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Earlham Institute
Decoding Living Systems

GUIDELINES FOR SUBMISSION OF DNA AND RNA FOR THE EARLHAM INSTITUTE (EI)

DNA and RNA quality and quantity are the two most critical factors in the success of sequencing projects. If you cannot meet EI's specification for DNA and RNA or have questions and concerns, please contact EI's project managers at projects@earlham.ac.uk.

In this form, the following areas will be covered:

- Quality and quantity assessment for DNA, RNA and libraries
- Sample quantity and concentration for the various NGS platforms
- Sample delivery information
- A list of kits recommended for DNA and RNA isolation
- How to assess DNA/RNA quality if using NanoDrop

Quality and Quantity Assessment

DNA

EI recommends that DNA is quantified by fluorometry using either a Qubit fluorometer (Invitrogen) with QUANT-iT dsDNA assay (Broad Range or High Sensitivity) or the Pico Green assay (Invitrogen). A NanoDrop reading is also required to determine if there are any contaminants present in the sample.

In addition, DNA can also be run on a 0.8-1% agarose gel to assess quantity and quality.

DNA samples should be free of RNA so an RNase treatment is highly recommended (treat the samples with RNase A at the beginning of your prep during the pellet re-suspension (20 ul of a 20 mg/ml solution)).

For large insert libraries, DNA should be of high molecular weight (ideally >40 kb). EI will use the Agilent Tapestation Genomic assay to determine the sample size range.

Note: Please see Appendix 1 for a list of kits that can be used for DNA and Appendix 2 provide guidelines on how to interpret NanoDrop results.

RNA

RNA should be supplied pure and free of DNA (DNase treated) and chemical contaminants (especially organic solvents).

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RNA quality can be assessed by either running it on a 0.8-1% agarose gel or preferably on a Bioanalyzer RNA chip (See examples in the figure below). The RIN number (RNA integrity number) should be of 8 or higher for mammalian samples, and 7 or above for non-mammalian and plant samples. Although we advise discussing any more unusual sample types (shown on the Bioanalyzer result page).

RNA quantity should be estimated using fluorometry, preferably using Qubit fluorometer with QUANT-iT RNA assay (Invitrogen). If this method is not available, please note that spectrophotometry methods can provide inaccurate measurements due to contaminating chemicals.

Note: Please see Appendix 1 for a list of kits that can be used for RNA isolation.

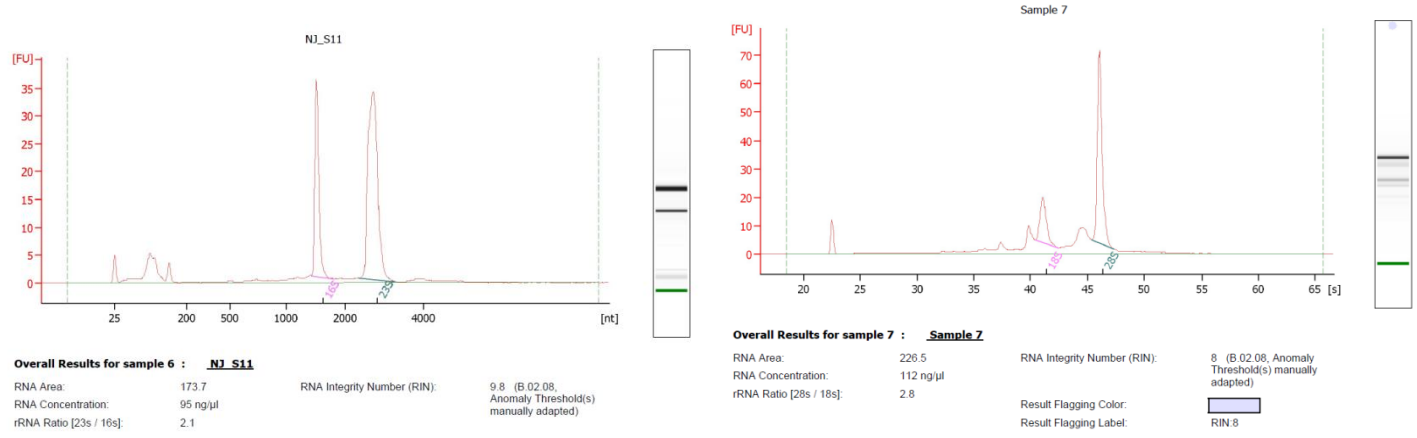

Figure: The left figure shows the profile of a good quality RNA sample from bacteria and the right figure shows good quality RNA from a plant.

