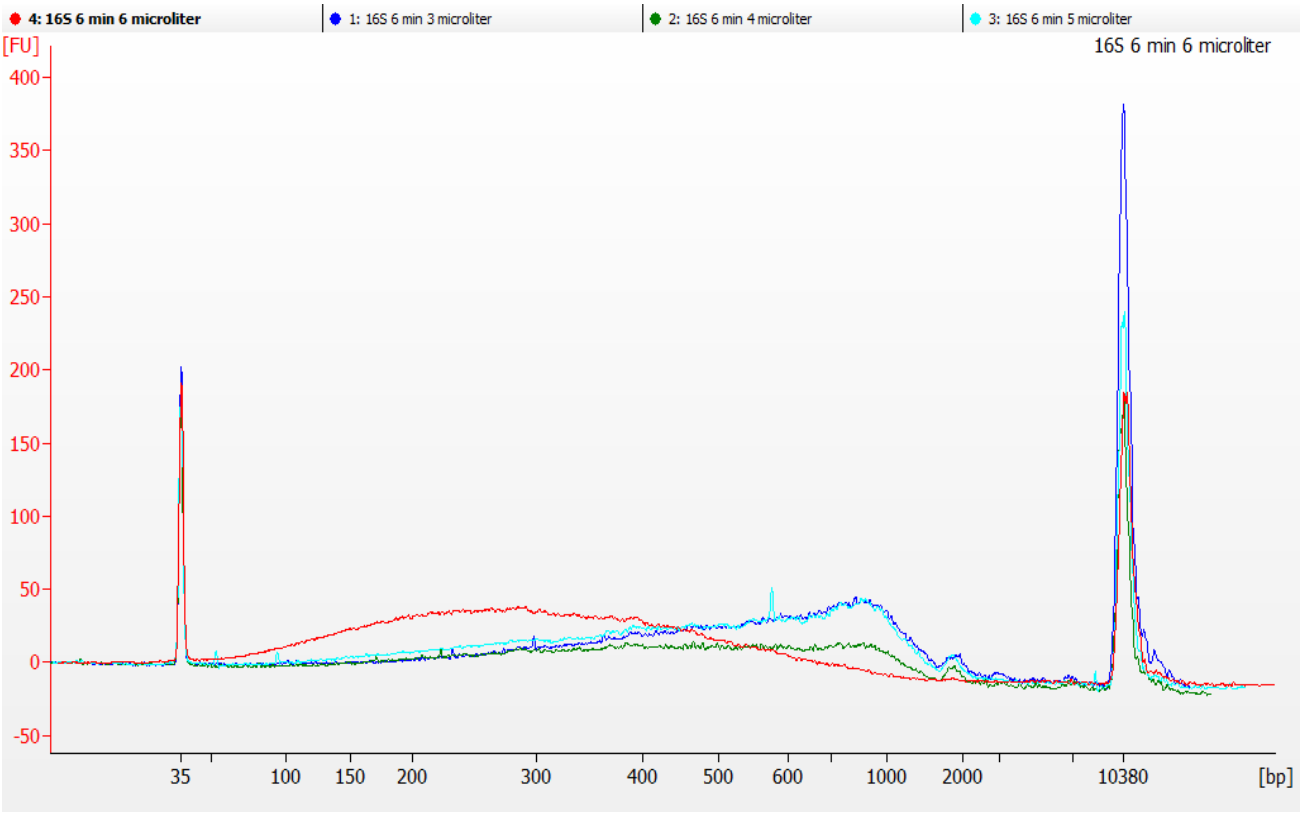
Supplement 2: Shearing patterns during Library preparation

Pre-shearing for Spiked-DNA-GP: 5 microliter was chosen because there was little difference between 5 and 6 microliter at 400 bp, although the shearing of 6 microliter was better. However 5 microliter means less 16S ribosomal DNA and more DNA from the actual sample. (top graph and table)

Bottom table: The amounts of DNA according to Qubit and Bioanalyzer measurements. The Qubit measurement of 16S ribosomal amplicon was 10 ng/microliter. For the Qubit, this was multiplied by the amount of microliters used i.e. (10 ng/microl * volume). The Bioanalyzer samples were dissolved in 50 microliters, which was multiplied by the concentration in pg/microliter (conc*50).

