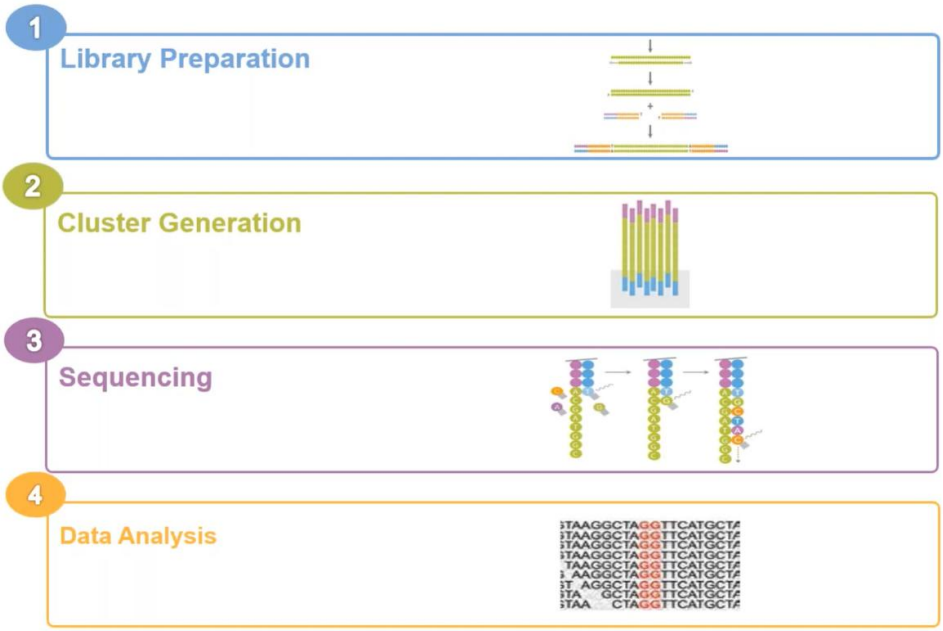
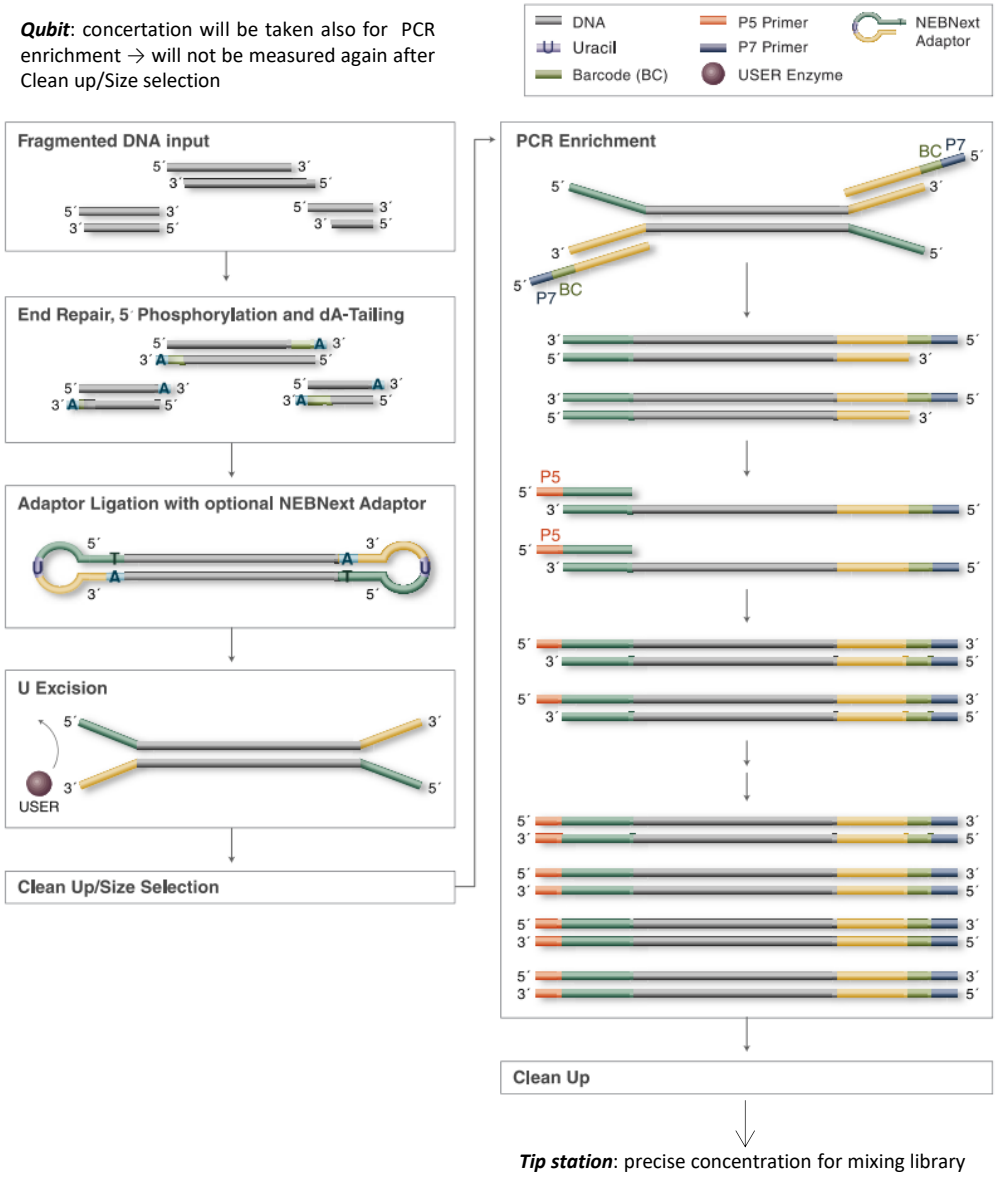
Illumina Sequencing Workflow



Library preparation: scheme

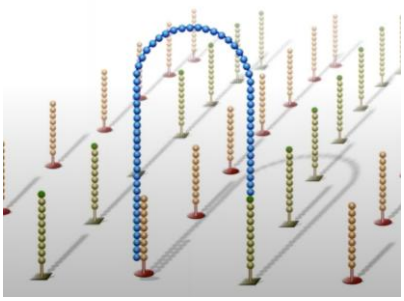
Figure 1. Workflow demonstrating the use of NEBNext Ultra II DNA Library Prep Kit for Illumina

