

Fire Monkey: fast long-read DNA extraction

RevoluGen has designed Fire Monkey, a fast, easy-to-use spin column that extracts optimal DNA for long-read sequencing and is already being used in a number of applications.

Spin columns quickly extract DNA from a wide range of samples using a simple, low-cost process. Those characteristics make the protocol the go-to DNA extraction technique. However, standard spin columns shear DNA into small fragments, forcing researchers who want to sequence long-reads to use slower, more expensive extraction kits. In Fire Monkey, RevoluGen has developed a product that brings the established benefits of spin columns to long-read sequencing.

Like conventional spin columns, Fire Monkey runs on standard lab equipment and delivers DNA immediately ready for library preparation in around 1 hour. That is where the similarities end. At each step in the process, Fire Monkey prevents the shearing of high-molecular-weight (HMW) DNA. The result is extracts that are significantly longer than those delivered by standard spin columns.

Fire Monkey is far from the first extraction kit to improve upon the DNA lengths extracted by standard spin columns. The need for longer fragments has driven the proliferation of techniques that eliminate the spinning step that causes spin columns to shear DNA. Those techniques have their own downsides though.

Many HMW DNA extraction kits take hours or even days, and the need to perform a separate size selection step adds to the wait. Some kits require specialized lab equipment or make multiplexing hard or impossible, driving up costs. In some cases, existing kits yield a high proportion of short extracts, which are preferentially sequenced, resulting in short reads despite the presence of long fragments. Other kits yield DNA that is so long it blocks pores and otherwise lowers throughput.

RevoluGen has designed Fire Monkey to be free of those shortcomings. The extraction kit yields a high percentage of fragments of 100 kb and longer,

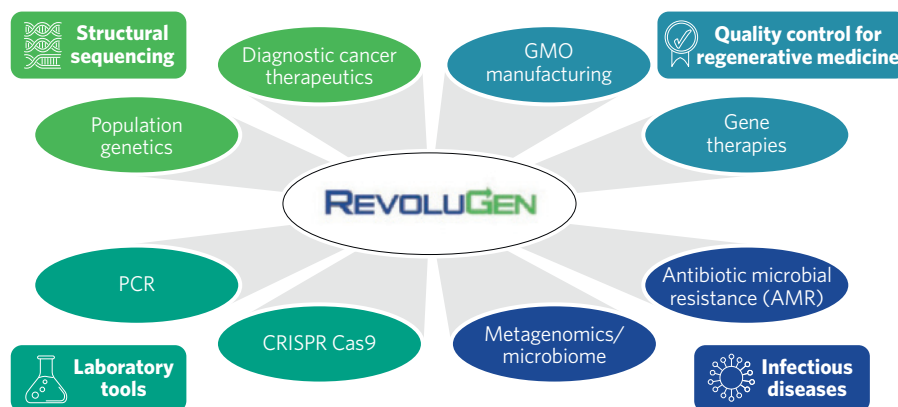


Fig. 1 | FireMonkey applications across diverse and growing DNA sequencing markets. GMO, genetically modified organism; PCR, polymerase chain reaction.

ensuring long strands of DNA are sequenced. All the DNA is neither too short nor too long, resulting in a specific 'Goldilocks' distribution, filling up sequencing throughput capacity with the longest reads that can be sequenced efficiently and ensures optimal coverage.

Size distribution optimization is supported by Fire Flower, a spin column protocol built into the Fire Monkey protocol that, when used separately, removes DNA fragments that are 10 kb or shorter in 15 minutes. Applied to a sample with 50% long extracts, Fire Flower delivers an output that contains 80% long DNA fragments. The kit, which is fully compatible with all extraction systems, delivers even greater improvements when used on samples that feature a smaller percentage of long extracts.

The application of Fire Monkey

The ability of Fire Monkey and Fire Flower to facilitate long-read sequencing positions RevoluGen to serve a large number of growth markets (Fig. 1). Early adopters of the kits have already begun to validate their potential in a number of applications.

Early use cases have demonstrated the power of using a spin column to extract long DNA fragments. As Fire Monkey is as quick and easy as standard spin columns, one group used the kit to extract DNA for use in long-read sequencers and combined the data from short reads from the same type of sample to understand antibiotic resistance in *Salmonella* strains¹. The short-read and long-read runs identified single-nucleotide mutations (or single-nucleotide polymorphisms) and the deletion of 250 base pairs. Short-read sequencers find single-nucleotide mutations with high accuracy but are ill-equipped to detect such large deletions. Yet, the benefits of spin column extraction mean samples are often only

prepared for short-read sequencing. Fire Monkey, with its familiar spin column workflow, makes it trivial for researchers to extract DNA suitable for both short-read and long-read sequencing from a sample. Other groups are using Fire Monkey to build the reference databases for rare *Salmonella* isoforms by extracting DNA for long and short sequencing from the same biological sample².

RevoluGen is further improving Fire Monkey, notably by developing an automated protocol that will use a 96-well plate and centrifuge to eliminate manual steps while increasing extraction yield of DNA and reducing the time and costs of the process. Through such efforts, RevoluGen will move closer to establishing the Fire Monkey–Fire Flower protocol as a simple, globally scalable product.

The realization of that vision will bring big benefits. Equipped with a spin column that supports long reads, researchers will be able to extract DNA for short-read sequencing, store the extracted sample and use the same material for a long-read sequencing run if needed later. This option will do more than spare researchers the inconvenience, errors and costs of re-extracting DNA from raw samples; it will facilitate greater use of long-read sequencing, potentially opening the door to breakthroughs in a wide range of fields.

1. Zhang, C. Z. et al. *J. Antimicrob. Chemother.* **75**, 2773–2779 (2020).
2. Gao, R. et al. *Microbiol. Resour. Announc.* **9**, e00280–20 (2020).

We have found Fire Monkey to be much faster and easier to work with than alternate DNA extraction technologies and, most importantly, it delivers a true step-change in length of DNA, thus enabling multiplexed long read sequencing from ONT's Minion

Gemma Langridge, Group Leader, Quadram Institute

CONTACT

Erling Refsum, COO
RevoluGen Ltd
Ascot, Berkshire, UK
Tel: +44 1457 857103
Email: info@revolugen.co.uk