TABLE S1 List of HPV types detectable by TypeSeq and Linear Array.

HPV Genotype	Genotype Detectable by Assay	
	TypeSeq	**Linear Array**
3	Yes	No
6	Yes	Yes
11	Yes	Yes
13	Yes	No
16	Yes	Yes
18	Yes	Yes
26	Yes	Yes
28	Yes	No
30	Yes	No
31	Yes	Yes
32	Yes	No
33	Yes	Yes
34	Yes	Yes (as 64)[a]
35	Yes	Yes
39	Yes	Yes
40	Yes	Yes
42	Yes	Yes
43	Yes	No
44	Yes	Yes (as 55)[a]
45	Yes	Yes
51	Yes	Yes
52	Yes	Yes
53	Yes	Yes
54	Yes	Yes
56	Yes	Yes
58	Yes	Yes
59	Yes	Yes
61	Yes	Yes
62	Yes	Yes
66	Yes	Yes
67	Yes	Yes
68	Yes	Yes (lineages C-F only, formerly “68b”)
69	Yes	Yes
70	Yes	Yes
71	Yes	Yes
72	Yes	No
73	Yes	Yes
74	Yes	No
76	Yes	No
81	Yes	Yes
82	Yes	Yes
83	Yes	Yes
84	Yes	Yes
85	Yes	No
86	Yes	No
87	Yes	No
89	Yes	Yes
90	Yes	No
91	Yes	No
97	Yes	No
114	Yes	No

[a]HPV64 has been re-classified as HPV34, and HPV55 is now HPV44.

