Agilent

Trusted Answers

Research Citation Collection

Selected publications featuring the Agilent Fragment Analyzer systems

See How Easy Sample Quality Control Can Be

Your nucleic acid samples are the foundation of your experiment—so make sure they're reliable. Agilent Fragment Analyzer instruments provide a comprehensive, objective assessment of your nucleic acid quantity, quality, and integrity that can be quickly and seamlessly integrated into any workflow.

Knowing the quality of your samples prior to downstream analysis means you'll repeat less work and gain the ability to optimize your workflows. With the Fragment Analyzer systems, you will also benefit from incorporating automation into your sample QC workflow. The result is better workflow efficiency so you can spend time on the results that matter.

In this compendium, we've provided eight peer-reviewed publications to illustrate the utility and versatility of the Fragment Analyzer systems in areas such as human disease research, agricultural genomics, conservation biology, and more. See how researchers working in these key application areas were able to easily incorporate sample QC steps into their workflows for cfDNA analysis, mitochondrial DNA research, RNA sequencing, and beyond!

Table of contents

A Cost-Effective RNA Extraction Technique from Animal Cells and Tissue Using Silica Columns

J. Biol. Methods **2017**, *4*(2), 72

Authors

Mario D. Escobar and Jason L. Hunt

Synopsis

RNA is commonly extracted by phenol-chloroform, producing hazardous by-products, or with expensive, commercially available silica spin column kits. In this publication, the authors have developed a new, cost-effective, RNA extraction method using generic silica columns and readily available reagents. The Fragment Analyzer system was used to determine the integrity of the RNA and the presence of degradation. The new RNA extraction protocol resulted in intact RNA with no degradation (Figure 2). In addition, the publication states that the RNA quality number (RQN) has been shown to correlate with the RNA integrity number (RIN) from the Bioanalyzer system. The Fragment Analyzer system verified that the new RNA extraction protocol produces a high yield of quality RNA.

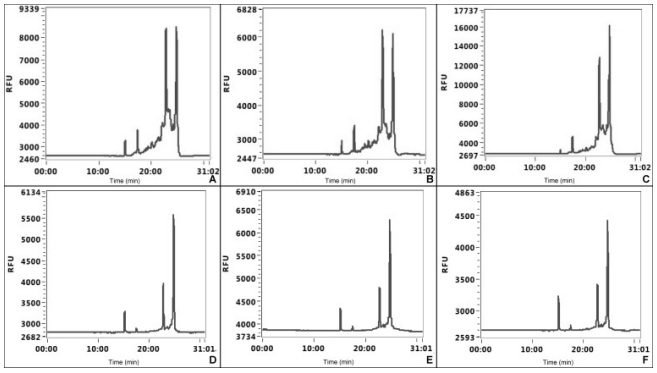

Figure 2. RNA fragment analysis. (A-C) Tissues samples 1 - 3. (D-F) Three independent cell cultures. This figure has been reproduced from Escobar *et al.*

Escobar, MD; Hunt, JL. A cost-effective RNA extraction technique from animal cells and tissue using silica columns.
J Biol Methods **2017**;*4*(2):e72. http://www.jbmethods.org/jbm/article/view/184/168

Hybridization Capture Using RAD Probes (hyRAD), a New Tool for Performing Genomic Analyses on Collection Specimens

PLoS One **2016**, *11*(3)

Authors

Tomasz Suchan, Camille Pitteloud, Nadezhda S. Gerasimova, Anna Kostikova, Sarah Schmid, Nils Arrigo, Mila Pajkovic, Michal Ronikier and Nadir Alvarez

Synopsis

In this paper, the authors present an alternative to targeted restriction-associated-DNA (RAD) sequencing, hybridization RAD (hyRAD), designed for sequencing of degraded or historical samples that are not well represented with RAD sequencing due to their smaller fragment lengths. For this approach, a modified double-digestion RAD protocol is applied to high-quality samples in order to generate hybridization-capture probes. The probes are then used for enrichment of the fragments of interest in the shotgun libraries prepared from the degraded samples. To generate the probes, a size-selection step was utilized to narrow down the size of the fragment used and remove the adaptor sequences. Then, the fragments are biotinylated. Both the probe and the final capture-enriched library were analyzed using the Fragment Analyzer system (Figure S1) to confirm size selection and library quality.

