결과 보고서

Sequencing Report

Project No. 2025MY073 **Reporting Date** 2025/08/21

Sample Information		Sequencing Information	
Species	Plant	**Library prep kit**	MGIEasy FS DNA Library Prep kit
Sample type	gDNA	**Platform**	MGI DNBSEQ-G400
Sample count	14	**Read type**	Paired-end 100 cycles

Customer Information 김상태 님 / 성신여자대학교

(주)엘에이에스 T. 02-3486-0911~2 | F. 031-986-1913 | www.las.kr

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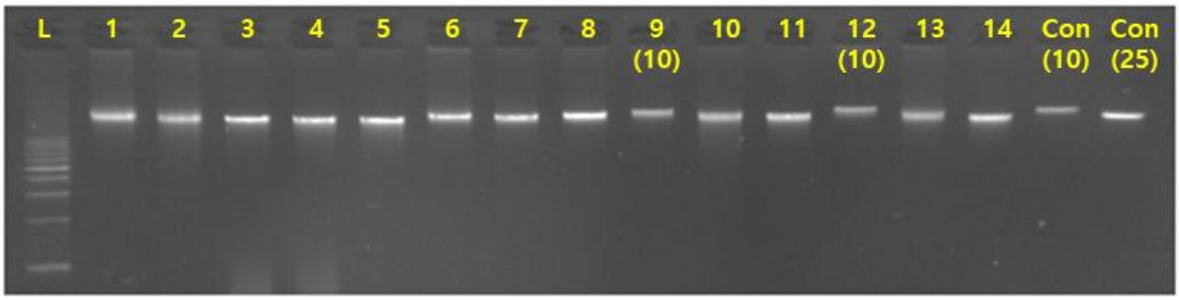
Sample Quality Control

▪ Summary

No.	Sample ID.	Trinean			Picogreen	Volume	Amount	결과
		DNA Conc. (ng/ul)	A260/ 280	A260/ 230	Conc. (ng/ul)	(ul)	(ng)	
1	C-len	718.75	1.86	2.43	325.41	17	12218.75	Pass
2	C-dis	1569.20	1.90	2.43	351.91	17	26676.40	Pass
3	C-boh	531.50	2.12	2.41	84.72	17	9035.50	Pass
4	C-bxm	319.85	2.07	2.34	75.31	17	5437.45	Pass
5	C-kuj	51.40	1.93	2.28	37.13	17	873.80	Pass
6	C-kob	150.95	1.82	2.48	66.87	17	2566.15	Pass
7	C-lig	195.65	1.89	2.45	48.87	17	3326.05	Pass
8	C-cili	394.60	1.84	2.27	206.22	17	6708.20	Pass
9	C-che	62.30	1.85	1.59	23.97	17	1059.10	Pass
10	L-acr	60.30	1.80	2.03	39.08	17	1025.10	Pass
11	V-car	61.65	1.95	1.43	77.89	17	1048.05	Pass
12	P-cuc	29.10	1.66	0.92	19.75	17	494.70	Pass
13	H-hon	203.80	1.83	1.93	117.76	17	3464.60	Pass
14	D-pan	97.85	1.85	1.93	98.04	17	1663.45	Pass