Qubit: concertation will be taken also for PCR enrichment → will not be measured again after Clean up/Size selection



Note
DNA fragments with sequencing adapters

Tip station: precise concentration for mixing library



Note
Clean Up/Size Selection

- Fragments should not be too big – they might break during "Bridge amplification"; it is better with a new illumina
- possibility of smaller fragments higher amplification; possibility of attached more .... ; Fragments should not be too big – they might break during "Bridge amplification"; it is better with a new illumina

Low Plexity Pooling Guidelines

Illumina uses a red laser/LED to sequence A/C and a green laser/LED to sequence G/T. For each cycle, both the red and the green channel need to be read to ensure proper image registration (i.e. A or C must be in each cycle, and G or T must be in each cycle). If this color balance is not maintained, sequencing the index read could fail. The following table lists some valid combinations that can be sequenced together. Note: for 1-plex (no pooling), use any i7 primer with any i5 primer. In this case it is important to select “0” index reads in the Illumina Experiment Manager.

CAUTION: Sufficient primers are provided to generate 96 different samples if each i5 primer is used only once with each i7 primer.

If using subsets of i5 and i7 primers multiple times, you may have to readjust primer pairs to be able to generate 96 samples.

GOOD

i7 PRIMERS		i5 PRIMERS	
i7-PCR Index 1	A T T A C T C G	i5-PCR Index 3	C C T A T C C T
i7-PCR Index 2	T C C G G A G A	i5-PCR Index 4	G G C T C T G A
i7-PCR Index 3	C G C T C A T T	i5-PCR Index 5	A G G C G A A G
i7-PCR Index 4	G A G A T T C C	i5-PCR Index 6	T A A T C T T A
	✓✓✓✓✓✓✓✓		✓✓✓✓✓✓✓✓

BAD

i7 PRIMERS		i5 PRIMERS	
i7-PCR Index 1	A T T A C T C G	i5-PCR Index 2	A T A G A G G C
i7-PCR Index 2	T C C G G A G A	i5-PCR Index 4	G G C T C T G A
i7-PCR Index 3	C G C T C A T T	i5-PCR Index 6	T A A T C T G A
i7-PCR Index 4	G A G A T T C C	i5-PCR Index 8	G T A C T G A C
	✓✓✓✓✓✓✓✓		✓✓✘✓✓✘✓✘

Library preparation

12.11.24

1. Quibit: measure concentration

Qubit™ dsDNA Quantification Assay Kits

- Already mix buffer with dye
- Cat: Q32854
- LOT: 2201615

Q32853	dsDNA Quantitation, Broad Range	4 to 2000 ng	500 Reactions

Two-point calibration:

- S1 and S2 (lower and upper point calibration)

Dilution

- Samples: 1 µL + 199 µL
- Input BioID: dilution was too high → dilution of inputs 1:10 and than 1 µL + 199 µL again

Workflow

- samples on ice
- thaw at r.t. (but keep on the ice rest of the time)
- "flick the samples" – to homogenize
- spin down (short spin at r.t. centrifuge)
- Mix with buffer (pipet first buffer; 199; and than sample; 1;
- quick vortex
- incubation (r.t.; 3 min)
- measure

Data

- Download on USB

Notes Qubit and library prep 13.11.

Down-stream for library prep

- 1st clean up – possibility of smaller fragments higher amplification; possibility of attached more …. ; Fragments should not be too big – they might break during "Bridge amplification"; it is better with a new Illumina
- Amplification step as law as possible
- Table 1.1
- ----------

Qubit: put samples order other way around

- 1. make it in correct order
- 2. if low concentration (unmeasured on Qubit) – try to calculate from lin reg. from the similar concentration of samples

Qubit

- Concentration – total amount of material (in 15 µL: what left in epi after qPCR and Qubit)
  - Input: 50 ng from 1:10 dilution
  - Samples 15 µL … just take it all

Workflow:

1. End repaired
   table 1.1
   TE buffer
2. Adaptor dilution
   table 2.2
   table 2.1
3. Purification (first clean up), if samples with low concentration– skip size selection
4. PCR cy
   table 4.1

- Tape station – measurement of concertation – can perform qPCR again

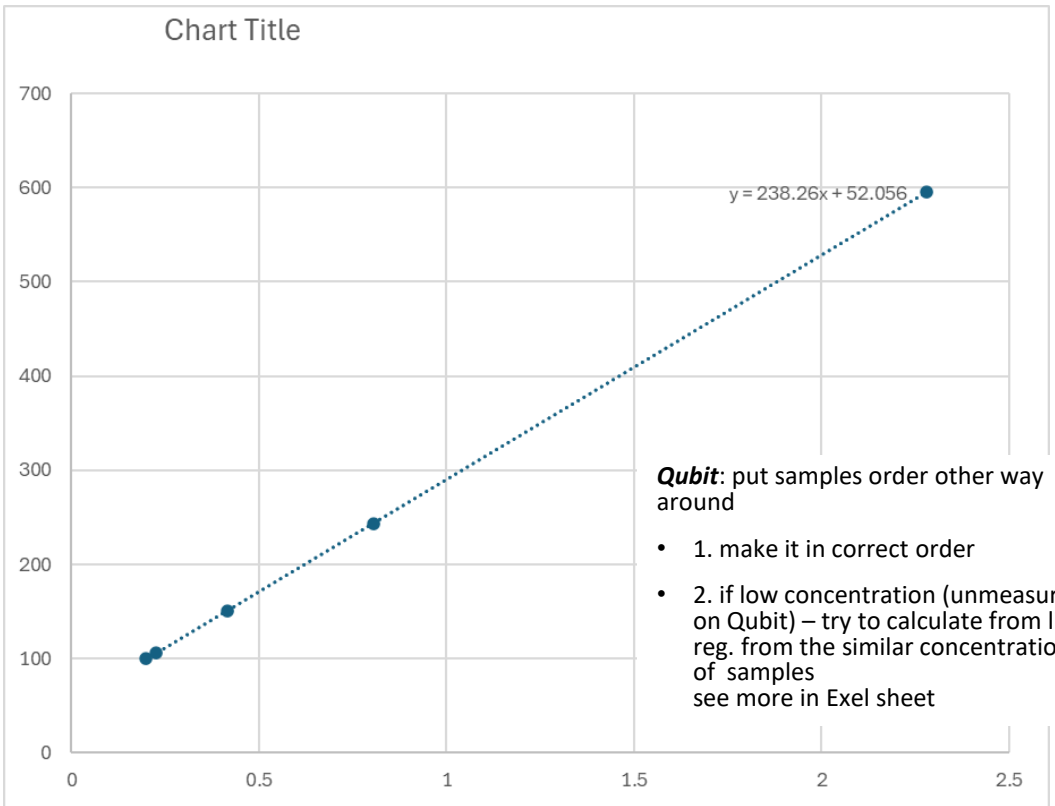
- One barcode use for 3 samples (more or less)

