

Supplemental Information

The Importance of Earth Reference Controls in Spaceflight -Omics Research: Characterization of Nucleolin Mutants from the Seedling Growth Experiments

Aránzazu Manzano, Alicia Villacampa, Julio Sáez-Vásquez, John Z. Kiss, F. Javier Medina, and Raúl Herranz

SUPPLEMENTARY INFORMATION

Transparent Methods

Seedling growth conditions

Seeds of *Arabidopsis thaliana* wild-type (WT) ecotype Columbia (Col 0) and two mutant lines of nucleolin protein, *nuc1-2*: Salk_002764 (Pontvianne et al., 2010) and *nuc2-2*: GABI178D01 (Durut et al., 2014), were surface sterilized with 70% (v/v) ethanol (Sigma#270741) for 4 min, rinsed twice with 95% (v/v) ethanol for 1 min and dried in a laminar flow cabin. Then, 28 selected seeds were affixed onto a sterile nitrocellulose membrane (VWR#28149-472) with 1% (w/v) Guar Gum (Sigma#G-4129) and the membranes with seeds were affixed in turn on blotter paper (Whatman 17 CHR, Fisher Scientific#3017-915) previously soaked in 1/2 MS (Murashige and Skoog's medium, Duchefa Biochemie#M0221) growth medium and placed on the cassette (culture chamber, CC) base. The culture chamber volume is around 6-8 mL.

These samples belong to the ground reference control (*1ggr*) at nominal Earth gravity to be used as baseline for a larger spaceflight experiment (Seedling Growth). The seedlings grew in the European Modular Cultivation System (EMCS) Engineering Reference Model (ERM), located in the Norwegian User Support and Operations Centre (N-USOC) of the European Space Agency (ESA) at Trondheim, Norway. The EMCS was an incubator on the ISS that was able to hydrate the seeds and control atmospheric conditions as humidity, O₂, CO₂ and ethylene levels, also providing videorecording and image capture facilities (Brinckmann, 1999, 2005; Brinckmann and Schiller, 2002). Standard EMCS Experiment Containers (ECs) need to be complemented with an Experiment Unique Equipment (EUE) providing specific experimental requirements. In our case, we used the "Tropi" EUE, developed by NASA, consisting of culture chambers (CC), or cassettes, providing semiautomatic hydration and three independent LEDs systems: White LEDs in the top, or lateral 1:1 Red/Blue LEDs that can be operated independently (Correll et al., 2005; Kiss et al., 2007). Each EC could accommodate five "Tropi" cassettes.

Ground control began with the hydration of the cassettes, and the seedlings grew for 6 days at 22 °C and in a controlled atmosphere. The first 4 days (96 h) of growth all seedlings were illuminated with a photoperiod regime (16 h white light, 30-40 $\mu\text{mol}/\text{m}^2\text{s}$ and 8h

darkness) and the last 2 days (48 h) half of them were kept in darkness and the other half were photostimulated with unidirectional red light ($19 \mu\text{mol}/\text{m}^2\text{s}$, a lower intensity was used for lateral photostimulation only, to ease the comparison with samples in darkness). This experimental timeline followed the one we used in our spaceflight experiments (Vandenbrink et al. 2019; Herranz et al. 2019) After the growth period, the seedlings were collected from the cassettes and frozen in tubes with RNAlater (Ambion#AM7020) at -80°C . Once frozen (stable state), they were transported to Madrid (Spain) for processing.

RNA extraction and Sequencing

Total RNA was extracted independently in three replicates (pooling 8-10 seedlings from two different CC) per each experimental condition using a commercial kit and following manufacturer's instructions (MACHEREY-NAGEL, 740949.250). This kit includes one digestion step with DNase for 15 min at room temperature. The quantity and quality RNA was measured in the Bioanalyzer 2100 expert_Plant_RNA nano with Agilent RNA 6000 Nano Kit (Agilent Technologies#5067-1511). Data S1 includes the RNA quality report for the red-light photostimulated samples labelled as 21-29 corresponding to Col-0-RL, nuc1-2-RL and nuc2-2-RL. Similar RNA extraction yields and quality indicators as RIN/RQI index were obtained in other experiments performed in Ground Based Facilities without the constraints of spaceflight hardware (Manzano et al., submitted). Samples comprise the same genotypes Col-0, nuc1-2 and nuc2-2 in 1g control and microgravity simulation conditions.

Once we determined that we had sufficient quantity, the samples with a RNA Integrity Number (RIN) >7 were sequenced on the Illumina HiSeq2500 sequencer from Genomics Unit at the Centre for Genomic Regulation (CRG core facilities, Spain) with stranded RNA read type and 50bp read length. Eighteen total RNA samples were used to generate eighteen sequencing libraries using the Illumina TruSeq RNA Library Preparation Kit (Illumina, USA). Samples were individually indexed. The samples then were combined at equimolar proportions into two pools. Each pool was loaded onto two lanes of a flow cell. Sequencing was performed until the 25 million reads per sample objective were reached ($27,5 \pm 1$ millions of sequence obtained).

RNASeq samples processing was made using Galaxy (<https://usegalaxy.org/>) (Afgan et al., 2018). Reads quality was check with FASTQC and fragments were filter using Trim Galore! (Krueger, 2015) with default settings. Reads were aligned to Arabidopsis TAIR10 genome (<https://www.arabidopsis.org>) using RNA STAR (Galaxy Version 2.7.2b) (Dobin et al., 2013) and gene counts were obtained with FeatureCounts (Galaxy Version 1.6.3) (Liao et al., 2014). This transcriptional dataset has been submitted to NASA's GENELAB database (Ray *et al.*, 2019), and it will be released with the reference GLDS-313 (<https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-313>, DOI: 10.26030/0g0m-dj21).

Functional analysis

Statistical analyses of differential gene expression were conducted utilizing DESeq2 (Ray *et al.*, 2019) as part of the Galaxy Version 2.11.40.2 tool (Love et al., 2014). A multiple-test corrected p-value (q-value; Benjamini and Hochberg, 1995) of 0.05 was employed. In order to observe the replicates dispersion and the general differences between samples Principal Component Analysis was performed using iDEP.91 (Ge et al., 2018). Once the Fold Change (FC) and the corrected p-value for each of the comparisons were obtained, we identified the number of genes that are common by various comparisons or unique to each of them using Venn diagrams (Bardou et al., 2014). The filters used to determine the differentially expressed genes (DEG) that give us information on the effect of red light versus darkness in each of the genotypes comparisons (WT-RL_WT-DN, nuc1-2-RL_nuc1-2-DN, nuc2-2-RL_nuc2-2-DN) were corrected p-value <0.05 and FC>1.5. In contrast, the filter applied to the comparisons that show us the differences between genotypes for both illuminations (darkness: nuc1-2-DN_WT-DN, nuc2-2-DN_WT-DN, nuc1-2-DN_nuc2-2-DN and red light: nuc1-2-RL_WT-RL, nuc2-2-RL_WT-RL, nuc1-2-RL_nuc2-2-RL) was p-adj<0.05.

The next step was to determine in which biological processes the common and non-common genes are involved by utilizing the Metascape multi-gene-list meta-analysis tool by selecting Custom Analysis with GO Molecular Function, GO Biological Processes and GO Cellular components in Enrichment Analysis. The same criteria used for DEG p values were used for the list comparisons in Venn diagrams. Metascape provides a clustered heatmap

with top enriched clusters and their enrichment patterns across multiple gene list (Zhou et al., 2019).

