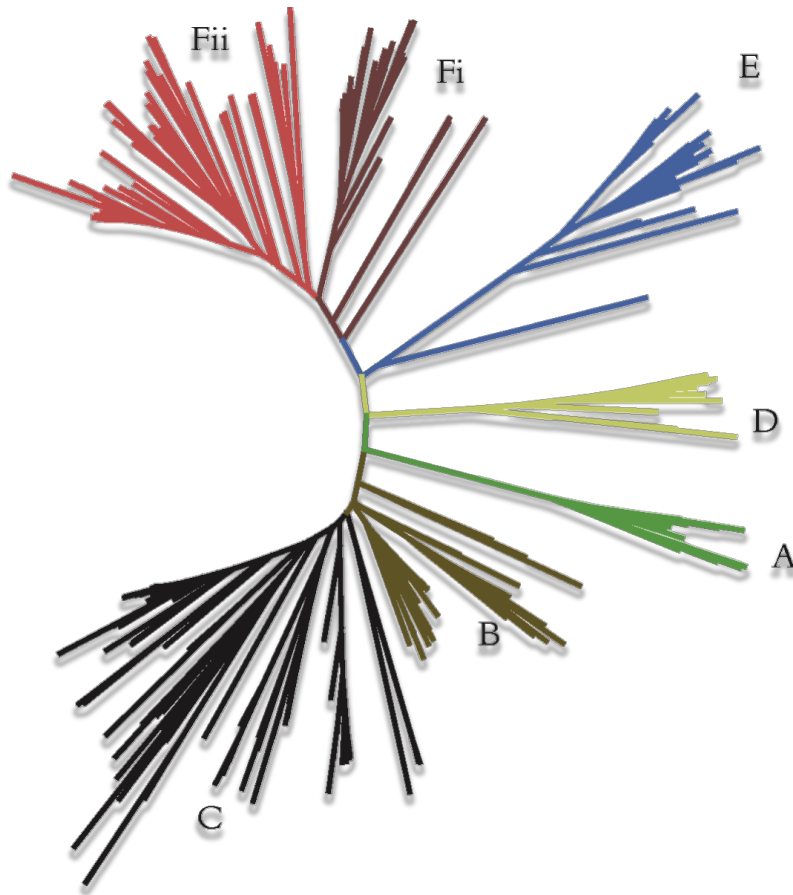
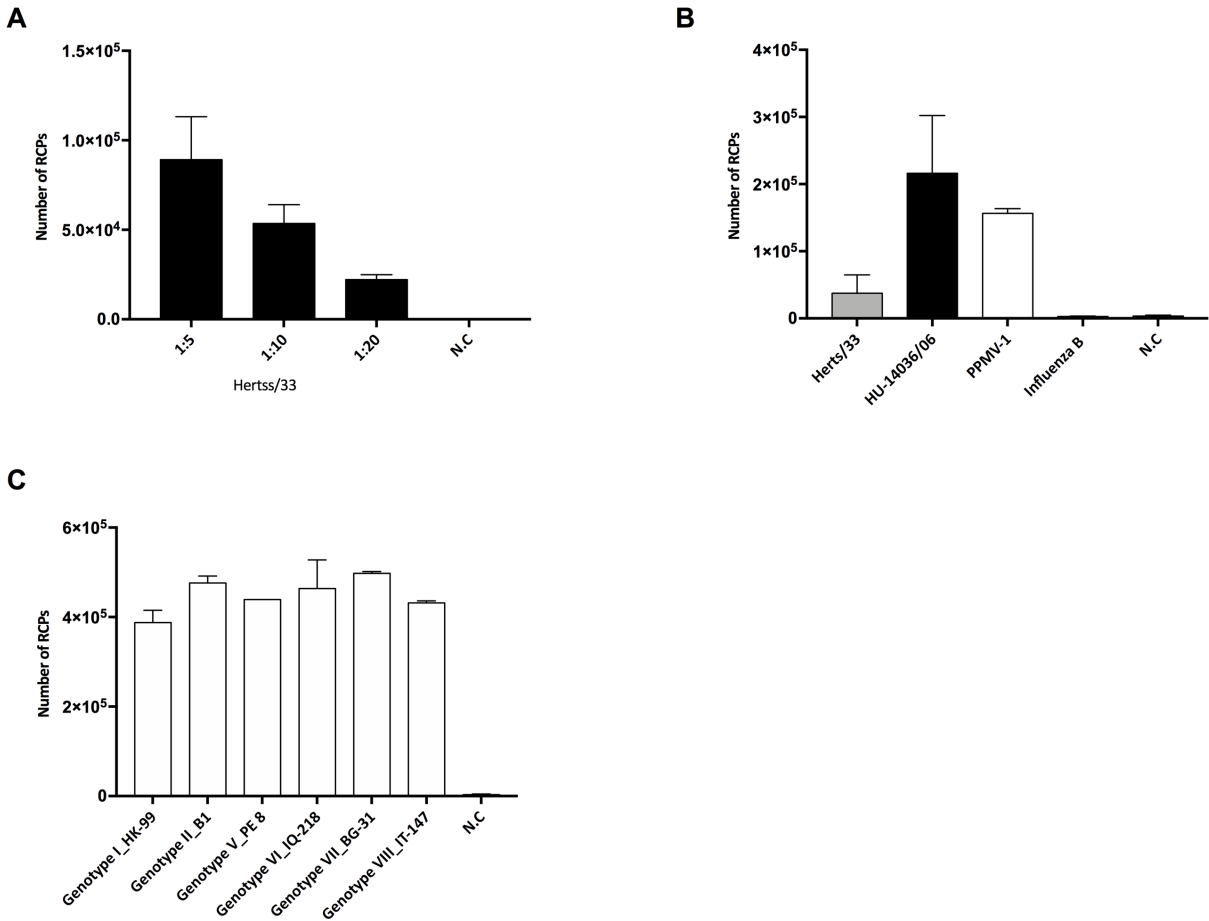