2. Library preparation: Export from Qubit

19.11. 24

Concentration used for calculation

1x dsDNA High Sensitivity	265	ng/mL	53	ng/uL	1	200	53.26	24581.26	Blue	Green	13013.47	
1x dsDNA High Sensitivity	217	ng/mL	43.4	ng/uL	1	200	53.26	24581.26	Blue	Green	10609.37	
1x dsDNA High Sensitivity	4.03	ng/mL	0.806	ng/uL	1	200	53.26	24581.26	Blue	Green	243.61	
1x dsDNA High Sensitivity	2.08	ng/mL	0.416	ng/uL	1	200	53.26	24581.26	Blue	Green	151.19	
1x dsDNA High Sensitivity	269	ng/mL	53.8	ng/uL	1	200	53.26	24581.26	Blue	Green	13162.79	
1x dsDNA High Sensitivity	288	ng/mL	57.6	ng/uL	1	200	53.26	24581.26	Blue	Green	14124.28	
1x dsDNA High Sensitivity	1	ng/mL	0.2	ng/uL	1	200	53.26	24581.26	Blue	Green	99.95	
1x dsDNA High Sensitivity	11.4	ng/mL	2.28	ng/uL	1	200	53.26	24581.26	Blue	Green	595.43	
1x dsDNA High Sensitivity	81.4	ng/mL	16.3	ng/uL	1	200	53.26	24581.26	Blue	Green	3992.29	
1x dsDNA High Sensitivity	98.8	ng/mL	19.8	ng/uL	1	200	53.26	24581.26	Blue	Green	4838.17	
1x dsDNA High Sensitivity	Out of range	ng/mL	0.08	ng/uL	1	200	53.26	24581.26	Blue	Green	71.73	
1x dsDNA High Sensitivity	1.13	ng/mL	0.226	ng/uL	1	200	53.26	24581.26	Blue	Green	105.99	
1x dsDNA High Sensitivity	62.4	ng/mL	12.5	ng/uL	1	200	53.26	24581.26	Blue	Green	3066.83	
1x dsDNA High Sensitivity	33.9	ng/mL	6.78	ng/uL	1	200	53.26	24581.26	Blue	Green	1680.93	
1x dsDNA High Sensitivity	22.3	ng/mL	4.46	ng/uL	1	200	53.26	24581.26	Blue	Green	1119.78	
1x dsDNA High Sensitivity	7.98	ng/mL	1.6	ng/uL	1	200	53.26	24581.26	Blue	Green	432.44	
1x dsDNA High Sensitivity	4.74	ng/mL	0.948	ng/uL	1	200	53.26	24581.26	Blue	Green	277.59	
1x dsDNA High Sensitivity	2.39	ng/mL	0.478	ng/uL	1	200	53.26	24581.26	Blue	Green	165.64	
	Chart Title											0.08257366

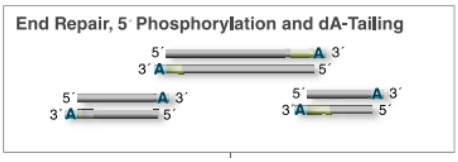


Qubit: put samples order other way around

- 1. make it in correct order
- 2. if low concentration (unmeasured on Qubit) – try to calculate from lin reg. from the similar concentration of samples
  see more in Exel sheet

2. Library preparation: End Prep

14.11. 24



Starting Material: 500 pg–1 µg fragmented DNA. NEB recommends that DNA be sheared in 1X TE. If the DNA volume post shearing is less than 50 µl, add 1X TE to a final volume of 50 µl. Alternatively, samples can be diluted with 10 mM Tris-HCl, pH 8.0 or 0.1X TE.

1. NEBNext End Prep

1.1. Add the following components to a sterile nuclease-free tube:

COMPONENT	VOLUME
• (green) NEBNext Ultra II End Prep Enzyme Mix	3 µl
• (green) NEBNext Ultra II End Prep Reaction Buffer	7 µl
Fragmented DNA	50 µl
Total Volume	60 µl

… calculation in Excel sheet; input from dilution 1:10; samples whole

1.3. Place in a thermal cycler, with the heated lid set to ≥ 75°C, and run the following program:
30 minutes @ 20°C
30 minutes @ 65°C
Hold at 4°C

End Prep

- ∗ Use tips with filter for whole procedure
- ∗ Mixing in individual PCR tubes (with flat cap, not the round – they are safer)
- Prepare 10 mM Tris pH8
- Master mix (for 18 + 1 samples); stock from company - green cap epi: NEB buffer (133) + enzyme (57)
  - ∗ if enzyme precipitate: quick vortex
  - ∗ Enzyme – do not vortex; it is viscose
- ***Workflow:***
  1. Pipet Tris – fill up to 50 µL total of volume – based to calculation (table, slide 9)
  2. Pipet sample
  3. Master mix: pipet buffer
  4. Preheat PCR machine (can be done also before, but has to be ready after mixing everything)
  5. Master mix: pipet enzyme

2. Library preparation: Adaptor Ligation

14.11. 24

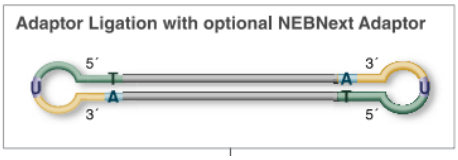


Table 2.1: Adaptor Dilution

INPUT	ADAPTOR DILUTION (VOLUME OF ADAPTOR: TOTAL VOLUME)	WORKING ADAPTOR CONCENTRATION
1 µg–101 ng	No Dilution	15 µM
100 ng–5 ng	10-Fold (1:10)	1.5 µM
less than 5 ng	25-Fold (1:25)	0.6 µM

Note: The appropriate adaptor dilution for your sample input and type may need to be optimized experimentally. The dilutions provided here are a general starting point. Excess adaptor should be removed prior to PCR enrichment.

