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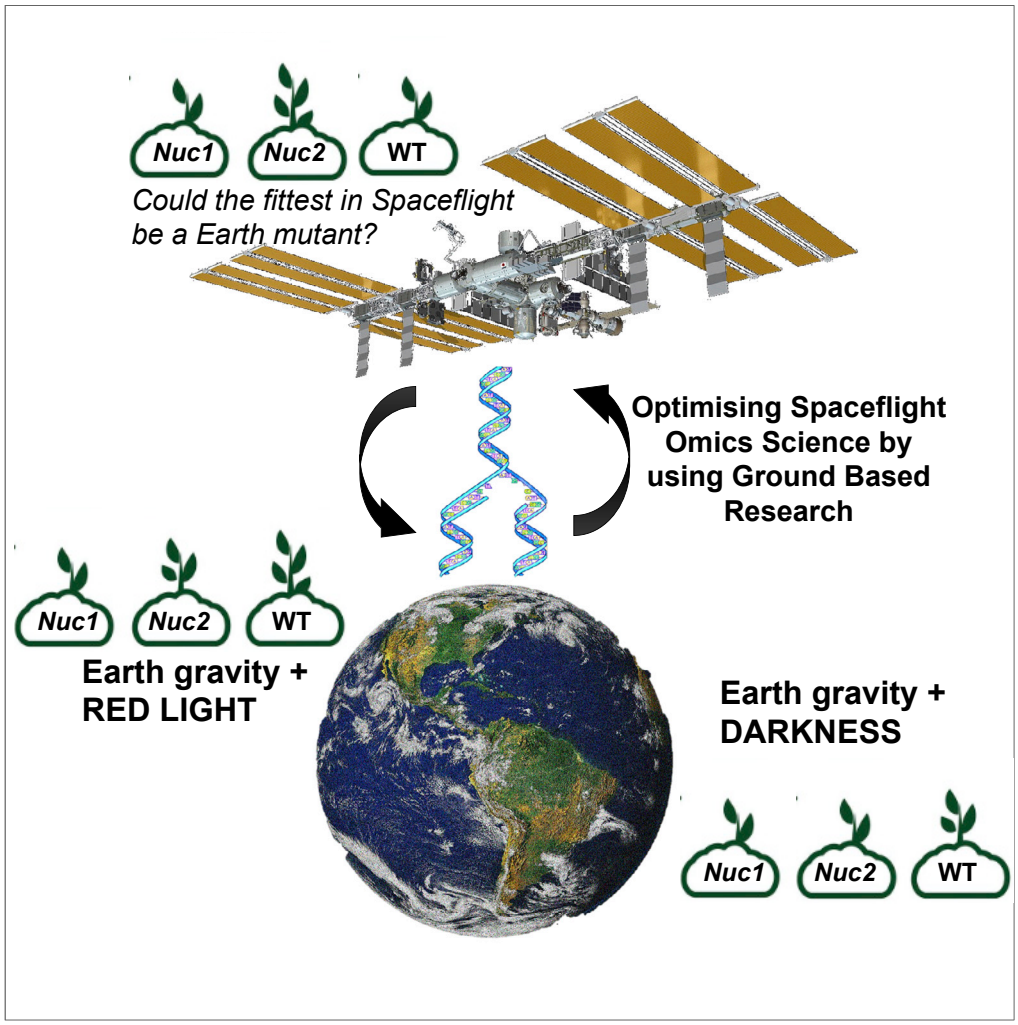

Aránzazu Manzano, Alicia Villacampa, Julio Sáez-Vásquez, John Z. Kiss, F. Javier Medina, Raúl Herranz

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HIGHLIGHTS

Ribosome synthesis is a target of spaceflight stressor effects on plant development

Nucleolin mutants promote a differential response to light/ darkness stress

Red light and NUC2 may counteract the spaceflight alterations in gene expression

Ground controls are important for the interpretation of spaceflight -omics experiments

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

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SUMMARY

Understanding plant adaptive responses to the space environment is a requisite for enabling space farming. Spaceflight produces deleterious effects on plant cells, particularly affecting ribosome biogenesis, a complex stress-sensitive process coordinated with cell division and differentiation, known to be activated by red light. Here, in a series of ground studies, we have used mutants from the two *Arabidopsis* nucleolin genes (*NUC1* and *NUC2*, nucleolar regulators of ribosome biogenesis) to better understand their role in adaptive response mechanisms to stress on Earth. Thus, we show that nucleolin stress-related gene *NUC2* can compensate for the environmental stress provided by darkness in *nuc1* plants, whereas *nuc2* plants are not able to provide a complete response to red light. These ground control findings, as part of the ESA/NASA Seedling Growth spaceflight experiments, will determine the basis for the identification of genetic backgrounds enabling an adaptive advantage for plants in future space experiments.

INTRODUCTION

Space exploration will soon include new human missions to the Moon as a first step in the human exploration of Mars. Recent studies in human (i.e., NASA Twin Study, Garrett-Bakelman et al., 2019) and mammalian systems (rodent missions, Beheshti et al., 2019; Ronca et al., 2019) are paving the way to understand the effects of the microgravity environment on human physiology, but human life in space will rely on the essential role of plants in bioregenerative life support systems (Zabel et al., 2016), not only as a source of water, food, and removal of CO_2 but also by providing a terrestrial-like environment for the psychological well-being of astronauts.

Due to their sessile condition, plants have to promote adaptive responses to cope with changes in environmental conditions. Light plays multiple roles in the mechanisms of these adaptive responses. On the one hand, light is the source of energy by means of photosynthesis and regulates indirectly cell proliferation and cell growth via the central regulator TOR kinase through the expression of S-phase genes, and also ribosome biogenesis (Caldana et al., 2013; Xiong et al., 2013; Sablowski and Carnier Dornelas, 2014). In addition, light is a major driver in the establishment of the patterns of plant growth and development, by means of phototropism and photomorphogenesis. In playing this role, light is associated with gravity as one of the major tropistic cues, such that gravitropism and phototropism (and the interaction between them) are essential modulators of plant development (Vandenbrink et al., 2014). At the cellular and molecular levels, plant development relies on the activity of cell growth and proliferation taking place in the meristems, which supply differentiated cells and are influenced by the tropistic cues (Perrot-Rechenmann, 2010).

Darkness is indeed a stress condition for plants. Several articles describe that in the dark, apical meristem proliferation is arrested in the G1 and G2 phases of the cell cycle (López-Juez et al., 2008; Mohammed et al., 2017), but in the root meristem, light induces the production of flavonols and other metabolites leading to

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the reduction of cell proliferation (Silva-Navas et al., 2016). This reduction could be related to the sugar starvation that results from the inability to perform photosynthesis.

However, red light has a stimulating effect on ribosome biogenesis and cell proliferation. An increase in the mitotic index, in the expression of some regulators of these processes at both gene expression and protein levels, and in post-translational modifications of some protein factors has been described in plants irradiated with red light (Tong et al., 1997; Reichler et al., 2001).

The absence of gravity (weightlessness, or microgravity, as it exists in free-fall, or in spaceflight) is also, by itself, a stress condition for plants, and specifically for the functions of meristematic cells (Matía et al., 2010; Boucheron-Dubuisson et al., 2016). An experiment performed in the International Space Station (ISS) in which *Arabidopsis thaliana* seedlings grew for 4 days in darkness resulted in an increase in the rate of cell proliferation and a decrease in the cell growth rate, estimated by the activity of ribosome biogenesis in the nucleolus, compared with the 1*g* control (Matía et al., 2010). As the coordination of these two activities defines meristematic competence, the effect of the lack of tropistic stimuli, particularly of gravity signals, may result in serious alterations of the developmental pattern of the plant, as was also shown in simulated microgravity experiments (Boucheron-Dubuisson et al., 2016).

The Seedling Growth (SG) experiments, recently performed in the ISS, aimed at unraveling the link between phototropism and gravitropism, using the weightless environment of spaceflight (Vandenbrink et al., 2019; Herranz et al., 2019). In the first SG experiment, *A. thaliana* seedlings corresponding to wild-type (WT) ecotype *Landsberg erecta* (Ler) and two phytochrome mutants (*phyA* and *phyB*) known to be involved in phototropism (Kiss et al., 2003; Molas and Kiss, 2008) were grown in space for 6 days and photostimulated for the last 2 days, revealing differential blue and red light phototropism in space (Vandenbrink et al., 2016). The analysis of the expression of regulatory genes of cell cycle and ribosome biogenesis showed that red light irradiation produced a significant reversion of the uncoupling of cell proliferation and cell growth caused by microgravity in darkness (Matía et al., 2010) and, consequently, a compensation of the loss of meristematic competence (Valbuena et al., 2018).

In view of these results from our previous space experiments, we decided to focus on the cellular process of ribosome biogenesis for the successive spaceflight experiments of the SG project, with the purpose of testing the separate and synergistic effects of the light and gravity tropistic signals on specific molecular and cellular components of this process. Ribosome biogenesis, which takes place in the nucleolus, represents the most complex multi-step process that the cell must perform and is one of the most intricately regulated and controlled (Sáez-Vásquez and Medina, 2008; Pelletier et al., 2018). Therefore, its regulation must adapt to the environmental conditions in which the cell finds itself and coordinate with other cellular processes, such as cell division and differentiation. Several studies have described and used the nucleolus as a major stress sensor, using stress-induced changes in the organization and composition of this organelle (Mayer and Grummt, 2005; Boulon et al., 2010; Lewinska et al., 2010; Kalinina et al., 2018).

Briefly, ribosome biogenesis consists of the transcription of 45S rRNA genes (45S rDNA) containing the sequences of 18S, 5.8S, and 25S rRNAs, followed by the multi-step cleavage of 45S pre-rRNA to produce, in association with 5S rRNA and ribosomal proteins (RPs), the mature ribosomal subunits, which are then exported to the cytoplasm and assembled as mature ribosomes.

In addition to RPs, hundreds of non-ribosomal proteins (NRPs), or nucleolar proteins and small nucleolar RNAs are required for ribosome biogenesis, playing regulatory roles (Sáez-Vásquez and Delseny, 2019). Among NRPs, nucleolin is the most abundant protein of the nucleolus, where it plays a key role in the different steps involved in ribosome biogenesis, including RNA polymerase (Pol) I transcription, processing of pre-rRNA (Ginisty et al., 1999; Roger et al., 2003), and assembly and nucleocytoplasmic transport of ribosome particles (Bouvet et al., 1998). Moreover, nucleolin has been even implicated in other functions, with or without collateral relationship with ribosome biogenesis (Angelov et al., 2006; Ma et al., 2007; Mongelard and Bouvet, 2007; Stepiński, 2012).

Animal and yeast genomes encode a single nucleolin gene, whereas plants offer various examples of gene multiplicity. In *A. thaliana*, two genes encoding nucleolin proteins have been described: *NUC1* and *NUC2* (Pontvianne et al., 2007, 2010). The *NUC1* gene is highly and ubiquitously expressed in normal growth

conditions and NUC1 protein is required to inhibit *NUC2* gene expression at the transcriptional level and may also influence the accumulation of NUC2 protein. In contrast, *NUC2* is a functional protein-coding gene developmentally controlled in most plant tissues and organs (Durut et al., 2014). NUC1 and NUC2 proteins localize in the nucleolus.

Disruption of *NUC1* gene (*nuc1-2* mutant) leads to severe defects in plant growth and development. At the molecular level, this mutant produces *NUC2* expression, nucleolus disorganization, rDNA (NOR) heterochromatin decondensation, pre-rRNA accumulation, de-repression of specific rDNA variants, and demethylation of some sequences of rDNA (Pontvianne et al., 2010).

In contrast, disruption of *NUC2* gene (*nuc2-2* mutant) has much weaker effects. The *nuc2-2* mutant seedlings grow quite similarly to WT plants, but flowering occurs later. Knockout of the *NUC2* gene induces expression of some rDNA variants and hypermethylation of some sequences of pre-rRNA. In addition, NUC2 is required for the stability of rDNA variants' copy number (Durut et al., 2014).

In this article, in a series of ground studies to complement space experiments, we have exposed *A. thaliana* (ecotype Columbia) WT, *nuc1-2* and *nuc2-2* seedlings to two different illumination regimes at the beginning of the plant development (6 days from germination). In addition to highlighting the importance of Earth reference controls in spaceflight -omics experiments, our goal was to better understand the responses of nucleolin mutants to red light stimulation for further uses in space experiments under altered gravity conditions. The differential adaptive responses to the light are shown, suggesting that stress-related mutants may show a reduced response to environmental stress. In the long term, these results may lead to more efficient agriculture if an exaggerated response may reduce plant development under suboptimal environmental conditions.

