CUT&RUN for nuclei using the CUTANA™ ChIC/CUT&RUN Kit

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methods

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We use this protocol and it's working

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ABSTRACT

Modified CUTANA™ ChIC/CUT&RUN Kit protocol using nuclei from brain tissue.

GUIDELINES

Taken from the Cutana ChIC/CUT&RUN Kit (https://www.epicypher.com/products/epigenetics-reagents-and-assays/cutana-chic-cut-and-run-kit) for more information.

MATERIALS

- NEXTFLEX Rapid DNA-Seq Kit 2.0 Bundle **PerkinElmer Catalog #NOVA-5188-12**
- KONTES Dounce Tissue Grinders **Kimble Chase Catalog #KT885300-0002**
- Qubit® dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q32854**
- HALT phosphatase and protease inhibitor cocktail (100x) **Thermo Fisher Scientific Catalog #78442**
- 2 ml LoBind Tubes **Eppendorf Catalog #0030108078**
- 1.5 mL LoBind tubes **Eppendorf Catalog #022431021**
- Cutana ChIC/CUT&RUN Kit **EpiCypher Catalog #14-1048**

Roche Complete Protease Inhibitor EDTA-Free tablets **Merck MilliporeSigma (Sigma-Aldrich) Catalog #5056489001**

Molecular Grade Water **ATCC Catalog #60-2450**

Sera-Mag SpeedBeads Carboxylate-Modified Magnetic Particles **GE Healthcare Catalog #44152105050350**

MACS SmartStrainers 30um **Miltenyi Biotec Catalog #130-098-458**

NEBNext Ultra II DNA Library Prep Kit for Illumina **NEB Catalog #E7645S**

NEBNext Ultra End Repair/dA-Tailing Module - 24 rxns **New England Biolabs Catalog #E7442S**

HEPES **Fisher Scientific Catalog #BP310**

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8787-50ML**

Glycerol, 1000ml **Promega Catalog #H5433**

Magnesium Chloride **Fisher Scientific Catalog #AC223210010**

High Sensitivity D1000 ScreenTape **Agilent Technologies Catalog #5067-5584**

High Sensitivity D1000 Reagents **Agilent Technologies Catalog #5067-5585**

Equipment

new equipment	NAME
Qubit 2.0 Fluorometer instrument	BRAND
Q33226	SKU
with Qubit RNA HS Assays	SPECIFICATIONS

Equipment

4200 TapeStation System NAME

Electrophoresis tool for DNA and RNA sample quality control. TYPE

TapeStation Instruments BRAND

G2991AA SKU

https://www.agilent.com/en/product/automated-electrophoresis/tapestation-systems/tapestation-instruments/4200-tapestation-system-228263 LINK

Equipment

Thermomixer C NAME

Eppendorf BRAND

2231000667 SKU

https://www.pipette.com/2231000667-Promotion-Eppendorf-ThermoMixer-C-with-24x1-5-mL-SmartBlock-and-ThermoTop LINK

	10mM	Tris pH 8.0
	250mM	sucrose
	25mM	KCl
	5mM	MgCl2
	0.10%	Triton X-100
	0.50%	RNasin Plus RNase inhibitor Promega Catalog #N2611
	0.20%	1X protease Inhibitor Promega Catalog #G6521
	0.1mM	DTT

HB

SAFETY WARNINGS

Regular lab safety rules apply. Ensure the use of googles during the usage of dry ice and formaldehyde.

BEFORE START INSTRUCTIONS

The pre-experimentation steps are important
Also it is important that all the primers have been ordered and reconstituted beforehand.

pre-experimentation

1 All steps should be performed on ice or at 4 °C. 1m

2 Pre-chill all Dounces and pestles to 4 °C in a fridge. Or just leave on ice for a while. 10m

3 Pre-chill all tubes.

For each sample you are processing, you will need:

(i) One 2 mL 2 ml LoBind Tubes **Eppendorf Catalog #0030108078** per sample

(ii) Three 1.5 mL LoBind tubes **Eppendorf Catalog #022431021** per sample

(iii) one PCR tube per sample

(iv) One 50 ml Falcon tube **Contributed by users** for filtration step per sample

4 Prepare buffers.

i) Homogenization buffer

ii) 25X protease inhibitor

iii) wash buffer (see step 5)

iv) CP buffer (see step 5)

v) antibody buffer (see step 5)

vi) Nuclear Buffer (see step 26)

