210304 Hi-C libraries WT KO // DMSO NUT // 1-2 BR

WEDNESDAY, 3/3/2021

Quantification by Qubit of 1st week libraries from Cambridge:

Table1

	A	B
1	Sample	ng/ul
2	WT DMSO 1	1050
3	WT DMSO 2	587
4	WT NUT 1	2360
5	WT NUT 2	3050
6	KO DMSO 1	930
7	KO DMSO 2	780
8	KO NUT 1	2410
9	KO NUT 2	2690

Quantification differ from Biola's old notes. Our quantifications are 1.5x-2x more concentrated.

THURSDAY, 3/4/2021

Start of 2nd week low rcPCHi-C protocol. Samples processed by Blanca U. (WT DMSO 1 + WT NUT 1) and Alberto R. (WT DMSO 2 + WT NUT 2) under Llroenç's supervision

Grab **15ug** instead of 10ug DNA (balancng between Biola's and Llorenç's concentration values) and top up to 164 with TLE.
Mix:

164 ul Hi-C library
2 µl 10 mg/ml BSA
20 µl 10x NEBuffer 2
4 µl 10 mM dATP
10ul (30 Units) T4 DNA polymerase (NEB M0203S)

[[200 µl TOTAL]]

Incubate the mixture at 20°C for 4 hours without shaking.
The previous reaction is stopped by adding 4 µl 0.5 M EDTA pH 8.0

To purify the DNA, top up with TLE up to 300 ul of final volume and preform a phenol pH 8.0:chloroform (1:1) extraction followed by ethanol precipitation.

300ul sample
750ul ice cold 100 % EtOH
30ul sodium acetate pH 5.2
2ul GlycoBlue

The supernatant is discarded and DNA pellets is re-suspended 130 µl ddH2O

Removal of biotin at non-ligated DNA ends

Note: Some biotinylated fragments will not have been ligated. To avoid pulling them down later, biotin-dATP is removed from these un-ligated ends using the exonuclease activity of T4 DNA polymerase.

Mix:
164 ul Hi-C library contacting between 5 to 10 ug (**correct with TLE if used sample for controls!**)
2 µl 10 mg/ml BSA,
20 µl 10x NEBuffer 2
4 µl 10 mM dATP,
10ul (30 Units) T4 DNA polymerase (NEB M0203S)
200 µl of total volume

Incubate the mixture at 20°C for 4 hours without shaking.

The previous reaction is stopped by adding 2 or 4 µl 0.5 M EDTA pH 8.0 if started with less than 5 ug or 5-10 ug

To purify the DNA, top up with TLE up to 300 ul of final volume and preform a phenol pH 8.0:chloroform (1:1) extraction followed by ethanol precipitation.
300ul sample
750ul ice cold 100 % EtOH
30ul sodium acetate 3M pH 5.2
2ul GlycoBlue

Incubate 1 h at -80°C

Centrifuge 30 min at full-speed

Wash 1x with 70% etoh
The supernatant is discarded and DNA pellets is resuspended 130 µl ddH2O

Sonication and end repair

Sonication

1. Divide the sample into COVARIS cuvettes (ref Covaris 520045 or Covaris 520077) (up to 10µg of sample per cuvette = 130µl)

Settings for the COVARIS M220 to sonicate centered in 400 bp:
Duty Factor 10%
Peak Incident Power (w) 50
Cycles per Burst 200
Time (seconds) 70
Max temperature 10ºC

FRIDAY, 3/5/2021

End Repair

Do this step on ice.

Add: Per 10µg of library

(130µl sonicated sample)

10x ligation buffer 18 µl
dNTP mix 2.5 mM each 18 µl
T4 DNA Pol (M0203L) 6.5µl
T4 PNK (M0201L) 6.5µl
Klenow (M0210 L) 1.3µl (6.5u)
(180ul total volumen)

Incubate at 20°C for 30 min.

To purify products from supernatant 1x volume. We Added 180µl SPRI beads (if you process 10µg of library). Incubate for 10 min at room temperature. Discard supernatant, wash 3x with 1ml fresh 70% EtOH (second wash do on the magnet without resuspending the beads). Discard EtOH.
****SPRI beads have to be pre-warm at room temp before use***

Air-dry the beads at 37°C until the 1st crack.

Eluted the library with 35,7µl TLE, vortex, incubate for 10 min at room temperature, vortex again, soft spin, place on the magnet, transfer supernatant to a new tube and place on the magnet again to remove any leftover beads. Transfer supernatant into new tube.

