AMPure XP Reagent cleanup App on Biomek NGeniuS System

App Template Version 1.0.1

BECKMAN COULTER Life Sciences

2024-GBL-EN-105071-v1

App Template Description

The AMPure XP PCR purification system utilizes Beckman Coulter's SPRI paramagnetic bead technology for high-throughput purification of PCR amplicons. AMPure XP Reagent utilizes an optimized buffer to selectively bind DNA fragments 100 bp and larger to paramagnetic beads. Excess primers, nucleotides, salts, and enzymes can be removed using a simple washing procedure. The result is a more purified PCR product. Input sample volume for the app template is restricted to 20 µL. The app template supports commonly used bead ratios throughout the NGS applications space including .6X, .8X, 1.0X, 1.2X, 1.8X, and 3.0X. User-tunable timings, wash parameters, and elution volumes from 10-50 µL in 10 µL steps are also supported. Default app template settings follow guidance provided in the AMPure XP PCR Purification Instructions for Use [B37419.AB]. Parameter selections such as low elution volumes, excessive wash cycles, and extended drying times may result in reduced recovery. Refer to the AMPure XP PCR Purification Instructions for Use for best practices, guidance, and frequently asked questions. This app template is intended for use with the following Beckman Coulter Life Sciences products: A63880-AMPure XP 5 mL, A63881-AMPure XP 60 mL, A63882-AMPure XP 450 mL.

Document ID: 2023-GBL-EN-101342-v2

Scoping

Scoping

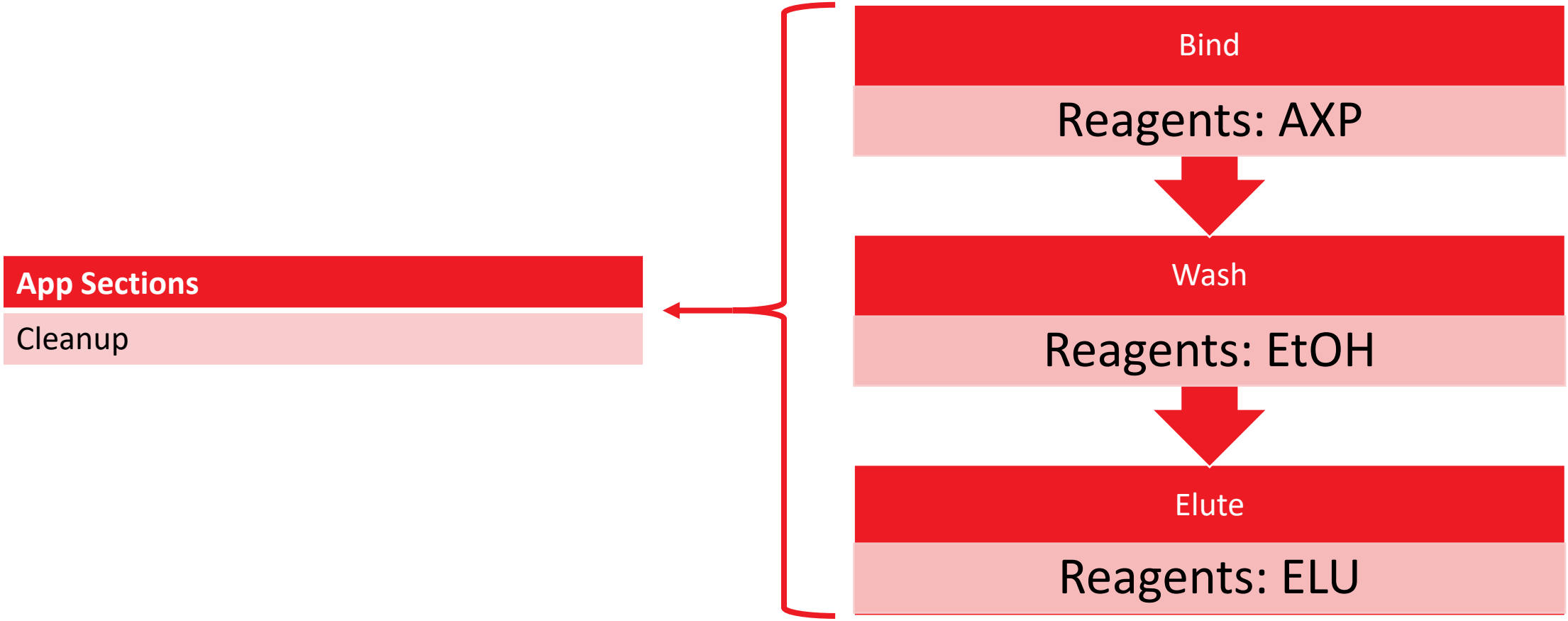
- Author
  - Beckman Coulter Life Sciences
- Kit
  - N/A
- Supported
  - 20 µL sample input
  - Selected bead ratios from 0.6X – 3.0X
  - “Left side” cleanup (i.e. removal of small nucleic acid fragments)
- Excluded
  - Use as size selection
  - Two sided cleanups
  - “Right side” cleanup (i.e. removal of large nucleic acid fragments

