

Urinary cell-free DNA as a noninvasive liquid biopsy for hepatocellular carcinoma

Amy K. Kim¹, Selena Y. Lin², Hsin-Ni Liu³, Surbhi Jain², Terence P. Gade⁴, Fwu-Shan Shieh², James Hamilton¹, Hie-Won Hann⁵, Ting-Tsung Chang⁶, Dmitry Goryunov², Zhili Wang², Ying-Hsiu Su^{3,*}

¹Division of Gastroenterology and Hepatology, Johns Hopkins School of Medicine. Baltimore, MD 21205, USA

²JBS Science Inc., Doylestown, PA 18902, USA

³Baruch S. Blumberg Institute, Doylestown, PA 18902, USA

⁴Department of Radiology, University of Pennsylvania Hospital, Philadelphia, PA 19104, USA

⁵Division of Gastroenterology and Hepatology, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA

⁶Department of Internal Medicine, National Cheng Kung University Medical College, Center of infectious Disease and Signaling Research, National Cheng Kung University, Tainan 701, Taiwan

Corresponding Author: Ying-Hsiu Su, Baruch S. Blumberg Research Institute, 3805 Old Easton Rd, Doylestown, PA 18902; Tel: 215-489-4949; Fax: 215-489-4920; E-mail: Ying-hsiu.su@bblumberg.org; ORCID: <https://orcid.org/0000-0003-0969-0215>.

Table S1. Clinical information of HBV patients for comparisons of HBV DNA fragment sizes in urine

	Hepatitis (n=14)	Cirrhosis (n=13)	HCC (n=17)
Age (years)	53.2 (35-70)	58.5 (46-72)	65.1 (42-82)
Gender (M:F)	11:3	10:3	12:5
Serum HBV DNA (log IU/ml)			
<1.0 or UD	14	11	4
1-10	0	0	0
>10-10 ²	0	1	2
>10 ² -10 ³	0	0	3
>10 ³	0	0	5
NA	0	1	3

UD, undetectable; NA, not available

Table S2. Clinical information of 15 HCC patients for HCC-targeted NGS study.

Patient ID	Age	Gender (M/F)	Serum AFP (ng/ml)	HBV (+/-)	HCV (+/-)	Cirrhosis (Yes/No)	Alcoholic (Yes/No)	Primary or recurrent HCC	Stage of tumor (BCLC)	Tumor size (cm)
HCC-008	64	M	4.5	-	+	No	No	Recurrent	B	2.6
HCC-030	57	M	n/a	-	+	Yes	No	Primary	B	2.3
HCC-054	68	F	5	-	-	Yes	No	Primary	C	0.9
HCC-057	62	M	n/a	-	+	Yes	No	Primary	B	2
HCC-058	71	M	n/a	-	+	Yes	Yes	Primary	B	2
HCC-060	70	F	5	+	-	Yes	No	Recurrent	B	2.9
HCC-063	69	M	2.8	-	-	Yes	No	Recurrent	B	2.3
HCC-068	68	M	5.6	-	-	Yes	Yes	Primary	B	2.2
HCC-069	65	F	5	-	+	Yes	No	Recurrent	A	2
HCC-079	80	M	8.3	-	-	No	No	Primary	A	2.5
HCC-277	56	M	6	-	-	Yes	No	Primary	A	1.8
HCC-297	63	M	5.3	-	-	Yes	No	Primary	B	4.4
HCC-326	55	M	9.5	-	-	Yes	Yes	Primary	A	2.8
HCC-335	69	M	7.5	-	-	Yes	No	Primary	B	2.6
HCC-724	70	M	3.4	-	n/a	n/a	n/a	Recurrent	n/a	3.6

n/a, not available

Table S3. The 24 target genes of JBS HCC panel.

