ChIP-nexus protocol
http://www.nature.com/nbt/journal/v33/n4/full/nbt.3121.html

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Zeitlinger Lab, December 2014
Stowers Institute for Medical Research

In ChIP-nexus (Chromatin-Immunoprecipitation with nucleotide resolution using exonuclease digestion, unique barcode and single ligation) is a ChIP-exo protocol ¹ that makes use of a unique barcode to identify duplicate reads and a novel library preparation strategy that is adopted from iCLIP ².

In the ChIP-nexus protocol, the same amount of cells, formaldehyde fixation and antibodies are used as in regular ChIP-seq experiments. Since many parts of the protocol (Part A, B and D) are based on standard ChIP-seq protocols, their description is relatively brief and references are provided for more information. During the chromatin immunoprecipitation step (Part C), the DNA fragments are ligated to Nexus adapters and ChIP-exo treatment is performed. After the DNA has been purified, the preparation of the ChIP-Nexus library and recommendations for sequencing and data processing (Part E) are described.

An updated protocol may be found at http://research.stowers.org/zeitlingerlab

Prepare reagents as described in the reagents section.
A. Preparation of cross-linked cells

This part of the protocol is described in more detail by Orlando et al. \(^3\), Zeitlinger et al. \(^4\) and Sandmann et al. \(^5\)

**Tissue culture cells**

Harvest up to \(~50\) million cells and transfer to a 15 ml tube. Adjust volume to 10 ml using standard media.

Add 270 µl of 37% formaldehyde and fix for 10 min.

Add 1 ml of 2.5 M glycine in PBS and quench for 2 min.

Pellet at 200 x g for 5 min at 4 °C.

Remove media and wash cells with 10 ml cold PBS. Pellet cells again at 200 x g for 5 min at 4 °C.

Optional: freeze the cross-linked cells in liquid nitrogen and store at –80 °C.

**Drosophila embryos**

Harvest, dechorionate and wash embryos.

Fix up to \(~1\) g embryos in

- 2.3 ml Fix Buffer (50 mM Hepes, 1 mM EDTA, 0.5 mM EGTA, 100 mM NaCl) (total volume with embryos should be \(\leq 3\) ml)
- 130 µl Formaldehyde (final concentration in water phase 1.8 %)
- 7.5 ml n-heptane

Shake vigorously at room temperature for 15 min.

Stop fixation by washing with 250 mM glycine in PBT. Wash twice with \(~15\) ml PBT.

Resuspend in \(~2\) ml PBT and transfer embryos into pre-weighed microfuge tubes.

Centrifuge 30 s at high speed and remove excess PBT. Re-weigh tube and determine weight of embryos.

Optional: freeze the cross-linked sample in liquid nitrogen and store at –80 °C.
B. ChIP set up

This protocol is described by Lee et al. but uses buffers derived from Chanas et al or Orlando et al.

**Bead preparation**

Make block solution: 0.5% BSA (w/v) in PBS (e.g. 250 mg BSA in 50 ml PBS). Store at 4 °C (keeps for several days).

Use **100 µl Dynabeads** (e.g. Protein A or G) per ChIP. Aliquot each 100 µl into a separate microfuge (e.g. in Eppendorf Safe-Lock to prevent evaporation) or pool Dynabeads in a 15 ml tube.

Wash 3 times with block solution.

Resuspend beads in 800 µl block solution per ChIP and add ~10 µg of Ab per ChIP.

Incubate on rotator at 4 °C for at least 3 h or overnight

**Preparation of chromatin extract**

This protocol is for up to ~50 million cells or ~1 g embryos.

Get cross-linked sample and place on ice (~10 million tissue culture cells per ChIP, 50-100 mg Drosophila embryos per ChIP).

Make fresh protease inhibitors by dissolving one tablet (Roche - complete EDTA-free protease inhibitor cocktail) in 2 ml ddH2O. This makes a 25x solution.