A novel mutation tolerant padlock probe design for multiplexed detection of hypervariable RNA viruses

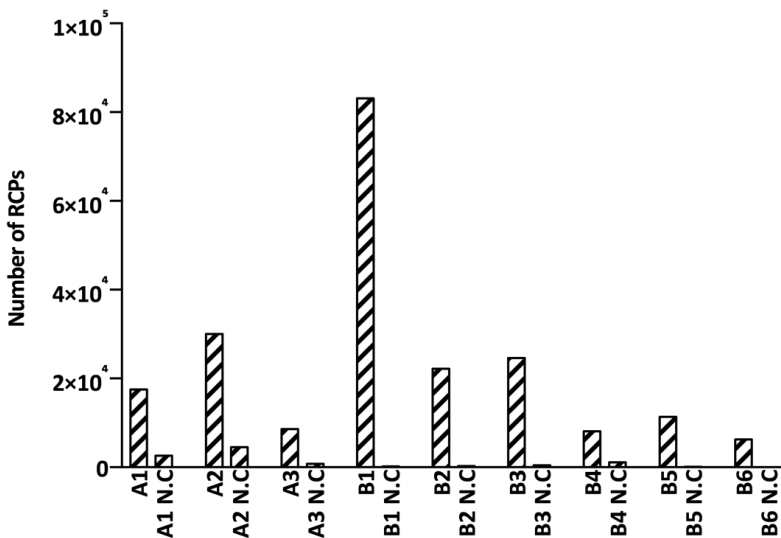
Sibel Ciftci¹, Felix Neumann¹, Iván Hernández-Neuta¹, Mikhayil Hakhverdyan², Ádám Bálint³, David Herthnek¹, Narayanan Madaboosi^{1*}, Mats Nilsson^{1*}



