Supplemental Table 1. Patient and specimen characteristics for the clinical plasma samples tested in this study.

Sample #	Patient #	Age	Viral load[a]	Diagnosis	CMV status	Anti-CMV drugs	Reference Laboratory Test[b]	NGS: DRM (% variant)	Predicted Resistance	NGS: Putative Mutant (%variant)
AA8_20	Patient-01	65	1753	HSCT	D-/R+	GCV, FOS, CDV	unable to amplify (+21day)	NONE	NA	NONE
AA7_03	Patient-01	65	820	HSCT	D-/R+	GCV, FOS, CDV	unable to amplify (+1 day)	NONE	NA	NONE
AA5_17	Patient-03	43	899	HSCT	D-/R+	GCV, FOS	NO	NONE	NA	NONE
AA7_19	Patient-04	61	918	LUNG	R-	VCV, GCV	NO	NONE	NA	UL54: K513R (99.5), R1037C (99.0); UL97: NONE
AA7_09	Patient-05	37	11157	HIV/AIDS	NA	VCV, GCV, CDV	NO	NONE	NA	NONE
AA8_13	Patient-05	37	6258	HIV/AIDS	NA	VCV, GCV, CDV	NO	NONE	NA	NONE
AA7_01	Patient-06	74	770	LUNG	UNKNOWN	VCV, GCV	NO	NONE	NA	NONE
AA7_21	Patient-07	47	4258	LIVER	R+	VCV	NO	NONE	NA	UL54: M637I (98.1); UL97: NONE
AA5_24	Patient-08	16	493	HSCT	R-	VCV	NO	NONE	NA	UL54: G531S (100); UL97: NONE
AA5_21	Patient-09	20	394	KIDNEY	UNKNOWN	VCV	NO	NONE	NA	NONE
AA8_01	Patient-10	71	12584	HSCT	D-/R+	VCV, FOS	NO	NONE	NA	NONE
AA7_18	Patient-11	56	5371	HSCT	D+/R+	VCV	NO	NONE	NA	NONE
AA8_07	Patient-11	56	7506	HSCT	D+/R+	VCV	NO	NONE	NA	NONE
AA5_23	Patient-12	49	706	HSCT	D+/R+	VCV, GCV, FOS	NO	NONE	NA	UL54: R1037C (100); UL97: NONE
AA8_10	Patient-13	57	6685	HSCT	D+/R+	FOS, GCV	NO	NONE	NA	NONE
AA7_17	Patient-13	57	5831	HSCT	D+/R+	FOS, GCV	NO	NONE	NA	NONE
AA8_15	Patient-14	66	2719	HSCT	R+	VCV, GCV	NO	NONE	NA	NONE
AA7_22	Patient-15	71	3663	LUNG	UNKNOWN	VCV, GCV	unable to amplify (+6 days)	NONE	NA	NONE
AA8_06	Patient-15	71	1831	LUNG	UNKNOWN	VCV, GCV	unable to amplify (-5 days)	NONE	NA	NONE
AA8_05	Patient-16	67	2191011	HEART	UNKNOWN	FOS	Negative (-3 days)	NONE	NA	NONE
AA8_04	Patient-17	67	1247	HSCT	D-/R+	VCV, GCV,	NO	NONE	NA	UL54: H556R (21.4), G974S (21.5); UL97: NONE
AA7_23	Patient-18	27	130337	OTHER	NA	VCV, GCV	NO	NONE	NA	NONE
AA8_11	Patient-19	42	731	HSCT	R+	GCV	NO	NONE	NA	UL54: V873A (31.9), H465Y (55.1); UL97: NONE
AA7_20	Patient-20	66	50337	KIDNEY	R+	VCV	NO	NONE	NA	NONE
AA5_19	Patient-21	57	1030	HSCT	UNKNOWN	VCV	NO	NONE	NA	UL54: R800C (98.1%), P642L(50.9%); UL97: NONE

