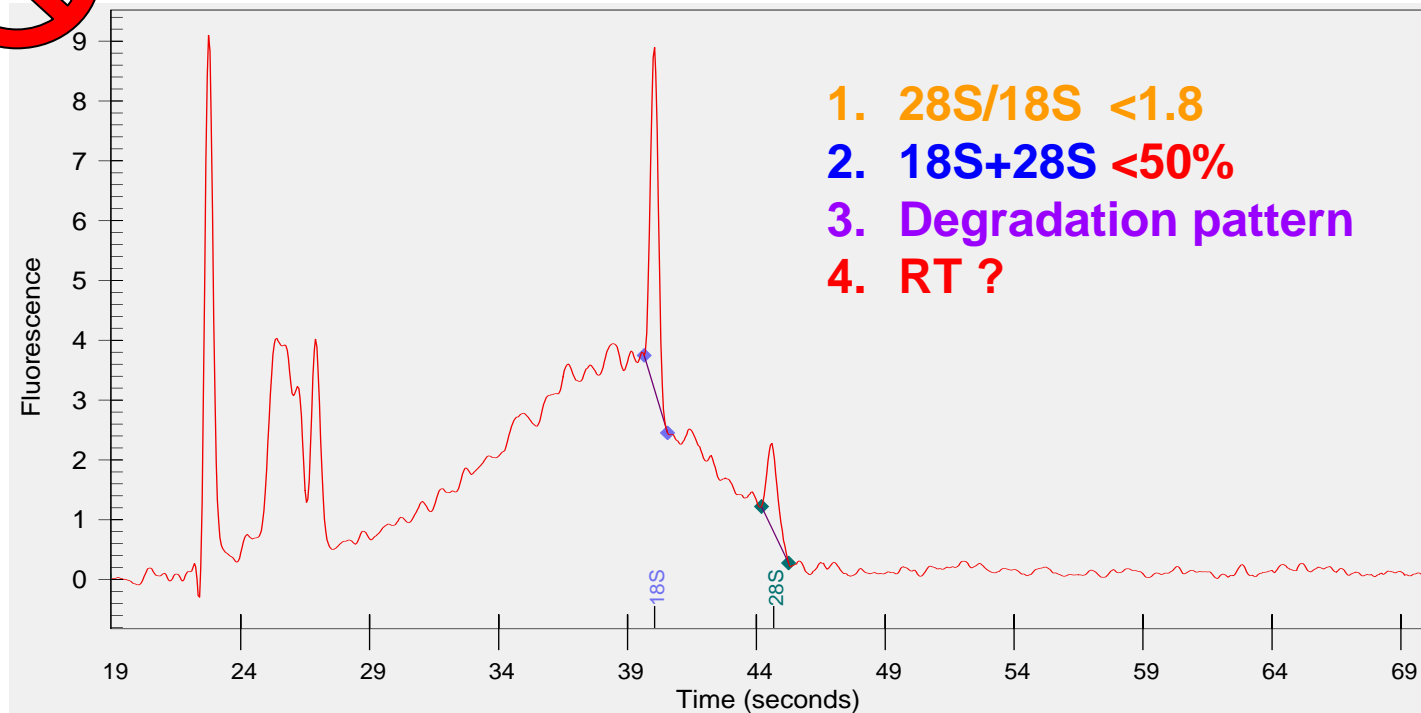
rRNA Ratio [28S / 18S]

117.51
1.83

1. 28S/18S >1.8
2. 18S+28S <50%
3. Degradation pattern



Total RNA from Arabidopsis root



N317_Total-RNA-Nano_02015_2004-08-31_12-02-11 Sample 12

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area
1	18S	39.65	40.55	5.15	3.73
2	28S	44.20	45.25	1.52	1.10

