

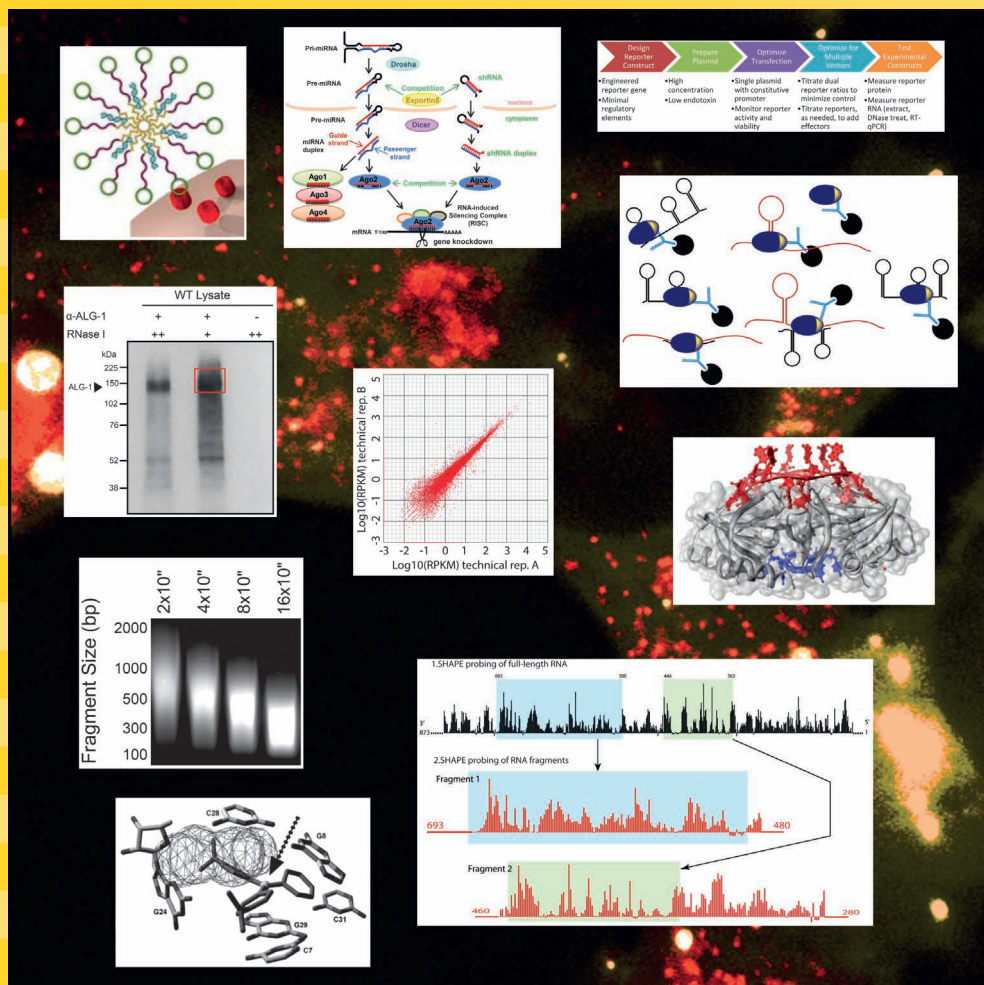
METHODS

FOCUSING ON RAPIDLY DEVELOPING TECHNIQUES

Non-coding RNA Methods

Editor

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Guest Editor's Introduction

Editorial for “Non-coding RNA methods”