- **dATP tailing, biotin pull-down and adapter ligation, library amplification and double size selection**

dATP tailing

Do this step on ice and carry on until the library amplification
Add: Per 10ug in 35,7ul Per 20µg of library in 71,4ul
10x NEB2 5 ul 10 µl
10mM dATP 2,3 ul 4,6 µl
Klenow (exo-) (M0212L) 7 ul 14 µl
(50ul total volume) (100ul total volume)

Incubate at 37ºC for 30 min.

To inactivate Klenow, incubate at 65ºC for 20 min, gentle spin and cool on ice.

Top up to 300ul of TLE

Directly proceed biotin pull-down until before PCR amplification at least.

- **Biotin pull-down and adapter ligation**

Make up beads washing buffers 1xTB, 1x NTB & 2x NTB:

- 1x TB (Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA (AM9260G, Invitrogen), 1M NaCl, 0.05% Tween20 (Sigma Aldric;h P9416-100ML)

Need >1.2ml 1xTB per sample
To make 5ml of TB:
3.958ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl, 12.5µl 20% Tween

- 1x NTB (no Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA, 1M NaCl

Need >800µl 1xNTB per sample

To make 5ml of 1x NTB:
3.97ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl

- 2x NTB (no Tween buffer): 10mM Tris-HCl pH8.0, 1mM EDTA, 2M NaCl

Need 300µl 2xNTB per sample
To make 1ml of 2x NTB:
588µl H_2O, 10µl 1M Tris-HCl, 2µl 0.5M EDTA, 400µl 5M NaCl

Take 150µl Dynabeads MyOne Streptavidin C1 beads (Fisher; 65001) per sample (5µg in 300µl) and wash the beads using prepared buffers. For each wash: Resuspend beads in the buffer, rotate for 3 min at room temp at 5 rpm and soft spin. Place beads on magnet for 2 min and remove supernatant.

1. Wash 150µl beads twice with 400µl 1xTB (this number of beads are for processing up to 20ug of library)
2. Re-suspend beads in 300µl 2x NTB
3. Combine beads with 300µl Hi-C library (up to 20µg) and incubate at room temp for 15 min with rotation 5 rpm.
4. Place beads on magnet, remove supernatant and wash with 400µl 1x NTB (beads form a dusty layer around the epp. Remove by pipetting some supernatant onto the front wall).
5. Wash beads in 100µl 1x ligation buffer.
6. Re-suspend beads in 50µl 1x ligation buffer (NEB B0202 10x, dilute in water to 1x).
7. Add 4µl of pre-annealed adapter mix and 4µl NEB T4 Ligase 400U/µl (1200-1400U total) OR 1µl NEB T4 Ligase 2000U/µl (M0202T). Incubate at room temp for 2 h.
8. Place beads on magnet, remove supernatant and wash twice with 400µl TB rotating 2 min between washes.
9. Wash with 200µl 1x NTB.
10. Wash with 100µl and then 50 µl 1x NEB2.
11. Finally re-suspend in 50µl 1x NEB2.

Final PCR
Mix on ice!!!!

50ul of beads with library
250ul of master mix (contains also enzyme) (NEB; M0531S or Life technologies; F531S)
12 ul of 25uM of Primer Mix from BGI
188 ul of H2O

1. PCR program:

98ºC 40´´
98ºC 10´´
64ºC 30´´ 8 cycles
72ºC 30´´

Transfer samples into the PRC machine when it rises the 98ºC

Collect reactions and separate beads on the magnet. Keep supernant and make a final volume of 500ul if some ul were lost.

Table2

	A	B
1	Sample	Index BGI
2	WT DMSO 1	47
3	WT DMSO 2	**49**
4	WT NUT 1	48
5	WT NUT 2	**50**

Double-sided size-selection on SPRI beads and PCR purification

To select fragment size 200-700bp (majority 250-550bp), perform two sequential SPRI selections: first with **0.4x** beads (to remove high molecular weight DNA), followed by **1x** beads (to select for desired fragment size 200-700bp):

1. Make sure SPRI beads are at room temperature. Vortex them before use.
2. Add 200µl of beads to 500ul of library (0.4x).
3. Mix with pipette, vortex, rotate at 5 rpm for 10 min at room temp.
4. Place on magnet for 5 min and transfer supernatant (700µl) to a new LoBind epp (you library is in the supernatant, leaving behind beads binding high molecular weight DNA).
5. Concentrate the beads: to use for the second size selection for adding an excess of beads in a low volume (we want to have 750µl-worth of beads in only 300µl). Actually, the volume of polyethylene glycol is important for the size selection reaction.