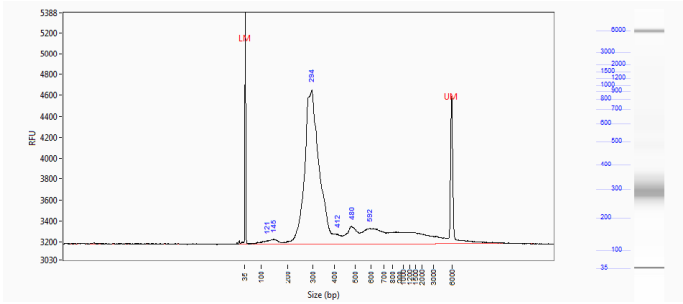

Figure S1. Profile of the RAD-probes precursor, the RAD-seq library. Left panel, X-axis: fragment size (semi-log scale); Y-axis: fragment density (Relative Fluorescent Units). Right panel: gel-like representation of the left panel. This figure has been reproduced from Suchan *et al*.

Suchan T; Pitteloud C; Gerasimova NS; Kostikova A; Schmid S; Arrigo N; et al. Hybridization Capture Using RAD Probes (hyRAD), a New Tool for Performing Genomic Analyses on Collection Specimens. *PLoS ONE*. **2016**. 11(3):e0151651.
https://doi.org/10.1371/journal.pone.0151651

BRB-Seq: Ultra-Affordable High-Throughput Transcriptomics Enabled by Bulk RNA Barcoding and Sequencing

Genome Biol. **2019**, *20*(1), 71

Authors

Daniel Alpern, Vincent Gardeux, Julie Russeil, Bastien Mangeat, Antonio C. A. Meireles-Filho, Romane Breysse, David Hacker, and Bart Deplancke

Synopsis

This publication details the development of a new approach for highly multiplexed RNA profiling, combining high-throughput, single-cell transcriptomics with RNA-Seq into a new method: bulk RNA barcoding and sequencing (BRB-Seq). The Fragment Analyzer system and the RQN quality metric were used to study the reliability of BRB-Seq for low-quality RNA samples that are normally not recommended for sequencing. Aliquots of samples with high and low RQN scores were fragmented (Figure S5) and sequenced. Even highly degraded samples with an RQN of 2.2 were able to detect 75% of the genes that were detected in the high-quality samples (RQN >8.9), indicating that BRB-Seq allows reliable differential gene expression. Total RNA integrity and final NGS library quality was assessed on the Fragment Analyzer systems. Electropherograms of final libraries prepared with different tagmentation and RT enzymes are shown thought the paper as examples of how the authors used the Fragment Analyzer to help optimize the BRB-Seq method.

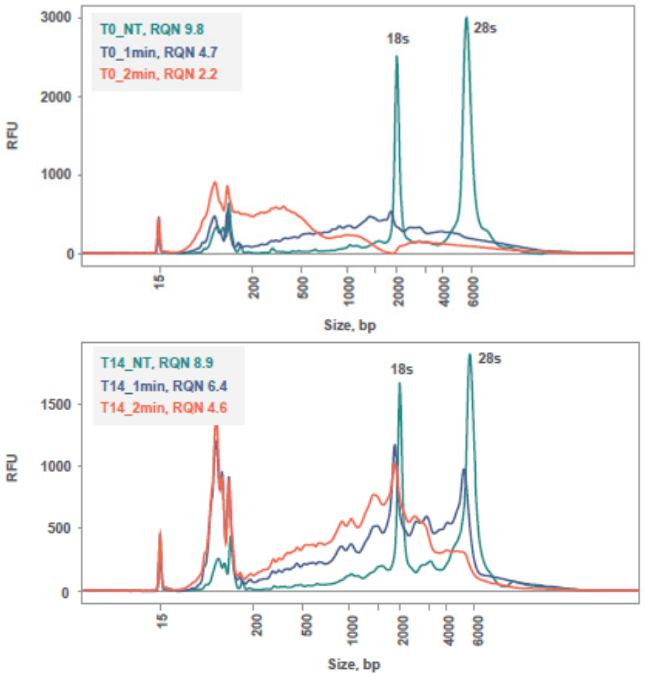

Figure S5. The RNA fragment size profiles of intact samples and their degraded counterparts after one or two minutes of fragmentation. This figure has been reproduced from Alpern *et al*.