Add protease inhibitor cocktail to Lysis Buffer (e.g. A1) to make sufficient 1x solution for all washes. Keep on ice.

Resuspend cross-linked sample in 5 ml Lysis Buffer and transfer to 7 ml Wheaton Dounce homogenizer. *Work with embryos on ice from now on.*

Dounce with each pestle until homogenized (this may take 5-40 times for each pestle dependent on type and concentration of sample).

Transfer homogenate to 15 ml tube and spin for 3 min at 3000 g at 4 °C.

Discard supernatant and add 5 ml Lysis Buffer. Resuspend the pellet by gently pipetting with a wide bore tip. *Do not vortex.* Spin for 3 min at 3000 g at 4 °C. Wash pellet two more times in 5 ml Lysis Buffer (three washes total).
Add protease inhibitor cocktail to ChIP Buffer (e.g. A2) on ice.

Wash sample with 5 ml ChIP Buffer and remove supernatant. Resuspend the sample in ChIP Buffer to obtain a volume of 300 µl per ChIP. Using a wide bore tip, transfer each 300 µl to a fresh microfuge tube.

Sonicate in Bioruptor on HIGH (30 sec on/30 sec off) for 15-30 min. It should give a fragment size distribution peaking at 100 – 500 bp.

Spin at 4 °C for 15 min at max speed and transfer supernatant to a clean tube. This is the whole cell extract (WCE) or Input for the ChIP.

Set aside 50 µl WCE as control and store at -20°C.

**Incubate ChIP**

Finish preparing the antibody-bound Dynabeads (can be done during the 15 min spin of chromatin extracts above): wash 3 times in 1 ml block solution.

If not done already, aliquot beads for each ChIP into a separate microfuge (e.g. Eppendorf Safe-Lock). Remove supernatant.

Add 300 µl Input to each microfuge tube. 
*Note: adding further ChIP Buffer may help for some ChIPs.*

Incubate over night at 4 °C with rotation.
C. ChIP-exo treatment

This protocol is based on Rhee et al. but uses the Illumina protocol for adapter ligation. The end repair and dA-tailing steps can also be performed using buffers and enzymes from Illumina kits (e.g. NEBNext end repair module and NEB dA tailing module).

Note that the protocol is optimized for 100 µl Dynabeads, which have a ~5 µl volume after removing liquids. If a different volume is used, water volumes need to be adjusted accordingly.

For all wash steps: Wash buffers A-D, 10 mM Tris washes and ChIP samples are kept on ice (or work in cold room). Wash volumes are 1 ml per sample with only a brief hand inversion to resuspend the beads for each wash. Washing too long may result in loss of sample. Place samples on magnetic rack to retain beads while removing wash buffers.

For all incubations: Resuspend the beads every ~15 min by gently tapping the tubes.

Wash ChIP samples with buffers A, B, C, D, then 10 mM Tris pH 7.5

End Repair
Add master mix:

<table>
<thead>
<tr>
<th></th>
<th>1x</th>
<th>µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 DNA Ligase Buffer (pH7.5)</td>
<td>5 µl</td>
<td></td>
</tr>
<tr>
<td>10mM dNTP’s</td>
<td>2 µl</td>
<td></td>
</tr>
<tr>
<td>T4 DNA Polymerase</td>
<td>2.5 µl</td>
<td></td>
</tr>
<tr>
<td>DNA Polymerase I, Large (Klenow) fragment</td>
<td>0.5 µl</td>
<td></td>
</tr>
<tr>
<td>T4 polynucleotide Kinase</td>
<td>2.5 µl</td>
<td></td>
</tr>
<tr>
<td>dH₂O</td>
<td>32.5 µl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45 µl</td>
<td></td>
</tr>
</tbody>
</table>

Mix gently and incubate at 12 °C for 30 min.