RESULTS

Overall Transcriptional Profile Effects Confirm a Suitable Quality of Plant Material and Clustering of the Replicates

Two environmental conditions (light) are compared here, both of them as part of the SG Earth control reference experiment using the European Modular Cultivation System (EMCS). Seedlings were germinated in the same spaceflight hardware during 4 days with a day/night cycle of illumination, and then half of the samples were exposed to 2 days of darkness and the other group to 2 days of continuous red light (Figure 1A). After 6 days growth in the culture chamber (CC), the experiment was completed and the phenotypes of the three mutants were observed (Figure 1B, Supplemental Information, Figure S1). Seedling growth was homogeneous within each cassette, with clearly smaller seedlings in the case of the *nuc1-2* mutant. No clear phenotypical differences were observed in the root system physiology between darkness- and red light-stimulated samples (including the absence of red phototropism at 1*g* conditions). The phenotype was compared with a previous experiment with the same duration and genotypes but in petri dishes and with a 6-day photoperiod illumination profile (Manzano et al., 2020 submitted). Despite the effects of the TROPI cassette volume, the overall phenotype of the seedlings is also very similar in both studies.

The quality of the replicates and overall similarities among the illumination regimes and different plants used is shown by principal-component analysis (PCA, Figure 1C and Supplemental Information, Data S1). First, a clear difference between the transcriptional profile of the WT, *nuc1-2,* and *nuc2-2* plants exposed to darkness or red light photostimulation during the last 2 days of growth is shown by principal component 1 (PC1). Second, the differences between the genotypes can be observed in principal component 2 (PC2), where the *nuc1-2* genotype has the most disrupted nucleolus phenotype, in that the *nuc2-2* mutant is closer to the WT. The effect is clearer in the dark samples, because the red light stimulation produces more similarities among the three genotypes. Last, all the biological replicates included in the study cluster together according to the experimental condition, strengthening the statistical validity of the study. The use of three biological replicates (including up to 10 seedlings each) is enough considering the difficulties in increasing this number due to the availability of spaceflight research capabilities.

Global Effect of Red Light Photostimulation Differs in Each *Arabidopsis thaliana* Line

To understand the effect of the two lighting regimes (red light and darkness) on the transcriptional status, we compared seedlings with the differential illumination during the last 2 days of the plant growth period for each of the lines used (WT, *nuc1-2,* and *nuc2-2*).

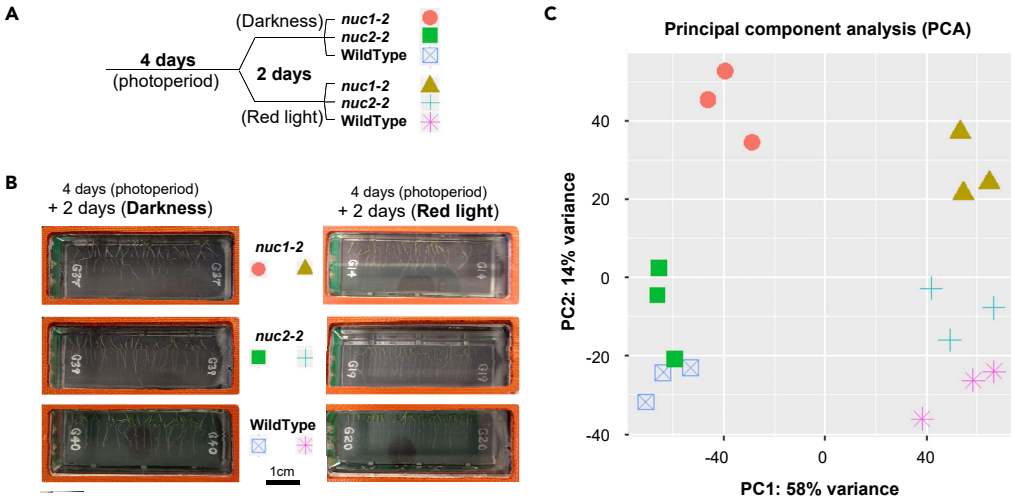

Figure 1. The Seedling Growth 2 Ground Reference Experiment

(A) Experimental design including the illumination profile for each sample including color code used as key.

(B) Images of 6-day-old seedlings (WildType, *nuc1-2,* and *nuc2-2,* under the two illumination options) inside the CC just before collection for freezing (additional photos are provided as Supplemental Information Figure S1). Scale bar, 1 cm (the gridded membrane has clearly defined grid lines spaced at 3.1 mm). The labels on the membrane represent the cassette # in the ground control.

(C) Principal-component analysis (PCA) of the 18 samples (three replicates per condition) using read counts data from FeatureCounts. This diagram gives an overview of the similarities and dissimilarities between samples and the experimental conditions' overall effects (see Data S1 for an RNA quality report on the red light samples). All replicates are consistently grouped according to their experimental conditions, and two clear PC1 (for illumination conditions during the last 2 days of growth: darkness and red light) and PC2 (for genetic background: WT, *nuc1-2,* and *nuc2-2*) are observed.

The number of differentially expressed genes (DEGs) in the WT is 1,428 genes, of which 1,067 have their expression activated and 361 have it repressed. In the *nuc1-2* mutant, the total number of DEGs is lower (1,001), mainly at the expense of the up-regulated genes (633 are over-expressed and 368 are repressed). The number of DEGs from the comparison (red light versus darkness) in the *nuc2-2* mutant is very similar as in the WT: a total of 1,017 up-regulated genes and 339 down-regulated genes, making a total of 1,365 DEGs (Figure 2).

Venn diagrams illustrate the group of DEGs that is affected in a single genotype or shared by others (Figure 2). Red light up-regulated genes affected only 298 genes in the WT, 134 genes in *nuc1-2* mutant, and 199 genes for *nuc2-2* mutant. On the other hand, the WT has 34 up-regulated genes in common with *nuc1-2* but a large collection of 353 genes in common with *nuc2-2* (note that the total number of up-regulated genes common for the three genotypes is 382), and only 83 genes for both nucleolin mutants (Figure 2B).

Among DEGs down-regulated by the red light compared with the dark, there is a similar number of unique DEGs for each of them; 139 genes for the WT, 142 genes for the *nuc1-2* mutant, and 104 genes for the *nuc2-2* mutant. In this case, the number of DEGs down-regulated in the three lines is 125 genes, without any of the pair comparisons reaching that level (the *nuc1-2* and *nuc2-2* mutants have 44 and 53 genes in common with WT, respectively, whereas the two nucleolin mutants share 57 down-regulated genes, Figure 2C).

In summary, the line with the least number of DEGs, when we compare plants illuminated with red light with those kept in darkness, is the *nuc1-2* mutant. As the down-regulation response seems similar in all genotypes, the global effect is mainly due to a higher number of up-regulated DEGs in both *nuc2-2* and WT genotypes. In fact, we can easily extrapolate from Figure 2B and compare the gene lists, including the common responses to red light (382 genes) and the list of up-regulated genes not detected in the *nuc1-2* phenotype (353 genes). The main difference between these lists is shown by the functional analysis "GO Enrichment" included in Figure 2. All genotypes show the obvious photosynthesis/light-harvesting response together with increased Calvin cycle activity, but the *nuc1-2* genotype is the only one lacking

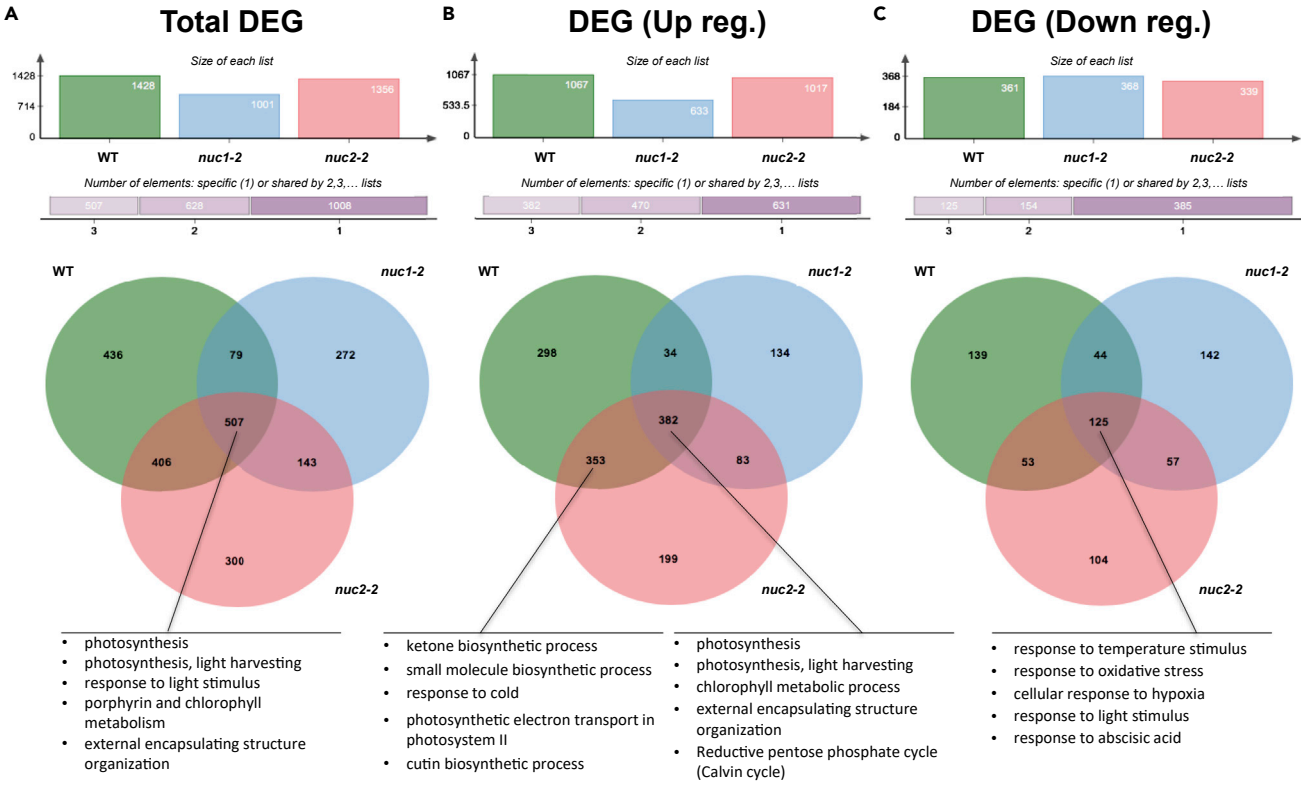

Figure 2. Differentially Expressed Genes (DEG p adj<0.05, Red Light Photostimulation versus Darkness) in WT and Nucleolin Mutants (*nuc1-2* and *nuc2-2*)

(A) Venn diagram comparing all DEGs and the five most significant gene ontology (GO Enrichment) categories in the common DEG in the three genotypes.

(B) Venn diagram comparing up-regulated DEGs and the five most significant gene ontology (GO Enrichment) categories in the common DEGs in the WT and *nuc2-2* genotypes and in the three genotypes, respectively.

(C) Venn diagram comparing down-regulated DEGs and the five most significant gene ontology (GO Enrichment) categories from common DEGs in the three genotypes.

an increased secondary metabolism, together with a response to stress elements (cold) observed in both WT and *nuc2-2*. These results suggest that the presence of the NUC1 protein is necessary for the known effect of red light in the stimulation of cell proliferation, but the NUC2 protein is not an important player in that response.

A complementary functional analysis has been done using the full lists of up- or down-regulated genes in each genotype, obtained by means of a Heatmap GO Enrichment. Up-regulated gene lists reveal that red light photostimulation versus darkness mainly leads to an unequivocal activation of genes involved in different phases of photosynthesis, together with processes that involve external encapsulation, secondary metabolism, and drug catabolism in all three *A. thaliana* lines examined (Supplemental Information, Figure S2A). Other significantly affected categories are the GO involving cell wall modification, pathogenesis, responses to insects, and defense mechanisms to bacteria. In addition to the mentioned GO groups, red light activates different biosynthetic processes (ketone, cutin, and small molecules) and the response to stimuli, such as cold and UV light, in the WT and *nuc2-2* mutant lines. Solely in the WT line, an enrichment in cell division-related genes (microtubule-based movement, cell cycle, meiotic cell cycle) is produced. In the case of down-regulated GO groups, red light photostimulation reduces expression of genes involved in the response to different stimuli (temperature, hypoxia, light, abscisic acid, oxidative stress) as well as the circadian rhythm of plants (Supplemental Information, Figure S2B). Additional down-regulated genes involved in the response to stress appear in WT and *nuc2-2* comparison (heat and osmotic stress), in the WT and *nuc1-2* comparison (cold acclimation, response to karrikin, hormone metabolism, and cell wall modification), or *nuc1-2* only (response to gibberellin). The two nucleolin mutant lines share a repression of the amino acid metabolism (alpha-amino acid catabolic process and leucine degradation).