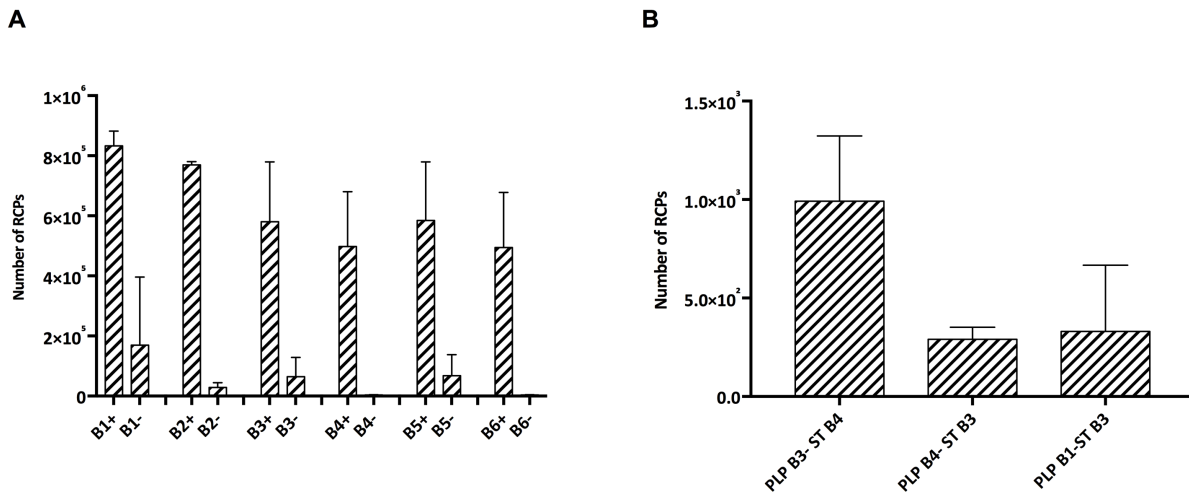
SI, Fig. S1: Phylogenetic tree construction over N-gene of IBV, and its groups (A, B, C, D, E, Fi, Fii).



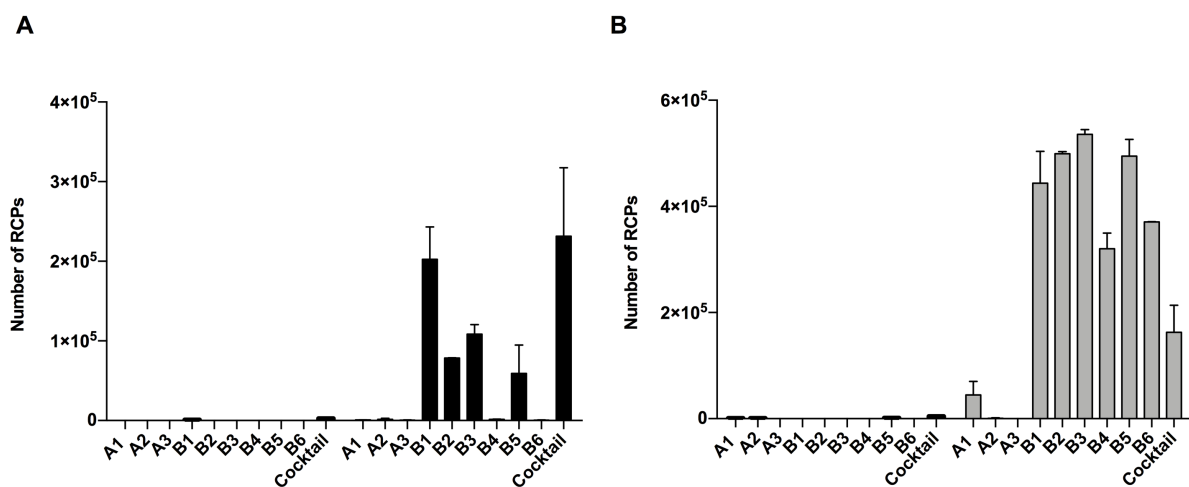
SI, Fig. S3: Optimization of RT-C2CA experiments. **A.** Different concentrations of cDNA from Herts/33 synthesized using SP (B1) and amplified using PLP (B1). **B.** 1:40 dilution of cDNA from different strains (along with Influenza B virus as specificity control), synthesized using RP and amplified using cocktail PLP. **C.** 1:10 dilution of cDNA from different strains, synthesized and amplified as in **B.**