Add the following components directly to the End Prep Reaction Mixture:

COMPONENT	VOLUME
End Prep Reaction Mixture (Step 1.3 in Section 1)	60 µl
• (red) NEBNext Adaptor for Illumina**	2.5 µl
• (red) NEBNext Ultra II Ligation Master Mix*	30 µl
• (red) NEBNext Ligation Enhancer	1 µl
Total Volume	93.5 µl

* Mix the Ultra II Ligation Master Mix by pipetting up and down several times prior to adding to the reaction.

** The NEBNext adaptor is provided in NEBNext Oligo kits. NEB has several oligo options which are supplied separately from the library prep kit. Please see www.neb.com/oligos for additional information.

Adaptor Ligation

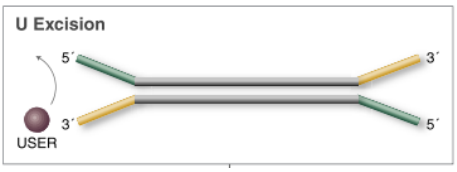
- Pipet adaptor separately to sample
- Prepared master mix for the rest components
- Adaptor:
  - dilution 1:10 : 10 µL of adaptor stock + 90 µL Tris (10mM; pH8)
  - dilution 1:25 : 4 µL of adaptor stock + 96 µL Tris (10mM; pH8)
- Before pipetting: check if PCR finished – take samples, keep the lid open to cool down

Note

- Pair-end … more information, for 3D genome organization etc
- Single-end … we will do

U Excision with USER enzyme

- Just add enzyme directly to reaction and continue …



2.5. Add 3 µl of • (red or blue) USER® Enzyme to the ligation mixture from Step 2.3.

Note: Steps 2.5. and 2.6. are only required for use with non indexed NEBNext Adaptor. USER enzyme can be found in most NEBNext oligo kits. If you are using the indexed UMI adaptor, USER is not needed. Please see corresponding manual for use with UMI on the NEB #E7395 product page under the protocols, manuals, and usage tab.

2. Library preparation: Clean Up without size selection and PCR 14.11. 24
3. TAPEstation

Clean Up/Size Selection

Clean Up/without Size Selection

Note

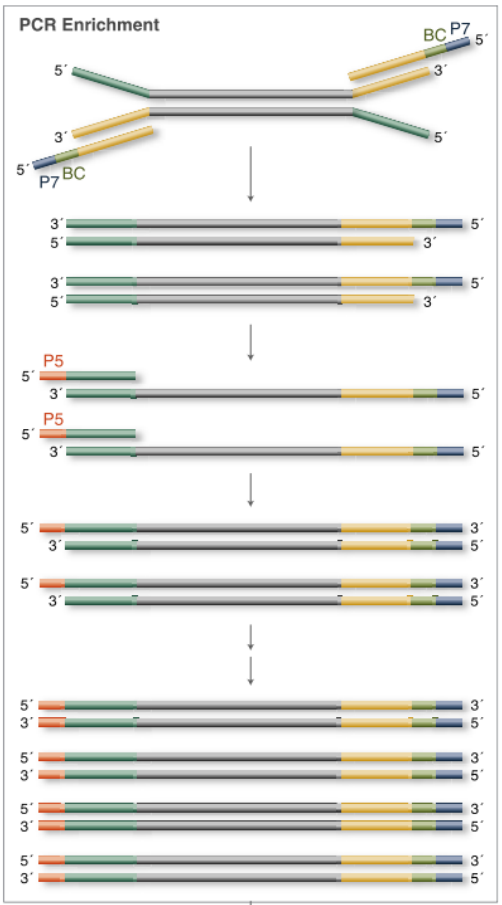
- Beads:
  - resuspend on the roller + vortex well before starting
  - do not cut tip while pipetting, use the filter one

3B. Cleanup of Adaptor-ligated DNA without Size Selection (for input ≤ 50 ng)

The following section is for cleanup of the ligation reaction. If your input DNA is > 50 ng, follow the size selection protocol in Section 3A.

3B.8. Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry.



Clean Up ✗

Genomic center ✗

PCR

- Appendix A: Principle for Use and Pooling Guide https://www.neb.com/en/protocols/2015/01/23/setting-up-the-pcr-reaction-e7600
- Multiplex Oligos for Illumina (Dual Index Primers) (ChIP-seq, CUT&RUN) Protocol https://www.cellsignal.com/learn-and-support/protocols/multiplex-oligos-illumina-dual-index

TAPEstation (Genomic center)

- To check more precise concertation of chromatin – after PCR, but still with primers (additional peaks)
- Buffer D1000; because of the fragments are smaller than 1000bp (but still enough concentration)
- Max 16 samples/run

1. Flick and spin down samples
2. Pipet buffer (3 µL)
3. Pipet sample (1 µL)
4. Shake (special one next to machine) – for the strips already set for 1 min
5. Spin down
6. Place to the machine

Software

- Choose: "electronic ladder"
- Scale to sample
- Define range: usually 200 – 800 bp*
  *we change to 150 – 800 bp, based on results
- File → create report

PCR

- Additional steps base to results from TAPEstation
- Just put mixture to PRC machine – the primers and should be still fine

