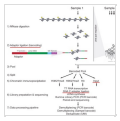

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MINUTE ChIP

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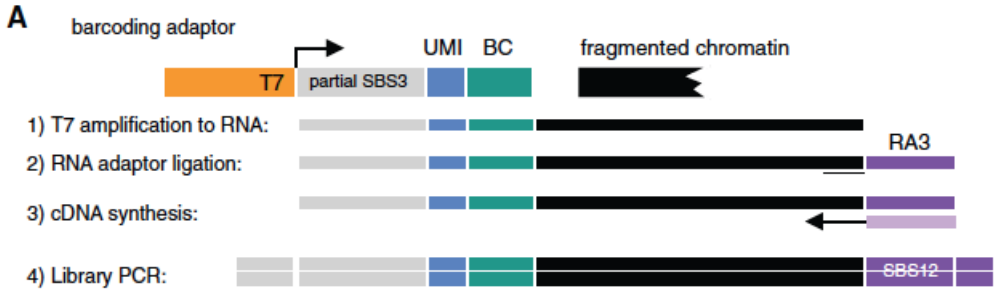
1 Works for me dx.doi.org/10.17504/protocols.io.8nkhvcw

Banushree Kumar

ABSTRACT

The need for quantitative ChIP-seq has been widely appreciated, and a number of quantitative methods have been proposed. Mint-ChIP, developed by the Bernstein lab provides a streamlined one-pot chromatin barcoding and a post ChIP linear amplification that requires only one adaptor per chromatin fragment. Here, we introduce unique molecule (unique molecular identifier [UMI]) counting and paired-end mapping of the chromatin fragments to this method, which we then termed MINUTEChIP for multiplexed indexed unique molecule T7 amplification end-to-end sequencing. For paired-end mapping, we modified the linear amplification strategy introduced in Mint-ChIP by priming the cDNA synthesis from a 30 adaptor (RA3) ligated onto the amplified RNA, thus maintaining the original genomic fragment length, retrieving atypical mononucleosomal fragment length for histone ChIP. We have also optimized the ligation conditions and stoichiometry of adaptors to chromatin to enable sequencing of the input. We show that MINUTE-ChIP is a sensitive method with a large linear dynamic range for accurately quantifying relative differences in genome-wide histone modification patterns across multiple pooled samples.



Schematic of MINUTE adaptor design for barcoding chromatin. One-sided ligation of adaptor comprising T7 RNA polymerase promoter (T7), random 6-bp sequence (UMI), and 8-bp barcode (BC) is sufficient for subsequent linear amplification. cDNA is primed from a ligated RNA adaptor (RA3). SBS3 and SBS12 designate standard Illumina read1 and read2 sequencing primers.

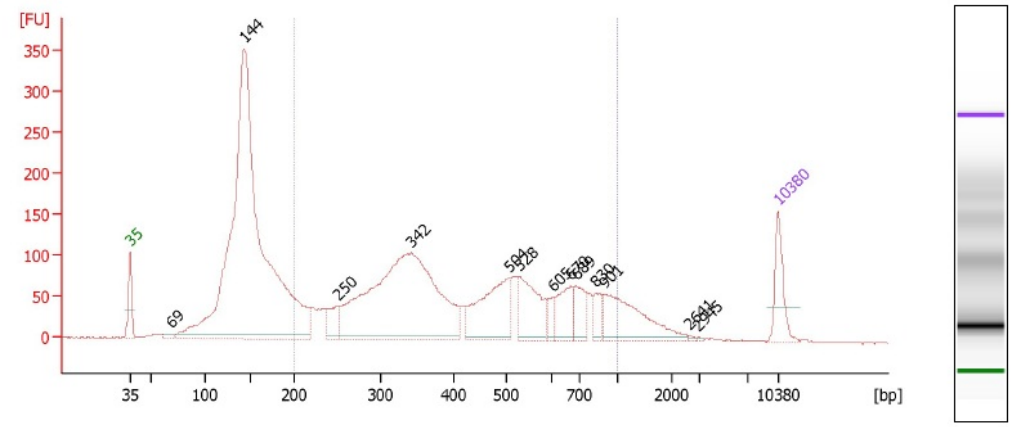
EXTERNAL LINK

https://www.cell.com/cell-reports/fulltext/S2211-1247(19)31094-0#secsectitle0025

PIIS2211124719310940.pdf

GUIDELINES

Optimal fragmentation of the genomic DNA is crucial for the success of the protocol. Hence, the concentration of MNase to be used needs to be titrated based on cell type and density in a pilot experiment.