Alpern, D; Gardeux, V; Russeil, J *et al*. BRB-seq: ultra-affordable high-throughput transcriptomics enabled by bulk RNA barcoding and sequencing. *Genome Biol.* **2019**, 20, 71. https://doi.org/10.1186/s13059-019-1671-x

Liquid Biopsy: A New Source of Candidate Biomarkers in Amyotrophic Lateral Sclerosis

Ann. Clin. Transl. Neurol. **2018**, *5*(6), 763–768

Authors

Maite Mendioroz, Leyre Martínez-Merino, Idoia Blanco-Luquin, Amaya Urdánoz, Miren Roldán and Ivonne Jericó

Synopsis

The use of liquid biopsy for diagnosis and monitoring the progression of amyotrophic lateral sclerosis (ALS) was explored in this publication. Cell-free DNA (cfDNA) was isolated from ALS patients and controls for downstream analysis. Concentration, quality, and size distribution of the cfDNA was assessed using a Fragment Analyzer system with the HS Large Fragment 50 kb kit (DNF-464). Extracted cfDNA displayed a typical profile with three nucleosomal peaks (Figure 1A). After cfDNA library preparation and sequencing, the authors identified a novel differentially methylated mark in the RHBDF2 gene in ALS patients compared to controls. Thus, they concluded that plasma may serve as a source of potential epigenetic biomarkers for neurodegenerative disorders.

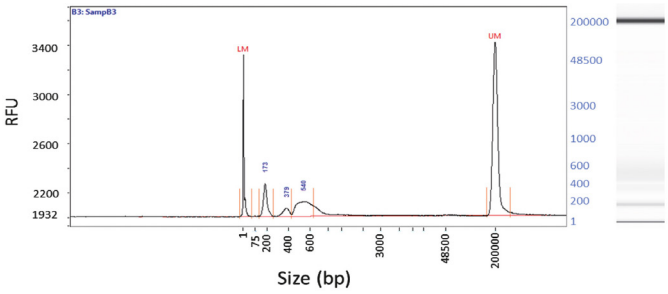

Figure 1A. *RHBDF2* DNA methylation levels in cfDNA in ALS. (A) The diagram represents the typical pattern of cfDNA with peaks at around 165 bp, 350 bp and 565 bp after performing capillary electrophoresis on a Fragment Analyzer system. This figure has been modified and reproduced from Mendioroz *et al*.

Quality Assurance After a Natural Disaster: Lessons from Hurricane Sandy

Biopreserv. Biobank. **2018**, *16*(2), 92–96

Authors

Collin Dickerson, Yanshen Hsu, Sandra Mendoza, Iman Osman, Jennifer Ogilvie, Kepal Patel, and Andre L. Moreira

Synopsis

Hurricane Sandy was a category 1 hurricane that caused havoc in the greater Caribbean and then made its way up the United States eastern seaboard in October of 2012, causing widespread power outages. This study examines the quality of biospecimens stored at a biobank in New York after the power outages caused by Hurricane Sandy. Nucleic acids were extracted from samples that had been collected in 2012 before the power outage and compared to new samples received in 2013. Analysis of the nucleic acids with the Fragment Analyzer system, a capillary electrophoresis instrument, showed no significant difference in concentration, size, or quality. Integrity of DNA samples was analyzed using the genomic quality number (GQN), reporting a GQN of 8.0 for samples before and after the power outage (Figure 1). RNA was assessed with the RNA quality number (RQN), ranging from 8.1–8.3 and the 28Ss/18S ratio of rRNA at 1.83–1.87 for before and after samples, respectively. The Fragment Analyzer system confirmed that samples maintained their quality and were not degraded because of freezers losing electricity during the power outage.