SI, Fig. S4: PLP validation by RT-C2CA assay on their respected synthetic targets (100 fM) along with their negative controls (water instead of synthetic target).



SI, Fig. S5: RT-C2CA assay performed using synthetic targets (ST) at a concentration of 100 fM and cocktail PLP for the specific performance of the PLPs used. **A.** Use of specific PLPs, alongside showing the results after the elimination of the respective PLP from the cocktail (e.g., B1+ uses cocktail PLP for detecting B1 synthetic target; B1- has elimination of B1 PLP from the cocktail). **B.** Cross-reactivity of PLPs on synthetic targets.



SI, Fig. S6 Performance of individual and cocktail PLPs for C2CA validation using **A.** Herts/33 and **B.** HU-14036/06, along with their respective negative controls (water instead of template). cDNA (dilution of 1:20) synthesized by SP (B1 for Herts/33 and B3 for HU-14036/06) + RP was used.

SI, ST1. Synthetic targets used in this study

Name	5' modification	Sequence
NDV.A1	Biotin	TGCGAGAGGGGGWATTGAGGGGCTTTGCCAGAA
NDV.A2	Biotin	AAGACAGAGAGACTATCAGRTCAGATACTTTCTTCATCTA
NDV.A3	Biotin	TATATCGAGTGTCTCCATATGTCCATATATCAAATGACT
NDV.B1	Biotin	CAGGGAAATGGGGTCATTCARGATAGCATATCCTT
NDV.B2	Biotin	TGCAAAAGTATTGTCTTAAYTGGAGATATCAGACAGTCAA
NDV.B3	Biotin	GTGAAAACACTGTAATGTCYGTGTGCCAACATTGCATC
NDV.B4	Biotin	AGATTATGCCCGGAAYAGAAGCTGGTCACCTTTGACAGG
NDV.B5	Biotin	CACCTTACATTACATATCYAATGATTTCYCAAAGGCTATT
NDV.B6	Biotin	TGACTCGTGCTCAACAAAAATTSTAYATGAAAACCATAGG