4.1 Homogenization buffer (HB);

	A	B
	10mM	Tris pH 8.0
	250mM	sucrose
	25mM	KCl
	5mM	MgCl2
	0.10%	Triton X-100
	0.50%	RNasin Plus RNase inhibitor Promega Catalog #N2611
	0.20%	1X protease Inhibitor Promega Catalog #G6521
	0.1mM	DTT

Homogenization buffer

RNasin Plus RNase inhibitor **Promega Catalog # N2611**

1X protease inhibitor **Promega Catalog #G6521**

4.2 ii) 25X protease inhibitor

1 protease inhibtor tablet (Roche) in 2 mL water (25X stock)

Store remaining 25X stock for 12 weeks at -20 °C

Roche Complete Protease Inhibitor EDTA-Free tablets **Merck MilliporeSigma (Sigma-Aldrich) Catalog #5056489001**

DAY 1 - Section I - Buffer Prep

30m

5

	A	B	C	D	E	F	G	H	I
	# of Samples	1X	2X	3X	4X	5X	6X	8X	16X
	Wash Buffer –								
	Pre-wash buffer	1.8 ml	3.6 ml	5.4 ml	7.2 ml	9 ml	10.8 ml	14.4 ml	28.8 ml
	25X Protease inhibitor	72 ul	144 ul	216 ul	288 ul	360 ul	432 ul	576 ul	1.15 ml
	1 M Spermidine	0.9 ul	1.8 ul	2.7 ul	3.6 ul	4.5 ul	5.4 ul	7.2 ul	14.4 ul
	**1% Triton X-100	0.02 ul	0.038 ul	0.056 ul	0.074 ul	0.092 ul	0.10 ul	0.146 ul	0.30 ul
	** 0.05% SDS	0.015 ul	0.19 ul	0.028 ul	0.037 ul	0.046 ul	0.05 ul	0.073	0.15 ul
	Cell Permeabilization buffer –								
	Wash Buffer	1.4 ml	2.8 ml	4.2 ml	5.6 ml	7 ml	8.4 ml	11.2 ml	22.4 ml
	5% Digitonin	2.8 ul	5.6 ul	8.4 ul	11.2 ul	14 ul	16.8 ul	22.4 ul	44.8 ul
	Antibody Buffer –								
	Cell Permeabilization buffer	100 ul	200 ul	300 ul	400 ul	500 ul	600 ul	800 ul	1.6 ml
	0.5 M EDTA	0.4 ul	0.8 ul	0.16 ul	0.32 ul	0.64 ul	0.128 ul	3.2 ul	6.4 ul

buffer scaling calculations

Wash buffer - store at RT for use on Day 1

CP buffer - store at 4° for use on Day 2

Antibody buffer - store on ice for use on Day 1

6 Example of making solutions:

Add 1.8 mL of **Pre-Wash Buffer** per sample to a single conical tube labeled **Wash Buffer**
Add 72 µL µL of 25X protease inhibitor stock per sample to the **Wash Buffer**
Add 0.9 µL ul of 1M Spermindine per sample to the **Wash Buffer**

Room temperature

7 Transfer 1.4 mL of **Wash Buffer** per sample into a new conical tube labelled **Cell Permeabilization Buffer**
Add 2.8 µL of ***5% digitonin*** to CP buffer

Store the remaining **Cell Permeabilization Buffer** at 4 °C Overnight (for Day 2 use).

8 Transfer 100 µL per sample of **Cell Permeabilization Buffer** into a new tube labelled "**Antibody Buffer**"

Add 0.4 µL [M] 0.5 Molarity (m) **EDTA** per sample

Store final buffer on ice.

Section II - Bead Activation

30m

9 Gently resuspend the **ConA Beads** by pipetting.

Transfer 11 µL/sample to a 1.5 mL tube for batch processing.

NOTE: Batch processing at this step is recommended to improve sample handling. If a 1.5 mL tube magnet is not available, the beads can be processed individually (10 µL/ sample) in the provided 8-strip PCR tubes using a compatible 8-strip magnet.

10 Place the tube on a magnet until slurry clears and pipette to remove sup.

IMPORTANT: For all steps involving magnetic racks, take care to avoid disturbing the immobilized beads with pipette tips.

11 To avoid drying the beads, immediately add 100 µL/sample cold **Bead Activation Buffer**.

Pipette gently to mix.

