**SPRI Bead QC protocol**

Updated: 10/1/2025

**Goal:** perform quality control on a new batch of SPRI beads. This will include:

* Confirm that beads purify DNA and estimate efficiency of recovery
* Assess impact of varying ratio of beads:sample on size of DNA purified
* Confirm absence of DNase and RNase activity [RNase protocol pending]

**Materials**

* New SPRI beads
* Existing SPRI beads for comparison (could be commercial)
* 50 bp DNA ladder (NEB #N3236S), diluted in gel loading buffer
* Magnet racks
* 70-75% ethanol
* Water or TE for elution
* Agarose gel

**Protocol**

* Purify NEB 50 bp DNA ladder using a range of ratios of beads:ladder per the table below. Follow our lab’s standard SPRI bead purification protocol. Elute in 12 µL H2O. Do this using the new beads. In parallel, do this using an existing batch of known-good beads.

|  |  |  |
| --- | --- | --- |
| **Beads:sample ratio** | **Diluted 50 bp DNA ladder (µL)** | **Beads (µL)** |
| 0.6:1 | 10 | 6 |
| 0.8:1 | 10 | 8 |
| 1:1 | 10 | 10 |
| 1.2:1 | 10 | 12 |
| 1.6:1 | 10 | 16 |
| 2:1 | 10 | 20 |

* Run 10 µL of each of the purified DNAs out on an agarose/EtBr gel (+ 2 µL 6x orange G loading buffer). As a control, run 10 µL of unpurified 50 bp DNA ladder in one or more lanes (the already diluted 50 bp DNA ladder).
* Image the gel and save the image to a USB flash drive
* Write up your results in a 1-page Word document (or google docs, etc):
  + Your name, the date, “QC of SPRI bead batch MM/DD/YYYY”  
     (date of SPRI bead batch)
  + Annotate gel lanes: what are ratios, which beads are new, which are old, what are ladder sizes
  + Print out 1-page document and tape to fridge door where we store SPRI beads.

**Interpretation of results**

**Absence of DNase activity.** If there is DNase activity present in the beads, this could be evident by smearing of ladder bands that have been purified.

**Efficiency of recovery:** compare the intensity of bands in the purified ladder lanes to the unpurified lanes to estimate an efficiency of recovery.

**Ratios and size cutoffs:** Note the impact of different ratios of beads:sample. This is useful information if you are using beads to selectively remove small nucleic acid molecules of different sizes.

**NEB 50 bp ladder**

[**https://www.neb.com/en-us/products/n3236-50-bp-dna-ladder**](https://www.neb.com/en-us/products/n3236-50-bp-dna-ladder)

A close-up of a dna test

Description automatically generated

50 bp DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel. Mass values are for 1 µg/lane. [source NEB]

**Background**

**Principle**: Solid-phase reversible immobilization (SPRI) beads are an effective, convenient, and reasonably priced way to purify DNA and/or RNA. DNA binds to magnetic SPRI beads under certain chemical conditions (e.g. ~10% PEG and 1M NaCl) and can be washed and eluted in water or other low salt buffer.

A diagram of a glass with a magnet and a few objects

Description automatically generated

*Image: Beckman Coulter*

Longer DNA molecules bind preferentially to SPRI beads and the lower cutoff of binding can be varied by varying the ratio of beads/buffer:sample. This is useful because it enables us to selectively purify larger DNA molecules, which avoids possibly unwanted small molecules, like primer-dimers that were generated during PCR. The gel image below shows a DNA ladder that has been “cleaned up” using SPRI beads at the indicated ratio of beads:ladder [e.g.: ratio of 2.5x = 2.5 volumes beads per volume ladder].

A close-up of a test

Description automatically generated

*Image: http://core-genomics.blogspot.com/*

Kapa Pure Beads are one of several commercially available SPRI bead products. These are relatively expensive, but it is not difficult to prepare your own SPRI beads. We follow a home brew recipe based on the “serapure” protocol, see: https://ethanomics.files.wordpress.com/2012/08/serapure\_v2-2.pdf