Stage 2 Re-Coding PCR Example – HPV52 universal forward priming region

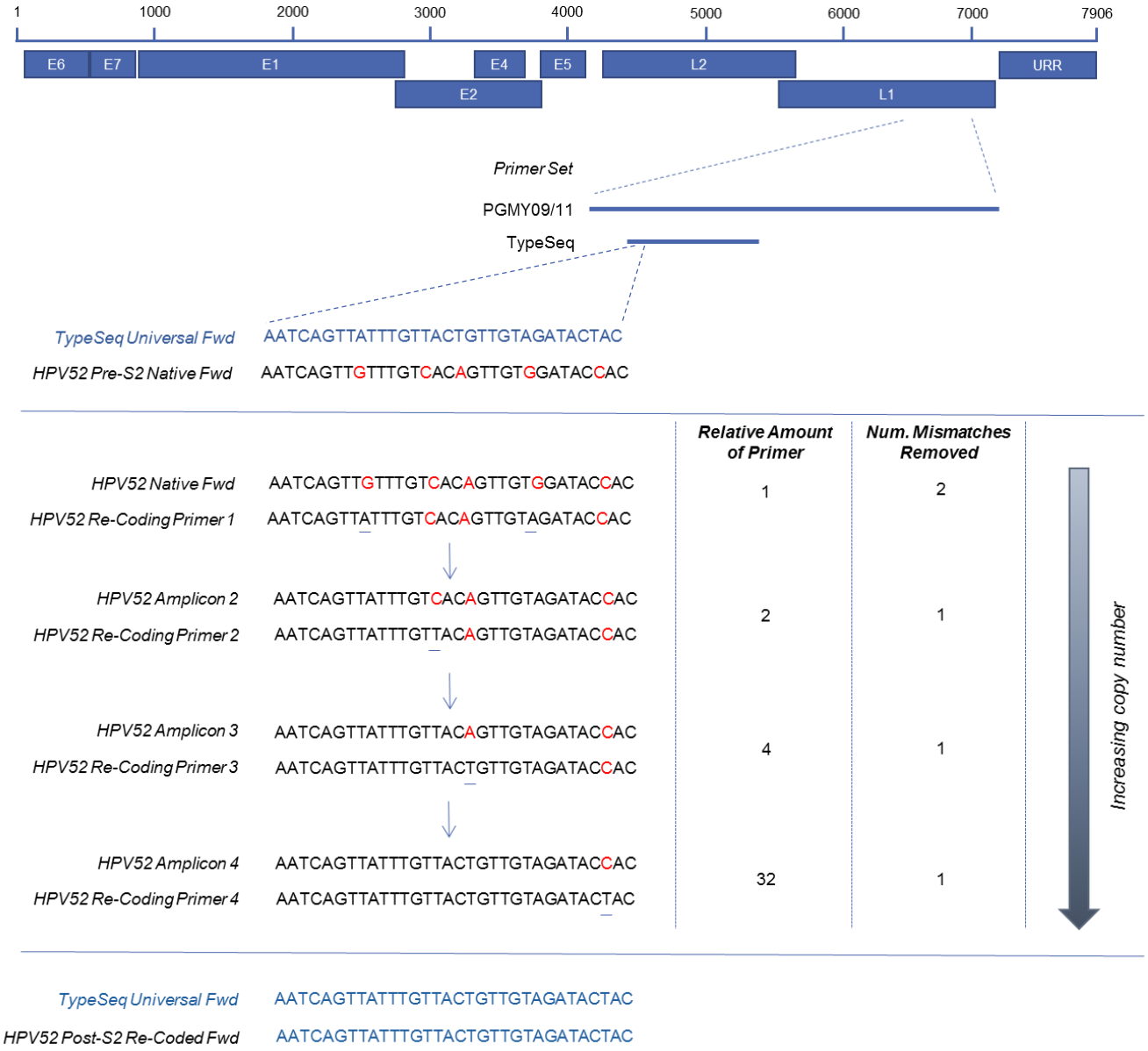

FIG S1 An example of the TypeSeq Stage 2 Re-Coding PCR process for the forward universal priming region of HPV52, performed to improve compatibility with the TypeSeq universal forward primer. The locations within the L1 gene of the HPV genome of the PGMY09/11 consensus priming region used in Linear Array and of the TypeSeq universal target region are shown. After S1 amplification with type-specific primers, the universal priming regions of each HPV amplicon are amplified with S2 re-coding primers under constant, low stringency conditions. Each of the 170 S2 primers is designed to be partially

homologous to a template strand (either the S1 products, or newly-synthesized S2 strands), with 1 or 2 mismatches homologous to the universal primer sequence rather than the native HPV type's sequence. S2 primer amounts are deliberately low, so that when primers homologous to a target sequence have been depleted, remaining unincorporated primers with the closest homology will then preferentially anneal. With each new primer incorporated, the native HPV sequence is gradually modified to be more homologous to the universal primer, with copy number also increasing in tandem with universal primer homology. Fwd = forward.

A **STAGE 1 PCR COMPOSITION**

COMPONENT	Vol 1x (µL)
Nuclease-free water	0.45
Polyethylene Glycol 8000 (15%)	4.00
Tetramethylammonium Chloride (5 M)	0.24
$MgSO_4$ (100 mM)	0.144
10 mM dNTPs	0.42
10x Thermopol Buffer	1.20
BSA (non-acetylated; 50 mg/mL)	0.096
Stage 1 Primer Pool (50 µM)	0.23
RNase H2 (2 U/uL)	0.02
Vent (exo-) DNA Polymerase (2 U/µL)	0.20
Genomic DNA	5.00
Final Volume	**12**

STAGE 1 CYCLING CONDITIONS

STEP	TEMP	TIME	
Initial Denaturation	95°C	2 min	
Denaturation	95°C	10 sec	**x 2 cycles**
Annealing & Extension	60°C	2 min	
Denaturation	95°C	10 sec	**x 43 cycles**
Annealing & Extension	62°C	4 min	
Final Extension	68°C	7 min	
Hold	4 - 10°C	∞	

B **STAGE 2 PCR COMPOSITION**

COMPONENT	Vol 1x (µL)
Nuclease-free water	1.48
Polyethylene Glycol 8000 (15%)	2.00
$MgCl_2$ (25 mM)	1.08
10 mM dNTPs	0.20
5x OneTaq Standard Buffer	2.00
Stage 2 Primer Pool (25 µM)	0.21
OneTaq Hot Start DNA Polymerase (5 U/µL)	0.035
Stage 1 reaction (diluted)	3.00
Final Volume	**10**

