Small Amount DNA Solexa Library Construction– Aug 2009

A. End-Repair

Reagent: End-It DNA End-Repair Kit (Epicenter Cat. No. ER0720)

1 Use ~0.5 ng (if you have enough DNA, ~50ng DNA could be used) as starting materials in 34 µl of 10mM Tris pH8.0 (EB). Save any left over sample at -20 – clearly label all tubes

	µl
DNA	34
10X End-repair Buffer	5
2.5mM dNTPs	5
10mM ATP	5
END-IT enzyme mix	1
Total vol.	50

2 Incubate for 45 min at room temperature.
3 Minelute PCR purification Kit (Qiagen).
4 Add 5 vol of PB to samples. Add samples to minelute columns.
5 Wash with 750ul PE. Turn vacuum off.
6 Spin tubes 2 min max speed to remove excess PB in filter.
7 Add 32ul EB to center of membrane
8 2 min at RT – spin 2m at max speed to elute samples.

B. Addition of an 'A' Base to the 3' End of the DNA fragments

Reagent: Klenow(3'-5' exo-) (New England Biolabs, Cat.# M0212L)

	µl
DNA from section A	32
10X Klenow Buffer	5
1mM dATP	10
Klenow (3'-5' exo-) (5U/ul)	3
Total vol.	50

1 Incubate for 30min. at 37C.
2 Minelute purification. Elute in 10 µl of EB twice. Speedvac to 4 µl.

C. Ligation of Adapters to the Ends of the DNA Fragments

Reagent: DNA ligase (New England Biolabs, M2200S)

	µl
DNA from section B	4
2X Ligase Buffer	5
Adapter Oligo mix (1:10 in H2O)	0.5
DNA Ligase (1U/µl)	0.5
Total vol.	10

1 Incubate for 15min. at room temperature.
2 QIAquick PCR purification Kit (Qiagen) to eliminate unligated adapters. Elute in 24µl of EB.

D. Enrichment of adapter-modified DNA fragments by PCR

	µl
DNA from section C	23
2X Phusion DNA polymerase (Finnzymes)	25
PCR primer 1.1[25 uM]	1
PCR primer 2.1[25 uM]	1
Total vol.	50

1 Amplify using the following PCR protocol:
- 30 sec at 98C
- [10 sec at 98C, 30sec at 65C, 30 sec at 72 C] 18 cycles
- 5 min at 72C
- Hold at 4C

E. Gel Purification of the Products From the PCR Reaction

Purpose: To remove remaining primers, and select a size-range of templates to go on the sequencing platform. Purify up to 2-3 samples on a single gel to prevent cross-contamination. Load each sample over 4 lanes with 12.5ul of DNA per sample

- Prepare 2% agarose in 1X TAE buffer.
- Run gel at 120 V in cold room and sybr green staining.
- Excise bands from 275bp to 600bp.
- Purify DNA from agarose gel using Gel Extraction kit (Qiagen). Dice up gel, weigh samples and add 3 vol. of QC Solution. Incubate at RT for 20m. Add 1 original volume of Isopropanol and vortex quick. Minelute and Elute in 30µl of EB. Quantify with 2ul using the Qubit and run 4ul on a 1.2% FlashGel.