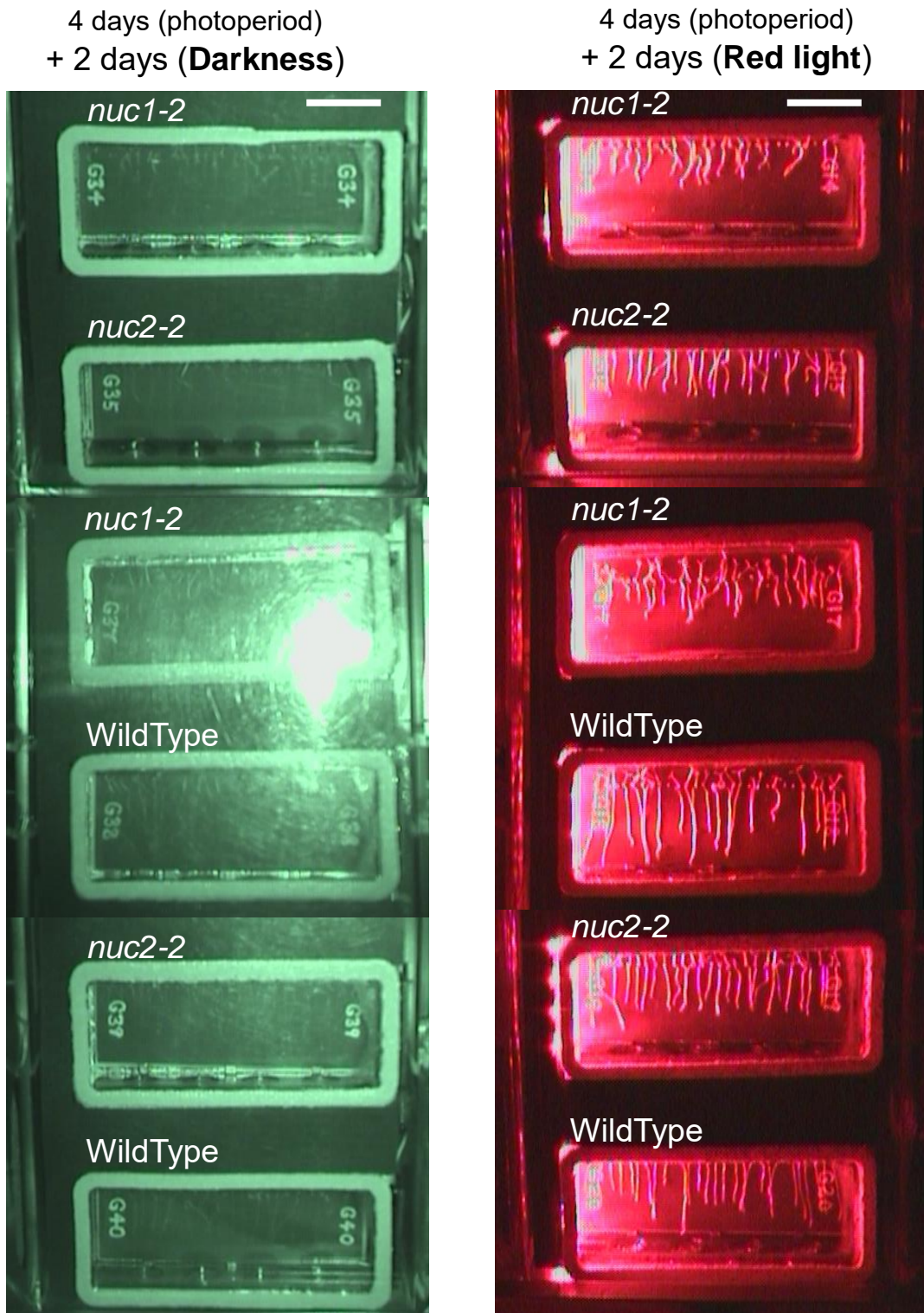
In addition, an enrichment analysis to visualize protein-protein association network was performed using STRING v.11. This tool uses a non-parametrical test (Aggregate Fold Change), that consist in calculate the average of all values provided by the user for the constituent genes and compare it against averages of randomized gene sets of the same size. Then, a multiple testing correction is applied separately within each functional classification framework (GO, KEGG, InterPro, etc.), according to Benjamini and Hochberg (1995). In addition to the functional classification frameworks, it performs a hierarchical clustering based on a confidence diffusion state distance matrix computed on the full organism-wide STRING network (Szklarczyk et al., 2019).

References

- Afgan, E. *et al.* (2018). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Research* 46, 537–544.
- Bardou, P., Mariette, J., Escudié, F., Djemeiel, C., and Klopp, C (2014). jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics* 15(293), 1–7.
- Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* 57(1), 289–300.
- Brinckmann, E. (1999). Spaceflight opportunities on the ISS for plant research - The ESA perspective. *Advances in Space Research* 24(6), 779–788.
- Brinckmann, E. (2005). ESA hardware for plant research on the International Space Station. *Advances in Space Research* 36(7), 1162–1166.
- Brinckmann, E., and Schiller, P. (2002). Experiments with small animals in BIOLAB and EMCS on the International Space Station. *Space Life Sciences: Biological Research and Space Radiation* 30(4), 809–814.
- Correll, M. J., Edelmann, R. E., Hangarter, R. P., Mullen, J. L., and Kiss, J.Zl. (2005). Ground-based studies of tropisms in hardware developed for the European Modular Cultivation System (EMCS). *Advances in Space Research* 36(7), 1203–1210.
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1), 15–21.
- Durut, N., Abou-Ellail, M., Pontvianne, F., Das, S., Kojima, H., Ukai, S., de Bures, A., Comella, P., Nidelet, S., Rialle, S., Merret, R., Echeverría, M., Bouvet, P., Nakamuta, K., and Sáez-Vásque, J. (2014). A duplicated NUCLEOLIN gene with antagonistic activity is required for chromatin organization of silent 45S rDNA in *Arabidopsis*. *The Plant Cell* 26(3), 1330–1344.
- Ge, S. X., Son, E. W., and Yao, R. (2018). iDEP : an integrated web application for differential

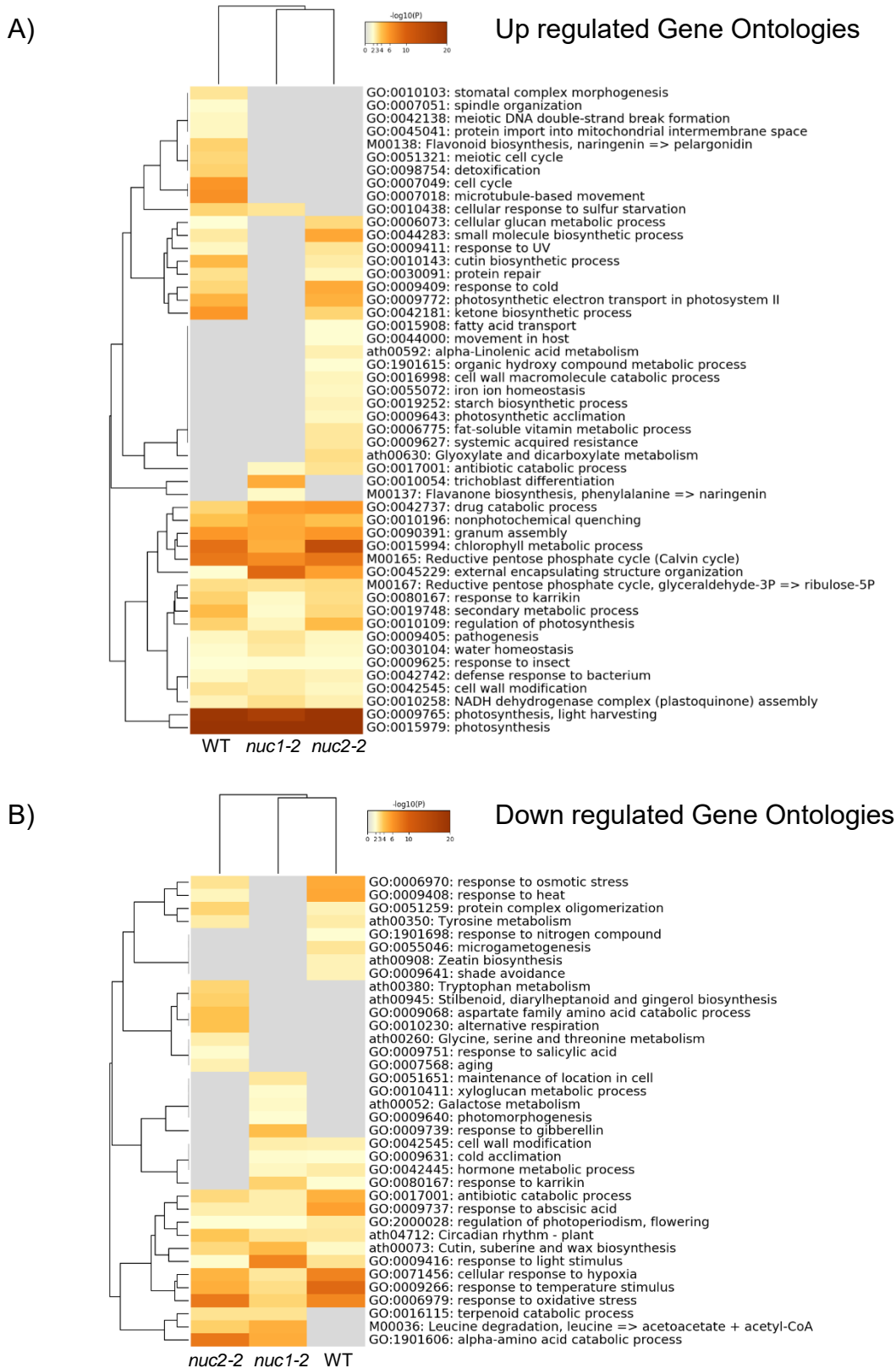
- expression and pathway analysis of RNA-Seq data. *BMC Bioinformatics*, 19(1), 534.
- Herranz, R., Vandenbrink, J. P., Villacampa, A., Manzano, A., Poehlman, W. L., Feltus, F. A., Kiss, J. Z., and Medina, F. J. (2019). RNAseq analysis of the response of *Arabidopsis thaliana* to fractional gravity under blue-light stimulation during spaceflight. *Frontiers in Plant Science* 10, 1–11.
- Kiss, J. Z., Kumar, P., Bowman, R. N., Steele, M.K., Eodice, M. T., Correll, M. J., and Edelmann, R. E. (2007). Biocompatibility studies in preparation for a spaceflight experiment on plant tropisms (TROPI). *Advances in Space Research* 39(7), 1154–1160.
- Krueger, F. (2015) ‘Trim galore’, A wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files, Available at https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/.
- Manzano, A, Pereda-Loth, V, de Bures, A., Sáez-Vásquez, J., Herranz, R., Medina, F.J. Interaction of light and gravity signals as a mechanism of counteracting alterations caused by simulated microgravity in proliferating plant cells. Under review. *American Journal of Botany*. doi: 10.21203/rs.3.rs-29236/v1
- Liao, Y., Smyth, G. K., and Shi, W. (2014). FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30(7), 923–930.
- Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15(12),1–21.
- Pontvianne, F., Abou-Ellail, M., Douet, J., Comella, P., Matía, I., Chandrasekhara, C., DeBures, A., Blevins, T., Cooke, R., Medina F. J., Tourmente, S., Pikaard, C. S., and Sáez-Vásquez, J. (2010), Nucleolin is required for DNA methylation state and the expression of rRNA gene variants in *Arabidopsis thaliana*. *PLoS Genetics* 6(11), 1–13.
- Ray, S., Gebre, S., Fogle, H., Berrios, D. C., Tran, P. B., Galazka, J. M., and Coste, S. V. (2019). GeneLab: omics database for spaceflight experiments. *Bioinformatics* 35(10), 1753–1759.
- Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., and von Mering, C. (2019). STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research* 47(D1), D607–D613.
- Vandenbrink, J. P., Herranz, R., Poehlman, W., Feltus, F. A., Villacampa, A., Ciska, M., Medina, F. J., and Kiss, J. Z. (2019). RNA-seq analyses of *Arabidopsis thaliana* seedlings after exposure to blue-light phototropic stimuli in microgravity. *American Journal of Botany* 106(11), 1466-1476.
- Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C., and Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications* 10(1), 1523.