Gene name
<i>ARID1A</i>
<i>APOB</i>
<i>LRP1B</i>
<i>NFE2L2</i>
<i>CTNNB1</i>
<i>BAP1</i>
<i>PIK3CA</i>
<i>ALB</i>
<i>TERT</i> promoter
<i>IL6ST</i>
<i>APC</i>
<i>ARID1B</i>
<i>PCLO</i>
<i>CDKN2A</i>
<i>ATM</i>
<i>ARID2</i>
<i>KMT2D</i>
<i>HNF1A</i>
<i>RB1</i>
<i>AXIN1</i>
<i>TSC2</i>
<i>TP53</i>
<i>KEAP1</i>
<i>RPS6KA3</i>

Table S4. Mutation detection by qPCR in matched urine and plasma of HCC patients (n=101).

Pt ID	Age	Gender	AFP ng/ml	TP53 mutation		CTNNB1 mutation		TERT mutation		Viral etiology (HBV/HCV)
				Plasma	Urine	Plasma	Urine	Plasma	Urine	
1	66	F	48,326	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	-
2	55	M	3	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	-
3	55	F	6,420	BLOD	BLOD	BLOD	BLOD	+	BLOD	HCV
4	30	M	1,640	BLOD	BLOD	BLOD	BLOD	+	BLOD	-
5	65	M	140	BLOD	BLOD	BLOD	BLOD	+	BLOD	HCV
6	59	M	901	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
7	54	M	72	BLOD	BLOD	BLOD	BLOD	+	BLOD	HBV
8	70	M	4	BLOD	BLOD	BLOD	BLOD	+	BLOD	-
9	70	M	13,522	BLOD	BLOD	BLOD	BLOD	+	BLOD	HCV
10	61	M	13	BLOD	BLOD	BLOD	BLOD	+	BLOD	-
11	53	M	38	BLOD	BLOD	+	+	+	+	HCV
12	53	M	58	BLOD	BLOD	BLOD	BLOD	+	+	HBV, HCV
13	70	M	35	BLOD	BLOD	BLOD	BLOD	+	BLOD	HBV, HCV
14	62	M	3.68	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
15	57	M	105.2	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
16	70	M	12.02	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HCV
17	63	M	3.26	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HCV
18	43	M	2.96	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
19	57	M	3.18	BLOD	BLOD	BLOD	BLOD	+	+	HBV
20	73	M	588.3	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
21	59	M	97.93	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
22	60	M	16.2	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
23	66	M	2.35	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	-
24	85	F	2.92	BLOD	BLOD	+	BLOD	+	+	-
25	69	F	44.8	+	BLOD	+	BLOD	BLOD	BLOD	HCV
26	60	M	<1.3	BLOD	BLOD	BLOD	BLOD	+	+	HBV
27	76	F	3	BLOD	BLOD	BLOD	+	+	+	HCV, HBV
28	52	M	1.8	BLOD	BLOD	BLOD	BLOD	BLOD	+	HBV
29	64	M	2.9	BLOD	+	BLOD	+	+	+	HBV
30	69	M	12	BLOD	BLOD	BLOD	BLOD	+	BLOD	HBV
31	57	M	79.85	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	HBV
32	69	F	3.6	BLOD	BLOD	BLOD	BLOD			HBV
33	79	F	1.6	BLOD	+	BLOD	BLOD			HBV
34	50	M	2	BLOD	+	+	+			HBV
35	50	M	2	BLOD	BLOD	BLOD	BLOD			HBV
36	90	M	270.6	BLOD	BLOD	BLOD	BLOD			-
37	52	M	38	BLOD	BLOD	+	BLOD			HCV
38	58	M	7.64	BLOD	BLOD	BLOD	BLOD			HBV

