How to Use Mag-Bind Beads to Remove Bacterial RNA from Plasmid DNA Minipreps

(Zongyue Zeng @ 08/26/2019; Commented by TCH)

NOTE: Carboxyl-coated paramagnetic beads such as AMPure, SPRISelect, and Omega Mag-Bind are frequently used in molecular biology to purify DNA from within reaction mixtures. Beads are typically supplied in a solution containing polyethylene glycol (PEG) and sodium chloride (NaCl). These components cause the negatively-charged DNA to condense and bind to the positively-charged beads. Once the supernatant is removed from the magnetized bead pellet, purified DNA is obtained by washing the beads in ethanol and eluting the DNA into elution buffer.

The nature of the reversible immobilization of DNA onto the beads is dependent upon the amount of PEG and NaCl in solution. Bead suppliers typically recommend a bead:sample ratio (v/v) of 1.8 to 1 (referred to as 1.8x) in order to recover DNA fragments above 100bp in length, at the exclusion of unincorporated dNTPs and primers. Using ratios lower than 1.8x means there is less PEG/NaCl in solution, which prevents small DNA fragments in the mixture from condensing and binding to beads in the solution. Thus, by using ratios other than the recommend 1.8x, DNA fragments of differing lengths will be preferentially bound, which permits the targeted the recovery of desired lengths of DNA. This behavior can be utilized to target a desired size cutoff, i.e. an approximate size division between the "small" and "large" fragments.

Protocol:

1. Shake or vortex the Mag-Bind® Total Pure NGS to resuspend any particles that may have settled. Allow Mag-Bind® Total Pure NGS to come to room temperature before use.
2. Concentrate the plasmid DNA (i.e., one miniprep) by ethanol precipitation, and dissolve it in 20µl ddH_2o and transfer to 200µl PCR tubes.
3. Add 8µl Mag-Bind beads into the DNA-containing 200µl PCR tubes. Pipet up and down 5-10 times or vortex for 30 seconds to mix well.
4. Let the tubes sit at room temperature for 5 minutes.
5. Spin it down briefly at low speed and put it on the magnetic separation device.
6. Aspirate and discard the clear supernatant. Do not disturb the Mag-Bind beads.
7. With the tubes are mounted on the magnet, add 200µl 70% ethanol.
8. Let the tubes sit at room temperature for 1 minute. It is not necessary to resuspend the Mag-Bind® Total Pure NGS.
9. Aspirate and discard the clear supernatant. Do not disturb the Mag-Bind beads.
10. Repeat Steps 7-9 for a second 70% ethanol wash. Leave the tubes on the magnetic separation device for 2-3 minutes to air dry the Mag-Bind beads. Remove any residual liquid with a pipette.
11. Remove the tubes from magnetic separation device. Add 50-100µL ddH_2O to elute plasmid DNA.
12. Pipet up and down 20 times or vortex for 30 seconds. Let the tubes sit at room temperature for 5 minutes
13. Place the tubes on a magnetic separation device. Let the tubes sit at room temperature until the Mag-Bind beads are completely absorbed by the magnet, and cleared from solution.
14. Transfer the cleared supernatant containing purified DNA to new tubes for downstream uses (e.g., transfection, DNA sequencing, or NGS).

Evaluation of Omega Mag-Bind® TotalPure NGS Beads for DNA Size Selection

By Maggie Weitzman, M.Sc. (University of Oregon / GC3F)

Disclaimer: Neither Maggie Weitzman, the University of Oregon, nor the Genomics & Cell Characterization Core Facility (GC3F) have any affiliation with Omega Bio-Tek or their products & services, nor are they being compensated for performing these tests or sharing this data.

BACKGROUND & OBJECTIVE

Carboxyl-coated paramagnetic beads such as AMPure, SPRISelect, and Omega Mag-Bind are frequently used in molecular biology to purify DNA from within reaction mixtures. Beads are typically supplied in a solution containing polyethylene glycol (PEG) and sodium chloride (NaCl). These components cause the negatively-charged DNA to condense and bind to the positively-charged beads. Once the supernatant is removed from the magnetized bead pellet, purified DNA is obtained by washing the beads in ethanol and eluting the DNA into elution buffer.

