

Supplementary Materials:

Table S2. FFPE Sample Details.

Sample ID	Oncomine Result	Tissue sampling method	Nucleated Cells per slide	Tissue Surface Area (mm2)	Tumor percent	Age	Gender
C101101	ALK Fusion	Lung	35714	75	70	71	M
C101114	NTRK Fusion	Lung	83333	150	90	66	M
C408258	EGFR exon 19 Deletion	Lung	13333	55	90	59	M
C408259	EGFR exon 19 Deletion	Lung	30000	150	100	66	M
C702031	EGFR T790M	Lung	25000	105	100	73	F
C702035	EGFR L858R	Lung	26316	110	95	65	M
C702040	KRAS G12C	Lung	10000	54	100	54	M
XX407334	EGFR exon 19 Deletion, KRAS G12C	Lung	1000	70	35	72	M
XX411434	KRAS G12C	Lung	>5000	143	95	68	M
XX532429	MET exon14 skipping	Lung	1000	36	75	72	F
XX662727	EGFR exon 19 Deletion, EGFR T790M	Lung	>5000	77	70	64	F
XX662730	ERBB2 exon 20 Insertion	Lung	3000	60	50	60	F
XX662731	ERBB2 exon 20 Insertion	Lung	1000	40	60	55	M
XX662733	EGFR L858R, MET exon14 skipping	Lung	2500	48	50	68	F
XX662757	EGFR T790M, EGFR L858R	Lung	3000	80	80	61	M
XX662765	ERBB2 exon 20 Insertion	Lung	1500	77	30	66	M
XX666465	EGFR G719X	Lung	UNKNOWN	UNKNOWN		71	M
XX668154	BRAF V600E	Lung	>7500	63	95	75	F
XX702673	ERBB2 exon 20 Insertion	Lung	3000	84	60	64	M
XX702713	ERBB2 exon 20 Insertion	Lung	UNKNOWN	UNKNOWN	30	69	F
XX704234	BRAF V600E	Lung	1500	100	70	67	M
XX706083	EGFR S768I, EGFR L858R	Lung	>5000	220	80	41	F
XX739460	EGFR exon 20 Insertion	Lung	UNKNOWN	UNKNOWN		35	M
XX739464	ROS Fusion	Lung	>5000	72	80	69	M
XX739467	EGFR exon 20 Insertion	Lung	UNKNOWN	UNKNOWN		35	M
XX739485	ROS Fusion	Lung	>5000	48	80	54	F
XX739596	RET Fusion	Lung	>5000	30	95	UNKNOWN	M

