

# Summary Report

Date: 2015.03.25

● Summary Table

Sample	Type	Expectation			Sanger			DNArails					
								SNV Calling Software (Samtools + Varscan)			DNArails		
		DEL19	T790M	L858R	DEL19	T790M	L858R	DEL19	T790M	L858R	DEL19	T790M	L858R
HS-H0	ALL	○ (2%)	○ (1%)	○ (3%)	○	○	○	S+V	S+V		○	○	○
HS-S1	WT							S					
HS-S2	DEL19	○ (4%)			○			S+V			○		
HS-S3	T790M		○ (6.5%)			○		S+V	S+V			○	
HS-S4	L858R			○ (6%)			○	S		V			○

● Recommendation

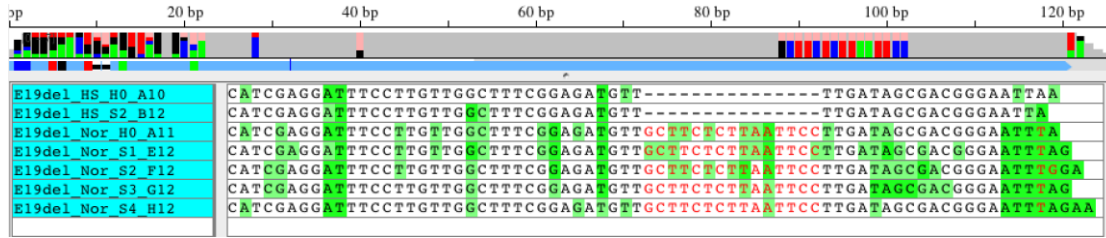
- Raw data from “FemtoPath mutation enrichment assays” plus “Illumina MiSeq” can significantly locate mutations from EGFR exon 19 deletions, exon 20 T790M, and exon 21 L858R by using DNArails structure variation detection tools (Gengnir tool software) auto-interpretation.
- Candidate sites (T790M and L858R) can solely detected by single-end sequencing from foreword strands. The performance is comparable to paired-end result.
- These ten samples for Illumina MiSeq analysis can achieve the same consistency with Sanger’s sequencing at the predefined cutoff.

● Cutoff Parameter:

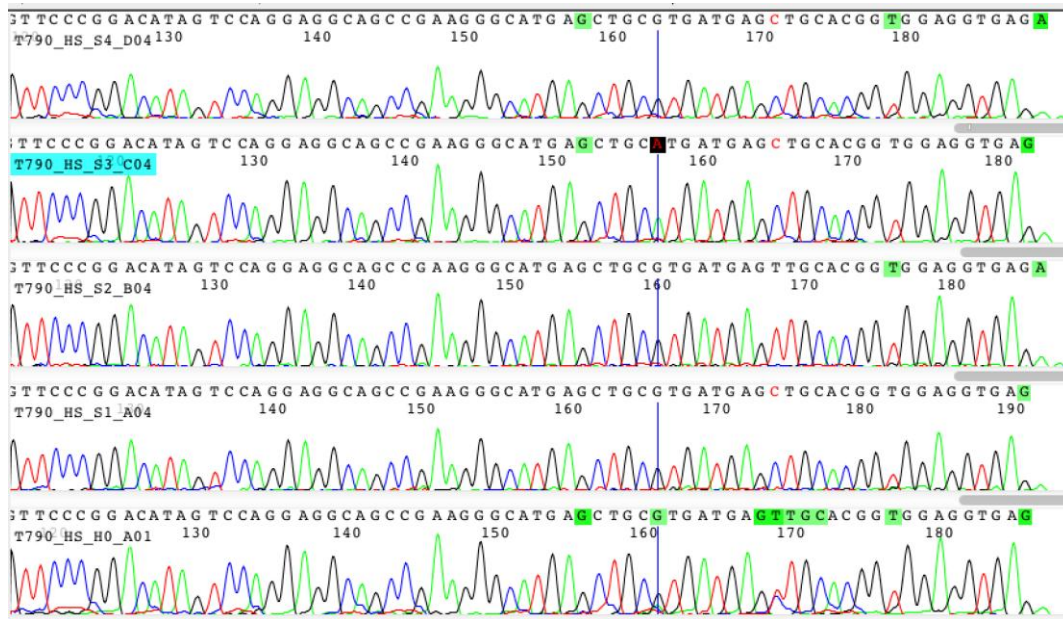
Locus	DEL19	T790M	L858R
LoD (sensitivity)	0.5%	1%	3%
Reading depth	>200	>200	>200
Frequency	>80%	>10%	>5%
Mutation times	>25	>25	>25