Figure S1. Photos from the twelve CC from the Seedling Growth Ground Reference Test used in this study (related to Figure 1)



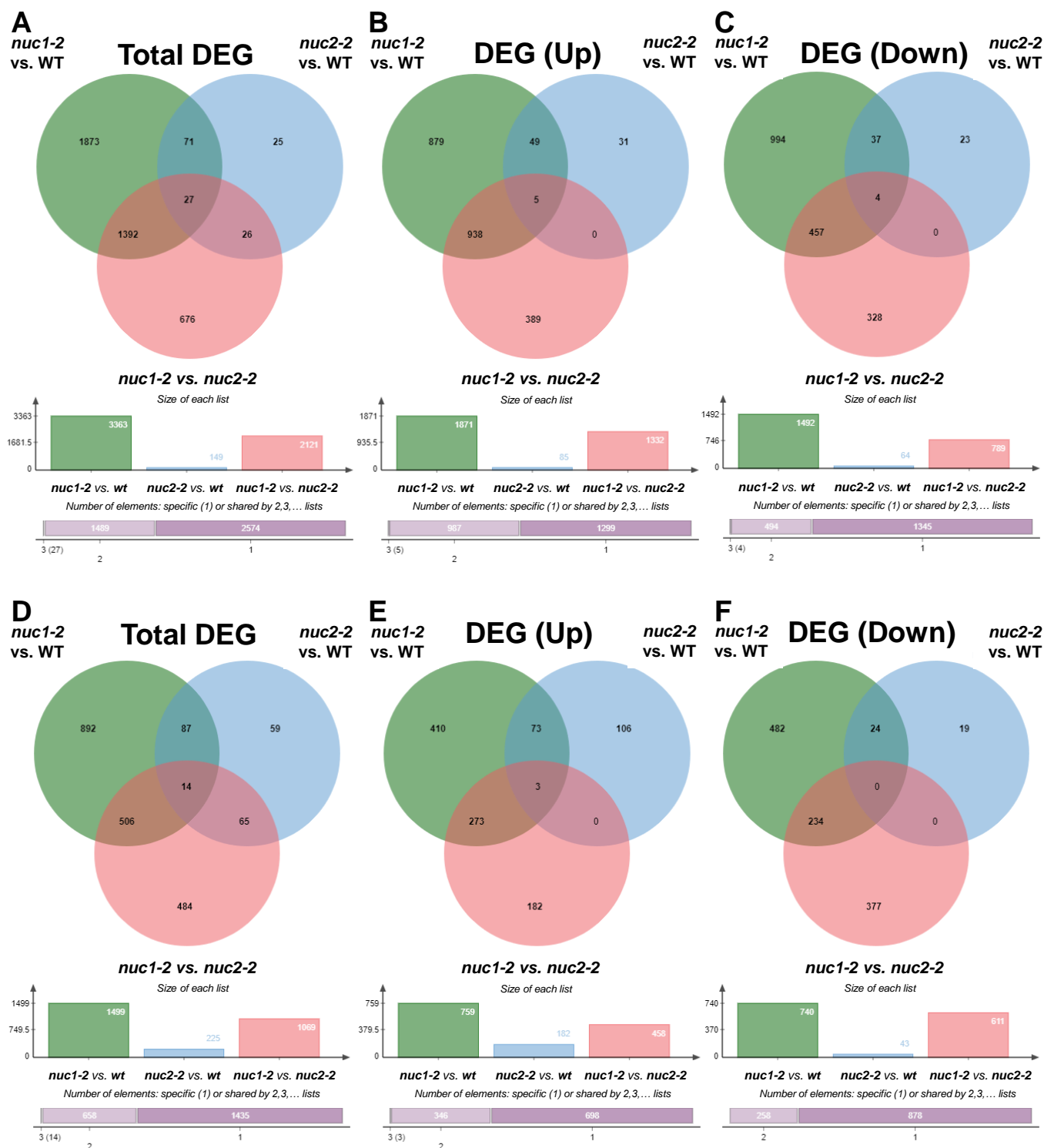
Images of the seedlings (WildType, *nuc1-2* and *nuc2-2*) were taken on the last day of cultivation during the experimental run under darkness (infrared videocapture provide not enough contrast to see all seedlings) or red light photostimulation. See Figure 1 for the final growth of the seedlings just before sampling for freezing. The scale bar in figure is 1 cm size (the gridded membrane has clearly defined grid lines spaced at 3.1 mm that were not observable in these photos).

Figure S2. Heatmap showing the top GO enrichment clusters for DEG in the WT and nucleolin mutants (*nuc1-2* and *nuc2-2*, related to Figure 4)



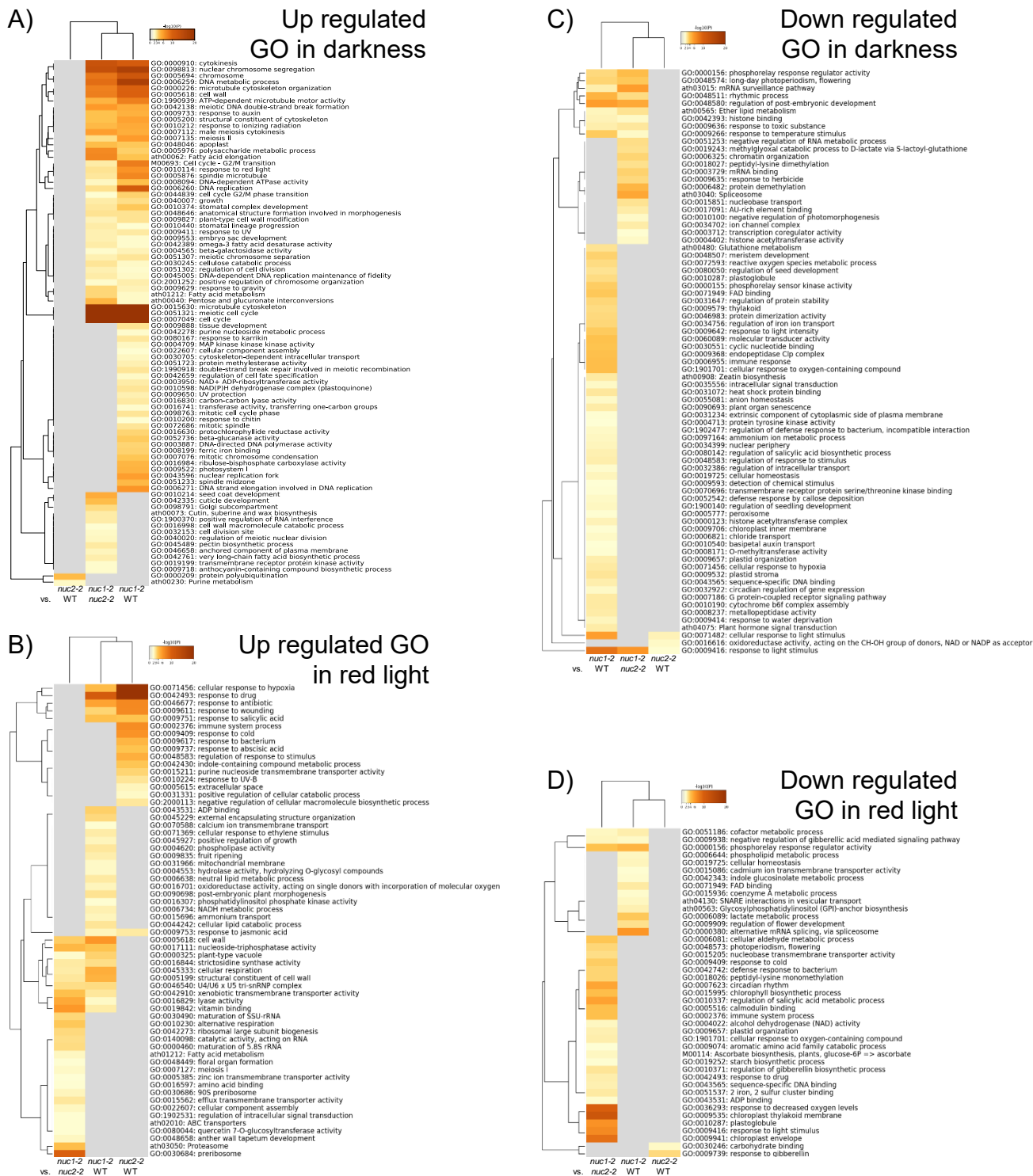
One row per cluster is shown, using a discrete color scale to represent statistical significance as $-\log_{10}(p \text{ value})$. Gray color indicates a lack of significance.

