

1 **Title: Bioanalyzer chips can be used interchangeably for many analyses of DNA or RNA**

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8 **Abstract**

9 The Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA) enables small-scale gel electrophoretic
10 separation of nucleic acids on a microfluidic chip. Shortage of chips and excess reagents is a common
11 issue. This report explored the compatibility of two commonly used Bioanalyzer reagents with three
12 Bioanalyzer chip types. Microfluidic electrophoretic separation of DNA and RNA using DNA High
13 Sensitivity and RNA 6000 Nano reagents, respectively, was successfully performed on multiple chip
14 types, following the assay-specific protocol. For RNA quality and next-generation sequencing library
15 size estimation, the Bioanalyzer chips tested can be used interchangeably. These findings will be
16 valuable for any laboratory using the Agilent Bioanalyzer in a shared facility.

17 **Methods summary**

18 Four RNA samples (from murine brain) and four DNA samples (RNA-seq libraries made using the
19 Illumina TruSeq Stranded mRNA kit, Illumina Inc., San Diego, USA) were run in triplicate using RNA
20 6000 Nano and DNA High Sensitivity (HS) reagents, respectively, on RNA 6000 Nano, DNA HS and
21 DNA 1000 chips. Quality and concentration of RNA samples, and concentration and size distribution
22 of DNA samples were tested. We demonstrate that any of the Bioanalyzer chips tested can be used
23 interchangeably with defined Bioanalyzer reagents for qualitative analysis, and can also be
24 reasonably quantitative, provided the protocol and software method for the corresponding assay
25 reagent kit are followed correctly.

26 **Main text**

27 The Agilent 2100 Bioanalyzer performs microfluidic electrophoretic separation on microfabricated
28 chips (1). In comparison to slab gel electrophoresis, the Bioanalyzer provides many advantages:
29 separation is quick; minimal sample volume is required (1 µl); user exposure to hazardous materials
30 is minimal; and the assessment of sample quantity and quality is not dependent on the user's
31 interpretation.

32 Results of nucleic acid sample separation are displayed on an electropherogram and gel-like image,
33 generated by the Bioanalyzer 2100 Expert Software (1). These provide a visualisation of sample
34 quality and quantity; for RNA, integrity is additionally assessed by a software algorithm which
35 produces an RNA integrity number (RIN) (2, 3). DNA samples, such as PCR products, restriction
36 digests, and plasmid digests, can be assessed with kits covering a vast range of product lengths.
37 Moreover, the additional high sensitivity reagents are particularly useful for library assessment prior
38 to next-generation sequencing.

39 A common problem in the laboratory is that of chip shortage with excess reagents, particularly in
40 laboratory service environments which experience both high usage and fluctuations in the demand
41 for different kit types. Therefore, an investigation into whether reagents can be used
42 interchangeably with different Agilent Bioanalyzer chips would be valuable for many researchers.
43 Anecdotal reports of using the wrong chip type have been noted previously (4). Others have
44 demonstrated the ability to re-use chips multiple times without detrimentally affecting results (5, 6).
45 We explored the compatibility of RNA 6000 Nano and DNA HS Bioanalyzer reagents with three chip
46 types, following the assay-specific protocol and using the assay-specific software.

47 The RIN and concentration of four RNA samples measured in triplicate (R1, R2, R3, and R4) were
48 assessed using the RNA 6000 Nano reagents and protocol, on RNA 6000 Nano, DNA HS and DNA
49 1000 chips. Importantly, the sticker displaying the chip layout was disregarded and the loading
50 pattern indicated in the assay-specific protocol was used. All chips were run on the Agilent 2100
51 Bioanalyzer using the Eukaryotic Total RNA Nano assay. Concentration and RIN of each RNA sample
52 were highly comparable between chips (inter-chip concentration: $p = 0.96$; RIN: ANOVA $p = 0.13$)
53 (see **Table 1 and Fig. 1**). Intra- and inter-chip variability for RNA RIN and concentration were very
54 similar. RIN and RNA concentration were both well within the normal variability expected of samples
55 submitted for RNA-seq experiments.

