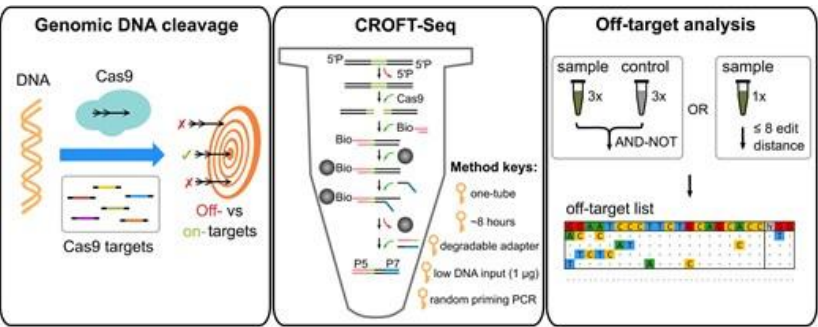


CROFT-Seq

BRIEF DESCRIPTION OF A TECHNOLOGY

CROFT-Seq (CRISPR nuclease **off**-target detection by **sequencing**) is a **sensitive**, **rapid**, and **cost-effective** assay for the genome-wide detection of CRISPR-Cas9 off-target sites *in vitro*.



PURPOSE

CROFT-Seq performs comparably in terms of specificity and sensitivity to the most commonly used SITE-Seq and CIRCLE-Seq methods; however, it is ~8-fold more cost-efficient and ~4-fold faster. Moreover, due to its unique reaction compositions and conditions, CROFT-Seq is suitable for performing the whole protocol in a single reaction tube, thus allowing for simple automation using liquid handling robots.

<i>In vitro</i> method	Genomic DNA input, µg	Hands-on time, min	Total time, h	Price, Eur/sample	Reads, mln/sample	Suitable for robotization
CROFT-Seq	1	45	8	7	~2-60	Fully (easy)
SITE-Seq	10	80	38	51	~0.62-2.46	Fully (difficult)
CIRCLE-Seq	25	154	36	66	~4-5	Fully (very difficult)

FIELDS OF APPLICATION

CROFT-Seq could be adapted for the off-target detection of other emerging genome editing tools, such as Cas12, TnpB, and base editors (under development).

TECHNOLOGY READINESS LEVEL

Validated in lab.

INTELLECTUAL PROPERTY

Patents: EP4471159B1; US12668891B2
JP patent application JP2024172618A
Applicants: Vilnius University (Lithuania)

INVENTORS

dr. Mindaugas Zaremba, dr. Paulius Toliušis, dr. Tautvydas Karvelis, dr. Giedrius Sasnauskas, dr. Tomas Šinkūnas, Algirdas Grybauskas, dr. Arūnas Šilanskas, dr. Evelina Zagorskaitė

PUBLICATIONS

Toliušis et al. (2026) *Synthetic Biology*,
<https://doi.org/10.1093/synbio/ysag006>



Vilnius
University

CONTACTS

- dr. Mindaugas Zaremba
Institute of Biotechnology
Life Sciences Center
Vilnius University
E-mail: mindaugas.zaremba@bti.vu.lt
Phone: +370 5 2234357
- dr. Aistė Vilkanauskytė
Innovation Office,
Vilnius University
E-mail: aiste.vilkanauskyte@cr.vu.lt
Phone: +370 5 2366296