Vortex beads stock, take 750µl of beads, place on magnet for 5 min, discard supernatant, vortex beads stock, add 300µl of beads to the dry beads and mix
Add 300µl of concentrated beads to 700µl of HiC library material.

1. Mix with pipette, vortex, place in room temp for 10 min, place on magnet for 5 min, discard supernatant (the HiC library is now attached to the beads).
2. Wash the beads 3 times with fresh 70% EtOH on the magnet without resuspending.
3. Dry at 37°C after the 1st crack.
4. Elute DNA from beads with 20,5µl TLE. HiC library will move from the beads to TLE.
5. Vortex the sample, place in room temp for 10 min, place on magnet for 5 min and take out the supernatant into a new LoBind tube.
6. Place again on the magnet and transfer supernatant to a new epp
7. Tapestation 1000 quantification of 1:4 dilution (25%) (0,5ul of library + 1,5ul of H2O)

Ignore first 2 lanes
D1 --> WT DMSO 1BR
N1 --> WT NUTLIN 1BR
D2 --> WT DMSO 2BR
NUT2 --> WT NUTLIn 2BR

030821_aRamos_HSD1000.pdf

Table3

	A	B	C	D
1	Sample	ng/ul	ng TOTAL	peak bp
2	DMSO 1	12	242	855
3	NUT 1	17	340	782
4	DMSO 2	0	0	-
5	NUT 2	6.1	122	764

WT DMSO 2BR does not show any sign of amplification. EtOH precipitation after biotin removal already showed dramatic loss of material.
This sample should be repeated.

TUESDAY, 3/16/2021

Start of 2nd week low rcPCHi-C protocol. Samples processed by Blanca U. (KO NUT 1) and Alberto R. (KO NUT 2).

Grab **15ug** instead of 10ug DNA (balancng between Biola's and Llorenç's concentration values) and top up to 164 with TLE.
Mix:

164 ul Hi-C library
2 µl 10 mg/ml BSA
20 µl 10x NEBuffer 2
4 µl 10 mM dATP
10ul (30 Units) T4 DNA polymerase (NEB M0203S)

[[200 µl TOTAL]]

Incubate the mixture at 20°C for 4 hours without shaking.

The previous reaction is stopped by adding 2 or 4 µl 0.5 M EDTA pH 8.0 if started with less than 5 ug or 5-10 ug

To purify the DNA, top up with TLE up to 300 ul of final volume and preform a phenol pH 8.0:chloroform (1:1) extraction followed by ethanol precipitation.
300ul sample
750ul ice cold 100 % EtOH
30ul sodium acetate 3M pH 5.2
2ul GlycoBlue

Incubate overnight at -20°C

WEDNESDAY, 3/17/2021

Centrifuge 30 min at 4ºC full-speed

Wash 1x with 70% etoh
The supernatant is discarded and DNA pellets is resuspended 130 µl ddH2O

- **Sonication**

1. Divide the sample into COVARIS cuvettes (ref Covaris 520045 or Covaris 520077) (up to 10µg of sample per cuvette = 130µl)

Settings for the COVARIS M220 to sonicate centered in 400 bp:
Duty Factor 10%
Peak Incident Power (w) 50
Cycles per Burst 200
Time (seconds) 70
Max temperature 10ºC

- **End Repair**

Do this step on ice.

Add: Per 10µg of library
(130µl sonicated sample)

10x ligation buffer 18 µl
dNTP mix 2.5 mM each 18 µl
T4 DNA Pol (M0203L) 6.5µl
T4 PNK (M0201L) 6.5µl
Klenow (M0210 L) 1.3µl (6.5u)
(180ul total volumen)

Incubate at 20°C for 30 min.

To purify products from supernatant add SPRI beads 1x volume. We Added 180µl SPRI beads (if you process 10µg of library). Incubate for 10 min at room temperature. Discard supernatant, wash 3x with 1ml fresh 70% EtOH (second wash do on the magnet without resuspending the beads). Discard EtOH.
****SPRI beads have to be pre-warm at room temp before use***

Air-dry the beads at 37°C until the 1st crack.

Eluted the library with 35,7µl TLE, vortex, incubate for 10 min at room temperature, vortex again, soft spin, place on the magnet, transfer supernatant to a new tube and place on the magnet again to remove any leftover beads. Transfer supernatant into new tube.