XX739627	EGFR exon 20 Insertion	Lung	UNKNOWN	UNKNOWN		44	F
XX739629	ERBB2 exon 20 Insertion	Lung	>5000	78	85	62	F
XX740118	EGFR exon 20 Insertion, RET Fusion	Lung	UNKNOWN	UNKNOWN		60	F
XX885918	RET Fusion	Lung	2000	81	45	73	M
XX927071	ALK Fusion, RET Fusion	Lung	2500	117	35	65	M
XY123837	EGFR exon 20 Insertion	Lung	2500	72	80	66	M
XY123841	ROS Fusion	Lung	2500	98	50	63	M
XY124711	ALK Fusion	Lung	>5000	162	60	58	M
XY124734	ALK Fusion	Lung	3500	160	50	65	M
XY124735	ROS Fusion	Lung	2000	75	50	58	M
XY125737	EGFR L858R	Lung	UNKNOWN	UNKNOWN		51	F
XY125739	ALK Fusion	Lung	3500	120	80	65	M
XY125745	EGFR L858R	Lung	2500	45	80	74	M
XY125888	MET exon14 skipping	Lung	UNKNOWN	UNKNOWN		47	M
XY125894	EGFR T790M, EGFR L858R	Lung	2500	72	50	69	M
XY125903	ERBB2 Insertion	Lung	2500	119	65	62	M
XY166082	ERBB2 Insertion	Lung	2500	72	70	68	F
XY204535	RET Fusion	Lung	2500	78	50	70	F
XY214570	NTRK Fusion	Lung	1500	114	40	58	M
XY214595	NTRK Fusion	Lung	>5000	45	90	77	F
XY214643	EGFR S768I	Lung	>5000	85	85	67	F
XY214643	EGFR G719X	Lung	>5000	85	85	67	F
XY215016	KRAS G12C, NTRK Fusion	Lung	3500	55	75	68	M
XY215386	BRAF V600E	Lung	>5000	110	85	77	M
XY215673	EGFR exon 19 Deletion	Lung	>5000	104	75	82	F
XY215683	RET Fusion	Lung	3500	99	85	87	M
XY243852	EGFR L861Q	Lung	UNKNOWN	UNKNOWN		83	F
XY244180	EGFR exon 19 Deletion	Lung	2500	42	55	67	M
XY244238	ERBB2 Insertion	Lung	2500	30	50	67	F
XY244272	MET exon14 skipping	Lung	2500	91	65	66	M
XY244535	BRAF V600E	Lung	2500	160	50	33	F
XY244549	BRAF V600E	Lung	>5000	99	80	47	M
XY244553	BRAF V600E	Lung	>3000	120	90	49	M
XY244557	BRAF V600E	Lung	>7500	88	95	52	F
XY244565	BRAF V600E	Lung	>10000	112	100	53	M
XY244569	BRAF V600E	Lung	>5000	108	90	54	M
XY244572	ALK Fusion	Lung	UNKNOWN	UNKNOWN		55	M
XY244580	ALK Fusion	Lung	UNKNOWN	UNKNOWN		56	M
XY244590	EGFR exon 20 Insertion	Lung	>5001	72	85	58	M

XY244636	<i>BRAF</i> V600E	Lung	>5000	77	90	66	M
XY245166	<i>ALK</i> Fusion	Lung	UNKNOWN	UNKNOWN		61	M
XY245180	<i>EGFR</i> T790M, <i>EGFR</i> L858R	Lung	3500	24	50	66	M
XY245225	<i>ALK</i> Fusion	Lung	3500	66	65	67	F
XY245233	<i>ERBB2</i> Insertion	Lung	>5001	89	80	69	F
XY245263	<i>EGFR</i> exon 20 Insertion	Lung	3500	63	85	58	M
XY245278	<i>MET</i> exon14 skipping	Lung	3500	91	65	59	F
XY365121	<i>EGFR</i> S768I, <i>EGFR</i> G719X	Lung	3000	150	80	56	M
XY365134	<i>EGFR</i> exon 20 Insertion	Lung	UNKNOWN	UNKNOWN		62	M
XY365143	<i>EGFR</i> exon 20 Insertion	Lung	3000	100	60	59	M
XX662720	<i>EGFR</i> T790M	Lung	UNKNOWN	UNKNOWN		63	M

