The Simple Math

Yield is a function of sample volume and recovery rate

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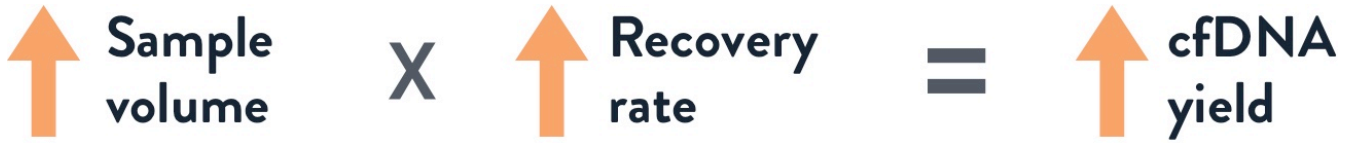
The formula for increased test sensitivity and result confidence



It’s simply biology and math…

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The Biology

- 1 mL of plasma = 8ng of cfDNA[1]
- 1 ng of cfDNA = 300 genomic equivalents[2]
- 83ng of cfDNA is required to achieve a highly confident result[3]
- 10 mutant copies are required to ensure reliable test results[4]

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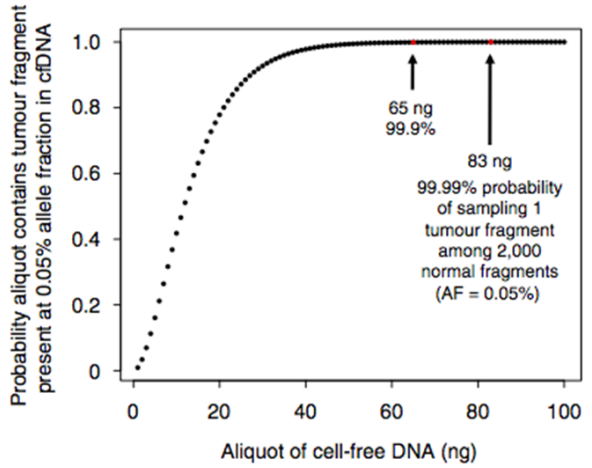
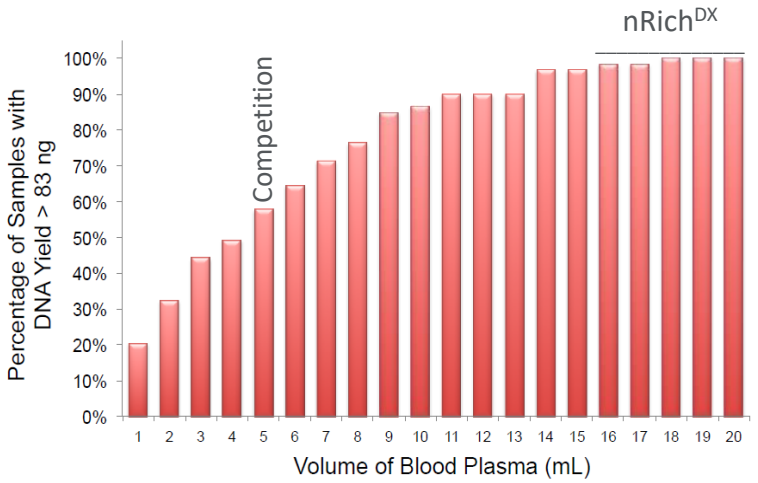


Probability of capturing tumor derived sequence as a function of amount of total ng of cfDNA at 0.05% prevalence of mutant.



[1] Valpione et al. (2018) "Plasma total cell-free DNA (cfDNA) is a surrogate biomarker for tumour burden and a prognostic biomarker for survival in metastatic melanoma patients." Eur J Cancer 88: 1–9

[2] Medina-Diaz et al. (2016) "Clinical evaluation of streck cell-free DNA blood collection tubes for liquid profiling in oncology." [abstract]. In: Proceedings of the 107th Annual Meeting of the American Association for Cancer Research; 2016 Apr 16-20; New Orleans, LA. Philadelphia (PA): AACR; Cancer Res 2016;76(14 Suppl):Abstract nr 3145.

[3] Nature Communications Volume 8, Article number: 15086 (2017)

[4] Genomic Precission, June 15, 2017 "How many target copies are present in your plasma DNA Sample?