12 Place the tube on a magnet until slurry clears and pipette to remove sup.

Repeat previous step for total of two washes.

13 Resuspend beads in 11 µL /sample cold **Bead Activation Buffer**.

NOTE: If not batch processing, use 10 µL/sample at this step. Proceed directly to Section III.

14 For each experimental condition, aliquot 10 µL /sample of activated bead slurry into separate **8-strip tubes**.

Keep on ice until needed.

Tissue preparation and Sample Preparation: Nuclei

1h

15 In the most sterile way possible, cut a small piece of tissue, around 50 mg, and leave it in the petri dish with a marking on the lid, in the dry ice. Weigh it and cut again if needed.

Make sure to use the ethanol to clean everything and be careful not to cut yourself.

16 Add 2 mL of 1X HB buffer into the dounce, which is sitting in the ice.

17 Place 20 mg frozen tissue into a pre-chilled 2 mL Dounce containing 2 mL cold 1x HB and let thaw for 00:05:00 .

18 Dounce with “A” loose pestle until resistance goes away (~10 strokes).

Put the A pestle into the beaker of water

19 Dounce with “B” tight pestle for 20 strokes.

Put the B pestle into the beaker of water

20 Pour everything from the dounce into a 30 um MACS smartstrainer which is sitting on top of a labelled 50 mL falcon tube sitting in ice.

MACS SmartStrainers 30um **Miltenyi Biotec Catalog #130-098-458**

21 Let it drip through for 00:15:00 15m

22 Transfer to a labelled lobind eppendorf tube, already cold from sitting in ice.

23 To pellet the nuclei, centrifuge 900 rpm, 4°C, 00:10:00 10m

23.1 Transfer the supernatant to a new tube without disturbing the pellet 10m

Repeat the centrifugation

24 Discard supernatant

Section III - Binding cells to activated beads 30m

25

	A	B
	Reagents	Amount
	HEPES buffer	20 mM

	A	B
	KCl (pH 7.9)	10 mM
	Triton X-100	0.1%
	Glycerol	20%
	MnCl2	1 mM

Nuclear extraction buffer

26 Prepare 410 µL / sample (+ extra dead volume) Nuclear Extraction Buffer fresh the day of use. Sterile Filter.

27 Add 1:10,000 dilution of [M] 1 Molarity (m) Spermindine and 1X Protease Inhibitor to the Nuclear Extraction Buffer. Place final buffer on ice.

200/10000 = 0.2 ul

28 Resuspend cells in 100 µL per sample 1X PBS. 3m

Set aside 10 µL cells for future analysis by Trypan blue staining (intact cell control).

Centrifuge for 600 rpm, 4°C, 00:03:00 . Remove and discard supernatant

*NOTE:For all steps, the ratio of buffer volumes; cells scales linearly. For example, use 1 mL buffer for 5 x 10^6 cells.

29 Resuspend cells in 200 µL per sample cold Nuclear Extraction Buffer.

30 Incubate 0 °C 10m (ice)

31 Centrifuge for 600 rpm, 4°C, 00:03:00 3m

Remove and discard sup.

The pellet should change in appearance from sticky, pale yellow (cells) to white and fluffy (nuclei).

32 Add 10 µL Trypan blue to the intact cell control (Step 4) and the isolated nuclei (previous step).

33 Load onto hemacytometer slide and examine under brightfield/phase microscope to determine whether nuclei have been efficiently isolated (Figure 12).

34 To cryopreserve nuclei, slowly freeze samples in isopropanol-filled chiller in -80 °C freezer.

34.1 It might be a good idea to add 300 µL of freezing medium to each tube for saving

	Hepes pH 7.5	10 mM	1 ml	1 M stock
	MgCl2	2 mM	200 ul	1 M stock
	KCl	25 mM	800 ul	3 M stock
	H20		98 ml	

Hyporonic Buffer N;

Mix 15 mL of buffer N containing 5 µL Halt protease inhibitor cocktail, [M] 1 millimolar (mM) DTT and [M] 1 millimolar (mM) PMSF with 35 mL of pure glycerol

PMSF P212121 Catalog #RP-P20270
DTT P212121 Catalog #SV-DTT

HALT Protease and Phosphatase Inhibitor Cocktail **Abcam Catalog #78440**

35 When ready to use samples for CUT&RUN, thaw nuclei quickly by placing on 37 °C block. Centrifuge down and reconstitute

36 Proceed to CUT&RUN ConA Bead conjugation step (Experimental Protocol, Step 10).

37 Resuspend cells in 105 µL /sample in RT **Wash Buffer**. Pipette to thoroughly resuspend.

Aliquot 100 µL washed cells to each 8-strip tube containing 10 µL of activated beads. Gently vortex or pipette to mix.