Scoping

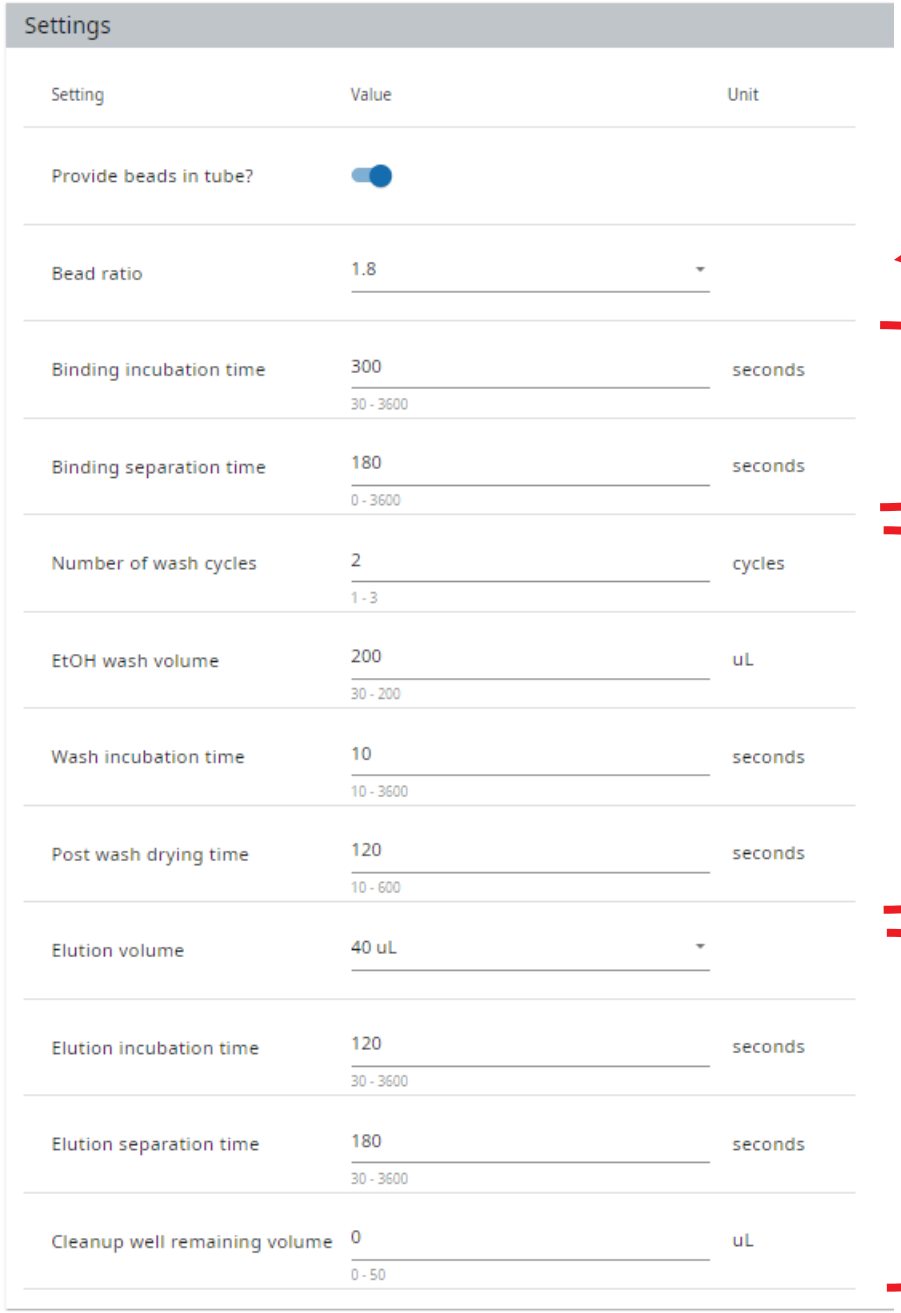
- Part numbers
  - A63882 - AMPure XP SPRI Reagent, 450 mL
  - A63881 - AMPure XP SPRI Reagent, 60 mL
  - A63880 - AMPure XP SPRI Reagent, 5 mL