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The Math: Sample volume matters more than recovery rate

10 mutant copies ÷ **0.03%** = **33,333**

95% reliable detection threshold for NGS | Mutant allele fraction for earliest detection of recurrence | total copies needed after sample prep

33,333 ÷ **300 copies/ng** = **>111 ng**

total copies needed after sample prep | total copies needed after sample prep | amount of cfDNA needed after sample prep

$$\frac{\text{>111 ng}}{\text{8 ng/mL} \times \text{80\%}} = \text{17-18 mL}$$

amount of cfDNA needed after sample prep

8 ng/mL: concentration of cfDNA in plasma

80% nRich DX: recovery rate

17-18 mL: blood plasma needed at 80% recovery rate

50% recovery rate requires 28 mL of plasma

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Biology + Math = nRichDX Revolution Solution

Typical nRich^{DX} yields

Sample Vol.	Total cfDNA	cfDNA Yield (ng) at Specified Recovery Rate					
(ml)	(ng)	50%	60%	70%	80%	90%	100%
1	8	4	5	6	6	7	8
2	16	8	10	11	13	14	16
3	24	12	14	17	19	22	24
4	32	16	19	22	26	29	32
5	40	20	24	28	32	36	40
6	48	24	29	34	38	43	48
7	56	28	34	39	45	50	56
8	64	32	38	45	51	58	64
9	72	36	43	50	58	65	72
10	80	40	48	56	64	72	80
11	88	44	53	62	70	79	88
12	96	48	58	67	77	86	96
13	104	52	62	73	83	94	104
14	112	56	67	78	90	101	112
15	120	60	72	84	96	108	120
16	128	64	77	90	102	115	128
17	136	68	82	95	109	122	136
18	144	72	86	101	115	130	144
19	152	76	91	106	122	137	152
20	160	80	96	112	128	144	160

Green = >95% Confident Result

	Total cfDNA (ng)	Copies of cfDNA	ctDNA by Mutant Allele Frequency							
			1%	0.20%	0.10%	0.050%	0.040%	0.030%	0.020%	0.010%
Competitor A	8	2,400	24	5	2	1	1	1	0	0
Competitor B	16	4,800	48	10	5	2	2	1	1	0
	24	7,200	72	14	7	4	3	2	1	1
nRich^{DX}	32	9,600	96	19	10	5	4	3	2	1
	40	12,000	120	24	12	6	5	4	2	1
	48	14,400	144	29	14	7	6	4	3	1
	56	16,800	168	34	17	8	7	5	3	2
	64	19,200	192	38	19	10	8	6	4	2
	72	21,600	216	43	22	11	9	6	4	2
	80	24,000	240	48	24	12	10	7	5	2
	88	26,400	264	53	26	13	11	8	5	3
	96	28,800	288	58	29	14	12	9	6	3
	104	31,200	312	62	31	16	12	9	6	3
	112	33,600	336	67	34	17	13	10	7	3
	120	36,000	360	72	36	18	14	11	7	4
	128	38,400	384	77	38	19	15	12	8	4
	136	40,800	408	82	41	20	16	12	8	4
	144	43,200	432	86	43	22	17	13	9	4
	152	45,600	456	91	46	23	18	14	9	5
	160	48,000	480	96	48	24	19	14	10	5

- Standard NGS - optimal cost, normal reimbursement
- Stretch NGS - high cost, normal reimbursement
- Borderline results - possibly no reimbursement
- No result

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Revolution System cfDNA extraction is highly efficient

Sample ID	Rxn Vol. [mL]	sDNA SPIKE (NG)	ng/rxn	ng/mL	Average (ng/mL)	Positive - Negative (ng/mL)	P-N / extraction
SS-190905-1	5	200	237.0	47.40	48 ng/mL	35	175
SS-190905-2			227.5	45.50			
SS-190905-3			252.5	50.50			
SS-190905-4			242.0	48.40			
SS-190905-5		0	65.5	13.10	13 ng/mL		
SS-190905-6			68.5	13.70			
SS-190905-7			63.0	12.60			
SS-190905-8			60.5	12.10			
				Efficiency		**88%**	**88%**

After accounting for the 35 ng/mL background (measured in samples 5-8), the Revolution System extracted 175 ng/reaction…an 88% recovery.