NOTE: Beads are prone to clumping. If your beads are clumped, continue to vortex and/or pipette mix to ensure even resuspension.

38 Incubate cell-bead slurry on the platform rocker for 00:10:00 at Room temperature to adsorb cells to beads. 10m

NOTE: Count cells by Trypan staining prior to incubation with ConA beads. After incubation with ConA beads, check the sup to ensure most cells have adsorbed to the beads.

38.1 I have been moving the tube to ice for 00:50:00 as an extra time incubation 50m

39

Sample Preparation: Cross-Linking (Optional) 30m

40 Labile targets or highly transient chromatin binding proteins may be improved by cross-linking

41 Transfer 500,000 cells or amount that you wish to use into a 1.5 mL lobind tube

42 Add formaldehyde directly to culture to achieve desired final concentration of formaldehyde. (recommended 0.1%-1%)

42.1 I use 16% formaldehyde in 200 µL of nuclear extraction buffer, which requires 13.75 µL of formaldehyde.

43 Incubate Room temperature 10m on 10m

Equipment	
Bio RS-24 Mini-rotator	NAME
mini-rotator	TYPE
BioSan	BRAND
RS-24	SKU
https://biosan.lv/products/-bio-rs-24-mini-rotator-for-test-tubes-with-timer/	LINK

44 Quench the fixation by adding [M] 2.5 Molarity (m) glycine to a final concentration of [M] 125 millimolar (mM)

In this case it would be 11 µL

44.1 600 rpm, 4°C, 00:03:00

44.2 Discard supernatant

Section IV - ANTIBODY BINDING

18h

45 If using a multi-channel pipette (recommended) place a multi-channel reagent reservoir on ice.

IMPORTANT: If processing > 8 samples (more than 1 x 8-strip tube), for subsequent wash steps remove & replace sups for a single strip before processing the next strip. This avoids bead dry out during wash steps.

NOTE: Multi-channel pipetting is highly recommended through the rest of the experiment. This helps to avoid bead dry out, improves yield, and increases experimental throughput.

46 Place the 8-strip tubes on an 8-strip tube magnet (high volume setting) until slurry clears.

Pipette to remove sup, taking care to avoid disturbing the immobilized beads with pipette tip.

47 Immediately add 50 µL cold **Antibody Buffer** to each sample and gently vortex and/or pipette mix to prevent beads from drying.

48 (Optional)
Add 2 µL SNAP-**CUTANA K-MetStat Panel** per 500,000 cells.
The samples designated for the positive (H3K4me3) and negative (IgG) control antibodies.

49 Add 0.5 µg antibody to each sample and gently vortex.

50 Incubate 8-strip tubes on rotator Overnight at 4 °C.

DAY 2 - Section IV - Antibody binding

10m

51 If using a multi-channel pipette (recommended) place a multi-channel reagent reservoir on ice.

Fill with **Cell Permeabilization Buffer.**

IMPORTANT: If processing > 8 samples (more than 1 x 8-strip tube), for subsequent wash steps remove & replace sups for a single strip before processing the next strip. This avoids bead dry out during wash steps.

52 Place the 8-strip tubes on magnet until slurry clears. Pipette to remove sup.

53 *While beads are on magnet*, add 200 µL cold Cell Permeabilization Buffer directly onto beads.

Pipette to remove supernatant

54 Repeat previous step for total of two washes, without removing 8-strip tubes from the magnet.

55 Add 50 µL cold Cell Permeabilization Buffer to each sample.

Gently vortex and/or disperse clumps by thorough pipetting.

Section V - BINDING OF PAG-MNASE

30m

56 Add 2.5 µL **pAG-MNase** (20x stock) to each sample.

Gently vortex/pipette mix.

NOTE: To evenly distribute enzyme across cells/nuclei, ensure beads are thoroughly resuspended by gentle pipetting with a P200.

57 Incubate samples for 00:10:00 at Room temperature on the platform shaker 10m

Return 8-strip tube to magnet.

Remove sup.

58 *While beads are on magnet*, add 200 µL cold **Cell Permeabilization Buffer** directly onto beads.