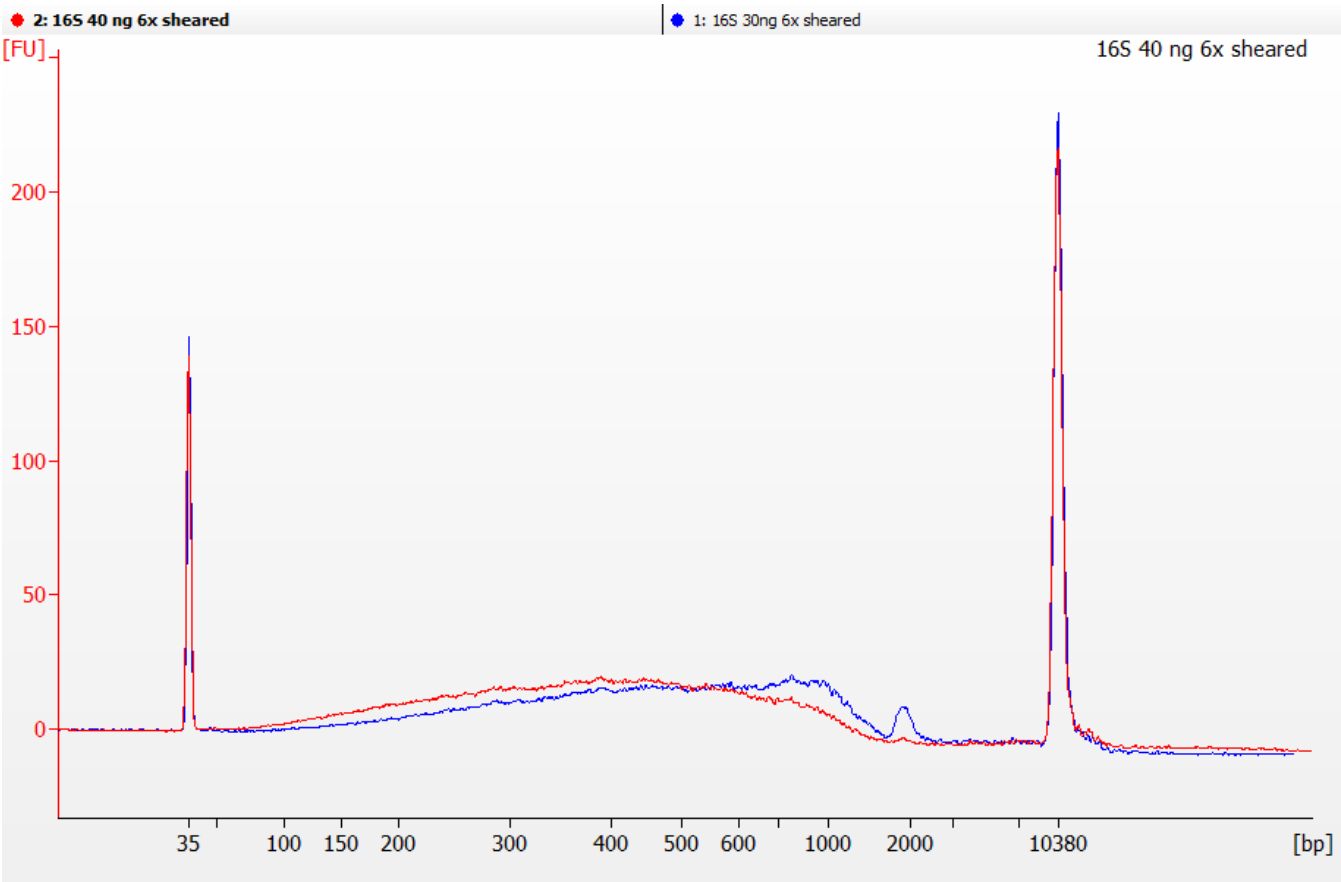


	From [bp]	To [bp]	% of Total	Average Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]
16S 6 min 3 microliter	75	8,000	97	678	428.47	1,645.80
16S 6 min 4 microliter	75	8,000	97	654	679.77	3,039.20
16S 6 min 5 microliter	75	8,000	98	603	822.4	3,634.90
16S 6 min 6 microliter	75	8,000	96	324	1,182.02	7,974.90