Figure S3. Differentially expressed genes (DEG p adj<0.05, in the three genotype pair-comparisons among WT, *nuc1-2* and *nuc2-2*, related to Figure 3)



A) Venn diagram comparing all DEG between plants exposed to darkness the last two days. B) Venn diagram comparing upregulated DEG between plants exposed to darkness the last two days. C) Venn diagram comparing downregulated DEG between plants exposed to darkness the last two days. D) Venn diagram comparing all DEG between plants exposed to red light stimulation the last two days. E) Venn diagram comparing upregulated DEG between plants exposed to red light stimulation the last two days. F) Venn diagram comparing downregulated DEG between plants exposed to red light stimulation the last two days.

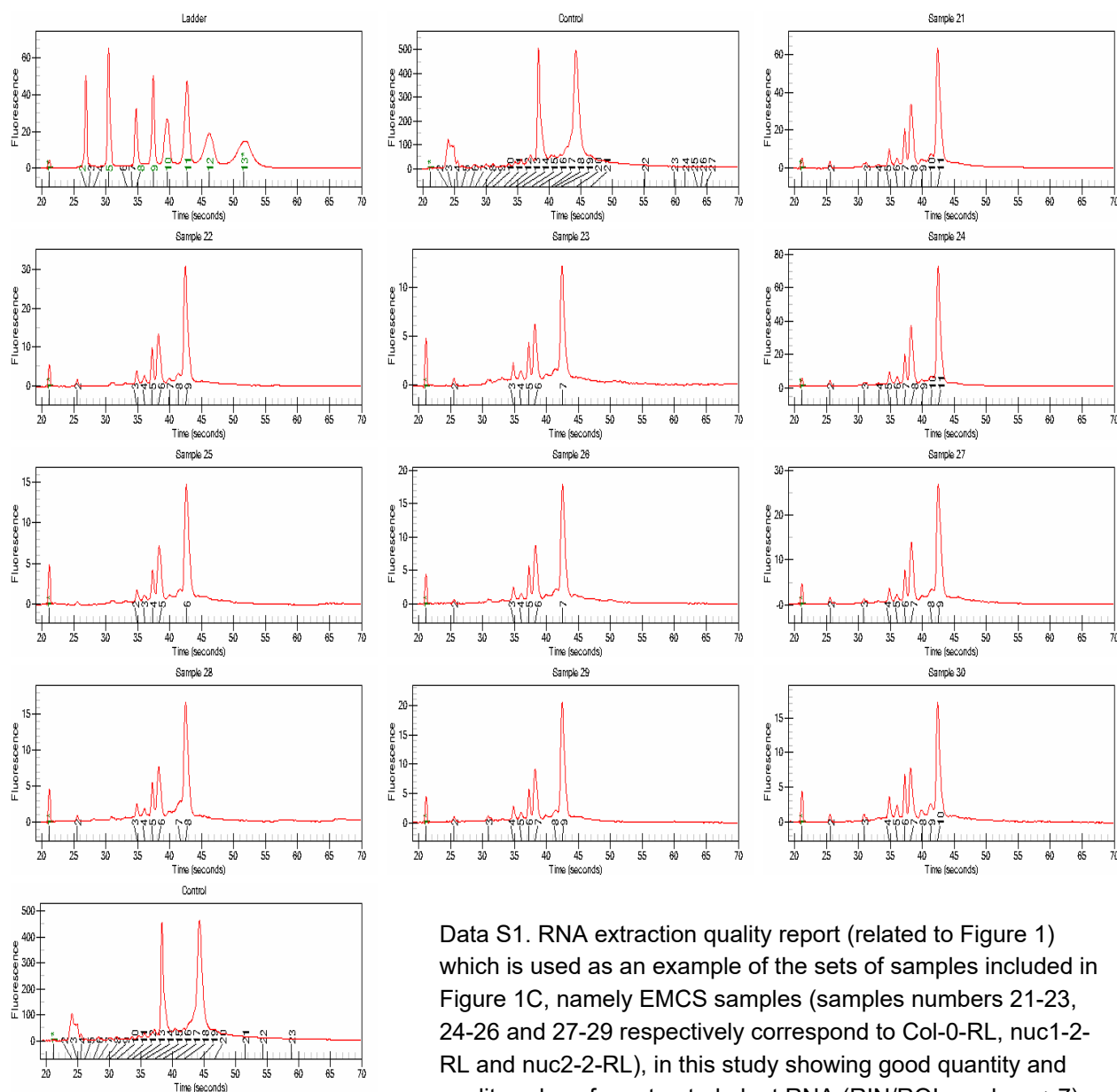
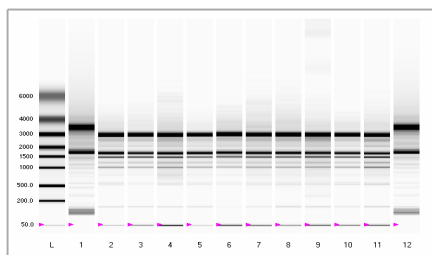
Figure S4. Heatmap showing the top Gene Ontologies enrichment clusters for DEG in the three genotype pair-comparisons among WT, *nuc1-2* and *nuc2-2* (related to Figure 4)



A) Up regulated in plants exposed to darkness the last two days. B) Up regulated in plants exposed to red light the last two days. C) Down regulated in plants exposed to darkness the last two days. D) Down regulated in plants exposed to red light the last two days. One row per cluster is shown, using a discrete color scale to represent statistical significance as $-\log_{10}(p)$ value. Gray color indicates a lack of significance.

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

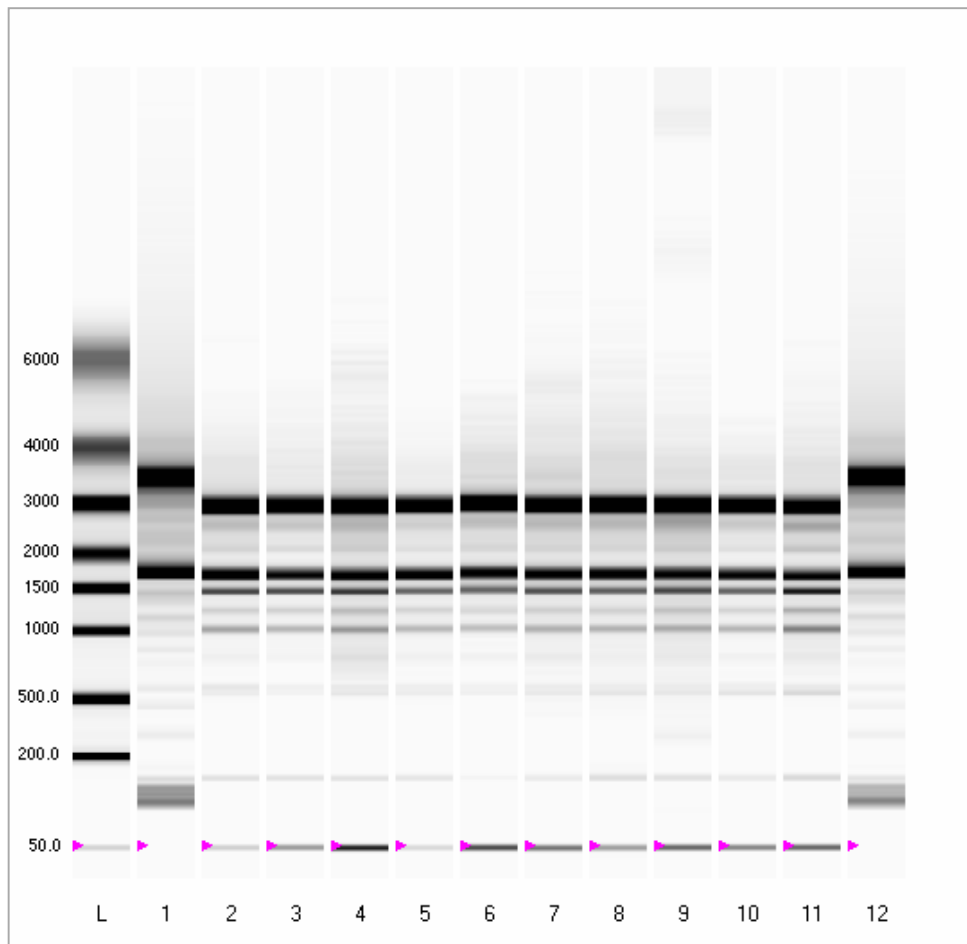
Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A



Data S1. RNA extraction quality report (related to Figure 1) which is used as an example of the sets of samples included in Figure 1C, namely EMCS samples (samples numbers 21-23, 24-26 and 27-29 respectively correspond to Col-0-RL, nuc1-2-RL and nuc2-2-RL), in this study showing good quantity and quality values for extracted plant RNA (RIN/RQI numbers >7).