The nature of the reversible immobilization of DNA onto the beads is dependent upon the amount of PEG and NaCl in solution. Bead suppliers typically recommend a bead:sample ratio (v/v) of **1.8 to 1** (referred to as **1.8x**) in order to recover DNA fragments above 100bp in length, at the exclusion of unincorporated dNTPs and primers. Using ratios lower than 1.8x means there is less PEG/NaCl in solution, which prevents small DNA fragments in the mixture from condensing and binding to beads in the solution. Thus, by using ratios *other than the recommend 1.8x*, DNA fragments of differing lengths will be preferentially bound, which permits the targeted the recovery of desired lengths of DNA. This behavior can be utilized to target a desired size cutoff, i.e. an approximate size division between the "small" and "large" fragments.

The objective of this report is to determine the specific DNA fragment sizes that can be recovered at various applied ratios of Omega Mag-Bind® TotalPure NGS magnetic beads.

MATERIALS

DNA Sources: O'GeneRuler **Low Range** DNA Ladder, 0.1 µg/µL (Thermo Fisher #SM1203, Lot 00593385)
Contains fragment sizes (bp): 25, 50, 75, 100, 150, 200, 300, 400, 500, 700

O'GeneRuler **100bp Plus** DNA Ladder, 0.1 µg/µL (Thermo Fisher #SM1153, Lot 00136737)
Contains fragment sizes (bp): 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000, 3000

O'RangeRuler **500bp** DNA Ladder, 0.05 µg/µL (Thermo Fisher #SM0643, Lot 00607550)
Contains fragment sizes (bp): 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000

Magnetic Beads: Mag-Bind **TotalPure NGS** Beads (Omega Bio-Tek #M1378-02, Lot TPN031417JCM22873)

Quantification: Quant-iT **dsDNA High Sensitivity** Assay Kit (Thermo Fisher #Q33120, Lot 1966973)

SpectraMax M5E **Microplate Reader** (Molecular Devices #M5E)

Fragment Analysis: High Sensitivity **NGS Fragment Analysis** Kit (Advanced Analytical #DNF-474-500)

Fragment Analyzer Automated CE System (Advanced Analytical)

METHOD

1. The 3 DNA ladders listed above were combined as follows:

+ 1 part of O'GeneRuler Low Range @ 0.1 µg/µL
+ 1 part of O'GeneRuler 100bp Plus @ 0.1 µg/µL
+ 2 parts of O'RangeRuler 500bp @ 0.05 µg/µL

= 4 parts of mixed ladder @ 66.67 ng/µL

2. The mixed ladder was diluted to approx. 15 ng/µL in Qiagen elution buffer (EB) (10 mM Tris-Cl, pH 8.5) and distributed into aliquots of 50 µL each

3. Aliquots of mixed ladder were size-selected using Omega Mag-Bind® TotalPure NGS beads with a standard bead cleanup protocol consisting of these main steps (see Table 1 & Figure 1 for more details):

    a. Mixing of 50 µL mixed ladder with Omega beads at 23 different beads:sample ratios (Table 1)
    b. Magnetization to separate the supernatant from the beads
    c. Removal of the supernatant
    d. Washing the beads twice with 150 µL of 80% ethanol
    e. Air drying the beads
    f. Eluting the DNA in 50 µL of Qiagen EB

4. From each of the 23 size selection reactions, both the **supernatant fraction** (containing smaller unbound fragments) and the **bead fraction** (containing larger bound fragments) were retained, and their bead cleanups were performed in parallel (Figure 1).

    Keeping both fractions in each reaction allows the separate recovery of both the small fragments and the large fragments on either side of the targeted size cutoff. Thus, each of the 23 ratio tests produced two separate eluted DNA samples.

    We establish the naming convention for these size-selection cleanups according to *which fragments are removed from the final eluted product:*

    - **Small Fragment Removal (SFR) Cleanup =**
        - *Removes smaller fragments* (in the supernatant)
        - *Keeps larger fragments* (bound to beads)

    - **Large Fragment Removal (LFR) Cleanup =**
        - *Removes larger fragments* (bound to beads)
        - *Keeps smaller fragments* (in the supernatant)
          NOTE: In order for the smaller fragments to be purified from the supernatant, a second addition of beads is required to bind the small DNA in solution (Figure 1).