56 The size distribution and concentration of four DNA samples (RNA-seq libraries; D1, D2, D3, and D4)
57 were assessed using the DNA HS reagents and protocol, on RNA 6000 Nano, DNA HS and DNA 1000
58 chips; again, the sticker displaying the chip layout was disregarded. All chips were run on the
59 Bioanalyzer using the DNA HS assay. Manual integration was used to label the prominent peak for
60 each RNA-seq library, enabling library length and concentration to be calculated. The average size
61 estimated across all Bioanalyzer chips was 290 bp (SD 6 bp) (**Table 2, and Fig. 1**), with a 40 bp range
62 across all samples (inter-chip library length: ANOVA $p = 0.69$). DNA concentration showed a 10-20 %
63 variation in concentration across chips (**Table 2**). Accordingly, care should be taken when
64 quantifying next-generation sequencing libraries using the Agilent Bioanalyzer; however, the
65 recommendation is to use quantitative PCR (7).

66 To assess assay reproducibility, which has been previously described by others (8,9), a pool of the
67 RNA samples described above, or a single DNA sample (RNA-seq library pool, SLX-10140) were
68 loaded as technical replicates in each well of duplicate chips, for all three chip types tested. Library
69 length and RIN were consistent between all chips (Fig. 2). RNA and DNA concentration data were
70 significantly different between chip types, although it was apparent that this was due to inter-chip
71 variability and not due to chip type (Fig. 2 and Supplementary data).

72 We determined the accuracy of our Bioanalyzer concentration measurements by comparison to
73 NanoDrop UV spectrophotometry (Nanodrop, DE, USA), Qubit (Thermo Fisher Scientific, MA, USA)
74 and Kapa qPCR (KAPA BioSystems, South Africa) measurements (Qubit and qPCR are the
75 recommended quantification methods for RNA and RNA-seq libraries, respectively). The Bioanalyzer
76 concentration measurement of the RNA pool was less precise (211 ng/ μ l, SD 102.89 ng/ μ l) than
77 Qubit (146 ng/ μ l, SD 6.24 ng/ μ l). The Bioanalyzer underestimated the concentration of our RNA-seq
78 library (1.25 ng/ μ l) when compared to qPCR (11.17 ng/ μ l) (Supplementary data). Our data support
79 the finding that Bioanalyzer quantification is more variable than other methods (9).

81 Our data confirm that, provided the assay-specific protocol is followed, the Bioanalyzer chip type
82 used is irrelevant for RIN and DNA size estimation, i.e. qualitative analysis of RNA and RNA-seq
83 libraries. It is important that the chip sticker is not used as a guide. Qubit and qPCR are
84 recommended for RNA and RNA-seq library concentration measurements; the variability in RNA and
85 DNA concentration measurements identified between Bioanalyzer runs supports this. These findings
86 will be applicable to an extensive number of research environments in which the Agilent 2100
87 Bioanalyzer is used for the assessment of nucleic acid samples; in particular, those facilities which
88 employ more than one type of kit and are high consumers of Bioanalyzer reagents.

89

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91 discussions; Sarah Vowler for help with statistical tests; and Cancer Research UK and the University
92 of Cambridge for funding the Genomics Core facilities through the Cambridge Institute grant.

93 **Figures and tables**

94 **Figure 1 legend:** Inter- and intra-chip consistency in the measurement of key sample parameters. A.
95 Overlaid electropherogram traces of all RNA and DNA Bioanalyzer runs. Four RNA or DNA samples
96 were run in triplicate on an RNA 6000 Nano, DNA High Sensitivity (HS) and DNA 1000 chip, according
97 to the RNA 6000 Nano or DNA HS kit protocol, respectively. Note that the lower fluorescence for the
98 DNA 1000 chip did not affect calculations of sample concentration or length. B. RNA integrity
99 number (RIN) of the four RNA samples and library size of the four RNA-seq libraries were determined
100 on each chip. UM = Upper marker; LM = Lower marker peak.

101 **Figure 2 legend:** Reproducibility of chip sample metrics. A technical replicate of RNA or DNA was
102 loaded onto duplicates of each chip type, and the RNA 6000 Nano or DNA HS kit protocols,
103 respectively, were followed. Tabulated data and results of statistical tests are given in the
104 Supplementary material.

