

GENOME SEQUENCING OF AmpliRun® CONTROLS – MOLECULAR CONTROLS

1. Introduction:

Vircell S.L. has performed genome sequencing for AmpliRun® - DNA/RNA Controls products. This advancement reinforces our commitment to quality and innovation.

2. Description of the action:

Next-generation sequencing (NGS) is an advanced technology that makes it possible to analyze large amounts of DNA or RNA, in a massively parallel manner. This technique has revolutionized the study of genomes by enabling rapid and accurate reading of millions of DNA sequences (1).

NGS is used to identify and characterize microorganisms, detect genetic variants and study their evolution (2). This technology allows us to advance in the genetic characterization of the AmpliRun® - DNA/RNA Controls product line. Vircell S.L.'s initiative aims to respond to a critical need of the scientific community: to guarantee complete traceability and quality in public genomic databases, which sometimes present inconsistencies in the metadata or inconsistencies in the reference genomes (3-4).

Therefore, Vircell S.L. has implemented a systematic strategy of whole genome sequencing and sequencing of genes of high importance in the molecular diagnosis of AmpliRun® products, using rigorous quality standards in sequencing methodologies and bioinformatics analysis.

This action seeks to:

- **Ensure authenticity and traceability:** each sequenced genome is directly linked to the physical controls in our catalog, eliminating uncertainties generated by incorrect or incomplete reference genomes.
- **Identify and resolve inconsistencies:** By comparing the generated sequence with public databases, we evaluate and correct discrepancies in metadata and genome assemblies.
- **Contribute to the improvement of public databases:** Genomes deposited at NCBI contribute to reducing errors in reference sequences and improve confidence in these tools.

Vircell S.L.'s commitment to quality and transparency ensures that customers receive reliable products, with accurate genetic information aligned with the current needs of biomedical research and molecular diagnostics.

3. Methodology:

Our process includes the following steps

- **Nucleic acid extraction:** If necessary, DNA/RNA from the was extracted using commercial kits following the manufacturer's instructions.
- **Library preparation:** A multiplex PCR was designed following an amplicon strategy to cover the whole genome or specific genes. Libraries were prepared from various amounts of previously amplified DNA/CDNA using a specific Illumina library preparation kit.

- **Sequencing:** Sequencing is performed on the iSeq100® System sequencer, using a read length of 2x150 bp.
- **Bioinformatics analysis:** Subsequently, a comprehensive bioinformatics analysis is performed to assemble the sequences and analyze the data obtained using the nf-core/viralrecon (5) and nf-core/bactmap (6) bioinformatics pipelines, depending on the type of microorganism, both within the Nextflow workflow (7). These analyses include sequence alignment against a reference genome, identification of genetic variants, and interpretation of the data taking into account the Fisher statistical test (8). Additionally, Interactive Genomics Viewer (IGV) (9) was used for data analysis and visualization.

This method guarantees accurate and reproducible results, providing our customers with detailed and reliable genetic data.

4. Results and Applications:

The obtained sequences have been deposited in the National Center for Biotechnology Information (NCBI) database. The NCBI committee has evaluated the submitted sequences and assigned an accession number. These accession numbers have been published on the Vircell S.L. website and are available below.

Table 1: List of microorganisms with published complete or partial genome sequences.

