# SOP: Solexa Paired-End Library Construction
# Date Modified: 08/19/2009
# Modified By: Molly Weaver (UW_NREMC)

## Initial Fractionation

- Samples need to be size fractionated to approximately 100-300bp range (for standard library).
- Library input amount is 50ng.

## End-repair

Materials:

- Fractionated input DNA
- NF Water
- T4 DNA ligase buffer with 10mM ATP (NEB #B0202S)
- dNTP mix, 10mM each (NEB #N0447S)
- T4 DNA polymerase, 3U/ul (NEB #M0203L)
- Klenow DNA polymerase, 5U/ul (NEB #M0210L)
- T4 PNK, 10U/ul (NEB #M0201L)
- Cooling block or PCR machine
- QIAquick PCR purification kit

1. Prepare the following reaction mix:

| | |
|---|---|
| Fractionated DNA | 18.75ul |
| T4 DNA ligase buffer with 10mM ATP | 2.5ul |
| dNTP mix | 1.0ul |
| T4 DNA polymerase | 1.25ul |
| Klenow DNA polymerase | 0.25ul |
| T4 PNK | 1.25ul |
| Total Volume | 25ul |

2. Incubate for 30min at 20$^{o}$C in cooling block.
3. Purify on one minElute column using Buffer PB. Elute in 16ul of Buffer EB.

## Addition of an 'A' Base to the 3' End of the DNA Fragments

Materials:

- DNA from last step
- 10X Klenow buffer (use NEB Buffer 2 that comes with Klenow exo$^{-}$)
- dATP, 1mM (Fermentas #R0141)
- Klenow exo$^{-}$ (3' to 5' exo minus), 5U/ul (NEB #M0212L)
- 37$^{o}$C Water bath
- QIAGEN MinElute kit

1. Prepare the following reaction mix:

| | |
|---|---|
| DNA from last step | 16.0ul |
| Klenow buffer | 2.5ul |
| dATP | 5.0ul |
| Klenow exo$^{-}$ (3' to 5' exo minus) | 1.5ul |
| Total Volume | 25ul |

2. Incubate for 30min at 37$^{o}$C
3. Purify on one MinElute column using Buffer PB. Elute in 10ul of Buffer EB.

## Ligation of PE Adaptors to the Ends of the DNA Fragments

Materials:

- DNA from last step
- 2X DNA ligase buffer (use buffer that comes with NEB Quick Ligation kit)
- Index PE Adapter oligo mix (from Solexa)
- DNA ligase, 1U/ul (NEB #M2200L)
- QIAquick PCR purification kit
- Low-bind tubes (Fisher #13-6987-91)
- **Use Filter Tips!!!**

1. Prepare the following reaction mix:

| | |
|---|---|
| DNA from last step | 10.0ul |
| DNA ligase buffer | 12.5ul |
| PE Adapter oligo mix (1:10 dil) | 1.25ul |
| DNA ligase | 1.25ul |
| Total Volume | 25ul |

2. Incubate for 15min at 20$^{o}$C in cooling block.
3. Purify on one minElute column using Buffer PB. Elute in 20ul Buffer EB into low-bind tube.

## Enrichment of the PE Adaptor-modified DNA Fragments by PCR

Materials:

- DNA from last step
- PCR grade water
- Phusion High-Fidelity PCR Master Mix with GC Buffer (NEB #F532L)
- PCR primer PE 1.0, 1:2 dilution (from Solexa)
- PCR primer PE 2.0, 1:2 dilution (from Solexa)
- PCR machine
- QIAquick gel purification kit
- **Use Filter Tips!!!**

1. Prepare the following PCR reaction mix:

| | |
|---|---|
| DNA | 5ul |
| Phusion DNA polymerase | 25ul |
| PCR primer PE 1.0 (1:2 dil) | 1ul |
| PCR primer PE 2.0 (1:2 dil) | 1ul |
| Water | 18ul |
| Total Volume | 50ul |

2. Amplify using the following PCR protocol:
    - 30” at 98$^{o}$C
    - [10”at 98$^{o}$C, 30” at 65$^{o}$C, 30” at 72$^{o}$C] for 16 cycles
    - 5min at 72$^{o}$C
    - Hold at 4$^{o}$C
3. Store excess post-ligation product at -20$^{o}$C.

## Final Fractionation and Clean-up

Materials:

- QIAGEN MinElute gel purification kit
- Isopropanol
- Low-bind tubes (Fisher #13-6987-91)
- **Use filter tips!!!**

1. Load entire enrichment product on a 2% agarose gel. Split sample into two lanes.
2. Purify by cutting out darkest band (approx 250-350bp) from gel. See gel image below for example. Pool fragments
3. Use the MinElute gel purification kit protocol with the following modifications to purify library.
4. Add 6x volume of Buffer QG and allow gel slice to melt at room temp for 30min with occasional vortexing.
5. Add 2X volume of Isopropanol and mix.
6. Load sample on a QIAGEN MinElute column with syringe adaptor and let sit for 15min to bind sample to column.
7. Wash with 500ul Buffer QG.
8. Add 750ul Buffer PE and let sit for five minutes.
9. Do two more washes with Buffer PE. Spin to dry column.
10. Add 15ul Buffer EB to column and let sit five minutes. Elute in low-bind tube.
11. Repeat previous step (final volume is 30ul).
12. Run 8ng on a 3% agarose gel to verify size fragments.
13. Quantify on Qubit. Use a 1:200 dilution and HS kit.