

CRISPR/Cas9 Methods: Preferences from the Field

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ABRF GERG website: <https://abrf.org/research-group/genome-editing-research-group-gerg>

Introduction

The Genome Editing Research Group (GERG) surveyed users of the CRISPR/Cas9 technology to help establish an understanding of preferred methods being used. As new core facilities are being formed to support the CRISPR/Cas9 technology, or existing cores have adapted their services to fit the technology into their workflows, considering what other cores are using is important. Questions regarding preferred guideRNA design tools, format of reagents, mutation analysis methods, and more were asked. In all, these survey results highlight some of the current trends in the genome engineering community and reveal what is being used day to day in the lab.

Survey

Survey Monkey platform was used to design and administer the survey. A total of 15 questions were written. The survey link was distributed through various online formats including:

- ABRF-GERG webpage
- ABRF Forum
- ABRF email blasts
- Twitter
- ISTT email blast
- Genome Engineering Google Group
- GERG member colleagues and institutions

Survey Data

A total of 75 responses were collected representing a variety of model organisms and both research labs and core facilities. All of the data can be viewed by scanning the QR code below. Additionally, a link to the results can be found on the GERG website.



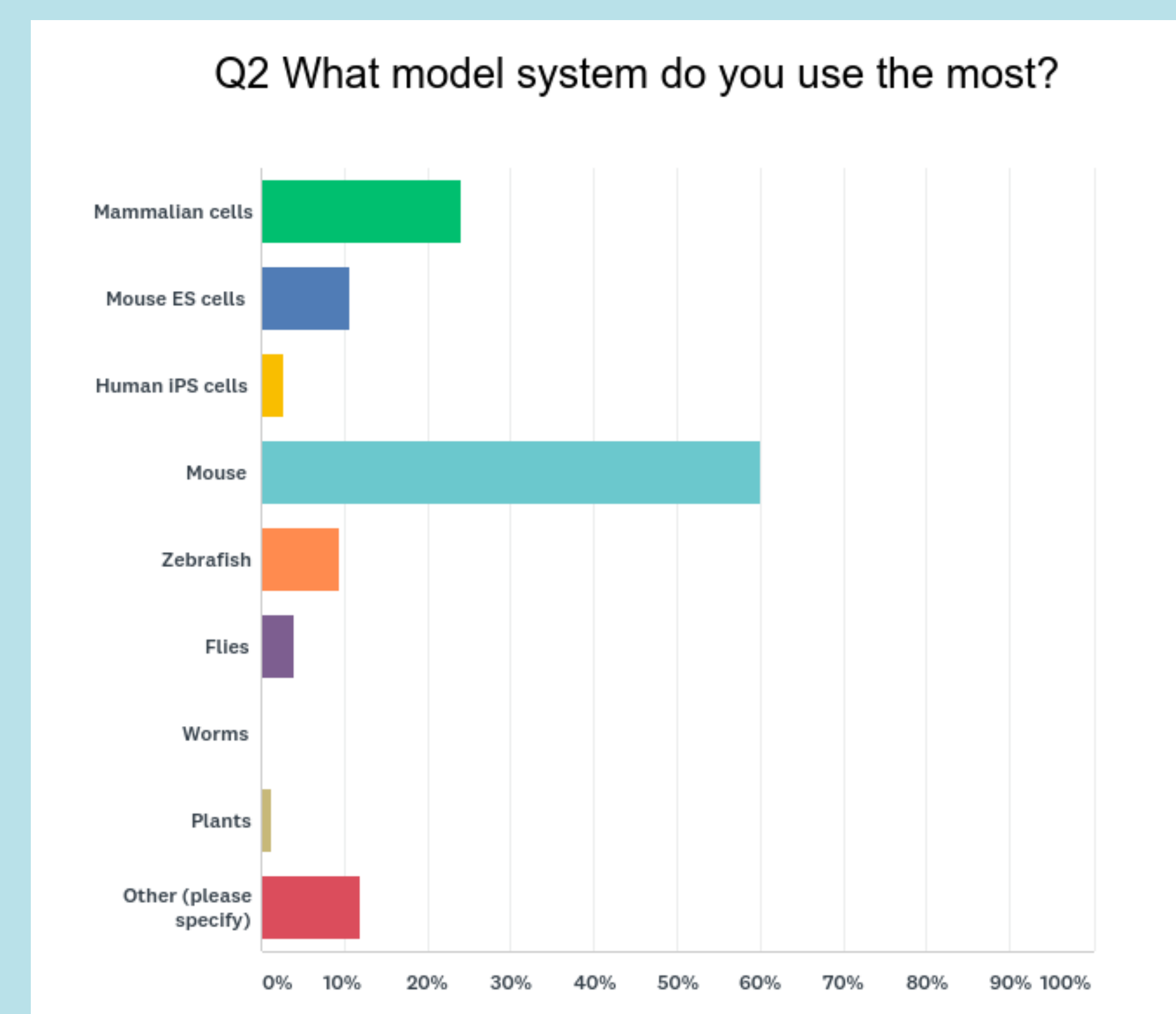
GuideRNA Design Tools

Question 1 asked what are the preferred guideRNA design tools. Eight choices were provided (in blue text) and 13 additional responses were entered by respondents.