FRIDAY, 3/19/2021

- **dATP tailing, biotin pull-down and adapter ligation, library amplification and double size selection**

dATP tailing

Do this step on ice and carry on until the library amplification
Add: Per 10ug in 35,7ul Per 20µg of library in 71,4ul
10x NEB2 5 ul 10 µl
10mM dATP 2,3 ul 4,6 µl
Klenow (exo-) (M0212L) 7 ul 14 µl
(50ul total volume) (100ul total volume)

Incubate at 37ºC for 30 min.

To inactivate Klenow, incubate at 65ºC for 20 min, gentle spin and cool on ice.

Top up to 300ul of TLE

Directly proceed biotin pull-down until before PCR amplification at least.

- **Biotin pull-down and adapter ligation**

Make up beads washing buffers 1xTB, 1x NTB & 2x NTB:

- 1x TB (Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA (AM9260G, Invitrogen), 1M NaCl, 0.05% Tween20 (Sigma Aldric;h P9416-100ML)

Need >1.2ml 1xTB per sample
To make 5ml of TB:
3.958ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl, 12.5µl 20% Tween

- 1x NTB (no Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA, 1M NaCl

Need >800µl 1xNTB per sample
To make 5ml of 1x NTB:
3.97ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl

- 2x NTB (no Tween buffer): 10mM Tris-HCl pH8.0, 1mM EDTA, 2M NaCl

Need 300µl 2xNTB per sample
To make 1ml of 2x NTB:
588µl H_2O, 10µl 1M Tris-HCl, 2µl 0.5M EDTA, 400µl 5M NaCl

Take 150µl Dynabeads MyOne Streptavidin C1 beads (Fisher; 65001) per sample (5µg in 300µl) and wash the beads using prepared buffers. For each wash: Resuspend beads in the buffer, rotate for 3 min at room temp at 5 rpm and soft spin. Place beads on magnet for 2 min and remove supernatant.

1. Wash 150µl beads twice with 400µl 1xTB (this number of beads are for processing up to 20ug of library)
2. Re-suspend beads in 300µl 2x NTB
3. Combine beads with 300µl Hi-C library (up to 20µg) and incubate at room temp for 15 min with rotation 5 rpm.
4. Place beads on magnet, remove supernatant and wash with 400µl 1x NTB (beads form a dusty layer around the epp. Remove by pipetting some supernatant onto the front wall).
5. Wash beads in 100µl 1x ligation buffer.
6. Re-suspend beads in 50µl 1x ligation buffer (NEB B0202 10x, dilute in water to 1x).
7. Add 4µl of pre-annealed adapter mix and 4µl NEB T4 Ligase 400U/µl (1200-1400U total) OR 1µl NEB T4 Ligase 2000U/µl (M0202T). Incubate at room temp for 2 h.
8. Place beads on magnet, remove supernatant and wash twice with 400µl TB rotating 2 min between washes.
9. Wash with 200µl 1x NTB.
10. Wash with 100µl and then 50 µl 1x NEB2.
11. Finally re-suspend in 50µl 1x NEB2.

Final PCR
Mix on ice!!!!

50ul of beads with library
250ul of master mix (contains also enzyme) (NEB; M0531S or Life technologies; F531S)
12 ul of 25uM of Primer Mix from BGI
188 ul of H2O

1. PCR program:

98ºC 40´´
98ºC 10´´
64ºC 30´´ 8 cycles

72ºC 30´´

Transfer samples into the PRC machine when it rises the 98ºC

Collect reactions and separate beads on the magnet. Keep supernant and make a final volume of 500ul if some ul were lost.

Table4

	A	B
1	Sample	Index BGI
2	KO NUT 1	43
3	KO NUT 2	45

Double-sided size-selection on SPRI beads and PCR purification

To select fragment size 200-700bp (majority 250-550bp), perform two sequential SPRI selections: first with **0.4x** beads (to remove high molecular weight DNA), followed by **1x** beads (to select for desired fragment size 200-700bp):

1. Make sure SPRI beads are at room temperature. Vortex them before use.
2. Add 200µl of beads to 500ul of library (0.4x).
3. Mix with pipette, vortex, rotate at 5 rpm for 10 min at room temp.
4. Place on magnet for 5 min and transfer supernatant (700µl) to a new LoBind epp (you library is in the supernatant, leaving behind beads binding high molecular weight DNA).
5. Concentrate the beads: to use for the second size selection for adding an excess of beads in a low volume (we want to have 750µl-worth of beads in only 300µl). Actually, the volume of polyethylene glycol is important for the size selection reaction.

