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Virus nomenclature below the species level: a standardized nomenclature for filovirus strains and variants rescued from cDNA

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Abstract Specific alterations (mutations, deletions, insertions) of virus genomes are crucial for the functional characterization of their regulatory elements and their expression products, as well as a prerequisite for the creation of attenuated viruses that could serve as vaccine candidates. Virus genome tailoring can be performed either by using traditionally cloned genomes as starting materials, followed by site-directed mutagenesis, or by *de novo* synthesis of modified virus genomes or parts thereof. A systematic nomenclature for such recombinant viruses is necessary to set them apart from wild-type and laboratory-adapted viruses, and to improve communication and collaborations among researchers who may want to use recombinant viruses or create novel viruses based on them. A large group of filovirus experts has recently proposed nomenclatures for natural and laboratory animal-adapted filoviruses that aim to simplify the retrieval of sequence data from electronic databases. Here, this work is extended to include nomenclature for filoviruses obtained in the laboratory via reverse genetics systems. The previously developed template for natural filovirus genetic variant naming, <virus name> (<strain>/)<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>, is retained, but

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we propose to adapt the type of information added to each field for cDNA clone-derived filoviruses. For instance, the full-length designation of an Ebola virus Kikwit variant rescued from a plasmid developed at the US Centers for Disease Control and Prevention could be akin to "Ebola virus H.sapiens-rec/COD/1995/Kikwit-abc1" (with the suffix "rec" identifying the recombinant nature of the virus and "abc1" being a placeholder for any meaningful isolate designator). Such a full-length designation should be used in databases and the methods section of publications. Shortened designations (such as "EBOV H.sap/COD/95/Kik-abc1") and abbreviations (such as "EBOV/Kik-abc1") could be used in the remainder of the text, depending on how critical it is to convey information contained in the full-length name. "EBOV" would suffice if only one EBOV strain/variant/isolate is addressed.

Filovirus reverse genetics

Filoviruses are characterized by having linear, nonsegmented, single-stranded RNA genomes of negative polarity. The current filoviruses taxonomy is summarized in Table 1 [1, 16, 17].

Seven groups have reported the establishment of filovirus reverse genetics systems in their laboratories [11] to rescue viruses directly related to wild-type or laboratory-adapted Ebola virus (EBOV) variant Mayinga [12, 27, 33, 34], wild-type Marburg virus (MARV) variant Musoke [5, 15], and Reston virus (RESTV) variant Pennsylvania [8]. A filovirus reverse genetics system was first described in 2001 by Volchkov et al. [34]. Subsequently, the established EBOV cDNA clone was mutated to evaluate the role of co-transcriptional editing in EBOV replication [34, 35]; particular phosphorylation sites of filoviral protein VP30 in transcription re-initiation/replication [21, 22]; viral protein VP35 in EBOV guinea pig adaptation [29]; and viral protein VP24 in nucleocapsid assembly [23] and EBOV rodent adaptation [24]. In addition, the established EBOV cDNA clone was used to create an enhanced green fluorescent protein (eGFP)-expressing reporter virus [21].

In 2002, Neumann et al. [27] established a similar reverse genetics system, demonstrating that EBOV could be rescued from plasmids encoding the EBOV genome or antigenome. Neumann's antigenomic system was then used to evaluate the importance of furin cleavage of the EBOV surface spike glycoprotein $GP_{1,2}$ on replication [27], the role of late-budding motifs located in viral protein VP40 in filovirus egress [28], the difference between wild-type and mouse-adapted EBOV [3], and the possibility of rescuing recombinant viruses using heterotypic support proteins [32]. Finally, a reporter gene (*eGFP*) was inserted into the EBOV genome for better tracking of infection [4].