5. All final eluted samples, along with the non-size selected mixed ladder, were characterized as follows:

    - **Quantified** to measure total DNA yield with Quant-iT dsDNA High Sensitivity Assay Kit
    - **Fragment analyzed** to determine recovery of the various DNA lengths with High Sensitivity NGS Fragment Analysis Kit

    These data were then normalized to the results from the 1.8x bead test (referred to as the "control" ratio as this is the ratio recommended by bead suppliers) to determine percent yields.

6. Each of the 23 tests were repeated independently, for a total of 2 replicates of each test condition. All data reported here are the means of the two replicates for each data point.

Table 1. Conditions used for size selection tests (*n*=2 for each condition). The control ratio of 1.8x (supplier recommended) is indicated in **red**.

Bead: Sample Ratio (v/v)	***1st DNA binding step***				***2nd DNA binding step***			
	Volume of **sample** used (µL)	Volume of **beads added** (µL)	Volume of **supernatant removed** (µL)	Fate of supernatant	Volume of **beads added** to the previous supernatant (µL) *in order to achieve the control bead:sample ratio of 1.8x*	Volume of **total PEG/NaCl** in solution (µL)	Volume of **final supernatant removed** (µL)	Fate of supernatant
0.30x	50	15	65	**Kept** and treated as a parallel sample for recovering **both the large & small fractions separately,** via a 2nd DNA binding step *[as per columns to the right → and indicated in Figure 1]*	75	90	140	**Discarded**
0.35x	50	17.5	67.5		72.5	90	140	
0.40x	50	20	70		70	90	140	
0.45x	50	22.5	72.5		67.5	90	140	
0.50x	50	25	75		65	90	140	
0.55x	50	27.5	77.5		62.5	90	140	
0.60x	50	30	80		60	90	140	
0.65x	50	32.5	82.5		57.5	90	140	
0.70x	50	35	85		55	90	140	
0.75x	50	37.5	87.5		52.5	90	140	
0.80x	50	40	90		50	90	140	
0.85x	50	42.5	92.5		47.5	90	140	
0.90x	50	45	95		45	90	140	
0.95x	50	47.5	97.5		42.5	90	140	
1.00x	50	50	100		40	90	140	
1.20x	50	60	110		30	90	140	
1.40x	50	70	120		20	90	140	
1.60x	50	80	130		10	90	140	
1.80x (control)	**50**	**90**	**140**	**Discarded**	--	--	--	
2.00x	50	100	150		--	--	--	
2.25x	50	112.5	162.5		--	--	--	
2.50x	50	125	175		--	--	--	
3.00x	50	150	200		--	--	--	