REFERENCE	PRODUCT	NO. NCBI ACCESS	LINK
MBC001	AMPLIRUN® ADENOVIRUS DNA CONTROL	PP386306	AMPLIRUN® ADENOVIRUS DNA CONTROL Vircell
MBC003	AMPLIRUN® SALMONELLA ENTERITIDIS DNA CONTROL	CP173222	AMPLIRUN® SALMONELLA DNA CONTROL Vircell
MBC007	AMPLIRUN® BORDETELLA PARAPERTUSSIS DNA CONTROL	CP173223	AMPLIRUN® BORDETELLA DNA CONTROL Vircell
MBC008	AMPLIRUN® BORDETELLA PERTUSSIS DNA CONTROL	CP142851	AMPLIRUN® BORDETELLA DNA CONTROL Vircell
MBC011	AMPLIRUN® CHLAMYDOPHILA PNEUMONIAE DNA CONTROL	CP160064	AMPLIRUN® CHLAMYDOPHILA PNEUMONIAE DNA CONTROL Vircell
MBC012	AMPLIRUN® CHLAMYDIA TRACHOMATIS DNA CONTROL	CP142852	AMPLIRUN® CHLAMYDIA TRACHOMATIS DNA CONTROL Vircell
MBC016	AMPLIRUN® CYTOMEGALOVIRUS DNA CONTROL	PP386308	AMPLIRUN® CYTOMEGALOVIRUS DNA CONTROL Vircell
MBC018	AMPLIRUN® COXIELLA BURNETII DNA CONTROL	CP177352	AMPLIRUN® COXIELLA DNA CONTROL Vircell
MBC019	AMPLIRUN® ENTEROVIRUS 71 RNA CONTROL	PP101991	AMPLIRUN® ENTEROVIRUS RNA CONTROL Vircell
MBC023	AMPLIRUN® HERPES SIMPLEX 1 DNA CONTROL	PQ569920	AMPLIRUN® HERPES SIMPLEX DNA CONTROL Vircell
MBC024	AMPLIRUN® HERPES SIMPLEX 2 DNA CONTROL	PP386309	AMPLIRUN® HERPES SIMPLEX DNA CONTROL Vircell
MBC025	AMPLIRUN® HHV-6 DNA CONTROL	PP921919	AMPLIRUN® HHV DNA CONTROL Vircell
MBC026	AMPLIRUN® ROTAVIRUS RNA CONTROL	PP101947	AMPLIRUN® ROTAVIRUS RNA CONTROL Vircell
		PP101948	
		PP101949	
		PP101950	
		PP101951	
		PP101952	
		PP101953	
		PP101954	
		PP101955	
		PP101956	

		PP101957		
MBC027	AMPLIRUN® YERSINIA ENTEROCOLITICA DNA CONTROL	CP173224		AMPLIRUN® YERSINIA DNA CONTROL Vircell
MBC028	AMPLIRUN® INFLUENZA A H1 RNA	Segment 1	PP825741	AMPLIRUN® INFLUENZA RNA CONTROL Vircell
		Segment 2	PP825742	
		Segment 3	PP825743	
		Segment 4	PP825744	
		Segment 5	PP825745	
		Segment 6	PP825746	
		Segment 7	PP825747	
		Segment 8	PP825748	
MBC029	AMPLIRUN® INFLUENZA A H3 RNA	Segment 1	PP815005	AMPLIRUN® INFLUENZA RNA CONTROL Vircell
		Segment 2	PP815006	
		Segment 3	PP815007	
		Segment 4	PP815008	
		Segment 5	PP815009	
		Segment 6	PP815010	
		Segment 7	PP815011	
		Segment 8	PP815012	
MBC030	AMPLIRUN® INFLUENZA B RNA CONTROL	Segment 1	PP815638	AMPLIRUN® INFLUENZA RNA CONTROL Vircell
		Segment 2	PP815639	
		Segment 3	PP815640	
		Segment 4	PP815641	
		Segment 5	PP815642	
		Segment 6	PP815643	
		Segment 7	PP815644	
		Segment 8	PP815645	

