THE EFFECT OF STORAGE TEMPERATURE ON DNA YIELD AND QUALITY: *FICUS CARICA* LEAVES SAMPLE

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Abstract. The storage of DNA under appropriate conditions is very crucial for scientific research since the quality and the quantity of DNA determines the quality and usability of downstream applications such as blotting, PCR (polymerase chain reaction) and DNA sequencing. The appropriate storage temperature depends on the intended use of DNA. In this study, we aim to determine the effect of the most commonly used storage temperatures (+4°C, -20°C and -80°C) on the quality and quantity of DNA by using DNA isolated from *Ficus carica* leaf samples. Our findings suggested that DNA with the highest quality was obtained from -80°C samples while DNA yield was the highest in the samples kept at +4°C which might reflect that the fragmentation of DNA at this temperature results in the highest yield. Collectively, our findings align with prior research advocating for cryogenic temperatures (e.g., -80 °C) as optimal for long-term DNA preservation and refrigerated conditions (e.g., +4 °C) for short-term utilization (spanning several weeks).

Keywords: *DNA absorbance, DNA isolation, DNA storage, Ficus carica, long-term and short-term preservation*

Introduction

DNA isolation procedures have been used frequently since their discovery (Murray and Thompson, 1980; Doyle and Doyle, 1987; Rogers and Bendich, 1989; Ye and Lei, 2023). There are numerous DNA isolation methods for different kinds of organisms, such as plants (Mavrodiev et al., 2021; Tiwari et al., 2021; De Silva et al., 2024), animals (Grela et al., 2021; Ozdemir et al., 2024) and bacteria (Bruggeling et al., 2021; Zhang et al., 2023). These methods may be divided into two main groups: commercially available kits (Wang et al., 2021) and homemade manual isolation protocols (Domínguez-Vigil et al., 2019; Kalendar et al., 2021). In plants, recently developed DNA isolation protocols include both modifications of traditional methods (Mavrodiev et al., 2021) and implementation of novel solvent systems (De Silva et al., 2024).

The yield and quality of the obtained DNA might be affected by various factors including tissue type and age, the amount of sample, storage conditions and extraction methods (Li et al., 2020; Salehi et al., 2023; Mitchell et al., 2023; Guillardín and MacKay, 2023). The type and the age of the plant tissue influence the yield and quality of isolated DNA. While young leaves usually contain fewer secondary metabolites and are more suitable for obtaining high-quality DNA, mature leaves often accumulate higher levels of secondary metabolites which decreases the quality of DNA (Bailey et al., 2022). In our study, we used healthy and young leaves of *Ficus carica* due to their big size and high content of source material.

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DNA should be stored under appropriate conditions in accordance with storage time. Generally, storage at +4 °C is recommended for short-term use, -20°C for medium-term use, and -80°C or in liquid nitrogen for longer periods of time (Tan et al., 2021; Landor et al., 2024). Recent studies have attempted to find the optimal storage conditions in terms of temperature. The quality and quantity of DNA in samples amplified immediately were comparable to the samples stored at -20°C and -80°C for 1 month before the PCR process (Kostadinovic et al., 2024). The cryostorage conditions should be carefully determined. The rate of freezing is another factor to consider, and rapid freezing may be more advantageous than slow freezing under some circumstances (Tan et al., 2021).

Various techniques such as gel electrophoresis, PCR (polymerase chain reaction) analysis, restriction enzyme digestion, fluorometric and chromatographic techniques might be used to determine the quality and quantity of DNA (Bunu et al., 2020; Bruijns et al., 2022; Wittmeier and Hummel, 2022), When deciding on the proper technique for the quantification of DNA, the characteristics of the biological material used for DNA extraction should also be considered. For instance, although spectrophotometry is a widely preferred technique due to its simplicity, speed, and cost-effectiveness (Wan et al., 2023), a recent study suggested that spectrophotometry might not be an optimal choice for quantification of DNA isolated from processed food due to its inaccuracy (Viljoen et al., 2022).

The ratio of absorbance values at 260 nm to 280 nm (A260/280) and the ratio of absorbance values at 260 nm to 230 nm (A260/230) are used to determine the purity of DNA. The ratio of A260/280 should lie between 1.8 and 2.0 for a pure DNA sample. An A260/280 ratio of less than 1.8 indicates protein contamination, whereas a ratio above 2.0 suggests RNA contamination (García-Alegría et al., 2020; Safeena et al., 2021). The A260/A230 ratio for pure DNA should be in the range of 2.0 to 2.2, and a lower ratio indicates contamination due to phenols, salts (i.e., ethylenediamine tetraacetic acid (EDTA)), proteins, or lipids (Lutz et al., 2023; Versmessen et al., 2024).

In this study, we wanted to find the optimal temperature for storage of DNA. We extracted DNA from *Ficus carica* leaves stored at either +4°C, -20°C, or -80°C and measured their concentrations along with their A260/280 and A260/230 ratios weekly for around 3 months. Additionally, we checked the integrity of DNA and performed PCR amplification to get some answers: (I) Which storage temperature is suitable for short- and long-term storage of DNA? (II) Is there a statistically significant change in DNA quality and quantity when stored at different temperatures?