RNA Area 138.05

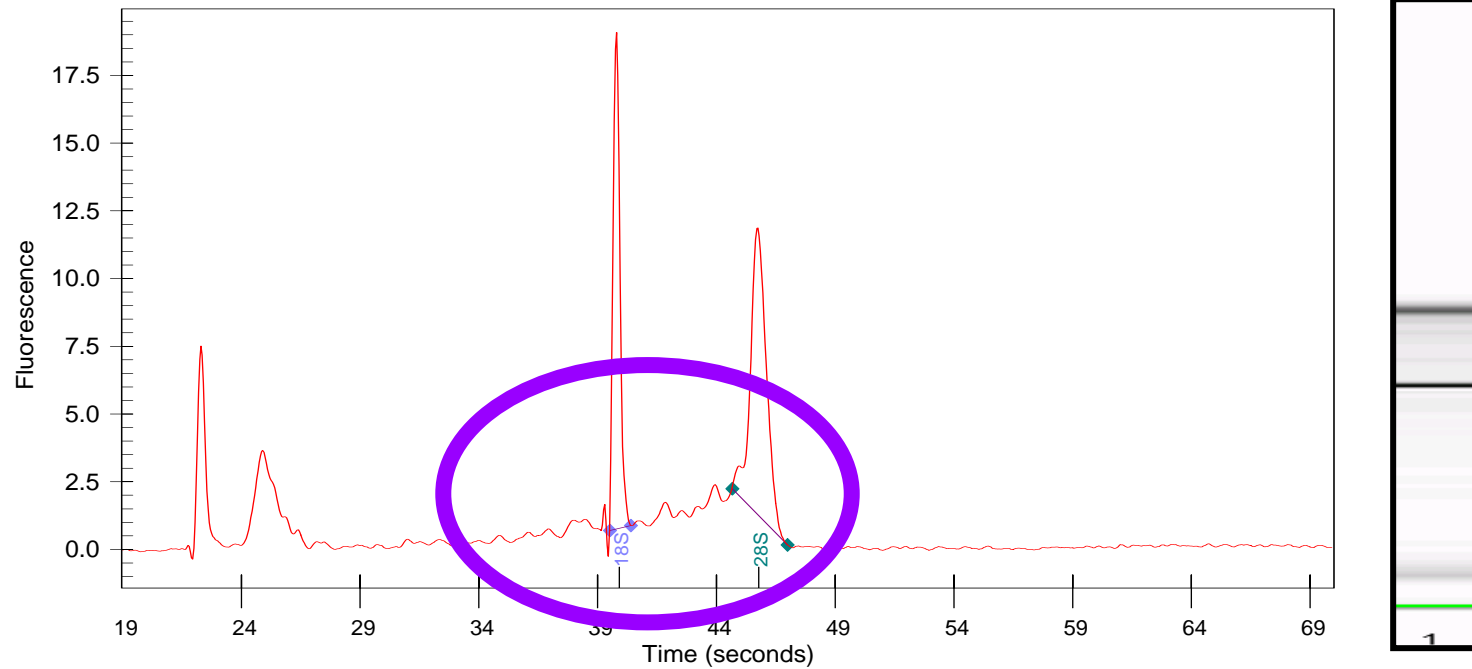
RNA Concentration(ng/ul)

150.13

rRNA Ratio [28S / 18S]

0.29

Total RNA from mouse tissue (brain)



IMB_Total-RNA-Nano_02015_2004-09-07_10-16-19

Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area
1	18S	39.52	40.40	15.63	17.67
2	28S	44.67	47.00	18.11	20.47

RNA Area 88.43

RNA Concentration(ng/ul) 88.54

rRNA Ratio [28S / 18S]