Vortex beads stock, take 750µl of beads, place on magnet for 5 min, discard supernatant, vortex beads stock, add 300µl of beads to the dry beads and mix

Add 300µl of concentrated beads to 700µl of HiC library material.

1. Mix with pipette, vortex, place in room temp for 10 min, place on magnet for 5 min, discard supernatant (the HiC library is now attached to the beads).
2. Wash the beads 3 times with fresh 70% EtOH on the magnet without resuspending.
3. Dry at 37°C after the 1st crack.
4. Elute DNA from beads with 20,5µl TLE. HiC library will move from the beads to TLE.
5. Vortex the sample, place in room temp for 10 min, place on magnet for 5 min and take out the supernatant into a new LoBind tube.
6. Place again on the magnet and transfer supernatant to a new epp
7. Tapestation 1000 quantification of 1:2 dilution (0,6ul of library + 0,6ul of H2O)

MONDAY, 3/22/2021

The tapestation results are:

032221_aRamos_D1000.pdf

The KO Nut BR2 has a peak at 1000bp instead of 600-700bp. We will repeat it.

WEDNESDAY, 3/24/2021

Start of 2nd week low rcPCHi-C protocol. Samples processed by Blanca U. (KO DMSO 1 and KO DMSO 2) and Alberto R. (KO NUT 2).

Grab **15ug** instead of 10ug DNA (balancng between Biola's and Llorenç's concentration values) and top up to 164 with TLE.

Mix:

164 ul Hi-C library
2 µl 10 mg/ml BSA
20 µl 10x NEBuffer 2
4 µl 10 mM dATP
10ul (30 Units) T4 DNA polymerase (NEB M0203S)

[[200 µl TOTAL]]

Incubate the mixture at 20°C for 4 hours without shaking.

The previous reaction is stopped by adding 2 or 4 µl 0.5 M EDTA pH 8.0 if started with less than 5 ug or 5-10 ug

To purify the DNA, top up with TLE up to 300 ul of final volume and preform a phenol pH 8.0:chloroform (1:1) extraction followed by ethanol precipitation.
300ul sample
750ul ice cold 100 % EtOH
30ul sodium acetate 3M pH 5.2
2ul GlycoBlue

Incubate 1h at -80°C

Centrifuge 30 min at 4ºC full-speed

Wash 1x with 70% etoh (the supernatant is discarded, add 500ul etoh and centrifugate 10min at full-speed)

The supernatant is discarded and DNA pellets is resuspended 130 µl ddH2O

• Sonication and end repair

Sonication

1. Divide the sample into COVARIS cuvettes (ref Covaris 520045 or Covaris 520077) (up to 10µg of sample per cuvette = 130µl)

Settings for the COVARIS M220 to sonicate centered in 400 bp:
Duty Factor 10%
Peak Incident Power (w) 50
Cycles per Burst 200
Time (seconds) 70
Max temperature 10ºC

THURSDAY, 3/25/2021

• End Repair

Do this step on ice.

Add: Per 10µg of library
(130µl sonicated sample)

10x ligation buffer 18 µl
dNTP mix 2.5 mM each 18 µl
T4 DNA Pol (M0203L) 6.5µl

T4 PNK (M0201L) 6.5µl
Klenow (M0210 L) 1.3µl (6.5u)
(180ul total volumen)

Incubate at 20°C for 30 min.

To purify products from supernatant add SPRI beads 1x volume. We Added 180µl SPRI beads (if you process 10µg of library). Incubate for 10 min at room temperature. Discard supernatant, wash 3x with 1ml fresh 70% EtOH (second wash do on the magnet without resuspending the beads). Discard EtOH.
****SPRI beads have to be pre-warm at room temp before use***

Air-dry the beads at 37°C until the 1st crack.

Eluted the library with 35,7µl TLE, vortex, incubate for 5 min at 37ºC, vortex again, soft spin, incubate for 5 min at room temperature, vortex again, soft spin, place on the magnet, transfer supernatant to a new tube.

- **dATP tailing, biotin pull-down and adapter ligation, library amplification and double size selection**

dATP tailing

Do this step on ice and carry on until the library amplification
Add: Per 10ug in 35,7ul

10x NEB2	5 ul
10mM dATP	2,3 ul
Klenow (exo-) (M0212L)	7 ul
(50ul total volume)	

Incubate at 37ºC for 30 min.

To inactivate Klenow, incubate at 65ºC for 20 min, gentle spin and cool on ice.

Top up to 300ul of TLE

Directly proceed biotin pull-down until before PCR amplification at least.

