DNA beads

Note that this protocol is based on Rebecca Heald's protocol: Heald et al., Nature 382 (1996), 420-425 https://www.nature.com/articles/382420a0

1-Digestion of MCP or pBS

<u>2xbio DNA: digest with BamHI only</u>
450µg DNA
120µl Buffer 3
12µl 100x BSA
45µl BamHI
water to 1200µl

<u>1xbio DNA: digest with BamHI/NotI</u>
450µg DNA
120µl Buffer 3
12µl 100x BSA
45µl NotI
45µl BamHI
water to 1200µl

divide into 100µl aliquots, incubate in PCR machine at 37C for at least 4hrs
combine all aliquots, add 35µl NotI, divide into 100µl aliquots & incubate at 37C in PCR machine at least 4hrs

Combine all aliquots, split into 3 tubes, ethanol precipitate (add 11% of volume of 3M NaOAc pH5.2 to each tube & 2volumes of absolute ethanol, incubate at least 20min at -20C). Spin in refrigerated centrifuge (13krpm, 10min). Take off supernatant with blue tip, spin in benchtop centrifuge for 15sec, take rest of supernatant off with gel loading tip.
Dry 5min 37C incubator, 5min on bench
Resuspend each pellet in 60µl TE. This can take a little bit. I usually add TE, and then incubate for 30min to several hours. You can do this at room temperature, or, if you're worried about nuclease contamination, in the cold room.

Measure DNA content by nanodrop of spec.

2-Klenow fill-in

Do multiple fill-in reactions in parallel:

<u>For both 1xbio and 2xbio DNA:</u>

DNA	50µg
Buffer 2	10µl
bio-dATP	12.5µl of 400µM stock, final concentration is 50µM
bio-dUTP	5 µl of 1 mM stock, final is 50µM

dGTP	to 50µM
thio-dCTP	1.4 µl of 3.6 mM, final is 50µM
Klenow	8µl
Water	to 100µl

Incubate at 37C in PCR machine for 3hrs

Purify each reaction on a GE nick column using 1x bead buffer (see below) as your buffer. You can reuse the column if you wash it with two column volumes of buffer after each sample. Combine all three eluates & measure DNA concentration

3-Binding to Dynabeads M280 streptavidin

I routinely bind at a ratio of 150µg of DNA to 1mg of beads. For the actual binding reactions, you may have to scale down, though.

Set up two binding reactions & one mock-binding reaction without DNA. I usually set up two reactions for binding ca. 50µg DNA to 30µl beads each:

7.5% PVA	to 2.5%
Tris pH 8	to 50mM
EDTA	to 2mM
DNA	50µg
NaCl	to bring everything to 1.5M NaCl final take into consideration that the DNA contains 2M NaCl

(PVA, polyvinyl alcohol)

Using this protocol, you keep the total volume as low as possible. Thus, the DNA is in higher concentration & can saturate the beads better

Take a bit & measure DNA concentration. (use the reaction without DNA as your blank). This is your "input" sample

Add all to ca. 30µl beads (pre-washed a couple of times in 1x Bead buffer). Try two different kinds of tubes: For one, use non-stick 1.5ml tubes, for the other one flat-bottom 2ml tubes

Rotate at room-temperature o/n.

Next day, capture beads on magnet, measure sup. Ideally you would have 95% or so bound. If substantially less than that, incubate longer. Determine how much DNA you have bound to each bead. 1µl Strepatividin beads stock suspension has ca. 6.7×10^5 beads. For comparison, one sperm is ca. 3.2pg of DNA

This is where you determine how clumpy your beads are. Ideally, your beads are monodisperse, but that never happens. You'll probably see that the beads have clumped up. If your beads are very clumpy, there's two things you can do. One thing is to pipet up and down many times. The other, more drastic, solution is to pass the beads a few times through a 25 gauge needle. Avoid keeping the beads pelleted too long as that very efficiently clumps them. Unfortunately, it's not quite clear why you sometimes get a lot of clumping & why sometimes you don't get that. One thing I've not

tried but which maybe worth trying is to pipet your binding reaction up & down a few times during binding (first time a few hours after you put it to rotation).

Wash the beads 3 times with 1x bead buffer, (transfer to new tube with first wash & last wash. Bring it to a 0.5ml non-stick tube with the last wash). Then wash 2x in SDB, resuspend in SDB to ca. 1µg DNA/µl. Have a look under the microscope to check how homogenously the beads are covered in DNA.

2x bead buffer:
4M NaCl
20mM Tris pH 7.75 @ 22C
2mM EDTA