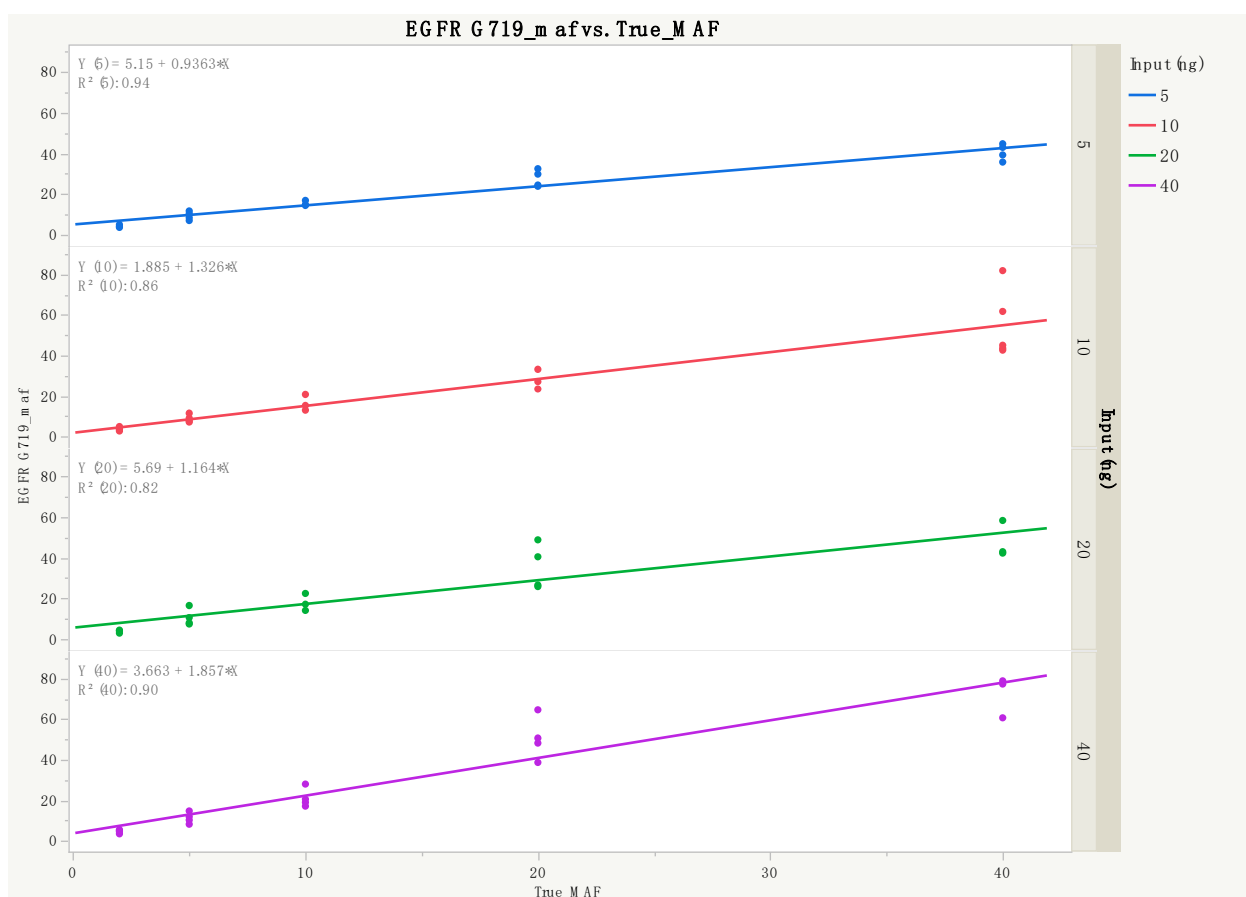


Figure S1: Known MAF vs estimated MAF at varying input. *EGFR* G719S was spiked in at 2, 5, 10, 20, and 40% known MAF (True_MAF) and plotted against the estimated MAF reported out by ChromaCode Cloud (*EGFR* G719_maf) at 5 ng, 10 ng, 20 ng, and 40 ng total DNA input. Input amount is defined in ng for each well as measured by Qubit. (n = 4 for all MAF/input combinations except 40% known MAF at 40 ng which n = 3)