BioA profile for 1x10^6 mESC cells digested in 100 µl mixture with 100 U of MNase.

For any native cell type, digestion condition that yields fragments of predominantly mono (150bp) to few di and tri nucleosomes is optimal for downtream steps of the protocol.

MATERIALS TEXT

Refer to "Key Resource Table" in the paper for catalogue of reagents required.

The T7 adapter and PCR barcode sequences listed below were designed based on the Mint-ChIP protocol.

van Galen P, Viny AD, Ram O, Ryan RJ, Cotton MJ, Donohue L, Sievers C, Drier Y, Liau BB, Gillespie SM, Carroll KM, Cross MB, Levine RL, Bernstein BE (2016). A Multiplexed System for Quantitative Comparisons of Chromatin Landscapes.. Molecular cell. https://doi.org/10.1016/j.molcel.2015.11.003

Additional unique T7 adapter and PCR barcode sequences can be provided on request.

FORWARD	
AdRan_BC01_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCT ACC AGG
AdRan_BC02_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCA TGC TTA
AdRan_BC03_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NGC ACA TCT
AdRan_BC04_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NTG CTC GAC
AdRan_BC05_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NAG CAA TTC

AdRan_BC06_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NAG TTG CTT
AdRan_BC07_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCC AGT TAG
AdRan_BC08_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NTT GAG CCT
AdRan_BC09_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NAC CAA CTG
AdRan_BC10_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NGG TCC AGA
AdRan_BC11_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NGT ATA ACA
AdRan_BC12_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NTT CGC TGA
AdRan_BC13_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NAG GGT ACT
AdRan_BC14_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCG GAC TAT
AdRan_BC15_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCT GCA CAA
AdRan_BC16_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NGA ATC GGT
AdRan_BC17_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NGT TGA GTC
AdRan_BC18_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NAA AGG GAC
AdRan_BC19_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCA CAG GTT

AdRan_BC20_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCC TAT GCA
AdRan_BC21_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NCT GTT GTG
AdRan_BC22_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NGA CCT ATC
AdRan_BC23_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NGA TAG TGC
AdRan_BC24_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NTA GCT GGA
AdRan_BC25_s	/5SpC3/GAA TTT AAT ACG ACT CAC TAT AGG GTA CAC GAC GCT CTT CCG ATC TNN NNN NTG ACA GTG
REVERSE	
AdRan_BC01_as	CCT GGT AGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC02_as	TAA GCA TGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC03_as	AGA TGT GCN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC04_as	GTC GAG CAN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC05_as	GAA TTG CTN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC06_as	AAG CAA CTN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC07_as	CTA ACT GGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC

AdRan_BC08_as	AGG CTC AAN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC09_as	CAG TTG GTN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC10_as	TCT GGA CCN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC11_as	TGT TAT ACN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC12_as	TCA GCG AAN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC13_as	AGT ACC CTN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC14_as	ATA GTC CGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC15_as	TTG TGC AGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC16_as	ACC GAT TCN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC17_as	GAC TCA ACN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC18_as	AAA GGG ACN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC19_as	AAC CTG TGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC20_as	TGC ATA GGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC21_as	CAC AAC AGN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC

AdRan_BC22_as	GAT AGG TCN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC23_as	GCA CTA TCN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC24_as	TCC AGC TAN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC
AdRan_BC25_as	CAC TGT CAN NNN NNA GAT CGG AAG AGC GTC GTG TAC CCT ATA GTG AGT CGT ATT AAA TTC

T7 ADAPTERS SEQUENCES (Includes T7 promoter; partial Illumina Primer SBS3; UMI; Barcode)

Ordered from IDT as individual oligos at a conc. of 500 µM each.
Combine equal amounts of each (ex:100µl + 100µl) and anneal with following program:
95°C for 3 minutes
94–79°C go down 1 degree per 10 seconds
79–69°C go down 1 degree per 30 seconds
69°C for 5 minutes
69–40°C go down 1 degree per 10 seconds
4°C forever
Annealed adaptor at conc. of 250 µM. Dilute further five fold to obtain working conc. of 50 µM.
Store at -20°C