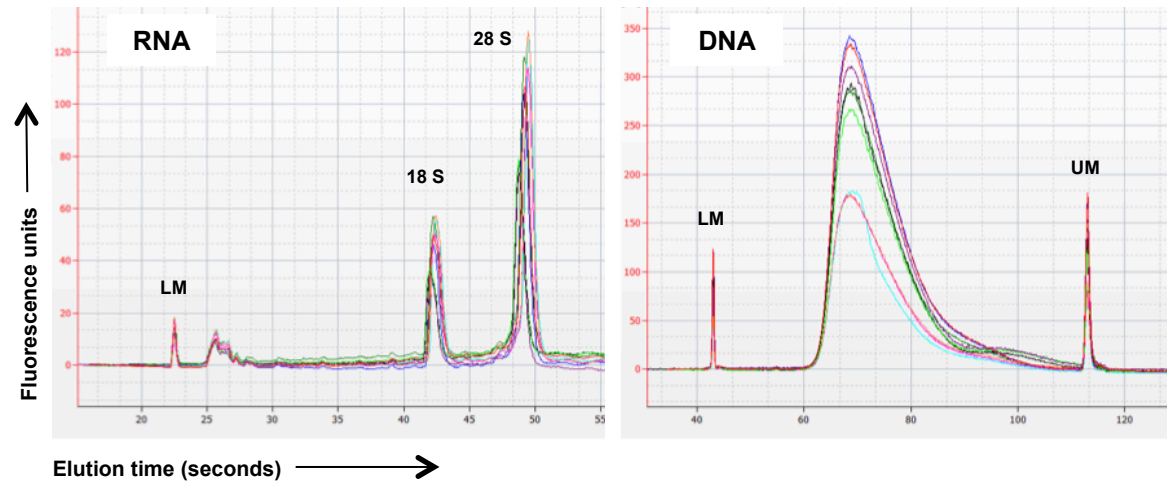
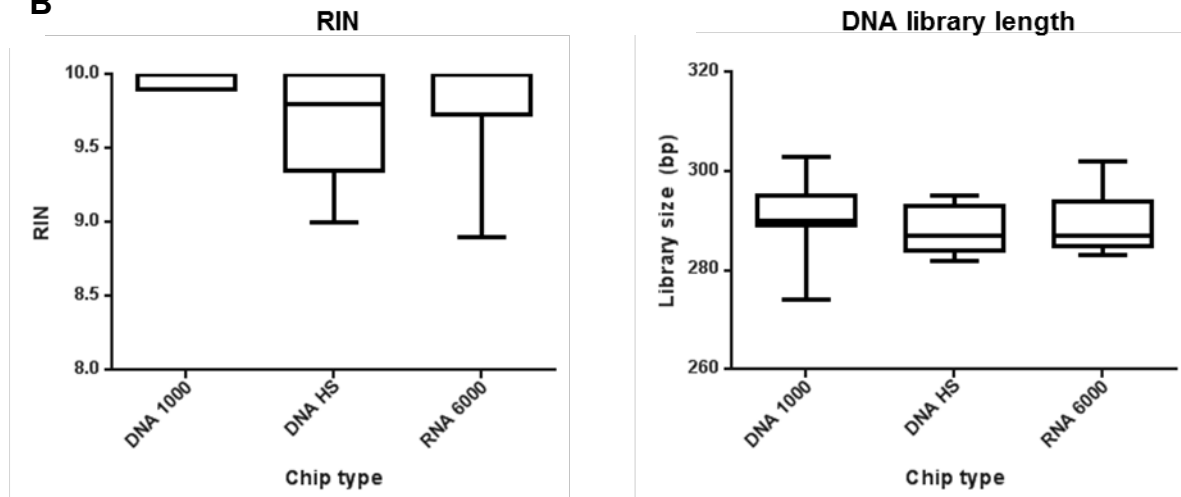
105 **Table 1 legend:** RNA data. Four RNA samples were run according to the RNA 6000 Nano protocol, on
106 each chip. RINs (upper) and sample concentration data (lower) (ng/ μ l) were determined. Sample R4
107 was excluded from the DNA HS chip, as the final three wells were used to validate ladder consistency
108 within a chip. M = mean.

109 **Table 2 legend:** DNA data. Four DNA samples (RNA-seq libraries) were run in triplicate according to
110 the DNA High Sensitivity (HS) protocol, on a DNA 1000, DNA HS and RNA 6000 Nano chip. Manual
111 integration was used to delete artefacts and select the desired peak; library length (upper) and
112 concentration data (lower) (ng/ μ l) were determined by the software. The DNA HS protocol uses 11
113 sample wells, therefore D4 was loaded in duplicate. M = median library length or mean
114 concentration.