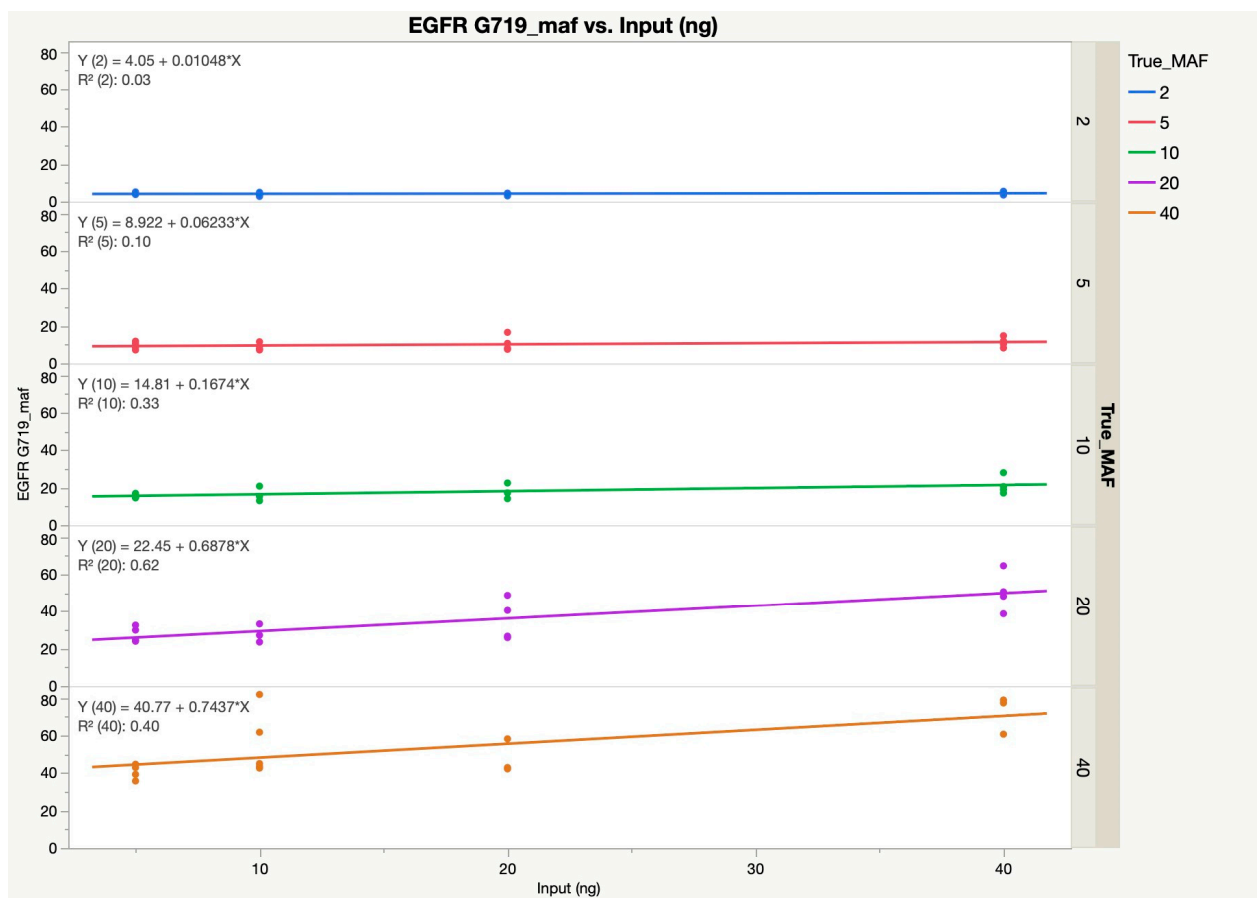


Figure S2: Estimated MAF vs input (ng) at varying known MAF. ChromaCode Cloud estimated MAF (EGFR G719_maf) was plotted against input (ng) for each known MAF. Input amount is defined in ng for each well as measured by Qubit. (n = 4 for all MAF/input combinations except 40% known MAF at 40 ng which n = 3)

Table S3: Parameters for In Silico Bioinformatic Analysis.

DNA Variants		
Variable	Input	
CosmicMutantExport.tsv.gz	Version	
v97	Download Date 08FEB2023	
Primary site	Lung	
	%in%c("adenocarcinoma",	
	"non_small_cell_carcinoma",	
	"squamous_cell_carcinoma",	
	"large_cell_carcinoma",	
Histology subtype	"large_cell_neuroendocrine_carcinoma",	
	"bronchioloalveolar_adenocarcinoma",	
	"acinar_adenocarcinoma",	
	"sarcomatoid_carcinoma")	
GRCh	38	
Mutation somatic status	%in%c("Reported in another cancer sample as somatic","Confirmed somatic variant")	
Filtering by Variant Type		
Variant Type	Filter Column	Input
	HGNC ID	3236 (EGFR)
	Mutation CDS	contains c.2185-c.2283
EGFR exon 19 deletion	Mutation Description	contains "Deletion", ignore.case=TRUE
	HGNC ID	3236 (EGFR)
	Mutation CDS	contains c.2284-c.2469
EGFR exon 20 insertion	Mutation Description	contains "Insertion", ignore.case=TRUE
	HGNC ID	3430 (ERBB2)
	Mutation CDS	contains c.2308-c.2493
	Mutation Description	contains "Insertion", ignore.case=TRUE
ERBB2 exon 20 insertion		
RNA Fusions		
Variable	Input	
CosmicFusionExport.tsv.gz	Version	
v97	Download Date 08FEB2023	
Primary site	Lung	

