Initial Library Preparation Bead Evaluation

I. Objective

To compare the performance of three batches of MagPure A4 XP (Magen #XP-50/XP-500) with AMPure XP (Beckman #A63881).

II. Materials

Comparison between three batches of MagPure A4 XP (Magen) and AMPure XP (A63881):

- AMPure XP (A63881)
- Three batches provided by Magen:
- HG050200, HE150200, HG070200

III. Experimental Design

1. For each batch of library preparation beads, three libraries were constructed using:

- Single size selection method
- Double size selection method

2. A total of 32 cfDNA samples were pooled into one tube, mixed thoroughly by vortexing, and then aliquoted into 28 tubes, each containing 30 μL.

3. Library yields were compared across different bead batches using the same library preparation method.

IV. Results

1. cfDNA Concentration

0.208 ng/μL

2. Library Construction Results

Bead Batch	Source	Library Prep Method	Sample ID	Adapter	Library Concentration (ng/μL)	Mean (ng/μL)
AmPure XP	Library preparation beads currently in use in the laboratory	Single size selection method	1	4	8.73	6.35
			2	5	6.24	
			3	6	4.08	
HG050200	Magen QC batch beads		4	7	8.23	7.99
			5	8	7.57	
			6	9	8.16	
HG070200			10	4	9.1	8.32
			11	5	6.77	
			12	4	9.1	
HE150200			13	4	9.1	8.32
			14	5	6.77	
			16	7	8.7	
AmPure XP	Library preparation beads currently in use in the laboratory	Double size selection method	17	8	4.32	3.39
			18	9	3.05	
			19	10	2.81	
HG050200	Magen QC batch beads		20	11	3.31	3.15
			21	12	2.84	
			22	13	3.31	
HG070200			23	14	2.99	3.71
			24	15	3.46	
			25	4	4.69	
HE150200			26	5	4.01	3.72
			27	6	3.54	
			28	7	3.62	

Microscale Recovery Efficiency Evaluation

Marker Recovery Experiment

Objective

To evaluate the recovery efficiency of XP magnetic beads at different ratios using a **25 ng, 50 bp purified DNA marker**.

Experimental Procedure

1. Add **20 ng of purified marker** into a **1.5 mL microcentrifuge tube**.
2. Adjust the volume to **100 µL** with nuclease-free water.
3. Add XP magnetic beads (different batches) at ratios of **0.7×, 0.8×, 0.9×, 1.2× and 1.5×**, respectively.
4. Vortex to mix thoroughly for **1 min**.
5. Incubate at room temperature for **10 min** to allow binding.
6. Place the tube on a magnetic stand for **5 min**.
7. Carefully remove and discard the supernatant.
8. Wash the beads twice with **500 µL of 70% ethanol**.
9. Briefly centrifuge and remove any residual ethanol.
10. Air-dry the beads for **10 min**.
11. Elute DNA in **50 µL of EB buffer**.

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			Yield	Recovery
A	Original sample dilution	0.502	0.0251	100%
AMPure XP	0.7	0.0975	0.004875	19%
	0.8	0.205	0.01025	41%
	0.9	0.274	0.0137	55%
	1.2	0.477	0.02385	95%
	1.5	0.475	0.02375	95%
HG050200	0.7	0.112	0.0056	22%
	0.8	0.219	0.01095	44%
	0.9	0.284	0.0142	57%
	1.2	0.468	0.0234	93%
	1.5	0.486	0.0243	97%
HG070200	0.7	0.106	0.0053	21%
	0.8	0.216	0.0108	43%
	0.9	0.286	0.0143	57%
	1.2	0.475	0.02375	95%
	1.5	0.485	0.02425	97%