MBC038	AMPLIRUN® PARAINFLUENZA 2 RNA CONTROL	PP101995	AMPLIRUN® PARAINFLUENZA RNA CONTROL Vircell
MBC039	AMPLIRUN® PARAINFLUENZA 3 RNA CONTROL	PP101994	AMPLIRUN® PARAINFLUENZA RNA CONTROL Vircell
MBC040	AMPLIRUN® MUMPS RNA CONTROL	PP818853	AMPLIRUN® MUMPS RNA CONTROL Vircell
MBC041	AMPLIRUN® RESPIRATORY SYNCYTIAL VIRUS (subtype A) RNA CONTROL	PP922776	AMPLIRUN® RESPIRATORY SYNCYTIAL VIRUS RNA CONTROL Vircell
MBC045	AMPLIRUN® TICK-BORNE ENCEPHALITIS VIRUS RNA CONTROL	PP862726	AMPLIRUN® TICK BORNE ENCEPHALITIS VIRUS RNA CONTROL Vircell
MBC046	AMPLIRUN® MEASLES RNA CONTROL	PP818854	AMPLIRUN® MEASLES RNA CONTROL Vircell
MBC048	AMPLIRUN® VARICELLA-ZOSTER VIRUS DNA CONTROL	PP386307	AMPLIRUN® VARICELLA-ZOSTER VIRUS DNA CONTROL Vircell
MBC050	AMPLIRUN® PARAINFLUENZA 4 A RNA CONTROL	PP101996	AMPLIRUN® PARAINFLUENZA RNA CONTROL Vircell
MBC051	AMPLIRUN® BK VIRUS DNA CONTROL	PP921918	AMPLIRUN® BK VIRUS DNA CONTROL Vircell
MBC055	AMPLIRUN® DENGUE 1 VIRUS RNA CONTROL	PP815631	AMPLIRUN® DENGUE RNA CONTROL Vircell
MBC056	AMPLIRUN® DENGUE 2 VIRUS RNA CONTROL	PP815632	AMPLIRUN® DENGUE RNA CONTROL Vircell
MBC057	AMPLIRUN® DENGUE 3 VIRUS RNA CONTROL	PP815633	AMPLIRUN® DENGUE RNA CONTROL Vircell
MBC058	AMPLIRUN® DENGUE 4 VIRUS RNA CONTROL	PP815634	AMPLIRUN® DENGUE RNA CONTROL Vircell
MBC061	AMPLIRUN® COXSACKIE B1 RNA CONTROL	PP025903	AMPLIRUN® COXSACKIE RNA CONTROL Vircell
MBC062	AMPLIRUN® COXSACKIE B5 RNA CONTROL	PQ570124	AMPLIRUN® COXSACKIE RNA CONTROL Vircell
MBC063	AMPLIRUN® COXSACKIE A6 RNA CONTROL	PQ530051	AMPLIRUN® COXSACKIE RNA CONTROL Vircell
MBC064	AMPLIRUN® ECHOVIRUS 5 RNA CONTROL	PP862731	AMPLIRUN® ECHOVIRUS RNA CONTROL Vircell
MBC065	AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL	PQ863711	AMPLIRUN® EPSTEIN-BARR VIRUS DNA CONTROL Vircell
MBC069	AMPLIRUN® WEST NILE VIRUS RNA CONTROL	PP862727	AMPLIRUN® WEST NILE VIRUS RNA CONTROL Vircell
MBC070	AMPLIRUN® STREPTOCOCCUS PNEUMONIAE DNA CONTROL	CP173225	AMPLIRUN® STREPTOCOCCUS PNEUMONIAE DNA CONTROL Vircell
MBC072	AMPLIRUN® ZIKA VIRUS RNA CONTROL	PP921916	AMPLIRUN® ZIKA VIRUS RNA CONTROL Vircell
MBC075	AMPLIRUN® NEISSERIA GONORRHOEAE DNA CONTROL	CP173226	AMPLIRUN® NEISSERIA GONORRHOEAE DNA CONTROL Vircell
MBC076	AMPLIRUN® BORRELIA BURGDORFERI DNA CONTROL	CP158509	AMPLIRUN® BORRELIA DNA CONTROL Vircell