In 2005, Towner et al. [33] published the expression of an additional transcription unit from a full-length EBOV genome. Towner et al. [33] created an eGFP-expressing EBOV and evaluated the role of several conserved $GP_{1,2}$ amino acid residues in EBOV cell entry [26] and of an interferon-response-inhibiting domain of VP35 [9, 10]. In 2012, Hoenen et al. [12] created a recombinant EBOV to study the intracellular localization of its viral polymerase L tagged with mCherry, to study the modulation of gene translation and virus replication by untranslated genomic regions [31], and to create a luciferase-expressing EBOV [13]. The eGFP-

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expressing EBOV full-length clone generated by Towner et al. was also used for evaluation of introduced point mutations into the various filovirus proteins [20].

The first MARV reverse genetics system was published in 2006 by Enterlein et al. [5]. Thus far, this system has been used to demonstrate that VP30 is required for MARV rescue [5], to characterize the MARV replication promoter [6], and to create an eGFP-expressing variant for drug screening purposes [30]. Krähling et al. [15] and Mittler et al. [25] followed a similar approach for the screening of various cell types as model systems to investigate MARV infections *in vitro* and to evaluate functions of the MARV $GP_{1,2}$ cytoplasmic tail in the viral life cycle.

Finally, Groseth et al. described a RESTV reverse genetics system in 2012 and created EBOV/RESTV chimeras to assess the role of $GP_{1,2}$ in virulence differences observed between the two viruses [8].

Systematic nomenclature for recombinant filoviruses

A large group of filovirus experts has recently established definitions and a consistent nomenclature for natural and laboratory-animal-adapted filovirus strains, variants, and isolates [18, 19]. This group also established templates for naming individual filovirus strains, variants, and isolates for a) Materials and Methods sections of manuscripts (full-length designations), b) alignment and phylogram figures (shortened designations), and c) flow-text (abbreviations) [18, 19]. These templates are generally organized in the order <virus name> (<strain>/)<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>. Suffixes point out whether a virus has been directly sequenced from a clinical specimen ("-wt"), has undergone tissue/cell culture passaging ("-tc"), is known from sequence fragments only ("-frag") or has been lost ("-hist") [19]. The suffix "-lab" is to be used when a filovirus was adapted in the laboratory to cells or animals it would not normally infect [18]. Accordingly, the double suffix "hist_lab" ought to be used if a filovirus was adapted to a non-natural host but is not available for study anymore.

Here, we propose extending this nomenclature to recombinant filoviruses experimentally derived from cDNA:

> Definition of "recombinant filovirus":
> A recombinant filovirus is any filovirus that has been rescued from a cDNA encoding its entire genome, i.e., any filovirus that is not derived directly from an ancestor virion infecting a cell, and any filovirus that ultimately evolved or is derived from a filovirus rescued from cDNA. The genome of a recombinant filovirus may be identical to that of a natural or laboratory animal-adapted filovirus, or it may contain artificially introduced mutations, insertions, deletions, or rearrangements not due to the evolution of the virus population in natural or laboratory organisms or derived tissue cultures. Virion-like particles (VLPs) that do not contain all filovirus elements known to be necessary to establish a self-sufficient infectious system (e.g., particles derived from filovirus "minigenomes" or filovirus genomes lacking individual vital genes) are not considered recombinant filoviruses.
>
> Definition of "recombinant filovirus strain":
> A recombinant filovirus strain is a genetically stable recombinant filovirus variant that causes a

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significantly different phenotype of infection compared to a wild-type or laboratory-adapted standard virus. The extent of genomic sequence variation is irrelevant for the classification of a variant as a strain.

Definition of "recombinant filovirus variant":
A recombinant filovirus variant is a recombinant filovirus that differs in its genomic sequence from that of wild-type or laboratory-adapted standard by $\leq$10 % (in its aligning parts) and does not necessarily cause a different phenotype of infection. All recombinant filovirus strains are recombinant filovirus variants, but most recombinant filovirus variants are not recombinant filovirus strains.