STAGE 2 CYCLING CONDITIONS

STEP	TEMP	TIME	
Initial Denaturation	94°C	2 min	
Denaturation	94°C	10 sec	**x 20 cycles**
Annealing	38°C	4 min	
Extension	65°C	40 sec	
Final Extension	65°C	5 min	
Hold	4 - 10°C	∞	

C **STAGE 3 PCR COMPOSITION**

COMPONENT	Vol 1x (µL)
Nuclease-free water	0.50
Polyethylene Glycol 8000 (15%)	2.00
$MgCl_2$ (25 mM)	0.28
10 mM dNTPs	0.20
5x OneTaq Standard Buffer	2.00
OneTaq Hot Start DNA Polymerase (5 U/µL)	0.02
Stage 3 Barcoded Primers (0.4 µM)	3.00
Stage 2 Exonuclease I-treated reaction	2.00
Final Volume	**10**

STAGE 3 CYCLING CONDITIONS

STEP	TEMP	TIME	
Initial Denaturation	94°C	2 min	
Denaturation	94°C	15 sec	**x 3 cycles**
Annealing	42°C	4 min	
Extension	65°C	40 sec	
Denaturation	94°C	15 sec	**x 13 cycles**
Annealing & Extension	65°C	1 min	
Final Extension	65°C	5 min	
Hold	4 - 10°C	∞	

FIG S2 Composition and thermal cycling conditions for TypeSeq Stages 1, 2 and 3.

TABLE S2 Analytic sensitivity of the TypeSeq assay for single and multiple HPV type reactions.[a]

Synthetic control HPV genotype	SINGLE HPV TYPE REACTIONS				MULTIPLE HPV TYPE REACTIONS		
	No. of positive samples/no. of samples tested at the specified HPV copy number per reaction			Analytical sensitivity of single type reactions	No. of positive samples/no. of samples tested at the specified HPV copy number per reaction in the presence of 50K copies of other types		Analytical sensitivity of low input multiple type mixture
	10	25	50		10	25	
3	6/6	6/6	6/6	10	6/6	6/6	10
6	6/6	6/6	6/6	10	6/6	6/6	10
11	6/6	6/6	6/6	10	6/6	6/6	10
13	6/6	6/6	6/6	10	6/6	6/6	10
16	6/6	6/6	6/6	10	6/6	6/6	10
18	6/6	6/6	6/6	10	6/6	6/6	10
26	6/6	6/6	6/6	10	6/6	6/6	10
28	6/6	6/6	6/6	10	6/6	6/6	10
30	6/6	6/6	6/6	10	6/6	6/6	10
31	6/6	6/6	6/6	10	6/6	6/6	10
32	6/6	6/6	6/6	10	6/6	6/6	10
33	6/6	6/6	6/6	10	6/6	6/6	10
34	6/6	6/6	6/6	10	6/6	6/6	10
35	6/6	6/6	6/6	10	6/6	6/6	10
39	6/6	6/6	6/6	10	4/6	6/6	25
40	6/6	6/6	6/6	10	6/6	6/6	10
42	5/6	6/6	6/6	25	4/6	6/6	25
43	6/6	6/6	6/6	10	6/6	6/6	10
44	6/6	6/6	6/6	10	4/6	6/6	25
45	6/6	6/6	6/6	10	6/6	6/6	10
51	6/6	6/6	6/6	10	6/6	6/6	10
52	6/6	6/6	6/6	10	6/6	6/6	10
53	6/6	6/6	6/6	10	6/6	6/6	10
54	6/6	6/6	6/6	10	5/6	6/6	25
56	6/6	6/6	6/6	10	6/6	6/6	10
58	6/6	6/6	6/6	10	6/6	6/6	10
59	6/6	6/6	6/6	10	6/6	6/6	10
61	6/6	6/6	6/6	10	6/6	6/6	10
62	6/6	6/6	6/6	10	6/6	6/6	10
66	6/6	6/6	6/6	10	6/6	6/6	10
67	6/6	6/6	6/6	10	6/6	6/6	10
68	6/6	6/6	6/6	10	6/6	6/6	10
69	6/6	6/6	6/6	10	6/6	6/6	10
70	6/6	6/6	6/6	10	6/6	6/6	10
71	6/6	6/6	6/6	10	6/6	6/6	10
72	6/6	6/6	6/6	10	6/6	6/6	10
73	6/6	6/6	6/6	10	6/6	6/6	10
74	6/6	6/6	6/6	10	5/6	6/6	25
76	6/6	6/6	6/6	10	5/6	6/6	25
81	6/6	6/6	6/6	10	6/6	6/6	10
82	6/6	6/6	6/6	10	6/6	6/6	10
83	6/6	6/6	6/6	10	6/6	6/6	10
84	6/6	6/6	6/6	10	6/6	6/6	10
85	6/6	6/6	6/6	10	6/6	6/6	10
86	6/6	6/6	6/6	10	6/6	6/6	10
87	6/6	6/6	6/6	10	6/6	6/6	10
89	6/6	6/6	6/6	10	6/6	6/6	10
90	6/6	6/6	6/6	10	6/6	6/6	10
91	6/6	6/6	6/6	10	6/6	6/6	10
97	5/6	6/6	6/6	25	5/6	6/6	25
114	6/6	6/6	6/6	10	6/6	6/6	10