39	56	F	22,366	BLOD	BLOD	BLOD	BLOD		HBV
40	72	M	1.5	BLOD	BLOD	BLOD	BLOD		HBV
41	69	M	105	BLOD	+			BLOD +	
42	61	M	3	BLOD	BLOD			BLOD +	HCV
43	58	M	30	BLOD	BLOD			+ +	HCV
44	64	M	151	BLOD	BLOD			BLOD +	HCV
45	66	F	577	BLOD	BLOD			BLOD +	HCV
46	78	M	5	BLOD	+			+ BLOD	-
47	58	M	336	BLOD	BLOD			+ BLOD	HCV
48	58	M	41.9	BLOD	+				HCV
49	72	F	4	BLOD	+			+ BLOD	HBV
50	60	M	2	BLOD	BLOD			BLOD BLOD	HCV
51	56	M	4.9	BLOD	BLOD				HCV
52	57	F	3.5	BLOD	BLOD			BLOD BLOD	HCV
53	71	F	10.3	BLOD	BLOD			BLOD +	HBV, HCV
54	74	F	11.8	BLOD	BLOD			+ BLOD	HCV
55	69	F	45.7	BLOD	BLOD			BLOD BLOD	-
56	84	F	34.7	BLOD	BLOD			BLOD BLOD	HCV
57	87	M	3.3	+	BLOD			+ BLOD	HBV
58	66	M	1.9	BLOD	BLOD			BLOD BLOD	HBV
59	57	F	591.4	BLOD	BLOD			+ BLOD	HCV
60	55	M	167.4	BLOD	BLOD			BLOD BLOD	HBV
61	76	M	n/a	BLOD	BLOD			BLOD BLOD	-
62	77	M	5	BLOD	BLOD			BLOD BLOD	HBV
63	68	M	1264	BLOD	BLOD			BLOD BLOD	-
64	53	M	5	BLOD	BLOD			BLOD BLOD	HBV
65	65	M	7.5	BLOD	BLOD			BLOD BLOD	HBV
66	76	F	833.6	BLOD	BLOD			BLOD BLOD	HCV
67	71	M	2.8	BLOD	BLOD			BLOD BLOD	HCV
68	62	F	n/a	BLOD	BLOD			BLOD BLOD	HBV
69	81	F	n/a	BLOD	BLOD			BLOD BLOD	HBV
70	77	M	12,179	BLOD	BLOD				-
71	67	M	6.7	BLOD	+				HBV
72	79	F	14.2	BLOD	BLOD				HCV
73	60	M	9.6	BLOD	BLOD				HBV
74	69	M	n/a	BLOD	BLOD				HBV
75	70	M	n/a	BLOD	BLOD				HBV
76	64	F	n/a	BLOD	BLOD				HBV
77	69	F	n/a	BLOD	BLOD				HCV
78	64	F	n/a	BLOD	BLOD				HBV
79	80	F	n/a	BLOD	BLOD				HBV
80	62	M	n/a	BLOD	BLOD				HBV
81	55	M	18,733	BLOD	BLOD			BLOD +	HBV, HCV
82	60	M	7.3			BLOD	BLOD	BLOD BLOD	HBV
83	68	F	430			BLOD	BLOD	+ +	HBV, HCV

84	33	M	4861		BLOD	BLOD	+	+	HBV
85	61	F	4.57		BLOD	BLOD	BLOD	BLOD	HBV
86	59	M	17		BLOD	+			HCV
87	79	M	1,596		+	BLOD			-
88	61	M	<1.3		BLOD	BLOD			HBV
89	64	M	3.42		+	BLOD			HBV
90	26	M	3				BLOD	+	-
91	69	M	104.5				BLOD	+	-
92	69	F	12,983				+	+	-
93	64	F	75,917				+	+	HBV
94	72	F	836,322				BLOD	BLOD	HBV
95	57	F	4,489				+	BLOD	HCV
96	69	M	1,103				BLOD	BLOD	-
97	55	M	595				BLOD	BLOD	HCV
98	71	F	n/a				BLOD	BLOD	HCV
99	60	M	11.4				BLOD	BLOD	-
100	61	M	8.2				BLOD	BLOD	HBV
101	72	M	361				+	BLOD	HBV

BLOD, below limit of detection; highlighted cells, positive detection; black cells, test not performed due to limited DNA quantity

Table S5. Global concordance by HCC-targeted NGS.