- Sanger

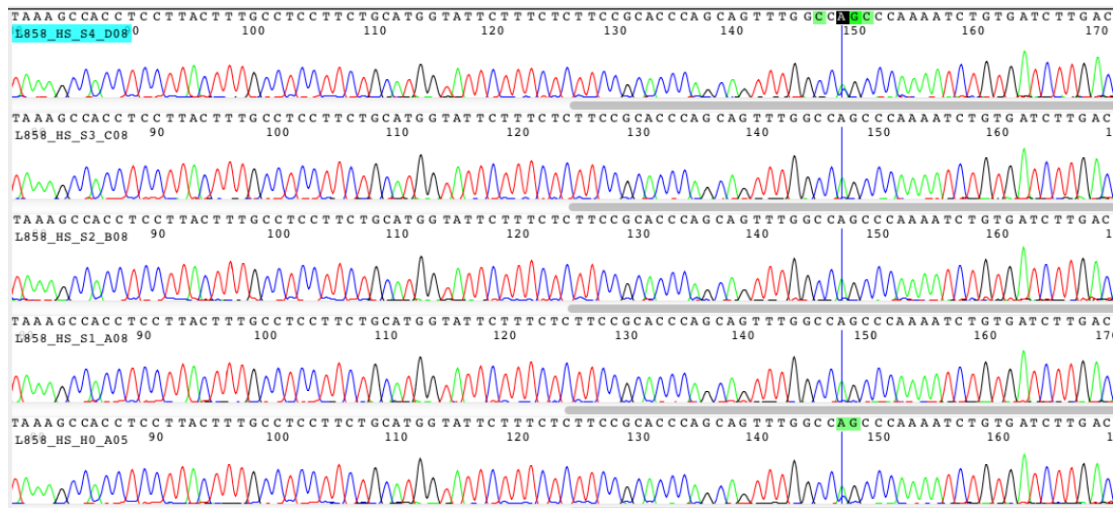
- Exon19DEL



- T790M



- L858R



- NGS

- Table. Frequency and Depth

Sample	Type	DEL19		T790M		L858R	
		Depth	Frequency	Depth	Frequency	Depth	Frequency
HS-H0	DEL19 T790M+L858R	32918	94.966%	107347	25.693%	157162	8.365%
HS-S1	WT	140	8.571%	75718	1.211%	281507	0.146%
HS-S2	DEL19	64971	94.86%	115471	0.413%	241589	0.16%
HS-S3	T790M	70	5.714%	135229	89.304%	231436	0.187%
HS-S4	L858R	93	8.602%	108699	1.188%	262977	16.633%
N-H0		53759	0.887%	81558	0.839%	107233	3.269%
N-S1		51514	0.01%	72149	0.148%	98871	0.136%
N-S2		123002	3.092%	67109	0.134%	136493	0.091%
N-S3		78360	0.038%	86771	6.994%	142039	0.076%
N-S4		163332	0.004%	108973	0.14%	96445	8.377%

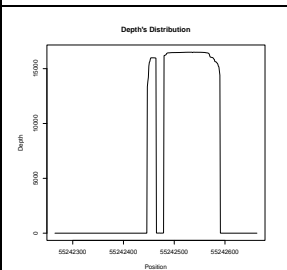
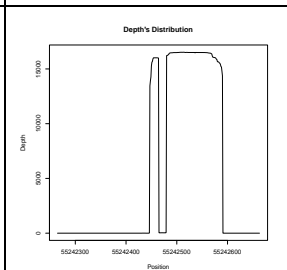
