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PCR and LAMP Assays for African Swine Fever Virus and Foot-and-Mouth Disease Virus

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> Intro ----------

- ASFV and FMDV are spreading worldwide
- Pathogen evolution can accelerate assay signature erosion
- We present an application of our automated *in silico* pipeline for prototyping and evaluation of new and existing PCR and LAMP assays for subspecies identification

> Methods ----------

- Compute reference k-mer set for target organisms using the BioVelocity® perfect hash algorithm on a large RAM machine
- Generate PCR assays candidates based on overlapping k-mer signature regions
- Generate LAMP assays using conserved regions with NEB® LAMP Primer Design Tool
- Evaluate assay performance based on sequence alignment using the PCR Signature Erosion Tool (PSET) against the BLAST+ nt and env_nt databases

> Results ----------

- Species/Serotype-level PCR assays
- Species-level LAMP assays
- Predicted average precision and sensitivity of **99% for PCR assays**

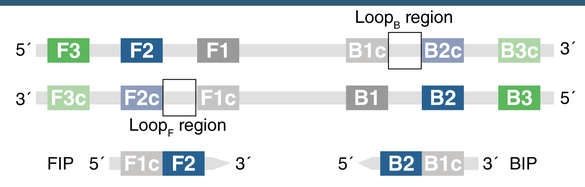
> Discussion ----------

- Pathogen evolution threatens outbreak response due to mutations in primer targets
- It is important to track the performance of existing assays and generate improved ones.
- Our pipeline is an efficient means to predict performance of hundreds of assays against millions of sequences.
- This work demonstrates its use towards the ongoing FMDV and ASFV outbreaks.

> Conclusion ----------

- PCR assays were automatically generated
- Process is semi-automated for LAMP assays, still need to run *in silico* evaluation
- Next steps include wet lab verification and field testing

Our automated pipeline generated **1,050 candidate PCR assays** with high predicted performance, including those targeting ASFV and FMDV serotypes.



SetID: ASFV_P8L135

	5′ pos	3′ pos	len	Tm	5′ dG	3′ dG	% GC	(required oligos are orange)
F3	973	991	19	60.11	-4.97	-4.55	53	GGGAAAAGGCACCACCATT
B3	1173	1191	19	59.27	-5.94	-5.88	53	CCGTTGATAACGGACACGT
FIP			39					TTAGGCGCGCTCAACAAGGTGGATAAGCCTTGCCGCACG
BIP			41					TACGGCGACCATTAAGGCCGATTAAACTTGGGGGACACGAC
LF	1021	1037	17	61.29	-7.02	-4.74	65	CCAGCGCCATCACAGAG
LB	1103	1123	21	65.48	-5.01	-5.90	57	GCAAAGCCCTATAGCCTGCAC
F2	1001	1018	18	60.59	-3.08	-7.51	61	GATAAGCCTTGCCGCACG
F1c	1048	1068	21	65.35	-4.93	-5.00	57	TTAGGCGCGCTCAACAAGGTG
B2	1142	1161	20	59.43	-3.01	-5.84	50	TTAAACTTGGGGGACACGAC
B1c	1081	1101	21	65.71	-6.26	-7.38	57	TACGGCGACCATTAAGGCCGA

SetID: FMDV_P1L214

	5′ pos	3′ pos	len	Tm	5′ dG	3′ dG	% GC	(required oligos are orange)
F3	14	33	20	60.11	-5.16	-5.08	50	AGGCTATCCTCTCCTTTGCA
B3	184	201	18	59.78	-4.14	-6.06	56	TTATGCGTCACCGCACAC
FIP			38					TCAGGTCCAGAGTGGACGGCCCGTGGGACCATACAGGA
BIP			41					CGAGTACCGGCGTCTCTTCGACACCCAACGCAGGTAAAGTG
LF	55	73	19	61.21	-6.25	-4.16	53	CTGCCACGGAGATCAACTT
LB	127	150	24	65.27	-5.84	-5.85	50	CAGGGTCTCTTTGAGATTCCAAGC
F2	36	53	18	59.79	-6.78	-5.25	61	CCGTGGGACCATACAGGA
F1c	79	98	20	65.53	-5.25	-7.03	65	TCAGGTCCAGAGTGGACGGC
B2	158	177	20	60.90	-6.01	-4.16	55	CACCCAACGCAGGTAAAGTG
B1c	99	119	21	65.36	-4.76	-5.04	62	CGAGTACCGGCGTCTCTTCGA

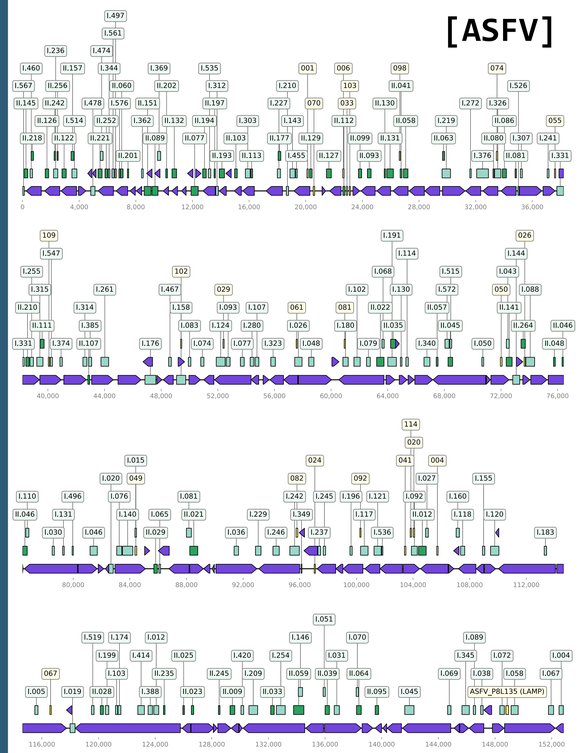
[Assay Regions]

Numeric/LAMP assay label target species.

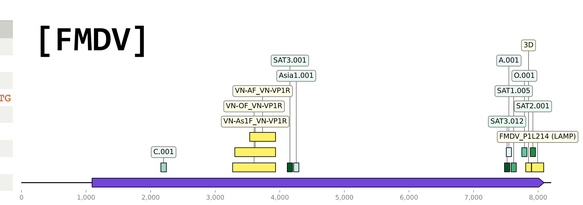
Prefix indicates serotype. From literature:

- **VN-*** (10.1016/j.jviromet.2011.04.027)
- **3D** (10.2460/javma.2002.220.1636)
- **I/II** (10.1111/tbed.14459)

[ASFV]



[FMDV]



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> References ----------

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figshare: Assay Files

ASFV

FMDV

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