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Clean up of Index PCRs using SPRI Beads V.2

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We use this protocol and it's working

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Abstract

This protocol describes the cleanup of index PCR products using SPRI bead-based size selection at a 1.4:1 bead-to-DNA ratio. The process includes bead binding, ethanol washes, DNA elution, and quantification using Qubit or other methods. The expected result is purified DNA libraries with concentration measurements for downstream analysis.

Protocol materials

- SpeedBeads magnetic carboxylate modified particles **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**GE45152105050250
- EB buffer **Qiagen Catalog #**19086
- 1.5mL DNA LoBind tubes **Eppendorf Catalog #**0030108051

Before start

This protocol is processed in the modern lab. Clean all surfaces with 5% bleach followed by 70% ethanol and all equipment with 70% ethanol before and after use.

Reagents and Materials

1 Before starting, make sure you have the following materials ready:

- SpeedBeads magnetic carboxylate modified particles **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**GE45152105050250

  (aka "SPRI beads"). Equilibrate to room temperature and vortex thoroughly before use. Can also use other types/brands of SPRI beads (e.g. AMPure XP Beads for DNA Cleanup).
- 80% ethanol (EtOH)
- EB buffer **Qiagen Catalog #**19086
- Magnetic rack
- 1.5mL DNA LoBind tubes **Eppendorf Catalog #**0030108051

2 Prepare **fresh 80% EtOH** in an appropriately sized tube (e.g. 15 mL or 50 mL falcon tube). You need 400µL per sample, and you have n samples.

	100% EtOH (µL)	ddH2O (µL)	per sample (µL)
	320	80	400
	n+1 * 320	n+1 * 80	n+1 * 400