Sample #	Patient #	Age	Viral load[a]	Diagnosis	CMV status	Anti-CMV drugs	Reference Laboratory Test[b]	NGS: DRM (% variant)	Predicted Resistance	NGS: Putative Mutant (%variant)
AA8_21	Patient-22	5M	967	LIVER	UNKNOWN	VCV, CDV,	NO	NONE	NA	NONE
AA8_08	Patient-23	58	6112	HSCT	D+/R+	GCV	NO	NONE	NA	UL54: P656S (20.1); A269V (33); UL97: NONE
AA8_16	Patient-24	57	1742	HSCT	R+	VCV	NO	NONE	NA	UL54: 2545:G/- del (52.4); UL97: NONE
AA7_07	Patient-25	63	16629	HSCT	D-/R+	GCV, FOS	negative (-5 days)	NONE	NA	NONE
AA7_06	Patient-25	63	11798	HSCT	D-/R+	GCV, FOS	negative (+2 days)	NONE	NA	NONE
AA8_09	Patient-26	23	4191	HSCT	D+/R+	GCV	negative (+8 days)	NONE	NA	NONE
AA8_12	Patient-26	23	1382	HSCT	D+/R+	GCV	negative (+2 days)	NONE	NA	NONE
AA7_04	Patient-27	63	5528	HSCT	D+/R+	VCV	NO	NONE	NA	NONE
AA8_22	Patient-28	44	1404	HSCT	D-/R+	FOS, GCV	NO	NONE	NA	UL54: T892A (29.6); A492T (21.7), UL97: NONE
AA7_05	Patient-28	44	2921	HSCT	D-/R+	FOS, GCV	unable to amplify (+1 day)	NONE	NA	NONE
AA8_17	Patient-28	44	1371	HSCT	D-/R+	VCV, GCV, CDV	unable to amplify (+7 days)	UL97: C592G (15.6)	GCV	NONE
AA7_08	Patient-28	44	933	HSCT	D-/R+	FOS, GCV	unable to amplify (0 days)	UL97: C592G (6.4)	GCV	NONE
AA8_23	Patient-29	33	3213	KIDNEY	UNKNOWN	VCV	NO	NONE	NA	NONE
AA7_10	Patient-29	34	3348	KIDNEY	UNKNOWN	VCV	NO	UL54: T503I (41.6), UL97: C603W (14.4)	GCV, CDV	NONE
AA5_18	Patient-30	26	717	HSCT	D+/R+	VCV, GCV, FOS	UL97: H520Q; UL54: VARIANT A692V (+17 days)	UL97_ H520Q (77.3)	GCV	UL54: A692V (98.9); UL97: NONE
AA5_22	Patient-31	62	727	HSCT	D-/R+	GCV, FOS	NO	NONE	NA	NONE
AA8_02	Patient-32	54	3270	HSCT	D+/R+	GCV, FOS	NO	NONE	NA	NONE
AA8_14	Patient-32	54	3618	HSCT	D+/R+	GCV, FOS	UL97: M460V (-9 days)	UL97: M460V (68.6), L595S (25.9)	GCV	NONE
AA7_24	Patient-33	68	8315	HSCT	D+/R+	GCV	NO	NONE	NA	NONE
AA7_02	Patient-34	51	1146	HSCT	UNKNOWN	VCV, GCV	NO	NONE	NA	NONE
AA8_19	Patient-35	66	3303	HSCT	D+/R-	GCV	NO	NONE	NA	NONE
AA8_03	Patient-35	66	6315	HSCT	D+/R-	GCV	NO	NONE	NA	NONE
AA8_18	Patient-35	66	1809	HSCT	D+/R-	GCV	NO	NONE	NA	NONE

[a]copies/mL plasma

[b] Shown in parentheses is the timing of the most recent genotypic drug resistance testing at a reference laboratory, if one was available within 21 days of the specimen tested in this study.

Supplemental Table 2. Distribution of primer pairs in the primer half-plate of the integrated fluidic circuit (IFC) of the Access Array.