Materials and methods

Plant material, DNA isolation and storage conditions

Young and healthy *Ficus carica* leaves were collected from a tree at Harran University, Osmanbey Campus garden, and dried in silica gel (Kurt et al., 2022). Dried plant material was kept at room temperature until DNA isolation. DNA isolation was done as described before (Kurt et al., 2022). Ten different leaves were prepared in triplicate, and the extracted DNA was kept either at +4°C, -20°C, or -80°C for approximately 3 months.

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Assessment of DNA yield and quality

Isolated double-stranded DNA was measured using a NanoPhotometer P-Class, P 300 (Implen). DNA quantity and quality were determined by the absorbance ratios (A260/280 and A260/230). The measurements were repeated three times for each sample.

Agarose gel analysis

We used 1.5% agarose gel to check the quality of our samples. Three samples from each temperature were selected, and 10 ng of each sample was loaded on the gel. GeneRuler 100 bp Opti-DNA ladder (Applied Biological Materials, Canada) was used as a marker, and BIO-RAD ChemiDoc MP (BIO-RAD, USA) was used to visualize the gel.

PCR amplifications

PCR amplifications were performed in 25 µl reactions containing 2 µl template DNA (10 ng/µl), 0.25 µl 10 × Taq DNA polymerase buffer (Thermo Fisher Scientific Inc.), 1 µl of each primer, 2.5 µl PCR buffer, 0.5 µl 10 mM dNTP, and 2 µl $MgCl_2$ (2.5 M). Forward and reverse primers for the trnL gene were used to amplify the DNA (Mansion et al., 2008).

PCR amplifications included 1 cycle of initial denaturation at 94 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 52.5 °C, 30 s at 72°C, and an ultimate 10 min elongation step at 72 °C (Mansion et al., 2008) using a thermal cycler (BIO-RAD, USA).

Statistical analysis

We measured the concentration and absorbance ratios of A260/280 and A260/230 in each sample weekly. For statistical analysis, the Shapiro-Wilk (Shapiro and Wilk, 1965) test was applied to determine the distribution pattern, and since our values gave a non-normal distribution, we performed the Kruskal-Wallis test (Kruskal and Wallis, 1952) to decide whether there was a significant change in terms of DNA yield and quality. For further analysis, the Dunn-Bonferroni test (Dunn, 1964) was used as a post-hoc test. For all of the statistical tests, the R statistical programming language was used (R Core Team, 2024). For the Shapiro- Wilk test and Kruskal-Wallis test, base R packages are used. For the Dunn-Bonferroni test, the package “dunn.test” was used (Dinno and Dinno, 2017). The graphics were created using the packages “tidyr” (Wickham and Wickham, 2017), “dplyr” (Wickham et al., 2023), “ggplot2” (Wickham, 2011), and “stringr” (Wickham, 2023).

Results

As the Shapiro-Wilk test indicated that our samples did not display normal distribution, the Kruskal-Wallis test was applied. The results of the Kruskal-Wallis test showed significant differences in DNA yield and quality between some of the samples stored at different temperatures. The Dunn-Bonferroni test was applied for further analysis, revealing significant differences in DNA yield stored at +4°C, -20°C, and -80°C (*Fig. 1*). The higher yield observed at +4°C might be attributed to the more concentrated DNA due to evaporation of water inside the sample.

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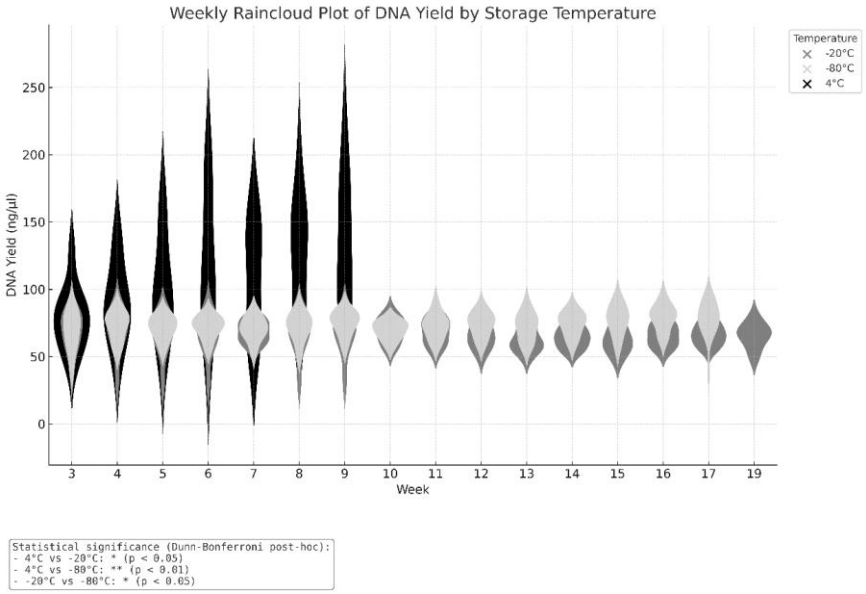

Figure 1. *The change in quantity of DNA stored at either +4◦C, -20◦C or -80◦C*

While significant differences in the A260/280 ratio were observed between -20°C and -80°C and between -80°C and +4°C, there were no significant differences in the A260/230 ratio at different temperatures (*Fig. 2*). The lack of significant differences in the A260/230 ratio at different temperatures suggests that interference of other contaminants was minimal, irrespective of storage conditions.