Sample Quality Control

- **Gel electrophoresis**



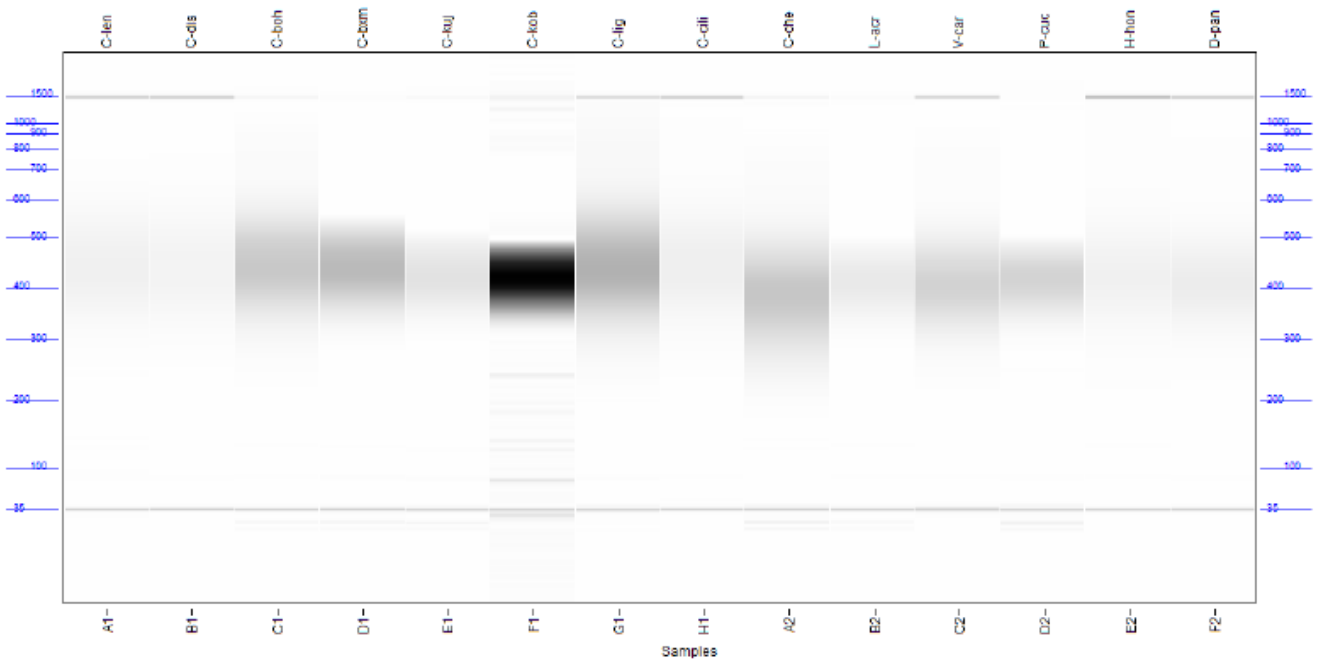
Library Construction

▪ Summary

No.	Sample ID.	Peak table			Region table					Adapter Index	Adapter Index Sequence
		Size (bp)	Conc. (ng/ul)	Molarity (nmol/l)	From (bp)	To (bp)	Average Size	Size distribution in CV(%)	Conc. (ng/ul)		
1	C-len	435	449.45	122.1	200	1000	447	19.6	122.05	41	TTAGATGCAT
2	C-dis	431	373.87	101.57	200	1000	447	20.67	101.48	42	GTCCAGAGCT
3	C-boh	439	7213.34	1994.67	200	1000	454	21.27	1989.29	43	CACGTGATAG
4	C-bxm	441	17372.84	4582.43	200	1000	435	12.18	4585.69	44	CCACTAGTCC
5	C-kuj	422	3690.12	939.72	200	1000	420	12.63	945.09	45	TGGACTTGGC
6	C-kob	423	8230.33	2080.93	200	1000	417	11.37	2109.12	46	GCTTGACAGG
7	C-lig	439	3001.34	835.41	200	1000	453	22.89	828.83	47	AAGACCTCTA
8	C-cili	436	498.29	134.46	200	1000	455	22.65	140.89	48	AGTTGCCATA
9	C-che	388	9641.19	2267.82	200	1000	388	23.19	2257.66	57	ATTCAACGGA
10	L-acr	416	1030.31	5.01	200	1000	407	11	1031.25	58	AACTGTACTG
11	V-car	408	1498.29	376.09	200	1000	413	20.6	375.73	59	GTACCTCAAT
12	P-cuc	422	15776.78	3931.45	200	1000	410	11.65	3947.57	60	GACTTCTAAT
13	H-hon	420	309.05	79.08	200	1000	437	25.42	82.88	61	TGAAGCGTTG
14	D-pan	412	536	136.82	200	1000	420	21.13	136.72	62	CGTGCGATCC

