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Genetica
Genome Analysis Facility

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| **Title:** | RNA quality measurement using capillary electrophoresis for RNAseq and Array-based expression studies. |
| **Protocol number:** | GAF ALG005 |
| **Version:** | Version 1 |
| **Date:** | July 1st, 2014 |
| **Author:** | P. van der Vlies |

| RNA quality measurement using capillary electrophoresis for RNAseq and Array-based expression studies. | GAF ALG005 |
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## Introduction

The Genome Analysis Facility of the Dept. Genetics UMCG (GAF) offers RNAseq and array based RNA expression profiling based on pure and non-degraded RNA. To facilitate researchers in their needs, the GAF offers this QC-measurement of the integrity of the total RNA sample with capillary electrophoresis.
This document contains:

- Information how to deliver RNA samples for  to the GAF for QC measurement with capillary electrophoresis
- Information about the output, and guidelines for interpretation

This measurement is performed by the GAF on a weekly basis, and is only intended as QC measurement prior to RNAseq or array based RNA expression studies. The GAF charges € 10 per sample for the service.

## Preparing your samples and shipping them

1. Perform a nanodrop measurement of the total RNA-solution
2. An aliquot of exactly 2 µl of the total RNA solution with a concentration of 5-5000 ng/µl should be transferred to 96-well plates (for instance: Greiner Bio-One, PCR-plate full skirted, order nr. 652270), starting with position A1, then B1, etc. Do not leave any blank wells between samples. All this RNA is consumed in this experiment.
3. The 96-well plates should be firmly closed, preferably with a Thermo-seal (*Thermo scientific order no. AB-0559*) using a heat-plate sealer. Alternatively, if a heat-plate sealer is not available, adhesive PCR foil seals (*Thermo scientific order no. AB-0626*) can be used. A unique plate name should be given to each 96-well plate, using a permanent marker to annotate each plate on both sides.
4. Record the sample IDs in a special XLS file according to the instructions below. The GAF doesn't accept samples with names of individuals.
5. Deliver the samples on dry ice to the Genome Analysis Facility lab or send your samples by courier to the Genome Analysis Facility laboratory at the Department Genetics, UMCG. (Eriba building, 5th floor)

## Documenting the sample and plate layout

The administration of all sample IDs and their corresponding wells in the 96-well plate should be recorded in an XSL file (Excel) (see figure 1). This XSL file should summarize all the required information:

- Researcher email address
- UMCG or RuG account number
- Sample number
- Plate name
- Well number
- Nanodrop concentration

Email address:.....

UMCG or RuG account nr/ kostenplaats: .....

| Nr. | PlateName | Well Nr. | NanodropConcentration (ng/ul) |
|---|---|---|---|
| 1 | Project 1 | A1 | 123 |
| 2 | Project 1 | B1 | 234 |
| 3 | Project 1 | C1 | 345 |
| 4 | Project 1 | D1 | 456 |
| 5 | Proi... | ... | 567 |
| | | | 678 |

*Figure 1. Example of the attached XSL file*

Please email the XLS file with your sample information to: b.sanjabi@umcg.nl

## Guidelines data analysis

The capillary electrophoresis of the total RNA sample is performed on the PerkinElmer LabChipGX. After finishing the analysis a PDF-file with the LabChip GX output is emailed to the researcher. In this way the GAF offers researchers the ability to verify the quality of the samples and prepare the samples for downstream experiments.

*Guidelines for analysis*

1. The **integrity** of the RNA is expressed in the RNA Quality score, this score reflects the ratio between the 28S and 18S ribosomal RNA peak area's in the electropherogram (see figure 2). The quality score is a value between 0 (bad quality RNA) and 10 (superiour quality RNA)

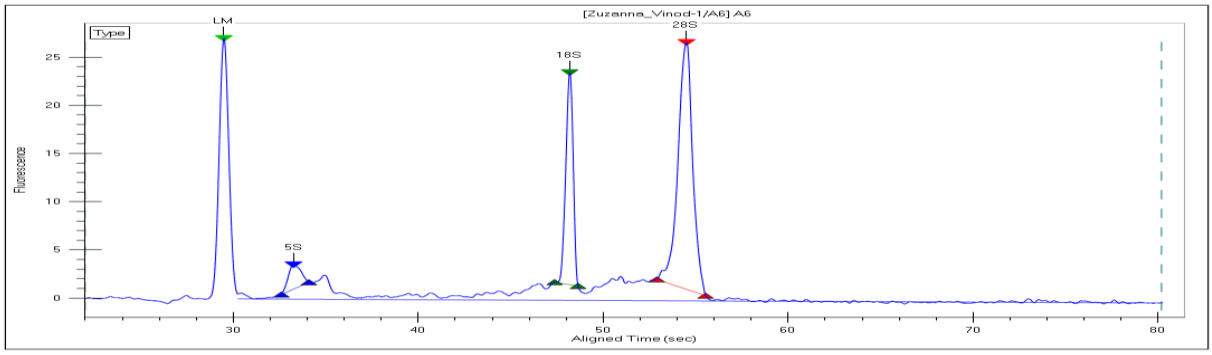


*Figure 2: LabChip GX electropherogram with Lower Marker peak and 5S, 18S and 28S ribosomal RNA peaks.*

2. The **concentration** of total RNA is quantified by measurement of peak area's. The concentration measurement by the LabChip GX is more precise than a nanodrop measurement due to the fact that this measurement is not influenced by contaminations.
3. The quality score and concentration of each sample is listed in a table, see figure 3

| Well Label | Sample Name | Total Conc. (ng/ul) | RNA Quality Score |
|---|---|---|---|
| C01 | 2616 | 258.71 | 9.4 |
| B02 | 2605 | 315.63 | 10.0 |
| B03 | 2606 | 213.02 | 9.8 |
| B04 | 2607 | 575.60 | [9.8] |
| B05 | 2608 | 212.16 | 9.6 |
| | | 470.04 | 9.9 |

*Figure 3: Labchip GX result table*

4. Thresholds for good quality downstream analysis are:

**Array based Expression analysis**

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| Volume | 15 µl |
| Minimal concentration | 20 ng/µl |
| LabChip Quality Score | >7 |
| More information in document | GAF E001 |

**RNAseq**

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|---|---|
| Volume | 50 µl |
| Minimal concentration | 20 ng/µl |
| LabChip Quality Score | >7 |
| More information in document | GAF S002 |

5. The GAF has a high success rate in case for samples with fewer starting material, however a successful experiment for these samples is not guaranteed. This counts only for samples where both 18S and 28S ribosomal peaks are visible in the LabChipGX electropherogram, indicating that the RNA is not degraded. The complexity of the RNA could be reduced in samples with limited starting material, resulting in a bias in the final output.

## Downstream Analysis

Continue with downstream analysis according document:

GAF E001:Guidelines Analysis of RNA Quantity and Quality for Array-Based Illumina Expression Studies
or
GAF S002: Guidelines Analysis of RNA Quantity and Quality for Next-Generation Sequencing Projects