RA3 adapter	/5rApp/AGA TCG GAA GAG CAC ACG TCT /3SpC3/
RT_primer	AGA CGT GTG CTC TTC CGA TCT

RNA 3' adapter* and Reverse transcription Primer

*This has special modifications and hence expensive.
Ordered from IDT, prediluted in TE buffer at **[M]100 Micromolar (µM)** .
Dilute the RA3 adapter further to **[M]10 Micromolar (µM)** and the RT_primer to **[M]20 Micromolar (µM)** . Store at -20°C, freeze/thawing is ok!

PCR_forward	AATGATACGGCGACCACCGAGATCTACACTCTT TCCCTACACGACGCTCTTCCGATCT

PCR_BC1	CAA GCA GAA GAC GGC ATA CGA GAT CCT GGT AGG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC2	CAA GCA GAA GAC GGC ATA CGA GAT TAA GCA TGG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC3	CAA GCA GAA GAC GGC ATA CGA GAT AGA TGT GCG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC4	CAA GCA GAA GAC GGC ATA CGA GAT GTC GAG CAG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC5	CAA GCA GAA GAC GGC ATA CGA GAT GAA TTG CTG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC6	CAA GCA GAA GAC GGC ATA CGA GAT TCG CAC CTG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC7	CAA GCA GAA GAC GGC ATA CGA GAT CTA ACT GGG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC8	CAA GCA GAA GAC GGC ATA CGA GAT AGG CTC AAG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC9	CAA GCA GAA GAC GGC ATA CGA GAT CAG TTG GTG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
PCR_BC10	CAA GCA GAA GAC GGC ATA CGA GAT TCT GGA CCG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT

PCR PRIMERS (Designed inhouse and includes essential Illumina primer P7 and SBS12 sequences.)

Resuspend all primers at 100 µM
Mix together for each primer :
25 µl forward primer (always the same)
25 µl barcoded primer
200 µl TE
To obtain **250 µl** primer mix, **10 Micromolar (µM)** each.
Store at -20°C, freeze/thawing is ok!

BUFFERS

2x Lysis buffer (Lyb buffer)
100mM Tris-HCl (1 M, pH 8.0)
0.2% Triton X-100 (25%)
0.1% sodium deoxycholate (DOC, 12.5%)
10mM CaCl2 (1 M)
H2O
Lysis dilution buffer
50mM Tris-HCl (1 M, pH 8.0)
150mM NaCl (5 M)
1% Triton X-100 (25%)
50 mM EGTA (0.5 M)

50 mM EDTA (0.5 M)
0.1% sodium deoxycholate (DOC, 12.5%)
H2O

ChIP elution buffer
10x TE buffer
0.1% SDS (10%)
300 mM NaCl (5 M)
H2O

ChIP wash buffers
RIPA buffer
0.1% DOC, 0.1% SDS, 1% Triton X-100, 10mM Tris-HCl pH 8.0, 1mM EDTA, 140 Mm NaCl
RIPA/High salt
0.1% DOC, 0.1% SDS, 1% Triton X-100, 10mM Tris-HCl pH 8.0, 1mM EDTA, 360 mM NaCl
LiCl Wash Buffer
250mM LiCl, 0.5% NP40, 0.5% deoxycholate, 1mM EDTA, 10mM Tris-HCl, pH 8.0
TE Buffer pH 8.0
10mM Tris-HCl pH 8.0, 1mM EDTA

LYSIS AND DIGESTION

1 Harvest 10^6 cells, spin, flash freeze the pellet.

Make sure cell pellet is frozen with as little excess liquid as possible

2 On the day of experiment resuspend in **50 µl** of ice cold PBS.

3 Add **50 µl** of MNase mix (final 2U/ul in 2xLyB for mouse embryonic stem cells).

MNase mix :
First add 20 **µ**l protease inhibitor to 1 ml 2x lysis buffer (2xLyB)
Stepwise dilute MNase (M0247S NEB 2000 U/ul) to 20U/µl and then 2U/µl in 2x LyB
Final working conc. = 2U/µl in 2xLyB.

