

Preparing samples for SOLiD sequencing:

A. Barcoded Adaptor Preparation

1. Oligos needed:

- Multiplex P1 Adaptor, 50 μ M
 - Multiplex Library PCR Primer 1, 50 μ M
 - Library PCR Primer 2, 50 μ M
 - Your color-balanced Multiplex P2 Adaptor/Barcodes
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- The adaptors must be annealed before they are ligated to your library (they need to be double stranded):
 - 96-well thermal cycler, GeneAmp 9700 or system with same ramp speed preferred
 - 5X T4 DNA Ligase Buffer
 - Multiplex P1 Adaptors A and B
 - Multiplex P2 Adaptor/Barcodes A and B

Prepare a 1 mM stock of each oligo.

For each set of P1 or P2 adaptors, mix equal volumes 1 mM oligos A and B.

Add enough 5X T4 DNA Ligase Buffer to get a final conc. of 1X Buffer.

Hybridize the oligos by running the following program:

Temp ($^{\circ}$ C)	Time (min)
95	5
72	5
60	5
50	3
40	3
30	3
20	3
10	3
4	∞

Store hybridized oligos at -20° C until ready for use.

B. Library Prep

1. Shear the DNA with your method of choice: procedures for the Bioruptor and Covaris are below.

*** Note: you must shear below 200 bp, optimal range is 100-110 bp. Use a sonicator where size can be estimated based on a standard curve, or size-select the sample on a 2% agarose gel.**

- 10 ng to 20 μ g of DNA
- Water or 1X Low TE Buffer

Bring the sample to a total volume of 100 μ L

If using the Bioruptor, have the power on high and do two cycles of 15 minutes each (30"on 30"off), using a 1.5 mL snap-top tube.

If using the Covaris, load sample into a Covaris microTube. Set to Bath temp: 5°C, Bath temp limit: 30 C, Mode: Frequency sweeping, Water quality testing: Off, 6 cycles of (Duty Cycle: 20%, Intensity: 5, Cycles/burst: 200, Time: 60 s). Remove the sample from the microTube and transfer to a 1.5 mL tube.

2. End Repair

- Sheared DNA (100 µL)
- 5x End-Polishing Buffer (40 µL)
- dNTP Mix, 10 mM (8 µL)
- End Polishing Enzyme 1, 10 U/µL (2 µL)
- End Polishing Enzyme 2, 5 U/µL (16 µL)
- Nuclease-free water (34 µL)

Mix in a 1.5 mL tube (total of 200 µL) and incubate at room temp for 30 minutes.

Purify with the PureLink or Qiagen PCR Purification kit.

Quantitate the purified DNA

3. Ligate adaptors

- Calculate the amount of adaptor needed: $X \mu\text{L of each adaptor needed} = (\text{pmol of DNA} * 30) / 50$
- Multiplex P1 Adaptor (ds), 50 pmol/µL (X µL)
- Multiplex P2 Adaptor w/ barcode (ds), 50 pmol/µL (X µL) **
- 5X T4 Ligase Buffer (40 µL)
- DNA (48-50 µL)
- T4 Ligase, 5 U/µL (10 µL)
- Nuclease-free water (variable)

Mix in a 1.5 mL tube (for a total of 200 µL) and incubate at room temp for 10 minutes.

Purify with the PureLink or Qiagen PCR Purification kit.

** To color-balance the barcodes, they must be run in sets of four.

4. Nick-translate and amplify

PCR reaction mix:

- Platinum PCR Amplification Mix (400 µL)
- Multiplex Library PCR Primer 1, 50 µM (10 µL)
- Library PCR Primer 2, 50 µM (10 µL)
- Adaptor-ligated purified DNA (48-50 µL)
- Nuclease-free water (variable)

Mix in a 1.5 mL tube (for a total of 500 µL). Pipette 125 µL of this reaction mix into 4 PCR tubes and set up 4 reactions for each sample.

Run the following PCR program with the minimum # of cycles:

Step	Temp. (°C)	Time	Amt. of DNA	# Cycles
Nick translate	72	20 min	10-100 ng	10
Denature	95	5 min		
Denature,	95	15 s	100 ng-1 µg	6-8
Anneal,	62	15 s		
Extend cycles	70	1 min	1-2 µg	4-6
Extend	70	5 min		
Hold	4	∞	2-20 µg	2-3

Pool the samples in a 1.5 mL tube.

Purify with the PureLink or Qiagen PCR Purification kit.

Quantitate the purified DNA

5. Gel-purify the library

- Use an Invitrogen E-gel 2% Size-Select gel or a 2% agarose gel to purify and size-select your library (remember to account for the length of the adaptors).
- Optional: if using the E-gel, purify with the PureLink or Qiagen PCR Purification kit.
- Quantify the library