Definition of "recombinant filovirus isolate":
A recombinant filovirus isolate is an instance of a particular recombinant filovirus strain or variant. Isolates can be identical or slightly different in sequence from each other. Both identical and slightly different isolates may represent a particular recombinant filovirus strain or variant.

Problems of and solutions for naming cDNA-clone-derived filoviruses

We propose to designate recombinant filovirus laboratory strains/variants/isolates according to the templates that were designed for natural filovirus and filovirus laboratory-animal-adapted strains/variants/isolates [18, 19]. Two opposing scenarios for filoviruses rescued from cDNA can be imagined, and both would have to be accommodated by a nomenclature system.

Naming of rescued versions of wild-type filoviruses and close derivatives

At one end of the spectrum lies a virus genome that has been assembled by cloning sequence fragments from a replicating wild-type or laboratory-adapted filovirus. The genome-encoding plasmid contains a sequence identical to the wild-type or laboratory animal-adapted consensus filovirus sequence and is available from GenBank. For instance, the virus to be cloned could be:

Full	Ebola virus H.sapiens-tc/COD/1995/Kikwit-9510621
Shortened	EBOV/Hsap/COD/95/Kik-9510621
Abbreviated	EBOV/Kik-9510621 [18]

The virus rescued from the plasmid created based on this parental virus may have a genomic sequence identical to that encoded by the plasmid or may contain a limited number of mutations. The name for this recombinant virus should ideally reflect its derivation, while at the same time clarifying that it is a recombinant virus. We therefore recommend such a virus to be called

Full	Ebola virus H.sapiens-rec/COD/1995/Kikwit-*isolate name*
Shortened	EBOV/Hsap/COD/95/Kik-*isolate name*
Abbreviated	EBOV/Kik-*isolate name*

The suffix "-rec" points towards the cDNA-cloned (recombinant) nature of the virus. The variant designation "Kikwit" implies that the rescued virus falls into the overall class of Kikwit isolates (i.e., the Kikwit variant), and a unique isolate name separates the new virus from already known isolates. The isolate name should be chosen by the team of researchers creating it. As explained previously, the <strain> field should remain empty except if the rescued virus fulfills the previously outlined criteria for a "strain" [18, 19]. We further advise that the full or near-complete sequence of the rescued virus be deposited in the GenBank database and, ideally, that the sequence is associated with a peer-reviewed publication. The "/Notes" field of the GenBank entry should be filled with information about the clone (for instance: "derived from GenBank #AF_1234.2" or specific mutations, deletions or insertions characteristic for the virus). Importantly, the isolate name should be short and succinct. The isolate name does not necessarily have to convey meaning, although, of course, it is preferable if it does.

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Table 1 Summary of the current filovirus taxonomy as endorsed by the ICTV *Filoviridae* Study Group and accepted by the ICTV

Current taxonomy and nomenclature (Ninth ICTV Report and updates) [1, 16, 17]	Previous taxonomy and nomenclature (Eighth ICTV Report) [7]
Order *Mononegavirales*	Order *Mononegavirales*
Family *Filoviridae*	Family *Filoviridae*
Genus *Marburgvirus*	Genus *Marburgvirus*
Species *Marburg marburgvirus*	Species *Lake Victoria marburgvirus*
Virus 1: Marburg virus (MARV)	Virus: Lake Victoria marburgvirus (MARV)
Virus 2: Ravn virus (RAVV)	
Genus *Ebolavirus*	Genus *Ebolavirus*
Species *Taï Forest ebolavirus*	Species *Cote d'Ivoire ebolavirus* [sic]
Virus: Taï Forest virus (TAFV)	Virus: Cote d'Ivoire ebolavirus [sic] (CIEBOV)
Species *Reston ebolavirus*	Species *Reston ebolavirus*
Virus: Reston virus (RESTV)	Virus: Reston ebolavirus (REBOV)
Species *Sudan ebolavirus*	Species *Sudan ebolavirus*
Virus: Sudan virus (SUDV)	Virus: Sudan ebolavirus (SEBOV)
Species *Zaire ebolavirus*	Species *Zaire ebolavirus*
Virus: Ebola virus (EBOV)	Virus: Zaire ebolavirus (ZEBOV)
Species *Bundibugyo ebolavirus*	
Virus: Bundibugyo virus (BDBV)	
Genus *Cuevavirus**	
Species *Lloviu cuevavirus**	
Virus: Lloviu virus (LLOV)	