Wash with buffers A-D, then 10 mM Tris pH 8.0

dA-Tailing
Add master mix:

<table>
<thead>
<tr>
<th></th>
<th>1x</th>
<th>µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x NEB Buffer 2 (pH7.9)</td>
<td>5 µl</td>
<td></td>
</tr>
<tr>
<td>1mM dATP</td>
<td>10 µl</td>
<td></td>
</tr>
<tr>
<td>Klenow exo- (5U/ µl)</td>
<td>3 µl</td>
<td></td>
</tr>
<tr>
<td>dH₂O</td>
<td>27 µl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45 µl</td>
<td></td>
</tr>
</tbody>
</table>

Incubate at 37°C for 30 min.

Wash with buffers A-D, then 10 mM Tris pH 7.5
**Nexus adapter ligation**

Add master mix:  
- 2x Quick Ligase buffer (pH 7.6)  
- Quick Ligase (2000 u/µl)  
- Nexus adapters (1 µM working stock)  
- dH₂O  

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>2x Quick Ligase buffer (pH 7.6)</td>
<td>25 µl</td>
</tr>
<tr>
<td>Quick Ligase (2000 u/µl)</td>
<td>5 µl</td>
</tr>
<tr>
<td>Nexus adapters (1 µM working stock)</td>
<td>1 µl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>14 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45 µl</td>
</tr>
</tbody>
</table>

Incubate at 25 °C for 60 min.  
*Note: the adapters can be diluted further to reduce adapter dimers*

**Wash with buffers A-D, then 10 mM Tris pH 8.0**

**5’ overhang fill in**

Add master mix:  
- 10× NEB buffer 2 (pH 8)  
- 10 mM dNTPs  
- Klenow (exo-)  
- dH₂O  

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>10× NEB buffer 2 (pH 8)</td>
<td>5 µl</td>
</tr>
<tr>
<td>10 mM dNTPs</td>
<td>1 µl</td>
</tr>
<tr>
<td>Klenow (exo-)</td>
<td>1 µl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>38 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45 µl</td>
</tr>
</tbody>
</table>

Incubate at 37 °C for 30 min.

**Wash with buffers A-D, then 10 mM Tris pH 8.0**

**5’ end trim**

Add master mix:  
- 10× NEB buffer 2 (pH 7.9)  
- 10 mM dNTPs  
- T4 DNA polymerase  
- dH₂O  

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>10× NEB buffer 2 (pH 7.9)</td>
<td>5 µl</td>
</tr>
<tr>
<td>10 mM dNTPs</td>
<td>1 µl</td>
</tr>
<tr>
<td>T4 DNA polymerase</td>
<td>1.5 µl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>37.5 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45 µl</td>
</tr>
</tbody>
</table>

Incubate at 12 °C for 5 min.

**Wash with buffers A-D, then 10 mM Tris pH 9.5**

**Lambda exonuclease digestion**

Add master mix:  
- Lambda Exonuclease (5 U/µl)  
- 10× Lambda Exonuclease buffer (pH 9.4)  
- 10% Triton-X  
- DMSO  
- dH₂O  

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambda Exonuclease (5 U/µl)</td>
<td>4 µl</td>
</tr>
<tr>
<td>10× Lambda Exonuclease buffer (pH 9.4)</td>
<td>10 µl</td>
</tr>
<tr>
<td>10% Triton-X</td>
<td>1 µl</td>
</tr>
<tr>
<td>DMSO</td>
<td>5 µl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>75 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>95 µl</td>
</tr>
</tbody>
</table>
Incubate at 37 °C for 60 min w/agitation (1000 rpm on thermomixer) or w/rotation.

**Wash with buffers A-D, then 10 mM Tris pH 7.9**

**RecJ f exonuclease digestion**

Add master mix:  
10× NEB Buffer 2  10 µl  
RecJf exonuclease (30 u/µl)  2.5 µl  
10% Triton-X  1 µl  
DMSO  5 µl  
dH₂O  76.5 µl  

95 µl

Incubate at 37 °C for 60 min w/agitation (1000 rpm on thermomixer) or w/rotation.

