

# FAST DISSECTION OF VIRAL INFECTION BY MINION SEQUENCER



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

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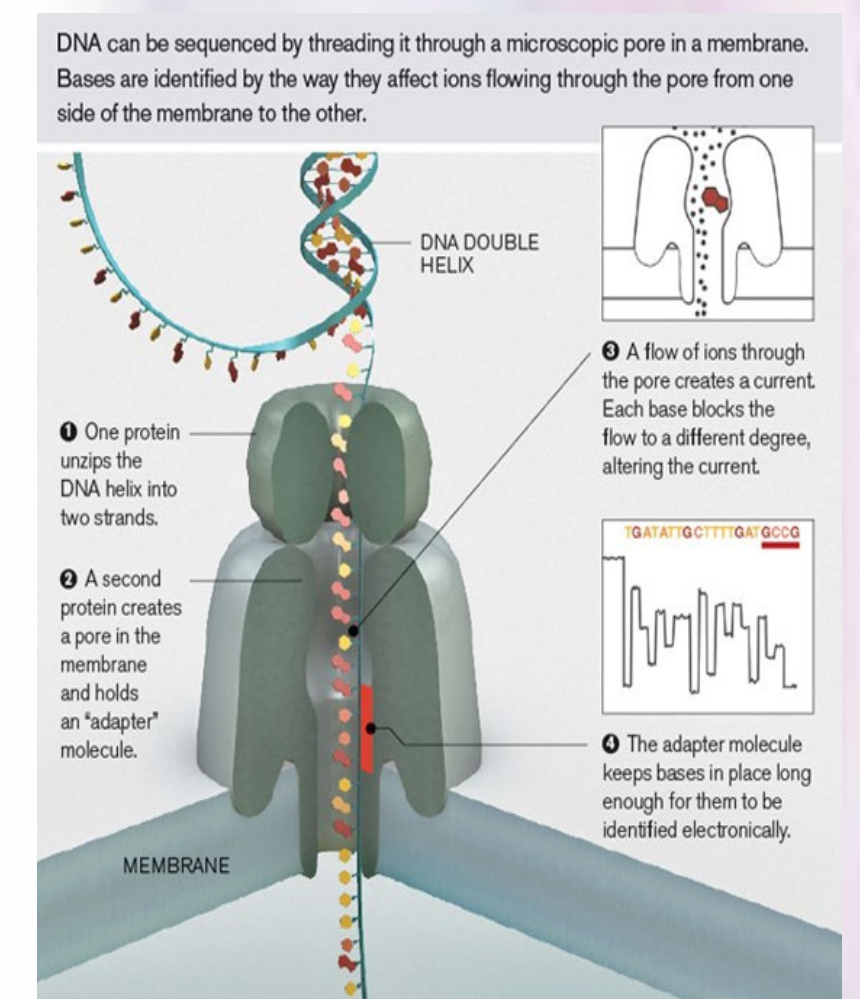
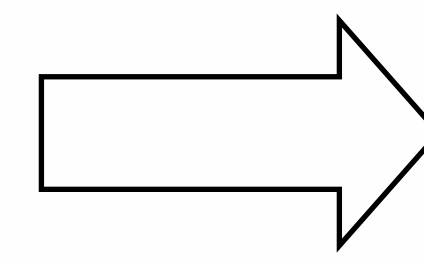
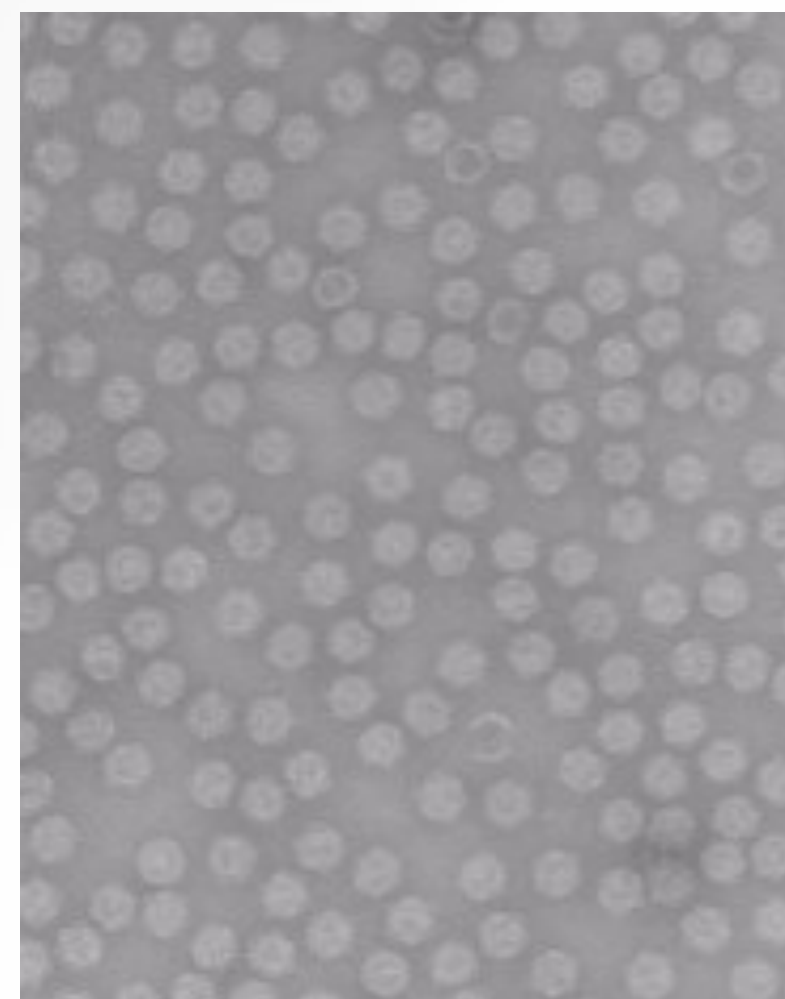
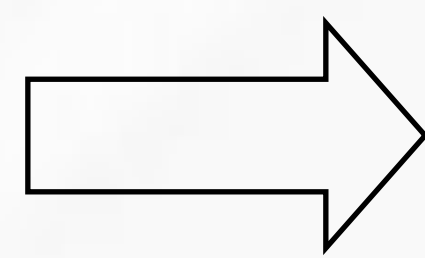
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## Background