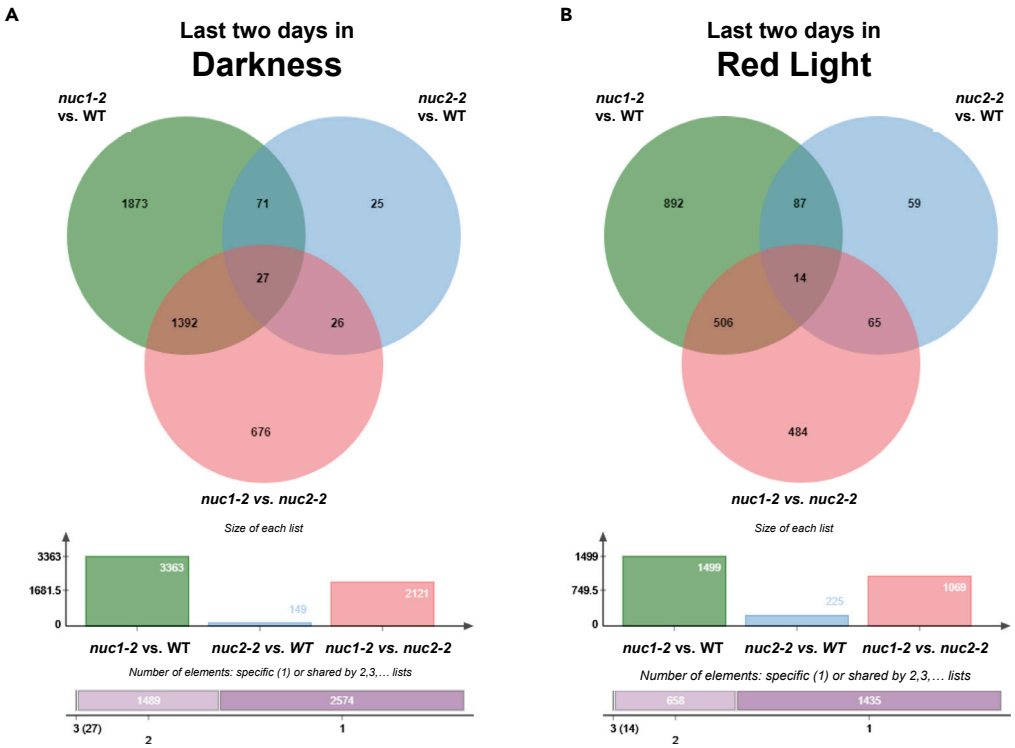

Figure 3. Differentially Expressed Genes (DEG p adj<0.05, in the Three Genotype Pair-Comparisons among WT, *nuc1-2*, and *nuc2-2*) within the Same Experimental Condition

(A) Venn diagram comparing all DEGs between plants exposed to darkness the last 2 days.

(B) Venn diagram comparing all DEGs between plants exposed to red light stimulation the last 2 days.

Dissecting Transcriptional Status for Each Genotype for Illumination Conditions at Normal Earth Gravity

To better understand the differences in the transcriptional status of each genotype that could be attributed to the illumination conditions designed to be used in the SG spaceflight experiment, we performed a series of comparisons between the different lines, namely, *nuc1-2* versus WT, *nuc2-2* versus WT, and *nuc1-2* versus *nuc2-2*, when they are either photostimulated with red light or kept in darkness for 48 h, after a growth period of 4 days under a 16 h/8 h white light photoperiod regime (regular growth condition).

The total number of DEGs when comparing the severe mutant *nuc1-2* with the WT is remarkable, with thousands of genes affected in the different illumination conditions. A similar result was obtained in the comparison of *nuc1-2* with *nuc2-2*. This number was much higher in the comparisons of the *nuc1-2* mutant with both the Col-0 and *nuc2-2* mutant in darkness: 3,363 genes and 2,121 genes, respectively. Under red light stimulation, the numbers dropped to half: 1,499 genes compared with the WT and 1,069 compared with *nuc2-2*, respectively. In contrast, the small number of DEGs between the WT and *nuc2-2* mild mutant (just 149 genes in darkness conditions) peaked to 225 when red light photostimulation was provided (Figure 3). The up- and down-regulated genes showed similar trends in this case (Supplemental Information, Figure S3).

The differential numbers in DEGs can be assigned to particular GO groups. Ontology analysis shows that both comparisons involving the *nuc1* mutant in the darkness produced an increase in the expression of genes mainly involved in cell division, such as cell cycle, meiotic cell cycle, microtubule cytoskeleton, nuclear chromosome segregation, cytokinesis, and cell cycle-G2/M transition (Supplemental Information, Figure S4A). Furthermore, in the *nuc1-2* versus WT and *nuc1-2* versus *nuc2-2* comparisons, categories of responses to different stimuli, namely, responses to auxin, red light, gravity, and UV and ionizing radiations, also appeared overrepresented. In the comparison *nuc1-2* versus *nuc2-2* in darkness, most functional categories (GO terms) appearing up-regulated in the *nuc1-2* mutant are related to the cell wall (cell wall

macromolecule catabolic process, pectin biosynthetic process), the cuticle (cuticle development and cutin, suberin, and wax biosyntheses), and the plasma membrane (anchored component of plasma membrane, very-long-chain fatty acid biosynthetic process, transmembrane receptor protein kinase activity).

The up-regulated processes when red light is applied are quite different (Supplemental Information, Figure S4B). In the *nuc1-2* versus WT and *nuc1-2* versus *nuc2-2* comparisons, DEGs appeared involved in cell wall (structural constituents of cell wall) and in development (positive regulation of growth, post-embryonic plant morphogenesis, cellular response to ethylene stimulus). In the *nuc1-2* versus *nuc2-2* comparison, many genes with a red-light-activated expression were involved in ribosome biogenesis (preribosome, maturation of SSU-rRNA, 90S preribosome, maturation of 5.8S RNA, ribosomal large subunit biogenesis).

The two nucleolin mutant lines relative to WT (*nuc1-2* versus WT and *nuc2-2* versus WT comparisons) in conditions of red light photoactivation have up-regulated genes involved in different response processes, such as the responses to hypoxia, drug, antibiotic, salicylic acid, and wounding. The number of response processes was increased in the *nuc2-2* versus WT comparison with the GO categories: responses to cold, bacterium, and abscisic acid.

The identification of common gene categories down-regulated in both darkness and red light conditions was more challenging (Supplemental Information, Figures S4C and S4D). In darkness, *nuc1-2* versus WT and *nuc1-2* versus *nuc2-2* comparisons were very similar and mainly related to developmental processes (regulation post-embryonic development, meristem development, regulation of seed development, plant organ senescence), mRNA quality (mRNA surveillance pathway), response to temperature (response to temperature stimulus, endopeptidase Clp complex, heat shock protein), and immune system (immune response, response to toxic substance, glutathione metabolism). In the comparisons indicated above, genes involved in long-day photoperiodism, flowering, rhythmic process, and response to light intensity were also less represented. In the same way, cellular responses to light stimulus category was over-represented in both nucleolin mutants with respect to WT and responses to light stimulus was a common GO category in all three comparisons. The gene categories repressed only in the *nuc1-2* versus *nuc2-2* comparison were involved in the spliceosome, protein demethylation, chromatin organization, mRNA binding, and histone acetyltransferase activity.

The gene categories with repressed expression under red light for any of the comparisons (*nuc1-2* versus *WT* and *nuc1-2* versus *nuc2-2*) were related to mRNA splicing (alternative mRNA splicing via spliceosome), light responses (response to light stimulus, photoperiodism flowering, circadian rhythm), photosynthesis (chlorophyll biosynthesis process, chloroplast envelope), and the immune system (immune system process, defense response to bacterium, regulation of salicylic acid metabolic process, Supplemental Information, Figure S4D).

Protein-Protein Interaction Networks Helps to Visualize the Differential Transcriptional State of *nuc1-2* Genotype in 1*g* Control References

Enrichment-PPI analysis (protein-protein interaction network) also showed that, in darkness, the mutant *nuc1-2* has up-regulated the expression of genes involved in the regulation of G2/M transition of the mitotic cell cycle, mitotic spindle assembly checkpoint, and auxin-activated signaling pathway, with respect to both the WT and the mutant *nuc2-2* (Figure 4). In the down-regulation side, *nuc1-2* mutant, when compared with WT, showed down-regulated genes involved in transcription regulator activity, sequence-specific DNA binding, defense response, and regulation of developmental process. In addition, the *nuc1-2* mutant has genes with repressed expression in darkness condition related to negative regulation of circadian rhythm, responses to light stimulus, and responses to jasmonic acid, when compared with WT and *nuc2-2* mutant (Figure 4). These results were in agreement with functional GO analysis (Supplemental Information, Figures S2 and S4).

DISCUSSION

Results from Spaceflight Experiments Can Have Several Limitations that Are Not Often Present in Other Biological -Omics Works

Major constraints of spaceflight experiments are the reduced amount of material and reproducibility, but other issues include the storage of samples before and after the experiment execution in space, causing staggered preparation and processing of the samples (Millar et al., 2010; Correll et al., 2013). In this

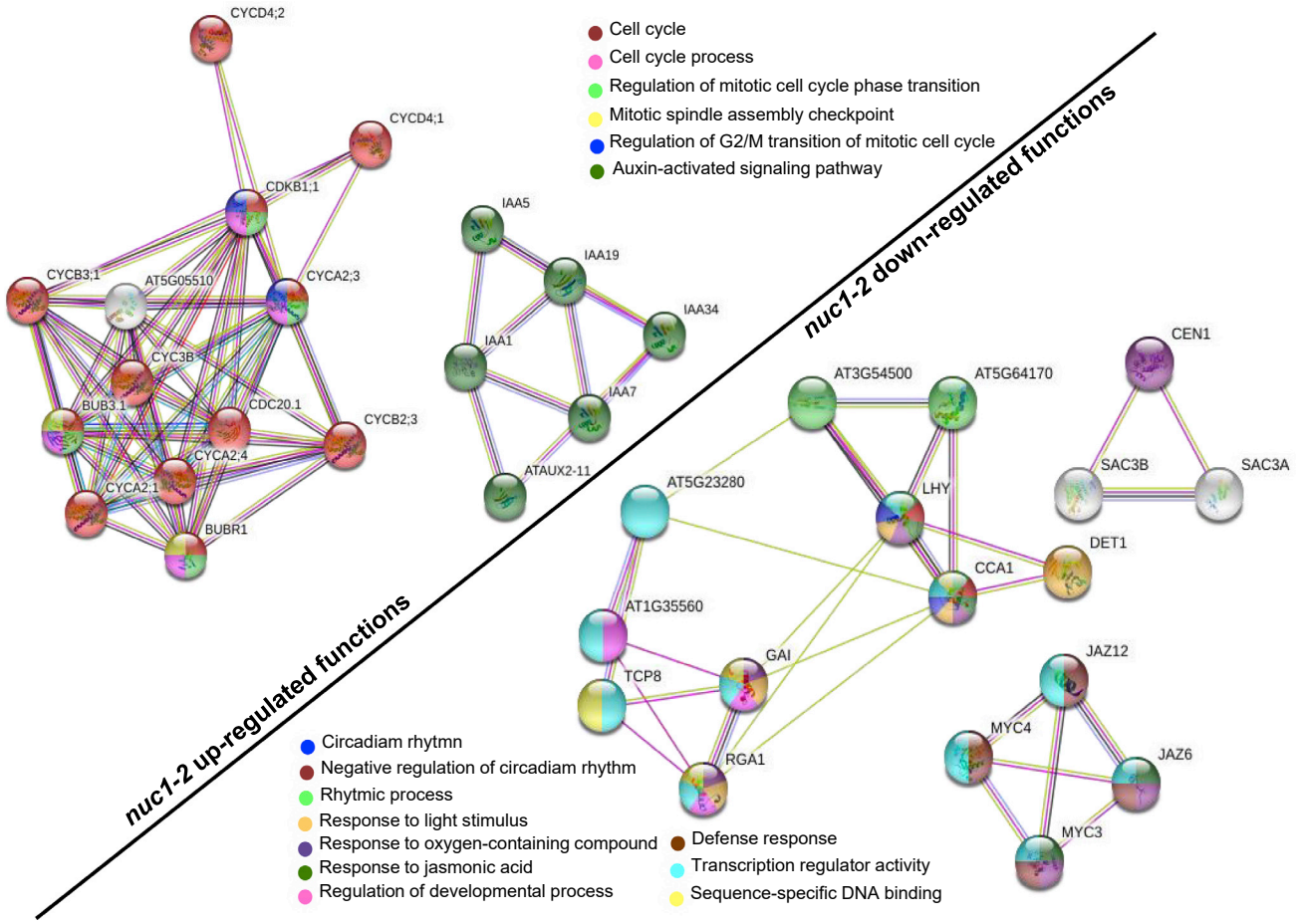

Figure 4. Protein-Protein Interaction Network of *Nuc1-2* Differential Response under Darkness Conditions

Enrichment network visualization for results from the over-represented gene lists in *Nuc1-2* versus the other genotypes. Gene in each node is represented by a chart indicating the functional category to which each one belongs (following the color legend). The networks show that processes such as cell cycle (left part or up-regulated genes), circadian rhythms, and stress responses (right part or down-regulated genes) are already affected in the 1×*g* control conditions as a reference for the Seedling Growth spaceflight experiments.

work, we have shown that, using the spaceflight hardware and procedures, we can perform a transcriptomic analysis with enough reliability to describe the differential transcriptional state of the nucleolin mutants under different light conditions. Three replicates could be considered low for current standards for animal -omics studies in Earth, but in our experiment each replica already represents the average of 10 plants. We show here that the replicates expected to be collected from the spaceflight experiment can be estimated to be sufficient for performing a sequencing study that allows clustering of the different genotypes and environmental conditions (Figure 1).