115

116 **References**

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A**B**

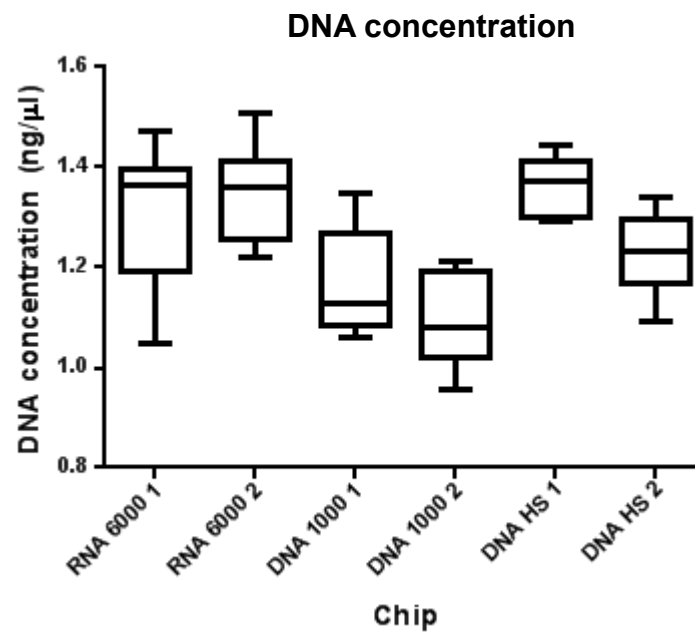
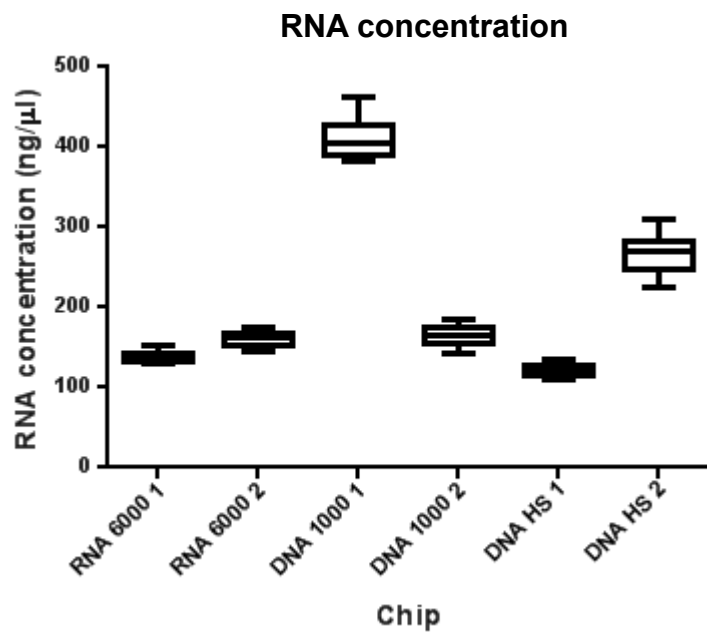
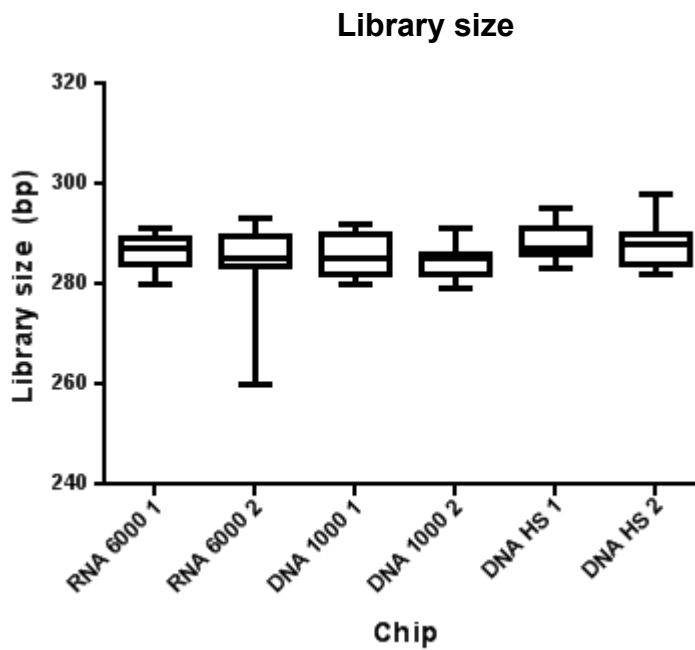
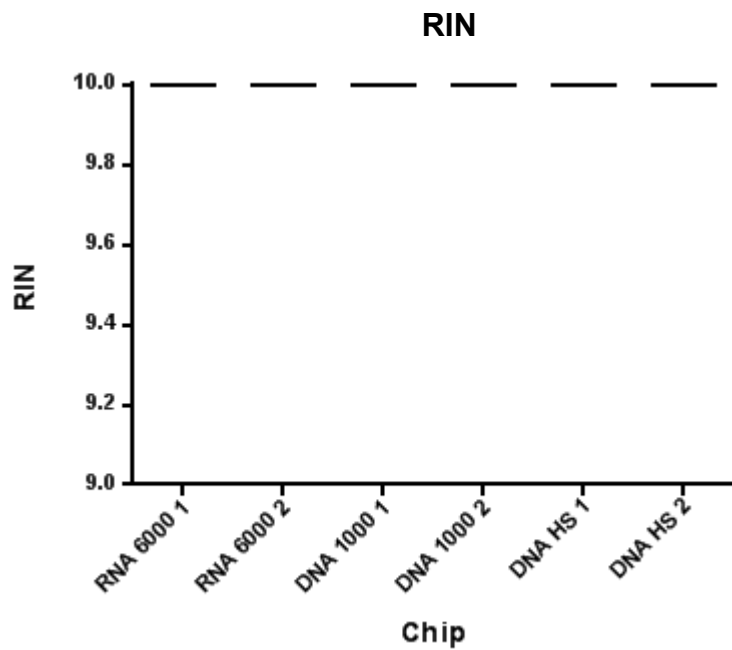