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Sample Quantity and Concentration

The quantity of DNA or RNA required for sequence library preparation is dependent on the application and the sequencing platform. Please note that the quantities listed in the table below are the recommended values as of June 2016. These may change with new protocols and advancements. If due to the nature of your sample or custom library preparation it is difficult to provide EI with the requested quantity/quality in this table, please get in touch with projects@earlham.ac.uk **before** starting your extractions or library construction to discuss options.

Table1: This table shows the quantity of DNA and RNA required per application and per platform.

ILLUMINA	
TruSeq RNA	Min 3-5 µg of total RNA (min 50 ng/µl in 60 µl) or 50-100 ng of ribo-depleted mRNA in 15 µl. RIN values should be of 8 or above for mammalian samples, and 7 or above for non-mammalian and plant samples.
Small RNA	Please contact projects@earlham.ac.uk for further information.
Genomic DNA	Min 2-5 µg of gDNA (min 35 ng/µl in 60 µl). Absorbance ratios of 260/280 1.6 – 2.0 nm, 260/230 1.8 – 2.4 nm.
ChIP seq	Min 10 ng of ChIP DNA.
Nextera	Min 50 ng of gDNA (min 2.5 ng/µl in 20 µl).
Nextera XT	Min 1 ng of gDNA (min 0.2 ng/µl in 5 µl).
Pre-made libraries	Min 10 nM in min 25 µl per pool of libraries. Any QC data such as Bioanalyzer traces or qPCR to be supplied if possible.
Mate Pair	Min of 20 µg of DNA of greater than 40kb in size.
PACBIO	
Large Insert Library (10-20kb)	Min 20 µg of gDNA (min 20 ng/µl) at a minimum molecular weight of 40kb. Absorbance ratios of 260/280 1.8 – 2.0 nm, 260/230 ~2 nm. Pulse field (or equivalent) images to be supplied if possible. Please contact projects@earlham.ac.uk to discuss further.
Amplicon DNA	Min 500 ng of amplicons (13 ng/ µl) of 2-6kb in size.

Other applications are currently being implemented and the table will be amended on an *ad hoc* basis. For enquiries about new applications please discuss your enquiries with EI's project managers.

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Sample delivery information

It is important that samples are sent using the following recommendations in order to avoid your samples becoming degraded or mislaid.

Delivery address:

Please send your samples to:

Sample Reception
Genomics Pipelines Group
Earlham Institute
Norwich Research Park
Norwich
NR4 7UG
UK
+44 1603 452900

How to send:

DNA can be sent in liquid form in low TE (10mM TRIS and 0.1mM EDTA, pH8), or deionised water and sent either on dry ice or packaged with ice or cool blocks. Please indicate on the delivery form what your sample has been re-suspended in. Please do not send the sample lyophilised.

If HMW DNA is being sent to EI and this is re-suspended in 1x TE buffer then it should remain stable if sent on ice blocks at around 4°C. In our experience if HMW gDNA is freeze thawed too many times then this can contribute towards fragmentation which is likely to lead to issues when attempting library construction/sequencing. EI recommends that HMW DNA is not sent on dry ice.

RNA should be sent on dry ice, ensuring there is enough dry ice to keep the samples frozen until delivery. Within the sample package the individual sample tubes should be contained within a plastic bag that will not degrade when subjected to dry ice temperatures/conditions (i.e. -80°C).

EI has had successful deliveries from courier companies such as DHL and FedEx but others may be used. **Please note, the Royal Mail prohibits dry-ice packages and must not be used for shipping samples.**

Ensure that your samples will arrive on a week day (before 3pm) and not at the weekend.

Sample labelling:

A sample information form will be sent to you when your project has been accepted. This form needs to be filled in and returned to EI project management.