MBC077	AMPLIRUN® BORRELIA GARINII DNA CONTROL	CP160381		AMPLIRUN® BORRELIA DNA CONTROL Vircell
MBC078	AMPLIRUN® BORRELIA AFZELII DNA CONTROL	CP160066		AMPLIRUN® BORRELIA DNA CONTROL Vircell
MBC082	AMPLIRUN® NOVEL INFLUENZA A H1N1 RNA CONTROL	Segment 1	OR565313	AMPLIRUN® INFLUENZA RNA CONTROL Vircell
		Segment 2	OR565314	
		Segment 3.I	OR565315	
		Segment 3.II	OR565316	
		Segment 4	OR565317	
		Segment 5	OR565318	
		Segment 6	OR565319	
		Segment 7	OR565320	
		Segment 8	OR565321	
MBC083	AMPLIRUN® RESPIRATORY SYNCYTIAL VIRUS (subtype B) RNA CONTROL	PP034516		AMPLIRUN® RESPIRATORY SYNCYTIAL VIRUS RNA CONTROL Vircell
MBC084	AMPLIRUN® MYCOPLASMA HOMINIS DNA CONTROL	CP160065		AMPLIRUN® MYCOPLASMA HOMINIS DNA CONTROL Vircell
MBC085	AMPLIRUN® MYCOPLASMA GENITALIUM	CP159789		AMPLIRUN® MYCOPLASMA GENITALIUM DNA CONTROL Vircell
MBC090	AMPLIRUN® CORONAVIRUS RNA CONTROL	PP810610		AMPLIRUN® CORONAVIRUS RNA CONTROL Vircell
MBC091	AMPLIRUN® RHINOVIRUS RNA CONTROL	PP101946		AMPLIRUN® RHINOVIRUS RNA CONTROL Vircell
MBC096	AMPLIRUN® VENEZUELAN EQUINE ENCEPHALITIS VIRUS RNA CONTROL	PP862732		AMPLIRUN® VENEZUELAN EQUINE ENCEPHALITIS RNA CONTROL Vircell
MBC097	AMPLIRUN® EASTERN EQUINE ENCEPHALITIS RNA CONTROL	PP862729		AMPLIRUN® EASTERN EQUINE ENCEPHALITIS RNA CONTROL Vircell
MBC098	AMPLIRUN® WESTERN EQUINE ENCEPHALITIS RNA CONTROL	PP862730		AMPLIRUN® WESTERN EQUINE ENCEPHALITIS RNA CONTROL Vircell
MBC099	AMPLIRUN® CHIKUNGUNYA VIRUS RNA CONTROL	PP862724		AMPLIRUN® CHIKUNGUNYA VIRUS RNA CONTROL Vircell
MBC100	AMPLIRUN® YELLOW FEVER VIRUS RNA CONTROL	PP025904		AMPLIRUN® YELLOW FEVER VIRUS RNA CONTROL Vircell

MBC101	AMPLIRUN® ST LOUIS ENCEPHALITIS VIRUS RNA CONTROL	PP054842	AMPLIRUN® ST LOUIS ENCEPHALITIS VIRUS RNA CONTROL Vircell
MBC102	AMPLIRUN® PARECHOVIRUS 1 RNA	PP862725	AMPLIRUN® PARECHOVIRUS RNA CONTROL Vircell
MBC103	AMPLIRUN® ZIKA VIRUS (ASIAN LINEAGE) RNA CONTROL	PP872156	AMPLIRUN® ZIKA VIRUS RNA CONTROL Vircell
MBC105-R	AMPLIRUN® HUMAN PARAINFLUENZA 1 RNA CONTROL	OR571883	AMPLIRUN® PARAINFLUENZA RNA CONTROL Vircell
MBC111	AMPLIRUN® NOROVIRUS RNA CONTROL	PP825703	AMPLIRUN® NOROVIRUS RNA CONTROL Vircell
MBC113	AMPLIRUN® RUBELLA RNA CONTROL	PP059123	AMPLIRUN® RUBELLA RNA CONTROL Vircell
MBC114	AMPLIRUN® ADENOVIRUS 41 DNA CONTROL	PP921917	AMPLIRUN® ADENOVIRUS DNA CONTROL Vircell
MBC125	AMPLIRUN® ENTEROVIRUS 68 RNA CONTROL	PP025902	AMPLIRUN® ENTEROVIRUS RNA CONTROL Vircell
MBC129	AMPLIRUN® TOSCANA VIRUS RNA CONTROL	Segment M	PP101988
		Segment S	PP101989
		Segment L	PP862728
MBC130	AMPLIRUN® ROSS RIVER VIRUS RNA CONTROL	PP101990	AMPLIRUN® ROSS RIVER VIRUS RNA CONTROL Vircell
MBC132	AMPLIRUN® MERS CORONAVIRUS RNA CONTROL	PP928422	AMPLIRUN® MERS CORONAVIRUS RNA CONTROL Vircell
MBC134	AMPLIRUN® JAPANESE ENCEPHALITIS RNA CONTROL	PP057999	AMPLIRUN® JAPANESE ENCEPHALITIS RNA CONTROL Vircell
MBC135	AMPLIRUN® CORONAVIRUS OC43 RNA CONTROL	PP862721	AMPLIRUN® CORONAVIRUS RNA CONTROL Vircell
MBC136-R	AMPLIRUN® CORONAVIRUS SARS (2003) RNA CONTROL	PP862722	AMPLIRUN® CORONAVIRUS RNA CONTROL Vircell
MBC142-R	AMPLIRUN® CORONAVIRUS NL63 RNA CONTROL	PP100766	AMPLIRUN® CORONAVIRUS RNA CONTROL Vircell
MBC144-R	AMPLIRUN® METAPNEUMOVIRUS RNA CONTROL	PP862723	AMPLIRUN® METAPNEUMOVIRUS RNA CONTROL Vircell
MBC147	AMPLIRUN® ASTROVIRUS RNA CONTROL	PQ530056	AMPLIRUN® ASTROVIRUS RNA CONTROL Vircell
MBC149	AMPLIRUN ECHOVIRUS 11 RNA CONTROL	PQ855417	AMPLIRUN® ECHOVIRUS RNA CONTROL Vircell
MBC150	AMPLIRUN® JC VIRUS DNA CONTROL	PQ530057	AMPLIRUN® JC VIRUS DNA CONTROL Vircell
MBC151	AMPLIRUN SAPOVIRUS RNA CONTROL	PQ570127	AMPLIRUN® SAPOVIRUS RNA CONTROL Vircell
MBC152	AMPLIRUN OROPOUCHE VIRUS RNA CONTROL	Segment L	PQ530048