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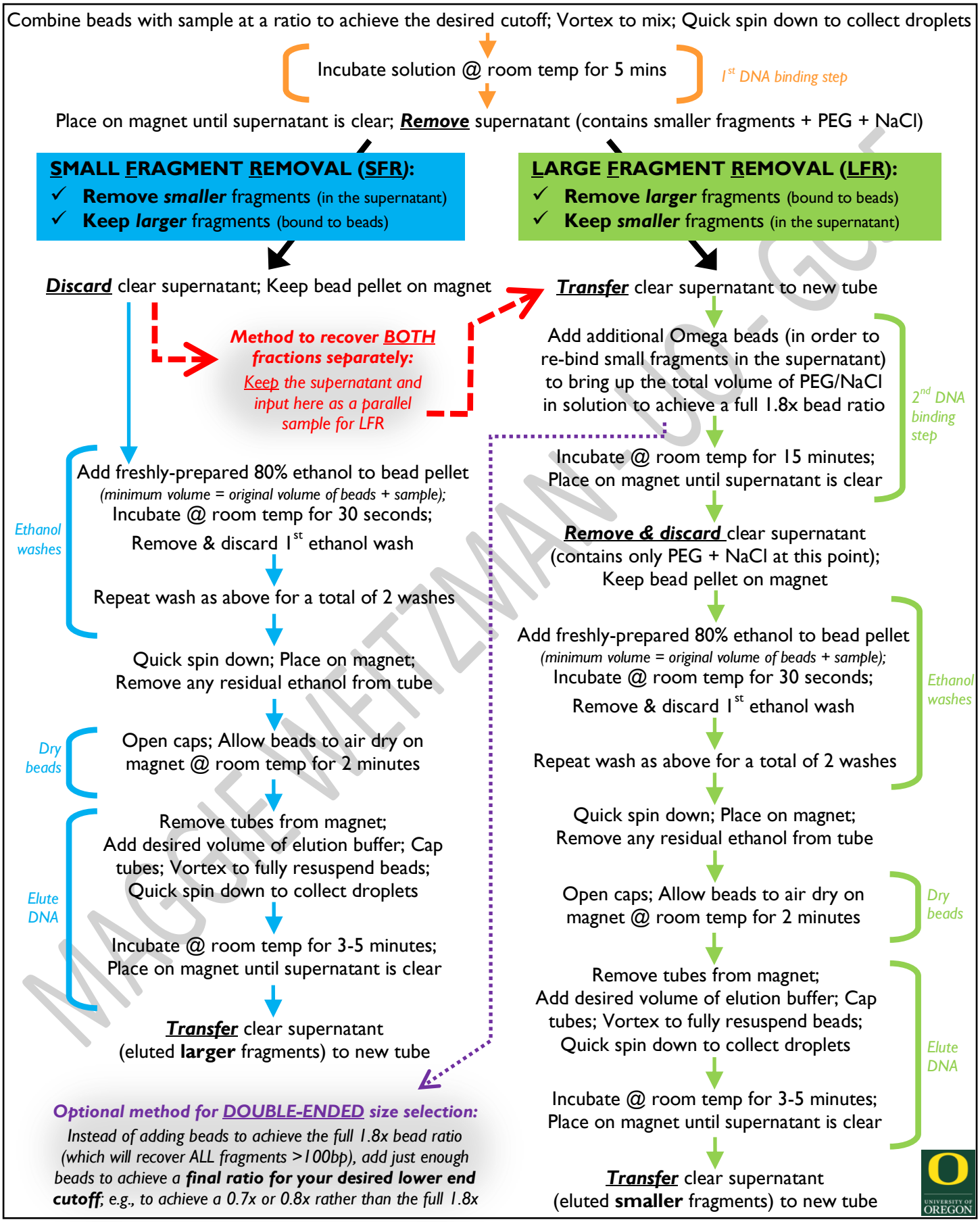
Figure 1. Diagram of method to perform SFR and LFR using Omega beads. The method used in this report to keep both fractions is indicated in **red**. An optional method to perform double-ended selection is in **purple**.



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RESULTS & DISCUSSION

Data from these tests indicate that different ratios of Omega Mag-Bind® TotalPure NGS beads can be used to precisely target any desired size cutoff (i.e. approximate size division between the "small" and "large" fragments) for the separation of DNA fragments ≤ 3000 bp. The recovery of the small or large fragments on either side of the cutoff is accomplished simply by keeping either the bead-bound fraction or the supernatant fraction (Figures 2 & 3; Table 2).