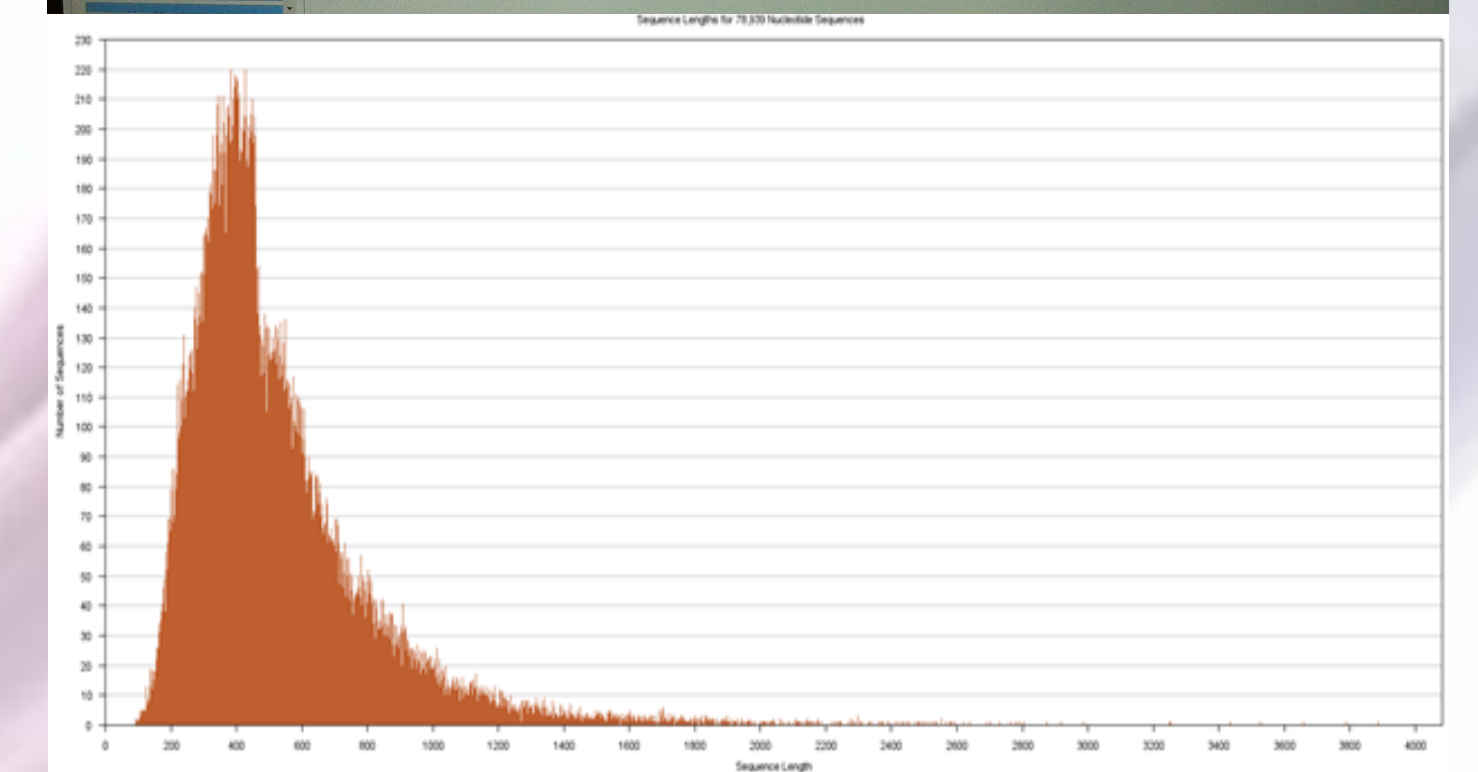
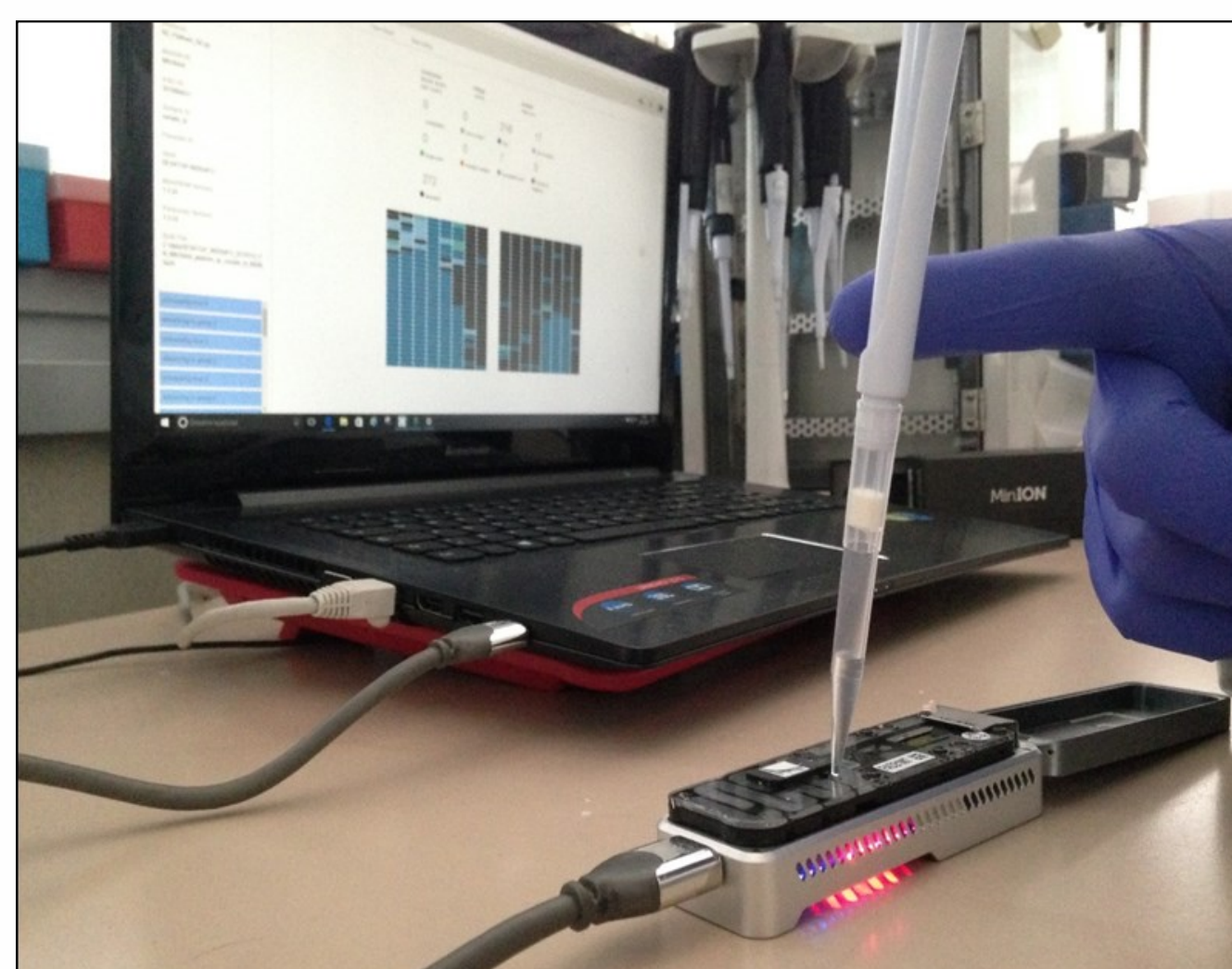