		Segment M	PQ530049	
		Segment S	PQ530050	
MBC153	AMPLIRUN MAYARO VIRUS RNA CONTROL	PQ530052		AMPLIRUN® MAYARO VIRUS RNA CONTROL Vircell

Table 2: List of microorganisms with published gene sequences of high molecular diagnostic importance.

REFERENCE	PRODUCT	GEN	NO. NCBI ACCESS	LINK
MBC002	AMPLIRUN® ASPERGILLUS FUMIGATUS DNA CONTROL	anxc4	PQ863734	AMPLIRUN® ASPERGILLUS DNA CONTROL Vircell
		bgt1	PQ863733	
		hsp70	PQ872347	
MBC010	AMPLIRUN® CANDIDA ALBICANS DNA CONTROL	18S	PQ845037	AMPLIRUN® CANDIDA ALBICANS DNA CONTROL Vircell
		pgk1	PQ863728	
		erg11	PQ863727	
MBC013	AMPLIRUN® CHLAMYDOPHILA PSITTACI DNA CONTROL	16S	PQ498793	AMPLIRUN® CHLAMYDOPHILA PSITTACI DNA CONTROL Vircell
		rpoB	PQ511241	
		ompA	PQ511242	
MBC020	AMPLIRUN® HAEMOPHILUS INFLUENZAE DNA CONTROL	16S	PQ497088	AMPLIRUN® HAEMOPHILUS INFLUENZAE DNA CONTROL Vircell
MBC021	AMPLIRUN® HAEMOPHILUS DUCREYI DNA CONTROL	dsrA	PQ570125	AMPLIRUN® HAEMOPHILUS DUCREYI DNA CONTROL Vircell
		hgbA	PQ570126	
MBC031	AMPLIRUN® LEGIONELLA PNEUMOPHILA	16S	PQ845011	AMPLIRUN® LEGIONELLA DNA CONTROL Vircell
		gyrB	PQ863730	
		rpoB	PQ863729	
MBC033	AMPLIRUN® LISTERIA MONOCYTOGENES DNA	plcA	PQ511237	AMPLIRUN® LISTERIA DNA CONTROL Vircell
		actA	PQ511238	
		hly	PQ511239	
MBC034	AMPLIRUN® MYCOBACTERIUM TUBERCULOSIS DNA CONTROL	rrs	PQ511243	AMPLIRUN® MYCOBACTERIUM DNA CONTROL Vircell
		embB	PQ511244	
		gyrA	PQ511245	
		gyrB	PQ511246	
		inhA	PQ511247	