30	crispr.mit.edu	2	flyCRISPR
20	CRISPOR	2	Cas Designer
8	Benchling	1	COSMID
7	IDT	1	ZiFit
6	CRISPRscan	1	EuPaGDT
5	chopchop	1	CRISPRdirect
5	Breaking-Cas	1	GT-Scan
4	ecrisp	1	WTSI Genome Editing
3	Broad sgRNA Designer	1	Synthego
2	CCTop	1	in house tool

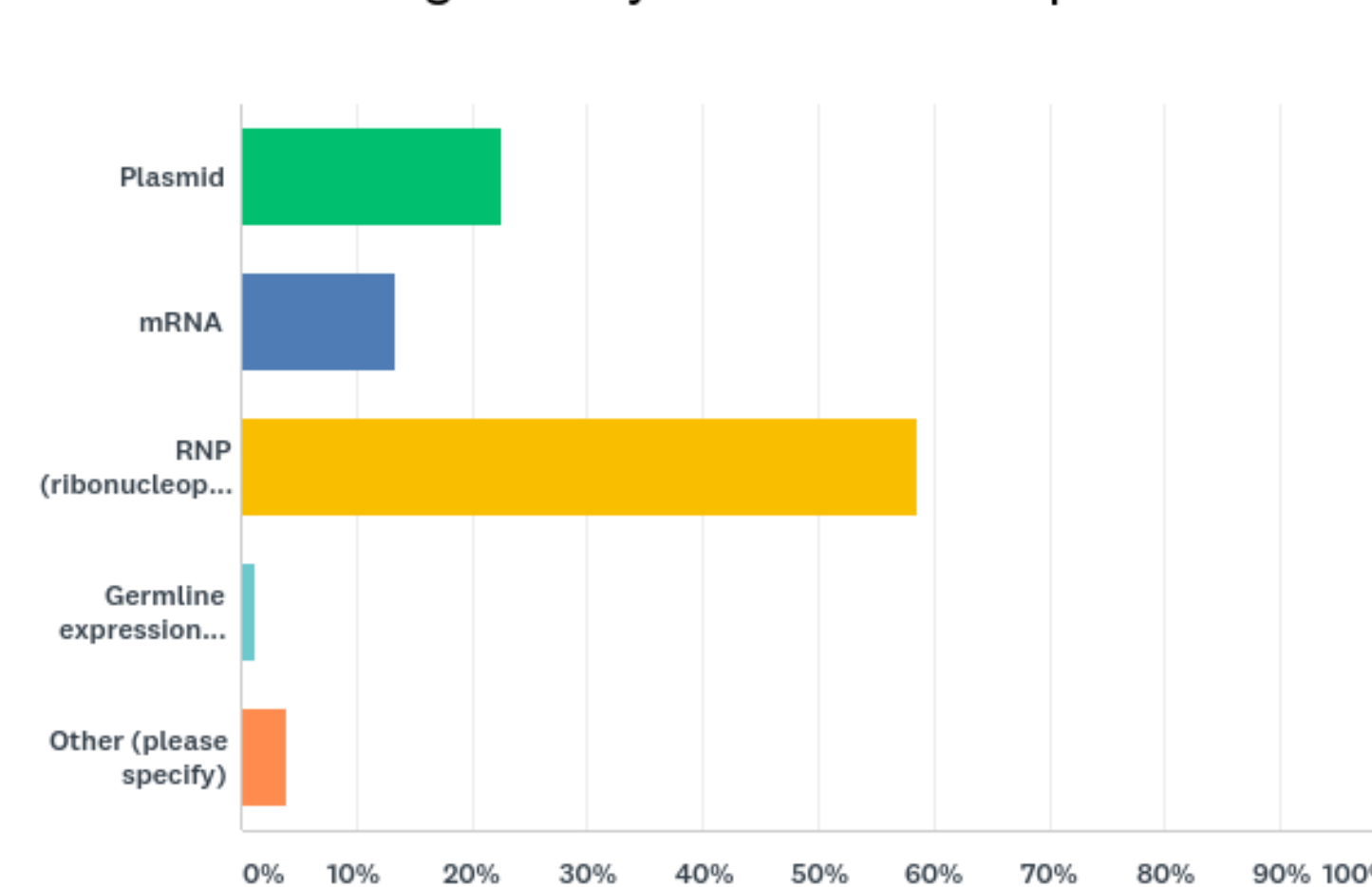
GuideRNA and Cas9 Format

The reagents can be delivered to cells in several formats and methods. This is often determined by the model organism or cell type being used. We asked which model systems were used the most. Mouse and mammalian cells were the most popular.