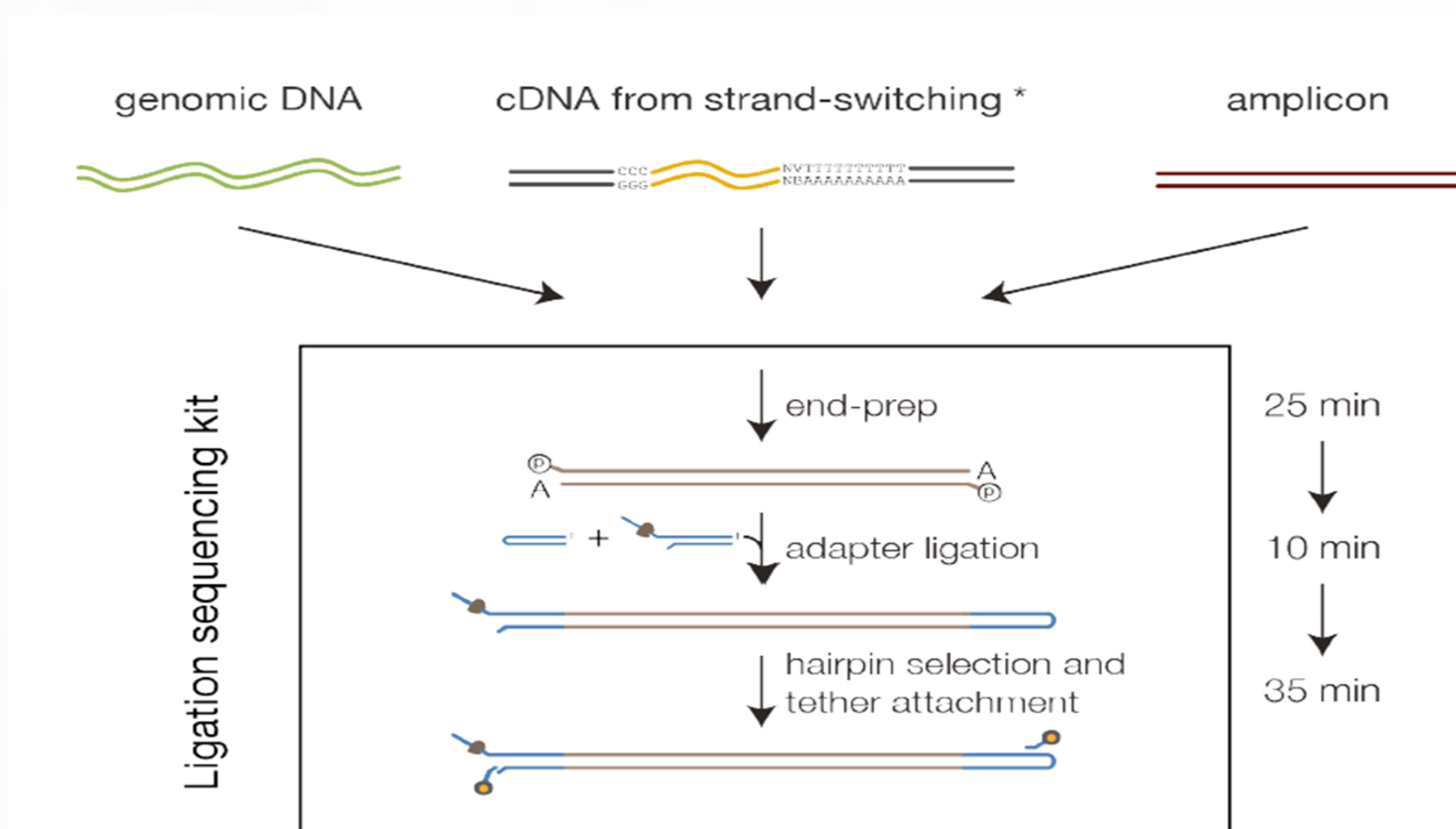
The MinION is a portable, real time, long-read, low-cost device that has been designed to bring easy biological analyses.