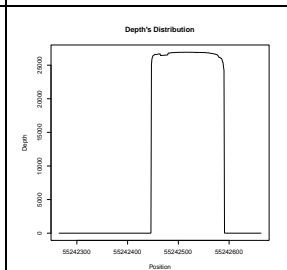
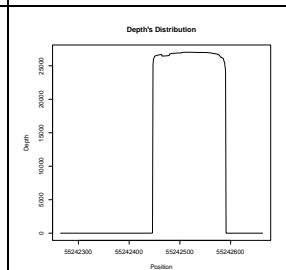
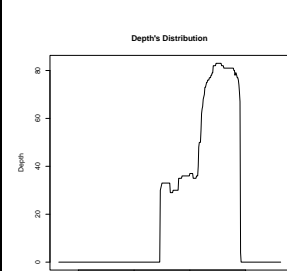
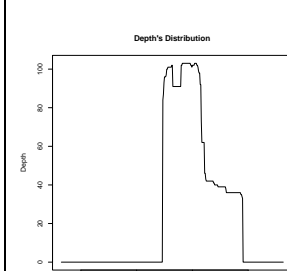
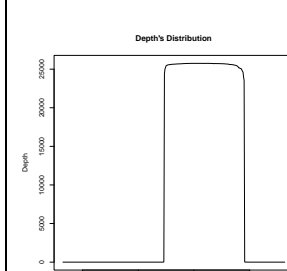
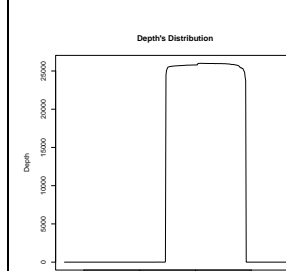
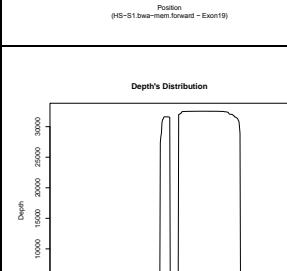
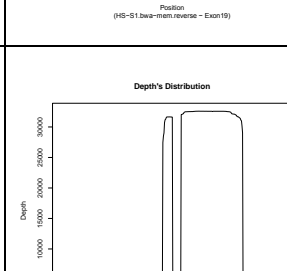
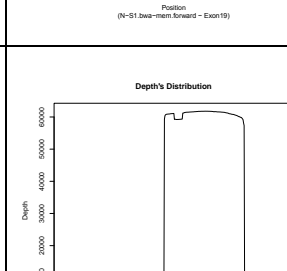
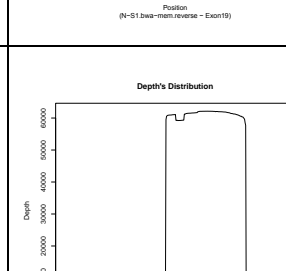
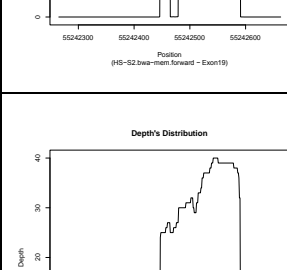
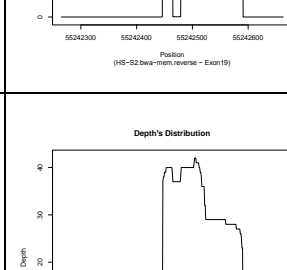
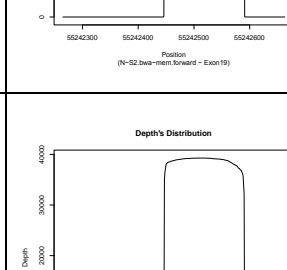
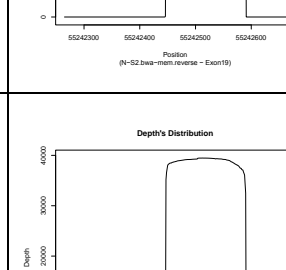
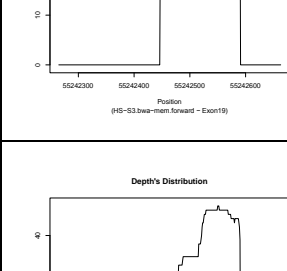
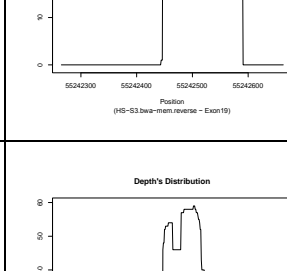
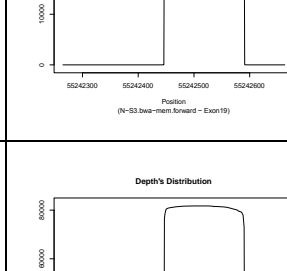
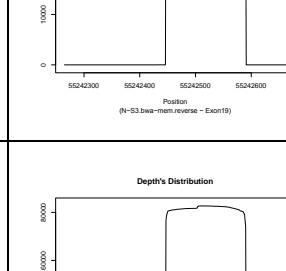
- Table. Forward-Reads' Frequency and Depth