Pipette to remove sup.

59 Repeat previous step for total of two washes without removing 8-strip tubes from the magnet.

60 Remove 8-strip tubes from the magnet.

Add 50 µL cold **Cell Permeabilization Buffer** to each sample.

Gently vortex and disperse clumps by pipetting.

Cover/ put away **Cell Permeabilization Buffer** for later use.

Section VI - TARGETED CHROMATIN DIGESTION AND RELEA... 3h

61 Place 8-strip tubes on ice.

Add 3 µL **Calcium Chloride** to each sample and gently vortex.

Ensure efficient digestion by making sure beads are thoroughly resuspended.

Gently pipette with a P200 if needed.

62 Incubate 8-strip tubes on rotator for 02:00:00 at 4 °C. 2h

63 Add 33 µL **Stop Buffer** to each sample.

Gently vortex to mix.

64 Prior to first use, reconstitute ***E. coli* Spike-in DNA** in 200 µL DNase free water.

***IMPORTANT: Lyophilized DNA pellet is invisible to the eye.**

Prior to opening, pellet DNA by quick spin in a benchtop microfuge.

After reconstitution, vortex tube on all sides to ensure complete resuspension.

65 Add 1-2 µL **Spike-in DNA** to each sample. Gently vortex to mix.

***NOTE: In general, aim for Spike-in DNA to comprise 0.5 – 5% (ideally closest to 1%) of total read counts in the sequencing data.**

Therefore, while 0.5 ng is a good starting amount for both high (e.g. H3K27me3) and low (e.g. H3K4me3) abundance targets, this may need to be adjusted higher or lower depending on the antibody used, target of interest, total DNA yield, and sequencing results.

66 Incubate 8-strip tubes for 00:10:00 at 37 °C in a thermocycler. 10m

66.1 (Optional - if Cross-Linking extra methodology only)

Place 8-strip tubes on magnet stand until slurry clears.

Transfer supernatants containing DNA to new 8-strip tubes

66.2 Reverse cross-links by adding 0.8 µL 10% SDS and 1 µL of [M] 20 µg/µL Proteinase K to each reaction.

Mix by vortexing

66.3 Overnight 55 °C
in a thermocycler

67 Quick spin in benchtop microfuge.

68 Place 8-strip tubes on a magnet stand until slurry clears.

Transfer sups containing CUT&RUN enriched DNA to 1.5 mL tubes and discard ConA Beads.

Section VII - DNA PURIFICATION

30m

69 Add 420 µL **DNA Binding Buffer** to each sample.

Mix well by vortexing.

70 For every sample, place a **DNA Cleanup Column** into a **DNA Collection Tube**.

Load each sample onto a column and label the top.

71 Centrifuge for 16.000 x g, 00:00:30 Room temperature

30s

Discard the flow-through.

Place the collection tube back on to the column.

NOTE: A vacuum manifold can be used in place of centrifugation.

For each step, add the indicated buffer, turn the vacuum on, and allow the solution to pass through the column before turning the vacuum off.

72 Prior to first use, add 20 mL ≥ 95% ethanol to **DNA Wash Buffer**.

Section VII (continued)

73 **SECTION VII: DNA PURIFICATION (~30 MIN), CONTINUED**

74 Add 200 µL **DNA Wash Buffer** to each sample column

75 Centrifuge for 16.000 x g, 00:00:30 , Room temperature 30s

Discard the flow-through.

Place the collection tube back on to the column.

76 Repeat for a total of two washes.

77 Discard the flow-through. 30s

Centrifuge one additional time for 16.000 x g, 00:00:30 to completely dry the column.

78 Transfer column to a clean pre-labeled 1.5 mL lobind tube, ensuring the column does not come into contact with the flow-through.

79 Elute DNA by adding 12 µL **DNA Elution Buffer**, taking care to ensure the buffer is added to the center of the column rather than the wall.

Tap the column + collection tube on the benchtop to ensure all droplets are absorbed onto the resin.

NOTE: 12 µL is recommended, however DNA can be eluted in 6 – 20 µL volumes depending on anticipated yield and desired final concentration.

Larger elution volumes, longer incubation times, and/or multiple rounds of elution may improve DNA yield. However, sample concentration will be reduced with larger total elution volume.

80 Let sit 00:05:00, then centrifuge for 16.000 x g, 00:01:00, RT. 6m

81 Vortex eluted material and use 1 µL to quantify the CUT&RUN-enriched DNA using the Qubit fluorometer as per the manufacturer's instructions.