- **Biotin pull-down and adapter ligation**

Make up beads washing buffers 1xTB, 1x NTB & 2x NTB:

- 1x TB (Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA (AM9260G, Invitrogen), 1M NaCl, 0.05% Tween20 (Sigma Aldric;h P9416-100ML)

Need >1.2ml 1xTB per sample
To make 5ml of TB:
3.958ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl, 12.5µl 20% Tween

- 1x NTB (no Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA, 1M NaCl

Need >800µl 1xNTB per sample
To make 5ml of 1x NTB:
3.97ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl

- 2x NTB (no Tween buffer): 10mM Tris-HCl pH8.0, 1mM EDTA, 2M NaCl

Need 300µl 2xNTB per sample
To make 1ml of 2x NTB:
588µl H_2O, 10µl 1M Tris-HCl, 2µl 0.5M EDTA, 400µl 5M NaCl

Take 150µl Dynabeads MyOne Streptavidin C1 beads (Fisher; 65001) per sample (5µg in 300µl) and wash the beads using prepared buffers. For each wash: Resuspend beads in the buffer, rotate for 3 min at room temp at 5 rpm and soft spin. Place beads on magnet for 2 min and remove supernatant.

1. Wash 150µl beads twice with 400µl 1xTB (this number of beads are for processing up to 20ug of library)
2. Re-suspend beads in 300µl 2x NTB
3. Combine beads with 300µl Hi-C library (up to 20µg) and incubate at room temp for 15 min with rotation 5 rpm.
4. Place beads on magnet, remove supernatant and wash with 400µl 1x NTB (beads form a dusty layer around the epp. Remove by pipetting some supernatant onto the front wall).
5. Wash beads in 100µl 1x ligation buffer.
6. Re-suspend beads in 50µl 1x ligation buffer (NEB B0202 10x, dilute in water to 1x).
7. Add 4µl of pre-annealed adapter mix and 4µl NEB T4 Ligase 400U/µl (1200-1400U total) OR 1µl NEB T4 Ligase 2000U/µl (M0202T). Incubate at room temp for 2 h.
8. Place beads on magnet, remove supernatant and wash twice with 400µl TB rotating 2 min between washes.
9. Wash with 200µl 1x NTB.
10. Wash with 100µl and then 50 µl 1x NEB2.
11. Finally re-suspend in 50µl 1x NEB2.

Final PCR
Mix on ice!!!!

50ul of beads with library
250ul of master mix (contains also enzyme) (NEB; M0531S or Life technologies; F531S)
12 ul of 25uM of Primer Mix from BGI
188 ul of H2O

1. PCR program:

98ºC 40´´
98ºC 10´´
64ºC 30´´ 8 cycles
72ºC 30´´

Transfer samples into the PRC machine when it rises the 98ºC

Collect reactions and separate beads on the magnet. Keep supernant and make a final volume of 500ul if some ul were lost.

Table5

	A	B
1	Sample	Index BGI
2	KO DMSO 1	42
3	KO DMSO 2	44
4	KO NUT 2	45

Double-sided size-selection on SPRI beads and PCR purification

To select fragment size 200-700bp (majority 250-550bp), perform two sequential SPRI selections: first with **0.4x** beads (to remove high molecular weight DNA), followed by **1x** beads (to select for desired fragment size 200-700bp):

1. Make sure SPRI beads are at room temperature. Vortex them before use.
2. Add 200µl of beads to 500ul of library (0.4x).

3. Mix with pipette, vortex, rotate at 5 rpm for 10 min at room temp.
4. Place on magnet for 5 min and transfer supernatant (700µl) to a new LoBind epp (you library is in the supernatant, leaving behind beads binding high molecular weight DNA).
5. Concentrate the beads: to use for the second size selection for adding an excess of beads in a low volume (we want to have 750µl-worth of beads in only 300µl). Actually, the volume of polyethylene glycol is important for the size selection reaction.

Vortex beads stock, take 750µl of beads, place on magnet for 5 min, discard supernatant, vortex beads stock, add 300µl of beads to the dry beads and mix
Add 300µl of concentrated beads to 700µl of HiC library material.