Sample	Type	DEL19		T790M		L858R	
		Depth	Frequency	Depth	Frequency	Depth	Frequency
HS-H0	DEL19 T790M+L858R	16443	95.60%	94755	25.72%	147682	8.26%
HS-S1	WT	36	11.11%	72392	1.20%	266028	0.15%
HS-S2	DEL19	32477	95.26%	105673	0.41%	227504	0.17%
HS-S3	T790M	30	6.67%	127955	89.40%	219279	0.20%
HS-S4	L858R	35	2.86%	102898	1.18%	254472	16.66%
N-H0		26874	0.89%	77862	0.85%	106416	3.27%
N-S1		25744	0.01%	69975	0.15%	98379	0.14%
N-S2		61492	3.12%	66084	0.14%	135938	0.09%
N-S3		39177	0.04%	84015	6.94%	141220	0.08%
N-S4		81703	0.00%	106714	0.14%	95736	8.36%

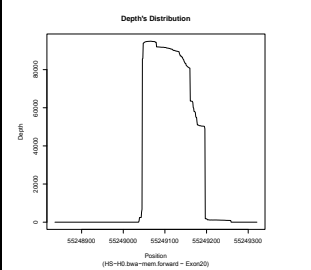
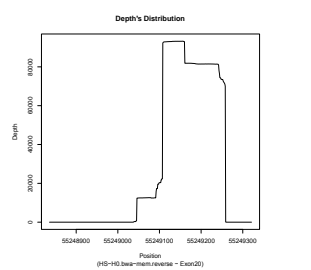
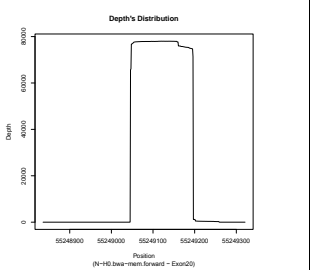
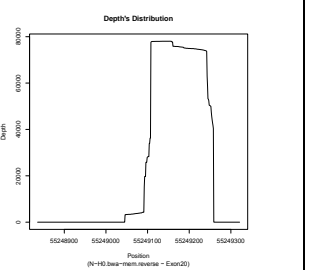
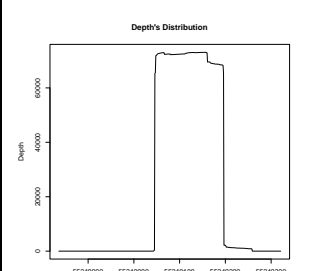
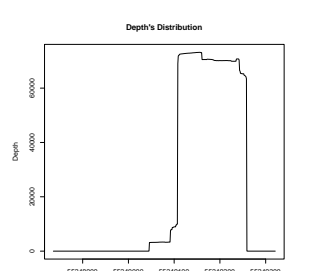
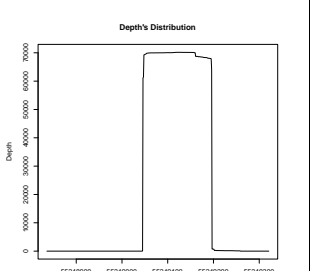
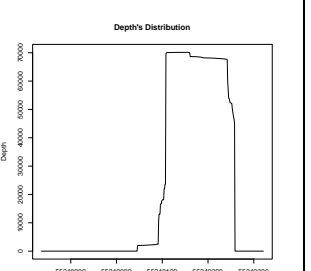
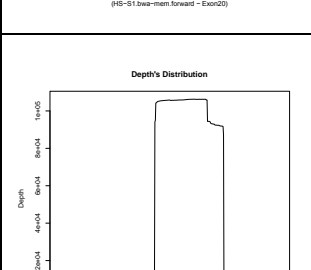
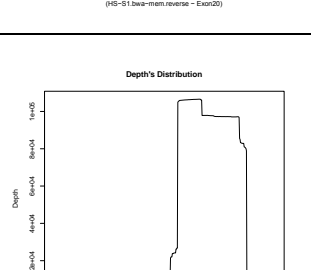
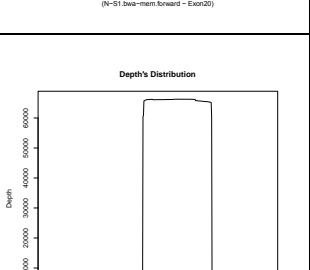
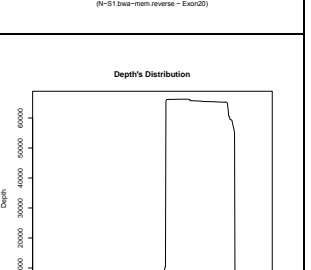
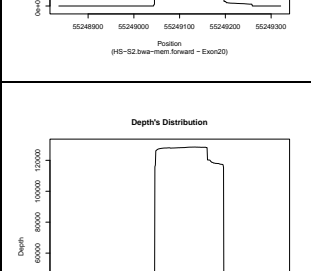
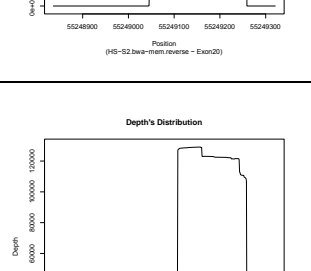
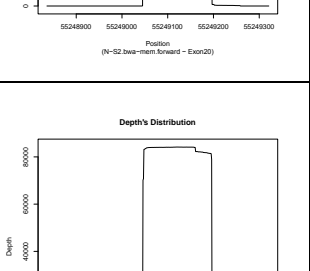
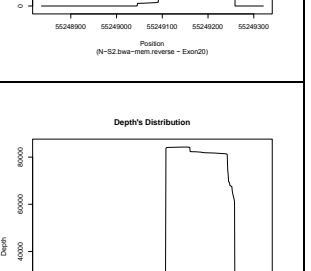
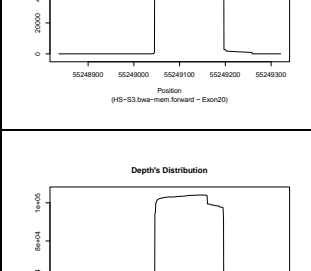
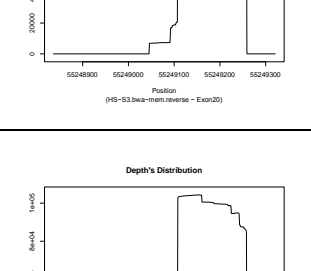
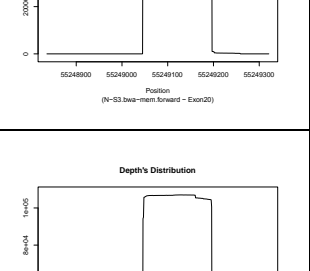
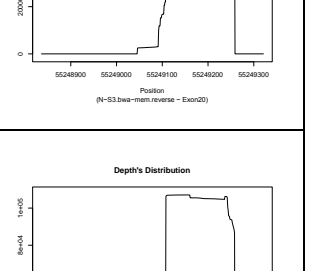
■ Table. Reverse-Reads' Frequency and Depth

Sample	Type	DEL19		T790M		L858R	
		Depth	Frequency	Depth	Frequency	Depth	Frequency
HS-H0	DEL19 T790M+L858R	16474	94.34%	12586	25.49%	9477	10.00%
HS-S1	WT	104	7.69%	3322	1.42%	15469	0.07%
HS-S2	DEL19	32493	94.46%	9792	0.41%	14080	0.04%
HS-S3	T790M	40	5.00%	7267	87.57%	12148	0.03%
HS-S4	L858R	58	12.07%	5791	1.28%	8495	16.00%
N-H0		26885	0.89%	3695	0.62%	804	3.11%
N-S1		25769	0.01%	2171	0.14%	486	0.21%
N-S2		61507	3.06%	1019	0.10%	540	0.00%
N-S3		39182	0.04%	2751	8.76%	809	0.00%
N-S4		81626	0.01%	2255	0.13%	698	9.89%