DNA (ng) in total shearing solution	
Bioanalzyer (conc*50)	**Qubit** (10ng/microl * volume)
21.4	30.0
34.0	40.0
41.1	50.0
59.1	60.0

Pre-shearing for Spiked-DNA-GP: Pre-shearing for Spiked-DNA-SP: 30 and 40 ng was used to test the effect of shearing. Shearing of 40 ng had the best shearing and given the fact that there was only one try with the actual sample, the safer option of 40 ng was opted.
Too little DNA could result in a graph too close to the baseline to distinguish the sample from said baseline. (top graph and table)

Bottom table: The amounts of DNA according to Qubit and Bioanalyzer measurements. The Qubit measurement of 16S ribosomal amplicon was 10 ng/microliter. For the Qubit, this was multiplied by the amount of microliters used i.e. (10 ng/microl * volume). The Bioanalyzer samples were dissolved in 50 microliters, which was multiplied by the concentration in pg/microliter (conc*50).

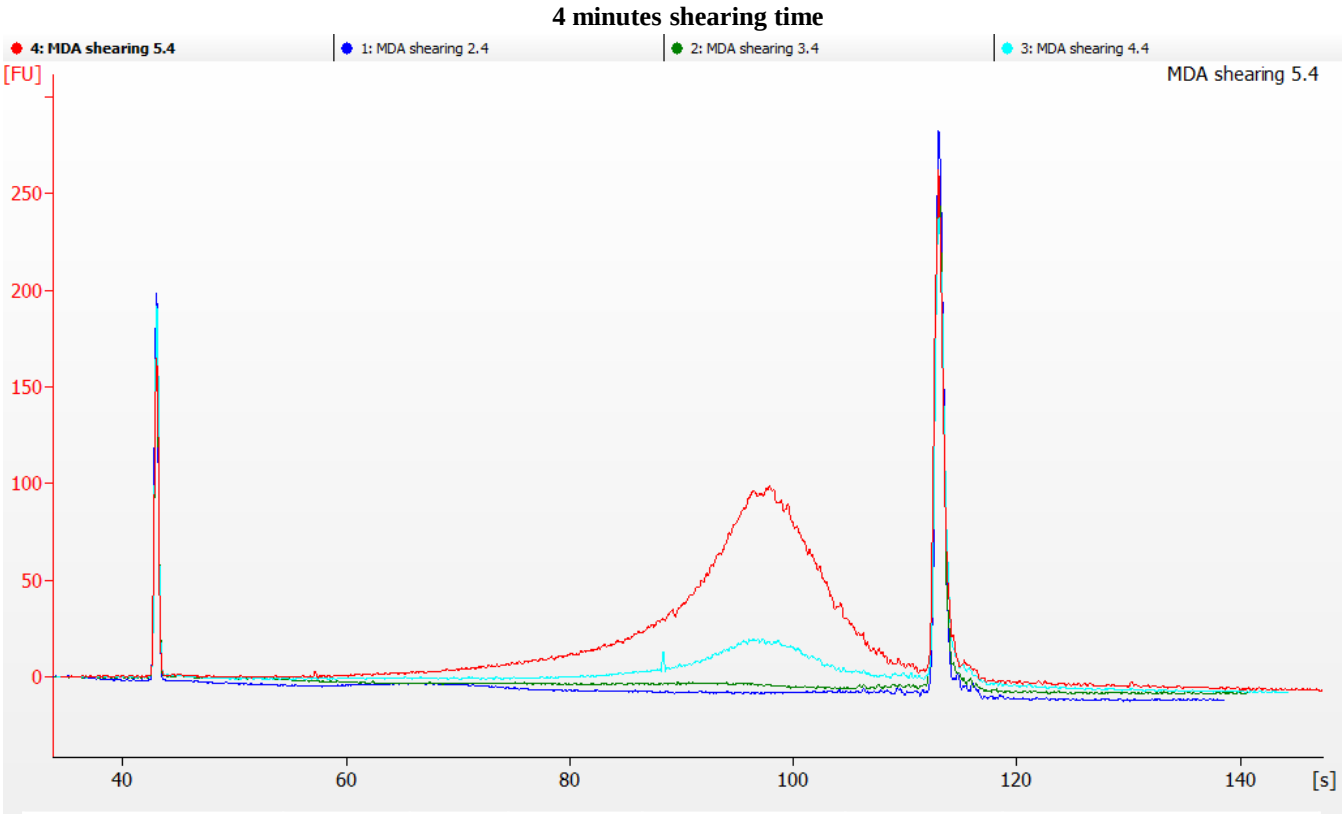


	From [bp]	To [bp]	% of Total	Average Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]
16S 30 ng 6x	75	8,000	97	706	538.04	2,306.60
16S 40 ng 6x	75	8,000	96	495	651.14	3,455.60