Source: Customer Evaluation Study

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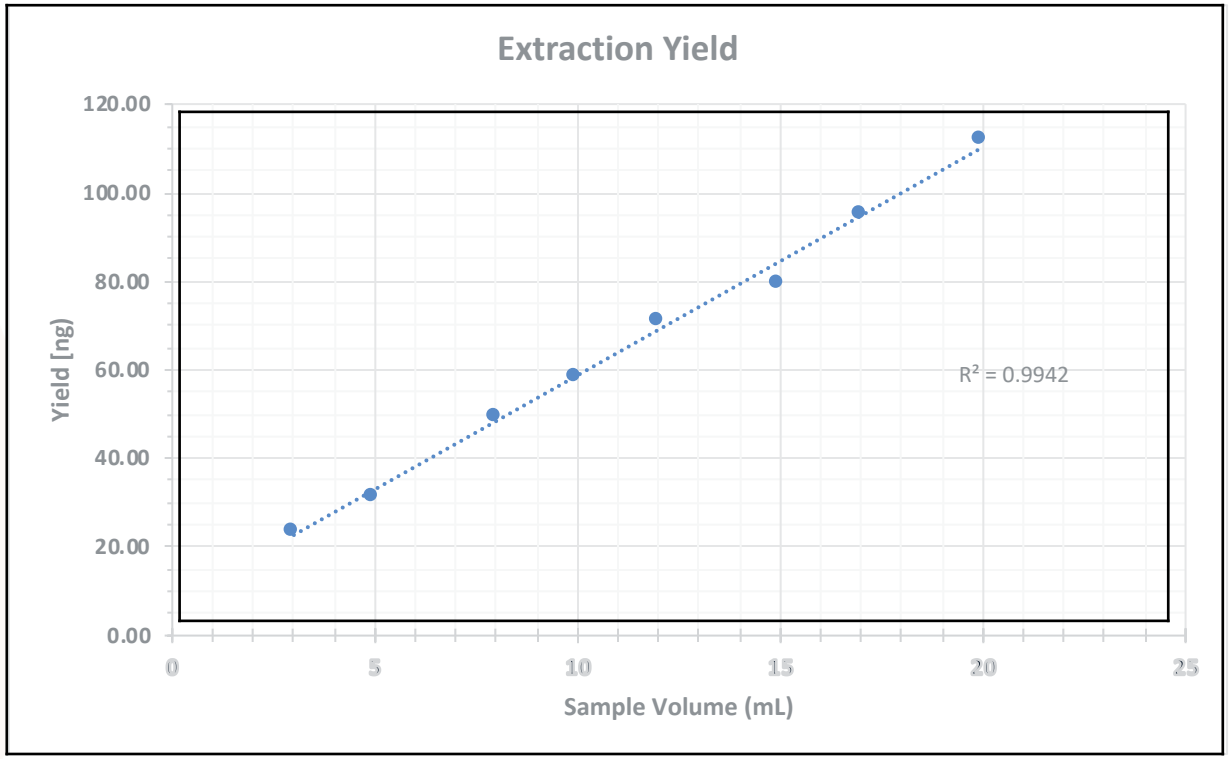
Revolution System cfDNA yield is proportionate across all sample volumes



All samples demonstrate **linear recovery rates** across the 3 mL-20 mL volume range

Source: Internal nRichDX Study

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Revolution System provides high cfDNA extraction efficiency for optimal mutation recovery

	BRAF MUTANT			
Sample ID	**SPIKE**	**Ct**	**Recovered**	**Efficiency**
IL-190819-1	1000	30.18	826	83%
IL-190819-2	1000	30.13	850	85%
IL-190819-3	1000	29.97	935	94%
IL-190819-4	1000	30.37	741	74%
IL-190819-5	1000	29.98	930	93%
				86%

- Spiked 1,000 copies of BRAF Mutant (BRAF_213_COSM475_Mutant) into 15 mL of Normal Human Plasma
- Extracted with nRichDX Revolution cfDNA Max 20 Kit
- Measured recovery with qPCR assay for BRAF Mutant
- Recorded Ct and calculated dose on BRAF Mutant standard curve