App Details

Sections Automated



App Settings



Default cleanup bead ratio

Adjust binding settings

Adjust wash settings

Adjust elution settings

Requested AMPure XP by run

	AMPure XP provided in 2 mL sarstedt tube																			
	Batch Size																			
Bead Ratio	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
0.6	97.8	116	134.2	152.4	170.6	183.8	197	210.2	223.4	236.6	249.8	263	276.2	289.4	302.6	315.8	329	342.2	355.4	368.6	381.8
0.8	115.4	138	160.6	183.2	205.8	223.4	241	258.6	276.2	293.8	311.4	329	346.6	364.2	381.8	399.4	417	434.6	452.2	469.8	487.4
1	133	160	187	214	241	263	285	307	329	351	373	395	417	439	461	483	505	527	549	571	593
1.2	150.6	182	213.4	244.8	276.2	302.6	329	355.4	381.8	408.2	434.6	461	487.4	513.8	540.2	566.6	593	619.4	645.8	672.2	698.6
1.8	203.4	248	292.6	337.2	381.8	421.4	461	500.6	540.2	579.8	619.4	659	698.6	738.2	777.8	817.4	857	896.6	936.2	975.8	1015.4
3	309	380	451	522	593	659	725	791	857	923	989	1055	1121	1187	1253	1319	1385	1451	1517	1583	1649

	AMPure XP provided in bulk reservoir																			
	Batch Size																			
Bead Ratio	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
0.6	2552.8	2566	2579.2	2592.4	2605.6	2618.8	2632	2645.2	2658.4	2671.6	2684.8	2698	2711.2	2724.4	2737.6	2750.8	2764	2777.2	2790.4	2803.6	2816.8
0.8	2570.4	2588	2605.6	2623.2	2640.8	2658.4	2676	2693.6	2711.2	2728.8	2746.4	2764	2781.6	2799.2	2816.8	2834.4	2852	2869.6	2887.2	2904.8	2922.4
1	2588	2610	2632	2654	2676	2698	2720	2742	2764	2786	2808	2830	2852	2874	2896	2918	2940	2962	2984	3006	3028
1.2	2605.6	2632	2658.4	2684.8	2711.2	2737.6	2764	2790.4	2816.8	2843.2	2869.6	2896	2922.4	2948.8	2975.2	3001.6	3028	3054.4	3080.8	3107.2	3133.6
1.8	2658.4	2698	2737.6	2777.2	2816.8	2856.4	2896	2935.6	2975.2	3014.8	3054.4	3094	3133.6	3173.2	3212.8	3252.4	3292	3331.6	3371.2	3410.8	3450.4
3	2764	2830	2896	2962	3028	3094	3160	3226	3292	3358	3424	3490	3556	3622	3688	3754	3820	3886	3952	4018	4084

Estimated Time of Completion

Samples	4	8	16	24
Reagent aliquot	00:09	00:13	00:14	00:15
Cleanup	00:34	00:34	00:50	01:05
Total ETC	00:43	00:47	01:04	01:20

Times (hours:minutes) calculated based on default settings of tube supplied beads, 3-minute bead binding time, and 2-minute elution and drying times using a 1.8X bead ratio and 40 µL elution volume.

Consumables

		Batch Size (samples)			
Consumable	Part number	4	8	16	24
RVs	C62705	3	3	3	3
Bulk Reservoirs	C62707	1	1	1	1
Lids	C62706	2	2	2	2
Millitips (boxes)	C59585	1	1	1	1
Microtips (boxes)	C62712	1	1	1	1
5.0 mL Sarstedt vial	60.611	0	0	0	0
2.0 mL Sarstedt vial	72.664	2	2	2	2
Seal plate	C70665	1	1	1	1

Consumables are assuming AMPure XP beads supplied in 2.0 mL Sarstedt vial.