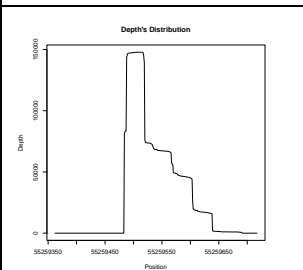
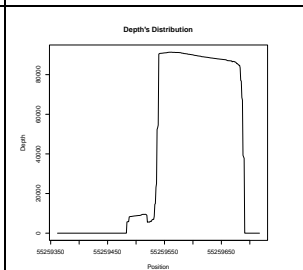
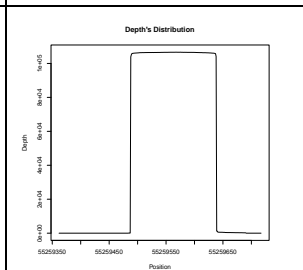
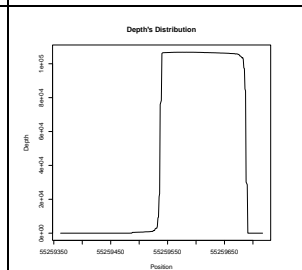
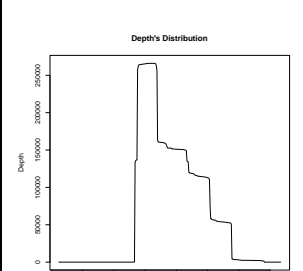
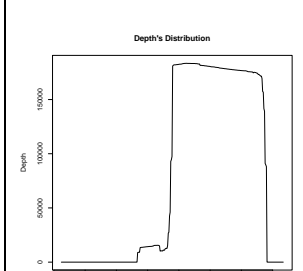
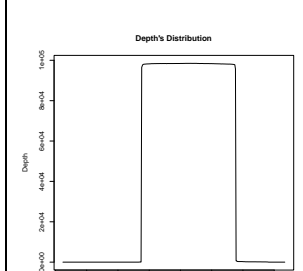
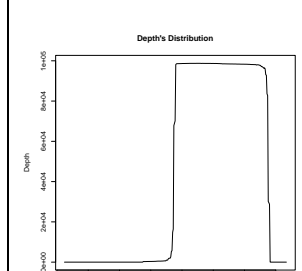
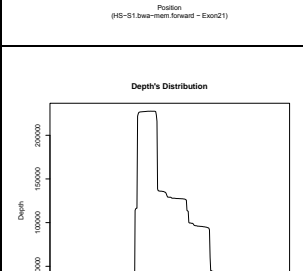
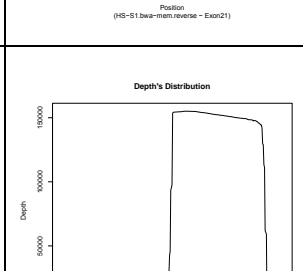
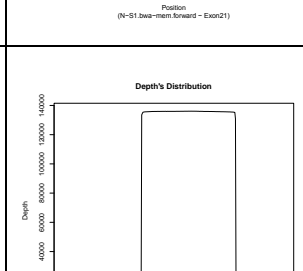
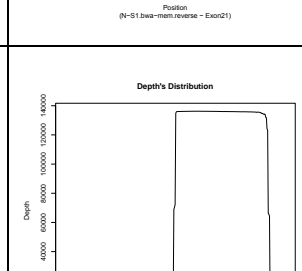
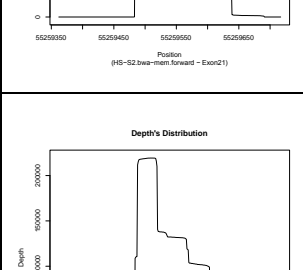
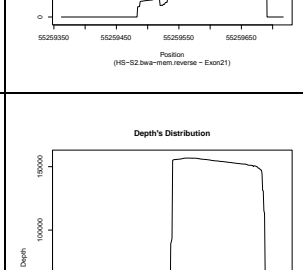
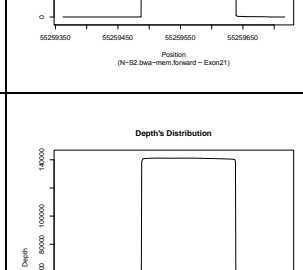
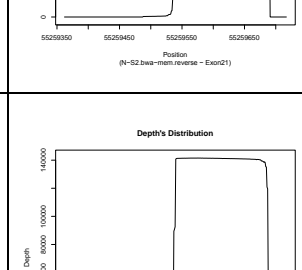
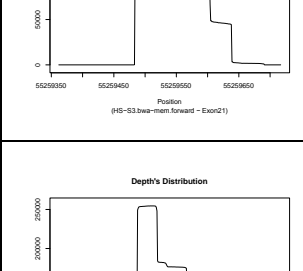
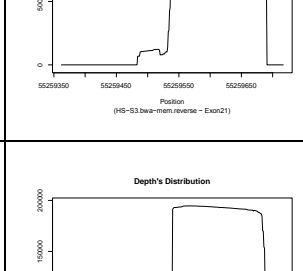
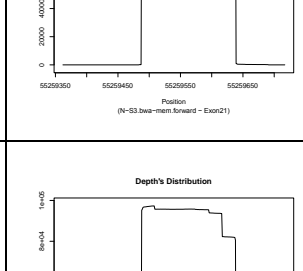
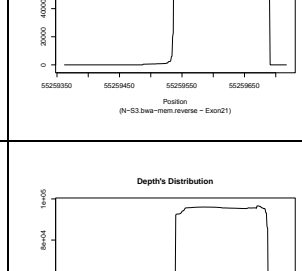
■ Depth's Distribution – Exon19

Sample	HS-*		N-*	
	Forward	Reverse	Forward	Reverse
H0	 <p>Depth's Distribution</p> <p>Position (HS-H0.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (HS-H0.bwa-mem.reverse - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-H0.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-H0.bwa-mem.reverse - Exon19)</p>
S1	 <p>Depth's Distribution</p> <p>Position (HS-S1.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (HS-S1.bwa-mem.reverse - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S1.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S1.bwa-mem.reverse - Exon19)</p>
S2	 <p>Depth's Distribution</p> <p>Position (HS-S2.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (HS-S2.bwa-mem.reverse - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S2.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S2.bwa-mem.reverse - Exon19)</p>
S3	 <p>Depth's Distribution</p> <p>Position (HS-S3.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (HS-S3.bwa-mem.reverse - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S3.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S3.bwa-mem.reverse - Exon19)</p>
S4	 <p>Depth's Distribution</p> <p>Position (HS-S4.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (HS-S4.bwa-mem.reverse - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S4.bwa-mem.forward - Exon19)</p>	 <p>Depth's Distribution</p> <p>Position (N-S4.bwa-mem.reverse - Exon19)</p>