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

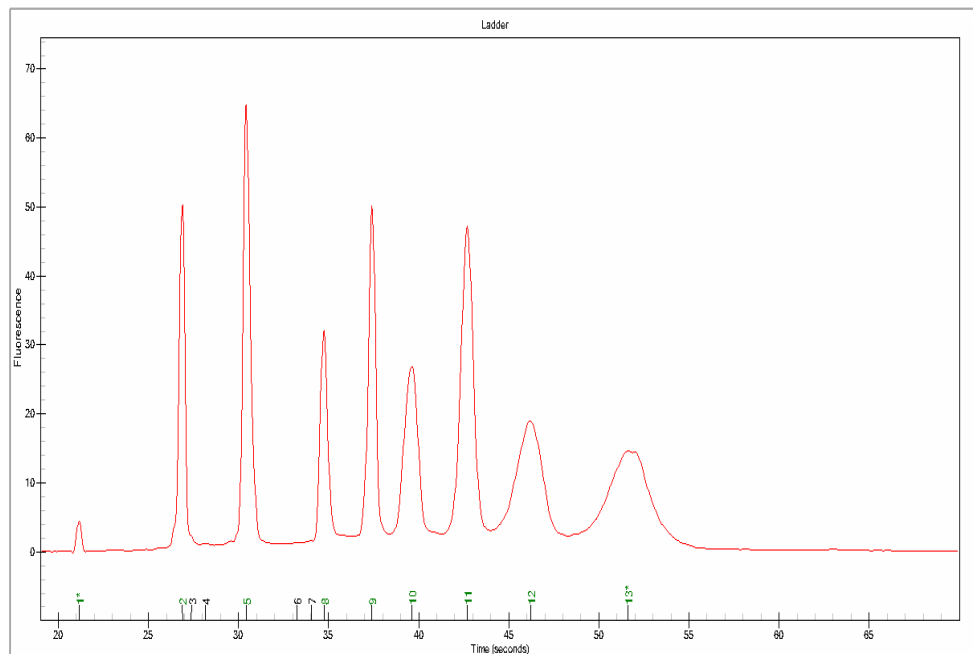
Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A



Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# Ladder









L

Well# Ladder

RNA Area: 681.27
 RNA Concentration: 160.00 ng/μl


Well# Ladder

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.47		0.31	1.37
	2	26.90	75.39		0.36	20.28
	3	27.35	2.43		0.29	0.67
	4	28.15	2.78		0.80	0.78
	5	30.45	112.32		0.43	34.20
	6	33.25	1.69		0.50	0.56
	7	34.05	2.51		0.65	0.85
	8	34.75	53.23		0.47	18.50
	9	37.40	74.05		0.45	27.69
	10	39.60	70.83		0.91	28.05

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

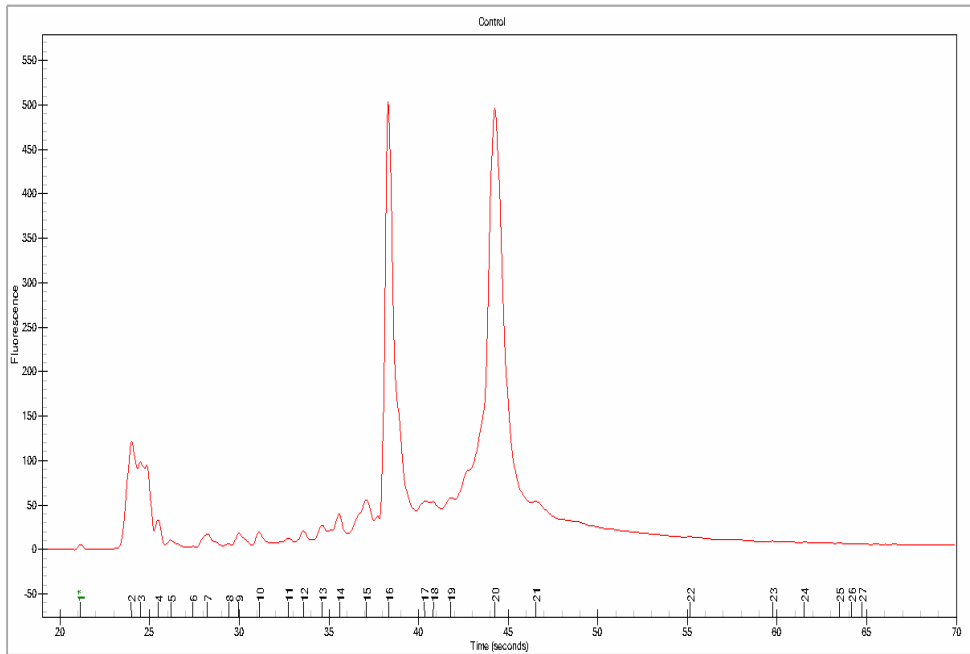
Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# Ladder

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
L	11	42.70	99.96		0.78	42.68
L	12	46.20	78.91		1.72	36.45
	13	51.60	82.57		2.56	42.61

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 1 Control

1

Well# 1 Control

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.75	39.70	704.26	15.97
2	28S	43.00	45.80	880.42	19.97

RNA Area: 4,409.13

RNA Concentration: 1,035.51 ng/μl

Ratio[28S/18S]: 1.25

RQI: 9.4

**Well# 1 Control**

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	7.51		0.31	1.59
	2	23.99	290.56		0.58	69.70
	3	24.48	271.46		0.70	66.46
	4	25.48	47.85		0.38	12.19
	5	26.18	23.97		0.73	6.28
	6	27.42	5.03		0.60	1.38
	7	28.22	47.33		0.68	13.36

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

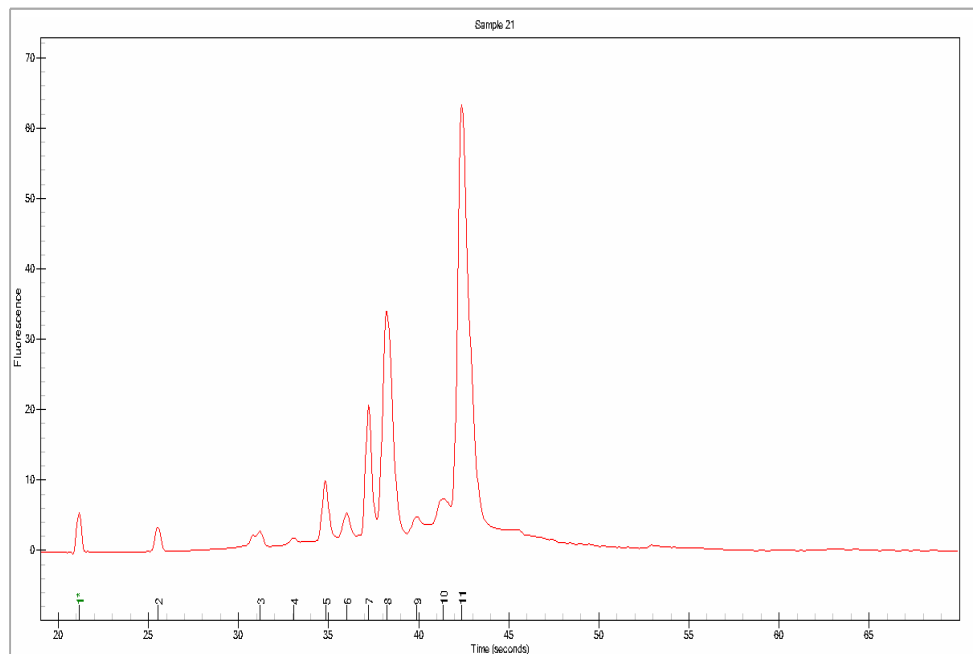
Well# 1 Control

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	29.41	7.30		0.50	2.15
	9	29.96	37.09		0.66	11.11
	10	31.10	33.45		0.53	10.40
	11	32.75	31.24		1.29	10.23
	12	33.59	31.18		0.57	10.47
	13	34.64	45.34		0.61	15.70
	14	35.58	73.15		0.83	26.03
	15	37.07	140.88		1.10	52.23
	16	38.32	916.66		0.49	351.25
	17	40.36	89.56		0.75	36.14
	18	40.81	70.17		0.60	28.64
	19	41.80	91.68		0.75	38.33
	20	44.24	1,570.41		0.93	694.76
	21	46.53	411.72		2.95	191.57
	22	55.14	31.91		2.14	17.60
	23	59.77	10.04		1.69	6.00
	24	61.51	7.71		1.94	4.74
	25	63.50	1.43		0.60	0.91
	26	64.15	0.83		0.45	0.53
	27	64.74	0.47		0.41	0.30

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 2 Sample 21



2

Well# 2 Sample 21

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.65	39.35	49.14	14.66
2	28S	41.80	43.85	97.28	29.02

RNA Area: 335.24
 RNA Concentration: 78.73 ng/μl
 Ratio[28S/18S]: 1.98
 RQI: 9.5



Well# 2 Sample 21

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	8.08		0.31	1.71
	2	25.55	5.18		0.37	1.32
	3	31.18	4.24		0.69	1.32
	4	33.06	1.00		0.38	0.33
	5	34.84	10.65		0.39	3.71
	6	36.02	5.13		0.45	1.85
	7	37.21	21.46		0.38	7.98