Ribonucleoprotein (RNP) complex was the preferred reagent format at 58%, plasmid at 22%, mRNA at 13%. For mouse users, RNP was preferred 80% of the time.

Q4 What reagent format do you most often use for the guideRNA and Cas9 delivery to the model organism you selected in question 2?



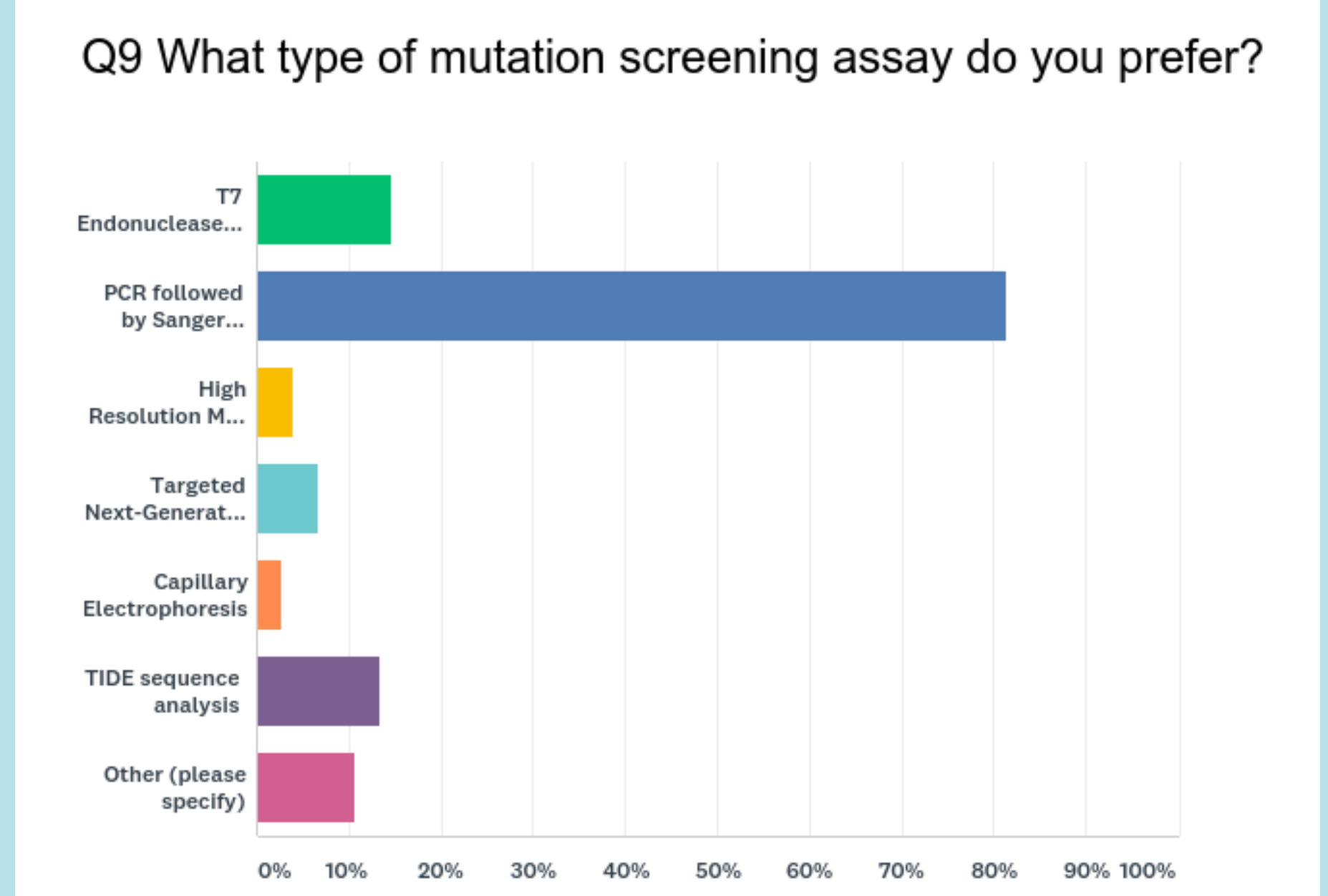
Delivery Methods

Multiple methods are being used for reagent delivery to both mammalian cells and mouse embryos. Lipofection and microinjection remain the most popular.