Demonstration Data (App Template Version 1.0.1)

Experimental Design for Demonstration Run Conditions

Experiment	Sample number	Input	Bead Ratio	Bead supply labware	Instrument	Operator
1	4	239 ng 100bp ladder	.6X	2 mL Tube	A	A
2	24	440 ng 100bp ladder	3X	Bulk	A	A
3	19	480 ng 100bp ladder	1.8X	2 mL Tube	A	A
4	19	386 ng 100bp ladder	1.8X	2 mL Tube	B	B

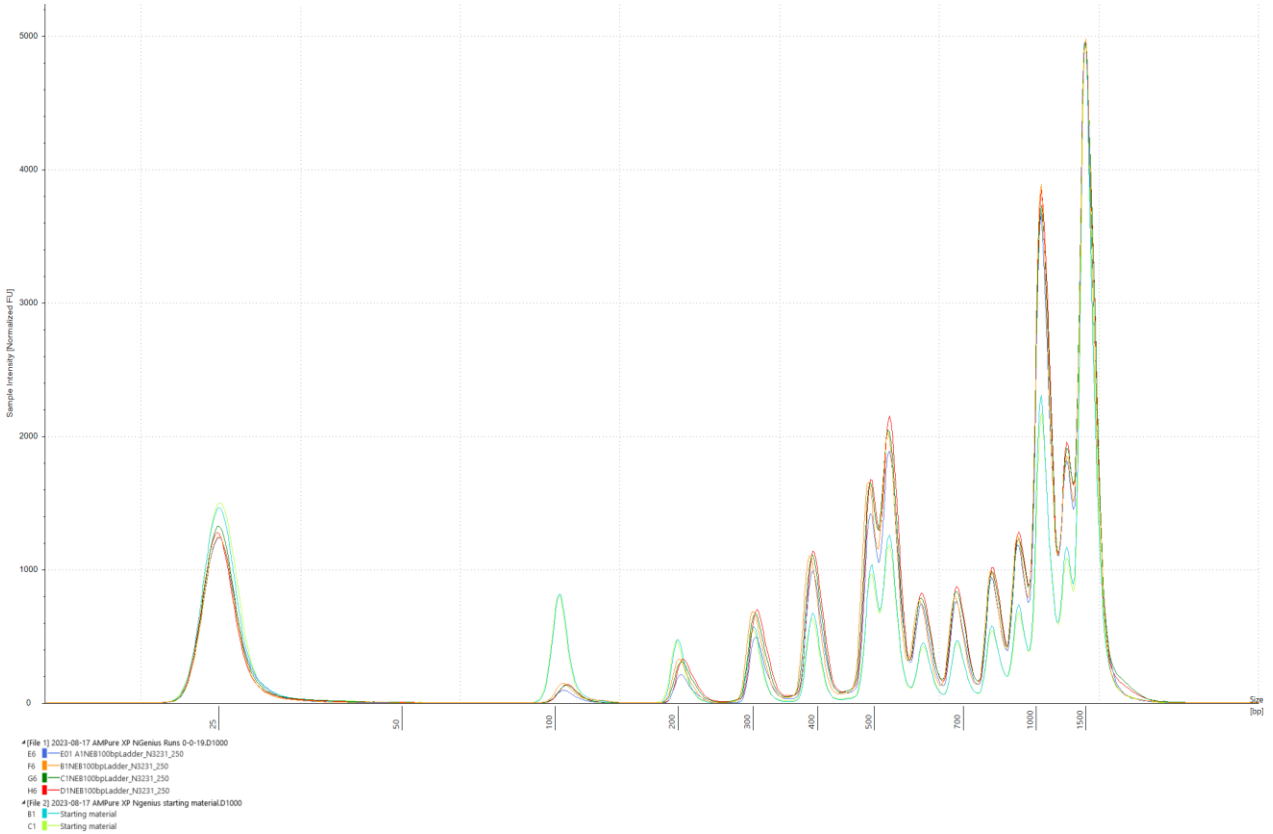
Bead Cleanup Pass Criteria:

- EXP2, EXP3, EXP4: Greater than 80% of input mass recovered as determined by D5000 ScreenTape
- EXP1: Greater than 60% recovery of peak-matched input at peaks 300-1000BP AND knockdown of 100 & 200 BP peaks to less than 3% of recovered material

AMPure XP Demonstration EXP01 - PASS

4 Samples, minimum bead ratio (0.6X), minimum elution volume (10 µL)

- >60% Recovery of input mass on matched peak-by-peak basis **for peaks 300-1000bp** on D1000 ScreenTape
- 100 and 200 BP peaks knocked down to less than 3% of recovered mass each peak



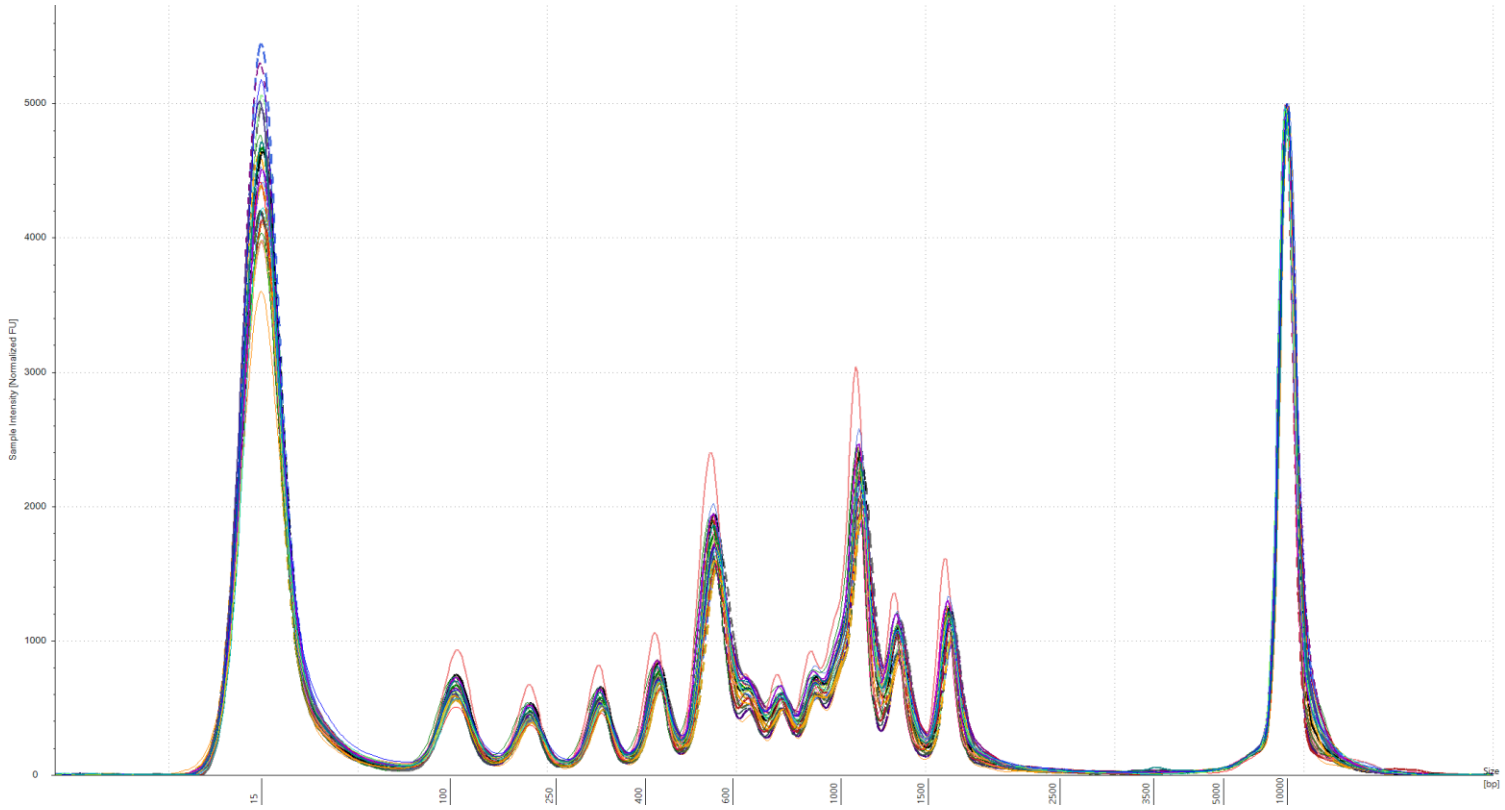
Nominal Size (bp)	Sample 1	Sample 2	Sample 3	Sample 4
100	N/A - Peak knocked down	N/A - Peak knocked down	N/A - Peak knocked down	N/A - Peak knocked down
200	N/A - Peak knocked down	N/A - Peak knocked down	N/A - Peak knocked down	N/A - Peak knocked down
300	Pass: 58.8%	Pass: 65.4%	Pass: 63.8%	Pass: 66.8%
400	Pass: 78.2%	Pass: 87.6%	Pass: 88.8%	Pass: 91.8%
500	Pass: 70%	Pass: 86.8%	Pass: 78.2%	Pass: 83.6%
530	Pass: 77.8%	Pass: 81.8%	Pass: 86.4%	Pass: 86.4%
600	Pass: 83.5%	Pass: 89.8%	Pass: 93.1%	Pass: 95.6%
700	Pass: 80.9%	Pass: 85.8%	Pass: 91.4%	Pass: 93.9%
800	Pass: 80.6%	Pass: 86.8%	Pass: 84%	Pass: 87.5%
900	Pass: 81.6%	Pass: 88.2%	Pass: 86.4%	Pass: 87%
1000	Pass: 80.9%	Pass: 86.6%	Pass: 82.9%	Pass: 85.9%