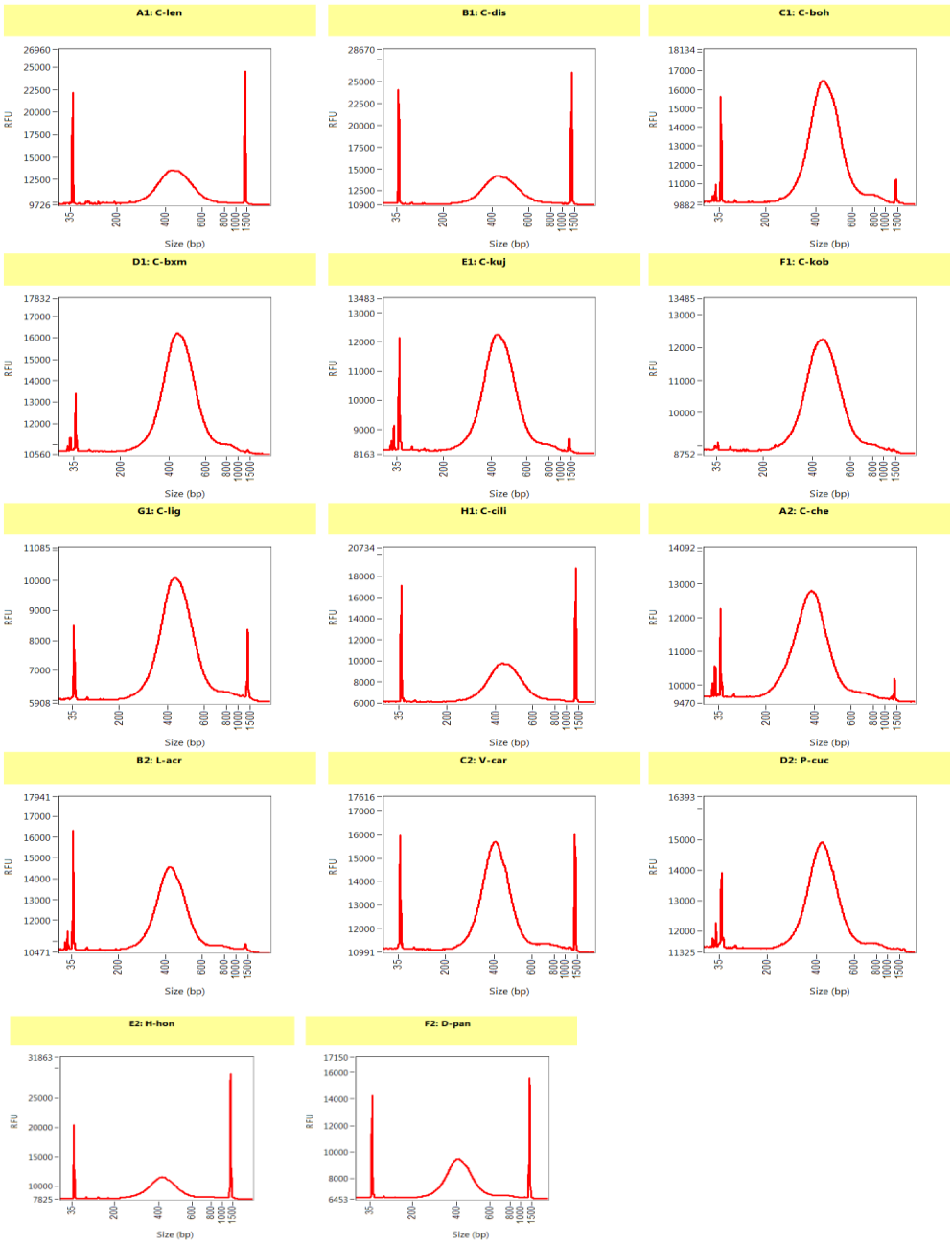
Library Construction

- **Gel electrophoresis**



Library Construction

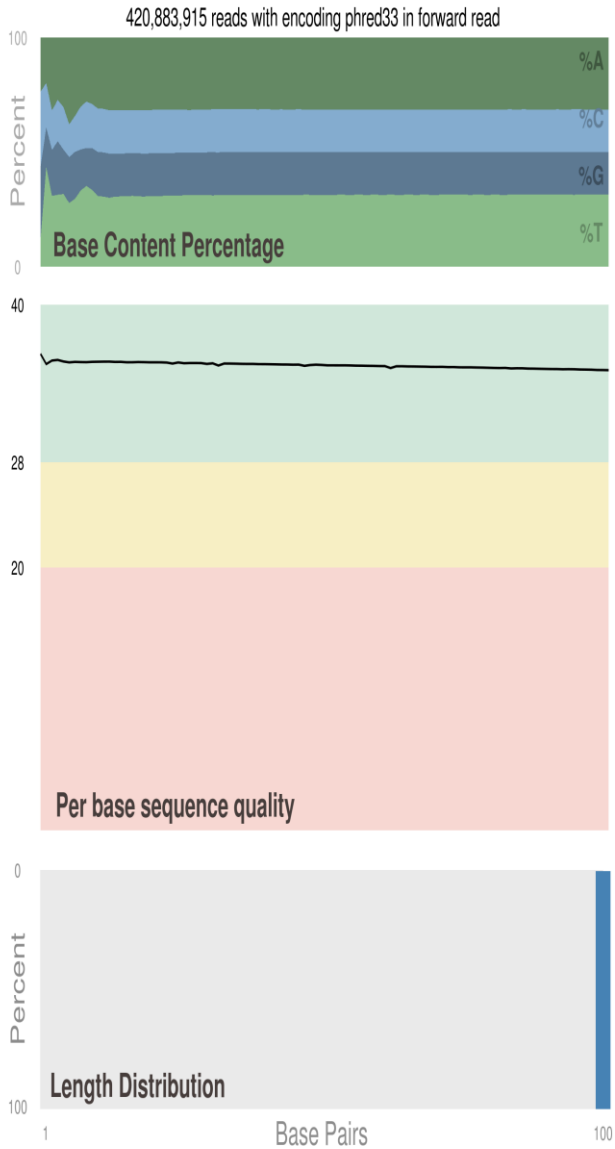
▪ Electropherogram

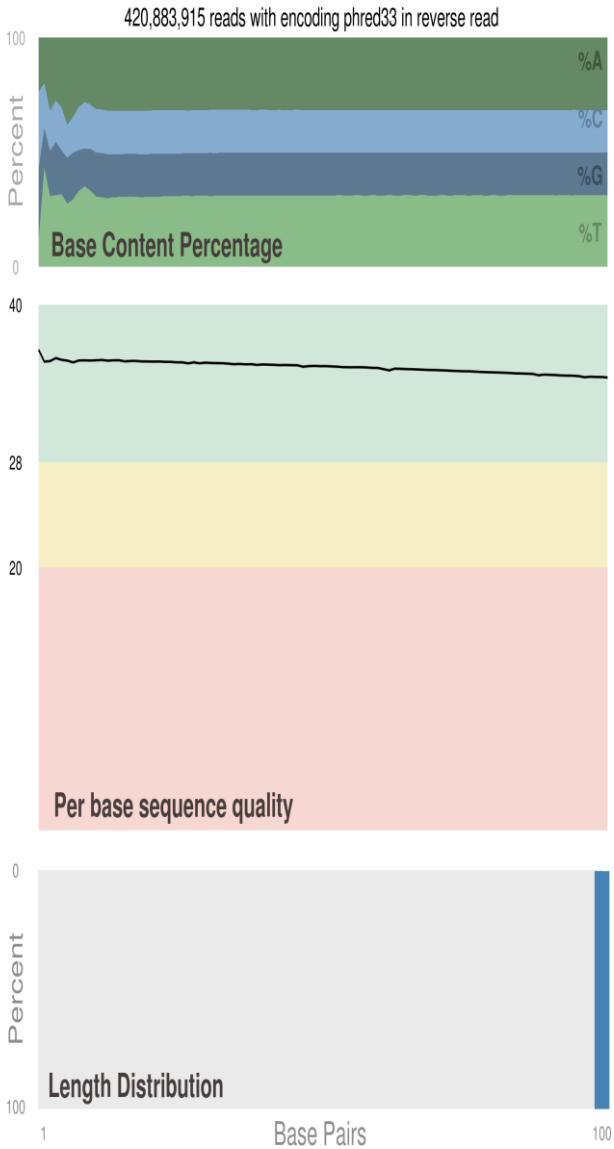


Sequencing Quality

▪ Project sequencing quality

Sample	Read-pairs	Yield (bases)	Over_Q30-Bases (%)	Mean-Quality-Score	Read-length	Run-type
total	420883915	84176783000	94.62	35.31	2x100	Paired-end