DNA (ng) in total shearing solution	
Bioanalzyer (conc*50)	**Qubit** (10ng/ml * volume)
26.9	30.0
32.5	40.0

Pre-shearing for MDA: Three shearing times were tested with 4 to 7 concentrations in order to determine the lower limits of the Bioanalyzer. From this, it appeared that quantities of DNA lower than 40 ng could approach baseline levels and are therefore not suitable for further steps.

Bottom table: The amounts of DNA according to Qubit and Bioanalyzer measurements. The Qubit measurement of MDA amplified DNA was 10,8 ng/microliter. For the Qubit, this was multiplied by the amount of microliters used i.e. (10 ng/microl * volume). The Bioanalyzer samples were dissolved in 50 microliters, which was multiplied by the concentration in pg/microliter (conc*50).



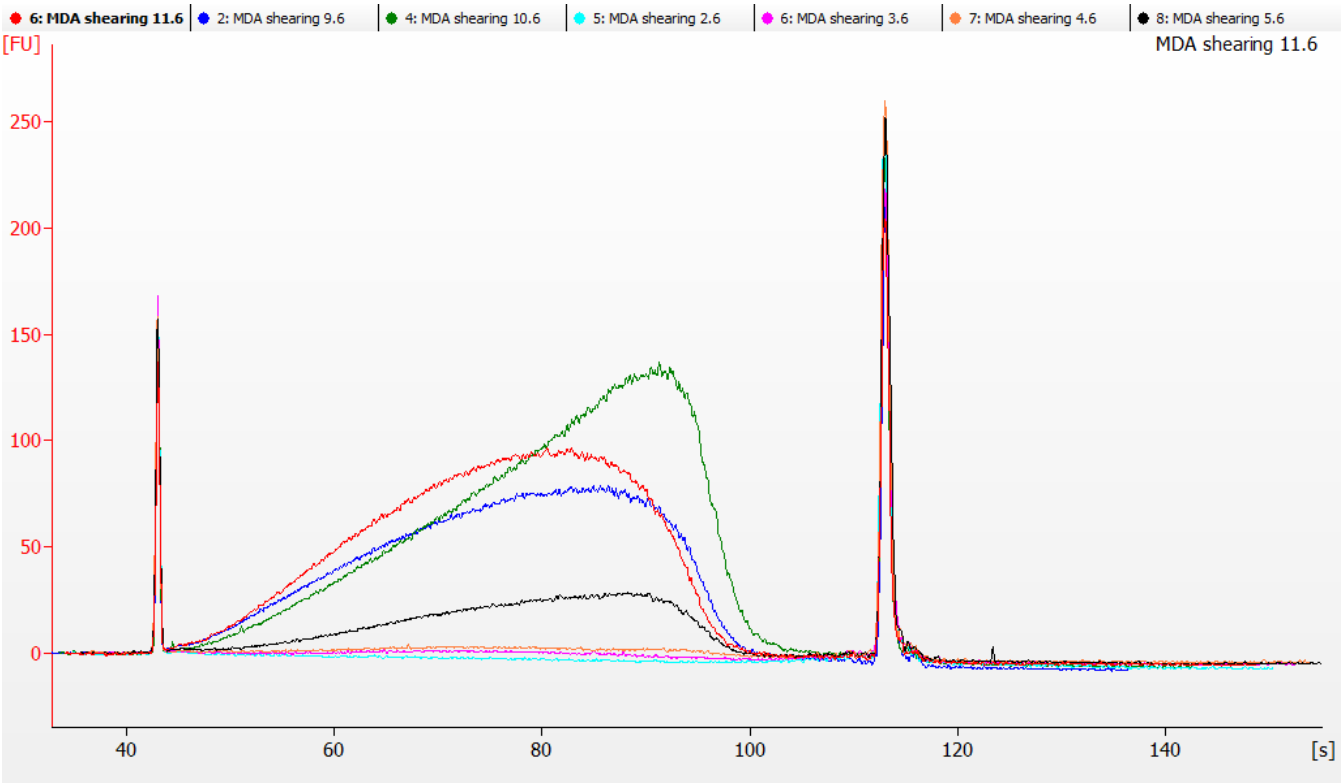
	From [bp]	To [bp]	% of Total	Average Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]
MDA 2.4 (21.6 ng)	75	8,000	28	4,980	1.78	2.5
MDA 3.4 (32.4 ng)	75	8,000	67	2,085	10.98	58.7
MDA 4.4 (43.2 ng)	75	8,000	91	1,581	171.74	440.7
MDA 5.4 (54.0 ng)	75	8,000	96	1,483	625.94	1,327.80