Histology subtype	%in%c("adenocarcinoma", "non_small_cell_carcinoma", "squamous_cell_carcinoma", "large_cell_carcinoma", "large_cell_neuroendocrine_carcinoma", "bronchioloalveolar_adenocarcinoma", "acinar_adenocarcinoma", "sarcomatoid_carcinoma")
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Filtering by Individual Fusion Type

Fusion Type	Filter	Input
ALK fusion subset of NSCLC fusion data	3'_GENE_NAME	ALK
RET fusion subset of NSCLC fusion data	3'_GENE_NAME	RET
ROS1 fusion subset of NSCLC fusion data	3'_GENE_NAME	ROS1
NTRK1 fusion subset of CosmicFusionExport*	3'_GENE_NAME	NTRK1
NTRK2 fusion subset of CosmicFusionExport*	3'_GENE_NAME	NTRK2
NTRK3 fusion subset of CosmicFusionExport*	3'_GENE_NAME	NTRK3

Table S4: Results of Empirical Analytical Inclusivity Testing by COSMIC ID for DNA and RNA targets.

	Target	Cosmic ID	Detected
1	ALK	COSF1051	2/2
2	ALK	COSF1056	2/2
3	ALK	COSF1058	2/2
4	ALK	COSF1060	2/2
5	ALK	COSF1064	2/2
6	ALK	COSF1127	2/2
7	ALK	COSF1257	2/2
8	ALK	COSF1264	2/2
9	ALK	COSF1276	2/2
10	ALK	COSF1296	2/2
11	ALK	COSF1300	2/2
12	ALK	COSF1381	2/2
13	ALK	COSF1430	2/2
14	ALK	COSF1461	2/2
15	ALK	COSF1541	2/2
16	ALK	COSF1544	2/2
17	ALK	COSF1612	2/2
18	ALK	COSF1614	2/2
19	ALK	COSF1616	2/2
20	ALK	COSF1620	2/2
21	ALK	COSF1625	2/2
22	ALK	COSF1712	2/2
23	ALK	COSF409	2/2
24	ALK	COSF413	0/2
25	ALK	COSF415	2/2
26	ALK	COSF421	2/2
27	ALK	COSF424	2/2
28	ALK	COSF426	2/2
29	ALK	COSF428	2/2
30	ALK	COSF434	2/2
31	ALK	COSF437	2/2
32	ALK	COSF439	2/2
33	ALK	COSF441	2/2
34	ALK	COSF444	2/2
35	ALK	COSF459	2/2
36	ALK	COSF460	2/2
37	ALK	COSF469	2/2
38	ALK	COSF477	2/2
39	ALK	COSF478	2/2
40	ALK	COSF487	2/2
41	ALK	COSF732	2/2