Clean UP ✗

- Beads as yesterday; 1:1

TAPEstation (Genomic center) ✗

- As previuous

✗ ***….. Will by done later***

In each column: all four bases should be presented, also for i5

Primer PCR	i7	i5	
i701/i508	A T T A C T C G	GTACTGAC	1
i701/i507	A T T A C T C G	CAGGACGT	2
i705/i506	A T T C A G A A	TAATCTTA	3
i709/i508	C G G C T A T G	GTACTGAC	4
i709/i507	C G G C T A T G	CAGGACGT	5
i709/i506	C G G C T A T G	TAATCTTA	6
i706/i508	G A A T T C G T	GTACTGAC	7
i706/i507	G A A T T C G T	CAGGACGT	8
i706/i506	G A A T T C G T	TAATCTTA	9
i711/i505	T C T C G C G C	AGGCGAAG	10
i711/i504	T C T C G C G C	GGCTCTGA	11
i712/i508	A G C G A T A G	GTACTGAC	12
i711/i503	T C T C G C G C	CCTATCCT	13
i712/i505	A G C G A T A G	AGGCGAAG	14
i712/i504	A G C G A T A G	GGCTCTGA	15
i705/i503	A T T C A G A A	CCTATCCT	16
i705/i505	A T T C A G A A	AGGCGAAG	17
i705/i504	A T T C A G A A	GGCTCTGA	18

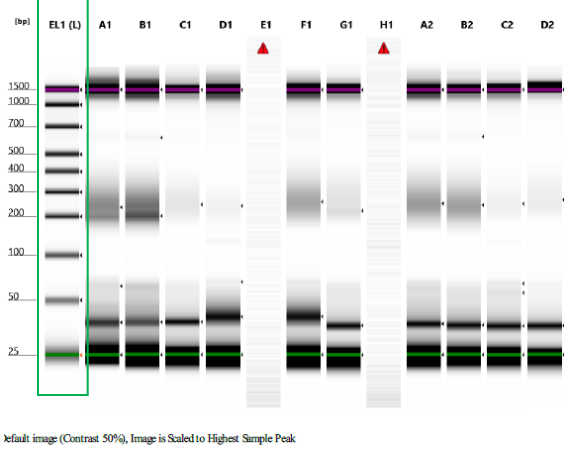
		Qubit concentration			Total amount m		Input v	ChIP	TE buff	Adaptor	PCR c	Primer PCR	i7	i5	
1	**R INP -**	53	ng/uL	dil 1:10			**0.9**		**49.1**	1:10	3	i701/i508	ATTACTCG	GTACTGAC	1
2	**R INP +**	43.4	ng/uL	dil 1:10			**1.2**		**48.8**	1:10	3	i701/i507	ATTACTCG	CAGGACGT	2
3	**R BIOID -**	0.806	ng/uL		12.09	ng		**15**	**35**	1:10	6	i705/i506	ATTCAGAA	TAATCTTA	3
4	**R BIOID +**	0.416	ng/uL		6.24	ng		**15**	**35**	1:10	7	i709/i508	CGGCTATG	GTACTGAC	4
5	**P INP -**	53.8	ng/uL	dil 1:10			**0.9**		**49.1**	1:10	3	i709/i507	CGGCTATG	CAGGACGT	5
6	**P INP +**	57.6	ng/uL	dil 1:10			**0.9**		**49.1**	1:10	3	i709/i506	CGGCTATG	TAATCTTA	6
7	**P BIOID -**	0.2	ng/uL		3	ng		**15**	**35**	1:25	8	i706/i508	GAATTCGT	GTACTGAC	7
8	**P BIOID +**	2.28	ng/uL		34.2	ng		**15**	**35**	1:10	5	i706/i507	GAATTCGT	CAGGACGT	8
9	**L INP -**	16.3	ng/uL	dil 1:10			**3.1**		**46.9**	1:10	3	i706/i506	GAATTCGT	TAATCTTA	9
10	**L INP +**	19.8	ng/uL	dil 1:10			**2.5**		**47.5**	1:10	3	i711/i505	TCTCGCGC	AGGCGAAG	10
11	**L BIOID -**	0.08	ng/uL		1.2	ng		**15**	**35**	1:25	10	i711/i504	TCTCGCGC	GGCTCTGA	11
12	**L BIOID +**	0.226	ng/uL		3.39	ng		**15**	**35**	1:25	8	i712/i508	AGCGATAG	GTACTGAC	12
13	**P INP**	12.5	ng/uL				**4.0**		**46.0**	1:10	3	i711/i503	TCTCGCGC	CCTATCCT	13
14	**L INP**	6.78	ng/uL				**7.4**		**42.6**	1:10	3	i712/i505	AGCGATAG	AGGCGAAG	14
15	**R INP**	4.46	ng/uL				**11.2**		**38.8**	1:10	3	i712/i504	AGCGATAG	GGCTCTGA	15
16	**P H2A**	1.6	ng/uL		24	ng		**15**	**35**	1:10	5	i705/i503	ATTCAGAA	CCTATCCT	16
17	**L H2A**	0.948	ng/uL		14.22	ng		**15**	**35**	1:10	6	i705/i505	ATTCAGAA	AGGCGAAG	17
18	**R H2A**	0.478	ng/uL		7.17	ng		**15**	**35**	1:10	7	i705/i504	ATTCAGAA	GGCTCTGA	18

- Dil 1:10 … input, because there was concentration to high; we use the diluted version
- Total amount: in 15 µL (left after qPCR and Qubit)
- Input = 50 / Qubit concentration
- TE buffer = 50 - Input

3. TAPEstation

19.11. 24

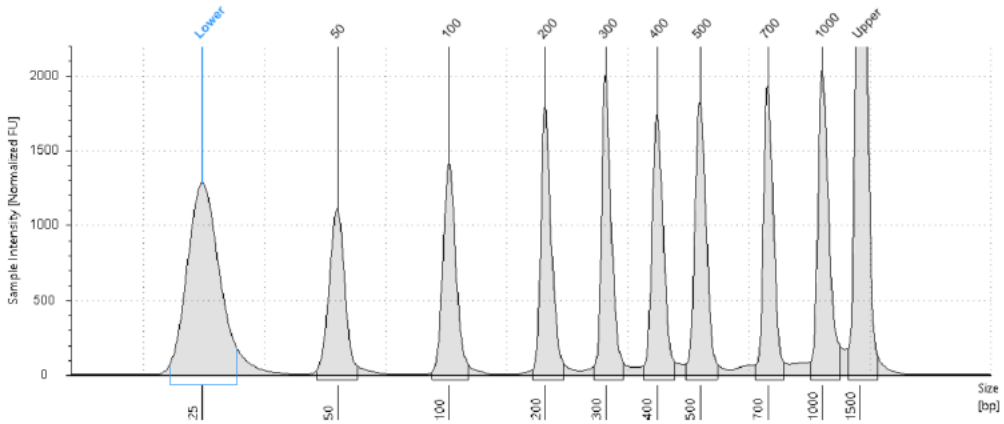
Electronic ladder



)efault image (Contrast 50%), Image is Scaled to Highest Sample Peak

If the sample do not pop up; but should be similar as other
change the position and re-measure or prepare again (BufferD1000 and sample)