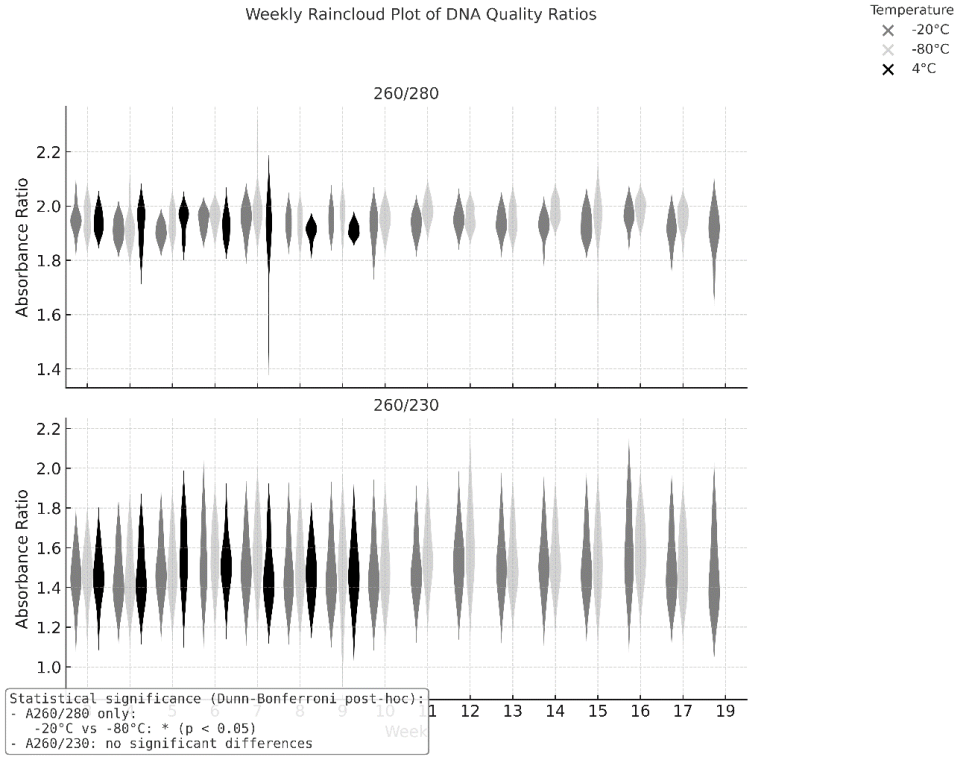

Figure 2. *The change in $A_{260/280}$ and $A_{260/230}$ ratios of DNA stored at either +4◦C, -20◦C or -80◦C*

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The descriptive statistic of DNA yield and quality parameters (A260/280 and A260/280 ratios) for each storage temperature condition are shown in *Table 1*. Dunn-Bonferroni Post-hoc comparison results are given in *Table 2*.

Agarose gel electrophoresis results indicated that some of the samples stored at -20°C and -80°C showed signs of DNA fragmentation, while those stored at +4°C remained intact (*Fig. 3*).

Table 1. *Summary Statistics for DNA Quality and Yield*

Variable	Statistic	4°C	-20°C	-80°C
Yield (ng/µl)	Min	39.4	22.96	27.6
	Max	1760.83	130.83	148.5
	Median	94.98	67.37	75.35
	Mean ± SD	165.09 ± 281.08	69.62 ± 23.26	75.67 ± 24.65
$A_{260/280}$	Min	1.55	1.73	1.66
	Max	2.02	2.04	2.22
	Median	1.93	1.94	1.96
	Mean ± SD	1.92 ± 0.07	1.93 ± 0.05	1.96 ± 0.06
$A_{260/230}$	Min	1.23	1.22	1.13
	Max	1.78	1.9	3.64
	Median	1.48	1.47	1.51
	Mean ± SD	1.49 ± 0.14	1.5 ± 0.15	1.54 ± 0.22

Table 2. *Dunn-Bonferroni Post-hoc Comparison Results*

Measurement	Comparison	Adjusted p-value	Significance
DNA Yield	4°C vs -20°C	0.023	*
	4°C vs -80°C	0.008	**
	-20°C vs -80°C	0.019	*
A260/280	-20°C vs -80°C	0.017	*
A260/230	All comparisons	> 0.05	ns

Adjusted p-values were calculated using the Dunn-Bonferroni method following Kruskal-Wallis tests. '*' indicates $p < 0.05$, '**' indicates $p < 0.01$, and 'ns' stands for non-significant