Due to logistical considerations, a specific constraint for plant space biology is the limited number of different mutants/genotypes that can be used in a spaceflight experiment in true microgravity. We have to be sure that the mutants of choice will provide valuable information. In that sense, the use of nucleolin mutants is a promising choice to provide insight into both the cell cycle and stress response mechanisms, apart from the ribosome biogenesis in which this protein is directly involved. These processes are known to be recurrently affected in space -omics experiments with plants (Choi et al., 2019; Ferl et al., 2015; Herranz et al., 2019; Johnson et al., 2017; Kruse et al., 2017; Paul et al., 2013, 2017). The analysis performed here allows us to know the specific functions assumed by the two nucleolin proteins of *A. thaliana* (NUC1 and NUC2) in different environmental conditions, such as red light photostimulation and darkness, affecting tropistic stimuli. This information can be considered as the 1*g* reference control required to obtain the best possible understanding of the changes produced by the spaceflight conditions on plant

development, and how the gravitational stress could be counteracted by changing phototropic stimuli. A longer-term goal is to use this knowledge for plant cultivation into bioregenerative life support systems.

The Transcriptomic Baseline for Nucleolin Mutants Is Different under Red Light Photostimulation and Darkness

Red light illumination mainly produces the activation of genes involved in photosynthesis, cell wall modification, drug catabolism, pathogenesis, and biotic stress response. Conversely, different abiotic stimuli responses (temperature, hypoxia, light, abscisic acid, oxidative stress) are down-regulated by the photostimulation treatment, as well as the circadian rhythm of plants. This transcriptional effect is similar in the three lines studied (Col-0 WT, *nuc1-2,* and *nuc2-2*), suggesting that it may be independent of the nucleolin protein functions. Therefore, the interpretation of transcriptional effects on plants' grown during spaceflight will be straightforward. In contrast, red light illumination produces a down-regulation of genes involved in the response to karritin, cold acclimation, and hormone metabolism in WT and *nuc1-2*, indicating that the NUC2 protein, and/or other proteins up-regulated in *nuc1-2* (Pontvianne et al., 2007), could participate in these functions. In addition, in the *nuc1-2* mutant, genes coding for response to gibberellin with the red light appear repressed. Gibberellin is a hormone involved in seed germination, bud and fruit formation, shoot longitudinal growth, and axial organ elongation (Hedden and Sponsel, 2015), which could support the relationship of defective plant growth and development with *NUC1* gene disruption.

Exposure to the dark during the last 2 days of cultivation of seedlings promotes gene categories mainly involved in processes of cell division and in the response to different stimuli (red light, UV, ionizing radiation, gravity, auxins). They are common in *nuc1-2* versus WT and *nuc1-2* versus *nuc2-2* comparisons and unique in *nuc1-2* versus WT comparison. These observations could indicate that, in a stress condition such as darkness, the NUC2 protein is capable of rescuing functions that the NUC1 protein performs under normal growth conditions. Indeed, a role of nucleolin in mitosis has been reported (de Carcer and Medina, 1999; Ma et al., 2007). Therefore, in the *nuc1* mutant, the NUC2 protein would be capable of performing functions necessary for plant survival, such as ribosome biogenesis regulation (Pontvianne et al., 2007), cell proliferation, and DNA repair. It is important to note that cell division GO was only significantly up represented in the WT when red light was provided (Figure 2B). In contrast, the gene categories down-regulated in darkness are mainly related to developmental processes, mRNA quality, response to temperature, and immune system. This may indicate that in the dark stress condition, the NUC2 protein is not capable of supplying the function of the NUC1 protein in these processes. These results, together with those previously described, indicate that the functional rescue of NUC1 by NUC2, when NUC1 is not expressed, would be only partial, which may be due to the structural difference between both proteins, including longer N-terminal acidic domain and less-conserved GAR domain I in the C terminal (Durut and Sáez-Vásquez, 2015).

Differential Role of Nucleolin Mutants' Transcriptomic Baseline May Offer New Insight in Spaceflight Experiments

Differential transcriptional response is observed when comparing the two nucleolin mutants. The categories of genes activated differentially in both mutants (*nuc1-2* versus *nuc2-2* comparison) are mainly involved in the cell wall and membrane systems. This finding is potentially interesting in space -omics to discriminate gravitropism from graviresistance mechanisms (Herranz and Medina, 2014) and could be related to the phenotype described in the *nuc1-2* mutant, in which a reduction in the cell number and a disorganization in every cell layer is observed in transversal sections of primary leaves (Pontvianne et al., 2007). Moreover, the gene categories repressed only in the above-mentioned comparison are involved in the spliceosome, protein demethylation, chromatin organization, mRNA binding, and histone acetyltransferase activity, which could be related to the described antagonist activity of these two proteins in pre-rRNA methylation and with their role in chromatin remodeling, RNA Pol I transcription, mRNA stability, and RNA/DNA metabolism (Pontvianne et al., 2010; Durut et al., 2014).

In the *nuc1-2* versus *nuc2-2* comparison under red light photostimulation, the ribosome biogenesis GO shows the most significant enrichment within the up-regulated genes (Supplemental Information, Figure S4B). This result indicates that the described activating effect of red light in this process depends more on the expression of NUC2 protein than on the NUC1 expression. Ultimately, the two nucleolin mutant lines, compared with WT, have genes up-regulated by red light involved in different response processes, such as the response to hypoxia, drug, antibiotic, salicylic acid, and wounding. These response

processes are extended in the *nuc2-2* versus WT comparison, including response to cold, bacteria, and abscisic acid. These results indicate that even though both nucleolar proteins, NUC1 and NUC2, are involved in response processes, the implication of NUC2 is higher as to the number of processes. Therefore, NUC2 has a greater response capacity to environmental signals, such as illumination with red light, thus being more sensitive.

A major challenge to be overcome in spaceflight -omics research is to differentiate and characterize which of the spaceflight environmental conditions (including not only microgravity and cosmic radiation but also the constraints imposed to the upload/download and storage processes in orbit, the gaseous atmosphere, among other factors) are responsible for each of the observed alterations in the transcriptome. According to our results, space -omics researchers need to pay special attention to the analysis of ground reference controls on Earth before conclusions can be made on the results obtained in spaceflight. In our particular example, the two nucleolin mutants are the perfect complement to the WT genotype to study how the NUC2 protein can replace the essential functions of NUC1 in orbit, and how light can modulate it, even rescuing WT transcriptional profiles in the *nuc1-2* mutant. The fact that *nuc2-2* mutant shows an increase in stress response GO when exposed to red light connects the red light stimulus with the modulation of the stress response. In parallel, the cell cycle and ribosome biogenesis functions required to be restored in microgravity are also light dependent in both the *nuc1-2* mutant (in Earth, as shown here) and the WT plants in orbit (Valbuena et al., 2018).

Limitations of the Study

Due to logistical constraints, this study lacks a continuously illuminated control set of samples performed simultaneously due to the absence of those samples in the spaceflight experiment as well as the reference experiment. The next step in this research is to analyze the samples from the space experiment in microgravity and the partial gravity conditions produced by the centrifuge in the EMCS on the ISS. We anticipate confirming that the duplicated nucleolin gene system works during spaceflight will lead us to discover novel mechanisms for plant adaptation to spaceflight conditions. Thus, this research will be eventually translated into better crops for life support systems in the human spaceflight ventures of the 21st century.

Resource Availability

Lead Contact

Further information and requests for materials should be directed to and will be fulfilled by the lead contact, Raúl Herranz (r.herranz@csic.es)

Materials Availability

Materials generated in this study are available from the lead contact with a completed materials transfer agreement.

Data and Code Availability

The GLDS-313 datasets generated during this study have been deposited and it is available at GENELAB repository (Ray et al., 2019). Original data have been deposited to (GENELAB: https://doi.org/10.26030/0g0m-dj21, https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-313)

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101686.

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AUTHOR CONTRIBUTIONS

Conceptualization, R.H. and F.J.M.; Methodology and Investigation, A.V., A.M., and R.H.; Writing – Original Draft, A.M. and R.H.; Writing – Review & Editing, A.V., F.J.M., J.Z.K., and J.S.-V.; Supervision and Funding Acquisition, F.J.M., J.Z.K., and J.S.-V.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

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

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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 (1*gg*r) 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, O_2, CO_2 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 ^{0}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 µmol/m^{2}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 μmol/m^2s, 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 ^{0}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 1*g* 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±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, In- terPro, 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).