SI, ST2. Table of oligonucleotides used for other avian viruses

Name	5' modification	Sequence
ILTV_PLP.1	Phosphate	GAAGGATTGATTTCTGGAACGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCGCCAACGTGAGCATCGTATAATAGCGTCTGGTCGATT
ILTV_PLP.2	Phosphate	CGACGTAAGAAGTACAACGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCGCCAACGTGAGCATCGTATACTCCGAATGGCCCGT
IBDV_PLP.1	Phosphate	GTCAAATTGTAGTTCGAGGTGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTCCGAGTGGTCACGAATACCCTGTGTCCCCACA
IBDV_PLP.2	Phosphate	CCACAGTCAAATTTGAGGTGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTCCGACTGGTCAGGTATACCTGACCCCTGTGTCCC
IBDV_PLP.3	Phosphate	CACTCAGTATCAATTTGTGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTCCGACTGGTCAGGTATAGCCAAGACGGTCCCTCT
IBDV_PLP.4	Phosphate	TTCTTGACGCGGCTCGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTCCGACTGGTCAGGTATAGTATGCAACCCGGCTCT
IBDV_PLP.5	Phosphate	TCTCTGCATGCACGTAGTGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTCCGACTGGTCAGGTATAGTATGCAACCCGGCTCT
IBV_PLP.1	Phosphate	CACCTGGTGAATGAACCTGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATAGATTAATGGGGTATTGATCAG
IBV_PLP.2	Phosphate	AAAGCATTGACCTCAGATGAGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATAGCAGGATGATGAAGTRGAT
IBV_PLP.3	Phosphate	GTTGCTGCAAAAGGGTGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATACAAGATGGTATAGTGTGG
IBV_PLP.4	Phosphate	AATGAGGARGGTATTAAGGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATATTTGGTATGACAAGATG
IBV_PLP.5	Phosphate	AATGAGGARGGTATTAAGGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATAAATTTGGTATGACAAGATG
IBV_PLP.6	Phosphate	GTGTGGTTTCTGCAAAAGGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATATAGCCAAGATGGTATA
IBV_PLP.7	Phosphate	GTGTGGTTTCTGCAAAAGGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATATAGCCAAGATGGTATA
IBV_PLP.8	Phosphate	TTTTGGAAGTAGAGTACGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATATAGTACCATGCTGTGTCT
IBV_PLP.9	Phosphate	CGTACTAAAGTAAAGGGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATATTGATAAAGTTTTGGCCCT
IBV_PLP.10	Phosphate	CCTGATAATGAAATCTTAAAGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATATTGATAAAGTTTGGGTT
IBV_PLP.11	Phosphate	GACCTGAATTTGGGGTATTGTGTATGCAGCTCCTCAGTAATAGTGTCTTACCCTGCCGTGTGTGAGATAAACAGGACAGCCGCT
AIV_PLP.1	Phosphate	CRCTGGGCACGGTGTGTATGCAGCTCCTCAGTAATAGTGTCTTACAATCAGTCATACGAGCCGCTGCAGTCCYCYT

SI, ST3. Concentrations of cDNA samples used for assay optimization.

Primer and cDNA conditions	RP	SSP	SP mix	RP + SP mix
Genotype IV_Herts/33	3.8 ng/μL	0.800 ng/μL	3.16 ng/μL	4.64 ng/μL
Genotype VII_HU-14036/06	7.00 ng/μL	4.60 ng/μL	8.20 ng/μL	12.3 ng/μL

SI, ST4. List and details of viral strains used in the study.

	Strain Name	Genotype /virus	NCBI	Virulence*	cDNA (ng/ μ l)	Copy number/ μ l	Provider
BATCH-I	HK-99	I	EU604269	L	1.87		SVA (Sweden)
	B1	II	AF309418	L	4.40		
	Herts/33	IV	FJ687487	V	3.8		
	PE-8	V	EU604263	V	1.62		
	IQ-218	VI	EU604254	M	3.70		
	BG-31	VII	EU604250	V	5.16		
	IT-147	VIII	EU604262	V	3.18		
BATCH-II	Ulster 2C	I	AY562991	L		10 ⁴	NEBIH (Hungary)
	La Sota	II	AJ629062	L		10 ⁵	
	Ph/80	III	FJ687488	M		10 ⁴	
	Herts/33	IV	FJ687487	V		10 ⁵	
	HU-206/81	V	-	V		10 ⁵	
	HU-202/16**	VI	-	M		10 ⁵	
	HU-14036/06	VII	-	V		10 ⁴	
	IT-147/94	VIII	EU604262	V		10 ⁴	
Non-NDV strains	Cevac LT L	ILTV	-			10 ⁴	NEBIH (Hungary)
	Hipragumboro-CH/80	IBDV	-			10 ⁶	
	11518/10	IBV	-			10 ⁷	
	A/duck/Hungary/5	AIV	-			10 ⁶	
	4494/16 (H5N8)						
	B/Stockholm/5/2014 (Victoria)	Influenza B	-			2x10 ⁴	Karolinska institute (Sweden)

* L: Lentogenic M: Mesogenic V: Velogenic
** PPMV-1: Pigeon paramyxovirus type 1
(<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2566108/>)