■ Depth's Distribution – Exon20

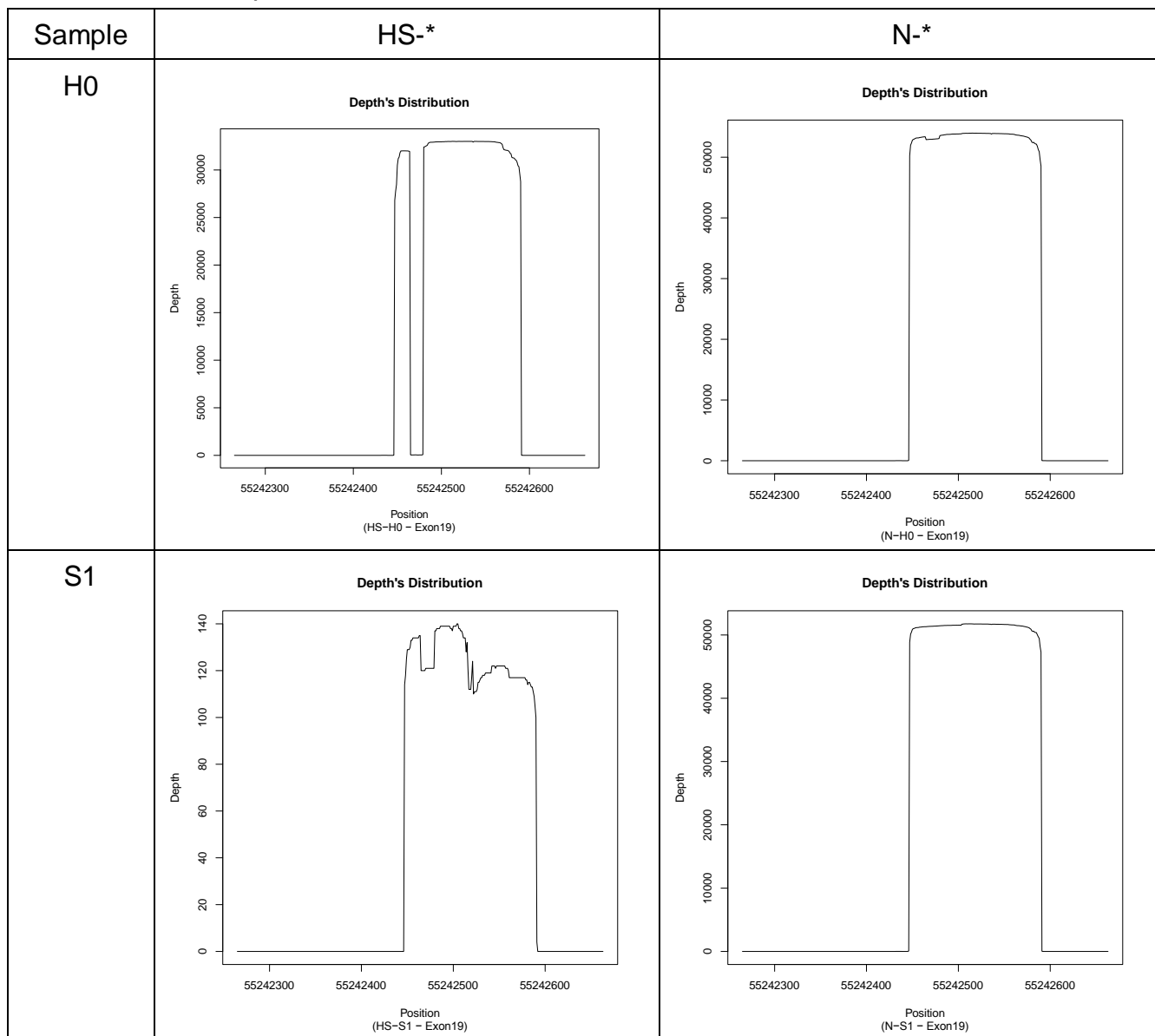
Sample	HS-*		N-*	
	Forward	Reverse	Forward	Reverse
H0				
S1				
S2				
S3				
S4				