**Wash three times with RIPA buffer**
D. Standard DNA purification

The protocol is based on Lee et al. 6

**Elute DNA**
To each 5 µl sample, add 100 µl elution buffer, vortex and shake in Thermomixer for 20 min at 65 ºC, 1000 rpm.

Spin briefly (1 min at top speed) and transfer 100 µl supernatant into new PCR tube containing 100 µl TE.

Reverse crosslinking: Incubate at 65 ºC for 6 h in thermocycler.

**DNA purification and precipitation**
Add 4 µl RNase A. Mix and incubate at 37 ºC for 2 h.

Add 2 µl proteinase K ([final] = 0.4 µg/µl). Mix and incubate at 55 ºC for 2 h.

Extract with 200 µl phenol. Extract with 200 µl phenol:chloroform:IAA.

Transfer to a new microfuge tube and add 3 µl of glycogen [10µg/µl] and 20 µl of 3 M NaOAc. Precipitate with 500 µl EtOH. Freeze at -80 ºC for 2 h.

Centrifuge at max speed for 20 min at 4 ºC and decant supernatant. Add 500 µl cold 70% EtOH, spin 5 min at 4 ºC. Air dry and resuspend pellets in 12 µl dH2O (1 µl for qPCR and 11 µl for library).

**Perform qPCR to check efficiency of ChIP**
Dilute 1 µl Nexus sample with 5 µl dH2O. Dilute 0.4 µl Input sample with 5 µl dH2O.

Make master mix: | 1x |
--- | --- |
2x Fast SYBR green | 10 µl |
Forward primer (3 µM stock) | 4 µl |
Forward primer (3 µM stock) | 4 µl |
Diluted Nexus sample or Input | 1 µl |
dH2O | 1 µl |
| 20 µl |

Run samples in qPCR machine and calculate enrichment for target region.
E. ChIP-nexus library preparation

The initial library preparation protocol is modified from König et al. ²

**Single-stranded DNA circularization**
Denature the 11 µl of Nexus sample by incubating at 95 °C for 5 min, then chill on ice.

Add master mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x CircLigase Buffer</td>
<td>1.5 µl</td>
</tr>
<tr>
<td>50 mM MnCl₂</td>
<td>0.75 µl</td>
</tr>
<tr>
<td>1 mM ATP</td>
<td>0.75 µl</td>
</tr>
<tr>
<td>CircLigase ssDNA Ligase (100 U/µl)</td>
<td>0.75 µl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>0.25 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>4 µl</td>
</tr>
</tbody>
</table>

Incubate at 60 °C for 1 h.

**Annealing of cut oligo**
To each 15 µl sample, add master mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>FastDigest Buffer</td>
<td>5 µl</td>
</tr>
<tr>
<td>Cut oligo (Nex_cut_BamHI) [10 µM]</td>
<td>1 µl</td>
</tr>
<tr>
<td>dH₂O</td>
<td>26 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>32 µl</td>
</tr>
</tbody>
</table>

Use oligo annealing program in thermocycler:
- 95 °C 1:00 min
- 25 °C 1:00 (1% ramp)
- 25 °C 30:00

**BamHI digestion and DNA precipitation**
To each 47 µl sample, add:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>FastDigest BamHI</td>
<td>3 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Incubate at 37 °C for 30 min.

To each sample, add 150 µl TE.

Transfer to a new microfuge tube containing 20 µl of 3 M NaOAC. Precipitate with 500 µl EtOH. Freeze at -80 °C for 2 h.

Centrifuge DNA at max speed at 4 °C for 30 min and decant supernatant.

Add 500 µl cold 70% EtOH, spin 5 min at 4 °C. Air dry and resuspend in 36 µl dH₂O.
**PCR Library amplification**

To each sample, add master mix:

- 1x Phusion buffer: 10 µl
- 5x Phusion buffer: 10 µl
- 10 mM dNTP: 1.5 µl
- Universal primer (Nex_primer_U) (10 µM): 1.0 µl
- Barcoded primer (e.g. Nex_primer_B01) (10 µM): 1.0 µl
- Phusion Polymerase: 0.5 µl

Total: 14 µl

**PCR program:**

1X 98 °C /30 sec
15-18X 98 °C/10 sec, 65 °C /30 sec, 72 °C /30 sec
1X 72 °C /5 min

Hold at 4 °C.

