Can We Use Historical Data to Predict Optimal Targeting Strategies for Efficient Knock-in Generation of Mouse Models ?

Aline Baur, Anna Rüegg, Sunwoo Chun, Annette Nebenius, Désirée Gronostay, Pawel Pelczar

Center for Transgenic Models, University of Basel, Basel, Switzerland

University of Basel

Abstract

Over the last few years, a multitude of CRISPR-based methods have been developed and used to generate knock-in mouse models. Is it possible to use historical data to predict the most effective targeting strategy for creating knock-ins at specific genomic loci? We conducted a comprehensive analysis of our transgenic core facility's data collected over the past three years. In this, we were focusing on the correlation between our success rate and the targeting techniques used, the conformation and DNA methylation status of the targeted locus as well as the assumed conformation of the inserted DNA fragment.

Results

Microinjection of 2-cell embryos 24h post-IVF yields the highest knock-in rates

Evaluating the efficiency of targeted integration with respect to varying embryo collection methods.

2-cell embryo injections performed 24 h post-IVF achieve significantly higher targeted integration rates than injections of frozen-thawed IVF 2-cell embryos or 1-cell embryos generated by superovulation and natural mating.

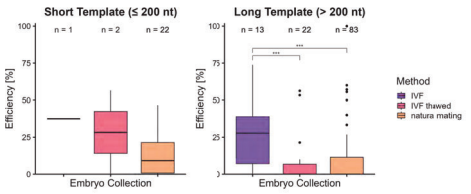

Fig 1: Relative knock-in efficiency for different embryo collection methods grouped by the size of the donor template

All targeting methods show similar efficiency of knock-in production

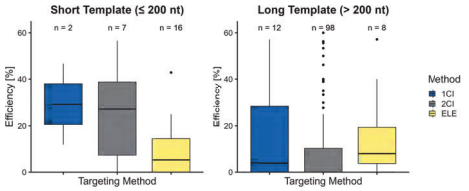

Fig. 2: Relative knock-in efficiency for different targeting methods grouped by the size of the donor template

Different targeting methods showed no significant difference in the efficiency of founder production.

It is to note, however, that electroporation was used mostly for shorter templates while 2-cell injections were performed mainly for longer templates and/or more "challenging" projects for which electroporation had failed (see Fig. 5).

All types of HDR templates attain comparable knock-in efficiencies

Comparing all knock-in templates focusing on the type of inserts used, we see a trend towards higher efficiencies with short ssDNA templates.

However, the observation could be biased by using the AAV and biotinylated-linear-dsDNA approach only for projects that failed using other strategies. In addition the length of the templates used varies between different template types (see Fig. 5).

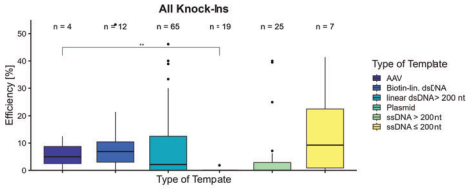

Fig 3: Relative knock-in efficiency for different types of donor template

HDR template length and knock-in efficiency do not correlate

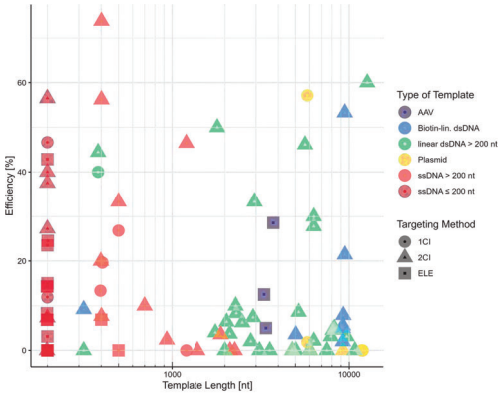

Fig 4: Relative knock-in efficiency against HDR template length with colors indicating the template type and shapes indicating the targeting method used.

For the majority of projects with an efficiency higher than 20%, 2CI was performed.
For dsDNA, the efficiency of integration is independent on the template size, whereas the integration efficiency of ssDNA appears to drop as its length increases.

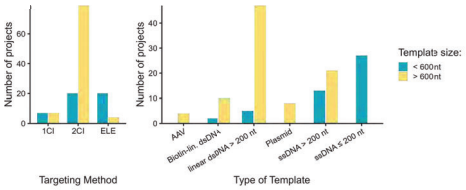

Fig. 5: Preferential use of 2CI, biotin-DNA and AAVs for "challenging" projects with long insertions leads to bias that obscures the true potential of these methods.

Impact of chromatin accessibility and histone modifications on the efficiency of founder production could depend on the insert size

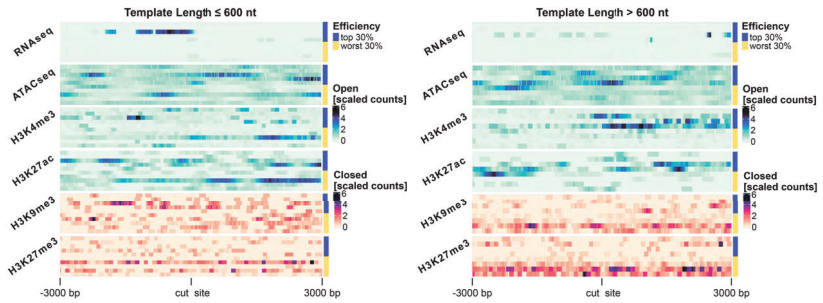

Fig. 6: Heatmap of scaled read counts for transcription, chromatin accessibility and histone modifications around the targeted sequence (+/- 3000 bp). The color of the scale indicates whether the mark is linked to an open (green-blue) or closed (orange-red) DNA configuration. The values for individual loci are sorted by efficiency and only the top (blue) and worst (yellow) 30% of all loci are displayed. The left plot displays the data for small inserts and the right plot for big inserts.

According to our data compiled for linear dsDNAs exceeding 600 nt, the knock-in efficiency does not correlate with template length or the method of template delivery (Fig 4). We therefore analyzed the chromatin conformation of genes that display a particularly high or low efficiency irrespective of the targeting strategy employed.

For inserts shorter than 600 nt, the DNA conformation at the target locus does not correlate with knock-in efficiency.
For inserts longer than 600 nt, a trend towards a higher integration efficiency in loci with open chromatin and active genes can be observed. Loci with a lower knock-in efficiency show higher read counts for closed chromatin conformation markers.

Analyzing the DNA conformation of the target locus might therefore be a useful step to improve targeting efficiency. Loci with an open chromatin structure can be efficiently targeted using simple standard approaches while more labor/cost intensive approaches like biotinylated-linear-dsDNA and AAV might be necessary for loci with closed chromatin conformation.

Conclusions

1. For long templates, microinjection of 2-cell IVF embryos is more efficient than using embryos after natural mating and frozen-thawed 2-cell IVF embryos.
2. In our hands, plasmid donor templates were less efficient at producing targeted insertions, whereas ssDNA integrated efficiently up to a size of ~ 600 nt.
3. The chromatin accessibility of the targeted locus might affect the success rate for integrating large DNA fragments (> 600 nt).

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Center for Transgenic Models
University of Basel
www.ctm-basel.ch