Well #	Primer pair(s)	Well #	Primer pair(s)	Well #	Primer pair(s)
1	POL_D23+PT_I23	9	POL_D23	17	POL_L11
2	POL_E44+PT_H22	10	POL_E44	18	POL_L11
3	POL_F45	11	POL_F54	19	POL_N22+POL_D23
4	POL_G45	12	POL_G54	20	POL_N22
5	POL_H65	13	POL_H65	21	PT_G45
6	POL_I46	14	POL_I54	22	PT_G65
7	POL_J56+PT_G45	15	POL_J56	23	PT_H22
8	POL_K23	16	POL_K23	24	PT_I23

Supplemental Table 3. UL54 mutations that have been confirmed phenotypically and were considered accepted drug resistance mutations in this study.

Mutation	Reference	Pubmed ID		Mutation	Reference	Pubmed ID		Mutation	Reference	Pubmed ID
Substitutions				**Substitutions**				**Substitutions**		
A987G	(1, 2)	8381637, 9621055		T700A	(2, 3)	8627655, 9621055		D413A	(4)	17157554
L957F*	(5)	21709106		F595I*	(5)	21709106		F412L	(6)	21295516
V946L*	(5)	21709106		D588E*	(2)	9621055		F412C	(2)	9621055
L862F*	(5)	21709106		D588N	(7, 8)	15634973, 11595579		F412S	(6)	21295516
G841A	(9)	17709468		S585A*	(5)	21709106		F412V	(2)	9621055
T838A	(7)	15634973		Q578H	(10, 11)	12637079, 18712833		F412L	(6)	21295516
A834P	(12)	17043128		T552N*	(5)	21709106		N410K	(13)	12825168
P829S*	(5)	21709106		L545W	(6)	21295516		N408S	(14)	23415883
T821I	(2)	9621055		L545S	(5, 15)	21709106, 9621055		N408K	(12)	17043128
T813S	(9)	17709468		C539R*	(5)	21709106		D301N	(13)	12825168
V812L	(5, 15)	21709106, 9721246		P522S	(2)	9621055				
A809V	(16)	9697736		P522A	(17)	18502683				
K805Q	(2)	9621055		I521T	(17)	18502683				
L802V*	(5)	21709106, 9621055		L516R	(13)	12825168				
L802M	(5)	21709106, 9621055		K513N	(15)	9721246		**Deletions**		
V787L	(5)	21709106, 12599051		K513E	(2)	9621055		del 981–982	(13)	12825168
V781I	(2, 8)	11595579, 9621055		T503I	(13)	12825168		del 524	(14)	23415883
L776M	(18)	18419454		L501I	(2)	9621055				
E756D	(13)	12825168		K500N	(5)	21709106				
E756Q	(19)	12599051		N495K	(20)	16856628				
E756K	(13)	12825168		P488R*	(5)	21709106				
V715M	(2, 3)	8627655, 9621055		D413E	(13, 21)	12825168, 20876378				

* the clinical significance of these mutations is unclear because of low levels of resistance in phenotypic assays or because the mutation has only been identified in laboratory experiments and not authenticated in clinical isolates or sequences.

Supplemental Table 4. UL97 mutations that have been confirmed phenotypically and were considered accepted drug resistance mutations in this study.

Mutation	Reference	Pubmed ID		Mutation	Reference	Pubmed ID
Substitutions				**Substitutions**		
L405P*	(22)	20385869		K599T	(23)	9864050
M460V	(22)	20385869, 9207351		C603S	(22)	20385869
M460T	(22)	20385869		C603R	(22)	20385869, 20138805
M460I	(24)	9207351		C603W	(22)	20385869, 9207351
V466G*	(25)	20138805		C607Y	(26)	11807689
H520Q	(26)	11807689		C607F	(26)	11807689
C592G	(22)	20385869				
A594T	(26)	11807689				
A594P	(27)	11750939				
A594E	(22)	20385869		**Deletions**		
A594G	(28)	9300382		del 591–594	(26)	11807689
A594V	(22, 24)	20385869, 9207351		del 591–607	(26)	11807689
L595S	(24)	9207351		del 595	(29)	7815545
L595W	(26)	11807689		del 595–603	(30)	10602745
L595F	(26)	11807689		del 600	(26)	11807689
E596G	(26)	11807689		del 601	(31)	15793144
G598S	(32)	11888658		del 601-603	(4)	17157554

*the clinical significance of these mutations is unclear because of low levels of resistance in phenotypic assays
or because the mutation has only been identified in laboratory experiments and not authenticated in clinical isolates or sequences.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Visualization of long-range PCR amplification products. An ethidium bromide stained agarose gel, showing long-range PCR products when the reference AD169 strain was used as template at serial dilutions resulting in 1200 (d1), 400 (d2), 133 (d3), and 44 (d4) copies/mL plasma. Primer sets I and II contain different primer pairs for UL54 and UL97 as described in Table 1. The arrows indicated UL54 and UL97 amplification products. A 1 kb plus ladder was used.