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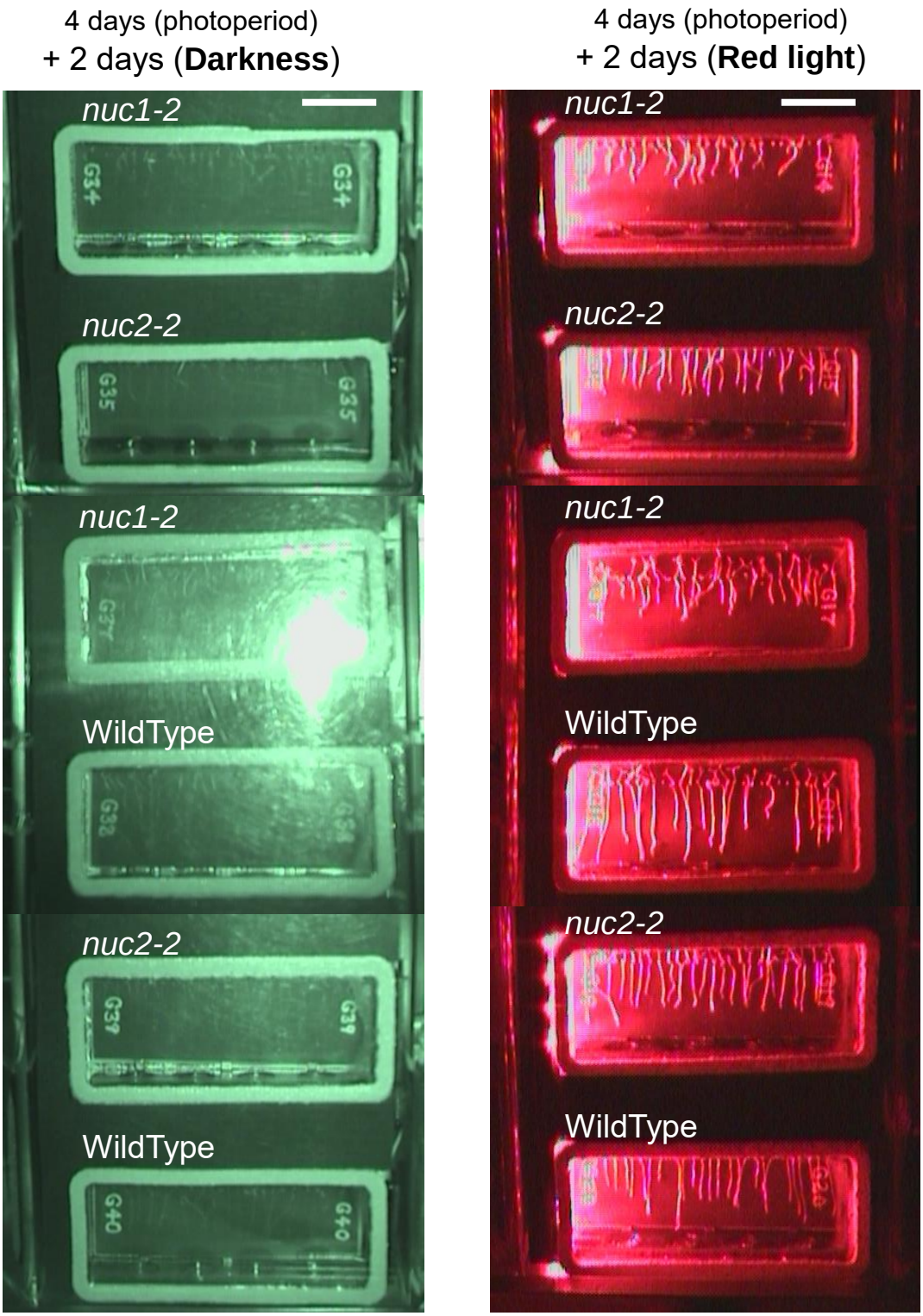
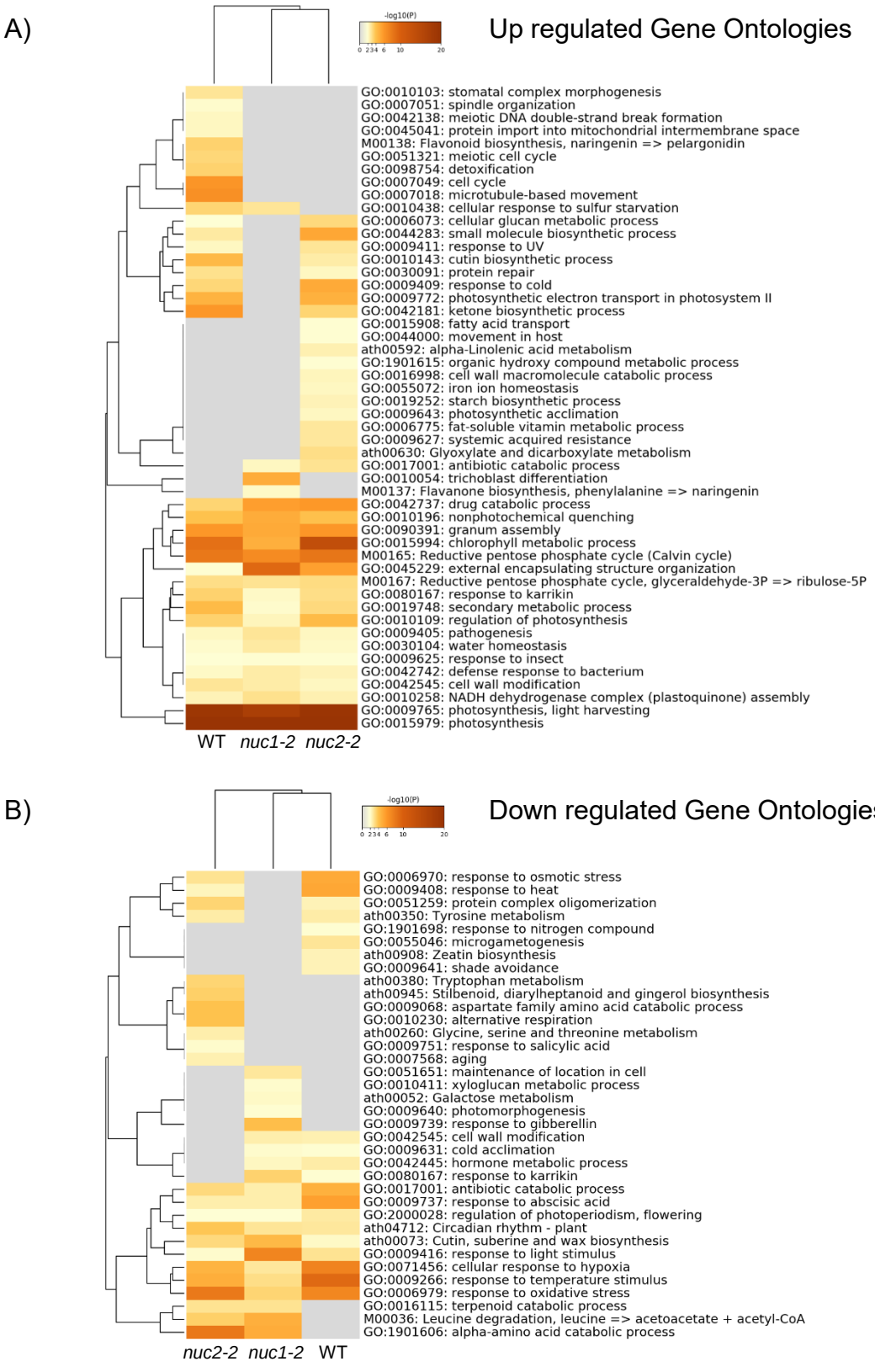
Figure S1. Photos from the twelve CC from the Seedling Growth Ground Reference Test used in this study (related to Figure 1)



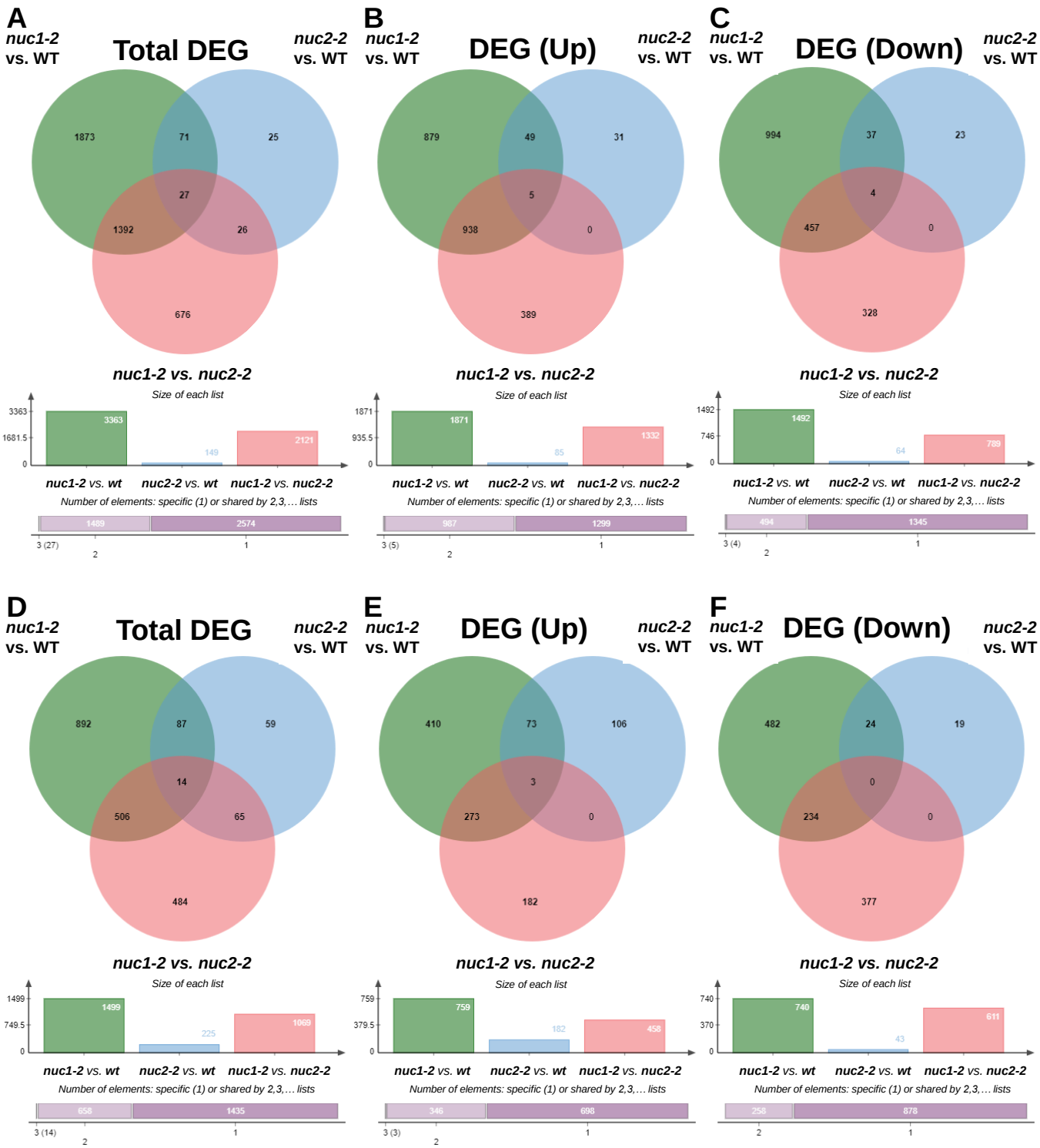
Images of the seedlings (WildType, *nuc1-2* and *nuc2-2*) were taken on the last day of cultivation during the experimental run under darkness (infrared videocapture provide not enough contrast to see all seedlings) or red light photostimulation. See Figure 1 for the final growth of the seedlings just 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)



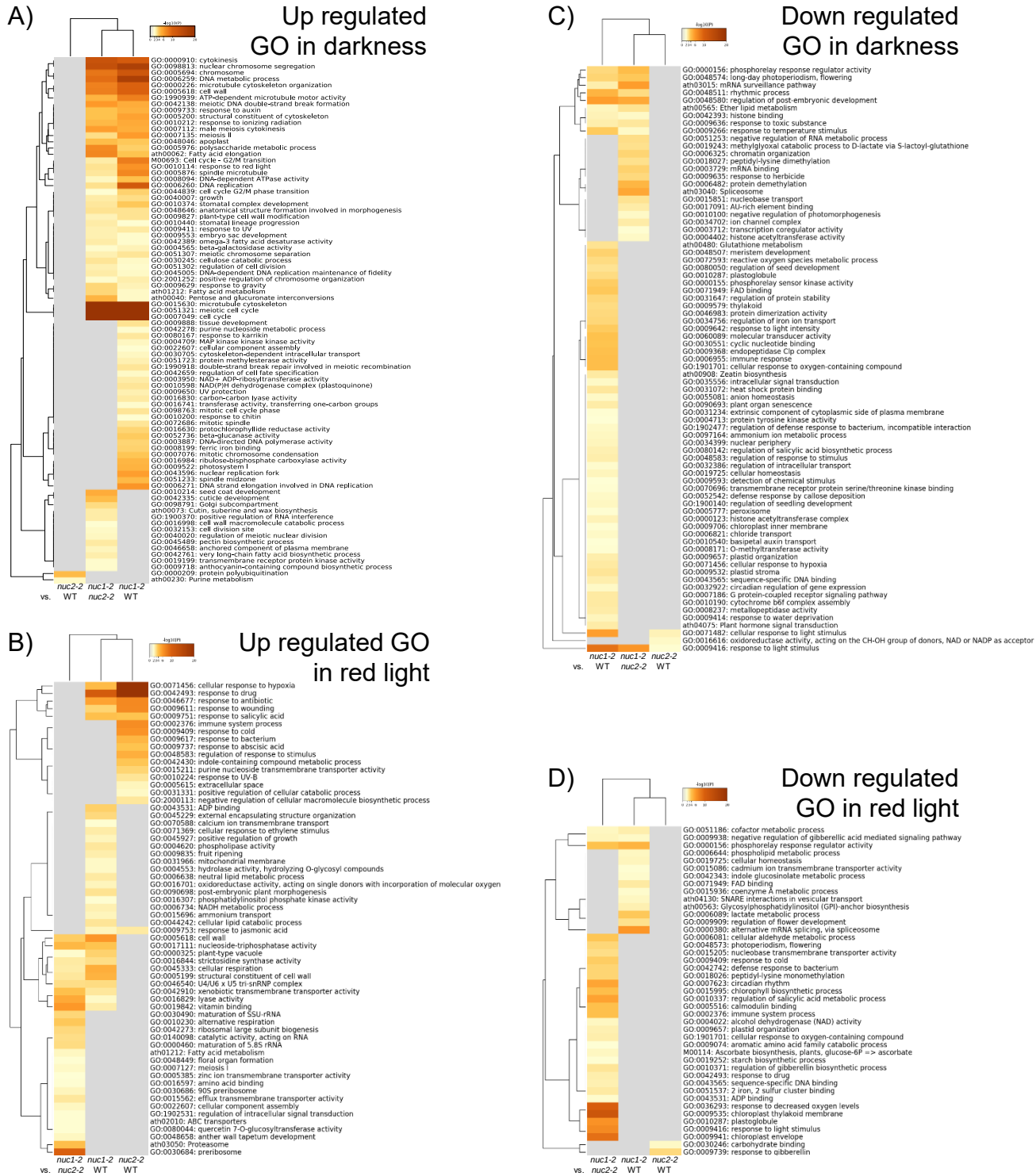
One row per cluster is shown, using a discrete color scale to represent statistical significance as -log10(p value). Gray color indicates a lack of significance.