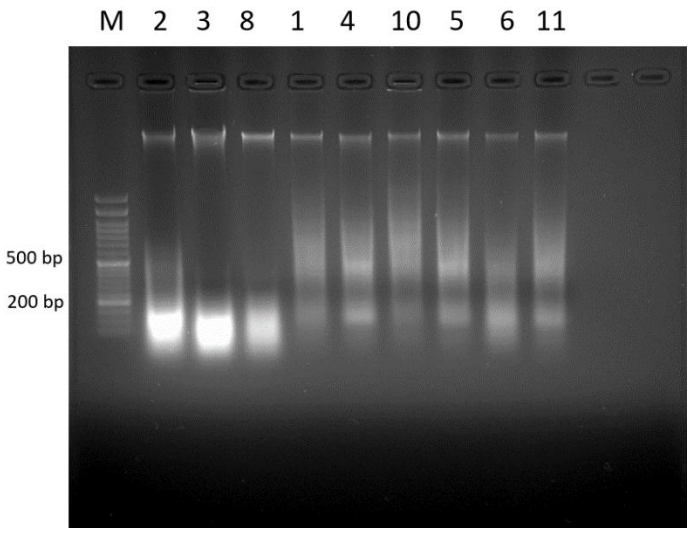

Figure 3. *Agarose gel electrophoresis result for the samples stored at +4°C (2,3,8), -20°C (1,4,10) and -80°C (5,6,11)*

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The PCR bands belonging to the trnL gene indicated that the over 500 bp region could be amplified successfully (*Fig. 4*). Despite the compromised DNA integrity in some samples, all of the samples could produce the desired band after PCR amplification regardless of storage temperature.

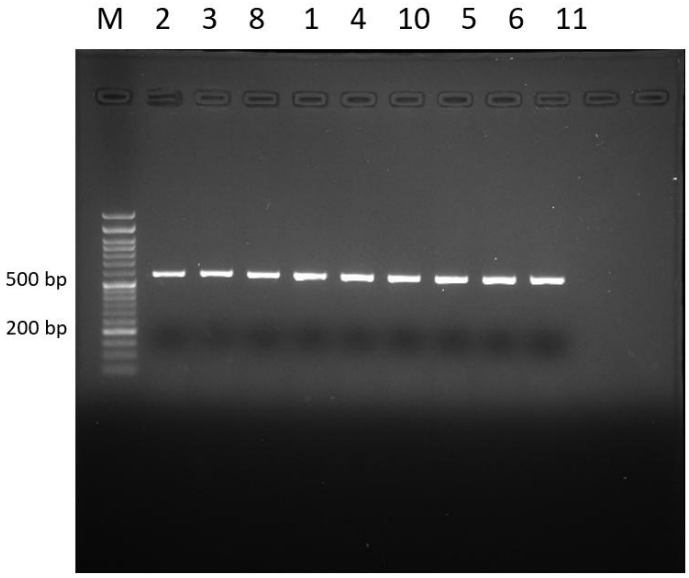

Figure 4. *PCR amplification result for the samples stored at 4°C (2,3,8), -20°C (1,4,10) and -80°C (5,6,11)*

Discussion

The quality and quantity of DNA are of great importance for downstream analyses such as PCR, sequencing, and cloning (Baptista et al., 2021; Karstens et al., 2021). In this study, we analyzed the effect of storage temperature on the quality and yield of DNA isolated from *Ficus carica* leaves. Our findings demonstrate significant differences in DNA quality and quantity between samples stored at different temperatures (+4°C, -20°C, and -80°C).

In addition to different storage temperatures, the buffers used for DNA extraction are also crucial for DNA quality and yield. In an earlier study, the effect of a particular buffer on the storage of DNA at different temperatures (-20 °C, 25 °C, 37 °C, and 50 °C) was examined, and the study found that samples stored in the storage buffer at room temperature gave comparable results to -20 °C controls in terms of DNA yield and quality (Howlett et al., 2014). In another study, when DNA was stored at different temperatures for 24 hours before isolation and a particular detergent-based lysis buffer was used for extraction, greater amounts of DNA were obtained at -20°C compared to higher temperatures (25 °C , 37 °C, and 50 °C) (Aloraer et al., 2017).

There is an increasing attempt to reduce the financial burden of storing at lower temperatures. In this study, the researchers attempted to optimize -20 °C storage conditions to find an alternative storage condition for DNA. The concentration and purity of DNA undiluted in ethanol and stored at -20°C for up to 12 months were found to be maintained at high levels compared to -80 °C (Hanzer and Duka, 2024). Another study examined the storage capability of −70 °C using tissue or cells from different organisms. The findings revealed that the stability of nucleic acids as well as prokaryotic

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communities was well preserved at −70 °C (Landor et al., 2024). In a recent study, air-drying at room temperature and freezing in liquid nitrogen (-30 °C) storage conditions gave the optimal results in terms of the quality and quantity of DNA (Shahzad et al., 2024). In line with these results, we found that lower storage temperatures, especially -80 °C, are more effective in preserving DNA quality. At the same time, the highest DNA yield was obtained from samples stored at +4 °C, which suggests that while the DNA may be more fragmented at this temperature, it might still be useful for short-term use when the financial burden of storage needs to be decreased.

In our study, significant differences in the A260/A280 ratio between -20 °C and -80 °C as well as between -80 °C and +4 °C indicated that DNA stored at -80 °C retained the highest quality. Our results suggest that while DNA quality was better preserved at lower temperatures, the DNA yield might be increased at higher temperatures. This could be due to slower DNA degradation at lower temperatures, resulting in less fragmentation and a lower optical absorption at 260 nm. DNA was stored after isolation, meaning that the samples themselves were not stored before extraction. As such, the hypothesis that higher temperature yields data results because of the easier extraction due to sample degradation does not apply here. Instead, the higher yield observed at higher temperatures (+4 °C) might be attributed to the more concentrated DNA due to evaporation of water inside the sample.