Table 1: RNA data

RNA Sample	DNA 1000				DNA HS				RNA Nano			
	1	2	3	M	1	2	3	M	1	2	3	M
R1	9.9	9.9	10.0	9.9	9.2	10.0	9.7	9.6	9.9	8.9	9.7	9.5
R2	9.9	9.9	9.9	9.9	10.0	9.0	10.0	9.7	9.8	10.0	9.2	9.5
R3	10.0	10.0	10.0	10.0	9.5	9.8	10.0	9.8	10.0	10.0	10.0	10.0
R4	10.0	10.0	10.0	10.0	n/a	n/a	n/a	n/a	10.0	10.0	10.0	10.0
R1	147.0	147.0	138.0	144.0	151.0	131.0	129.0	137.0	151.0	161.0	122.0	144.7
R2	213.0	189.0	216.0	206.0	223.0	270.0	206.0	233.0	193.0	157.0	256.0	202.0
R3	323.0	318.0	337.0	326.0	343.0	280.0	299.0	307.3	254.0	272.0	355.0	293.7
R4	222.0	231.0	265.0	239.3	n/a	n/a	n/a	n/a	227.0	238.0	261.0	242.0

Four RNA samples were run according to the RNA 6000 Nano protocol, on a DNA 1000, DNA HS and RNA 6000 Nano chip. RINs (upper) and sample concentration data (lower) (ng/μl) were determined. Sample R4 was excluded from the DNA HS chip, as the final three wells were used to validate ladder consistency within a chip. M = mean.

Table 2: DNA data

DNA Sample	DNA 1000				DNA HS				RNA Nano			
	1	2	3	Md	1	2	3	Md	1	2	3	Md
D1	274	284	291	284	287	285	282	285	286	285	283	285
D2	303	294	297	297	294	292	295	294	295	294	290	294
D3	290	290	290	290	282	285	284	284	286	285	287	286
D4	295	289	n/a	292	293	293	n/a	293	302	293	n/a	298
D1	2.4	2.1	2.1	2.2	1.9	2.0	2.0	2.0	2.7	2.6	2.5	2.6
D2	5.3	6.3	6.0	5.9	5.3	5.7	5.3	5.4	5.7	6.6	6.1	6.1
D3	2.0	1.9	1.9	1.9	1.8	1.7	1.8	1.8	2.3	2.5	2.3	2.4
D4	4.6	6.2	n/a	5.4	4.7	5.3	n/a	5.0	4.5	6.2	n/a	5.3

Four DNA samples (RNA-seq libraries) were run in triplicate according to the DNA High Sensitivity (HS) protocol, on each chip. Manual integration was used to delete artefacts and select the desired peak; library length (bp) (upper) and concentration data (lower) (ng/ μ l) were determined by the software. The DNA HS protocol uses 11 sample wells, therefore D4 was loaded in duplicate. Md = median.