* These taxa were approved by the ICTV Executive Committee in July 2013 but have not yet been ratified

Naming of rescued versions of complex mosaic or chimeric filoviruses

At the other end of the spectrum lies a (hypothetical) virus genome that has very little resemblance to any archived filovirus sequence. For instance, the rescued virus could be a mouse-adapted mosaic of two MARV, three EBOV, and two Lloviu (LLOV) genes; with two of those genes codon-optimized for expression in bat cells. This virus could contain one additional gene cassette expressing eGFP and have the mCherry open reading frame fused to VP40. All untranslated regions could be standardized to the same sequence, and two gene overlaps could have been resolved. The cDNA encoding the genome of this virus could have been assembled entirely from synthetic oligonucleotides.

It is important to keep such a mosaic virus in mind, as any nomenclature system should work prospectively, and filovirus cDNA clones will certainly become more complex with time. Less-complex filovirus genome mosaics have already been created to elucidate the mechanism behind EBOV adaptation to mice [3] or to find an answer to the question why RESTV does not seem to be virulent for humans [8]. Very complex mosaic and chimeric viruses have been created for other viruses (e.g., HIV-1) and are indicators for possible future developments in filovirology.

Three major problems arise in designating a mosaic virus. First, since such a virus is completely artificial, a parental (wild-type or laboratory-adapted) virus is not necessarily obvious. Consequently, variant and isolate names need to be chosen by the research team that created the virus. Second, because there is no parental virus, the "isolation host" field cannot contain an organism name. We therefore propose to fill this field with information about the cell type from which the virus was rescued. Third, depending on the complexity of the virus, it may not even be apparent whether the rescued entity is a Marburg virus, an Ebola virus or a Lloviu virus, or even whether it is a marburgvirus or an ebolavirus (note that the ICTV does not permit actual classification of recombinant viruses). To solve this problem, we propose to use the actual sequence cutoffs for the various filovirus taxa outlined in ref. 16 to determine which term should be used for the "virus name" field. For instance, if

- the new virus was rescued at USAMRIID in the USA in 2012 from Vero E6 cells,
- the genome of the virus fulfills the criteria of being a filovirus but differs from the type viruses of the type species of the three filovirus genera by $\geq$50 %,
- and assuming that the rescued virus, because of its extensive modification, fulfills the criteria for being a filovirus "strain" [18, 19],

then the name of this virus could be

Full	filovirus USAMRIID/Vero_E6-rec/USA/2012/Weirdo-abc2
Shortened	filovirus/USAMRIID/Vero_E6/USA/12/Weirdo-abc2
Abbreviated	filovirus/Wei-abc2

with "Weirdo" and "abc2" being the variant and isolate names chosen by the researchers who synthesized the genome and rescued the virus. Alternatively, if

- the new virus was rescued at DSTL in the UK in 2013 from HeLa cells,
- the genome of the virus fulfills the criteria of being a filovirus but differs from the type virus of the type species of the genus *Marburgvirus* (Marburg virus) by <50 % but >30 %,
- and assuming that the rescued virus does not fulfill the criteria for being a filovirus "strain" [18, 19],

then the name of this virus could be

Full	marburgvirus HeLa-rec/GBR/2013/Strange-abc3
Shortened	marburgvirus/HeLa/GBR/13/Strange-abc3
Abbreviated	marburgvirus/Strange-abc3

