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## Myers Lab Sequencing Library Protocol for Illumina or NEB Reagents

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DNA fragments recovered from ChIP's or reverse cross-linked chromatin are repaired, ligated to adapters, PCR-amplified and size selected to make the library for sequencing.

Reagents are supplied by Illumina in the DNA Library Construction Kit, or can be substituted from NEB and Finnzymes, except for the adapter oligo mix and the PCR primers, which can be ordered from Illumina.

NEB reagents:

- NEB N0447 – dNTP Deoxynucleotide Solution Mix
- NEB M0203 – T4 DNA Polymerase 3,000 units/mL
- NEB M0201 – T4 Polynucleotide Kinase 10,000 units/mL
- NEB M0210 – Klenow DNA Polymerase 5,000 units/mL
- NEB M0212 – Klenow Fragment (3' to 5' exo-) 5,000 units/mL
  - Supplied with 10X NEBuffer2 used for dA addition step
- NEB M0202 – T4 DNA Ligase
  - Supplied with 10X DNA ligase buffer. This buffer is used for the end-repair and the adapter ligation steps of the protocol.

Finnzymes:

- F531 – Phusion High-Fidelity PCR Master Mix

DNA in EB can be stored in the freezer at any step.

DNA in agarose can be stored overnight at 4°C.

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### END REPAIR

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*Be sure to use Klenow (E. coli DNA polymerase large fragment) and not the Klenow exo-polymerase for this step.*

Mix in a PCR tube on ice:

10  $\mu$ L 10X T4 DNA ligase buffer (with 10mM ATP)

4  $\mu$ L 10 mM dNTP mix (each @ 10 mM)

30  $\mu$ L recovered DNA fragments (200-500 ng)

45  $\mu$ L water (or any amount of water such that DNA volume + water = 75  $\mu$ L)

Mix, spin down briefly, and place on ice.

Add: 5  $\mu$ L T4 DNA polymerase

5  $\mu$ L T4 Polynucleotide Kinase

1  $\mu$ L Klenow DNA polymerase

Total volume = 100  $\mu$ L

Spin down briefly and incubate at 20°C in a PCR machine for 30 minutes.

Purify on one QIAquick PCR cleanup column with 2 consecutive elutions of 16  $\mu$ L 50°C EB.

Allow EB to sit on the filter in the column for 1 minute before spinning for 1 minute.

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## dA ADDITION

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*Be sure to use correct Klenow enzyme (3' to 5' exo-)*

Mix in a PCR tube on ice:

32  $\mu\text{L}$  end-repaired DNA fragments

10  $\mu\text{L}$  1mM dATP

5  $\mu\text{L}$  10X NEBuffer2 (or Klenow buffer from Illumina)

Mix, spin down briefly, and place on ice.

Add 3  $\mu\text{L}$  Klenow fragment (3' to 5' exo-)

Total volume = 50  $\mu\text{L}$

Spin down briefly and incubate at 37°C in a PCR machine for 30 minutes.

Purify on one QIAquick PCR cleanup column with 2 consecutive elutions of 21  $\mu\text{L}$  50°C EB.

Allow EB to sit on the filter in the column for 1 minute before spinning for 1 minute.

\*\*\*When using Illumina reagents, use Qiagen MiniElute columns and elute with 10  $\mu\text{L}$  50°C EB twice for a total of 20  $\mu\text{L}$  \*\*\*

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## ADAPTER LIGATION

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Mix in a PCR tube on ice:

	<b>NEB reagents (buffer is 10X)</b>	<b>Illumina reagents (buffer is 2X)</b>
DNA recovered from dA addition	42 $\mu\text{L}$	20 $\mu\text{L}$
Ligase buffer	5 $\mu\text{L}$	25 $\mu\text{L}$
Adapter oligo mix	1 $\mu\text{L}$	1 $\mu\text{L}$
T4 DNA ligase	2 $\mu\text{L}$	4 $\mu\text{L}$
Total volume	50 $\mu\text{L}$	50 $\mu\text{L}$

Spin down briefly and incubate at 20°C in a PCR machine for 15 minutes.

Purify on one QIAquick PCR cleanup column with 2 consecutive elutions of 16  $\mu\text{L}$  50°C EB.