4 Lyse cells on ice for **00:20:00** . Start preparing the enzyme mix for T7 adapter ligation.

5 Digest at **37 °C** for **00:10:00** **with agitation at 800 rpm.**

6 Transfer tubes **On ice**

T7 ADAPTER LIGATION

7 Prepare enzyme mix:

	Per 80 µl rxn
30 mM EGTA (0.5 M)	4.8
1× buffer (10×)	8.0
1.5 mM ATP (10 mM)	12.0
1× dNTP mix (10×)	8.0
End-Repair Enzyme mix	1.6
Fast-Link DNA ligase	1.6
5 mM sodium butyrate (1 M)	0.2
1× Protease inhibitor (100×)	0.4
Total volume	*36.6 µl*

7.1 QUICKLY add **36.6 µl** enzyme mix to **40 µl** of the digested cells. Then add **4 µl** of unique T7 adapter to each sample.
*Total volume ~80 **µ**l (40 **µ**l cells + 4 **µ**l T7 adapter + 36 **µ**l enzyme mix)*

7.2

Mix and incubate **with agitation at 800 rpm** at **Room temperature** for **02:00:00** and then at **16 °C** overnight.

The manufacturer's protocol for the end repair and blunt end ligation suggests 60 minutes incubation at room temperature.We have obtained satisfactory results with just 2 hours incubation at room temperature. The overnight incubation is therefore optional and was introduced more as a break point for the protocol.

8 Next day, add 1x protease inhibitor (100x) to Lysis dilution buffer and add **80 µl** of it to each sample in order to end reaction (Total volume per well: **160 µl**)

POOL AND SPLIT

9 Combine all samples into a single tube; spin down at **24000 rpm** for **00:10:00** at **4 °C** .

9.1

Transfer supernatant to a fresh tube.

Remember to save appropriate amount of the pool as input (Usually 5-10% of the volume used for the IPs)

IMMUNOPRECIPITATION

10 Precouple antibody to the magnetic beads

IP conditions such as amount of antibody to use, volume of pooled lysate to be added and duration of incubation can be optimized for individual experiments.

10.1 Was **50 µl** Protein G/A beads with 2x **1000 µl** PBS-T(0.1%)

10.2 Resuspend beads in **200 µl** of the same buffer and incubate with appropriate amount of antibodies for **01:00:00** at **Room temperature**

10.3 Wash coupled beads quickly (<1 min) with **200 µl** RIPA and resuspend in **50 µl** of Lysis dilution buffer.

10.4 Top up with 200 - 400 **µl** of the pooled lysate for each IP*, and rotate tube for **04:00:00** at **4 °C**

11 Wash

11.1 Magnetize samples and save supernatant at -20ºC

This supernatant can be used for checking IP efficiency or other troubleshooting

11.2

Resuspend, rotate for 5 mins, magnetize and aspirate :
2x with 500 µl ice cold RIPA buffer
1x with 500 µl ice cold RIPA/high salt buffer
2x with 500 µl ice cold LiCl wash buffer
1x with 200 µl ice cold TE (quick wash and change to new tube)

12 Elution from protein G beads and protein digestion

12.1 ⚠

Resuspend chromatin/antibody/beads in **97.5 µl** of ChIP elution buffer and **2.5 µl** of Proteinase K.

Remember to process the INPUT sample collected in Step 9.1, similar to the IPs from this step on.

12.2 Digest at **63 °C** for **01:00:00** (native chromatin) or overnight (to reverse crosslink fixed chromatin).

12.3 SPRI bead cleanup to isolate DNA (room temperature)

- Using magnet, transfer **100 µl** eluate to new wells. Add **100 µl** SPRI beads (1x, select >100 bp fragments)
- Mix by pipetting, wait **00:10:00**
- Magnetize for **00:05:00**
- Remove supernatant
- Wash 2x with **200 µl** 70% ethanol (magnetize 1 minute)
- Air-dry at RT for >= **00:05:00**
- Elute DNA in **12 µl** EB and measure **1 µl** with Qubit

There is no 'right' amount of ChIP DNA. Selective antibodies against low abundant targets will yield little DNA