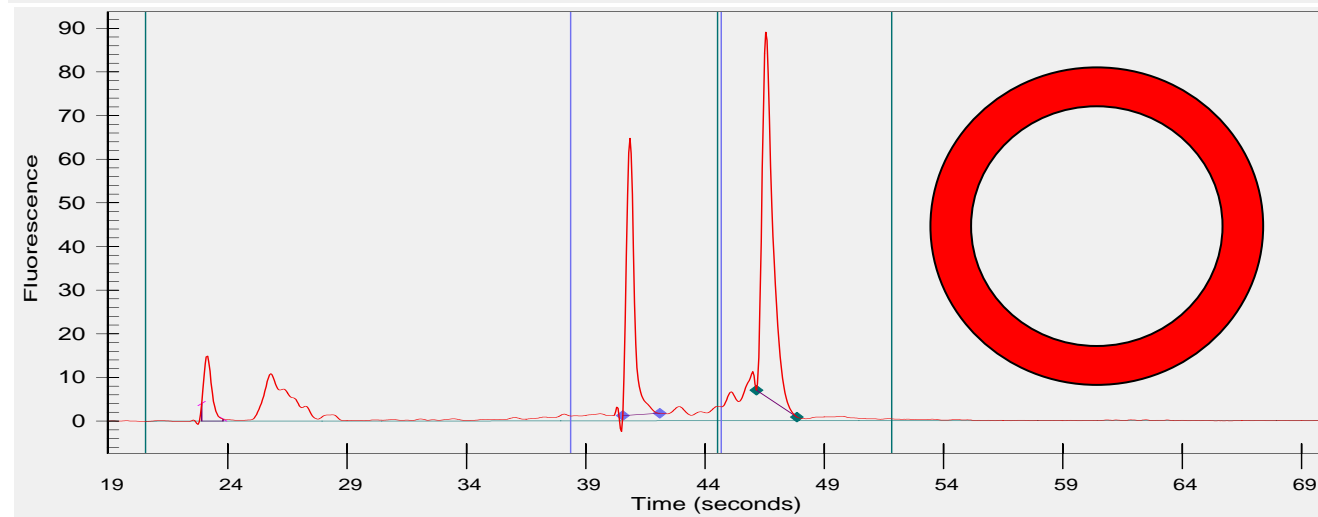
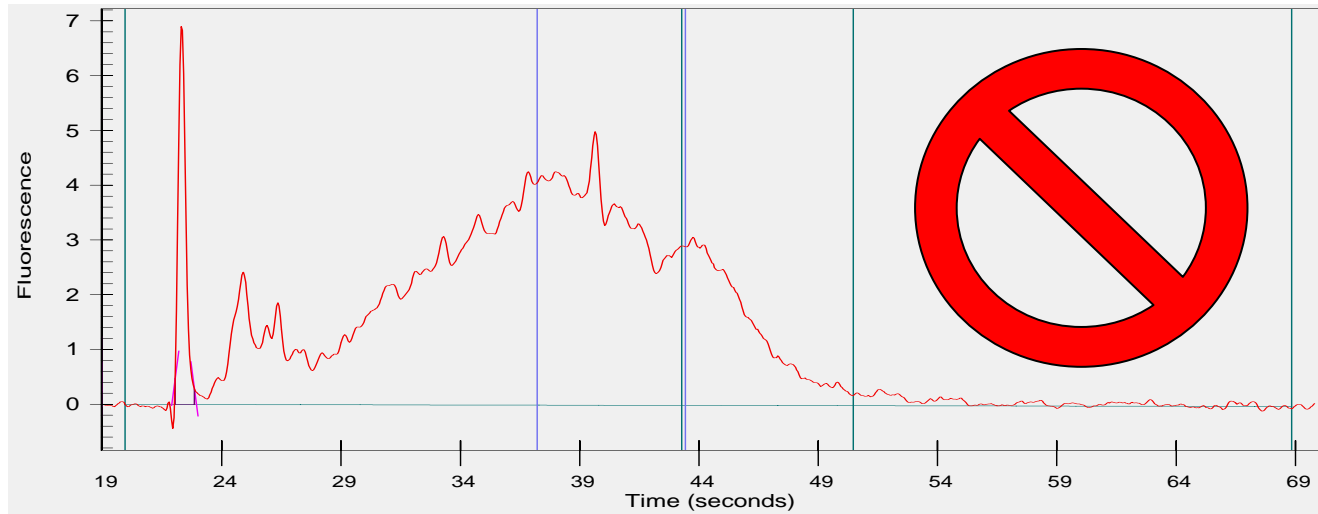
1.16