Allow EB to sit on the filter in the column for 1 minute before spinning for 1 minute.

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## LIBRARY AMPLIFICATION BY PCR

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Mix in a PCR tube on ice:

23  $\mu$ L DNA fragments

25  $\mu$ L Phusion DNA Polymerase Mix

1  $\mu$ L PCR primer 1.1 (Illumina) \*\*\*Note: although the Illumina protocol uses 1  $\mu$ L of each

1  $\mu$ L PCR primer 2.1 (Illumina) primer, we've been successful with 0.5  $\mu$ L.

Total volume = 50  $\mu$ L Change at your discretion.\*\*\*

Spin down briefly.

Amplify in thermal cycler using the following protocol:

98°C for 30 sec

25 cycles of: 98°C for 10 sec

65°C for 30 sec

72°C for 30 sec

72°C for 5 min

4°C hold

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## GEL PURIFICATION AND SIZE SELECTION

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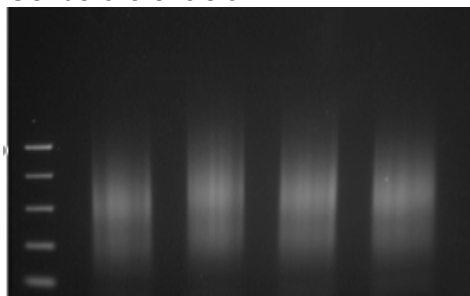
Gel purification removes the 90bp adapter dimer and isolates the 150-300bp fragments, allowing for higher density of productive clusters on the flow cell lanes.

Pour and run gel in the cold room. Pour a 1.5% NuSieve GTG agarose gel (we use Lonza agarose) in 1X TAE with EtBr (final concentration in gel is 0.4ug/mL). Use NEB PCR Marker (N3234L) as ladder. Load PCR products on gel and run gel at ~115 V (takes ~1.5 hours to run 6 cm). Image the gel before and after excision of the library. Cut out region from 150bp to 300bp.

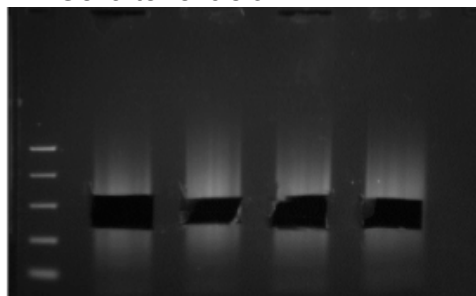
Use QIAquick Gel Extraction Kit with columns to extract DNA from the gel fragments. Follow the Qiagen protocol. Include the optional step of washing the column with 0.5 mL of Buffer QG before adding Buffer PE. Elute with 2 consecutive elutions of 16  $\mu$ L 50°C EB. Allow EB to sit on the filter in the column for 1 minute before spinning for 1 minute. Nanodrop for concentration.

Example gel:

Gel before excision



Gel after excision



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## SECOND PCR AMPLIFICATION

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Libraries sequence and store better if a short second round of PCR amplification is performed. This step brings the DNA concentration up, allows another round of enrichment for DNA fragments that contain sequences essential for sequencing and cleans up and residual agarose contamination.

Mix in a PCR tube on ice:

23  $\mu$ L DNA fragments

25  $\mu$ L Phusion DNA Polymerase Mix

1  $\mu$ L PCR primer 1.1 (Illumina)      \*\*\*Note: although the Illumina protocol uses 1  $\mu$ L of each

1  $\mu$ L PCR primer 2.1 (Illumina)      primer, we've been successful with 0.5  $\mu$ L.

Total volume = 50  $\mu$ L      Change at your discretion. \*\*\*

Spin down briefly

Amplify in thermal cycler using following protocol:

98°C for 30 sec

15 cycles of: 98°C for 10 sec

65°C for 30 sec

72°C for 30 sec

72°C for 5 min

4°C hold

Purify on one QIAquick PCR cleanup column with 2 consecutive elutions of 16  $\mu$ L 50°C EB. Allow EB to sit on the filter in the column for 1 minute before spinning for 1 minute.

Nanodrop recovered DNA for concentration.

Library is ready to put on flow cell for sequencing.