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.

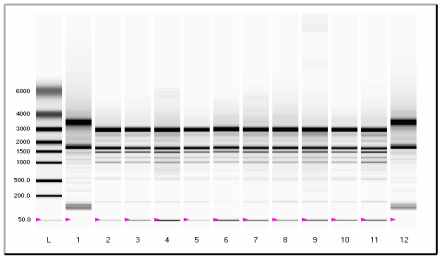
Figure S4. Heatmap showing the top Gene Ontologies enrichment clusters for DEG in the three genotype pair-comparisons among WT, *nuc1-2* and *nuc2-2* (related to Figure 4)

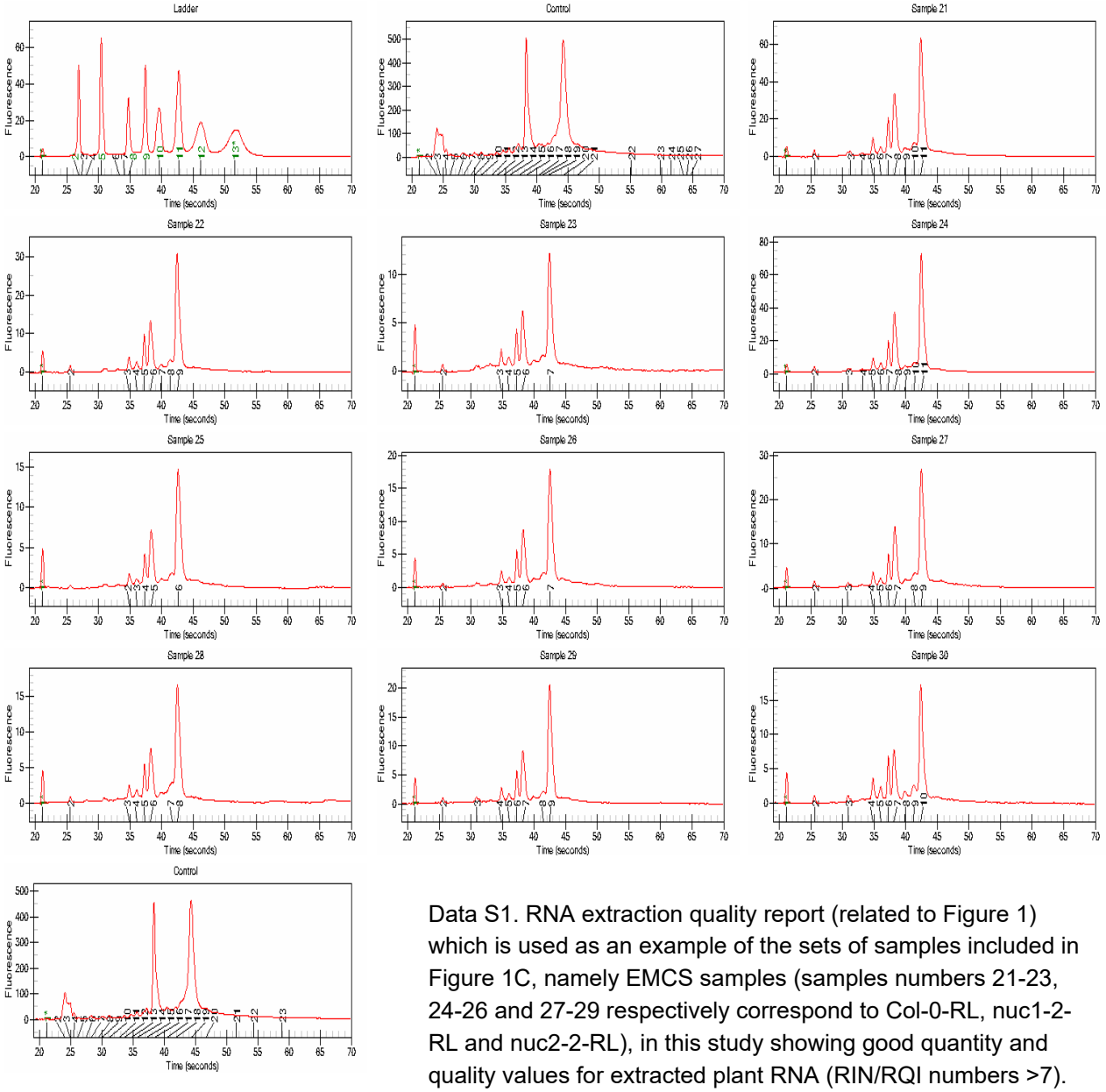


A) Up regulated in plants exposed to darkness the last two days. B) Up regulated in plants exposed to red light the last two days. C) Down regulated in plants exposed to darkness the last two days. D) Down regulated in plants exposed to red light the last two days. One row per cluster is shown, using a discrete color scale to represent statistical significance as -log10(p value). Gray color indicates a lack of significance.

Data S1. RNA extraction quality report (related to Figure 1).

Project:	Javier Medina	**Acq. Analyst:**	DefaultUser
Assay:	Eukaryote Total RNA StdSens	**Acq. Time:**	12/21/2018 8:51:32 AM
Run:	Experion RNA SS 21-12-18(3)	**Signature:**	N/A
Run Version:	N/A		

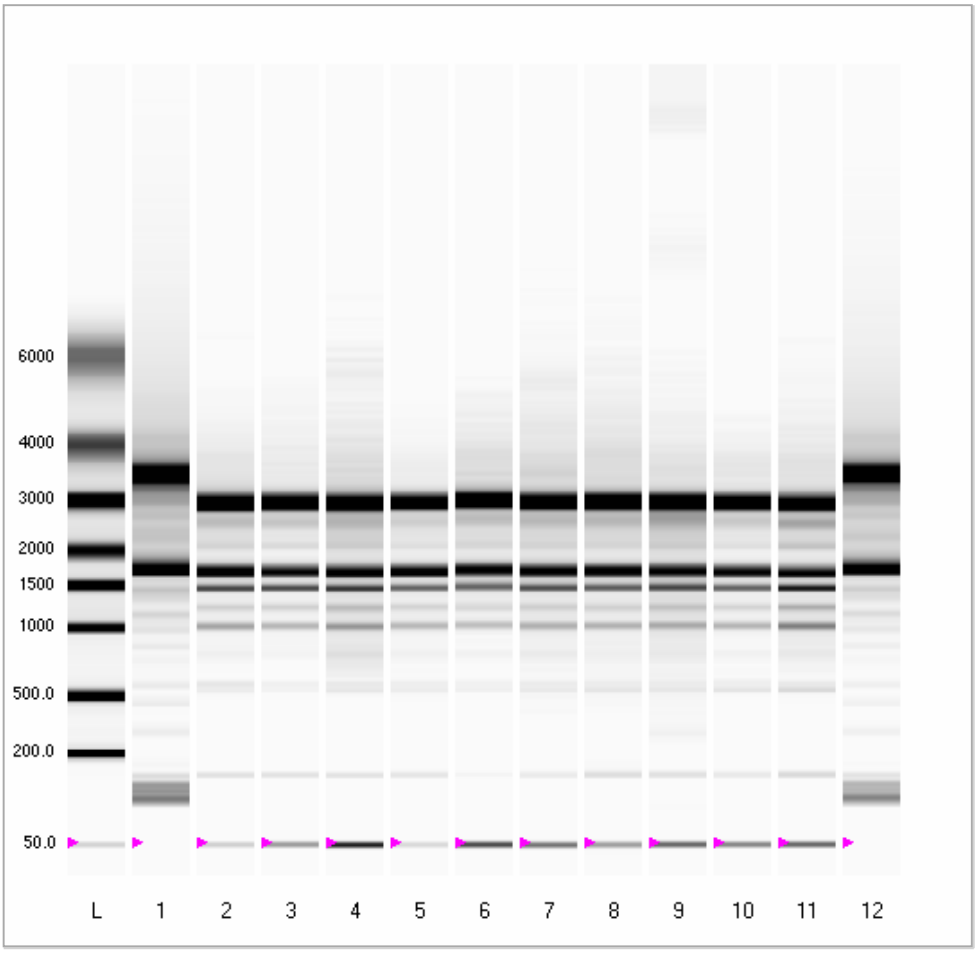




Data S1. RNA extraction quality report (related to Figure 1) which is used as an example of the sets of samples included in Figure 1C, namely EMCS samples (samples numbers 21-23, 24-26 and 27-29 respectively correspond to Col-0-RL, nuc1-2-RL and nuc2-2-RL), in this study showing good quantity and quality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

Virtual Gel Report

Project:	Javier Medina	**Acq. Analyst:**	DefaultUser
Assay:	Eukaryote Total RNA StdSens	**Acq. Time:**	12/21/2018 8:51:32 AM
Run:	Experion RNA SS 21-12-18(3)	**Signature:**	N/A
Run Version:	N/A		

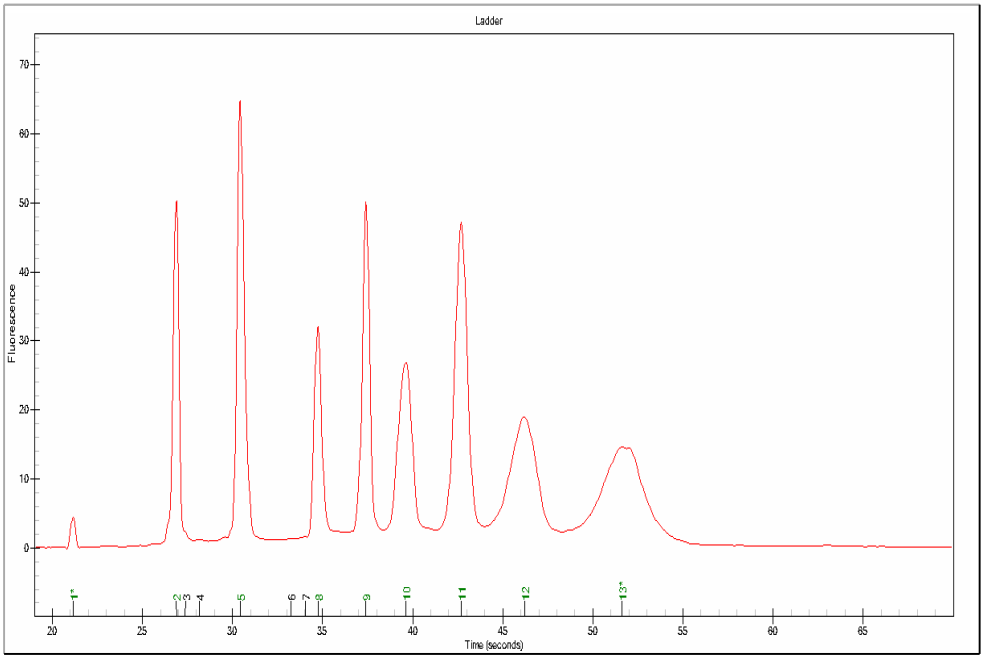


Egram, Gel Lane and Result Table Report

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
L	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
L	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
L	8	34.75	53.23		0.47	18.50
L	9	37.40	74.05		0.45	27.69
L	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