Previous reports examining the effect of repeated freeze-thaw cycles on the quality and quantity of DNA are conflicting. While some studies found no deterioration in DNA yield and quality after up to 100 freeze-thaw cycles (Safarikova et al., 2021), earlier studies reported a decline in both DNA quality and quantity after 18 cycles (Shao et al., 2012). We also subjected our samples stored at -20 °C and -80 °C to multiple freeze-thaw cycles, which might contribute to the DNA degradation that was observed in our experiments.

Conclusion

Future studies could investigate the effect of repeated freeze-thaw cycles on DNA integrity, as well as explore intermediate temperatures of storage, which could provide further insights into optimizing DNA preservation. These studies could have valuable implications for downstream applications, including but not limited to PCR, sequencing, and STR typing.

In conclusion, our findings reinforce the importance of low temperatures for the long-term storage of isolated DNA to ensure both quality and usability in downstream applications. Our results suggest that storage temperature significantly affects the quality and yield of DNA, with -80°C providing the best condition for preserving DNA integrity, while higher temperatures may lead to increased fragmentation and reduced overall quality. Careful management of storage conditions is essential for maintaining high-quality DNA for molecular analyses.

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APPENDIX

Supplementary Table 1. *Initial concentrations of samples used for DNA gel electrophoresis*

Sample Number	Temperature (°C)	Yield
1	+4	67,6
1	-20	63,0
1	-80	63,9
2	+4	130,2
2	-20	124,3
2	-80	118,8
3	+4	76,0
3	-20	73,9
3	-80	74,2
4	+4	78,4
4	-20	153,2
4	-80	74,2
5	+4	100,9
5	-20	86,9
5	-80	87,0
6	+4	33,9
6	-20	32,2
6	-80	30,3
8	+4	55,3
8	-20	55,5
8	-80	57,8
9	+4	79,2
9	-20	77,9
9	-80	78,7
10	+4	85,2
10	-20	75,8
10	-80	80,1
11	+4	65,4
11	-20	65,5
11	-80	66,7

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Supplementary Table 2. *DNA Yield and Quality Measurements by Storage Condition. This table presents all measured data from Ficus carica leaf samples stored at 4°C, -20°C, and -80°C. Grouped values include DNA yield, and absorbance ratios A260/280 and A260/230*

Sample no.	Date	+4°C			-20°C			-80°C		
		Yield	260/280	260/230	Yield	260/280	260/230	Yield	260/280	260/230
1	17/01/22	65.46	1.89	1.23	61.86	1.87	1.22	61.53	1.91	1.25
	24/01/22	77.88	1.96	1.36	64.83	1.91	1.32	66.4	1.89	1.35
	31/01/22	85.55	1.97	1.39	65.62	1.9	1.36	63.6	1.86	1.37
	07/02/22	113.78	1.96	1.41	63.87	1.92	1.35	68.88	1.96	1.42
	14/02/22	155.33	1.84	1.31	62.32	1.94	1.34	66.75	1.96	1.44
	21/02/22	159.07	1.91	1.37	64.9	1.92	1.34	64.6	1.91	1.39
	28/02/22	103.0	1.95	1.39	65.27	1.95	1.35	70.0	1.96	1.4
	07/03/22	NA	NA	NA	59.35	1.9	1.32	65.55	1.94	1.39
	14/03/22	NA	NA	NA	59.38	1.93	1.36	68.68	1.97	1.42
	21/03/22	NA	NA	NA	59.2	1.94	1.36	68.97	1.94	1.43
	28/03/22	NA	NA	NA	58.88	1.93	1.36	62.32	1.92	1.4
	04/04/22	NA	NA	NA	58.37	1.92	1.36	68.7	1.96	1.42
	11/04/22	NA	NA	NA	55.37	1.93	1.34	73.6	1.94	1.42
	18/04/22	NA	NA	NA	58.9	1.95	1.38	79.0	1.98	1.44
	25/04/22	NA	NA	NA	59.67	1.91	1.35	67.5	1.92	1.37
	29/04/22	NA	NA	NA	60.65	1.88	1.33	69.37	1.94	1.38
	09/05/22	NA	NA	NA	56.17	1.9	1.32	NA	NA	NA
2	17/01/22	129.0	1.97	1.6	118.44	1.96	1.59	121.89	1.99	1.61
	24/01/22	142.83	2.0	1.68	130.83	1.96	1.66	128.67	1.93	1.68
	31/01/22	167.5	1.97	1.72	122.83	1.95	1.69	125.5	1.91	1.72
	07/02/22	188.17	1.87	1.63	122.67	1.98	1.69	124.33	2.01	1.74
	14/02/22	1215.5	2.02	1.74	118.83	2.01	1.7	126.33	2.01	1.8
	21/02/22	199.33	1.9	1.64	125.67	1.99	1.69	131.0	1.98	1.72
	28/02/22	218.0	1.9	1.72	123.0	2.0	1.67	135.33	2.02	1.74
	07/03/22	NA	NA	NA	129.0	1.99	1.67	126.5	2.0	1.72
	14/03/22	NA	NA	NA	110.87	1.99	1.69	133.83	2.02	1.75
	21/03/22	NA	NA	NA	115.73	1.98	1.68	134.83	2.0	1.78
	28/03/22	NA	NA	NA	123.67	2.0	1.71	132.0	2.0	1.75
	04/04/22	NA	NA	NA	121.9	1.98	1.72	131.83	2.02	1.75
	11/04/22	NA	NA	NA	122.0	2.0	1.72	127.72	2.0	1.76
	18/04/22	NA	NA	NA	124.38	2.02	1.75	148.5	2.02	1.78
	25/04/22	NA	NA	NA	130.67	1.99	1.73	147.67	1.99	1.74
	29/04/22	NA	NA	NA	129.5	1.98	1.72	145.83	1.98	1.73
	09/05/22	NA	NA	NA	130.5	2.0	1.73	NA	NA	NA
3	17/01/22	79.13	1.96	1.46	75.5	1.95	1.47	75.88	1.99	1.5
	24/01/22	84.58	1.95	1.49	79.08	1.93	1.48	78.93	1.92	1.52
	31/01/22	95.37	1.97	1.56	75.23	1.9	1.52	72.22	1.97	1.54
	07/02/22	119.88	1.95	1.57	73.8	1.95	1.53	74.22	1.97	1.57
	14/02/22	151.67	1.91	1.54	77.75	1.97	1.52	70.15	1.99	1.62
	21/02/22	143.83	1.93	1.54	78.68	1.96	1.5	77.85	1.97	1.56
	28/02/22	161.67	1.92	1.53	78.42	1.96	1.5	79.08	1.99	1.55
	07/03/22	NA	NA	NA	75.95	1.95	1.49	75.53	1.97	1.56