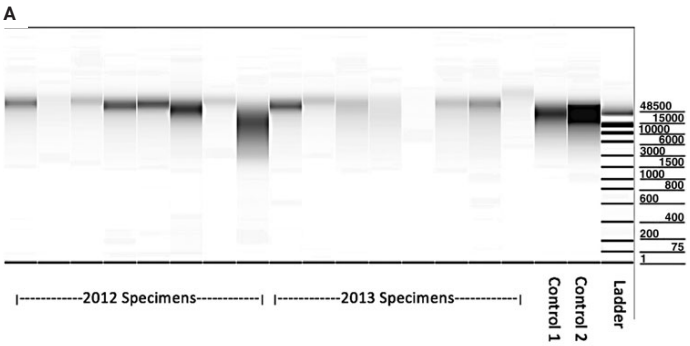

Figure 1. (A) Results of capillary electrophoresis of extracted DNA from select 2012 and 2013 specimens compared with a ladder of known nucleotide lengths and two control DNA samples with known high fidelity. RNA samples not shown. This figure has been modified and reproduced from Dickerson *et al.*

Optimizing ddRADseq in Non-Model Species: A Case Study in *Eucalyptus dunnii* Maiden

Agronomy **2019**, *9*(9), 484

Authors

Natalia Cristina Aguirre, Carla Valeria Filippi, Giusi Zaina , Juan Gabriel Rivas, Cintia Vanesa Acuña, Pamela Victoria Villalba, Martín Nahuel García, Sergio González, Máximo Rivarola, María Carolina Martínez, Andrea Fabiana Puebla, Michele Morgante, Horacio Esteban Hopp, Norma Beatriz Paniego and Susana Noemí Marcucci Poltri

Synopsis

NGS library preparation protocols for different types of sequencing are well established, but many are not optimized for non-model species. In this study, the authors discuss the steps they went through to optimize a double-digest, restriction site-associated DNA sequencing (ddRADseq) protocol for a non-traditional model organism, *Eucalyptus dunnii*, and how to scale up the protocol for high-throughput experiments. The paper shows that the Fragment Analyzer systems were used to visualize the profile of the samples after digestion with different enzyme combinations and the quality of the final NGS library. The optimized protocols were given as supplementary materials. In these protocols, they show examples of electropherograms from the Fragment Analyzer systems for several steps throughout the library preparation process, including initial verification of gDNA integrity (Figure 2), following enzyme digestion, and the final size-selected library. The Fragment Analyzer systems were used to QC the initial samples and throughout the entire library preparation workflow for quality, size, and concentration.

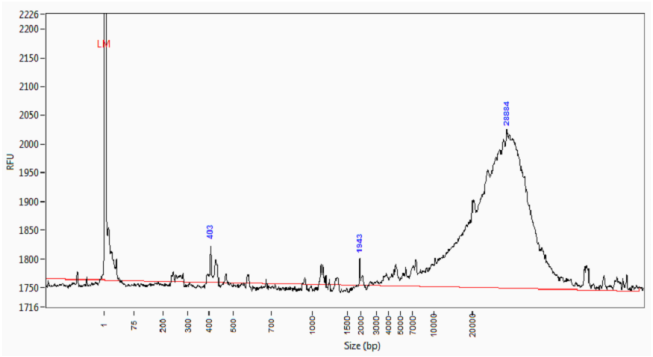

Figure 2 (Supplementary, Protocol 1). Fragment Analyzer for verification of the *E. dunnii* genomic DNA integrity. Molecular weight: 28884 bp. This figure has been reproduced from Aguirre *et al*.

Aguirre, NC; Filippi, CV; Zaina, G; Rivas, JG; Acuña, CV; Villalba, PV; García, MN; González, S; Rivarola, M; Martínez, MC; Puebla, AF; Morgante, M; Hopp, HE; Paniego, NB; Marcucci Poltri, SN Optimizing ddRADseq in Non-Model Species: A Case Study in Eucalyptus dunnii Maiden. *Agronomy* **2019**, 9, 484. https://doi.org/10.3390/agronomy9090484

Transcriptomics of Arabidopsis sperm cells at single-cell resolution

Plant Reprod. **2019**, *32*(1), 29–38

Authors

Chandra Shekhar Misra, Mário R. Santos, Mariana Rafael-Fernandes, Nuno P. Martins, Marta Monteiro and Jörg D. Becker

Synopsis

This study combines FACS (fluorescent-activated cell sorting) with single-cell RNA-Seq to study the transcriptome profile of single sperm cells from *Arabidopsis* to examine differentially expressed genes. The RNA from single-sorted sperm cells underwent reverse transcription, pre-amplification, and cDNA purification. A Fragment Analyzer system was utilized to confirm the size-distribution of the amplified single cell cDNA (Figure 3A-B). The cDNA was then used as a template for RT-PCR, and if amplifiable, was used to create a single-cell RNA-Seq library. The final library was also analyzed using a Fragment Analyzer system to confirm the library size before sequencing (Figure 3D). QC analysis allowed the authors to choose only the best cDNA samples and libraries for sequencing.