1. 28S/18S <1.4

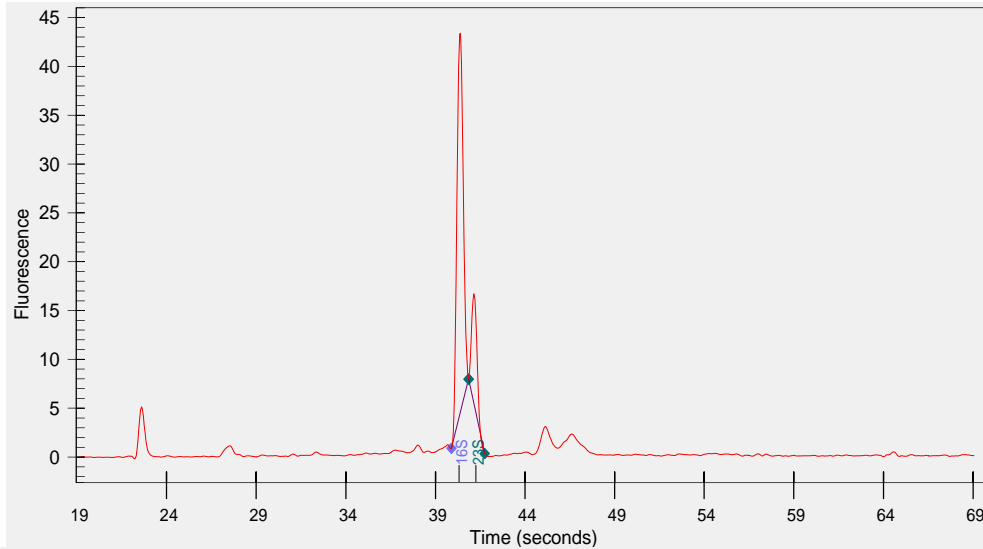
2. 18S+28S <40%

3. Degradation pattern

Total RNA from mouse tissue (brain)



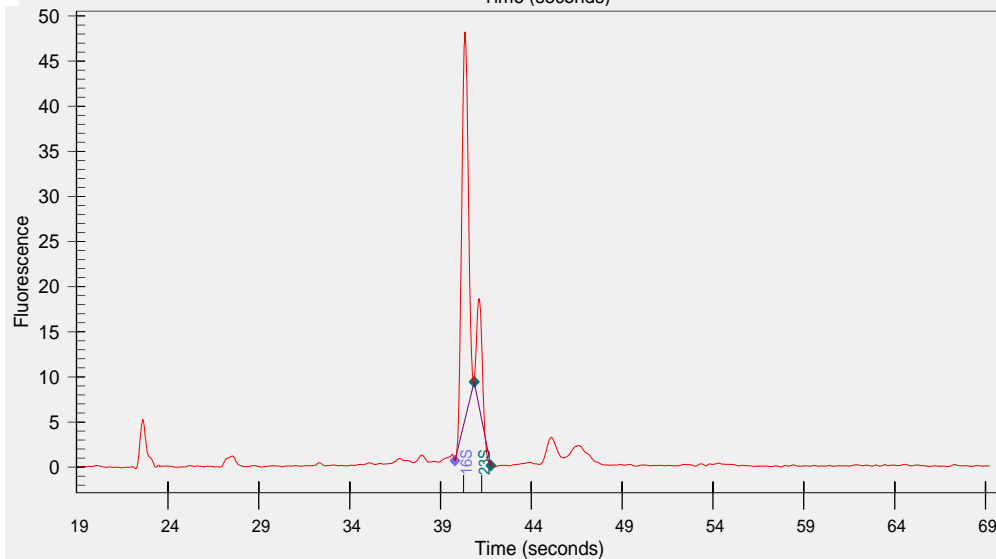
Total RNA from Insect Sf21 tissue culture