EL1: Electronic Ladder



Sample Table

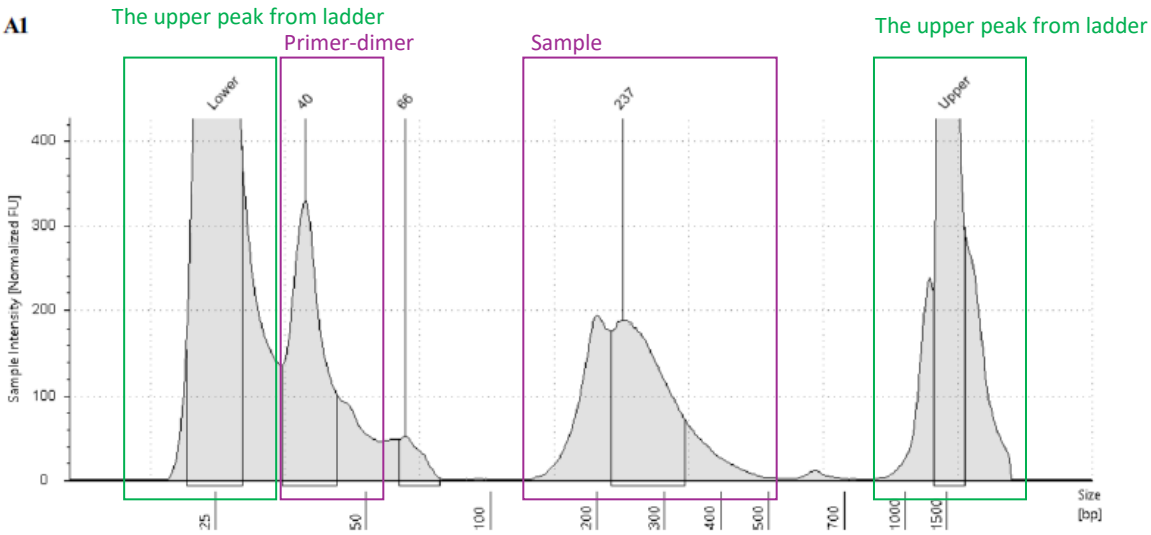
Well	Conc. [ng/µl]	Sample Description	Observations
EL1	20.3	Electronic Ladder	Ladder

Peak Table

Size [bp]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	Peak Molarity [nmol/l]	%Integrated Area	Peak Comment	Peak	Observations
25	5.22	-	321	-		-	Lower Marker
50	2.25	-	69.3	11.11		1	
100	2.37	-	36.5	11.71		2	
200	2.47	-	19.0	12.20		3	
300	2.55	-	13.1	12.56		4	
400	2.57	-	9.87	12.66		5	
500	2.71	-	8.33	13.36		6	
700	2.46	-	5.41	12.15		7	
1000	2.89	-	4.44	14.25		8	
1500	6.50	6.50	6.67	-		-	Upper Marker

2. Library preparation: End Prep

19.11. 24

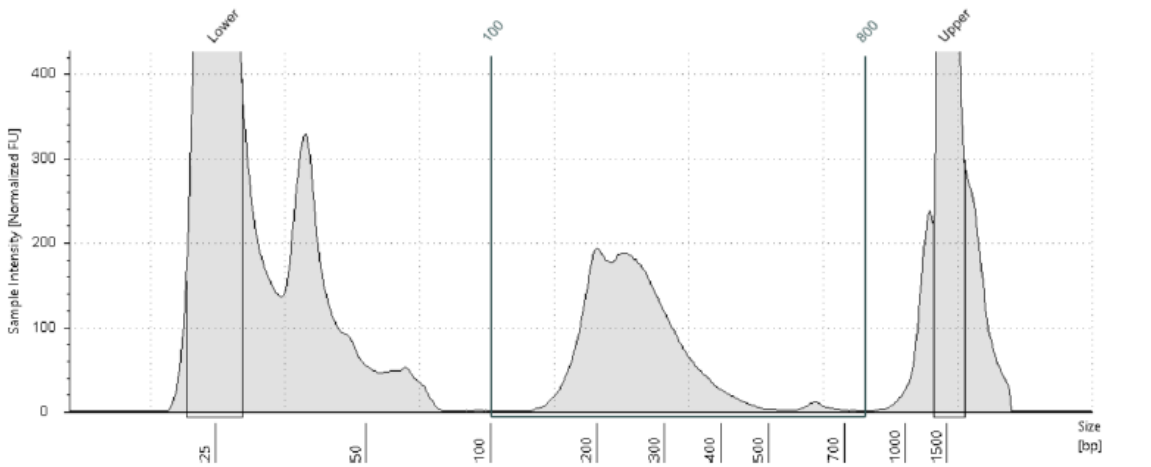


Sample Table

Well	Conc. [ng/µl]	Sample Description	Observations
A1	2.36		

Peak Table

Size [bp]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	Peak Molarity [nmol/l]	% Integrated Area	Peak Comment	Peak	Observations
25	9.19	-	566	-		-	Lower Marker
40	1.14	-	44.2	48.32		1	
66	0.131	-	3.08	5.55		2	
237	1.09	-	7.08	46.13		3	
1500	6.50	6.50	6.67	-		-	Upper Marker



Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total	Region Comment	Color
100	800	263	1.98	12.5	36.13		