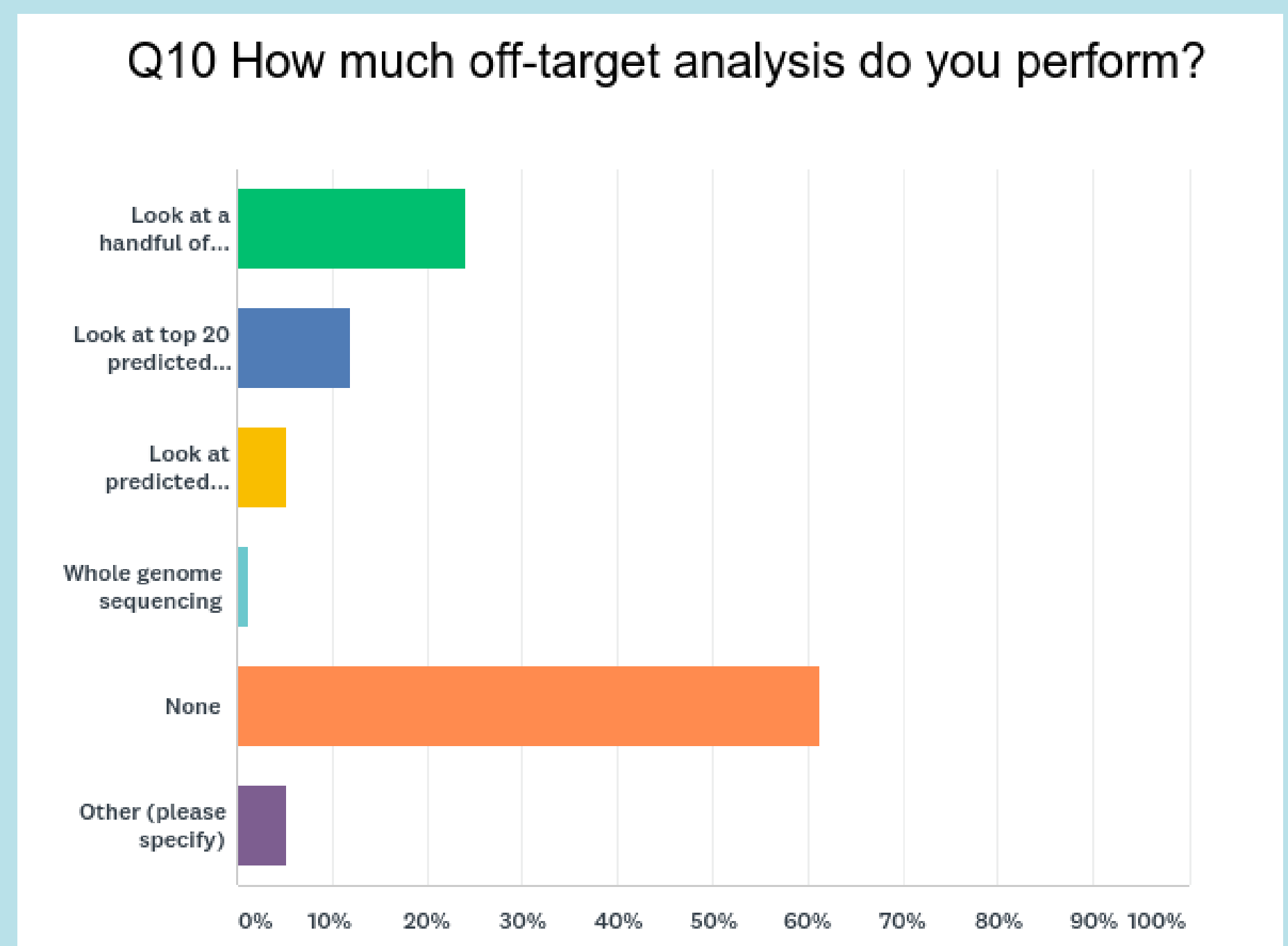
Delivery Methods	%
Lipofection	62
Lentivirus	23
Nucleofection	15
Embryo microinjection	62
Electroporation	10
Embryo in-vivo electroporation	2

Mutation Detection

For 81% of all survey takers, PCR followed by Sanger sequencing was the highest ranked mutation analysis method.



When looking for off-target mutations, 61% responded they perform no off-target analysis. Only 6% look at predicted off-targets by next-generation sequencing or whole genome sequencing.



Acknowledgements

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