rRNA	%_of_total_Area
1	41.44
2	9.99

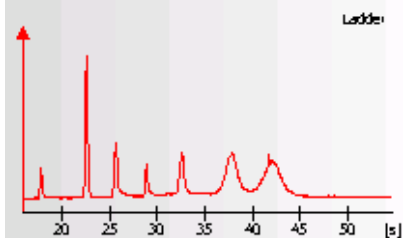
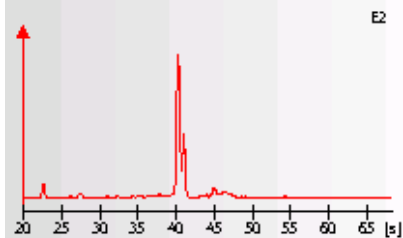
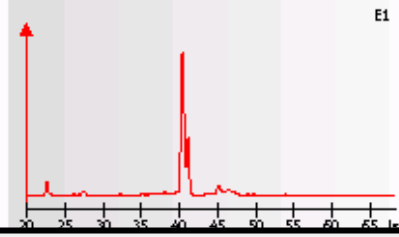
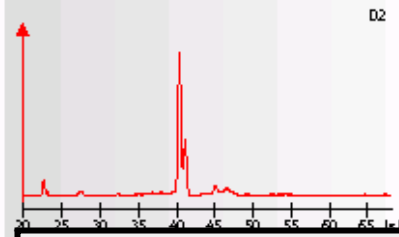
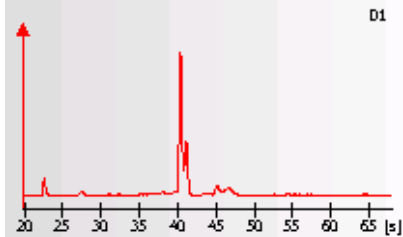
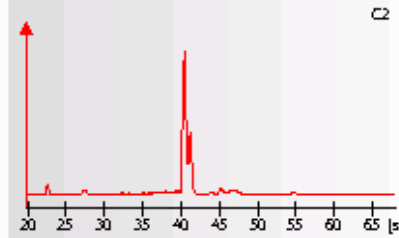
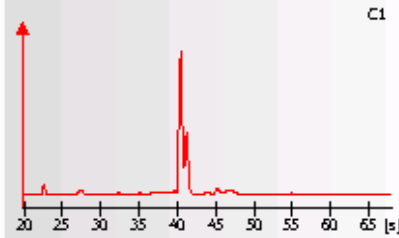
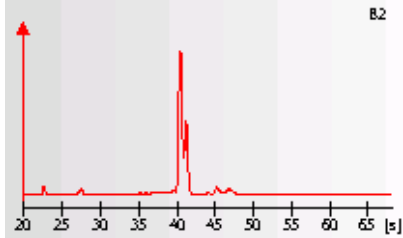
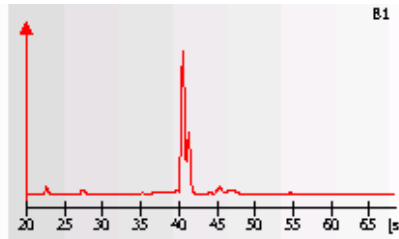
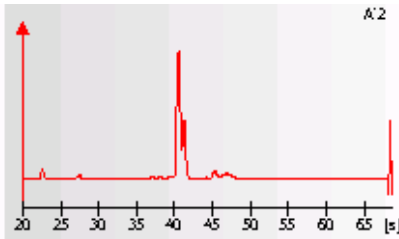
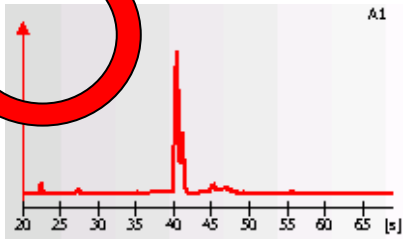
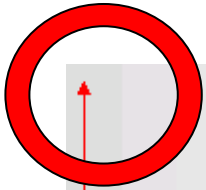
rRNA Ratio **0.24**