42	EGFR Del	COSM12370	2/2
43	EGFR Del	COSM12382	2/2
44	EGFR Del	COSM12383	2/2
45	EGFR Del	COSM12384	2/2
46	EGFR Del	COSM12422	2/2
47	EGFR Del	COSM12678	2/2
48	EGFR Del	COSM13556	2/2
49	EGFR Del	COSM24267	0/2
50	EGFR Del	COSM26038	2/2
51	EGFR Del	COSM26704	0/2
52	EGFR Del	COSM26718	2/2
53	EGFR Del	COSM28517	2/2
54	EGFR Del	COSM6218	2/2
55	EGFR Del	COSM6225	2/2
56	EGFR Del	COSM6255	2/2
57	EGFR Del	COSM6256	2/2
58	EGFR Ins	COSM12376	2/2
59	EGFR Ins	COSM12378	2/2
60	EGFR Ins	COSM12380	2/2
61	EGFR Ins	COSM12381	2/2
62	EGFR Ins	COSM12427	0/2
63	EGFR Ins	COSM13428	2/2
64	EGFR Ins	COSM18432	2/2
65	EGFR Ins	COSM26720	0/2
66	ERRB2	COSM12552	2/2
67	ERRB2	COSM12553	0/2
68	ERRB2	COSM12558	2/2
69	ERRB2	COSM26681	2/2
70	ERRB2	COSM6438151	0/2
71	G719X	COSM6252	2/2
72	G719X	COSM6253	2/2
73	NTRK	COSF1323	2/2
74	NTRK	COSF1324	2/2
75	NTRK	COSF1326	2/2
76	NTRK	COSF1329	2/2
77	NTRK	COSF1446	2/2
78	NTRK	COSF1448	1/2
79	NTRK	COSF1534	2/2
80	NTRK	COSF1536	2/2
81	NTRK	COSF1653	2/2
82	NTRK	COSF823	2/2
83	RET	COSF1230	2/2
84	RET	COSF1232	2/2
85	RET	COSF1234	2/2

86	RET	COSF1236	2/2
87	RET	COSF1253	2/2
88	RET	COSF1262	2/2
89	RET	COSF1340	2/2
90	RET	COSF1481	2/2
91	RET	COSF1491	2/2
92	RET	COSF1498	2/2
93	RET	COSF1503	2/2
94	RET	COSF1507	2/2
95	RET	COSF1509	2/2
96	RET	COSF1511	2/2
97	RET	COSF1513	2/2
98	RET	COSF1515	2/2
99	RET	COSF1519	2/2
100	RET	COSF1521	2/2
101	RET	COSF1525	2/2
102	RET	COSF1687	2/2
103	ROS1	COSF1139	2/2
104	ROS1	COSF1188	2/2
105	ROS1	COSF1196	2/2
106	ROS1	COSF1198	2/2
107	ROS1	COSF1198	2/2
108	ROS1	COSF1202	2/2
109	ROS1	COSF1259	2/2
110	ROS1	COSF1261	2/2
111	ROS1	COSF1265	2/2
112	ROS1	COSF1267	2/2
113	ROS1	COSF1269	2/2
114	ROS1	COSF1273	2/2
115	ROS1	COSF1278	2/2
116	ROS1	COSF1280	2/2
117	ROS1	COSF1478	2/2
118	ROS1	COSF1627	2/2
119	ROS1	COSF1631	2/2
120	ROS1	COSF1637	2/2
121	ROS1	COSF1639	2/2
122	ROS1	COSF1641	2/2
123	ROS1	COSF1643	2/2
124	ROS1	COSF1645	2/2
125	ROS1	COSF1647	2/2
126	ROS1	COSF1663	2/2
127	ROS1	COSF1671	2/2
128	ROS1	COSF1673	2/2

Table S5: Internal Control performance in clinical samples by sample source vendor.

Vendor	All Wells Pass	1+ Well IC Failure	% with 1 Failure
Vendor A	3	5	63%
Vendor B	29	0	0%
Vendor C	53	16	23%

Table S6: Fusion partners for discordant resolution.

ID	Fusion Coordinates
1101	chr2:299621113, chr2:29446553 chr2:29498458, chr2:29498378
1114	chr18:45085964, chr1:156851219 chr17:9138266, chr9:156851219 chr17:9138266, chr9:87475930
3837	chr6:117659858, chr6:117658503
5739	chr1:156849914, chr1:156851249
4595	chr9:87367700, chr9:87356807 chr9:87367000: chr9:87317074 chr9:87285856: chr9:87317138
7566	chr6:117723136, chr6:117718279

S3. 2-D plots of representative dPCR data generated during the Analytical Sensitivity study.