2. Library preparation: End Prep

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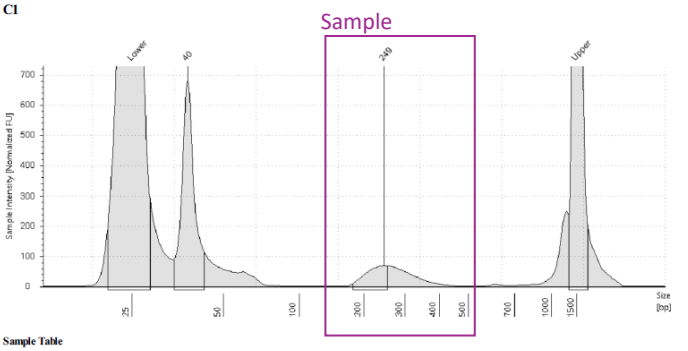
Examples
Still OK

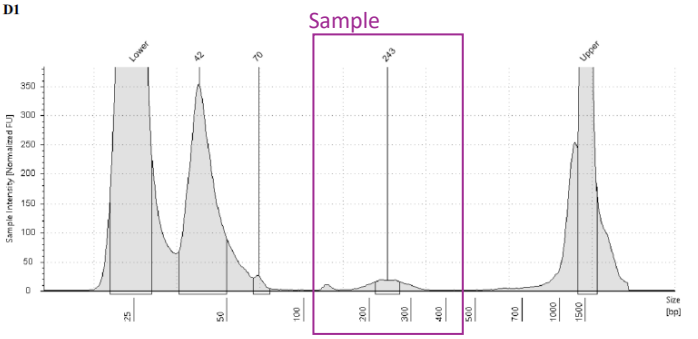
C1



Sample Table

Well	Conc. [ng/µl]	Sample Description	Observations
C1	1.93		

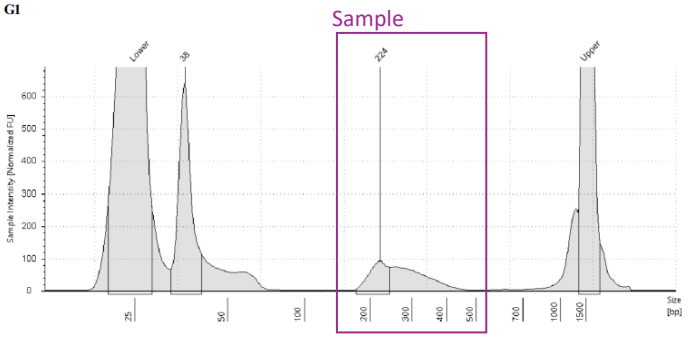
D1



Sample Table

Well	Conc. [ng/µl]	Sample Description	Observations
D1	1.83		

G1

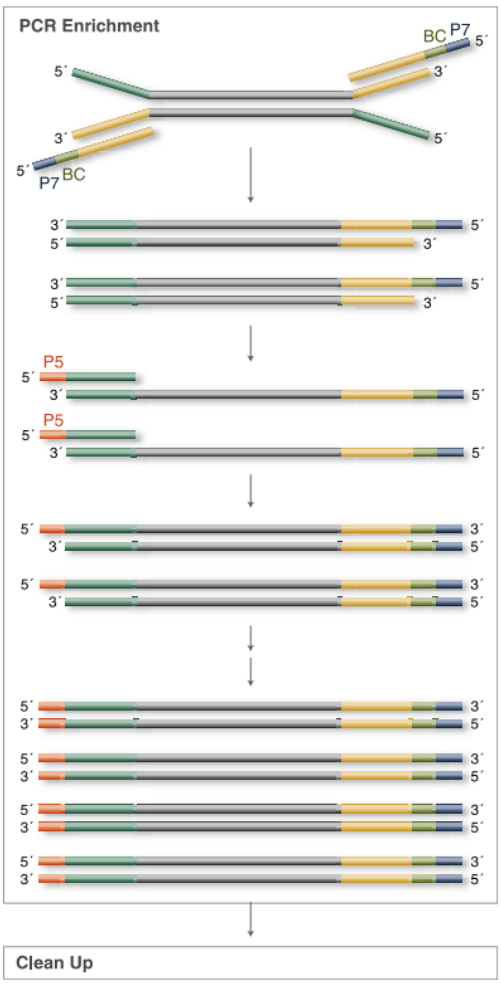


Sample Table

Well	Conc. [ng/µl]	Sample Description	Observations
G1	1.61		

4. Library preparation: additional PCR and second Clean Up

14.11. 24



Genomic center

PCR

- Additional steps base to results from TAPEstation
- Just put mixture to PRC machine – the primers and should be still fine

Clean UP

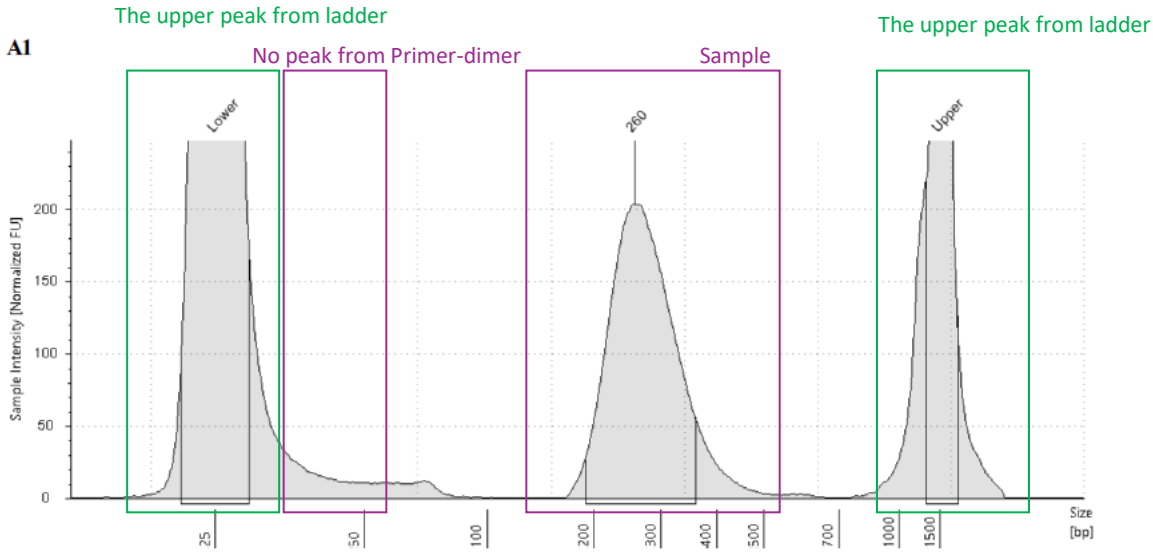
- Beads as yesterday; 1:1

TAPEstation (Genomic center)