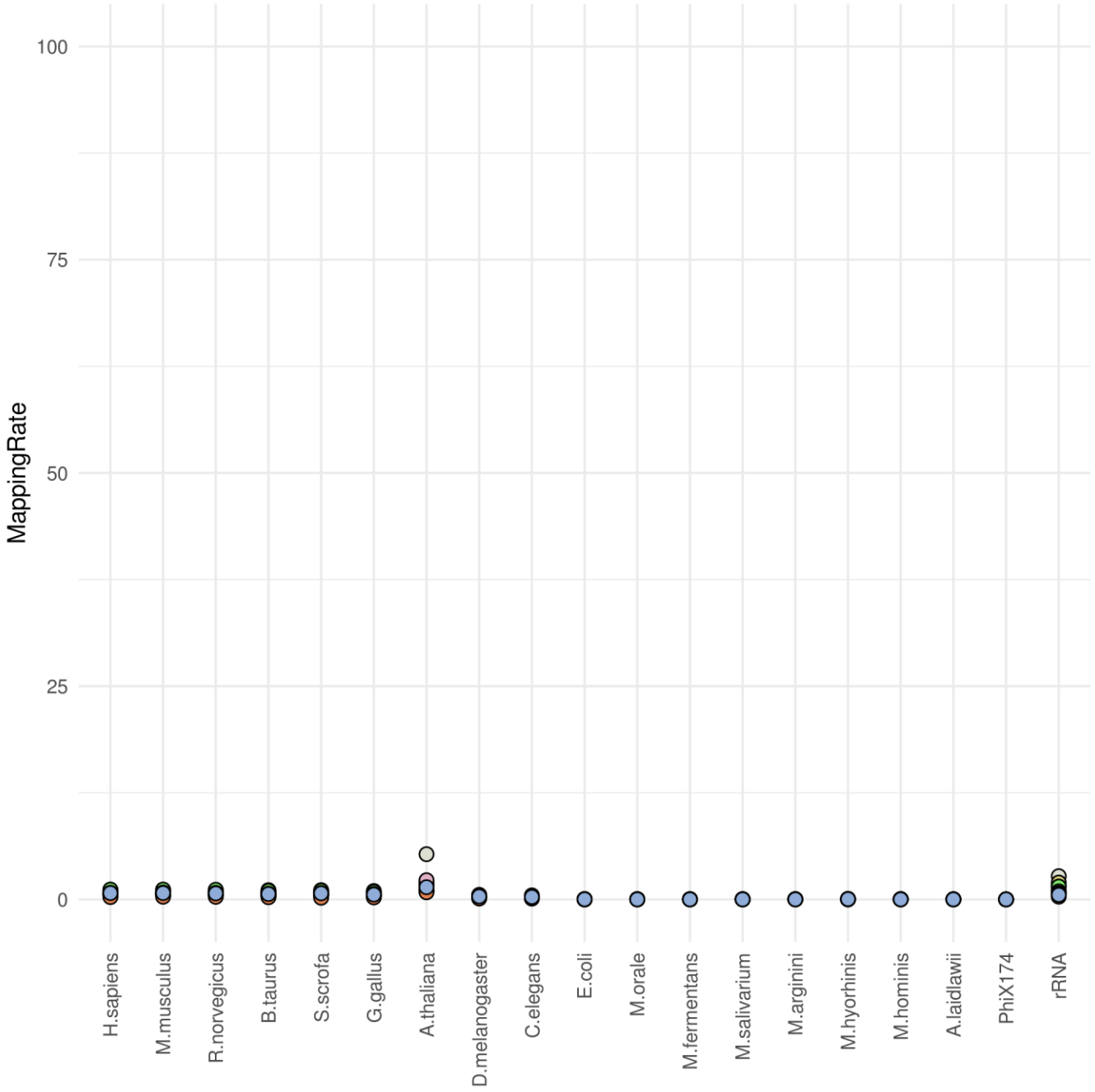
Sequencing Quality

▪ Sample sequencing quality

Sample	Read-pairs	Yield (bases)	Over_Q30-Bases (%)	Mean-Quality-Score	Read-length	Run-type
C-boh	27321944	5464388800	94.05	35.1	2x100	Paired-end
C-bxm	32568419	6513683800	93.55	34.95	2x100	Paired-end
C-che	33769454	6753890800	95.8	35.65	2x100	Paired-end
C-cili	30756850	6151370000	94.45	35.25	2x100	Paired-end
C-dis	29989682	5997936400	94.1	35.15	2x100	Paired-end
C-kob	28565183	5713036600	93.85	35.05	2x100	Paired-end
C-kuj	25271357	5054271400	93.95	35.1	2x100	Paired-end
C-len	24909011	4981802200	94.2	35.15	2x100	Paired-end
C-lig	30703704	6140740800	94.05	35.15	2x100	Paired-end
D-pan	33025623	6605124600	95.35	35.55	2x100	Paired-end
H-hon	26903997	5380799400	95.25	35.6	2x100	Paired-end
L-acr	32275407	6455081400	95	35.4	2x100	Paired-end
P-cuc	31172036	6234407200	95.6	35.75	2x100	Paired-end
V-car	33651248	6730249600	95.5	35.55	2x100	Paired-end

Sequencing Quality

- **Fastq_screen**



Experimental Workflow

Sample QC