Figure S3A. T790M positive partitions (green), Internal Control (hgDNA) positive partitions (blue), along with calibrator and negative partitions.

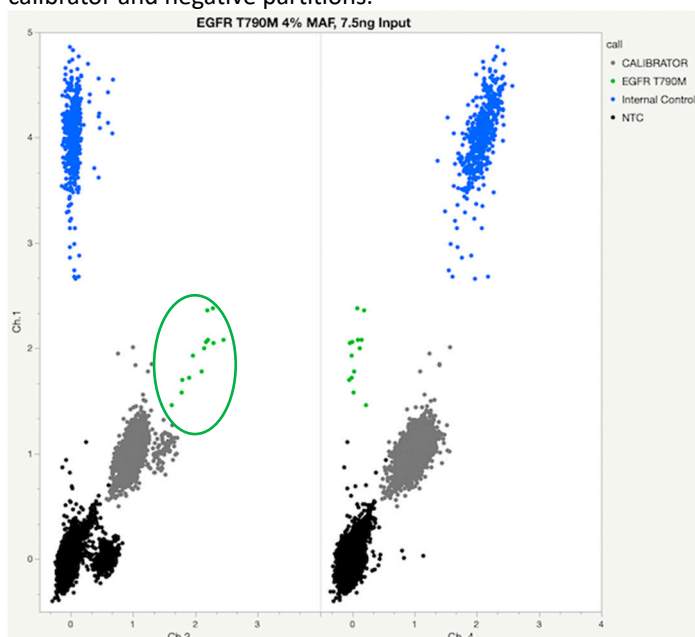


Figure S3B. ROS1 positive partitions (green), Internal Control (hgRNA) positive partitions (blue), along with calibrator and negative partitions.

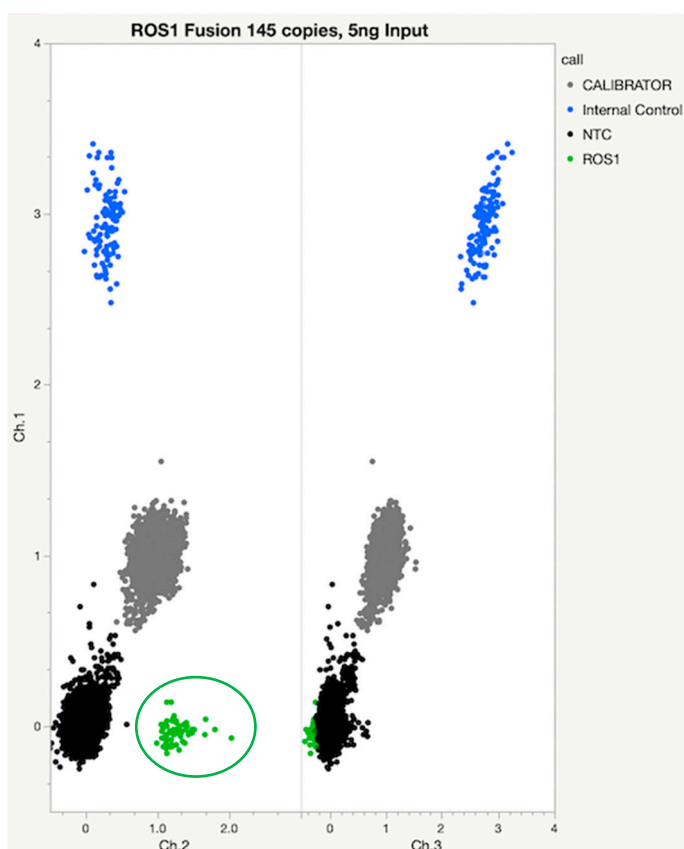


Table S7. Institutional Review Board Statement.

IRB/Ethic Committee Name	Protocol/IRB #	Approval Date
BioChain Institute Inc. IRB #1 - Biomedical	IRB00008283	6/9/21
Vanderbilt University IRB	IRB 010294	10/15/22
Advarra IRB	CR00425931	3/6/23
	Pro00051469	3/6/23