Proposed designations of recombinant filoviruses

Full-length designation

<virus name> (<strain>/)<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>

- the **virus name** should be given in full, as outlined recently [16, 17]. For instance: "Marburg virus," "Ebola virus," "Sudan virus". Depending on sequence divergence, the virus name could also be "ebolavirus", "marburgvirus", "cuevavirus" or "filovirus"
- the **strain** field should contain the abbreviation of the institute at which the strain was developed (for institute designations, see ref. [18]). The field should remain empty if the virus in question does not fulfill the previously outlined criteria for a filovirus "strain" [18, 19]
- the **isolation host** should be provided in one word in the format "First letter of genus name.full name of species descriptor" of the host, but remain unitalicized to denote the fact that the virus was isolated from an entity and not from a taxon [2]. For instance: "H.sapiens" (member of the species *Homo sapiens*). Laboratory mice and some other laboratory animals cannot be assigned to a species. Consequently, this field should be filled with the official strain designation of the animal used for the experiments – in the case of laboratory mice and laboratory rats in accordance with the most recent "Guidelines for Nomenclature of Mouse and Rat Strains", e.g. "C57BL/6" or "BALB/c" [14]. In the case of a completely artificial filovirus, the field should contain the name of the cell line from which the virus was rescued. For instance: "Vero_E6" or "HEK_293T" (spaces replaced with "_")
- the **country of sampling** field should contain the same information as provided in the field for the natural (wild-type) virus. In the case of a completely artificial filovirus, the field should pertain to the country in which the virus was created
- the **year of sampling** field should contain the same information as provided in the field for the natural (wild-type) virus. In the case of a completely artificial filovirus, the field should pertain to the year in which the virus was created
- the **genetic variant designation-isolate designation** field should contain the same information as provided in the same field for the natural (wild-type) virus, connected by a hyphen to a laboratory isolate descriptor. The isolate descriptor should be unique to separate the new virus from already known isolates. For instance: "Kikwit-abc1". In the case of a completely artificial filovirus, a unique variant name should be chosen. For instance, "Weirdo"

Examples for full-length designations of isolates in the methods sections of manuscripts:

"Ebola virus H.sapiens-rec/COD/1995/Kikwit-abc1" or "filovirus USAMRIID/Vero_E6-rec/USA/2012/Weirdo-abc2".

Shortened designation

<virus name abbreviation> (<strain>/)<isolation host-suffix>/<country of sampling>/<year of sampling>/<genetic variant designation>-<isolate designation>

- the **virus name** abbreviation should be accepted by the ICTV *Filoviridae* Study Group, as outlined recently [16, 17]. For instance: "MARV," "EBOV," "SUDV". Depending on sequence divergence, the virus name could also be "ebolavirus", "marburgvirus", "cuevavirus" or "filovirus" (no abbreviations)
- the **strain** field should contain the abbreviation of the institute at which the strain was developed (for institute designations see ref. [18]). The field should remain empty if the virus in question does not fulfill the previously outlined criteria for a filovirus "strain" [18, 19]
- the **isolation host** should be provided in a four-letter format "First letter of genus name.first three letters of

species descriptor" of the host. For instance: "C.por" (member of the species *Cavia porcellus*). Laboratory mice and some other laboratory animals cannot be assigned to a species. Consequently, this field should be filled with the official strain designation abbreviation of the animal used for the experiments – in the case of laboratory mice and laboratory rats in accordance with the most recent "Guidelines for Nomenclature of Mouse and Rat Strains". For instance, "B6" for C57BL/6 mouse strains or "C" for "BALB/c" mouse strains [14]. In the case of a completely artificial filovirus, the field should contain the name of the cell line from which the virus was rescued. For instance: "Vero_E6" or "HEK_293T" (spaces replaced with "_")