This work has been exploited by the remarkable potential of MinION for the identification of filamentous fungi and plant virome, never tried before.



## Material and methods

DNA libraries were generated from total RNA after ribosomal RNA depletion (RiboMinus™ Plant Kit), dsRNA (Valverde *et al.*, 1990) or VANA (Candresse *et al.*, 2014) from tobacco, rose, tomato, fig, cabbage, pomegranate or *F. culmorum* strains and subject to analysis in pools of 10-15 samples.

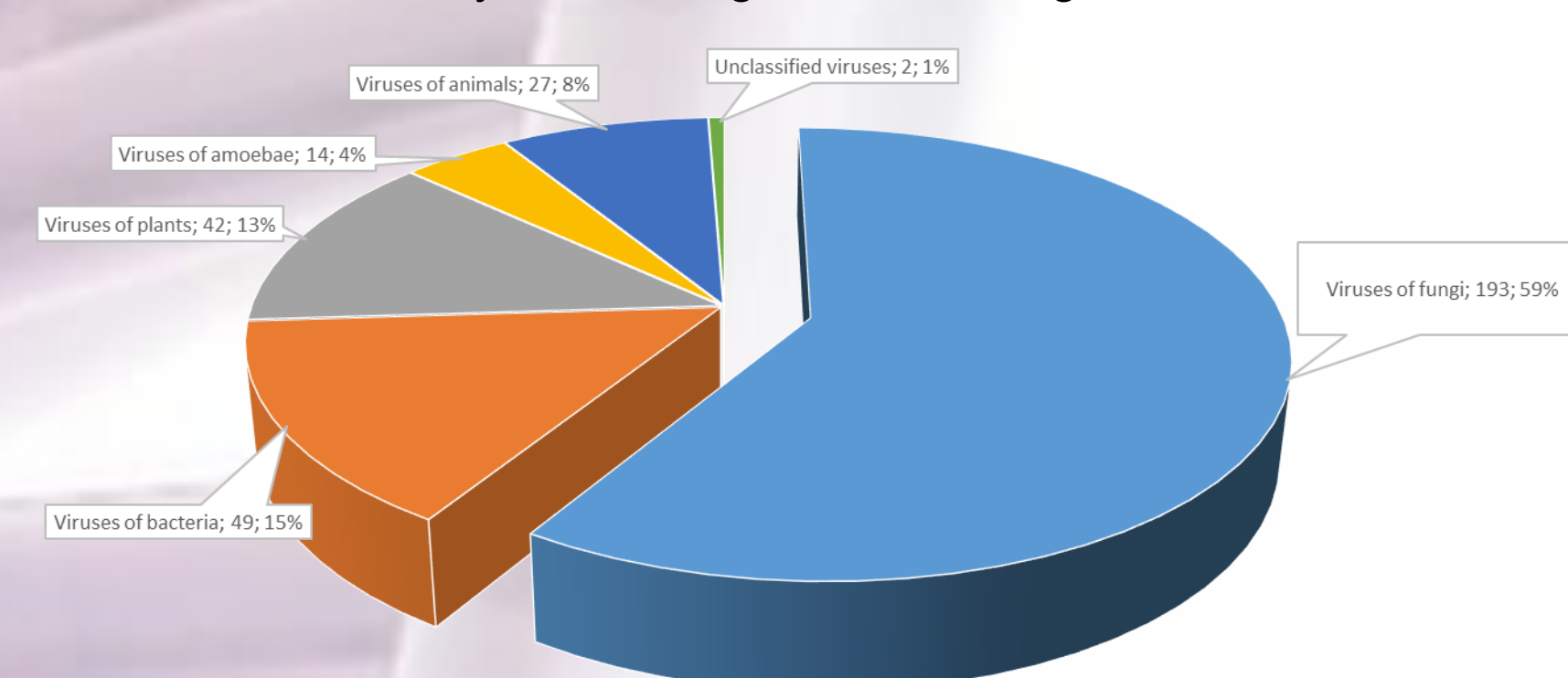


## Results and discussion

The MinION sequencer can analyze several amplicons from small sequences to 50kb without difficulty. This type of third generation sequencer is useful for investigating the presence of pathogens by first screening of total nucleic acids (TNA).

Input: Pool of 13 *F. culmorum* strains (dsRNA)  
Run: 2h  
Reads: 78.939

Analysis of contigs with viral origin



## Why choose the MinION?

- Saving time and money;
- Quick and easy preparation of cDNA libraries;
- Reusable nanopore chip;
- Analysis of a single filament in real time;
- A community and a technology that is constantly growing and evolving.

Candresse *et al.*, 2014. Appearances Can Be Deceptive: Revealing a Hidden Viral Infection with Deep Sequencing in a Plant Quarantine Context. PLOS ONE, 9 (7); 1-13.  
Valverde *et al.*, 1990. Analysis of double-stranded RNA for plant virus diagnosis. Plant Dis. 74; 255-258.

Input: Pool of 10 plant samples (VANA)

Run: 4h 20min  
Reads: 210.085

Virus	Target gene (main)	Identity (%)	Virus	Contigs	Range query coverage (%)	Range identical site (%)