DNA (ng) in total shearing solution	
Bioanalzyer (conc*50)	**Qubit** (10,8 ng/ml * volume)
0.09	21.6
0.55	32.4
8.87	43.2
31.3	54.0

Pre-shearing for MDA: Three shearing times were tested with 4 to 7 concentrations in order to determine the lower limits of the Bioanalyzer. From this, it appeared that quantities of DNA lower than 40 ng could approach baseline levels and are therefore not suitable for further steps.

Bottom table: The amounts of DNA according to Qubit and Bioanalyzer measurements. The Qubit measurement of MDA amplified DNA was 10,8 ng/microliter. For the Qubit, this was multiplied by the amount of microliters used i.e. (10 ng/microl * volume). The Bioanalyzer samples were dissolved in 50 microliters, which was multiplied by the concentration in pg/microliter (conc*50).

6 minutes shearing time



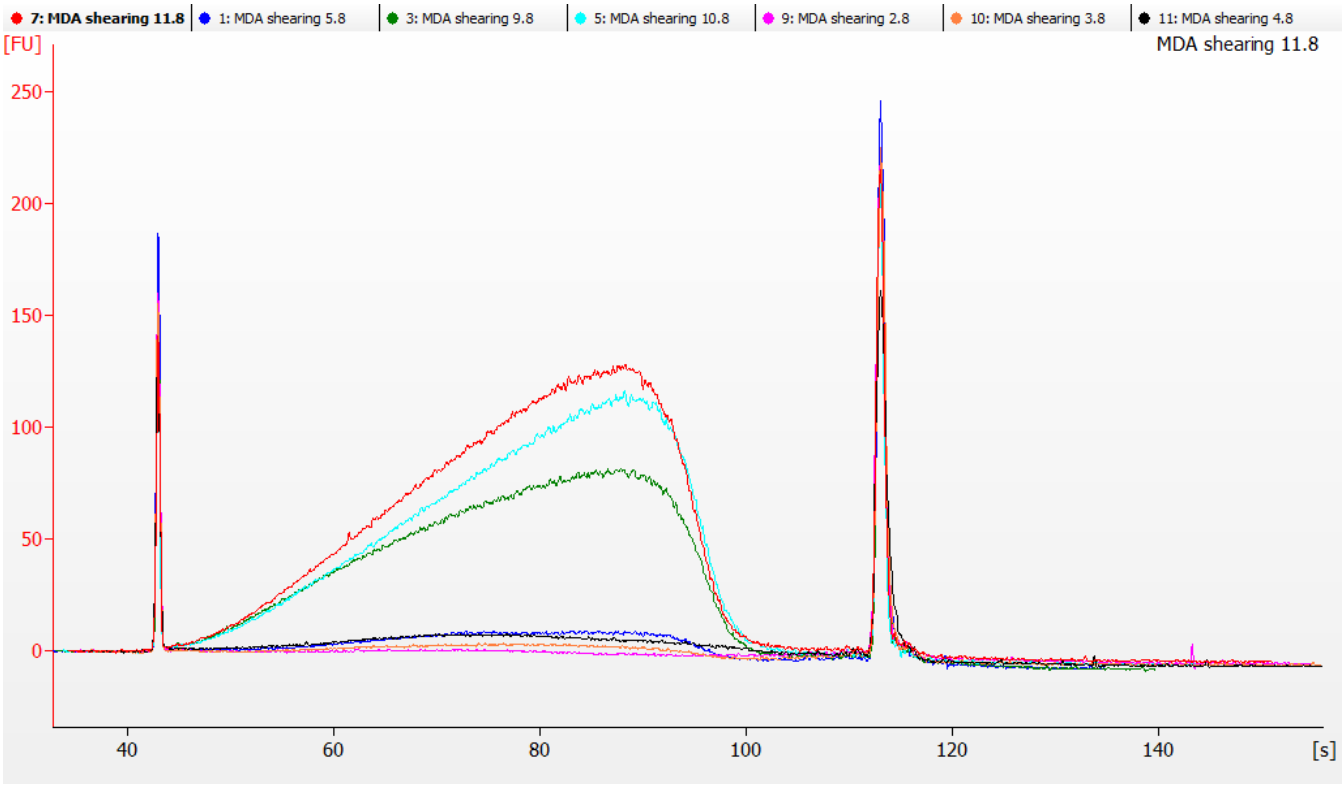
	From [bp]	To [bp]	Corr. Area	Average Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]
MDA 2.6 (21.6 ng)	75	8,000	37.4	3,148	18.62	131.3
MDA 3.6 (32.4 ng)	75	8,000	109.8	1,191	59.92	320.5
MDA 4.6 (43.2 ng)	75	8,000	233.2	880	117.03	639.1
MDA 5.6 (54.0 ng)	75	8,000	1,158.40	580	581.37	2,699.30
MDA 9.6 (97.2 ng)	75	8,000	3,810.40	510	2,387.84	12,435.80
MDA 10.6 (108 ng)	75	8,000	4,749.30	612	2,675.53	11,634.60
MDA 11.6 (118.8 ng)	75	8,000	4,266.80	463	2,764.75	14,475.80

DNA (ng) in total shearing solution	
Bioanalzyer (conc*50)	**Qubit** (10,8 ng/ml * volume)