[a] Testing was performed on 500 bp synthetic dsDNA control fragments in a minimum of 2 batches, for a total of 6 replicates per condition.

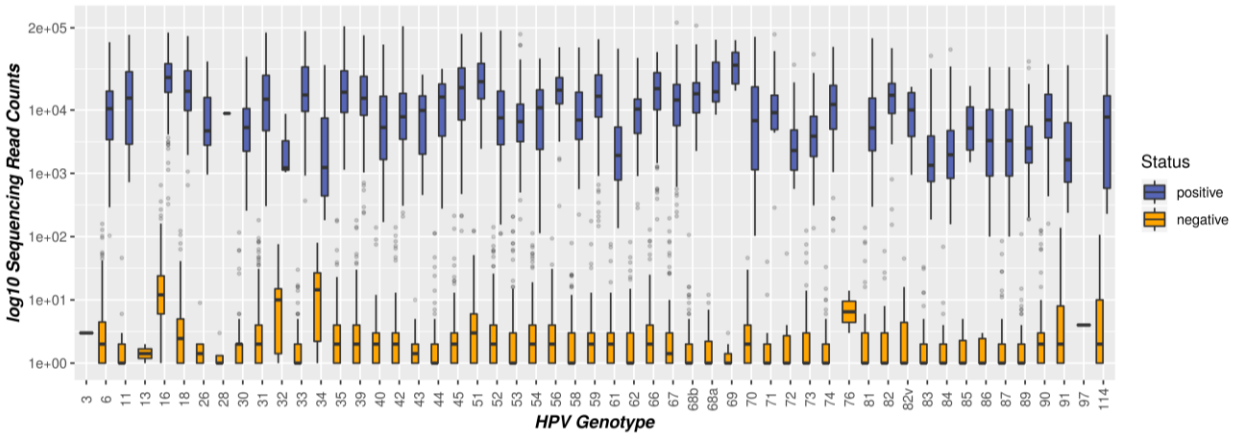

FIG S3 TypeSeq sequencing read count signal-to-noise plot for 849 clinical specimens. Read count distributions are shown on a $\log_{10}$ scale for specimens grouped by positive (blue) or negative (orange) type status. HPV3, 13, 76 and 97 were negative for all specimens. A single specimen was positive for HPV28. HPV68a (lineages A and B), 68b (lineages C-F), 82, and 82v are uniquely identifiable by TypeSeq and individual results are shown.