Position Call		Plasma	Urine
negative (reference)		942,253	934,576
positive (variant)		17,837	25,514
total		960,090	960,090
positive concordant		6,506	
negative concordant		923,245	
Concordance, %	overall ^a	96.8	
	positive ^b	17.7	
	plasma-positive ^c	36.5	
	urine-positive ^d	25.5	

^a. percentage of positive and negative concordance over total positions ((6,506+923,245)/960,090)

^b. percentage of positive concordance over total positive variants (6,506/(17,837+24,514-6,506))

^c. percentage of positive concordance over plasma variants (6,506/17,837)

^d. percentage of positive concordance over urine variants (6,506/25,514)

Table S6. Somatic (VAF<0.4) variant call rates by target in the 15-patient HCC cohort.

Target Gene	Plasma	Urine
<i>TERT</i> promoter	0.2023	0.2189
<i>KMT2D</i>	0.0367	0.0468
<i>TP53</i>	0.0275	0.0348
<i>ARID1A</i>	0.0242	0.0355
<i>KEAP1</i>	0.0232	0.0320
<i>AXIN1</i>	0.0199	0.0276
<i>PCLO</i>	0.0199	0.0250
<i>CDKN2A</i>	0.0121	0.0259
<i>NFE2L2</i>	0.0193	0.0185
<i>TSC2</i>	0.0120	0.0221
<i>HNF1A</i>	0.0113	0.0226
<i>ARID1B</i>	0.0098	0.0210
<i>ALB</i>	0.0119	0.0170
<i>APC</i>	0.0111	0.0164
<i>LRP1B</i>	0.0108	0.0166
<i>APOB</i>	0.0087	0.0168
<i>BAP1</i>	0.0075	0.0169
<i>ATM</i>	0.0101	0.0138
<i>ARID2</i>	0.0075	0.0145
<i>CTNNB1</i>	0.0093	0.0122
<i>RB1</i>	0.0085	0.0128
<i>IL6ST</i>	0.0056	0.0124
<i>PIK3CA</i>	0.0051	0.0122
<i>RPS6KA3</i>	0.0070	0.0070