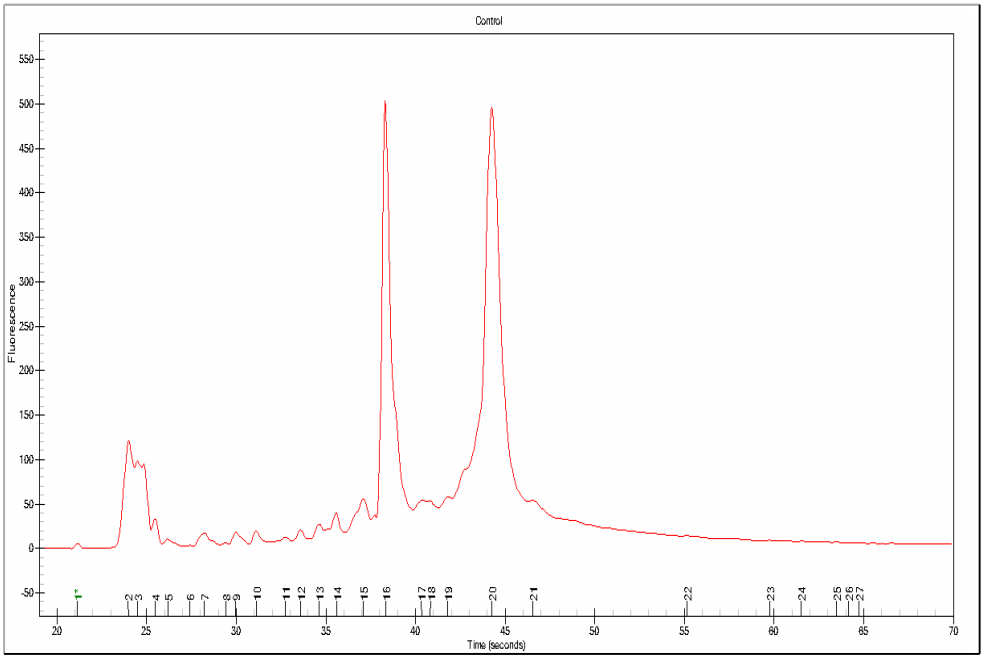
Well# Ladder

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

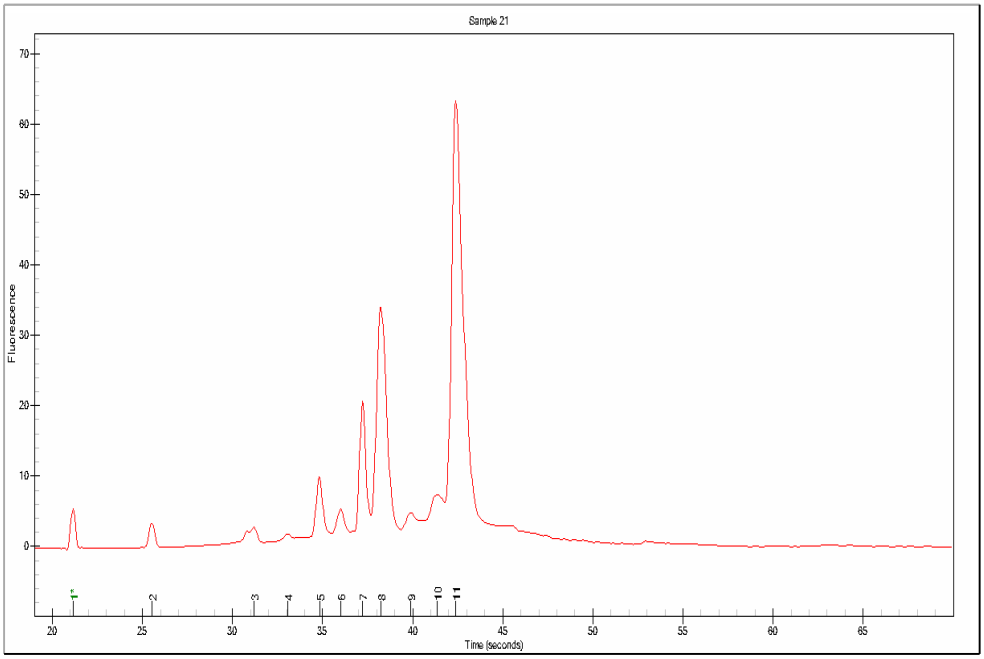
Well# 1 Control

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

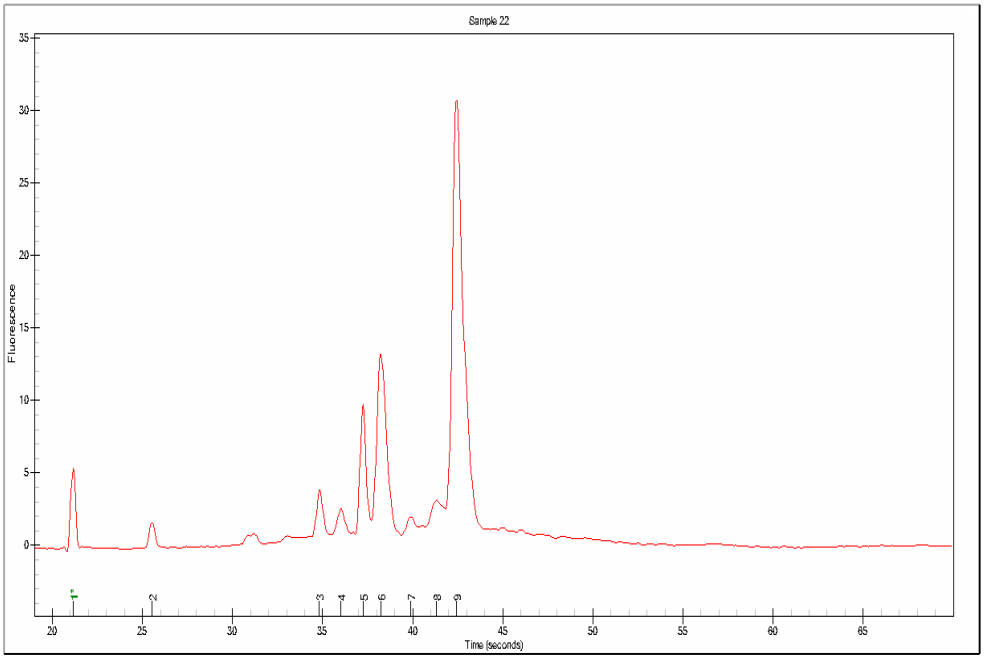
Well# 2 Sample 21

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

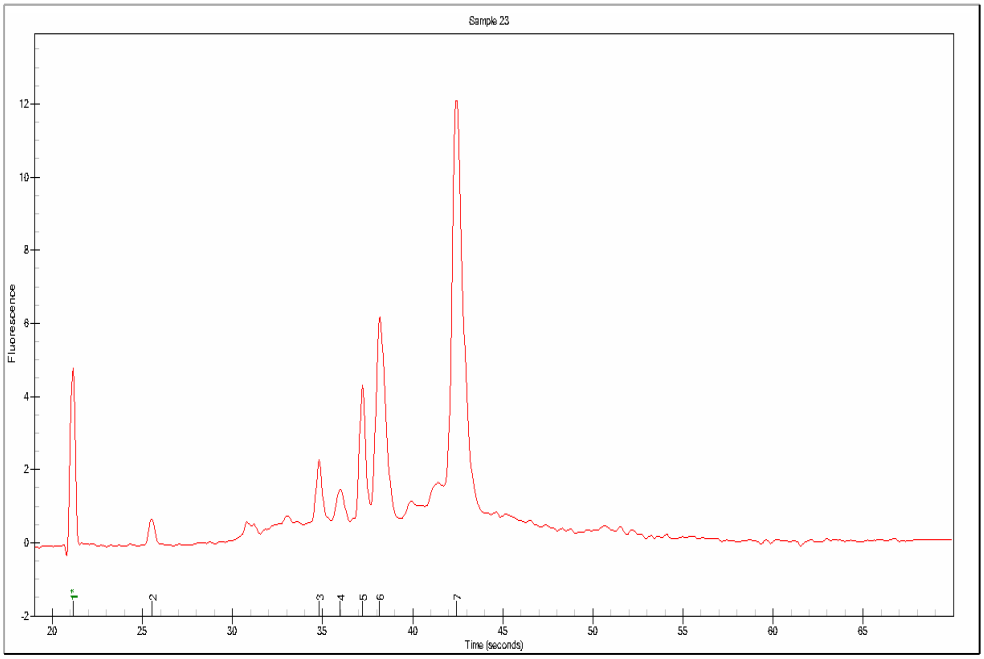
Well# 3 Sample 22

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

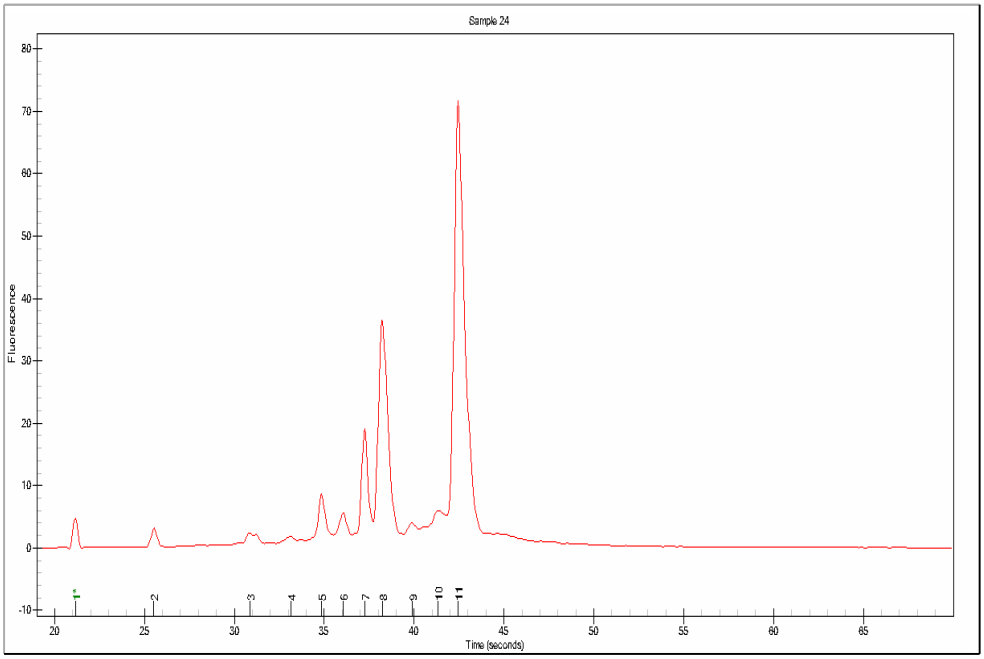
Well# 4 Sample 23

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

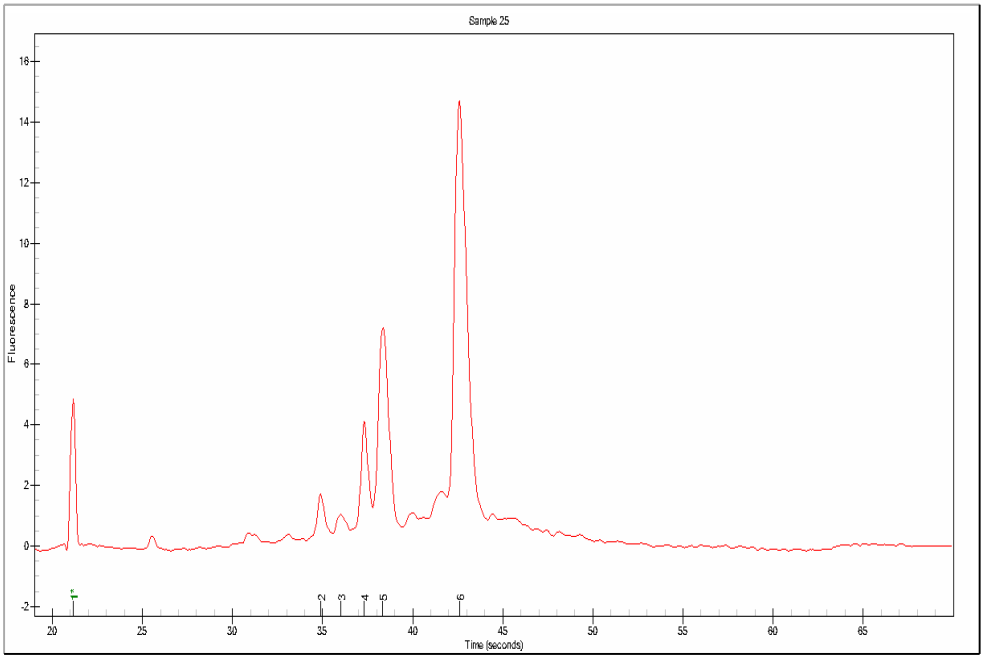
Well# 5 Sample 24

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

Egram, Gel Lane and Result Table Report

Project:	Javier Medina	**Acq. Analyst:**	DefaultUser
Assay:	Eukaryote Total RNA StdSens	**Acq. Time:**	12/21/2018 8:51:32 AM
Run:	Experion RNA SS 21-12-18(3)	**Signature:**	N/A
Run Version:	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