- the **country of sampling** field should contain the same information as provided in the field for the natural (wild-type) virus. In the case of a completely artificial filovirus, the field should indicate the country in which the virus was created
- the **year of sampling** field should contain the same information as provided in the field for the natural (wild-type) virus. In the case of a completely artificial filovirus, the field should indicate the year in which the virus was created
- the **genetic variant designation-isolate designation** should contain the same information as provided in the field for the natural (wild-type) virus, connected by a hyphen to an isolate abbreviation, e.g., "Kik-abc1". The isolate descriptor should be unique to separate the new virus from already known isolates. In the case of a completely artificial filovirus, an abbreviation of the unique variant name should be chosen. For instance "Wei"

Examples for the shortened designations of isolates in the methods sections of manuscripts:

"EBOV H.sap/COD/95/Kik-abc1" or "filovirus/USAMRIID/Vero_E6/USA/12/Weirdo-abc2".

Abbreviation

<virus abbreviation>/<genetic variant designation-isolate designation>

- the **virus abbreviation** should be one accepted by the ICTV *Filoviridae* Study Group, as outlined recently [16, 17]. For instance: "MARV," "EBOV," "SUDV". Depending on sequence divergence, the virus name could also be "ebolavirus", "marburgvirus", "cuevavirus" or "filovirus" (no abbreviations)
- the **genetic variant designation-isolate designation** should contain the same information as provided in the field for the natural (wild-type) virus, connected by a hyphen to an isolate abbreviation, e.g., "Kik-abc1". The isolate descriptor should be unique to separate the new virus from already known isolates. In the case of a completely artificial filovirus, an abbreviation for the unique variant name should be chosen. For instance "Wei"

Examples for abbreviations in the text of a manuscript: "EBOV/Kik-abc1" or "filovirus/Wei-abc2" (if other isolates of the same genetic strain/variant/isolate are addressed in the same article); or simply "EBOV" or "filovirus" (if the article only addresses work with one particular genetic strain/variant/isolate).

Usage of designations

We suggest that full-length isolate designations always be used once in the Materials and Methods section of a manuscript next to the taxonomic placement of the closest natural isolate (family, species) and its passaging history. Examples of placement of these designations are:

> Vero E6 cells in 24-well plates were infected for 1 h with Ebola virus H.sapiens-rec/COD/1995/Kikwit-abc1 (GenBank #AF1234; derived from an Ebola virus of the family *Filoviridae*, species *Zaire ebolavirus*) at an MOI of 0.5, 1, or 5. Virus was created by transfecting BSR T7/5 cells with plasmids encoding the viral genome and proteins NP, VP35, VP30, L, and T7 RNA polymerase as described previously.

or

> HEK 293T cells in 6-well plates were infected for 1 h with filovirus USAMRIID/Vero_E6-rec/USA/2012/Weirdo-abc2 (henceforth "Weirdo") at an MOI of 5. The genome of Weirdo (GenBank #AF5678) was synthesized commercially by company X, and the virus was rescued by following standard filovirus rescue protocols using Vero E6 cells.

We urge investigators not to use taxon (italicized) names elsewhere in the manuscript, as artificially created organisms currently do not have taxonomic standing. We further suggest using only the virus abbreviation or a designation determined by the authors in the remainder of the manuscript text (in the examples above, "EBOV" or "Weirdo") after proper introduction as long as no other version of the same virus or another filovirus of the same species is addressed. We recommend using abbreviations in cases where several variants or isolates of one filovirus are addressed:

> Here we demonstrate that infection of rhesus monkeys with EBOV/Kik-abc1 results in clinical signs indistinguishable from animals infected with EBOV/Kik-GFP1.

In the example above, it would also be unproblematic to only differentiate between "abc1" and "GFP1" viruses in the flow of text. Of course, the authors would be free to introduce their own abbreviations for the viruses used for the sake of manuscript writing. We propose to limit the use of shortened designations to phylograms and sequence alignments or to replace them with abbreviations if space is limited.

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