somagenics

RealSeq®-AC application note: Size selection with Novex Gels

Materials needed:

1. QIAquick PCR purification kit (Qiagen #28104)
2. MinElute PCR purification kit (Qiagen #28004)
3. 6% Novex TBE PAGE gel 1.0 mM 10-well (ThermoFisher #EC6265BOX)
4. Orange DNA loading Dye (6X) (ThermoFisher #R0631)
5. O'RangeRuler 10 bp DNA ladder (ThermoFisher #SM1313)
6. SYBR-Gold Nucleic Acid Gel stain (ThermoFisher #11494)
7. RNase-free Disposable Pellet Pestles (FisherScientific #12-141-368)
8. Corning Costar Spin-X Centrifuge Tube Filters

Purify PCR products using Qiagen PCR purification kit.

- Add 500 µl of Buffer PB to PCR sample and mix
- Add 10 µl of 3 M Sodium Acetate (sample should turn yellow)
- Place a Qiagen spin column in a provided 2 ml collection tube
- Apply the sample to the column and centrifure for 30-60 s
- Discard flow-through. Place column back into the same tube
- To wash, add 0.75 ml Buffer PE to column and centrifuge for 30-60 s
- Discard flow-through and place column back in the same tube. Centrifuge the column for additional 5 mins with lid open
- Place column in a clean 1.5 ml microcentrifuge tube
- To elute DNA, add 30 µl of H_2O to the center of the membrane, let the column stand for 1 min, and then centrifuge the column for 1 min.
- Quantify samples with a Nanodrop

Size-select products using 6% Novex TBE gels

- Mix the purified PCR product with 6x Orange Loading dye
- Load 2.5 µl of 10 bp O'Range ruler on the 6% TBE gel
- Load three wells each with a volume corresponding to 100 ng of purified product
- Run the gel for 40 minutes at 120 V or until the bromophenol blue band reaches the bottom of the gel.
- Remove the gel from the apparatus and stain the gel with SYBR Gold nucleic acid gel stain for 10 min and view the gel on a UV transiluminator
- The ~145 bp band corresponds to adapter-ligated constructs from ~21 nt RNA fragments. Cut the band corresponding to the desired insert size.
- Place gel slices in one 1.5 ml tube and add 200 µl of water
- Crush the gel slices with the RNase-free disposable pellet pestles
- Vortex horizontally for at least 2 hours at room temperature
- Transfer the eluate and gel debris to the top of a SpinX column
- Centrifuge the filter for 2 min at > 13,200 rpm
- Use a MinElute column to purify and concentrate sample
  - Add 5 volumes of Buffer PB to sample and mix
  - Add 10 µl of 3 M Sodium Acetate (sample should turn yellow)
  - Place a MinElute spin column in a provided 2 ml collection tube
  - Apply 600 µl of the sample to the MinElute column and centrifure for 30-60 s
  - Discard flow-through. Place the MinElute column back into the same tube
  - Apply remainder 600 µl of the sample to the MinElute column and centrifure for 30-60 s

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Tel: (831) 426-7700 Fax: (831) 420-0685

- Discard flow-through. Place the MinElute column back into the same tube
- To wash, add 0.75 ml Buffer PE to the MinElute columns and centrifuge for 30-60 s
- Discard flow-through and place the MinElute column back in the same tube. Centrifuge the column for additional 5 mins with lid open

- Place MinElute column in a clean 1.5 ml microcentrifuge tube
- To elute DNA, add 10 µl of EB buffer to the center of the MinElute membrane, let the column stand for 1 min, and then centrifuge the column for 1 min.

- Quantify library with Agilent Bioanalyzer/TapeStation or qPCR

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