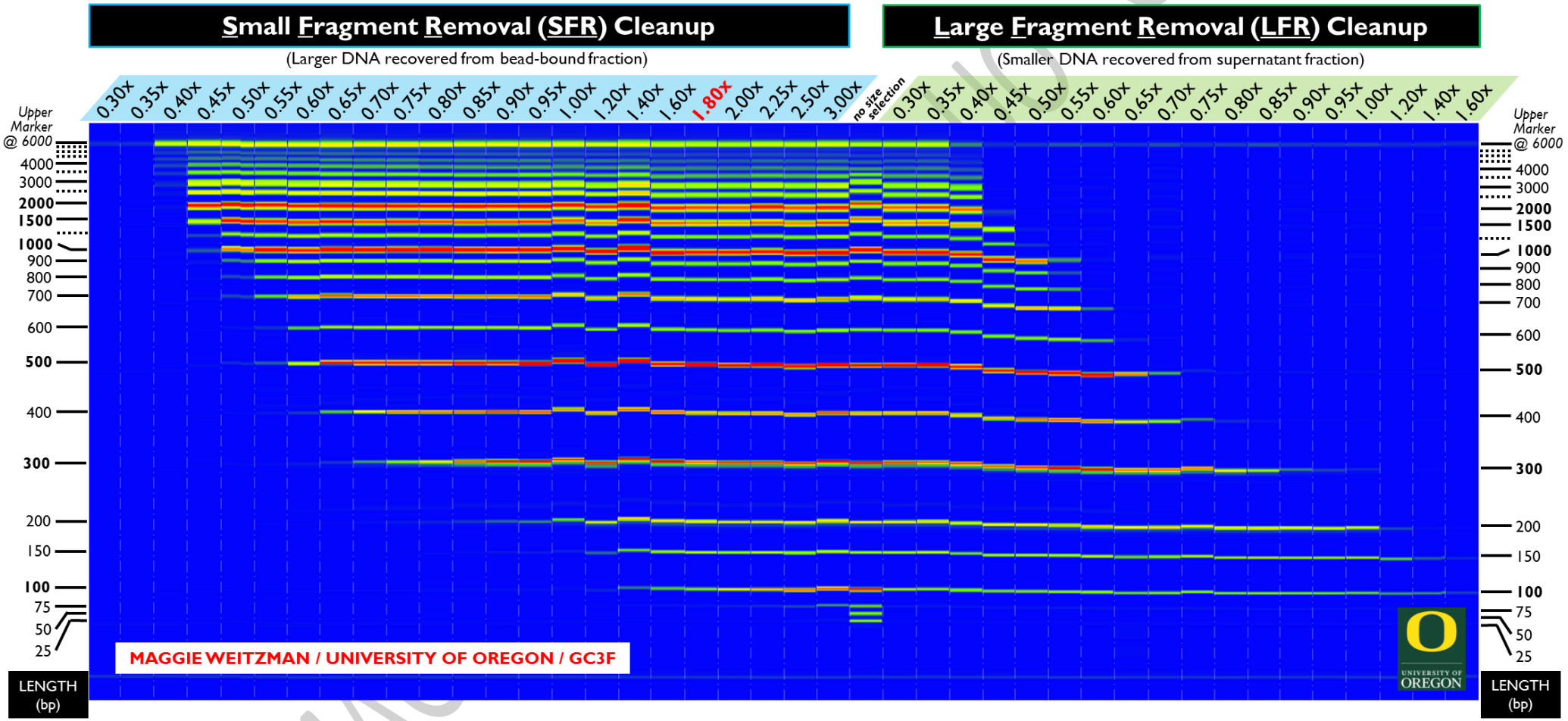

Figure 2. Pseudogel image from the fragment analysis of DNA recovered after size selection. *Note: Data from only 1 of the 2 test replicates is shown here.*

Table 2. Heatmap of percent of DNA removed for various fragment lengths (versus 1.8x control, in **red**). *n*=2 for each condition

Note: Negative percentages are reported as 0%.