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

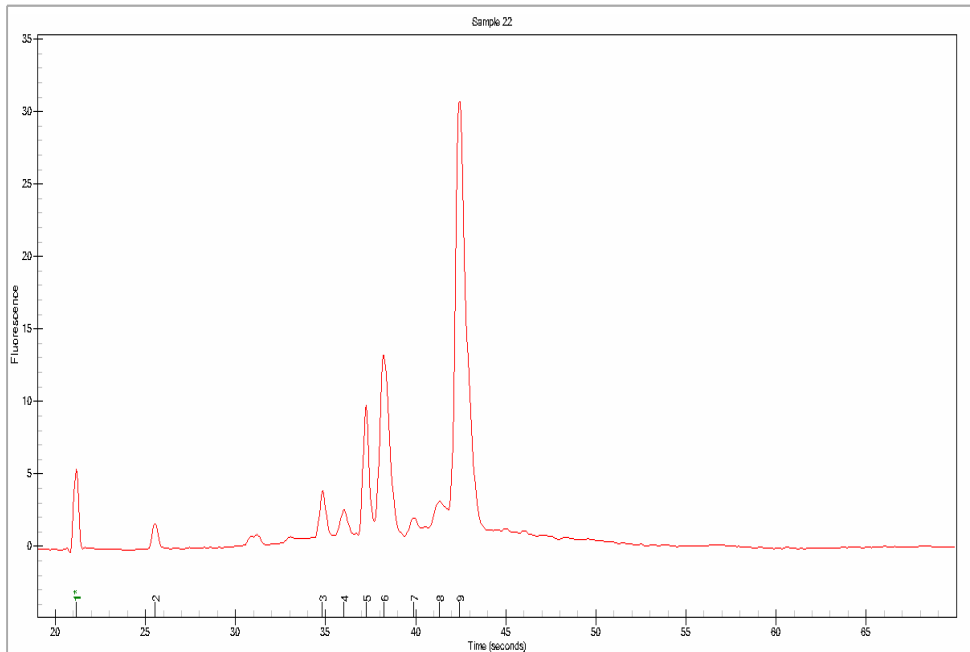
Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 2 Sample 21

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	38.25	55.04		0.62	21.05
	9	39.88	4.96		1.05	1.98
	10	41.36	9.45		0.84	3.91
	11	42.40	108.28		0.68	45.91

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 3 Sample 22


3

Well# 3 Sample 22

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.70	39.35	18.76	13.28
2	28S	41.80	43.85	46.67	33.02

RNA Area: 141.34
RNA Concentration: 33.19 ng/μl
Ratio[28S/18S]: 2.49
RQI: 9.7

**Well# 3 Sample 22**

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	8.04		0.31	1.70
	2	25.53	2.58		0.37	0.66
	3	34.82	3.77		0.38	1.31
	4	36.00	2.50		0.46	0.90
	5	37.23	9.70		0.37	3.61
	6	38.22	20.42		0.59	7.80
	7	39.89	1.26		0.48	0.50

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

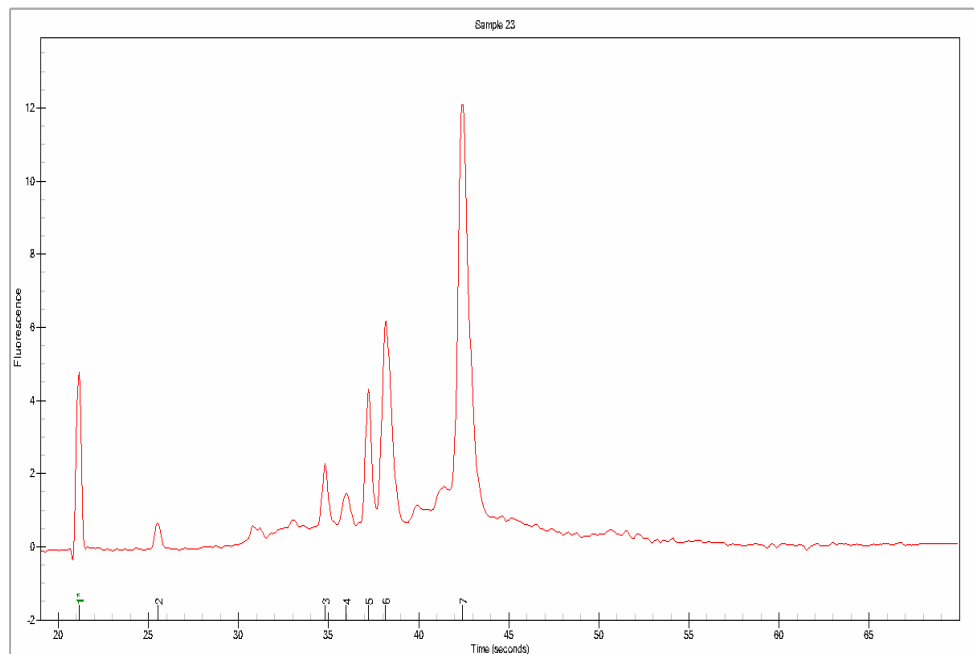
Well# 3 Sample 22

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	41.32	3.89		0.83	1.61
	9	42.45	50.41		0.63	21.40

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 4 Sample 23



4

Well# 4 Sample 23

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.65	39.30	8.16	10.98
2	28S	41.75	43.80	17.68	23.79

RNA Area: 74.32
 RNA Concentration: 17.45 ng/μl
 Ratio[28S/18S]: 2.17
 RQI: 9.1



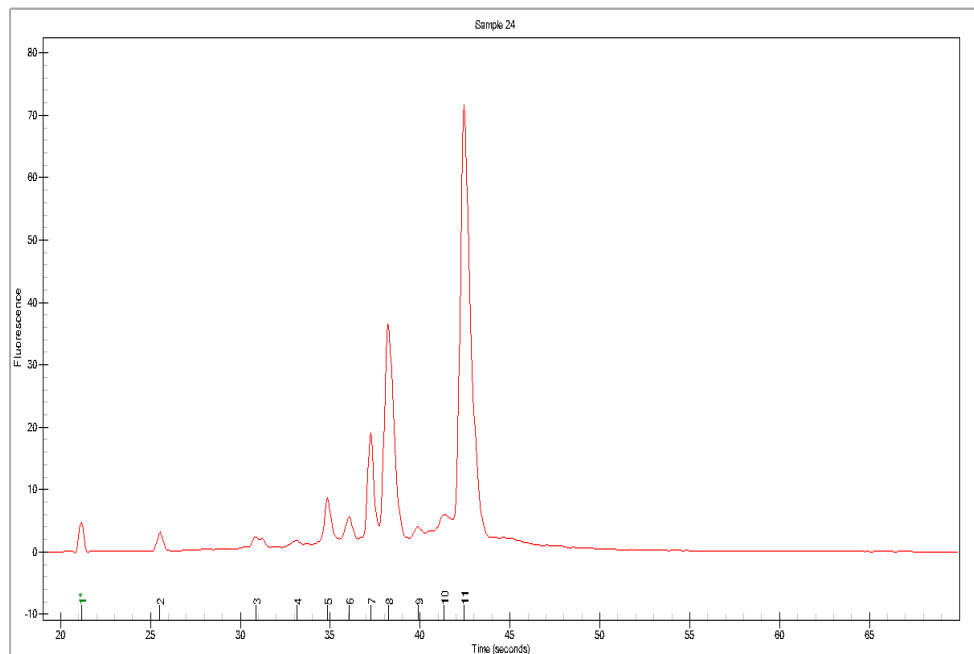
Well# 4 Sample 23

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.95		0.31	1.47
	2	25.54	1.08		0.38	0.28
	3	34.80	1.85		0.36	0.64
	4	35.96	0.95		0.44	0.34
	5	37.23	3.58		0.36	1.33
	6	38.16	8.03		0.56	3.06
	7	42.45	17.69		0.62	7.51

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 5 Sample 24



5

Well# 5 Sample 24

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.70	39.35	50.42	16.23
2	28S	41.85	43.85	104.06	33.49

RNA Area: 310.74
 RNA Concentration: 72.98 ng/μl
 Ratio[28S/18S]: 2.06
 RQI: 9.5



Well# 5 Sample 24

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.48		0.30	1.37
	2	25.52	4.48		0.36	1.14
	3	30.85	3.32		0.70	1.02
	4	33.13	1.17		0.42	0.39
	5	34.83	9.66		0.40	3.37
	6	36.04	5.88		0.46	2.12
	7	37.26	19.92		0.38	7.42

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

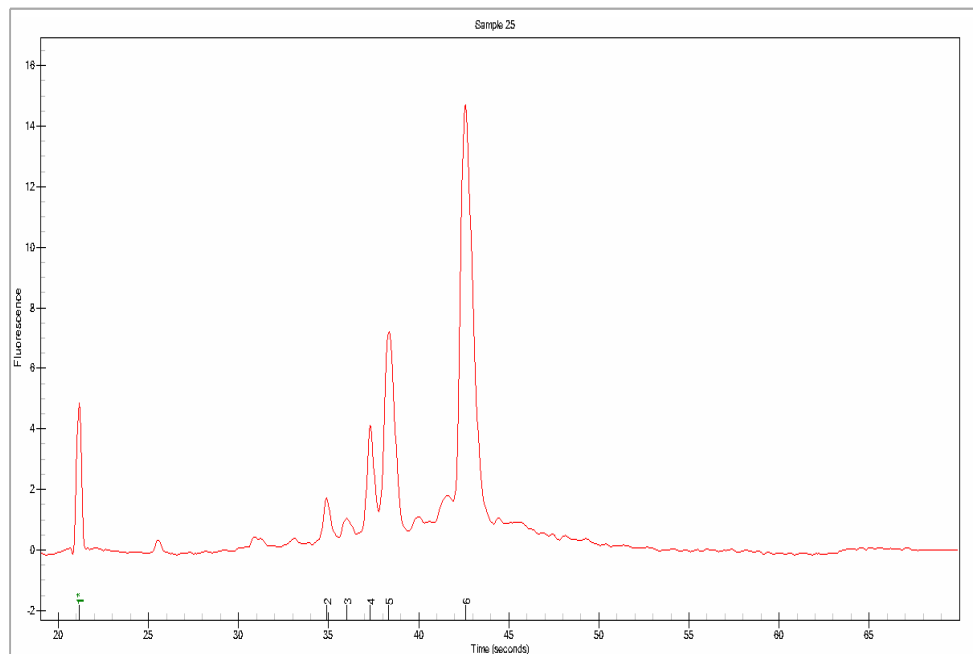
Well# 5 Sample 24

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	38.23	56.75		0.57	21.69
	9	39.87	2.95		0.63	1.18
	10	41.33	7.61		0.84	3.15
	11	42.45	111.94		0.60	47.51