- As previuous

5. TAPEstation: PRC after purification

19.11. 24



Region Table

From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total	Region Comment	Color
100	800	283	1.94	11.0	62.11		

Compact Region Table

WellId	Sample Description	From [bp]	To [bp]	Average Size [bp]	Conc. [ng/µl]	Region Molarity [nmol/l]	% of Total	Region Comment	Color
A1		100	800	283	1.94	11.0	62.11		
B1		100	800	274	1.90	11.2	62.12		
C1		100	800	318	2.41	12.5	66.42		
D1		100	800	372	0.972	4.59	42.91		
F1		100	800	355	2.20	10.5	66.36		
G1		100	800	353	3.46	16.4	75.03		
A2		100	800	309	3.02	16.0	73.22		
B2		100	800	305	2.38	12.6	70.87		
C2		100	800	387	3.94	17.6	71.46		
D2		100	800	361	2.57	12.0	67.99		

2. Library preparation: End Prep

19.11. 24

TAPEstation (Genomic center)

- To check more precise concertation of chromatin – after last Clean up, samples without primers
- Buffer D1000;

1. Flick and spin down samples
2. Pipet buffer (3 µL)
3. Pipet sample (1 µL)
4. Shake (special one next to machine) – for the strips already set for 1 min
5. Spin down
6. Place to the machine

Software

- Choose: "electronic ladder"
- Scale to sample
- File
  → include compact peak
  → include compact
- Define range 100 – 800 bp*
  *because these samples do not contain primers after Clean up
- File → create report

Barcode 1 … i7
Barcode 2 … i5

From the final concentration calculate Pooling in Illumina Pooling Calculator

- https://support.illumina.com/help/pooling-calculator/pooling-calculator.htm
- Plexity: number of samples
- Different, import value
- nM
- 4 nM
- 100 µL …. Total volume

- Copy values (the concentration) form TAPEstation
- Copy Calculation to Excel (Total Pool Volume): Library 1 -XX

Pooling with someone

- Agree on primers
- Has to be compatible:
  - Similar size of fragments
  - Smear
    + another "contamination"
  - Better not to pool samples from different samples concentration

illumina

Pooling Calculator

Library Plexity	18
Do the libraries have the same concentration?	○ Same ○ Different ◉ Different, import values ○ Different, import values and names
Unit of Measure for Library	◉ nM ○ ng/µl
Pooled Library Concentration (nM)	4
Total Pooled Library Volume (µl)	100
Description (optional)	

Enter values separated by commas, spaces, tabs, or returns.
For example: 1.500 1.672 1.781

How to Calculate Illumina Read Length

All Illumina sequencing reagents feature a certain number of sequencing cycles. These cycles are directly related to sequencing read length. Because one base is sequenced per cycle, the total number of cycles indicates the maximum number of bases that can be sequenced. You can use sequencing reagents to generate single continuous reads or for paired-end sequencing in both directions. (For example, a 300-cycle kit can be used for a 1 × 300 bp single-read run or a 2 × 150 bp paired-end run.)

bfabrik (Genomic center)

- samples contains trans genes: "no"
- 1 lane 1.58 flocel: 750 000pair read end; aprox. 41*10^6/one ChIP
- Paired-end 150 bp

illumina

Pooling Calculator

Library Plexity						
18						
Do the libraries have the same concentration?						
Different, import values						
Unit of Measure for Library						
nM						
Pooled Library Concentration (nM)						
4						
Total Pooled Library Volume (µl)						
100						
Description (optional)						
	Library Con	Library Concentration (nM)	Library Volu	10 mM Tris-	Pooling Volume (µl)	
10 mM Tris-HCl, pH 8.5				0		
Total Pool Volume	100					
Library 1		11	4.1	7	5.5	
Library 2		11.2	4	7.1	5.5	
Library 3		12.5	3.6	7.5	5.5	
Library 4		4.59	9.8	1.3	5.5	
Library 5		9.44	4.8	6.3	5.5	
Library 6		10.5	4.3	6.8	5.5	
Library 7		16.4	2.7	8.4	5.5	
Library 8		12.8	3.5	7.6	5.5	
Library 9		16	2.8	8.3	5.6	
Library 10		12.6	3.5	7.6	5.6	
Library 11		17.6	2.5	8.6	5.6	
Library 12		12	3.7	7.4	5.6	
Library 13		17.6	2.5	8.6	5.6	
Library 14		15.7	2.8	8.3	5.6	
Library 15		13.8	3.2	7.9	5.6	
Library 16		25.2	2	10.7	5.6	
Library 17		27	2	11.6	5.6	
Library 18		27.9	2	12.1	5.6	
Mon Nov 18 2024						

				Pool 4 nM	
		Concentration Tapestation (nM)	Library Volume (µl)	10 mM Tris-HCl, pH 8.5 (µl	Pooling Volume (µl)
1	**R INP -**	11	4.1	7	5.5
2	**R INP +**	11.2	4	7.1	5.5
3	**R BIOID -**	12.5	3.6	7.5	5.5
4	**R BIOID +**	4.59	9.8	1.3	5.5
5	**P INP -**	9.44	4.8	6.3	5.5
6	**P INP +**	10.5	4.3	6.8	5.5
7	**P BIOID -**	16.4	2.7	8.4	5.5
8	**P BIOID +**	12.8	3.5	7.6	5.5
9	**L INP -**	16	2.8	8.3	5.6
10	**L INP +**	12.6	3.5	7.6	5.6
11	**L BIOID -**	17.6	2.5	8.6	5.6
12	**L BIOID +**	12	3.7	7.4	5.6
13	**P INP**	17.6	2.5	8.6	5.6
14	**L INP**	15.7	2.8	8.3	5.6
15	**R INP**	13.8	3.2	7.9	5.6
16	**P H2A**	25.2	2	10.7	5.6
17	**L H2A**	27	2	11.6	5.6
18	**R H2A**	27.9	2	12.1	5.6