The bright future of (non-coding) RNAs: methods light the way

The human genome project took 13 years and ~\$1/base pair for a total of \$3 billion to complete [1]. When the entire sequence became available a decade ago, the hope was that a complete understanding of how a human cell lives and dies would soon follow. Instead, we learned that the number of protein-coding genes is much smaller than previously thought – perhaps less than 21,000 [2] – making us not very distinct in this regard from soil-dwelling nematode worms. However, the remaining over 98% of the genome in its majority codes for unique RNAs – referred to as non-coding RNAs, or ncRNAs – whose functions we are now slowly discovering as a basis for understanding cellular life. Yet, the legacy of the human genome project goes well beyond giving us foundational sequence information, as it also pushed the development of sequencing methods – in particular shotgun sequencing – to the fore and effectively spawned the field of bioinformatics to cope with the flood of emerging data. After a decade where we saw the cost for sequencing an entire human genome drop precipitously to \$3000 – 1 ppm, or part-per-million of the original cost – entirely new avenues of inquiry have opened up, for example, into understanding human genetic diversity by sequencing thousands of individual genomes [3]. In addition, as probably the most profound discovery arising from genome sequencing, both short and long ncRNAs have spawned a rejuvenation of the biotech industry, fueled by the ability of ncRNAs to control virtually all cellular processes. The associated rapid evolution of methods involving ncRNAs warrants renewed and sustained attention.

This special issue provides a current snapshot of a few exemplary ncRNA methods, spanning the entire gamut of ncRNA mediated applications, discovery of ncRNA interaction partners, physicochemical properties and cellular spatiotemporal distributions, in eukaryotes and in bacteria. First, Goldberg describes advances in techniques to deliver synthetic short ncRNAs termed short interfering RNAs (siRNAs) into cancer cells to silence undesired genes [4]. Delivery and specificity are the twin problems of siRNA-mediated gene silencing by RNA interference (RNAi), so the second review in the lineup by Lau and co-workers describes a method to design “organic” (read, “more natural”) small hairpin RNAs as RNAi effectors [5]. Goldstrohm and coworkers then summarize an optimized set of protocols to assess the efficacy of gene silencing [6]. Broughton and Pasquinelli switch gear to highlight protocols to identify RNA interaction partners of Argonaute proteins, main effectors of RNA silencing, by crosslinking and deep sequencing of the crosslinked RNA [7]. This is followed by an article from Nephew and coworkers on a new method for obtaining

purified RNA libraries for improved deep sequencing and observation of changes in expression pattern [8]. Next, two articles highlight the existence of ncRNAs in bacteria: First, Romby and coworkers focus on mapping ncRNA:mRNA interactions in the pathogenic *Staphylococcus aureus* using deep sequencing of substrates of RNase III, an endonuclease and family relative of Dicer, another key RNA silencing effector in eukaryotes [9]. Second, Faner and Feig summarize methods that similarly utilize Hfq protein, a chaperone of base pairing between small regulatory RNAs (sRNAs) and their target mRNAs, to discover sRNAs in bacteria [10]. Wierzbicki and coworkers then steer our attention towards plants by describing various immunoprecipitation methods for discovering long ncRNAs (lncRNAs) produced in *Arabidopsis thaliana* by RNA polymerase V [11].

Once the discovery phase is over, the biological and physicochemical properties of ncRNAs need to be uncovered, so the remaining three articles of this issue describe a subset of methods with this goal. First, Sanbonmatsu and coworkers describe a novel shotgun approach for determining the secondary structure of lncRNAs [12]. Second, Nilsen-Hamilton and co-workers illustrate how such biochemical analyses can be integrated with fluorescence and NMR spectroscopy, X-ray crystallography, and computational modeling based on molecular dynamics simulations to arrive at a more holistic view of structure–function–dynamics relationships [13]. Third, and finally, Walter and coworkers document a method termed iSHiRLoC, or intracellular Single-molecule High Resolution Localization and Counting, that allows one to characterize the spatiotemporal distributions of ncRNAs in their natural habitat, in single cells, at the single molecule level [14].

While a survey such as the one presented here by necessity is utterly incomplete, it was assembled with the hope that a sampling of ncRNA methods will inspire further accelerated development and publication in this rapidly expanding area of extraordinary biomedical potential, finally fulfilling the promise of the genome sequencing projects.

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