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Sample no.	Date	+4°C			-20°C			-80°C		
		Yield	260/280	260/230	Yield	260/280	260/230	Yield	260/280	260/230
	14/03/22	NA	NA	NA	77.33	1.95	1.52	74.93	1.99	1.59
	21/03/22	NA	NA	NA	77.65	1.96	1.52	78.82	1.99	1.62
	28/03/22	NA	NA	NA	74.88	1.95	1.53	73.98	1.97	1.57
	04/04/22	NA	NA	NA	73.45	1.94	1.54	76.4	2.0	1.59
	11/04/22	NA	NA	NA	77.62	1.95	1.54	79.5	1.99	1.61
	18/04/22	NA	NA	NA	75.0	1.97	1.57	84.37	2.01	1.64
	25/04/22	NA	NA	NA	77.37	1.94	1.55	87.42	2.0	1.59
	29/04/22	NA	NA	NA	75.23	1.93	1.55	82.17	1.98	1.6
	09/05/22	NA	NA	NA	72.77	1.94	1.54	NA	NA	NA
	17/01/22	75.53	1.96	1.41	73.7	1.94	1.42	73.39	1.97	1.43
	24/01/22	94.58	1.96	1.45	77.33	1.92	1.41	75.72	2.05	1.42
	31/01/22	99.08	1.96	1.48	71.27	1.9	1.44	77.12	1.98	1.46
	07/02/22	93.05	1.96	1.47	78.85	1.96	1.47	78.38	1.97	1.46
	14/02/22	137.02	1.89	1.4	73.13	1.96	1.45	72.97	1.97	1.48
	21/02/22	135.17	1.92	1.43	76.43	1.95	1.44	77.82	1.95	1.46
	28/02/22	156.65	1.88	1.38	77.47	1.96	1.44	78.58	1.98	1.45
	07/03/22	NA	NA	NA	74.93	1.96	1.43	69.62	1.95	1.43
4	14/03/22	NA	NA	NA	75.65	1.95	1.45	75.78	1.99	1.48
	21/03/22	NA	NA	NA	69.68	1.94	1.44	75.15	1.94	1.49
	28/03/22	NA	NA	NA	66.9	1.94	1.48	75.63	1.96	1.46
	04/04/22	NA	NA	NA	67.13	1.94	1.49	78.9	2.0	1.48
	11/04/22	NA	NA	NA	63.08	1.94	1.48	78.12	1.97	1.49
	18/04/22	NA	NA	NA	68.1	1.95	1.51	80.0	2.0	1.51
	25/04/22	NA	NA	NA	67.75	1.92	1.5	80.78	1.98	1.48
	29/04/22	NA	NA	NA	69.85	1.92	1.47	79.32	1.95	1.48
	09/05/22	NA	NA	NA	67.64	1.9	1.38	NA	NA	NA
	17/01/22	91.38	2.0	1.55	79.06	1.99	1.54	79.51	2.03	1.57
	24/01/22	128.0	1.99	1.58	87.1	1.97	1.56	86.3	1.94	1.57
	31/01/22	154.17	1.97	1.64	81.2	1.94	1.57	82.33	2.0	1.59
	07/02/22	197.5	1.9	1.56	86.0	1.99	1.6	82.3	2.0	1.61
	14/02/22	1306.5	1.55	1.41	79.57	1.99	1.59	80.52	2.01	1.66
	21/02/22	153.18	1.94	1.51	83.52	1.98	1.56	86.48	1.99	1.6
	28/02/22	NA	NA	NA	83.5	1.99	1.57	86.33	2.01	1.6
	07/03/22	NA	NA	NA	82.78	1.96	1.57	77.4	1.99	1.6
5	14/03/22	NA	NA	NA	81.28	1.96	1.57	89.8	2.05	1.65
	21/03/22	NA	NA	NA	83.98	1.99	1.6	87.0	2.01	1.66
	28/03/22	NA	NA	NA	76.05	1.97	1.6	88.67	2.0	1.62
	04/04/22	NA	NA	NA	73.78	1.96	1.6	86.37	2.03	1.62
	11/04/22	NA	NA	NA	72.27	1.97	1.61	92.78	2.01	1.64
	18/04/22	NA	NA	NA	77.38	2.0	1.64	94.55	2.02	1.66
	25/04/22	NA	NA	NA	71.38	1.93	1.59	95.23	2.01	1.63
	29/04/22	NA	NA	NA	81.73	1.96	1.61	94.17	2.0	1.62
	09/05/22	NA	NA	NA	79.92	1.96	1.58	NA	NA	NA
6	17/01/22	42.04	1.9	1.65	38.75	1.91	1.64	39.46	1.88	1.67
	24/01/22	39.4	1.8	1.34	32.65	1.87	1.36	29.63	1.92	1.35