% OF DNA REMOVED				FRAGMENT LENGTH (bp)																			
				100	150	200	300	400	500	600	700	800	900	1000	1200	1500	2000	2500	3000	3500	4000	4500	5000	5500
BEAD:SAMPLE RATIO (v/v)	0.30x	APPROX. SIZE CUTOFF (bp) between "small" and "large" fragments	n/a	SMALL FRAGMENT REMOVAL (SFR) CLEANUP	99	100	100	100	100	100	100	100	100	99	100	99	100	100	100	100	99	100	99	99	99
	0.35x		n/a		100	100	100	100	99	100	100	100	100	100	100	99	100	100	99	100	99	99	99	99	99
	0.40x		3,000		99	100	100	100	100	100	100	100	100	100	100	99	99	98	95	86	71	52	39	30	48
	0.45x		1,000		100	100	100	100	100	99	99	98	98	96	94	80	48	1	0	0	0	0	0	0	0
	0.50x		700		99	100	100	100	99	98	96	92	82	66	50	9	0	0	0	0	0	0	0	1	3
	0.55x		500		99	100	99	99	96	95	85	68	38	20	12	9	7	7	8	15	10	11	9	12	9
	0.60x		450		99	99	99	98	91	78	44	19	8	7	5	7	7	5	6	6	9	7	8	2	0
	0.65x		350		99	99	99	95	73	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.70x		300		99	99	98	85	35	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.75x		280		97	99	96	76	23	15	6	5	5	6	4	5	7	5	2	5	6	5	2	4	11
	0.80x		260		98	99	96	59	17	22	13	13	13	13	12	12	14	12	11	12	15	16	14	17	23
	0.85x		240		98	97	90	27	0	13	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
	0.90x		220		97	96	84	15	2	9	4	3	3	4	2	3	6	3	4	5	7	9	5	7	4
	0.95x		200		97	95	77	5	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1.00x		175		96	93	65	11	9	15	10	9	9	9	8	3	10	8	4	5	7	8	9	15	3
	1.20x		150		90	67	12	0	0	19	0	0	0	0	0	0	0	0	0	0	0	8	2	6	0
	1.40x		125		72	27	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		10	8
	1.60x		100		46	15	8	8	9	12	10	10	10	10	10	12	11	12	12	14	14	15	13	15	15
	1.80x		100		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0.30x		n/a	LARGE FRAGMENT REMOVAL (LFR) CLEANUP	0	3	4	5	6	4	9	8	8	8	7	10	8	9	11	10	11	16	9	19	14
	0.35x		n/a		14	10	9	12	13	13	14	15	14	15	14	17	16	16	18	18	20	22	14	20	25
	0.40x		3,000		21	19	18	20	20	22	20	21	19	20	19	21	20	21	24	31	44	57	67	75	77
	0.45x		1,000		21	13	12	15	15	21	18	18	19	20	22	34	59	95	99	99	99	99	99	99	99
	0.50x		700		28	19	18	18	21	30	26	29	36	48	61	89	98	100	100	100	99	99	99	99	99
	0.55x		500		26	14	11	11	13	20	24	35	67	85	93	99	99	99	99	99	99	99	99	99	98
	0.60x		450		24	13	11	12	18	36	51	88	97	99	99	99	99	99	99	99	99	99	99	99	99
	0.65x		350		36	22	21	25	40	70	93	99	100	100	100	100	100	100	99	99	99	99	99	99	99
	0.70x		300		27	14	13	23	65	94	98	99	99	99	99	99	99	99	99	99	98	98	98	97	98
	0.75x		280		29	17	0	38	85	98		99	99	99	99	99	99	99	99	99	99	99	99	99	99
	0.80x		260		33	13	0	54	95	100	100	100	100	100	100	100	100	100	100	100	99	100	100	100	100
	0.85x		240		29	8	12	72	97	99	98	99	100	100	99	100	99	99	99	99	99	99	100	100	100
	0.90x		220		38	19	30	88	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
	0.95x		200		45	22	36	93	99	100	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
	1.00x		175		40	16	44	97	99	99	99	99	99	98	99	99	99	99	99	99	99	99	99	99	99
	1.20x		150		51	40	88	99	99	99	99	99	99	97	99	99	99	99	99	99	99	99	99	99	99
	1.40x		125		60	72	97	99	99	99	99	99	99	99	100	99	99	99	99	99	99	99	99	100	100
	1.60x		100		81	91	99	100	100	100	99	99	100	100	100	100	100	100	100	100	100	100	100	100	100

% OF DNA REMOVED
99 to 100
96 to 98
90 to 95
80 to 89
70 to 79
60 to 69
50 to 59
40 to 49
30 to 39
20 to 29
15 to 19
10 to 14
3 to 9
0 to 2

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RESULTS , cont'd

Summary of key findings:

When combining DNA yields from the SFR & LFR fractions (for the 18 ratios ≤1.8x), the following amounts of DNA were recovered (Table 3):

- **95-100% total recovery** for 13 of the 18 tests
- **91-94% total recovery** for 5 of the 18 tests

These yields indicate that Omega beads have a high DNA recovery rate and can be considered reliable for performing bead cleanups and size selection.

Less than 1% of the total DNA was retained when using a ratio under 0.40x (i.e. 0.35x or 0.30x). This is likely because there is simply not enough PEG/NaCl in solution to condense the DNA. Due to extremely low yields, these lowest bead ratios are not recommended for size selection.

