CRISPR Guide Characterization Strategies

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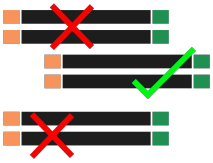


Guide Characterization Validates CRISPR Experiments

Guide characterization is a key step in gene editing experiments. The process of validating modifications in the genome helps investigators create strong causal relationships between specific mutations and phenotypic outcomes. This has relevance in basic research where experimental reproducibility remains a major concern. It is also critical to confirm on- and off-target effects as gene editing moves toward the clinic.

Next-Generation Sequencing Approaches to Guide Characterization

There are two ways to validate CRISPR experiments. The first involves “biased” detection, so-called because they are targeted to specific regions in the genome based on algorithmic prediction (such as Hsu 2013 or CFDoff-target analyses). Biased detection covers a broad range of techniques including mismatch cleavage assays, Sanger sequencing and targeted amplicon deep sequencing.

Targeted amplicon deep sequencing is a form of next-generation sequencing (NGS) and has many advantagesover Sanger and mismatch cleavage assays. Specifically, NGS can provide analysis of heterogenous samples in a high-throughput manner and yields highly sensitive, detailed and quantitative sequence data. This contrasts with the labor and inflexibility of Sanger and the imprecision of mismatch cleavage assays.

The other approach to guide characterization utilizes “unbiased” methods of detection. Unbiased assays search for evidence of CRISPR editing across the genome as opposed to bioinformatically predicted regions. For example, whole genome sequencing (WGS) allows the investigator to analyze comprehensive data from their model genome pre- and post-editing (Wang et al. 2014).

In recent years, several other unbiased detection assays have been developed to observe genome-wide editing events. These include cell-based methods IDLV (Gabriel et al. 2011, Wang et al. 2015, Osborn et al. 2016) (which can also be used *in vivo*), BLESS (Crosetto et al. 2013), GUIDE-seq (Tsai et al. 2015) and HTGTS (Frock et al. 2015) as well as the *in vitro* (cell-free) Digenome Seq (Kim et al. 2015). These techniques are still being improved and developed with the goal of even greater versatility and sensitivity.

The Danger of Missing Off-Target Events

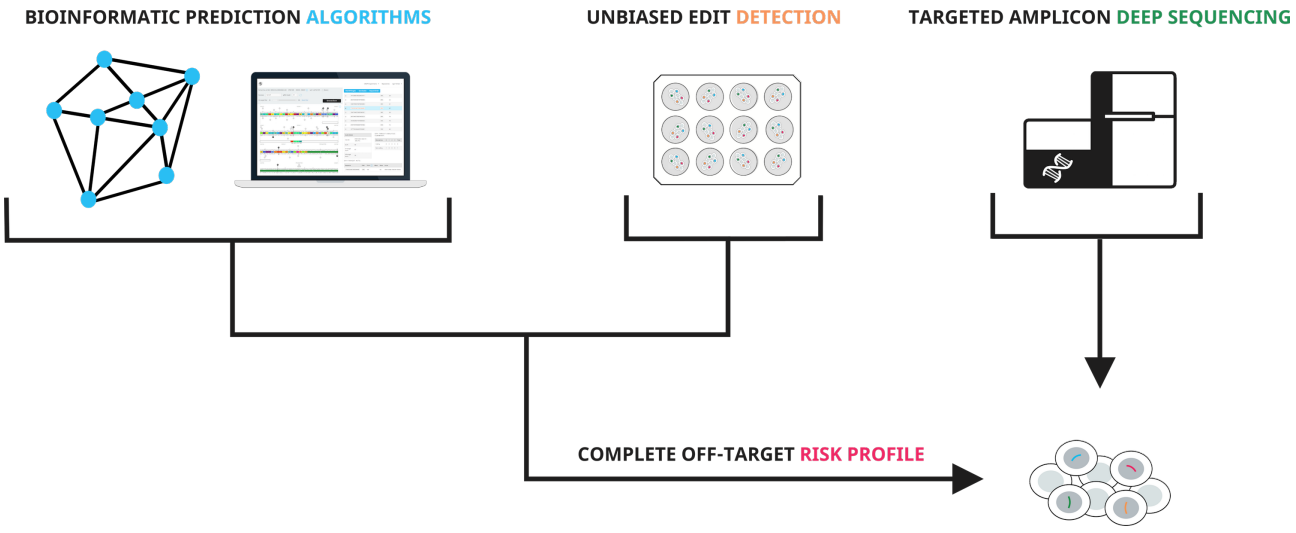
In general, unbiased strategies are not as sensitive as targeted amplicon deep sequencing. While deep sequencing can detect minor mutations occurring in just .01% of the population, unbiased sequencing only detects at a range of .1% (GUIDE-Seq, Digenome Seq) to 1% (IDLV). Additionally, they do not provide quantitative assessment of the frequency of off-target events. Detecting minor alleles caused by off-target events in a population is important both to associate an on-target edit with a particular phenotype and to detect potentially deleterious genomic modifications. This problem becomes even more pronounced as researchers attempt to translate CRISPR research and therapeutics into the clinic.

Nonetheless, unbiased sequencing has the distinct advantage of not foregoing the rest of the genome to only focus on predicted cleavage sites. If an investigator relies entirely on off-target scoring algorithms and targeted amplicon deep sequencing, there is a chance they might miss potential off-target events around the genome. This is because while algorithms provide a good starting place for analysis, they are not exhaustive. They rely on sequence-level information to make predictions about biological systems. This is a promising (and always improving) method of off-target analysis but it cannot be considered comprehensive.

Best Practices in the Lab and Clinic

As Tsai and Joung discuss in their 2016 *Nature review* of guide characterization methods, unbiased detection at increased sensitivity is critical for evaluating edits for clinical applications. Even the smallest change to the genome could have deleterious effects on a patient. Therefore, the .1% detection limit will need to be improved in order to ensure the health and safety of CRISPR therapeutics.

Tycko, Myer and Hsu suggest in their 2016 *Cell review* that the best option for researchers may be to combine biased and unbiased guide characterization approaches. This means using the latest sgRNA prediction algorithms to create an extensive (though inexhaustive) list of potential off-target sites. This report can be enhanced with an index of putative editing events as determined by techniques such as GUIDE-Seq. This creates an off-target risk profile which can then be verified using amplicon deep sequencing.



In combination, these approaches ensure that off-target events are validated with the most sensitive assays available. Targeted deep sequencing eliminates false positive results from both *in silico* prediction and unbiased detection. This will illuminate the precise sequence-level changes made to the genome.

Sources

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