Efficiency of nRichDX Revolution cfDNA Max 20 Kit

Source: Internal nRichDX Study

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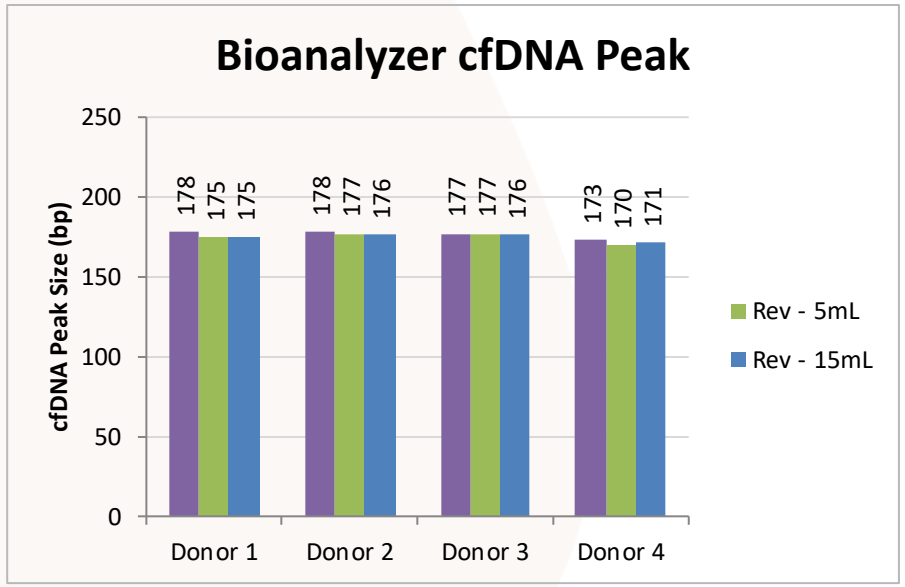
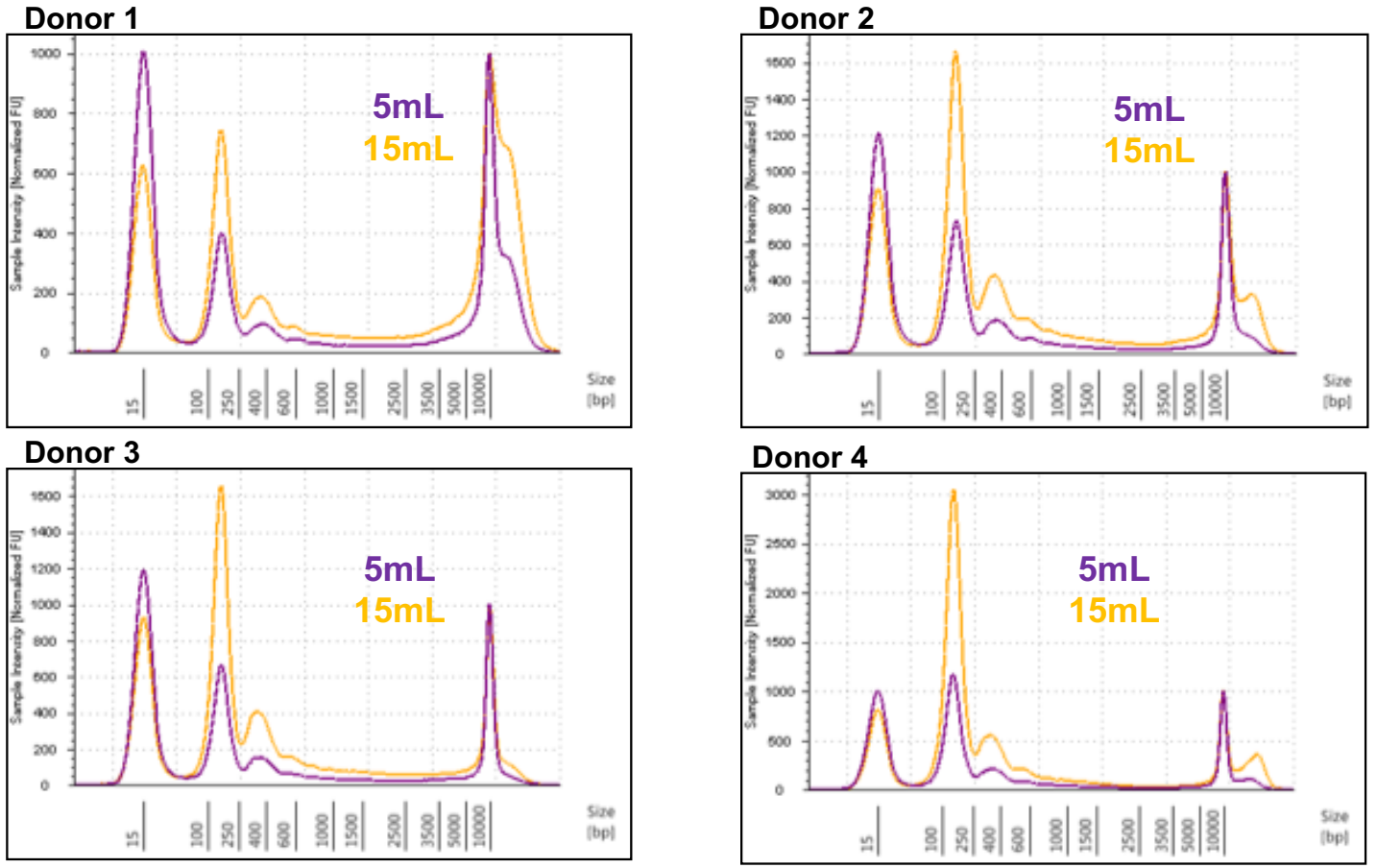
Revolution System proportionately extracts the actionable cfDNA across all sample volumes





Revolution System Comparative Recovery at 5 mL and 15 ml

Source: Customer Evaluation Study

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