The effect of size selection on the 6000 bp ladder band could not be evaluated because the Upper Marker reagent in the fragment analyzer kit (size 6000 bp) occurs at the same location on the electropherogram.

Table 3. Total DNA recovery for SFR, LFR, and summed fractions (versus 1.8x control, in **red**). *n*=2 for each condition

Bead: Sample Ratio (v/v)	Recovered from SFR (larger fragments)		Recovered from LFR (smaller fragments)		Sum of yields from SFR + LFR
	Mean DNA concentration (ng/µL) ± SD (*n*=2)	% Total DNA yield	Mean DNA concentration (ng/µL) ± SD (*n*=2)	% Total DNA yield	
0.30x	**0** ± 0	0%	**12.4** ± 0.3	98%	**99%**
0.35x	**0** ± 0	0%	**12.2** ± 0.1	97%	**97%**
0.40x	**1.0** ± 0.2	7%	**11.6** ± 0.3	92%	**99%**
0.45x	**4.0** ± 0.2	31%	**8.1** ± 0.2	64%	**95%**
0.50x	**5.7** ± 0.3	44%	**6.6** ± 0.1	52%	**96%**
0.55x	**6.8** ± 0.3	52%	**5.2** ± 0.1	41%	**93%**
0.60x	**7.8** ± 0.4	60%	**4.3** ± 0.1	34%	**93%**
0.65x	**8.8** ± 0.2	67%	**3.3** ± 0.1	26%	**93%**
0.70x	**10.3** ± 0.2	79%	**2.5** ± 0	20%	**99%**
0.75x	**10.2** ± 0.2	78%	**2.1** ± 0.1	16%	**95%**
0.80x	**10.7** ± 0.4	82%	**1.7** ± 0.1	14%	**96%**
0.85x	**11.4** ± 0.2	88%	**1.5** ± 0	12%	**100%**
0.90x	**11.2** ± 1.1	86%	**1.2** ± 0.1	9%	**95%**
0.95x	**11.5** ± 0.3	88%	**1.0** ± 0.2	8%	**96%**
1.00x	**11.8** ± 0.3	91%	**1.0** ± 0.1	8%	**98%**
1.20x	**12.5** ± 0.1	96%	**0.5** ± 0.1	4%	**100%**
1.40x	**11.9** ± 0.5	91%	**0.3** ± 0.0	2%	**93%**
1.60x	**11.7** ± 0.7	90%	**0.1** ± 0.1	1%	**91%**
1.80x (control)	**13.0** ± 0.6	100%	--	--	--
2.00x	**13.1** ± 0.7	101%	--	--	--
2.25x	**13.5** ± 0.6	103%	--	--	--
2.50x	**13.4** ± 0.5	103%	--	--	--
3.00x	**13.8** ± 0.3	106%	--	--	--

For most NGS library cleanups where the objective is to remove fragments <200bp (leftover primer, adapter dimers, small insert libraries, etc.), a bead ratio of **0.8x** would be adequate in most cases.

Ratios at 1.8x and below are intended to recover fragments *greater than 100 bp* in length (as per bead supplier protocols). To determine whether DNA fragments *less than 100 bp* can also be retained with Omega beads, several ratios *above* the recommended 1.8x were tested using the SFR method (Figure 1). It was found that more of the 25, 50, and 75 bp fragments could indeed be recovered when increasing the ratio above 1.8x. In fact, a 3.00x ratio recovered more than twice as many 25 bp fragments and nearly 6 times as many 75 bp fragments as a 1.8x ratio (Table 4).