- 1. rRNA ratio < 0.5**
- 2. rRNA% > 50%**
- 3. No degradation pattern**

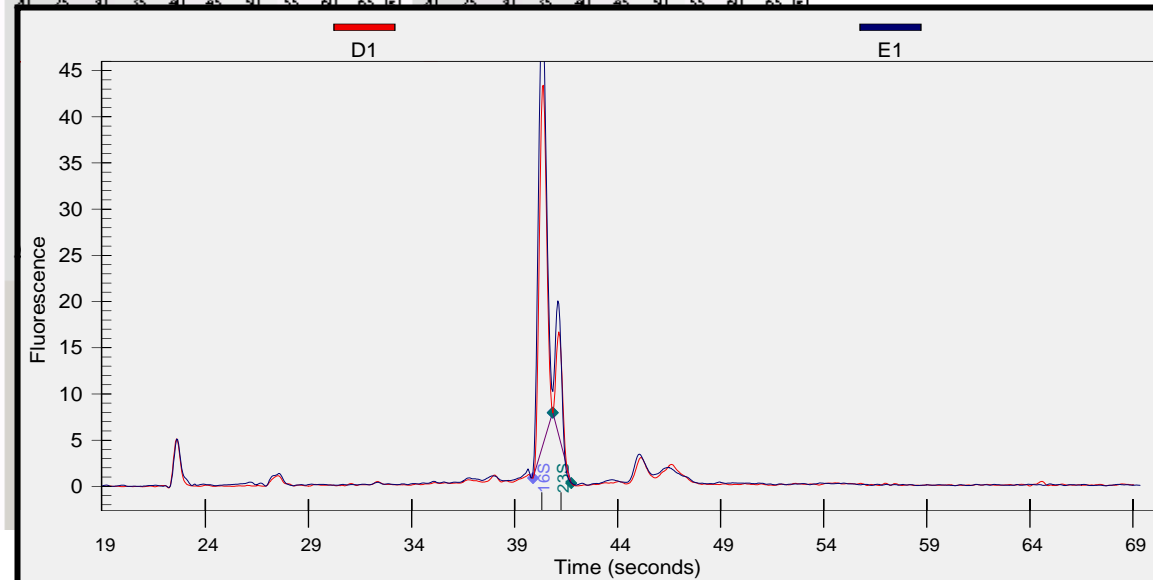


rRNA	%_of_total_Area
1	43.42
2	9.81

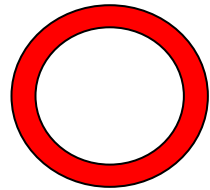
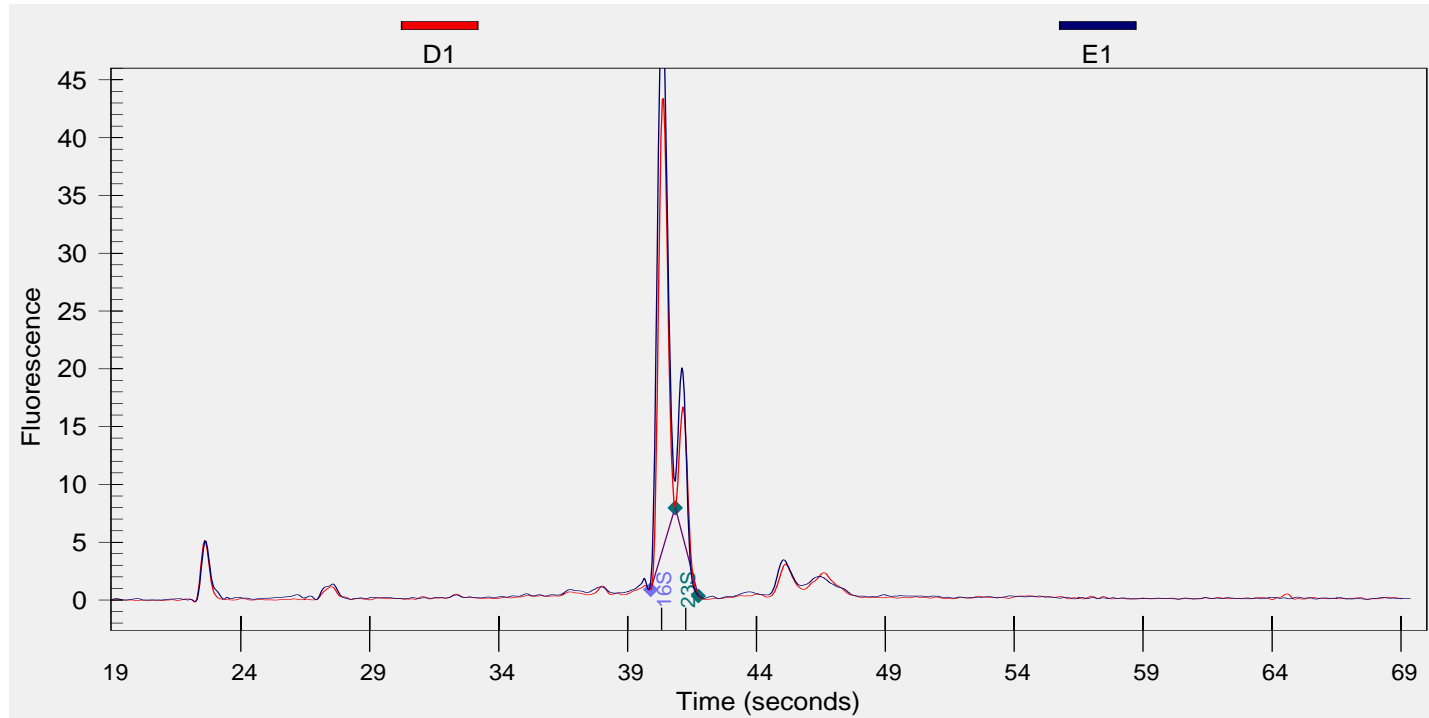
rRNA Ratio **0.23**