**Library DNA extraction with agarose gel**

Prepare a 2% agarose gel + Gel Red dye in 1X TAE buffer.

Load ~500 ng of 50 bp and 100 bp DNA ladders in two lanes of the gel.

Add 10 µl of 6X loading dye to 50 µl PCR library DNA and load the entire sample in another lane of the gel, leaving at least one empty lane between markers and sample.

Run gel at ~75 V until samples near end of gel.

View the gel on a Dark Reader transilluminator (to avoid UV exposure). Use a clean, sharp scalpel to precisely excise the band containing the library, avoiding the primer and adaptor dimers. Place gel slice into colorless microfuge.

Photograph the gel before and after excision.
Purification of DNA from gel slice

Purify the DNA from the agarose slices with a QIAGEN MinElute Gel Extraction Kit according to manufacturers’ instructions with slight modifications (in bold below): For example, the gel slice is dissolved at room temperature, not 50 °C, to avoid bias in GC content.

Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg gel = ~100 μl).

Incubate at room temperature until the gel slice is completely dissolved. Vortex every 2–3 min during incubation to help dissolve the gel.

Add 1 gel volume of isopropanol to the sample and mix by inverting.

Place a MinElute spin column in a provided 2 ml collection tube. Apply sample to the MinElute column and centrifuge for 1 min. Discard flow-through and place the MinElute column back into the same collection tube.

Wash with 500 μl Buffer QG. Repeat 3X.

Wash with 750 μl Buffer PE. Repeat 1X. Discard flow-through and centrifuge for 1 min to remove residual Buffer PE.

To elute, place each MinElute column into a clean 1.5 ml microcentrifuge tube. Add 12 μl Buffer EB (10 mM Tris-Cl, pH 8.5) to the center of the MinElute membrane. Let the column stand for 5 min, and then centrifuge for 1 min.

Bioanalyzer

Run samples on the Bioanalyzer to determine purity and size distribution.

Examples of Bioanalyzer results:
**Sequencing**
Sequence DNA samples on an Illumina HiSeq platform with the single-end sequencing primer over 50 cycles of extension according to manufacturer’s instructions.

For a typical transcription factor ChIP-nexus experiment in *Drosophila melanogaster* (and organisms with similarly sized genomes), we recommend targeting 10 million uniquely alignable reads.

Note that ChIP-nexus samples may give lower cluster numbers, which is likely due to adapter contamination. To avoid this, careful gel excision is recommended. If adapter contamination persists, reducing the concentration of adapters during ligation can help (but if too low, this may reduce the ligation efficiency). To improve cluster numbers in the presence of adapters, ChIP-nexus samples can be sequenced with non-ChIP-nexus samples (or PhiX Control) in the same lane. See the Illumina document “Using a PhiX Control for HiSeq® Sequencing Runs” for additional information.

**Data processing**
Keep reads passing the default Illumina quality filter (CASAVA v1.8.2).

Filter for the presence of the fixed barcode CTGA starting at read position 6.

Remove the random and fixed barcode sequences (read positions 1 through 9), while retaining the 5-bp random barcode sequence for each read separately.

Trim adapter sequences from the right end using the cutadapt tool.

Keep reads of at least 22 bp in length.

Align to appropriate reference genome using bowtie v1.0.011 and keep uniquely aligning reads with a maximum of 2 mismatches.

To remove ChIP-nexus duplicates, remove reads with identical alignment coordinates (chromosome, start position and strand) and identical random barcode using R and Bioconductor.

