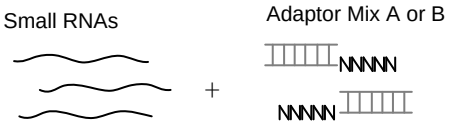
Workflow

Starting material

- Small RNA, purified using the flashPAGE™ Fractionator or PAGE, ***or***
- Total RNA that contains the small RNA fraction
  and
- Adaptor Mix A: for sequencing the 5' ends of small RNAs, ***or***
- Adaptor Mix B: for sequencing the reverse complement of the RNA



Hybridization and ligation to adaptor mix (8 hr)

On ice, mix RNA, Adaptor Mix, and Hybridization Solution.

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Incubate the sample at 65℃ for 10 min, then at 16℃ for 5 min.

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Add ligation reagents to each sample.

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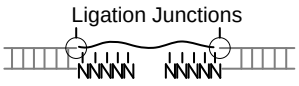
Incubate the sample at 16 ℃ for >8 hr in a themal *cycler*



Reverse transcription and RNase H digestion (1 hr)

On ice, add 20 μL RT Master Mix to each sample.

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Incubate at 42℃ for 30min

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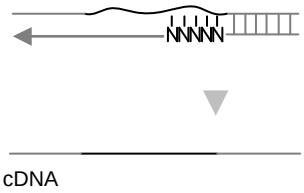
Add 1 μL RNase H to 10 μL cDNA and incubate at 37 ℃ for 30 min



cDNA library amplification (1 to 1.5 hr)

Dispense PCR Master Mix into wells of a PCR plate or tubes.

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Add RNase H-treated cDNA to each reaction mix, then run the PCR

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Run 5-10 μL PCR product on a native 6% polyacrylamide gel.

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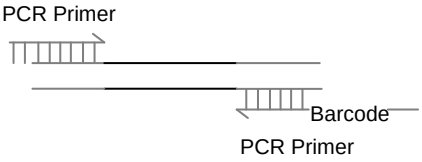
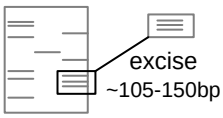
Evaluate the PCR products.



Amplified library cleanup and size selection by PAGE(3 to 4 hr)



SOLiD™ Sample Preparation and Sequencing

SOLiD™ System: start at the "Templated Bead Preparation" section of the SOLiD™ System insuctions(emulsion PCR)