- ✓ 100 & 200bp peaks knocked down < 3%
- ✓ Some knockdown on 300bp peak; expected
- ✓ 400-1000bp peaks > 60% recovery

AMPure XP Demonstration EXP02 - PASS

24 Samples, maximum bead ratio (3X), maximum elution vol (50 µL)

- >80% Recovery of input mass as determined by **D5000** ScreenTape



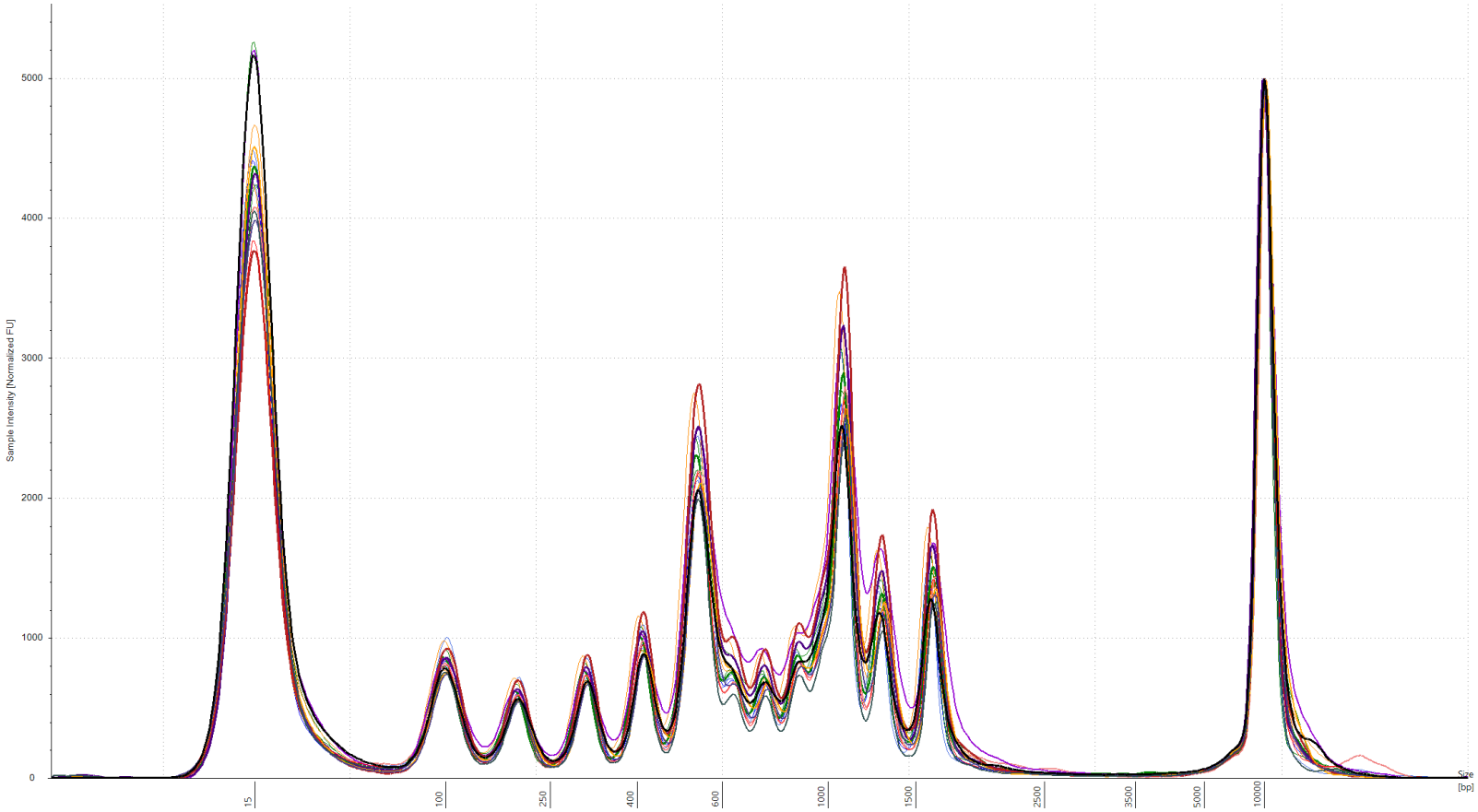
Sample Number	%Recovery
1	Pass - 110%
2	Pass - 115%
3	Pass - 111%
4	Pass - 115%
5	Pass - 116%
6	Pass - 124%
7	Pass - 128%
8	Pass - 113%
9	Pass - 116%
10	Pass - 99%
11	Pass - 96%
12	Pass - 115%
13	Pass - 104%
14	Pass - 124%
15	Pass - 116%
16	Pass - 120%
17	Pass - 101%
18	Pass - 100%
19	Pass - 97%
20	Pass - 100%
21	Pass - 111%
22	Pass - 97%
23	Pass - 92%
24	Pass - 118%
AVG	110%