1. rRNA ratio < 0.5
2. rRNA% < 40%
3. No degradation
4. Similar pattern
5. RT



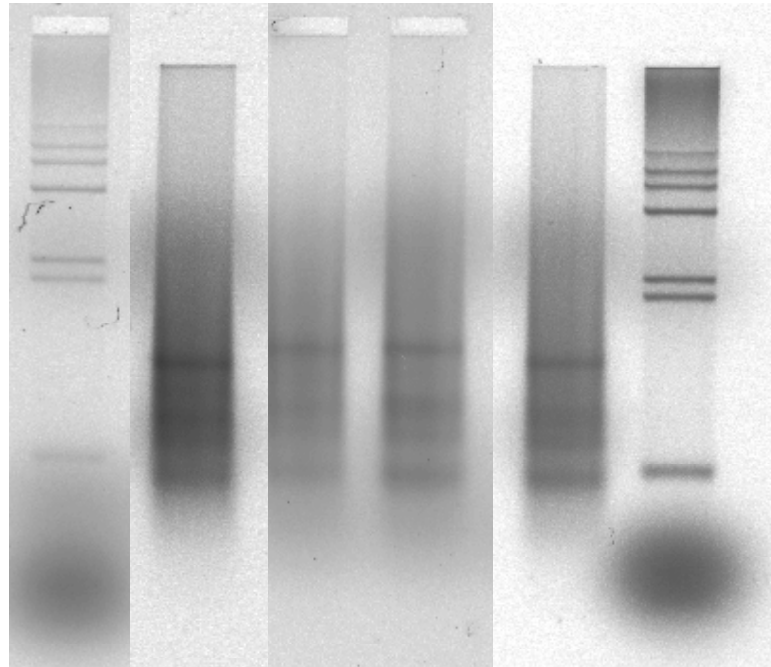
Electropherogram

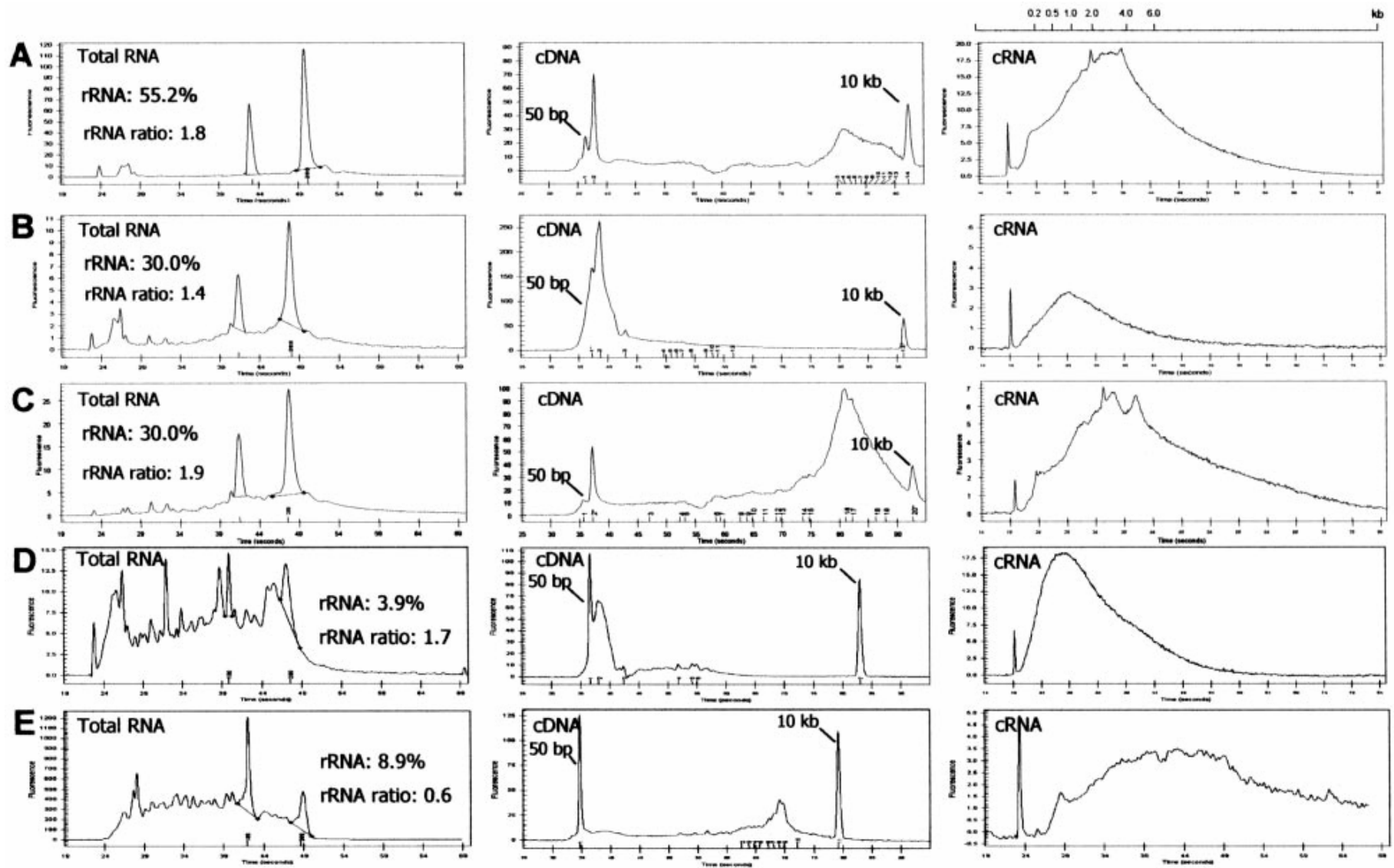


1. rRNA ratio < 0.5
2. rRNA% < 40%
3. No degradation pattern
4. Similar Electropherogram pattern

Lab/user	IMB	Date	2005-2-16
source	mouse	materials	Spinal cords
Labeling Method	Post-labeling RT 2005-2-1	Cy5 lot#	312564
		Cy3 lot#	313379
hybridization	yes	Note	
Slid ID	1228-1229		

Cy3 S9 C9 S10 C10 Cy5





- Fig. 1. Sample preparation QC criteria.
- We analyzed 1 L of the total RNA, cDNA, and cRNA samples for size distribution in the corresponding RNA 6000 Nano LabChip assay and DNA 7500 LabChip assay
- on the Agilent 2100 Bioanalyzer. Electropherograms are plotted for total RNA isolated from fresh harvested M12 cells from human prostate origin by the TRIZOL procedure (A); total RNA isolated from snap-frozen ovarian tumor sample 169 by the TRIZOL procedure (B); total RNA isolated from snap-frozen ovarian tumor sample 169 by the TRIZOL procedure followed by the RNeasy cleanup protocol (C); total RNA isolated by the TRIZOL procedure from frozen acute myelogenous leukemia bone marrow cells from sample 27 that were mishandled during the storage process (D); and total RNA isolated by the TRIZOL procedure followed by the RNeasy cleanup protocol from a snap-frozen liver tumor sample containing 80% necrosis, run on the RNA 6000 Pico LabChip assay (E).