Supplemental Figure 2. Average read coverage per nucleotide when 24 versus 8 specimens are sequenced. Sequencing data for the UL54 (A) and UL97 (B) genes are presented as the mean of total reads per nucleotide for the same 8 specimens when they are processed and sequenced alone (black line) or with 16 other specimens for a total of 24 samples in a single run (grey line).

Supplemental Figure 3. Read coverage per amplicon in the forward and reverse sequencing direction. The data are based on 24 patient plasma specimens in a single run. Data are presented as box whisker plots of the interquartile ranges (1.5 IQR, Tukey method). Amplicons beginning with the same letter have overlapping coverage (see Table 2). Green: Forward, Red: Reverse.

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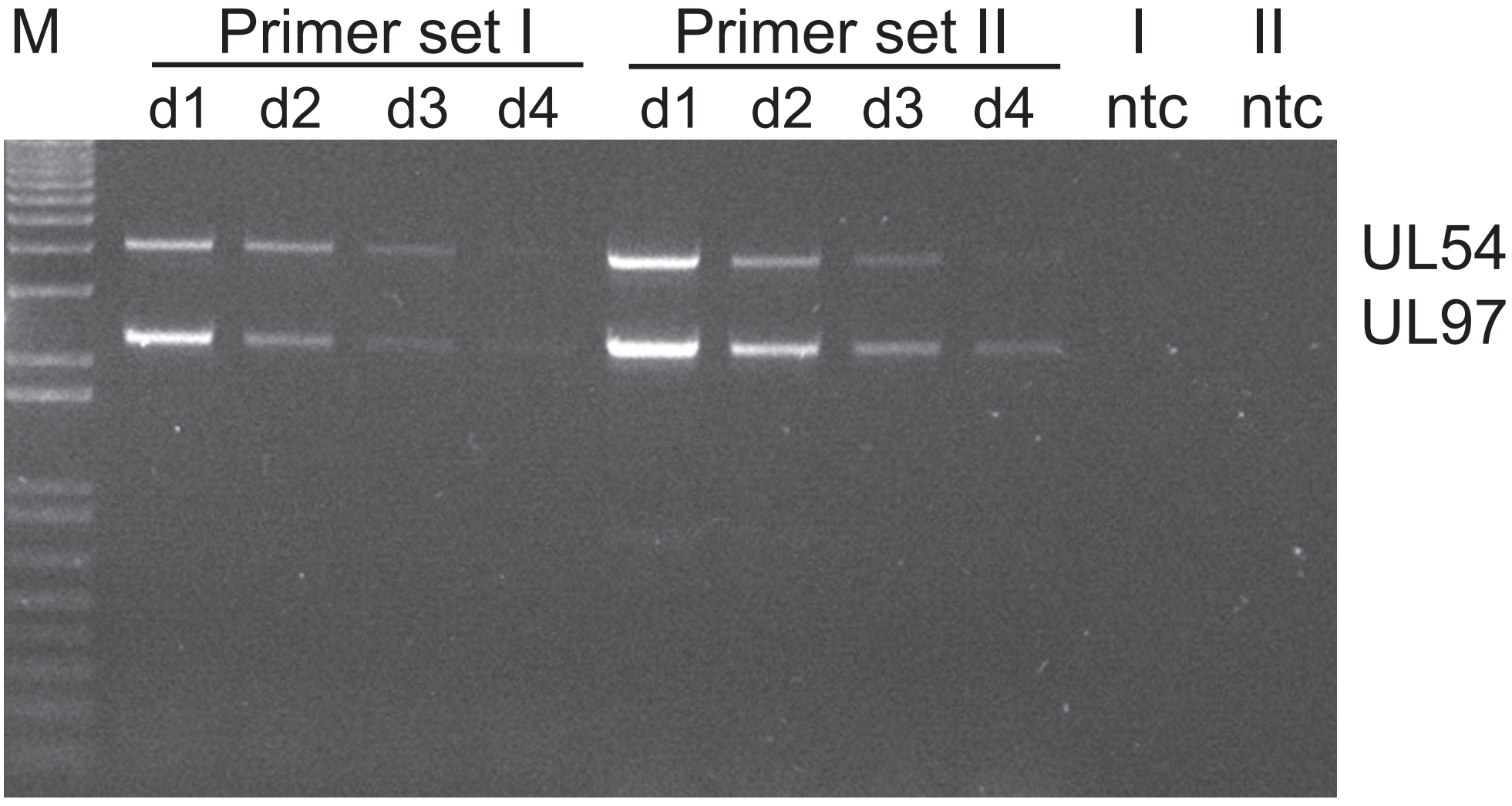
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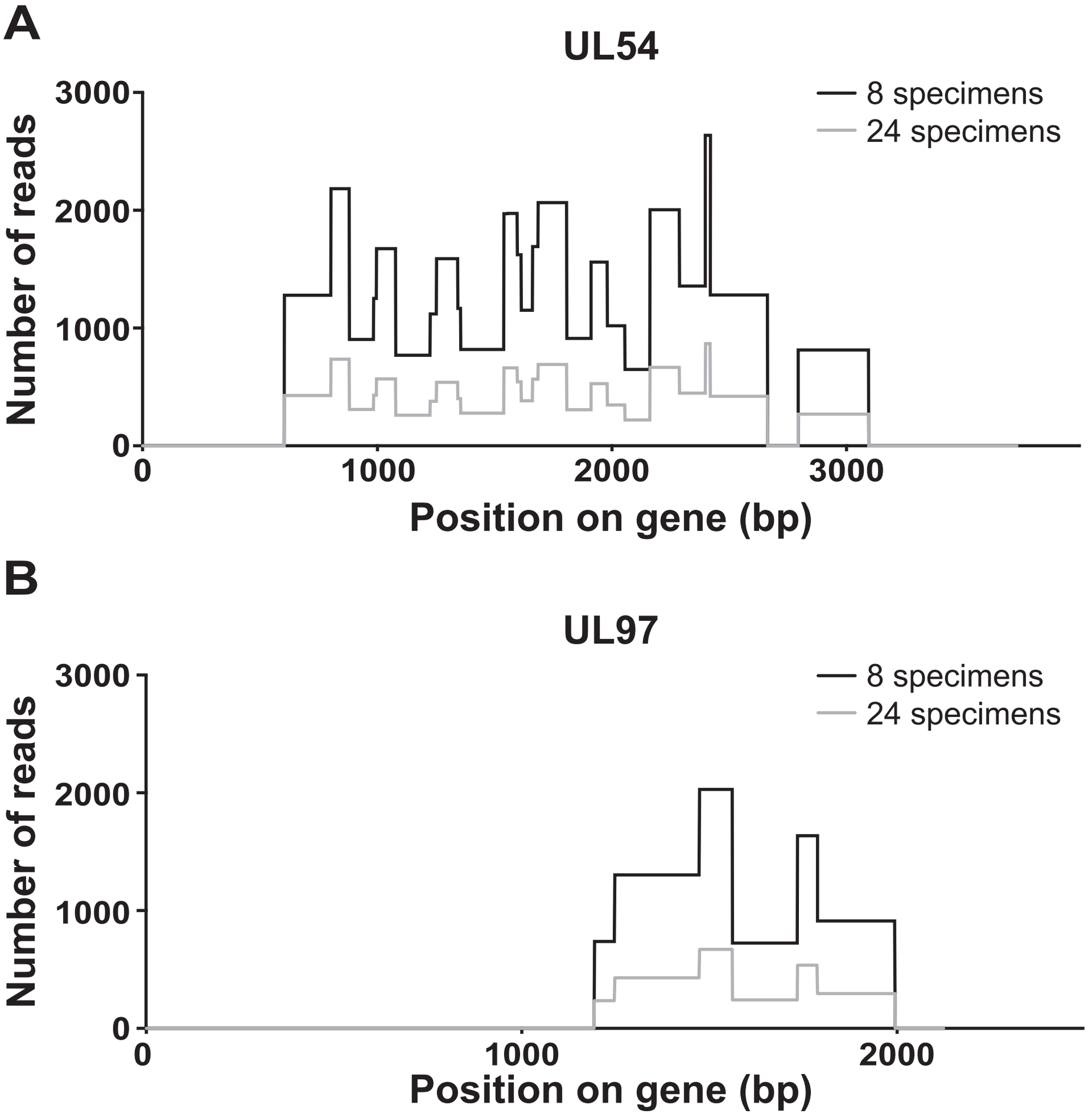
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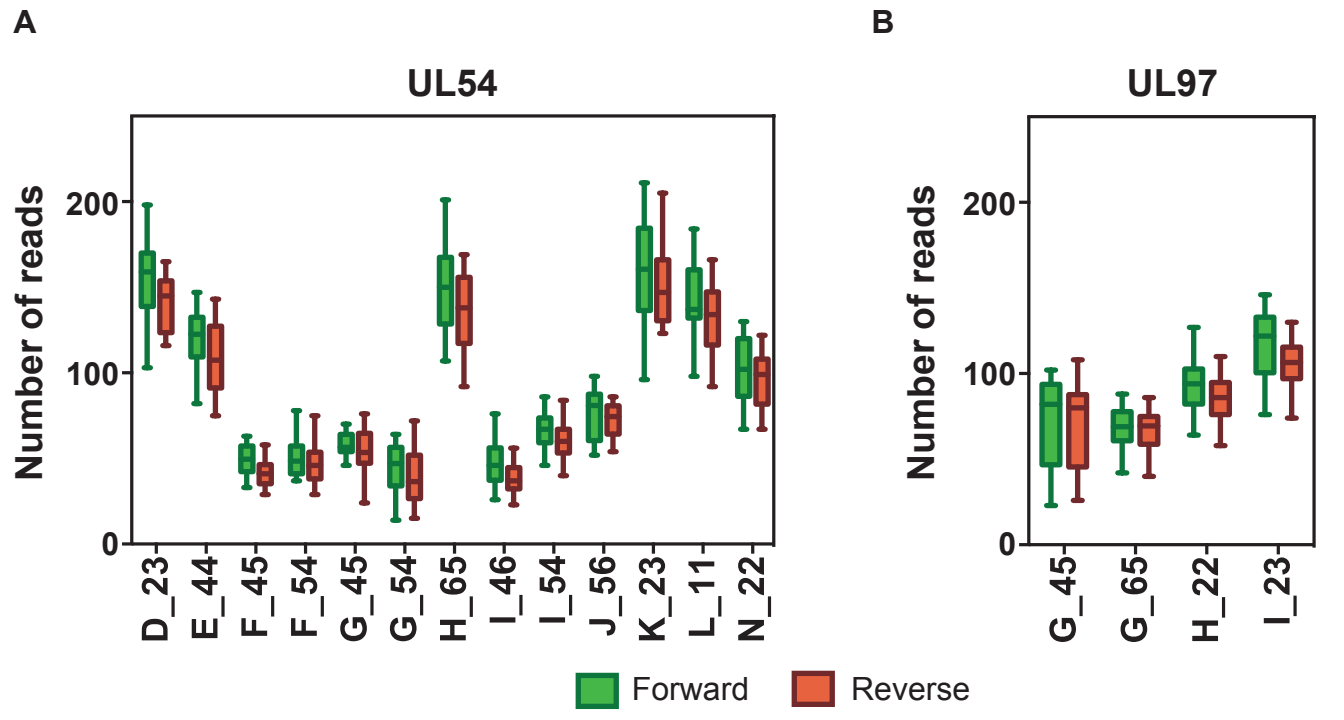
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