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 6 Sample 25



6

Well# 6 Sample 25

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.80	39.40	10.19	12.39
2	28S	41.90	44.05	24.24	29.47

RNA Area: 82.26
 RNA Concentration: 19.32 ng/μl
 Ratio[28S/18S]: 2.38
 RQI: 9.8



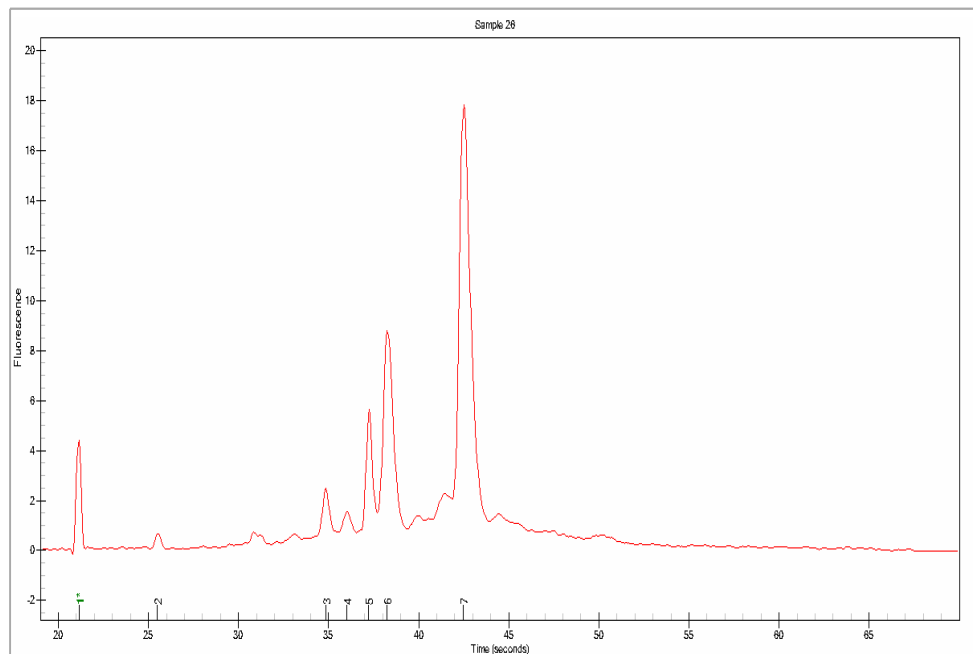
Well# 6 Sample 25

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.94		0.31	1.47
	2	34.90	1.85		0.41	0.65
	3	36.01	0.83		0.55	0.30
	4	37.31	4.27		0.42	1.59
	5	38.33	10.83		0.63	4.15
	6	42.59	25.77		0.74	10.98

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 7 Sample 26



7

Well# 7 Sample 26

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.70	39.35	12.02	12.03
2	28S	41.85	43.90	26.88	26.89

RNA Area: 99.94
 RNA Concentration: 23.47 ng/μl
 Ratio[28S/18S]: 2.24
 RQI: 9.3



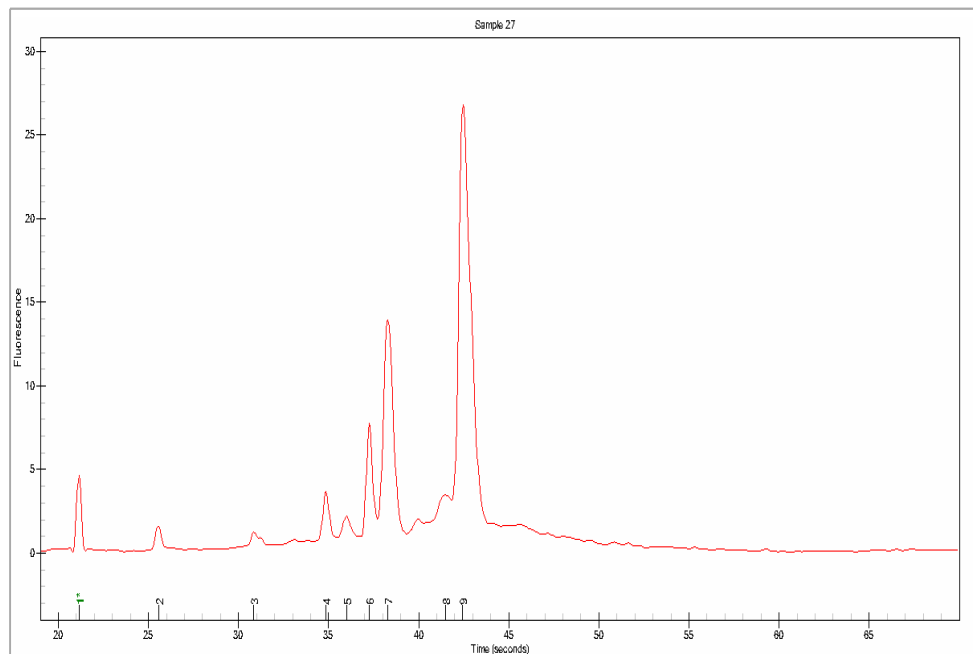
Well# 7 Sample 26

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.15		0.30	1.30
	2	25.50	0.81		0.36	0.21
	3	34.86	2.38		0.40	0.83
	4	36.02	0.98		0.44	0.35
	5	37.23	5.10		0.37	1.90
	6	38.24	12.44		0.59	4.76
	7	42.49	28.19		0.67	11.98

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 8 Sample 27



8

Well# 8 Sample 27

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.70	39.30	19.41	13.81
2	28S	41.85	43.90	42.07	29.95

RNA Area: 140.48
 RNA Concentration: 32.99 ng/μl
 Ratio[28S/18S]: 2.17
 RQI: 9.4



Well# 8 Sample 27

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.28		0.30	1.33
	2	25.55	2.03		0.37	0.52
	3	30.83	1.01		0.43	0.31
	4	34.85	3.33		0.38	1.16
	5	36.01	1.51		0.45	0.54
	6	37.27	6.98		0.37	2.60
	7	38.28	20.01		0.59	7.66

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

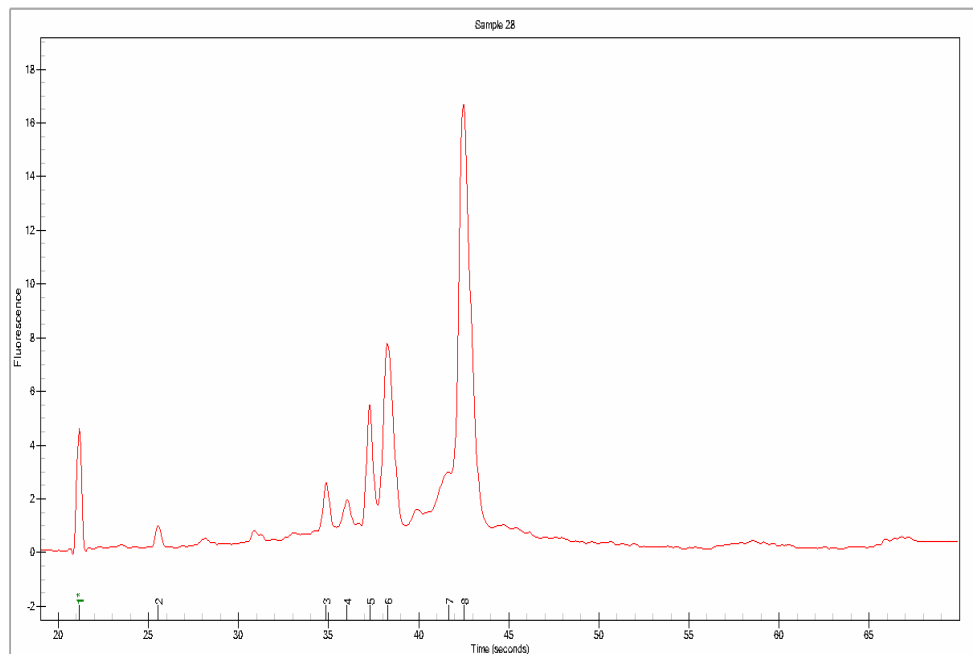
Well# 8 Sample 27

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	41.48	2.84		0.74	1.18
	9	42.45	45.42		0.73	19.28

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 9 Sample 28



9

Well# 9 Sample 28

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.70	39.25	10.07	11.32
2	28S	41.80	43.95	24.13	27.12

RNA Area: 88.96
 RNA Concentration: 20.89 ng/μl
 Ratio[28S/18S]: 2.40
 RQI: 9.2



Well# 9 Sample 28

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.36		0.30	1.35
	2	25.53	1.16		0.36	0.30
	3	34.86	2.06		0.38	0.72
	4	36.03	1.19		0.42	0.43
	5	37.29	4.82		0.39	1.80
	6	38.26	10.45		0.60	4.00
	7	41.67	2.71		0.72	1.13