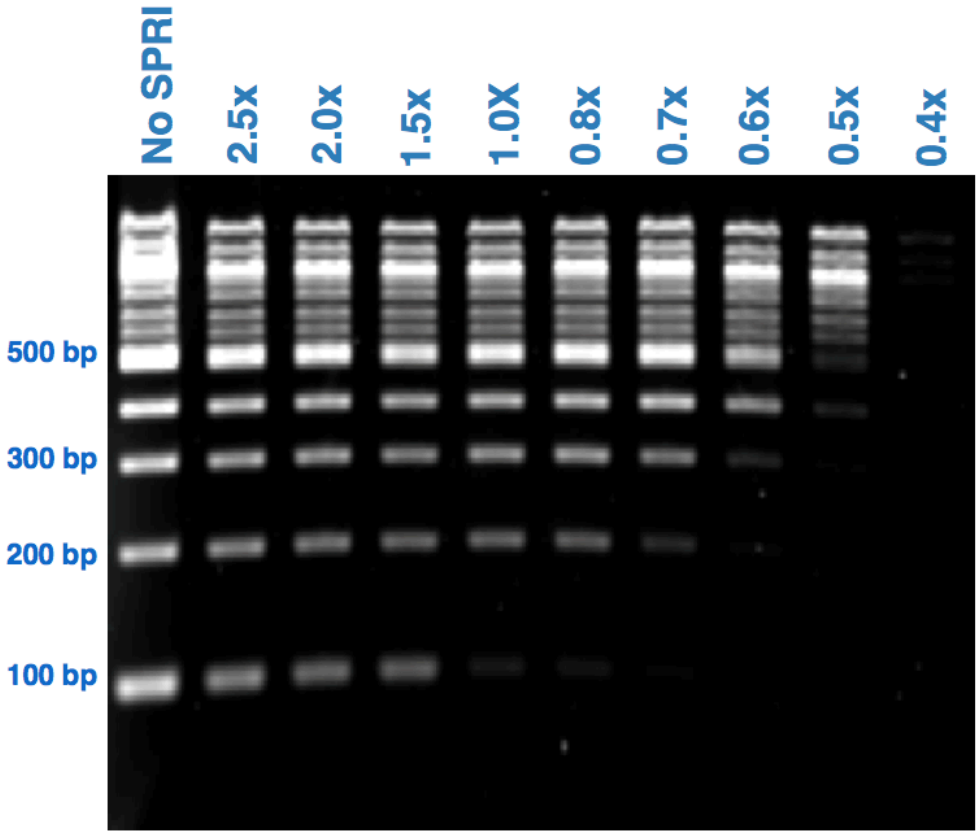
SPRI Bead Cleanup

5m

3

Note

- The following clean-up step is based on the ratio of beads: DNA = 1.4: 1.
- See the image below from the Broad Institute bootcamp (https://www.broadinstitute.org/genome-sequencing/broadillumina-genome-analyzer-boot-camp) to get an idea of what ratio of SPRI beads:DNA to use.
- The size of your library fragments depends on the size of your ancient DNA insert + adapters + indexes. For us, these are generally in the range of 160-350bp. Our adapters are 148bp in total (see our **Adapter-Splint Hybridization** protocol) which excludes the index sequences and DNA insert.



4 Add **70 µL SPRI beads** to a 1.5 mL tube.

5 Add **50 µL index PCR** product to the same tube. Mix them well by pipetting. Spin down very briefly in a microcentrifuge to collect beads - check that you did not pellet the beads at the bottom of the tube. If you did, resuspend by pipetting.

6 **Incubate** at room temperature for at least **5 minutes**. 5m

THIS IS A CRUCIAL STEP. This incubation allows the DNA to bind to the beads, so it's essential to avoid moving them and have enough time for the DNA to bind to the beads.

7 Place tubes on magnetic rack until **solution clears** (~2-5 min).

8 Remove supernatant using a pipette while the tubes are still in the magnetic rack (~110 µL).

Ethanol Wash

9 Add **200 µL 80% EtOH** while the tubes remain on the magnetic rack.

10 **Remove ethanol** carefully with a pipette. Be careful *not to remove any beads.*

11 Add another **200 µL 80% EtOH**.

12 **Remove ethanol** again.

13 Close lids **loosely.**

Drying

14 Open lids, **air-dry beads for ~2 minutes**. (Use timer)
- Important: **Do not over-dry, as this will destroy the DNA.**
- Use the small pipette to remove residual EtOH during these 2 minutes. Work quickly!

15 After drying for 2 min, close the lids.

Elution

16 Leave the tubes still in the magnetic rack. Add **25–32 µL EB buffer (typically 27 µL)** to elute the DNA.

17 **Remove the tubes from the magnetic rack**. Flick/vortex to resuspend.

18 **Incubate 10 minutes** at room temperature or 37°C. This is where the DNA is released from the beads into the elution buffer.

19 While waiting for the **10-minute incubation**, label new **LoBind tubes** - these are your final tubes**.**

20 Place the tubes **back in the magnetic rack** and wait until the beads pellet (the DNA is now in solution).

21 Transfer 25–32 µL (typically 25-26 µL) of supernatant to a clean, labeled LoBind tube. **Be careful not to transfer any beads**, as these will have negative effects on downstream applications, such as sequencing!

QC (Qubit or Fragment Analyser, or Bioanalyser etc.)

22 Following is the Qubit protocol to get the concentration of the libraries.
Typically, we would also run the indexed, cleaned libraries on a Fragment Analyzer (FA) or Bioanalyzer (BA) (TapeStation is also fine) to get the fragment size of the libraries, which is important when pooling libraries for sequencing. (Note that the FA, BA, and TapeStation have maximum concentration limits, so check their documentation and dilute your samples as needed, based on their Qubit concentrations.)

23 Prepare **Qubit working solution** (**WS**):

- Remove the Qubit standards from the fridge now to equilibrate to room temperature before use.

Assuming you have n samples, and 2 standards.

	A	HS buffer (µL)	Qubit HS Dye (µL)	per sample (µL)
	One sample	199	1	200
	n samples	199 * (n+2)	1 * (n+2)	200 * (n+2)

24 For samples:
Add **199 µL WS** to each Qubit tube + **1 µL library**. (You can use up to 5 µL of sample - just adjust the volume of WS accordingly. But even for aDNA libraries, we use only 1 µL.)

25 For standards:
Add **190 µL WS** + **10 µL standard 1**.
Add **190 µL WS** + **10 µL standard 2**.

26 Measure on Qubit:
Follow Qubit instructions to make measurements.
Set sample volume to 1 µL. Set output units to ng/µL.