- OD 측정 : Dropsense96 (Trinean)
- 형광 정량 : Picogreen (Invitrogen)
- Quality check : Gel Electrophoresis

Library prep.

- MGIEasy DNA Library Prep kit (MGI)
- Library QC : Bioanalyzer DNA 1000 Chip (Agilent Technologies)

Sequencing

- DNBSEQ-G400 (MGI)

Equipment & Reagent

▪ Library preparation

Consumables	Supplier
MGIEasy DNA Library Prep Kit	MGI
Agencourt AMPure XP	Beckman Coulter Genomics
2100 Bioanalyzer Desktop system	Agilent Technologies
Agilent DNA Chips	Agilent Technologies
Agilent DNA 1000 Reagents	Agilent Technologies

▪ Sequencing

Consumables	Supplier
DNBSEQ-G400 Sequencing System	MGI
DNBSEQ-G400RS Sequencing Flow Cell	MGI
DNBSEQ-G400RS High-throughput Sequencing Kit	MGI

Experimental Methods

▪ Library preparation

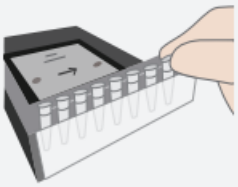
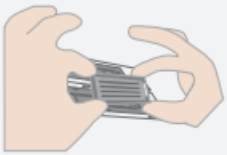
MGIEasy DNA Library Preparation Workflow
DNA fragmentation
Size selection and Normalization
End Repair and A Tailing
PCR enrichment and Normalization
Circularization
Digestion
DNB (DNA nanoball) preparation

