The Simple Math Sample prep yield is a function of input sample volume and recovery rate



© nRichDX

Biology and Math

The formula for increased test sensitivity and results confidence



The **Biology**

Successful analysis of rare alleles require better sample prep

- 1 mL of plasma = $8ng of cfDNA^1$
- 1 ng of cfDNA = 300 genomic equivalents²
- 83ng of cfDNA is required to achieve a highly confident result³
- 10 mutant copies are required to ensure reliable test results⁴



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 Diagnostic Precision [SeraCare blog], June 15, 2017 "How many target copies are present in your plasma DNA Sample? https://blog.seracare.com/ngs/how-many-target-copies-are-present-in-your-plasma-dna-sample

The Math

Increased recovery rate and sample volume required for rarest alleles



The nRichDX Revolution Solution Biology + math = sample prep for liquid biopsy

Sample Vol.	Total cfDNA	cfD	NA Yield	(ng) at S	pecified R	ecovery F	Rate		Total	Copies		ctDI	NA by I	Mutant	Allele	Freque	ency	
(ml)	(ng)	50%	60%	70%	80%	90%	100%		cfDNA (ng)	of cfDNA	1%	0.20%	0.10%	0.050%	0.040%	0.030%	0.020%	0.0
1	8	4	5	6	6	7	8	Competitor A	8	2,400	24	5	2	1	1	1	0	
2	16	8	10	11	13	14	16	Competitor B	16	4,800	48	10	5	2	2	1	1	
3	24	12	14	17	19	22	24		24	7,200	72	14	7	4	3	2	1	
4	32	16	19	22	26	29	32		32	9,600	96	19	10	5	4	3	2	
5	40	20	24	28	32	36	40		40	12,000	120	24	12	6	5	4	2	
6	48	24	29	34	38	43	48		48	14,400	144	29	14	7	6	4	3	
7	56	28	34	39	45	50	56		56	16,800	168	34	17	8	7	5	3	
8	64	32	38	45	51	58	64		64	19,200	192	38	19	10	8	6	4	
9	72	36	43	50	5.8	65	72		72	21,600	216	43	22	11	9	6	4	
10	80	40	48	56	64	72	80	ă	80	24,000	240	48	24	12	10	7	5	
11	29	44	53	62	70	70	88	L S	88	26,400	264	53	26	13	11	8	5	
12	06	49	55	67	77	96	06	<u> </u>	96	28,800	288	58	29	14	12	9	6	
12	90	40	50	70	11	00	90	<u> </u>	104	31,200	312	62	31	16	12	9	6	
13	104	52	62	13	83	94	104		112	33,600	336	67	34	17	13	10	D 7	
14	112	56	67	78	90	101	112		120	36,000	360	72	36	18	14	11	7	
15	120	60	72	84	96	108	120		128	38,400	384	77	38	19	15	12	8	
16	128	64	77	90	102	115	128		136	40,800	408	82	41	20	16	12	8	
17	136	68	82	95	109	122	136		144	43,200	432	86	43	22	17	13	9	
18	144	72	86	101	115	130	144		152	45,600	456	91	46	23	18	14	9	
19	152	76	91	106	122	137	152		160	48.000	480	96	48	24	19	14	10	
20	160	80	96	112	128	144	160			,500								

Green = >95% Confident Result

Standard NGS - optimal cost, normal reimbursement Borderline results - possibly no reimbursement

Stretch NGS - high cost, normal reimbursement

No result

Revolution cf/ctDNA Extraction is Highly Efficient Spike and recovery Assay: KRAS G12V PCR

Sample Description	KRAS Expected Copies	KRAS Copies Recovered	Standard Deviation	EFFICIENCY (%)
Sample 1 (5 mL)	111	105	6	95 %
Sample 2 (15 mL)	1224	1010	77	83%

Revolution cf/ctDNA Extraction is Highly Pure Low gDNA carryover



Revolution Yield is Consistent by Sample Volume Consistent cfDNA yield from 1mL - 20mL sample volume



Revolution Yield is Consistent by Sample Volume Consistent cfDNA yield from 1mL - 20mL sample volume - gel image



Revolution Yield is Consistent by Elution Volume





	Elution Volume (μL)	Total Yield (ng)	
	25	5.3	
5 mL	50	4.7	
	100	4.6	
	25	4.8	
15 mL	50	5.1	
	100	4.5	
Mean of a	4.8		

 Consistent cfDNA yield at 25ul, 50ul, and 100 ul elution volumes for 5 mL and 15 mL input sample volumes

How is Revolution Sample Prep Better?

The nRicher Cartridge - engineered for liquid biopsy



Don't Settle for Lower Yield and Sensitivity

- There's better sample prep for liquid biopsy applications
- See what higher yield and purity mean for your assay's sensitivity
- Email nRichDX at info@nrichdx.com