0.9	21.6
3	32.4
5.9	43.2
29.1	54.0
119.3	97.2
133.8	108.0
138.2	118.8

Pre-shearing for MDA: Three shearing times were tested with 4 to 7 concentrations in order to determine the lower limits of the Bioanalyzer. From this, it appeared that quantities of DNA lower than 40 ng could approach baseline levels and are therefore not suitable for further steps.

Bottom table: The amounts of DNA according to Qubit and Bioanalyzer measurements. The Qubit measurement of MDA amplified DNA was 10,8 ng/microliter. For the Qubit, this was multiplied by the amount of microliters used i.e. (10 ng/microl * volume). The Bioanalyzer samples were dissolved in 50 microliters, which was multiplied by the concentration in pg/microliter (conc*50).

8 minutes shearing time



	From [bp]	To [bp]	Corr. Area	Average Size [bp]	Conc. [pg/µl]	Molarity [pmol/l]
MDA 2.8 (21.6 ng)	75	8,000	110.5	1,495	60.96	302.7
MDA 3.8 (32.4 ng)	75	8,000	209.1	739	112.01	530.1
MDA 4.8 (43.2 ng)	75	8,000	477.1	787	287.73	1,489.60
MDA 5.8 (54.0 ng)	75	8,000	590.5	710	289.79	1,411.90
MDA 9.8 (97.2 ng)	75	8,000	3,732.90	542	2,124.21	10,631.70
MDA 10.8 (108 ng)	75	8,000	4,408.60	550	2,855.04	13,153.20
MDA 11.8 (118.8 ng)	75	8,000	4,933.00	533	2,898.54	13,630.20

DNA (ng) in total shearing solution	
Bioanalzyer (conc*50)	**Qubit** (10,8 ng/ml * volume)
3	21.6
5.6	32.4
14.3	43.2
14.5	54.0
106.2	97.2
142.8	108.0
145	118.8