1. Mix with pipette, vortex, place in room temp for 10 min, place on magnet for 5 min, discard supernatant (the HiC library is now attached to the beads).
2. Wash the beads 3 times with fresh 70% EtOH on the magnet without resuspending.
3. Dry at 37°C after the 1st crack.
4. Elute DNA from beads with 20,5µl TLE. HiC library will move from the beads to TLE.
5. Vortex the sample, place in room temp for 10 min, place on magnet for 5 min and take out the supernatant into a new LoBind tube.
6. Place again on the magnet and transfer supernatant to a new epp
7. Tapestation 1000 quantification of 1:2 dilution (0,6ul of library + 0,6ul of H2O)

Id samples
B1 = KO NUT 2
C1 = DMSO 1
D2 = DMSO 2

032621_bUrmeneta_D1000.pdf

THURSDAY, 4/8/2021

Start of 2nd week low rcPCHi-C protocol. Samples processed by Blanca U. (WT DMSO 2 and KO DMSO 1) and Alberto R. (KO DMSO 2).

Grab **15ug** instead of 10ug DNA (balancng between Biola's and Llorenç's concentration values) and top up to 164 with TLE.
Mix:

164 ul Hi-C library
2 µl 10 mg/ml BSA
20 µl 10x NEBuffer 2
4 µl 10 mM dATP
10ul (30 Units) T4 DNA polymerase (NEB M0203S)

[[200 µl TOTAL]]

Incubate the mixture at 20°C for 4 hours without shaking.

The previous reaction is stopped by adding 2 or 4 µl 0.5 M EDTA pH 8.0 if started with less than 5 ug or 5-10 ug

To purify the DNA, top up with TLE up to 300 ul of final volume and preform a phenol pH 8.0:chloroform (1:1) extraction followed by ethanol precipitation.
300ul sample
750ul ice cold 100 % EtOH
30ul sodium acetate 3M pH 5.2
2ul GlycoBlue

Incubate overnigh at -20°C

FRIDAY, 4/9/2021

Centrifuge 30 min at 4ºC full-speed

Wash 1x with 70% etoh (the supernatant is discarded, add 500ul etoh and centrifugate 10min at full-speed)

The supernatant is discarded and DNA pellets is resuspended 130 µl ddH2O

• Sonication and end repair

Sonication

1. Divide the sample into COVARIS cuvettes (ref Covaris 520045 or Covaris 520077) (up to 10µg of sample per cuvette = 130µl)

Settings for the COVARIS M220 to sonicate centered in 400 bp:
Duty Factor 10%
Peak Incident Power (w) 50
Cycles per Burst 200
Time (seconds) 70
Max temperature 10ºC

• End Repair

Do this step on ice.

Add:	Per 10µg of library (130µl sonicated sample)
10x ligation buffer	18 µl
dNTP mix 2.5 mM each	18 µl
T4 DNA Pol (M0203L)	6.5µl
T4 PNK (M0201L)	6.5µl
Klenow (M0210 L)	1.3µl (6.5u)
(180ul total volumen)	

Incubate at 20°C for 30 min.

To purify products from supernatant add SPRI beads 1x volume. We Added 180µl SPRI beads (if you process 10µg of library). Incubate for 10 min at room temperature. Discard supernatant, wash 3x with 1ml fresh 70% EtOH (second wash do on the magnet without resuspending the beads). Discard EtOH.
****SPRI beads have to be pre-warm at room temp before use***

Air-dry the beads at 37°C until the 1st crack.

Eluted the library with 35,7µl TLE, vortex, incubate for 5 min at 37ºC, vortex again, soft spin, incubate for 5 min at room temperature, vortex again, soft spin, place on the magnet, transfer supernatant to a new tube.

• dATP tailing, biotin pull-down and adapter ligation, library amplification and double size selection

dATP tailing

Do this step on ice and carry on until the library amplification

Add: Per 10ug in 35,7ul

10x NEB2	5 ul
10mM dATP	2,3 ul
Klenow (exo-) (M0212L)	7 ul

(50ul total volume)

Incubate at 37ºC for 30 min.

To inactivate Klenow, incubate at 65ºC for 20 min, gentle spin and cool on ice.

Top up to 300ul of TLE

Directly proceed biotin pull-down until before PCR amplification at least.

- **Biotin pull-down and adapter ligation**

Make up beads washing buffers 1xTB, 1x NTB & 2x NTB:

- 1x TB (Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA (AM9260G, Invitrogen), 1M NaCl, 0.05% Tween20 (Sigma Aldric;h P9416-100ML)

Need >1.2ml 1xTB per sample
To make 5ml of TB:
3.958ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl, 12.5µl 20% Tween

- 1x NTB (no Tween buffer): 5mM Tris-HCl pH8.0, 0.5mM EDTA, 1M NaCl

Need >800µl 1xNTB per sample
To make 5ml of 1x NTB:
3.97ml H_2O, 25µl 1M Tris-HCl, 5µl 0.5M EDTA, 1ml 5M NaCl