NB: Yields > 100% are possible due to quantitative variance of D5000 tape

AMPure XP Demonstration EXP03 - PASS

19 Samples, IFU bead ratio (1.8X), IFU elution vol (40 µL)

➢>80% Recovery of input mass as determined by **D5000** ScreenTape



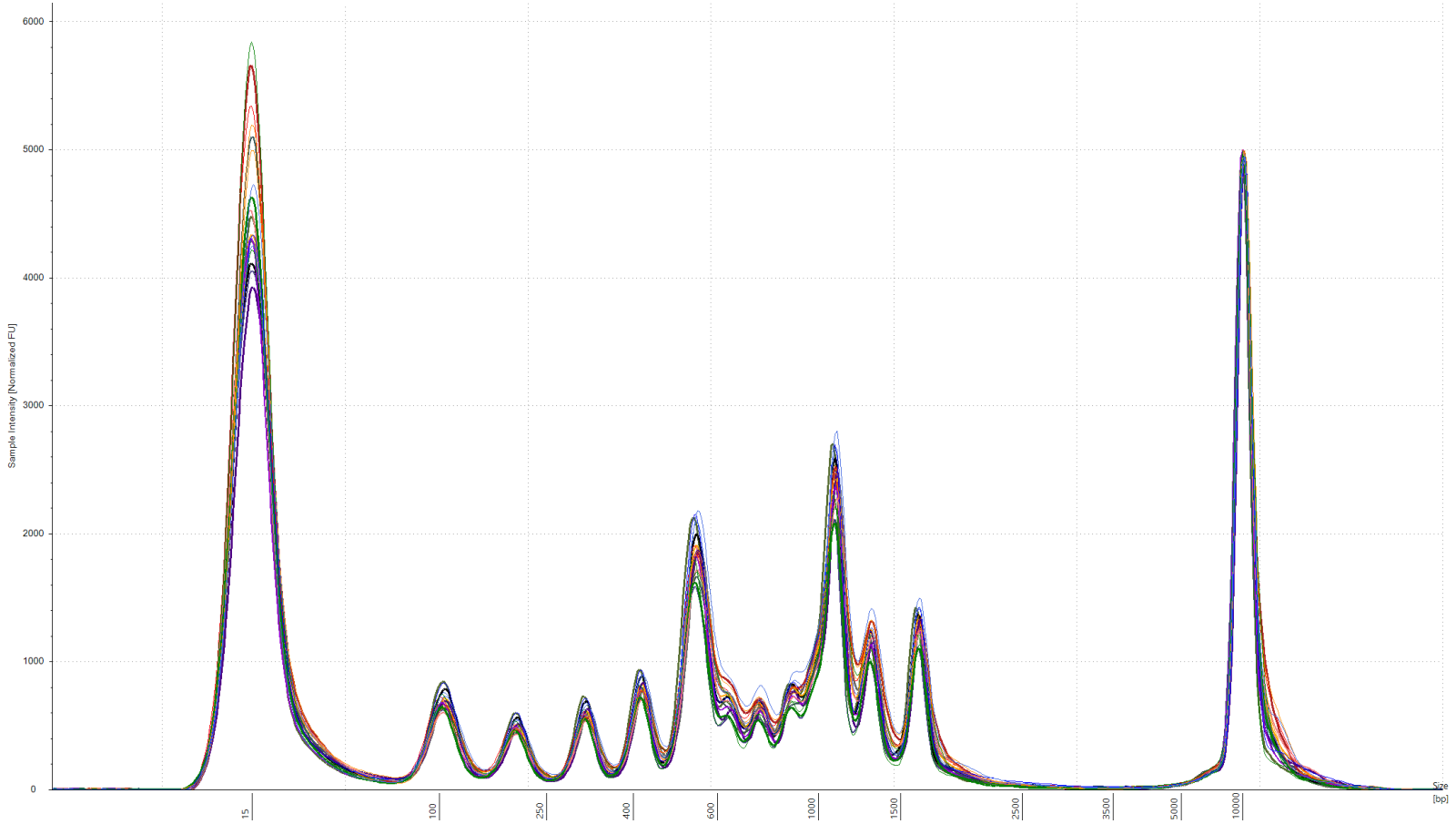
Sample Number	%Recovery
1	Pass - 128%
2	Pass - 110%
3	Pass - 108%
4	Pass - 107%
5	Pass - 107%
6	Pass - 103%
7	Pass - 100%
8	Pass - 123%
9	Pass - 129%
10	Pass - 94%
11	Pass - 85%
12	Pass - 94%
13	Pass - 96%
14	Pass - 86%
15	Pass - 108%
16	Pass - 102%
17	Pass - 103%
18	Pass - 98%
19	Pass - 107%
AVG	104%

NB: Yields > 100% are possible due to quantitative variance of D5000 tape

AMPure XP Demonstration EXP04 - PASS

19 Samples, IFU bead ratio (1.8X), IFU elution vol (40 µL) – Different operator, different instrument

➢>80% Recovery of input mass as determined by **D5000** ScreenTape



Sample Number	Conc (ng/uL)	%Recovery
1	12.3	Pass - 127%
2	12.5	Pass - 129%
3	12.4	Pass - 128%
4	10.3	Pass - 107%
5	11.6	Pass - 120%
6	9.48	Pass - 98%
7	9.97	Pass - 103%
8	10.9	Pass - 113%
9	10.7	Pass - 111%
10	10.1	Pass - 104%
11	10.8	Pass - 112%
12	12	Pass - 124%
13	11.4	Pass - 118%
14	11.3	Pass - 117%
15	9.58	Pass - 99%
16	10.4	Pass - 108%
17	11.2	Pass - 116%
18	10.9	Pass - 113%
19	11.9	Pass - 123%
AVG	11.0	114%

NB: Yields > 100% are possible due to quantitative variance of D5000 tape

Demonstration Summary

Demonstration Summary

- The AMPure XP App 1.0.1, written by Beckman Coulter Life Sciences, on the Biomek NGeniuS Next Generation Library Prep System cleans 20 µL of input libraries
- All conditions tested resulted in acceptable sample recovery suitable for NGS library cleanup
- Expected knockdown of small fragments was observed with reduced bead ratios

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