Following the above, a set of barcode stickers will also be sent to you. These need to be placed on your sample tubes prior to being sent. The stickers are suitable for pre-frozen tubes. **Samples without barcodes will not be processed.**

In the case of 8 or more samples being sent, a barcoded plate will be sent out to you. Your samples need to be placed in the wells corresponding to those indicated on your sample information form.

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Appendix 1: List of kits that can be used for DNA and RNA isolation

DNA

- DNA Isolation Kits, E.Z.N.A.® and E-Z 96® - VWR

DNA Isolation Kits allow for the isolation of genomic DNA from a variety of sample types, including blood, soil, stool, bacteria, yeast, insect, cultured cells, mouse tail, and fungal. Most kits are available in mini, midi, maxi, and 96-well formats. Isolated DNA can be used for restriction digest, PCR, southern blotting, and sequencing. Mag-Bind® Genomic DNA Kits can be adapted to most robotic liquid handling platforms.
https://uk.vwr.com/store/product/10925132/e-z-n-a-and-e-z-96-forensic-dna-kits

- PureLink™ Genomic DNA – Invitrogen

https://www.thermofisher.com/uk/en/home/life-science/dna-rna-purification-analysis/dna-extraction/genomic-dna-extraction/purelink-genomic-dna.html#

- PureLink™ Viral RNA/DNA Mini Kit

The PureLink™ Viral RNA/DNA Mini Kit is a nucleic acid purification system designed for fast and easy isolation of viral RNA or DNA from cell-free samples, such as serum, plasma, and cerebrospinal fluid
http://products.invitrogen.com/ivgn/product/12280050

- ChargeSwitch® Nucleic Acid Purification Technology - Invitrogen

ChargeSwitch® nucleic acid purification technology is the simplest, cleanest, and most effective means of purifying both DNA and RNA and can be configured in a range of product formats including simple manual purification methods as well as high throughput automated applications.
http://www.invitrogen.com/site/us/en/home/brands/Product-Brand/ChargeSwitch.html

- QIAGEN - Animal, Plant, Microorganism, and Other Samples

https://www.qiagen.com/gb/shop/product-finder/dna-preparation/

Plant Nuclear DNA preparation

Please contact projects@earlham.ac.uk for a copy of the protocol EI recommends for the isolation of plant nuclear DNA.

RNA

- RNA Isolation Kits, E.Z.N.A.® and E-Z 96® - VWR

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Kits for the isolation of total RNA or mRNA using manual or automated purification systems. Isolated RNA can be used for Northern blotting, RT-PCR, nuclease protection assays, *in-vitro* translation, and microarray analysis. Miniprep kits that use HiBind® spin columns use V-spin columns which feature an attached cap and a standard luer tip at the bottom for easy, leak-free connection to a vacuum manifold or luer hub needle. Midi and maxi columns feature a standard vacuum luer for faster processing. These columns can be used for both centrifugation and vacuum protocols.
https://uk.vwr.com/app/catalog/Product?article_number=M6731-01

- PureLink™ RNA Mini Kit - Invitrogen

The PureLink™ RNA Mini Kit provides rapid purification of total RNA from a wide range of cell and tissue types, including animal, plant, and yeast cells, bacteria, and blood.
http://products.invitrogen.com/ivgn/product/12183018A

- PureLink™ miRNA Isolation Kit - Invitrogen

The PureLink™ miRNA Isolation Kit is uniquely designed to purify cellular small (< 200 nt) RNA molecules, including tRNA, 5S rRNA, and 5.8S rRNA, and regulatory RNA molecules such as microRNA (miRNA) and short interfering RNA (siRNA).
http://products.invitrogen.com/ivgn/product/K157001

- QIAGEN - Total RNA Purification

http://www.qiagen.com/Products/Catalog/Sample-Technologies/RNA-Sample-Technologies/Total-RNA/

- Cambio – PowerPlant RNA Isolation kit

For fast and easy isolation of inhibitor-free total RNA from even the toughest plant and seed samples, including those high in polyphenols and polysaccharides.

http://www.cambio.co.uk/1383/6/products/powerplant-rna-isolation-kit/

Appendix 2: Assessing DNA/RNA quality using the NanoDrop

As absorbance measurements will measure any molecules absorbing at a specific wavelength, nucleic acid samples will require purification prior to measurement to ensure accurate results. Nucleotides, RNA, ssDNA, and dsDNA all will absorb at 260 nm and contribute to the total absorbance.