Split reads by strand orientation and calculate the genome-wide counts of the start positions (lambda exonuclease’s stop position) for each strand.
F. Reagents

Lysis Buffer and ChIP Buffer for Drosophila embryos and tissue culture cells
Derived from Chanas et al. 7

Lysis Buffer A1 (w/o PI)
- 15 mM HEPES pH 7.5
- 15 mM NaCl
- 60 mM KCl
- 4 mM MgCl2
- 0.5% Triton X100
- 0.5 mM DTT

ChIP Buffer A2 (w/o PI)
- 15 mM HEPES pH 7.5
- 140 mM NaCl
- 1 mM EDTA
- 0.5 mM EGTA
- 1% Triton X100
- 0.1% sodium deoxycholate
- 0.5% N-lauroylsarcosine
- 0.1% SDS

Protease inhibitors (PI): e.g. Roche - complete EDTA-free protease inhibitor cocktail

Alternative Lysis Buffer and ChIP Buffer for tissue culture cells

Orlando and Paro’s Lysis Buffer (w/o PI):
- 0.25% Triton X-100
- 10 mM Na-EDTA
- 0.5 mM EGTA
- 10 mM Tris, pH 8

ChIP Buffer (w/o PI):
- 10 mM Tris, pH 8
- 140 mM NaCl
- 1% Triton X100
- 0.1% sodium deoxycholate
- 0.5% N-lauroylsarcosine
- 0.1% SDS

Protease inhibitors (PI): e.g. Roche - complete EDTA-free protease inhibitor cocktail
**Wash and Elution Buffers**

**Wash Buffer A:**
- 10 mM TE
- 0.1% Triton X-100

**Wash Buffer B:**
- 150 mM NaCl
- 20 mM Tris-HCl [pH 8.0]
- 5 mM EDTA
- 5.2% sucrose
- 1.0% Triton X-100
- 0.2% SDS

**Wash Buffer C:**
- 250 mM NaCl
- 5 mM Tris-HCl (pH 8.0),
- 25 mM HEPES
- 0.5% Triton X-100
- 0.05% sodium deoxycholate
- 0.5 mM EDTA

**Wash Buffer D:**
- 250 mM LiCl
- 0.5% IGEPAL CA-630
- 10 mM Tris-HCl [pH 8.0]
- 0.5% sodium deoxycholate
- 10 mM EDTA

**Tris Buffers:**
- 10 mM Tris pH 7.5
- 10 mM Tris pH 8.0
- 10 mM Tris pH 9.5

**RIPA Buffer:**
- 50 mM HEPES pH 7.5
- 1 mM 0.5M EDTA
- 0.7% sodium deoxycholate
- 1% IGEPAL CA-630
- 0.5 M LiCl

**Elution buffer:**
- 50 mM Tris pH 8.0
10 mM EDTA
1% SDS

Reagents to order

<table>
<thead>
<tr>
<th>Vendor</th>
<th>Reagent</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>New England Biolabs</td>
<td>10X T4 DNA Ligase Buffer</td>
<td>B0202S</td>
</tr>
<tr>
<td></td>
<td>T4 DNA Polymerase</td>
<td>M0203S</td>
</tr>
<tr>
<td></td>
<td>DNA Polymerase, Large (Klenow) fragment [5U/µl]</td>
<td>M0210S</td>
</tr>
<tr>
<td></td>
<td>T4 Polynucleotide Kinase</td>
<td>M0201S</td>
</tr>
<tr>
<td></td>
<td>10x NEB Buffer 2</td>
<td>B7002S</td>
</tr>
<tr>
<td></td>
<td>100 mM dATP</td>
<td>N0440S</td>
</tr>
<tr>
<td></td>
<td>Quick ligase [2000 U/µl] with 2X buffer</td>
<td>M2200S</td>
</tr>
<tr>
<td></td>
<td>10 mM dNTPs</td>
<td>N0447S</td>
</tr>
<tr>
<td></td>
<td>Lambda exonuclease [5U/µl] with 10X buffer</td>
<td>M0262S</td>
</tr>
<tr>
<td></td>
<td>RecJf exonuclease [30 u/µl]</td>
<td>M0264L</td>
</tr>
<tr>
<td>Life Technologies</td>
<td>Proteinase K</td>
<td>25530-049</td>
</tr>
<tr>
<td>Epicentre</td>
<td>CircLigase w/1mM ATP, 50mM MnCl₂ &amp; buffer</td>
<td>CL4111K</td>
</tr>
<tr>
<td>Fisher Scientific</td>
<td>RNase A</td>
<td>EN0531</td>
</tr>
<tr>
<td></td>
<td>FastDigest BamHI with buffer</td>
<td>FERFD0054</td>
</tr>
<tr>
<td>Qiagen</td>
<td>QIAGEN MinElute Gel Extraction Kit</td>
<td>28604</td>
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### Nexus oligos to order