TABLE S3 TypeSeq reproducibility testing on 118 clinical specimens in duplicate.

HPV Genotype	Number of Specimens				% Prevalence		% Agreement	
	R1-/R2-	R1+ /R2-	R1-/R2+	R1+ /R2+	R1	R2	Total	Positive
Any HPV	0	2	0	116	100.0	98.3	98.3	98.3
Any HR-HPV[a]	13	0	1	104	88.1	89.0	99.2	99.0
16/18	73	0	1	44	37.3	38.1	99.2	97.8
3	118	0	0	0	0.0	0.0	100	NE
6	110	0	0	8	6.8	6.8	100	100
11	113	0	1	4	3.4	4.2	99.2	80.0
13	118	0	0	0	0.0	0.0	100	NE
16	85	1	0	32	28.0	27.1	99.2	97.1
18	101	0	2	15	12.7	14.4	98.3	88.2
26	116	0	0	2	1.7	1.7	100	100
28	118	0	0	0	0.0	0.0	100	NE
30	107	0	1	10	8.5	9.3	99.2	90.9
31	104	0	0	14	11.9	11.9	100	100
32	117	0	0	1	0.8	0.8	100	100
33	108	0	0	10	8.5	8.5	100	100
34	114	0	0	4	3.4	3.4	100	100
35	108	0	0	10	8.5	8.5	100	100
39	101	1	1	15	13.6	13.6	98.3	88.2
40	114	0	0	4	3.4	3.4	100	100
42	98	0	1	19	16.1	16.9	99.2	95.0
43	117	0	0	1	0.8	0.8	100	100
44	106	0	0	12	10.2	10.2	100	100
45	108	0	1	9	7.6	8.5	99.2	90.0
51	102	1	2	13	11.9	12.7	97.5	81.3
52	101	0	0	17	14.4	14.4	100	100
53	100	0	0	18	15.3	15.3	100	100
54	110	0	1	7	5.9	6.8	99.2	87.5
56	100	0	0	18	15.3	15.3	100	100
58	107	1	2	8	7.6	8.5	97.5	72.7
59	98	0	2	18	15.3	16.9	98.3	90.0
61	105	1	0	12	11.0	10.2	99.2	92.3
62	101	1	0	16	14.4	13.6	99.2	94.1
66	105	0	1	12	10.2	11.0	99.2	92.3
67	105	1	1	11	10.2	10.2	98.3	84.6
68	102	1	0	15	13.6	12.7	198.3	93.8
69	114	0	0	4	3.4	3.4	100	100
70	115	0	0	3	2.5	2.5	100	100
71	116	0	0	2	1.7	1.7	100	100
72	114	0	1	3	2.5	3.4	99.2	75.0
73	113	0	0	5	4.2	4.2	100	100
74	114	0	1	3	2.5	3.4	99.2	75.0
76	118	0	0	0	0.0	0.0	100	NE
81	110	0	0	8	6.8	6.8	100	100
82	110	0	0	8	6.8	6.8	100	100
83	112	1	1	4	4.2	4.2	98.3	66.7

TABLE S3 continued

HPV Genotype	Number of Specimens				% Prevalence		% Agreement	
	R1-/R2-	R1+ /R2-	R1-/R2+	R1+ /R2+	R1	R2	Total	Positive
84	109	0	1	8	6.8	7.6	99.2	88.9
85	118	0	0	0	0.0	0.0	100	NE
86	117	0	0	1	0.8	0.8	100	100
87	113	2	0	3	4.2	2.5	98.3	60.0
89	104	2	2	10	10.2	10.2	96.6	71.4
90	106	0	2	10	8.5	10.2	98.3	83.3
91	115	0	1	2	1.7	2.5	99.2	66.7
97	118	0	0	0	0.0	0.0	100	NE
114	114	1	0	3	3.4	2.5	99.2	75.0

[a] HR-HPV represents HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

R1 = replicate 1; R2 = replicate 2. NE = not evaluable.