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Sample no.	Date	+4°C			-20°C			-80°C		
		Yield	260/280	260/230	Yield	260/280	260/230	Yield	260/280	260/230
	31/01/22	42.73	2.0	1.79	31.35	1.92	1.47	30.8	1.91	1.38
	07/02/22	50.64	1.89	1.45	30.58	1.97	1.84	31.23	1.96	1.68
	14/02/22	55.83	1.94	1.38	28.71	1.85	1.52	31.7	2.22	3.64
	21/02/22	66.91	1.84	1.3	31.88	1.88	1.34	30.64	1.86	1.33
	28/02/22	98.79	1.89	1.23	31.84	1.89	1.29	31.75	1.87	1.29
	07/03/22	NA	NA	NA	29.61	1.8	1.38	27.6	1.88	1.33
	14/03/22	NA	NA	NA	26.48	1.91	1.45	27.64	1.97	1.54
	21/03/22	NA	NA	NA	24.98	1.96	1.54	30.38	1.91	1.95
	28/03/22	NA	NA	NA	25.61	1.92	1.56	32.76	1.88	1.44
	04/04/22	NA	NA	NA	24.76	1.91	1.53	32.76	1.96	1.46
	11/04/22	NA	NA	NA	23.04	1.9	1.43	29.58	1.66	1.31
	18/04/22	NA	NA	NA	26.71	1.98	1.9	30.41	1.98	1.68
	25/04/22	NA	NA	NA	26.03	1.85	1.36	30.91	1.88	1.28
	29/04/22	NA	NA	NA	27.08	1.8	1.33	38.34	1.85	1.31
	09/05/22	NA	NA	NA	22.96	1.73	1.38	NA	NA	NA
	17/01/22	56.62	1.91	1.39	56.22	1.93	1.36	56.77	1.91	1.42
	24/01/22	61.8	1.89	1.42	57.25	1.87	1.39	54.48	1.85	1.44
	31/01/22	64.03	1.9	1.5	57.38	1.87	1.44	54.93	1.91	1.48
	07/02/22	69.48	1.92	1.48	56.88	1.9	1.45	55.63	1.92	1.51
	14/02/22	71.22	1.94	1.48	55.5	1.93	1.44	53.22	1.94	1.55
	21/02/22	81.27	1.9	1.47	56.17	1.9	1.42	51.37	1.89	1.47
	28/02/22	90.42	1.92	1.45	55.82	1.92	1.42	57.22	1.92	1.48
	07/03/22	NA	NA	NA	55.38	1.9	1.43	57.05	1.93	1.5
8	14/03/22	NA	NA	NA	53.92	1.91	1.43	59.55	1.97	1.54
	21/03/22	NA	NA	NA	50.83	1.92	1.62	58.5	1.93	1.57
	28/03/22	NA	NA	NA	49.33	1.91	1.44	57.15	1.91	1.5
	04/04/22	NA	NA	NA	52.53	1.91	1.46	58.7	1.95	1.51
	11/04/22	NA	NA	NA	46.68	1.88	1.46	57.48	1.94	1.52
	18/04/22	NA	NA	NA	53.72	1.95	1.49	64.0	1.95	1.52
	25/04/22	NA	NA	NA	53.28	1.89	1.45	63.8	1.93	1.48
	29/04/22	NA	NA	NA	54.32	1.91	1.43	75.1	1.94	1.57
	09/05/22	NA	NA	NA	48.67	1.89	1.28	NA	NA	NA
	17/01/22	87.47	2.01	1.37	81.5	2.04	1.38	82.8	2.01	1.4
	24/01/22	85.13	1.99	1.7	75.37	1.95	1.66	77.6	1.94	1.69
	31/01/22	90.95	1.97	1.76	79.37	1.94	1.71	73.9	2.0	1.71
	07/02/22	110.95	2.01	1.76	64.82	1.98	1.75	74.87	1.99	1.72
	14/02/22	114.33	2.01	1.72	64.02	2.02	1.75	73.6	2.01	1.78
	21/02/22	138.93	1.93	1.67	68.15	2.0	1.74	75.15	1.99	1.72
9	28/02/22	176.5	1.9	1.61	68.82	2.02	1.74	79.2	2.02	1.72
	07/03/22	NA	NA	NA	65.45	1.99	1.75	75.77	2.01	1.73
	14/03/22	NA	NA	NA	61.8	1.98	1.74	74.47	2.03	1.77
	21/03/22	NA	NA	NA	60.12	2.02	1.8	77.82	2.0	1.78
	28/03/22	NA	NA	NA	57.22	2.0	1.79	79.37	2.02	1.75
	04/04/22	NA	NA	NA	55.87	1.96	1.77	78.28	2.03	1.75
	11/04/22	NA	NA	NA	55.23	2.0	1.78	81.95	2.02	1.78