<i>Grapevine leafroll-associated virus 1</i>	coat protein-like gene	72.2 - 100.0	<i>Alternaria alternata partitivirus 1</i>	31	18,2-98,9	32,0-80,0
<i>Tomato yellow leaf curl virus</i>	Coat Protein	72.6 - 100.0	<i>Botryosphaeria dothidea partitivirus 1</i>	30	10,8-92,5	37,7-80,8
<i>Tomato chlorosis virus</i>	heat shock 70-like protein (HSP70h)	73.5 - 96.4	<i>Botrytis cinerea partitivirus 1</i>	1	64,3	66,7
<i>Eggplant mottled dwarf virus</i>	RNA directed RNA Polymerase	74.4 - 94.4	<i>Colletotrichum partitivirus 1</i>	5	12,2-51,9	64,3-90,9
<i>American plum line pattern virus</i>	5'UTR	75.0 - 90.0	<i>Colletotrichum truncatum partitivirus 1</i>	1	43,6	53,3
<i>Cucumis melo endornavirus</i>	UvrD-like helicase C-terminal domain	80.3 - 88.3	<i>Discula destructiva virus 1</i>	2	27,1-42,2	64,0-74,4
<i>Pepper chlorotic spot virus</i>	RNA-dependent RNA polymerase	80.0 - 96.0	<i>Fusarium globosum mitovirus 1</i>	4	17,8-69,4	57,0-100,0
<i>Pitiosporum cryptic virus-1</i>	RdRp gene for RNA dependent RNA polymerase	70.6 - 100.0	<i>Fusarium poae alternavirus</i>	94	9,8-99,4	39,0-100,0
			<i>Fusarium poae mitovirus 1</i>	5	9,6-84,4	44,9-88,6
			<i>Fusarium poae negative-stranded virus 1</i>	4	14,5-90,9	27,2-36,8
			<i>Fusarium poae virus</i>	2	25,9-50,2	43,0-62,0
			<i>Fusarium poae virus 1-240374</i>	5	24,1-98,6	31,9-61,5
			<i>Grapevine associated partitivirus-1</i>	4	7,2-45,0	34,4-88,9
			<i>Magnaporthe oryzae RNA virus</i>	1	48,2	63,6
			<i>Ophiostoma mitovirus 1b</i>	1	33,2	61,4
			<i>Penicillium aurantiogriseum totivirus 1</i>	1	79,7	57,7
			<i>Penicillium spinulosum metavirus 1</i>	1	11,4	40,8