- 2x NTB (no Tween buffer): 10mM Tris-HCl pH8.0, 1mM EDTA, 2M NaCl

Need 300µl 2xNTB per sample
To make 1ml of 2x NTB:
588µl H_2O, 10µl 1M Tris-HCl, 2µl 0.5M EDTA, 400µl 5M NaCl

Take 150µl Dynabeads MyOne Streptavidin C1 beads (Fisher; 65001) per sample (5µg in 300µl) and wash the beads using prepared buffers. For each wash: Resuspend beads in the buffer, rotate for 3 min at room temp at 5 rpm and soft spin. Place beads on magnet for 2 min and remove supernatant.

1. Wash 150µl beads twice with 400µl 1xTB (this number of beads are for processing up to 20ug of library)
2. Re-suspend beads in 300µl 2x NTB
3. Combine beads with 300µl Hi-C library (up to 20µg) and incubate at room temp for 15 min with rotation 5 rpm.
4. Place beads on magnet, remove supernatant and wash with 400µl 1x NTB (beads form a dusty layer around the epp. Remove by pipetting some supernatant onto the front wall).
5. Wash beads in 100µl 1x ligation buffer.
6. Re-suspend beads in 50µl 1x ligation buffer (NEB B0202 10x, dilute in water to 1x).
7. Add 4µl of pre-annealed adapter mix and 4µl NEB T4 Ligase 400U/µl (1200-1400U total) OR 1µl NEB T4 Ligase 2000U/µl (M0202T). Incubate at room temp for 2 h.
8. Place beads on magnet, remove supernatant and wash twice with 400µl TB rotating 2 min between washes.
9. Wash with 200µl 1x NTB.
10. Wash with 100µl and then 50 µl 1x NEB2.
11. Finally re-suspend in 50µl 1x NEB2.

Final PCR
Mix on ice!!!!

50ul of beads with library
250ul of master mix (contains also enzyme) (NEB; M0531S or Life technologies; F531S)
12 ul of 25uM of Primer Mix from BGI
188 ul of H2O

1. PCR program:

98ºC 40´´
98ºC 10´´
64ºC 30´´ 8 cycles
72ºC 30´´

Transfer samples into the PRC machine when it rises the 98ºC

Collect reactions and separate beads on the magnet. Keep supernant and make a final volume of 500ul if some ul were lost.

Table6

	A	B
1	Sample	Index BGI
2	WT DMSO 2	49
3	KO DMSO 1	42
4	KO DMSO 2	44

Double-sided size-selection on SPRI beads and PCR purification

To select fragment size 200-700bp (majority 250-550bp), perform two sequential SPRI selections: first with **0.4x** beads (to remove high molecular weight DNA), followed by **1x** beads (to select for desired fragment size 200-700bp):

1. Make sure SPRI beads are at room temperature. Vortex them before use.
2. Add 200µl of beads to 500ul of library (0.4x).
3. Mix with pipette, vortex, rotate at 5 rpm for 10 min at room temp.
4. Place on magnet for 5 min and transfer supernatant (700µl) to a new LoBind epp (you library is in the supernatant, leaving behind beads binding high molecular weight DNA).
5. Concentrate the beads: to use for the second size selection for adding an excess of beads in a low volume (we want to have 750µl-worth of beads in only 300µl). Actually, the volume of polyethylene glycol is important for the size selection reaction.

Vortex beads stock, take 750µl of beads, place on magnet for 5 min, discard supernatant, vortex beads stock, add 300µl of beads to the dry beads and mix
Add 300µl of concentrated beads to 700µl of HiC library material.

1. Mix with pipette, vortex, place in room temp for 10 min, place on magnet for 5 min, discard supernatant (the HiC library is now attached to the beads).
2. Wash the beads 3 times with fresh 70% EtOH on the magnet without resuspending.
3. Dry at 37°C after the 1st crack.
4. Elute DNA from beads with 20,5µl TLE. HiC library will move from the beads to TLE.
5. Vortex the sample, place in room temp for 10 min, place on magnet for 5 min and take out the supernatant into a new LoBind tube.
6. Place again on the magnet and transfer supernatant to a new epp

MONDAY, 4/12/2021

1. Tapestation 1000 quantification of 1:2 dilution (0,6ul of library + 0,6ul of H2O)

Id samples
WT2 = WT DMSO Biological Replicate 2
KO1 = KO DMSO B.R. 1
KO2 = KO DMSO B.R. 2

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Summary of libraries p53

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