		katG	PQ511248	
		pncA	PQ511249	
		rpoB	PQ511250	
MBC035	AMPLIRUN® MYCOPLASMA PNEUMONIAE DNA CONTROL	16S	PQ498794	AMPLIRUN® MYCOPLASMA PNEUMONIAE DNA CONTROL Vircell
		23S	PQ498782	
		citadhesi na P1	PQ511253	
MBC042	AMPLIRUN® RICKETTSIA CONORII DNA CONTROL	23S	PQ498772	AMPLIRUN® RICKETTSIA DNA CONTROL Vircell
		sca4	PQ511234	
		gltA	PQ511235	
		ompA	PQ511236	
MBC043	AMPLIRUN® CLOSTRIDIUM DIFFICILE DNA CONTROL	tpi	PQ863732	AMPLIRUN® CLOSTRIDIUM DNA CONTROL Vircell
		tcdB	PQ863731	
MBC071	AMPLIRUN® STREPTOCOCCUS AGALACTIAE DNA CONTROL	16S	PQ498774	AMPLIRUN® STREPTOCOCCUS AGALACTIAE DNA CONTROL Vircell
		cps	PQ511240	
MBC073	AMPLIRUN® STAPHYLOCOCCUS AUREUS (mecA-) DNA CONTROL	16S	PQ845015	AMPLIRUN® STAPHYLOCOCCUS DNA CONTROL Vircell
		femA	PQ863719	
		coa	PQ863718	
		tuf	PQ863717	
MBC079	AMPLIRUN® TRICHOMONAS VAGINALIS DNA	actine	PQ511252	AMPLIRUN® TRICHOMONAS DNA CONTROL Vircell
MBC086	AMPLIRUN® MYCOBACTERIUM AVIUM DNA	16S	PQ845012	AMPLIRUN® MYCOBACTERIUM DNA CONTROL Vircell
		hsp65	PQ863723	
		embB	PQ863722	
MBC087	AMPLIRUN® MYCOBACTERIUM INTRACELLULARE DNA CONTROL	16S	PQ845016	AMPLIRUN® MYCOBACTERIUM DNA CONTROL Vircell
		rpoB	PQ863716	
		katG	PQ863715	
MBC089	AMPLIRUN® SHIGELLA FLEXNERI DNA CONTROL	16S	PQ845013	AMPLIRUN® SHIGELLA DNA CONTROL Vircell

		virF	PQ863721	
MBC108	AMPLIRUN® GARDNERELLA VAGINALIS DNA CONTROL	16S	PQ498792	AMPLIRUN® GARDNERELLA DNA CONTROL Vircell
		23S	PQ498781	
		cpn60	PQ511251	
MBC109	AMPLIRUN® TREPONEMA PALLIDUM DNA CONTROL	23S	PQ845036	AMPLIRUN® TREPONEMA DNA CONTROL Vircell
		polA	PQ863714	
MBC112	AMPLIRUN® UREAPLASMA UREALYTICUM DNA CONTROL	16S	PQ498795	AMPLIRUN® UREAPLASMA DNA CONTROL Vircell
		ureC	PQ511255	
MBC115	AMPLIRUN® ENTEROCOCCUS FAECALIS (vanB) DNA CONTROL	16S	PQ845014	AMPLIRUN® ENTEROCOCCUS DNA CONTROL Vircell
		vanB	PQ863720	
MBC118	AMPLIRUN® VIBRIO CHOLERAЕ DNA CONTROL	ompW	PQ863726	AMPLIRUN® VIBRIO CHOLERAЕ DNA CONTROL Vircell
		gbpA	PQ863725	
		rtxA	PQ863724	
MBC119	AMPLIRUN® GIARDIA INTESTINALIS DNA CONTROL	18S	PQ512127	AMPLIRUN® GIARDIA DNA CONTROL Vircell
		beta-giardine	PQ511257	
		gdh	PQ511258	
MBC126	AMPLIRUN® CRYPTOSPORIDIUM PARVUM DNA CONTROL	18S	PQ512126	AMPLIRUN® CRYPTOSPORIDIUM DNA CONTROL Vircell
		gp60	PQ511256	
MBC128	AMPLIRUN® HHV-8 DNA CONTROL	ORF26	PQ530053	AMPLIRUN® HHV DNA CONTROL Vircell
		ORF68	PQ530054	
		ORF73	PQ530055	
MBC133	AMPLIRUN® UREAPLASMA PARVUM DNA CONTROL	16S	PQ498796	AMPLIRUN® UREAPLASMA DNA CONTROL Vircell
		ureC	PQ511254	

5. Conclusions

Genome sequencing of AmpliRun® - products provides new functionality that will be of great value to our customers, providing essential genetic data for their research and applications. For more information, please do not hesitate to contact us.

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