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

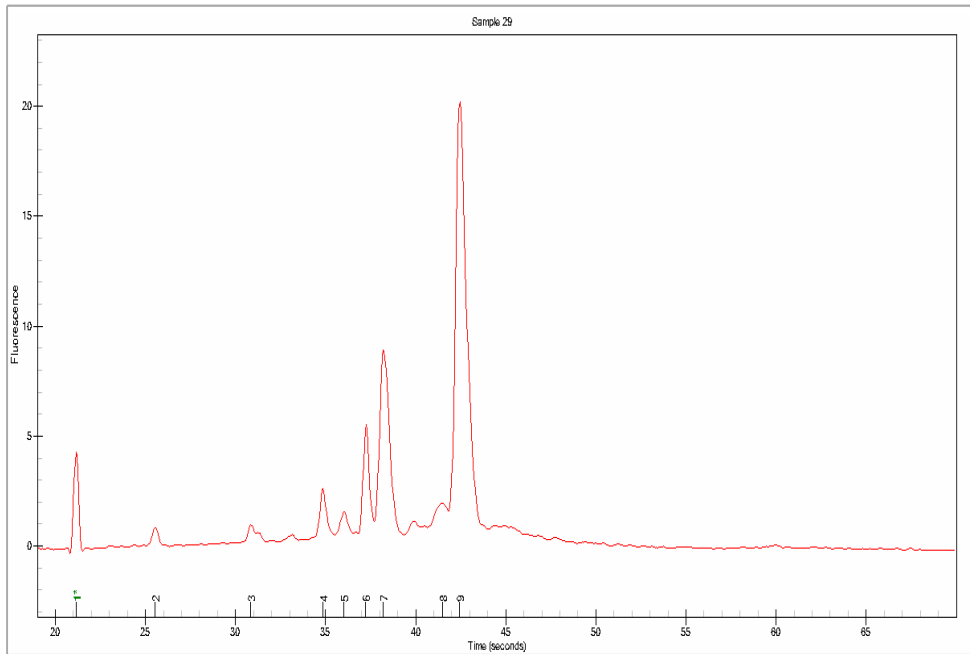
Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 9 Sample 28

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	42.49	28.10		0.70	11.94

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 10 Sample 29

10

Well# 10 Sample 29

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.70	39.35	12.71	12.54
2	28S	41.80	43.85	30.58	30.15

RNA Area: 101.42
 RNA Concentration: 23.82 ng/μl
 Ratio[28S/18S]: 2.41

RQI: 9.4

**Well# 10 Sample 29**

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.28		0.30	1.33
	2	25.54	1.26		0.39	0.32
	3	30.85	0.99		0.45	0.31
	4	34.84	2.68		0.38	0.93
	5	36.01	1.33		0.43	0.48
	6	37.23	5.25		0.37	1.96
	7	38.21	13.23		0.59	5.06

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

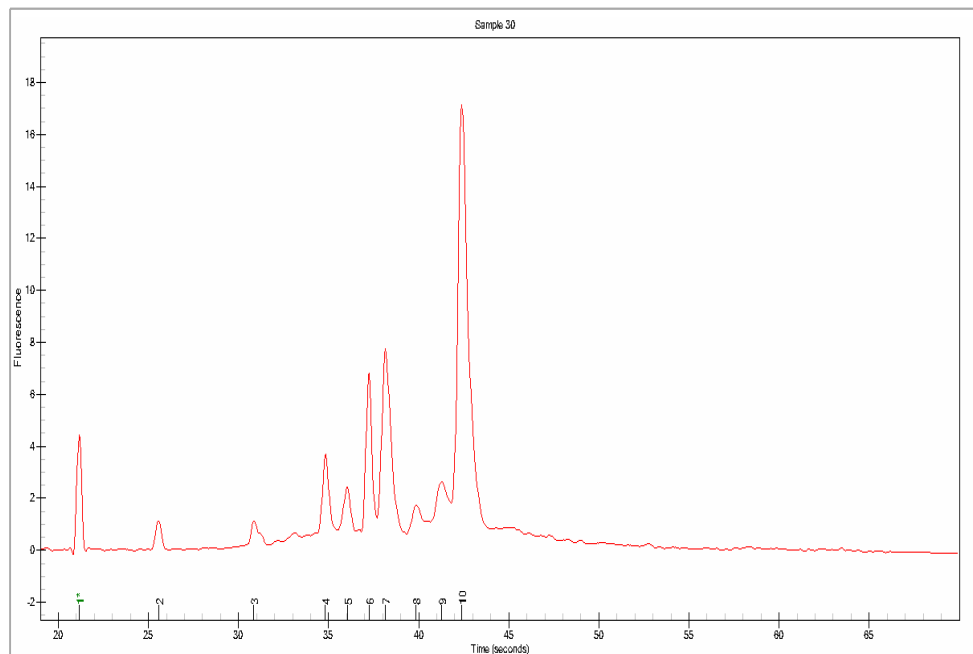
Well# 10 Sample 29

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	41.47	1.83		0.75	0.76
	9	42.45	32.37		0.65	13.74

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 11 Sample 30



11


Well# 11 Sample 30

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.65	39.30	9.79	10.47
2	28S	41.80	43.80	23.74	25.39

RNA Area: 93.50
 RNA Concentration: 21.96 ng/μl
 Ratio[28S/18S]: 2.43
 RQI: 9.0



Well# 11 Sample 30

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.29		0.30	1.33
	2	25.56	1.63		0.37	0.42
	3	30.84	1.48		0.45	0.46
	4	34.81	3.66		0.38	1.27
	5	36.03	2.23		0.42	0.80
	6	37.26	6.45		0.36	2.40
	7	38.14	10.80		0.54	4.12

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

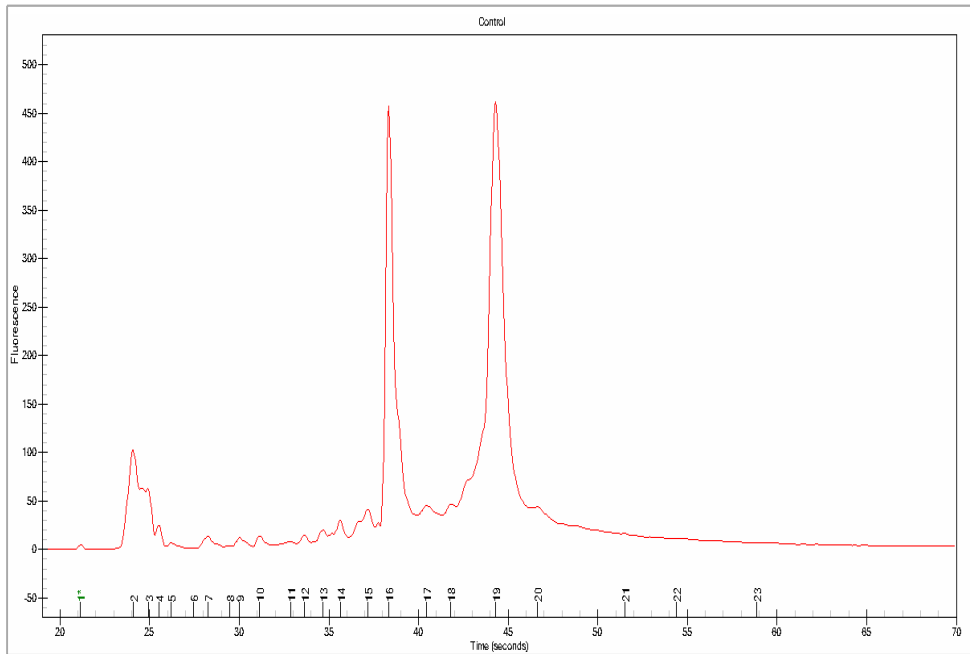
Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 11 Sample 30

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	39.85	1.31		0.55	0.52
	9	41.27	3.25		0.82	1.34
	10	42.40	26.15		0.59	11.09

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 12 Control

12

Well# 12 Control

Fragment Number	Fragment Name	Start Time	End Time	Area	% of Total Area
1	18S	37.75	39.65	607.21	16.93
2	28S	43.10	45.80	794.79	22.16

RNA Area: 3,586.20
 RNA Concentration: 842.24 ng/μl
 Ratio[28S/18S]: 1.31
 RQI: 9.6

**Well# 12 Control**

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	1	21.15	6.73		0.31	1.42
	2	24.09	261.59		0.67	63.01
	3	24.92	118.52		0.47	29.53
	4	25.51	31.68		0.34	8.08
	5	26.19	13.95		0.70	3.65
	6	27.47	1.70		0.54	0.47
	7	28.25	30.70		0.58	8.67

Project: Javier Medina
Assay: Eukaryote Total RNA StdSens
Run: Experion RNA SS 21-12-18(3)
Run Version: N/A

Acq. Analyst: DefaultUser
Acq. Time: 12/21/2018 8:51:32 AM
Signature: N/A

Well# 12 Control

Peak State	Peak Number	Mig. Time (secs)	Corrected Area	Comments	FWHM	Area
	8	29.47	3.41		0.44	1.00
	9	30.01	22.22		0.63	6.67
	10	31.14	20.68		0.46	6.44
	11	32.85	15.63		1.03	5.14
	12	33.63	20.16		0.52	6.78
	13	34.66	29.36		0.55	10.18
	14	35.64	52.25		0.54	18.62
	15	37.16	99.95		1.13	37.14
	16	38.33	774.43		0.46	296.87
	17	40.44	123.81		1.37	50.07
	18	41.81	73.03		0.78	30.54
	19	44.31	1,344.29		0.87	595.62
	20	46.61	226.00		2.58	105.34
	21	51.50	41.00		2.64	21.12
	22	54.39	25.28		2.84	13.75
	23	58.85	0.36		0.39	0.21