LIBRARY PREPARATION

13 Invitro Transcription (IVT)

13.1 Prepare IVT mix in PCR tubes as follows:

DNA from IP	8
NTP buffer mix (2x)	10
T7 RNA polymerase mix	2
Total volume	20 µl

It is advisable to take as much ChIP DNA as possible into the IVT reaction to maximize the library diversity

13.2 Incubate samples at **37 °C** overnight **16:00:00** **roughly**

13.3 Silane bead cleanup to isolate RNA (room temperature)

- Magnetize **15 µl** of Silane beads per sample (will bind up to 3 µg RNA).
- Wash with **60 µl** buffer RLT, magnetize, aspirate and resuspend beads in **60 µl** buffer RLT.
- Add **60 µl** washed beads to **20 µl** sample, wait **00:01:00**
- Add **48 µl** 100% EtOH (final: 0.6**x**), mix well, wait **00:05:00** (mix by pipetting at 2.5 min)
- Magnetize, wash twice with **200 µl** 70% EtOH
- Air-dry at RT for atleast **00:05:00**
- RNA eluted in **10 µl** EB
- Measure **1 µl** on Qubit

14 RNA 3' adapter ligation

14.1 Set up the ligation rxn as follows per sample:

RNA from IVT	8
RNA3 diluted adapter (10 µM)	1
RNA ligase buffer	2
T4 RNA ligase truncated	1
RNase OUT	1
Total Volume	13 µl

14.2 Incubate at **25 °C** for **02:00:00** .

15 Reverse Transcription

15.1 Move tube to ice and top up with the following components of the reverse transcription reaction:

RNA from above	13
RT primer (20µM)	4
Anneanling buffer	1
Total Volume	*18 µl*

Heat to **65 °C** for **00:05:00** and place on ice for **00:01:00**

1h 30m

15.2 Then add :

2x First-Strand Reaction Mix	20
SIII/RNase OUT Enzyme Mix	2
Total Volume	*40 µl*

Incubate in a thermocycler with following program :

25°C	10 min
50°C	50 min
85°C	5 min
4°C	∞

15.3

Add **1 µl** Rnase H, DNase free and incubate **00:15:00** at **37 °C** .
(this helps for accurate cDNA measurements)

15.4 SPRI bead cleanup to isolate DNA (room temperature)

- Add **59 µl** of nuclease free water to the cDNA mixture to make final volume 1 **0 µl**
- Add **100 µl** SPRI beads (1x select >100 bp fragments)
- Mix by pipetting, wait **00:10:00**
- Magnetize for **00:05:00**
- Remove supernatant
- Wash 2x with **200 µl** 70% ethanol (magnetize 1 minute)
- Air-dry at RT for >= **00:05:00**
- Elute DNA in **24 µl** of TE and measure conc. on Nanodrop.

16 Library PCR

16.1 Adjust cDNA conc. for all samples to be **150 ng** (or a minimum of 100 ng) in a total volume of **22.5 µl** with nuclease free water and set up following PCR reaction mix :

	Per rxn
cDNA + water	22.5
0.5 µM PCR primer mix (10 µM)	2.5
2x PCR master mix	25
Total Volume	50 µl

16.2

And set it up with the following cycling conditions:

Temperature	*Duration*	*Cycles*
95°C	3 min	1x
95°C	15 sec	
65°C	30 sec	4 x
72°C	30 sec	
95°C	15 sec	
72°C	60 sec	10 x
72°C	5 min	
4°C	∞	1x

16.3 SPRI bead cleanup to isolate DNA (room temperature)

We employed 1x to select for all >100bp fragments; no specific size selection.
But one can also size select at this step.

- Add **50 µl** of nuclease free water to the PCR mixture to make final volume **100 µl** .
- Add **100 µl** SPRI beads (1x select >100 bp fragments)
- Mix by pipetting, wait **00:10:00**
- Magnetize for **00:05:00**
- Remove supernatant
- Wash 2x with **200 µl** 70% ethanol (magnetize 1 minute)
- Air-dry at RT for >= **00:05:00**
- Elute DNA in **20 µl** of elution buffer.
- Measure **1 µl** on Qubit.

PREPARING LIBRARY FOR ILLUMINA

17