■ Depth's Distribution – Exon21

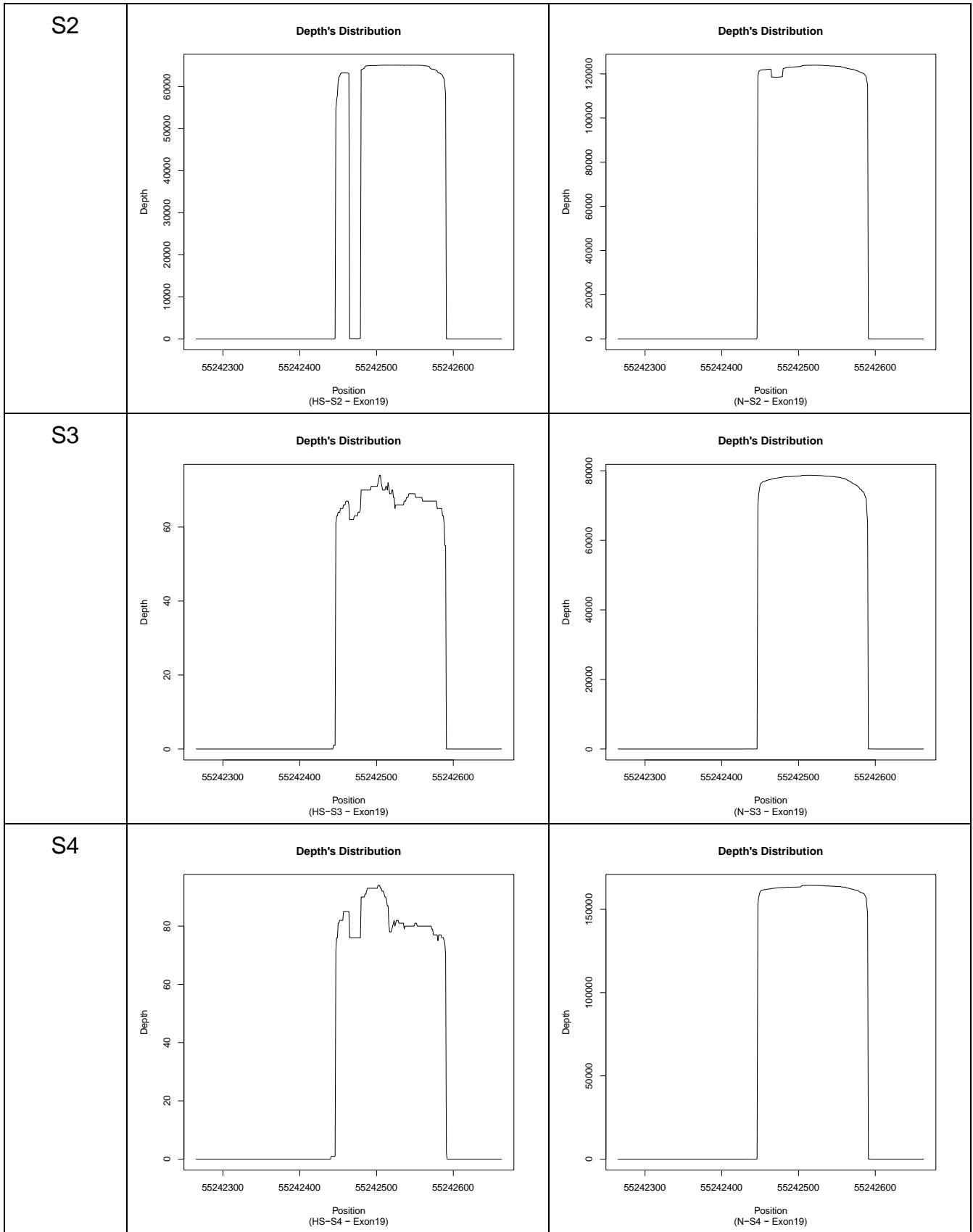
Sample	HS-*		N-*	
	Forward	Reverse	Forward	Reverse
H0				
S1				
S2				
S3				
S4				