260/280
The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

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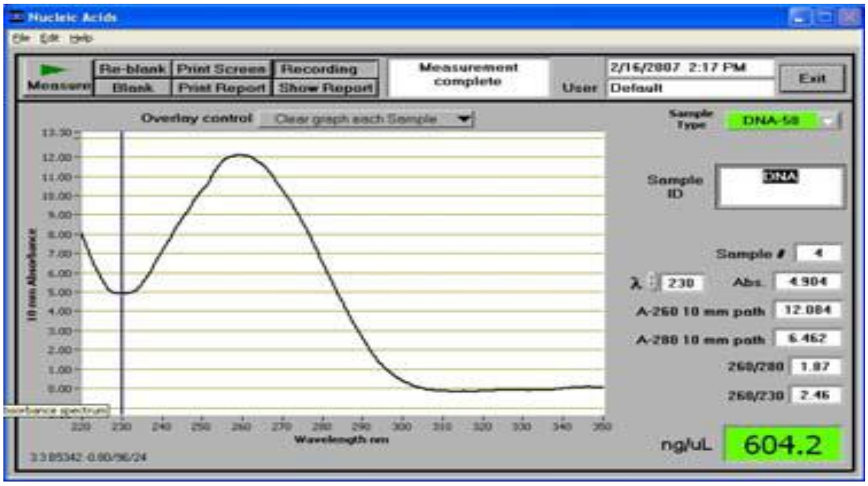
260/230
This ratio is used as a secondary measure of nucleic acid purity. The 260/230 values for "pure" nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2. If the ratio is appreciably lower than expected, it may indicate the presence of contaminants which absorb at 230 nm.

Change in sample acidity
Small changes in the pH of the solution will cause the 260/280 to vary. Acidic solutions will under-represent the 260/280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3.