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Sample no.	Date	+4°C			-20°C			-80°C		
		Yield	260/280	260/230	Yield	260/280	260/230	Yield	260/280	260/230
	18/04/22	NA	NA	NA	58.43	2.02	1.86	84.48	2.04	1.81
	25/04/22	NA	NA	NA	60.4	1.98	1.8	81.43	2.02	1.75
	29/04/22	NA	NA	NA	58.43	1.98	1.79	77.95	2.0	1.74
	09/05/22	NA	NA	NA	63.48	2.0	1.8	NA	NA	NA
	17/01/22	90.47	1.92	1.46	84.63	1.95	1.45	86.94	1.92	1.47
	24/01/22	105.27	1.86	1.29	81.28	1.86	1.28	80.02	1.85	1.28
	31/01/22	124.33	1.86	1.3	78.48	1.85	1.3	79.72	1.9	1.31
	07/02/22	178.33	1.86	1.3	77.18	1.89	1.31	75.17	1.89	1.32
	14/02/22	1760.83	1.94	1.32	72.92	1.89	1.29	82.67	1.92	1.34
	21/02/22	89.65	1.87	1.32	79.98	1.89	1.3	75.13	1.88	1.29
	28/02/22	NA	NA	NA	78.62	1.89	1.29	80.35	1.9	1.13
	07/03/22	NA	NA	NA	75.22	1.85	1.27	71.9	1.89	1.3
10	14/03/22	NA	NA	NA	72.6	1.86	1.3	78.88	1.92	1.33
	21/03/22	NA	NA	NA	70.53	1.89	1.32	80.5	1.9	1.35
	28/03/22	NA	NA	NA	60.22	1.86	1.31	77.85	1.9	1.33
	04/04/22	NA	NA	NA	64.52	1.83	1.29	81.07	1.93	1.32
	11/04/22	NA	NA	NA	66.22	1.87	1.32	88.27	1.92	1.34
	18/04/22	NA	NA	NA	70.68	1.88	1.31	79.83	1.93	1.35
	25/04/22	NA	NA	NA	66.73	1.83	1.3	90.88	1.93	1.35
	29/04/22	NA	NA	NA	75.53	1.84	1.3	92.15	1.92	1.36
	09/05/22	NA	NA	NA	68.62	1.84	1.3	NA	NA	NA
	17/01/22	62.59	1.94	1.47	61.19	1.95	1.46	62.73	1.93	1.5
	24/01/22	67.92	1.87	1.41	65.43	1.9	1.44	60.53	1.87	1.41
	31/01/22	66.9	1.88	1.49	58.7	1.88	1.45	67.3	1.94	1.49
	07/02/22	69.28	1.93	1.52	65.67	1.94	1.48	63.95	1.93	1.5
	14/02/22	69.03	1.96	1.49	63.15	1.94	1.48	64.38	1.96	1.54
	21/02/22	79.23	1.9	1.48	66.32	1.92	1.46	66.9	1.93	1.5
	28/02/22	88.28	1.93	1.47	66.95	1.92	1.45	66.78	1.96	1.48
	07/03/22	NA	NA	NA	67.6	1.91	1.46	63.62	1.94	1.49
11	14/03/22	NA	NA	NA	65.78	1.89	1.46	61.67	1.94	1.51
	21/03/22	NA	NA	NA	62.22	1.92	1.48	64.18	1.94	1.54
	28/03/22	NA	NA	NA	56.57	1.9	1.46	62.4	1.93	1.49
	04/04/22	NA	NA	NA	63.85	1.9	1.48	65.6	1.96	1.5
	11/04/22	NA	NA	NA	59.38	1.9	1.46	66.0	1.95	1.52
	18/04/22	NA	NA	NA	63.35	1.94	1.5	65.72	1.95	1.52
	25/04/22	NA	NA	NA	65.58	1.9	1.46	73.92	1.97	1.5
	29/04/22	NA	NA	NA	65.7	1.9	1.45	74.64	1.94	1.46
	09/05/22	NA	NA	NA	67.63	1.91	1.46	NA	NA	NA

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