Experimental Methods

▪ Sequencing

https://en.mgi-tech.com//Uploads/Temp/file/20221012/63461c1ccd634.pdf

DNBSEQ-G400 Sequencing Workflow

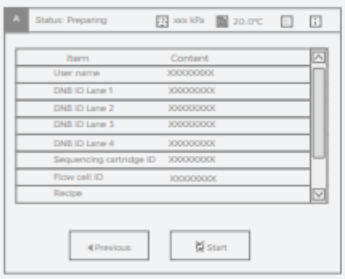
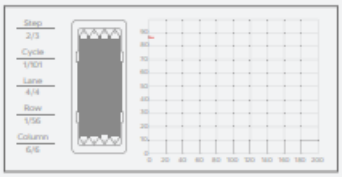
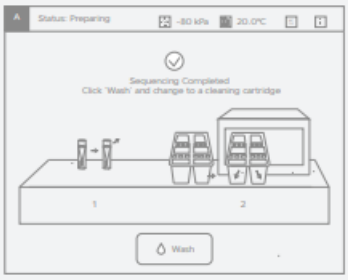
	Making DNB: use DNB preparation reagents for making DNB.
	Preparing a new Flow Cell: take out the Flow Cell from package and inspect to ensure the Flow Cell is intact.
	DNB loading: load the DNB onto sequencing Flow Cell.
	Preparing a new reagent cartridge: inspect and thaw the reagent cartridge and then load and mix the necessary reagents.
	Loading the Flow Cell: place the Flow Cell on the stage of the sequencer.
	Loading the reagent cartridge into the sequencer.

Experimental Methods

▪ Sequencing

https://en.mgi-tech.com//Uploads/Temp/file/20221012/63461c1ccd634.pdf

DNBSEQ-G400 Sequencing Workflow

	Starting sequencing: follow the user manual to enter sequencing information and start the run.
	Sequencing: monitor the sequencing run from the control software interface.
	Data analysis: the sequencer will automatically split barcode (if Split barcode is selected) and output FASTQ files when sequencing is completed.
	Device maintenance: perform device maintenance when sequencing is completed.

Experimental Methods

▪ Method for paper

Library preparation and Sequencing

gDNA (1 μg) was sheared using the S220 Ultra sonicator (Covaris). Library preparation was performed with MGIEasy DNA library prep kit (MGI) according to the manufacturer's instructions. Briefly, after size-selection of fragmented gDNA using AMPure XP magnetic beads, the fragmented gDNA was end-repaired and a-tailed at 37 °C for 30 min, and 65 °C for 15 min. Indexing adapter was ligated to the ends of the DNA fragments at 23 °C for 30 min. After cleanup of adapter-ligated DNA, PCR was performed to enrich those DNA fragments that have adapter molecules. Thermocycler conditions were as follows: 95 °C for 3 min, 7 cycles of 98 °C for 20 s, 60 °C for 15 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. The double stranded library is quantified using QuantiFluor ONE dsDNA System (Promega). The library is circularized at 37 °C for 30 min, and then digested at 37 °C for 30 min, followed by cleanup of circularization product. To make DNA nanoball (DNB), the library is incubated at 30 °C for 25 min using DNB enzyme. Finally, library was quantified by QuantiFluor ssDNA System (Promega). Sequencing of the prepared DNB was conducted on the DNBSEQ platform (MGI) with 150 bp paired-end reads.

Experimental Methods

▪ Adapter sequences for bioinformatics

- **Paired end read**

a1=AAGTCGGAGGCCAAGCGGTCTTAGGAAGACAA
# a1: Common Region of MGISEQ Adapter of Read 1

a2=AAGTCGGATCGTAGCCATGTCGTTCTGTGAGCCAAGGAGTTG
# a2: Common Region of MGISEQ Adapter of Read 2