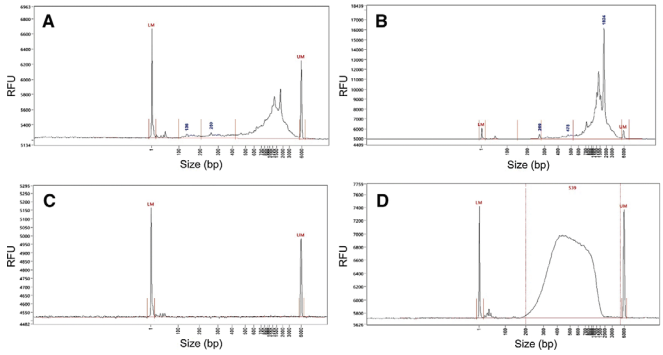

Figure 3. Representative electropherogram plots from the Fragment Analyzer showing various stages of single-cell RNA-seq library preparation. (A) Example of a good cDNA amplification from single cell with fragment distribution between 600 and 2500 bp, and an average size of 1500 to 2000 bp. (B) Example of a good cDNA amplification from bulk sample used as a positive control, with a similar fragment distribution. (C) Example of a negative (no cell) control. (D) Example of a typical fragmented and indexed Nextera library with a size distribution of 300 - 800 bp. Relative fluorescence units (RFU). This figure has been modified and reproduced from Misra *et al*.

Complete Mitochondrial Genomes of Eleven Extinct or Possibly Extinct Bird Species

Mol. Ecol. Resour. **2017**, *17*(2), 334–341

Authors

Jarl A. Anmarkrud and Jan T. Lifjeld

Synopsis

This paper discusses the use of museum specimens for collection of genetic material from species that are extinct or rare, and therefore difficult to obtain high-quality samples from for in-depth analysis. Genomic DNA extracted from these samples is often degraded, displaying a small size and low concentration. However, advances in sequencing technologies have made it possible to reconstruct mitochondrial genomes from low-quality samples such as museum specimens, enabling research on taxonomy, phylogenetics, biodiversity, and evolution. Anmarkrud and Lifjeld extracted gDNA from 11 museum specimens of extinct bird species and sequenced the mitochondrial genomes. The extracted gDNA was analyzed on a Fragment Analyzer system before NGS library preparation to determine the quality of the samples (Figure S2). Knowing the integrity and concentration of the samples enabled the author to determine which library preparation kit to use for each sample, since each kit provides recommendations for the DNA quality to use. *De novo* assembly of the short gDNA sequences from each specimen allowed for successful sequencing of the complete mitochondrial genomes, demonstrating that low-quality DNA can be successfully used in sensitive downstream applications with appropriate protocol adjustments.

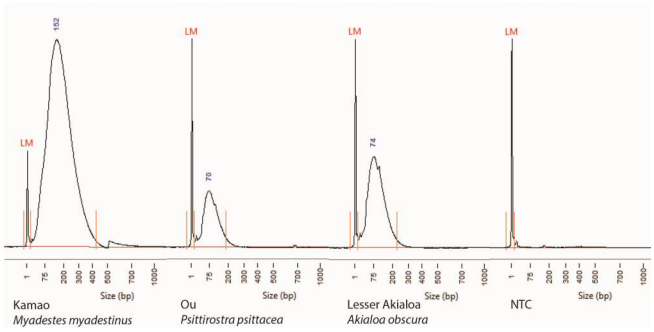

Figure S2. Fragment Analyzer trace of the DNA extract from selected study object. The lower marker (LM) peak is 1 bp and contains a constant concentration. The number above each peak represents the peak size (bp). The NTC is the two non-template controls combined. This figure has been modified and reproduced from Anmarkrud *et al*.

Learn more:
www.agilent.com/genomics/fragment-analyzer

Buy online:
www.agilent.com/chem/store

U.S. and Canada
1-800-227-9770
agilent_inquireies@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

For Research Use Only. Not for use in diagnostic procedures.

This information is subject to change without notice.

PR7000-7437

Published in the USA, September, 2020
5994-2351EN

Agilent
Trusted Answers