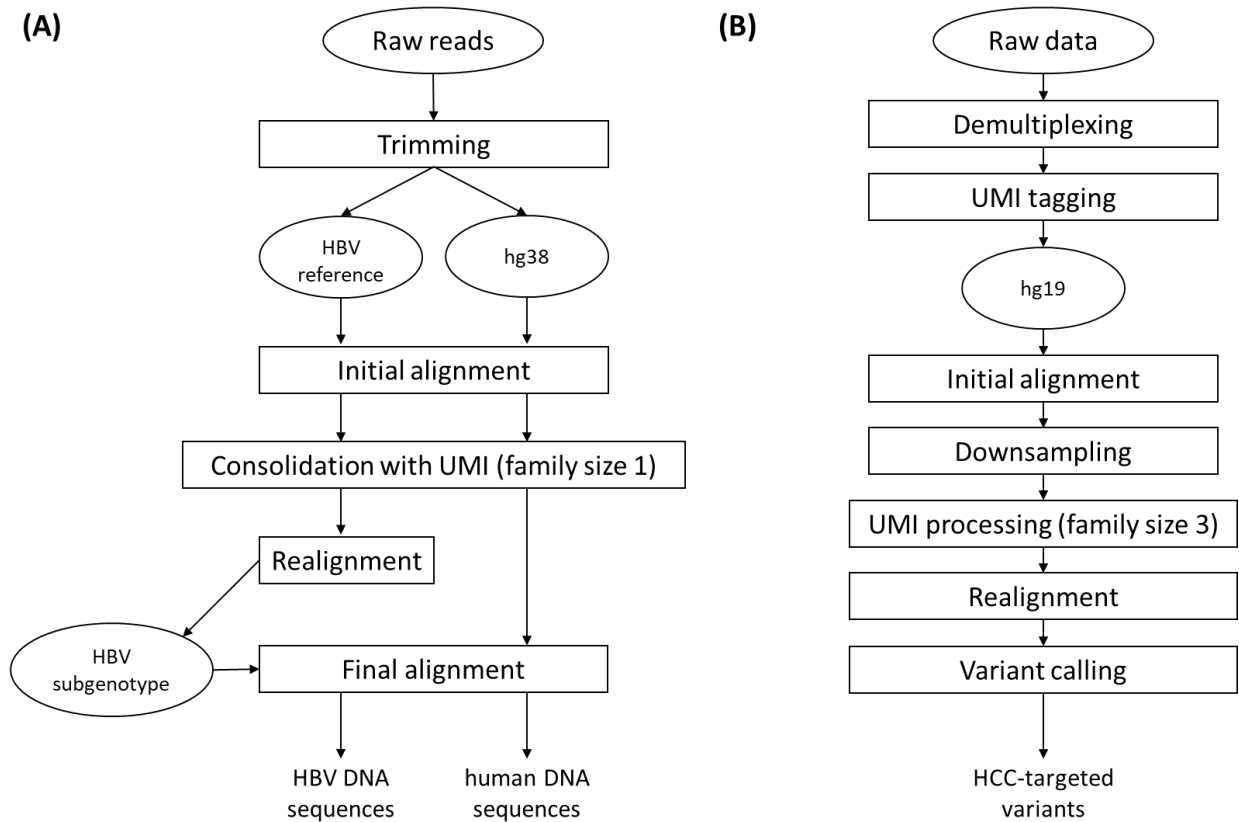


Figure S1. HBV-targeted (A) and HCC-targeted (B) NGS analysis workflow.

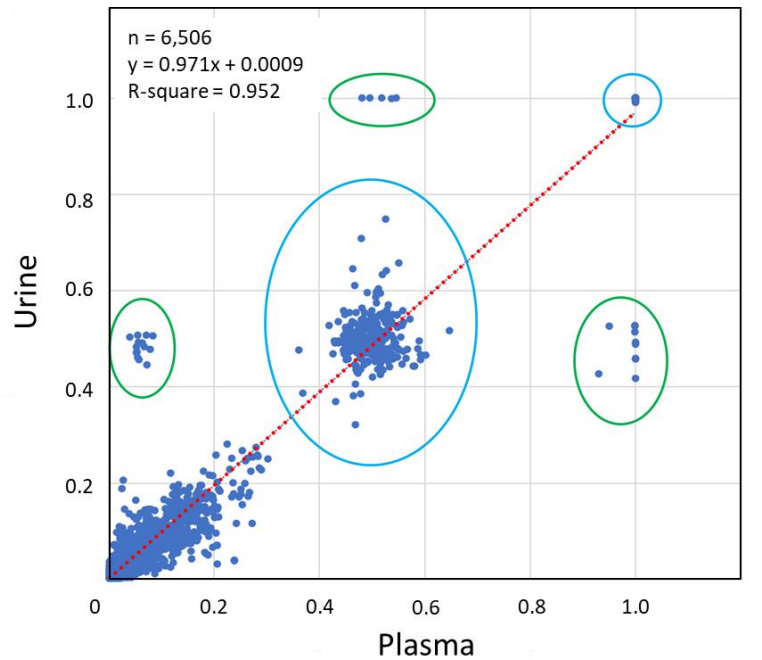


Figure S2. Correlation of variant allele frequencies (VAFs) of positive concordant variants detected by HCC-targeted NGS in plasma and urine cfDNA. Green circles indicate variants likely attributable to early embryonic somatic mutations and originating from circulating or uroepithelial cell gDNA. Blue circles indicate likely germline variants. Only variants supported by ≥ 3 unique reads (UMI families) were included in the analysis. n, number of double-positive positions.