## Appendix

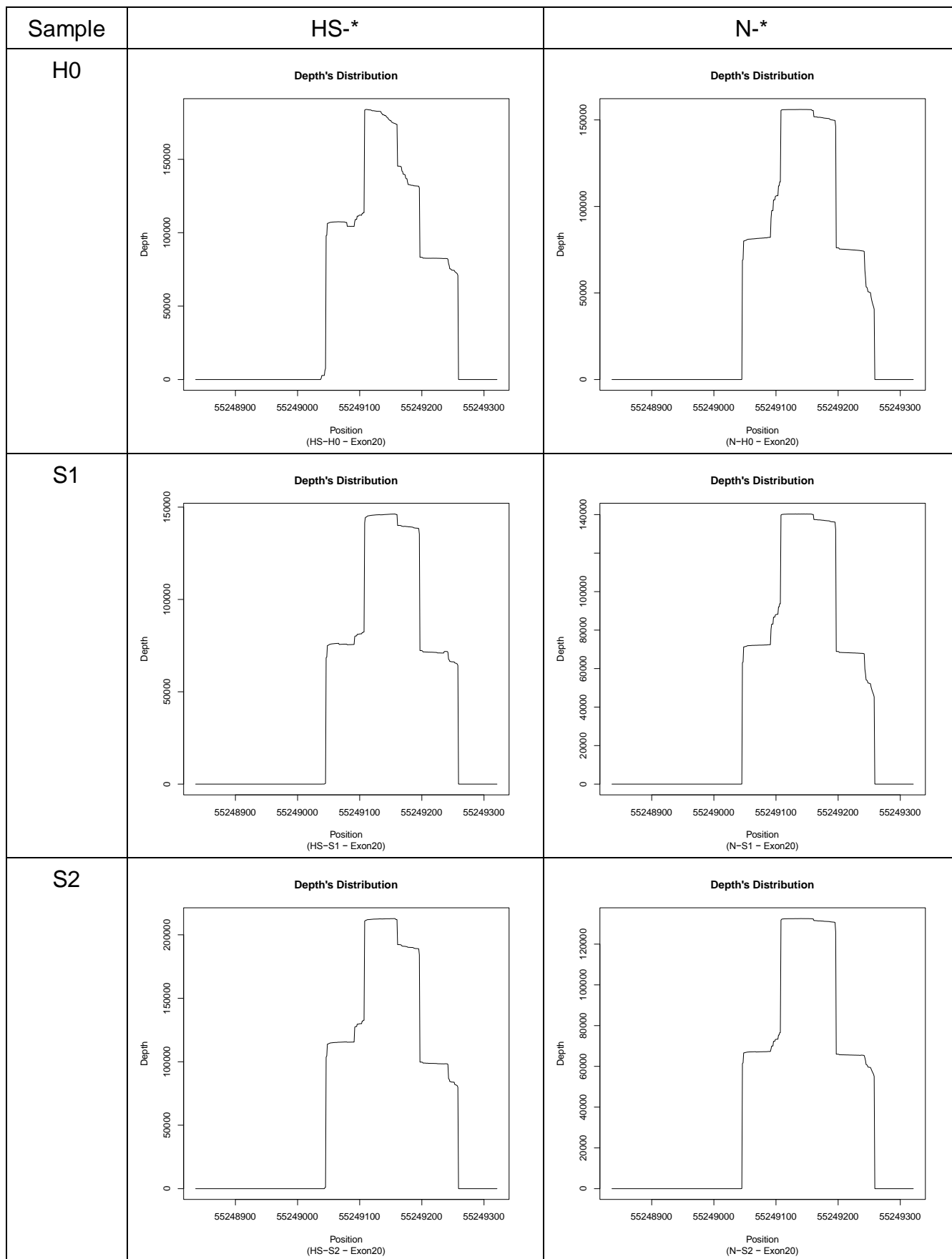
## ■ Depth's Distribution – Exon19

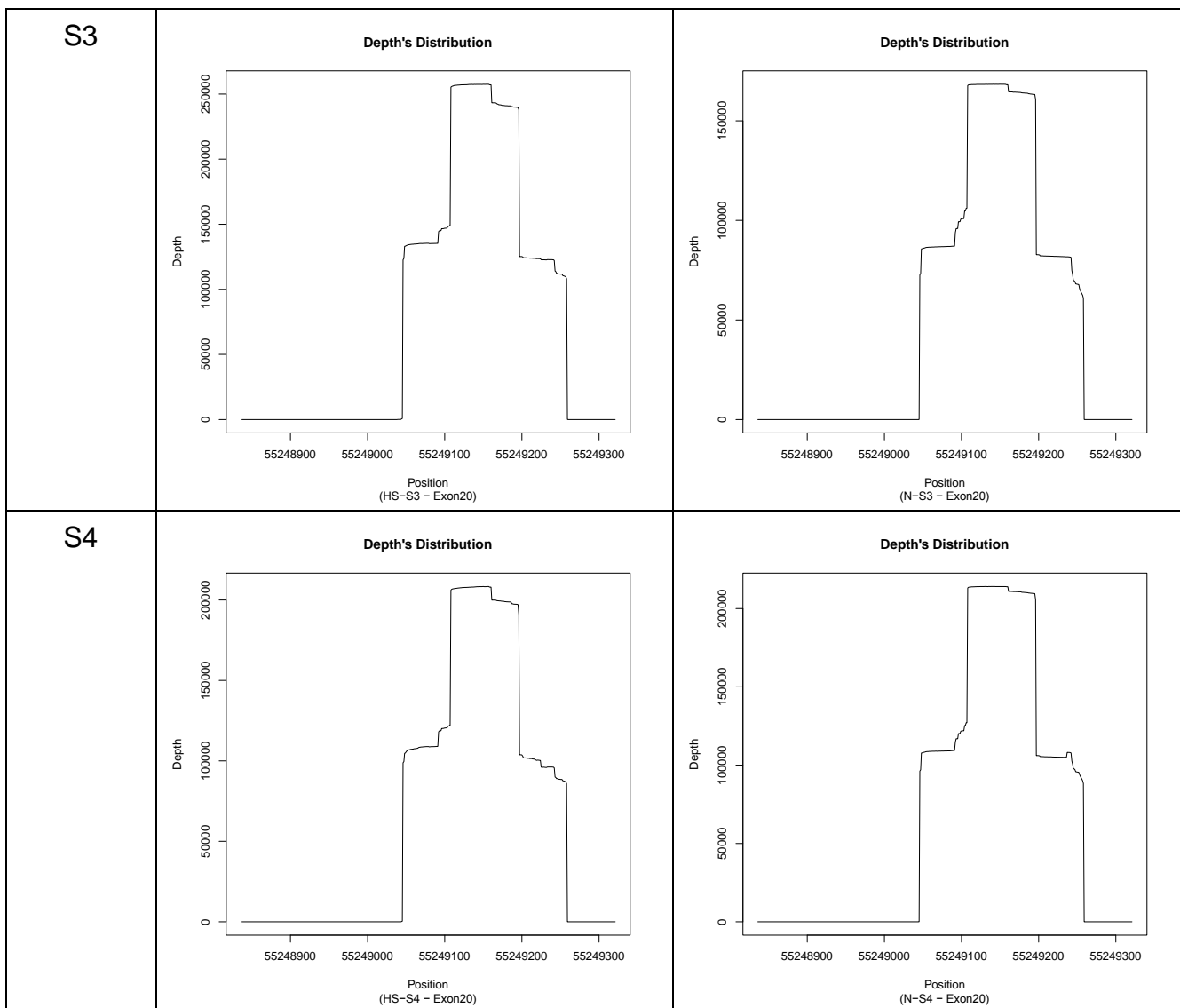






■ Depth's Distribution – Exon20





■ Depth's Distribution – Exon21