EXAMPLES OF NANODROP PROFILES

A typical trace file of nucleic acid



EDTA, carbohydrates and phenol all have absorbance near 230 nm

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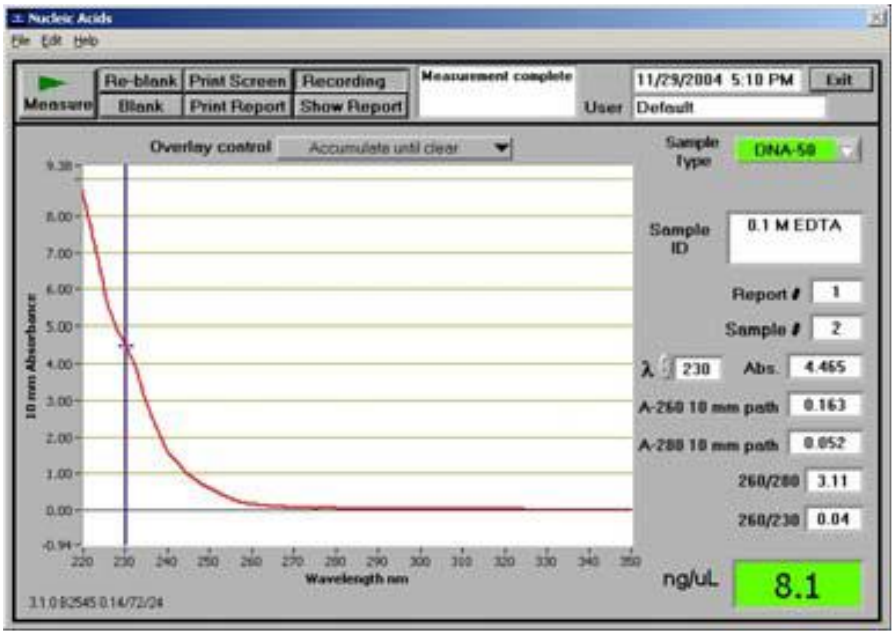
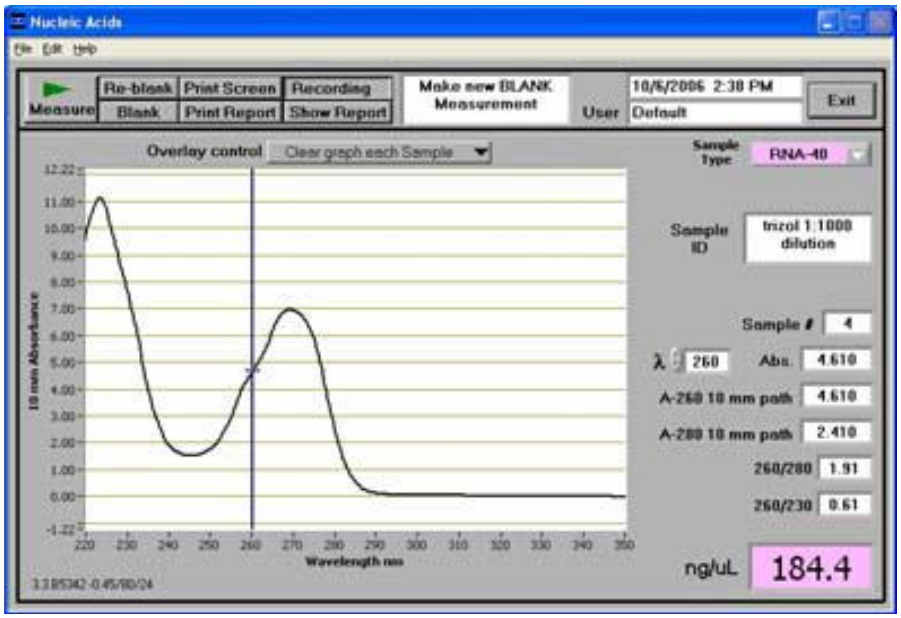


The TRIzol reagent is a phenolic solution which absorbs in the UV both at 230 nm and ~270 nm



Guanidine HCL used for DNA isolations will absorb at ~230 nm

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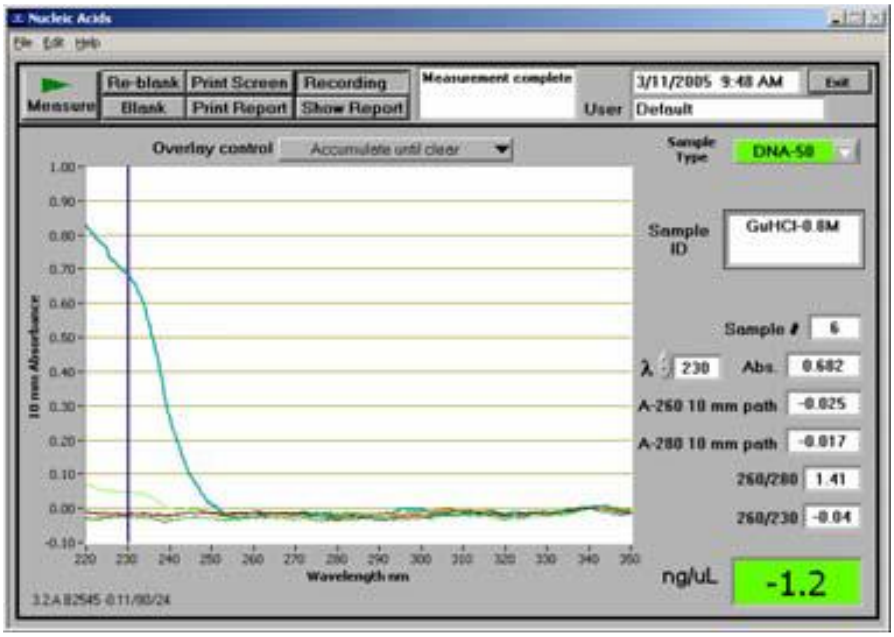

Guanidine isothiocyanate, used for RNA isolations will absorb at ~260 nm