See **Quality Control Checks** section for typical DNA yields.

82 CUT&RUN DNA can be stored at -20 °C for future processing.

Section VIII - Library Preparation 18m

83 1) End Prep

Starting Material: 500 pg – 1 µg fragmented DNA. We recommend 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 [M] 10 millimolar (mM) Tris-HCl, pH 8.0 or 0.1X TE.

84 **NEBNext End Prep**

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

	A	B
	Reagent	Amount
	NEBNext Ultra II End Prep Enzyme Mix	7 ul
	NEBNext Ultra II End Prep Reaction Buffer	3 ul
	DNA	50 ul
	Volume	60 ul

End Prep Reaction

85 Set a 100 µL or 200 µL pipette to 50 µL and then pipette the entire volume up and down at least 10 times to mix thoroughly.

Perform a quick spin to collect all liquid from the sides of the tube.

Note: It is important to mix well. The presence of a small amount of bubbles will not interfere with performance.

86 Place in a thermocycler, with the heated lid set to ≥ 75 °C, and run the following program:

	A	B
	Time	Temperature
	30 minutes	20°
	30 minutes	65°
	Hold	4°

End Prep Incubation

If necessary, samples can be stored at –20°C; however, a slight loss in yield (~20%) may be observed. We recommend continuing with adaptor ligation before stopping.

87 Serapure purification 18m

- Add 1.8X Speed beads to samples (108 µL beads to 60 µL reaction)
- Flick to mix
- Incubate for 00:10:00 at room temperature
- Use the magnet to collect the DNA-beads
- Discard supernatant
- Wash with 70% ethanol
- Dry at 37 °C for 00:03:00
- Elute with 20 µL TE buffer
- Incubate for 00:05:00 at room temperature
- Use the magnet to collect the DNA-beads
- Collect supernatant into a new tube

88 Universal Adapter ligation

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

	A	B
	Reagent	Amount
	End Prep Reaction Mixture	20
	NEBNext Quick T4 DNA Ligase	1 ul
	NEBNext Ligation Buffer	2.5 ul
	NextFlex unique adaptor	2.5 ul
	Total Volume	26 ul

Universal Adapter ligation

NEXTFLEX Rapid DNA-Seq Kit 2.0 Bundle **PerkinElmer Catalog #NOVA-5188-12**

88.1 Or one can skip the purification and run the ligation suggested in the NEB protocol

	A	B

	A	B
	Reagent	Amount
	End Prep Reaction Mixture	60 ul
	NEBNext Ligation Enhancer	2.5 µl
	NEBNext Ligation Master Mix	30 ul
	NEBNext Adaptor	1 µl
	Total volume	93.5 µl

Universal Adapter ligation

89 Incubate the reaction for 00:10:00 at room temperature. 10m

90 Serapure purification 18m

- Add 0.8X Speed beads to samples (20 µL Speed beads to 25 µL reaction)
- Flick to mix
- Incubate for 00:10:00 at room temperature
- Use the magnet to collect the DNA-beads
- Discard supernatant
- Wash with 70% ethanol
- Dry at 37 °C for 00:03:00
- Elute with 21 µL TE buffer
- Incubate for 00:05:00 at room temperature
- Use the magnet to collect the DNA-beads
- Collect supernatant into a new tube

91 PCR and primer indexing

	A	B
	Reagent	Amount
	Next Flex primer mix	5 ul
	NEBNext Master Mix	25 ul

	A	B
	Sample	20 ul

92 PCR and primer indexing according to the following cycling parameters:

	A	B	C
	Tempurature	Time	Cycle
	98°	45 seconds	1
	98°	15 seconds	14
	60°	10 seconds	-
	72°	1 minute	-
	72°	10 minutes	1
	4°	hold	1

93 Serapure purification 18m

- Add 1.0X Speed beads to samples (50 µL Speed beads to 50 µL reaction)
- Flick to mix
- Incubate for 00:10:00 at room temperature
- Use the magnet to collect the DNA-beads
- Discard supernatant
- Wash with 70% ethanol
- Dry at 37 °C for 00:03:00
- Elute with 20 µL TE buffer
- Incubate for 00:05:00 at room temperature
- Use the magnet to collect the DNA-beads
- Collect supernatant into a new tube

94 Qubit and Tapestation (D1000) analysis