RESULTS , cont'd

Table 4. Yield of small DNA fragments for ratios above the 1.8x control (in **red**). *n*=2 for each condition

Bead: Sample Ratio (v/v)	% yield of small fragment lengths *(versus 1.8x control)*				% yield of small fragment lengths *(versus non-size selected ladder)*			
	25 bp	50 bp	75 bp	100 bp	25 bp	50 bp	75 bp	100 bp
1.80x (control)	100%	100%	100%	100%	2%	0%	7%	34%
2.00x	118%	123%	149%	143%	2%	0%	10%	48%
2.25x	98%	70%	236%	207%	2%	0%	16%	70%
2.50x	146%	102%	344%	238%	3%	0%	24%	80%
3.00x	209%	163%	587%	273%	4%	1%	41%	92%

However, even a ratio of 3.00x could only recover 4% of the 25 bp fragments that were present in the original non-size selected DNA ladder (Table 4). Thus, while increasing the bead ratio beyond the recommended 1.8x can provide a higher recovery of smaller fragments, it is still a relatively small recovery when compared to the total amount of small DNA present in the original sample. For applications where recovery of these small fragments is more critical, increasing the ratio even further beyond 3.00x could result in higher yields.

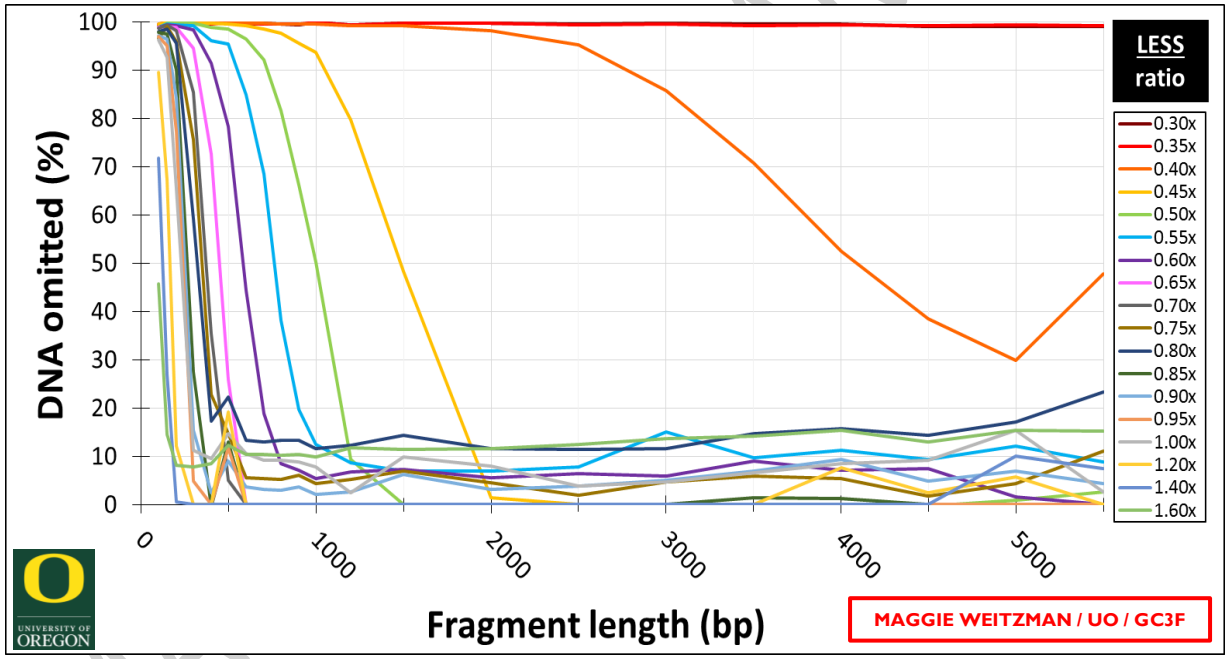

Figure 3. Percent of DNA removed at various fragment lengths for SFR tests (versus 1.8x control, in **red**). *n*=2 for each condition

Note: Negative percentages are reported as 0%

Extrapolating from these data, ratios at smaller increments than the 0.5x increments shown here could be used to target cutoffs at intermediate intervals. For example, a ratio of 0.57x would result in an expected cutoff around 600bp. At the GC3F, we routinely use such smaller increment ratios for size selecting DNA.

We also routinely combine the SFR and LFR methods into a double-ended size selection protocol (Figure 1), such that the two size cutoffs can be made in a single tube. Removing DNA from both ends of the spectrum allows the targeted recovery of fragments from only within a specific range (e.g., from 400-800bp, from 275-625bp, etc.). We find Omega beads to be immensely useful and an essential part of our NGS workflows!