<table>
<thead>
<tr>
<th>Name</th>
<th>Identity</th>
<th>Modification</th>
<th>Barcode</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nex_adapter_UBamHI</td>
<td>Adaptor: universal</td>
<td>5’ phosphate</td>
<td>/</td>
<td>/SPath/GATCGGAAGACGACTGCCAGCTCTGGATCCACGAGCCCTTC</td>
</tr>
<tr>
<td>Nex_adapter_BN5BamHI</td>
<td>Adaptor: barcoded</td>
<td>5’ phosphate</td>
<td>TCAGNNNNN</td>
<td>/SPath/TCAGNNNNNAGATCGGAAGACGCTCTGGATGAGCATGCCAGCTCTGGATCCACGAGCCCTTC</td>
</tr>
<tr>
<td>Nex_cut_BamHI</td>
<td>Oligo for digestion</td>
<td>/</td>
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| Nex_primer_U            | Primer: universal | 3’ phosphoro-thioate bond | /            | AATGTACAGGGCGCTACAGCTACGAGATCGTGATGACTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA...
Prepare Nexus oligos

For 50 µM Nexus adapters, anneal:

- Nex_adapter UBamHI (200 µM) 25 µl
- Nex_adapter BN5BamHI(200 µM) 25 µl
- 10x TE 10 µl
- 5M NaCl 1 µl
- dH2O 49 µl
- Total 100 µl

Create an adapter annealing program on the thermocycler:
- 95 °C 5:00 min
- 25 °C 1:00 (1% ramp)
- 25 °C 30:00

For the adapter ligation step, dilute this stock 1:50 to obtain a working stock of 1 µM Nexus adapters (avoid repeated freeze thawing).

For the cut oligos, as well as for the primer set for the Nexus PCR library preparation, make aliquots of 50 µM stock solution. Dilute 1:5 to obtain a working stock solution of 10 µM.

qPCR primers

Choose a target region based on literature or ChIP-seq data, as well as a negative control (e.g. from a gene desert region, which is unlikely enriched).

Design primers for qPCR such that the product size is small (~60 bp is recommended but 100-150 bp products also work).

Make a 3 µM stock solution for each primer.

Other reagents and equipment checklist

- Dynabeads (e.g. protein A or protein G)
- Bovine serum albumin (BSA)
- Phenol
- Phenol:chloroform:IAA
- 100% Ethanol (EtOH)
- 70% EtOH
- Glycogen [10 µg/µl]
- 3M NaOAC
- Agarose
- Gel Red dye
- 6X loading dye
- TAE buffer
SYBR green

Microfuges (e.g. Sure-Lock Eppendorff)
PCR tubes
15 ml tubes (e.g. Falcon)
50 ml tubes (e.g. Falcon)
Disposable scalpels
7 ml Wheaton Dounce homogenizer

Diagenode Bioruptor or other sonicator
Thermocycler (e.g Eppendorff)
qPCR machine
Agilent 2100 Bioanalyzer
Thermomixer
Magnetic racks
Rotating rack
Gel apparatus for agarose gel
Refrigerated benchtop centrifuge (e.g Eppendorff)

References