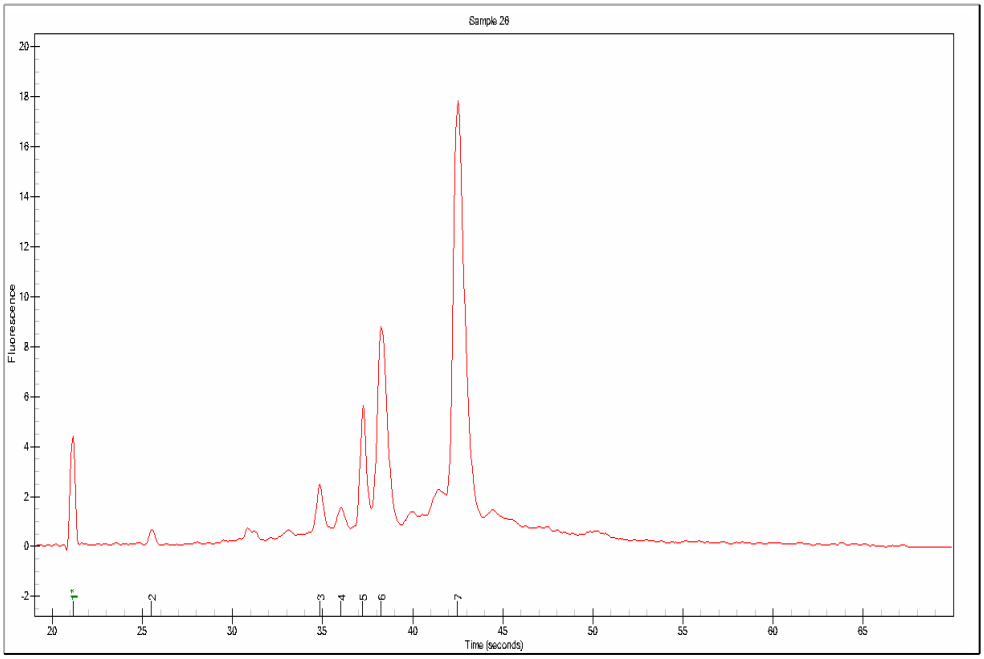
Well# 6 Sample 25

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

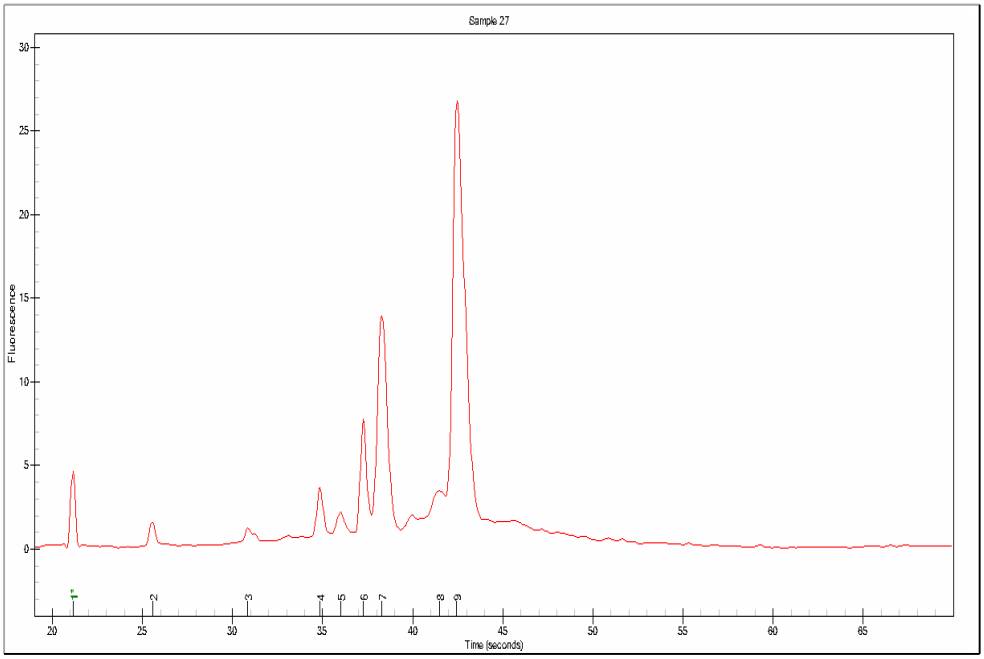
RQI: 9.3

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

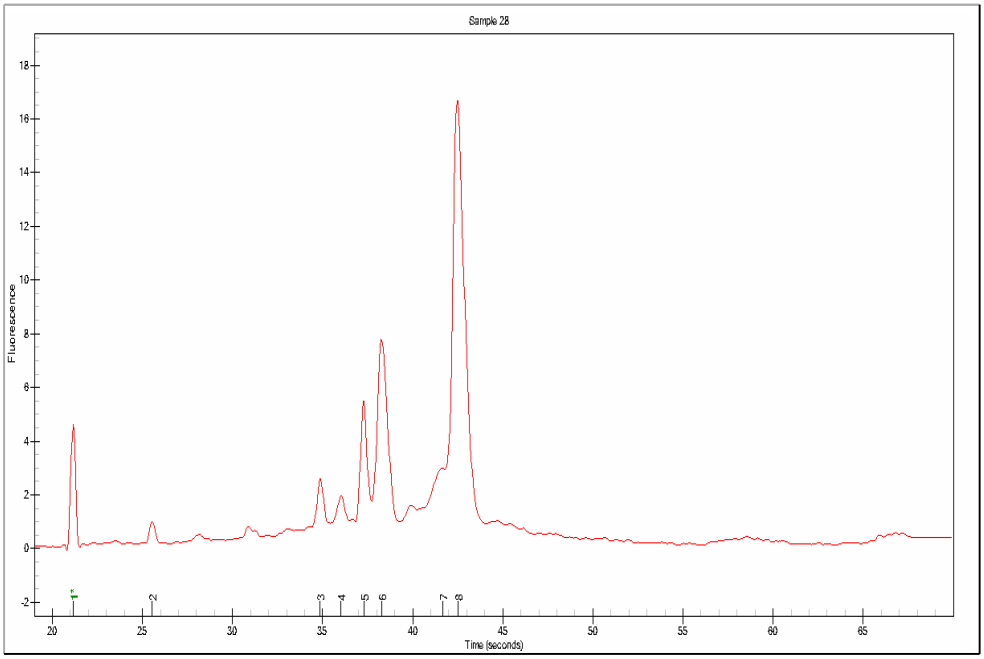
Well# 8 Sample 27

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	**Acq. Analyst:**	DefaultUser
Assay:	Eukaryote Total RNA StdSens	**Acq. Time:**	12/21/2018 8:51:32 AM
Run:	Experion RNA SS 21-12-18(3)	**Signature:**	N/A
Run Version:	N/A		

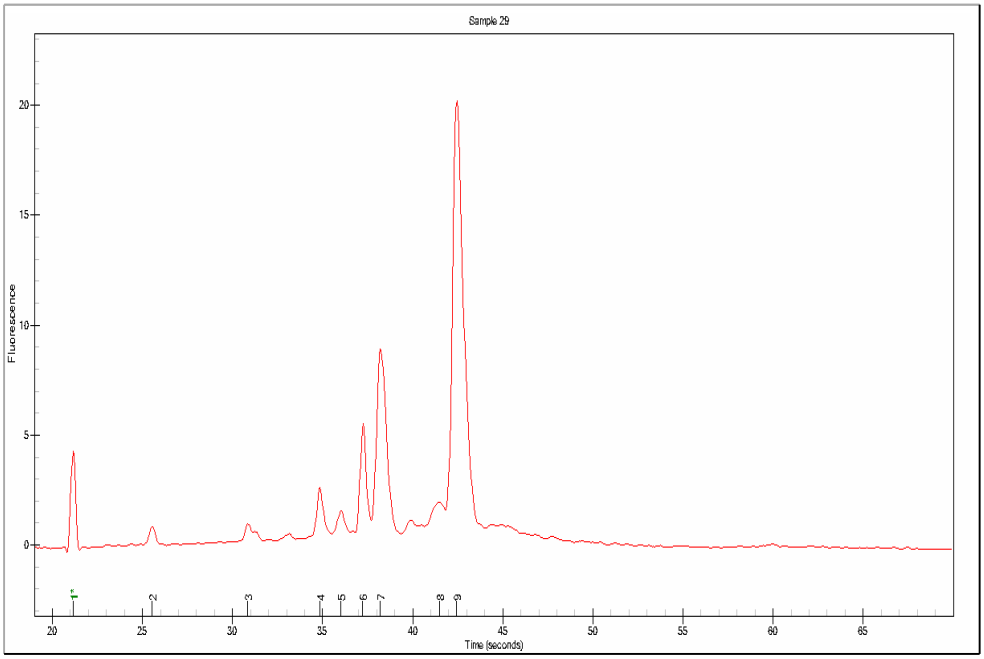
Well# 9 Sample 28

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	**Acq. Analyst:**	DefaultUser
Assay:	Eukaryote Total RNA StdSens	**Acq. Time:**	12/21/2018 8:51:32 AM
Run:	Experion RNA SS 21-12-18(3)	**Signature:**	N/A
Run Version:	N/A		

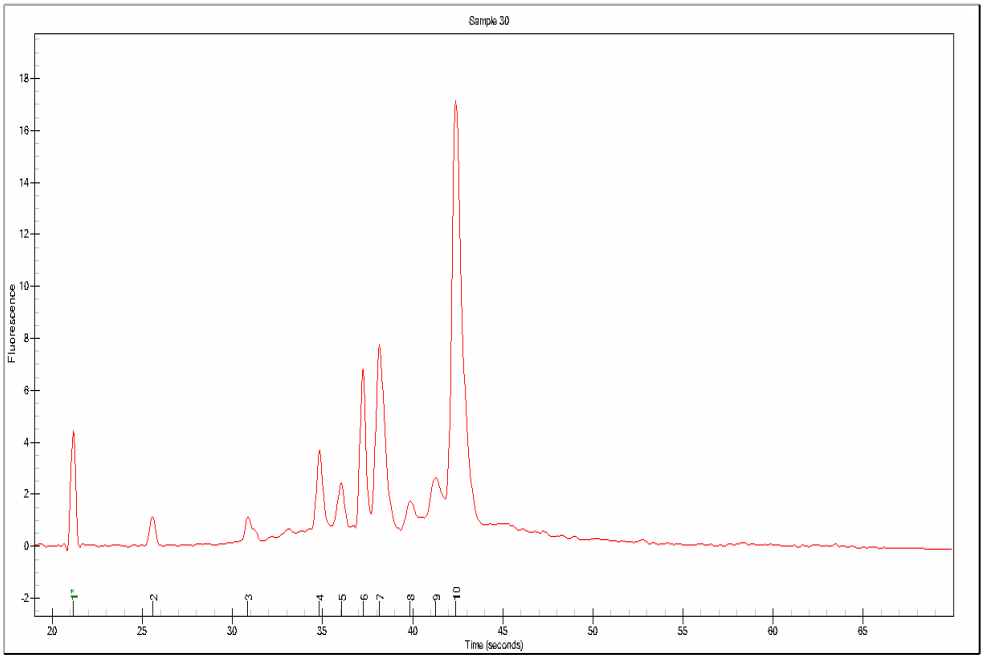
Well# 10 Sample 29

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

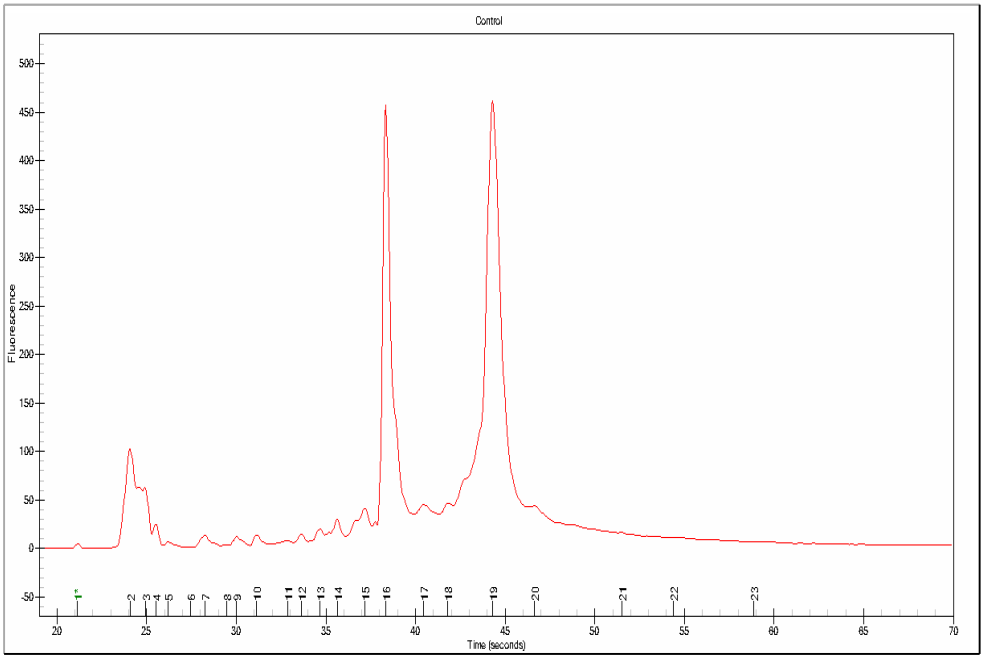
Well# 11 Sample 30

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