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Transcriptome-wide measurement of translation by ribosome profiling

Nicholas J. M[c]Glincy, Nicholas T. Ingolia

Department of Molecular and Cell Biology, Center for RNA Systems Biology, California Institute for Quantitative Biosciences, University of California, Berkeley, 16 Barker Hall # 3202, Berkeley, CA 94720-3202, USA

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ABSTRACT

Translation is one of the fundamental processes of life. It comprises the assembly of polypeptides whose amino acid sequence corresponds to the codon sequence of an mRNA's ORF. Translation is performed by the ribosome; therefore, in order to understand translation and its regulation we must be able to determine the numbers and locations of ribosomes on mRNAs *in vivo*. Furthermore, we must be able to examine their redistribution in different physiological contexts and in response to experimental manipulations. The ribosome profiling method provides us with an opportunity to learn these locations, by sequencing a cDNA library derived from the short fragments of mRNA covered by the ribosome. Since its original description, the ribosome profiling method has undergone continuing development; in this article we describe the method's current state. Important improvements include: the incorporation of sample barcodes to enable library multiplexing, the incorporation of unique molecular identifiers to enable to removal of duplicated sequences, and the replacement of a gel-purification step with the enzymatic degradation of unligated linker.

1. Introduction

Translation is one of the fundamental processes of life – it describes the process by which the genetic information within the nucleotide sequence of an mRNA is converted into physical agency in the form of a protein. Indeed, in the yeast *Saccharomyces cerevisiae*, protein comprises approximately half the dry weight of a cell [1,2], indicating that translation is a major biosynthetic activity, consuming approximately half the energy expended during rapid growth. Translation is performed by a mega-dalton ribonucleoprotein complex called the ribosome. The ribosome first identifies the region within an mRNA that encodes a protein (the Open Reading Frame, ORF), and then reads that ORF three nucleotides (one codon) at a time, assembling a polypeptide whose amino acid sequence corresponds to the codon sequence of the ORF. While we have an understanding of the general mechanism of translation, much remains to be determined. Furthermore, we have comparatively little understanding of the regulation of translation in difference physiological contexts, and how its dysfunction leads to disease.

Ribosome profiling is a method that leverages high-throughput sequencing and bioinformatics to determine the transcriptome-wide locations of ribosomes in a cell lysate. The basis of ribosome profiling is that, at a given moment, each ribosome covers a short fragment (~28 nt) of the mRNA that it is translating. In a cell lysate, this results in that short fragment of mRNA being protected from digestion with exogenous RNases (see Wolin & Walter [3], and references therein). Thus, for an organism for which we know the sequence of the transcriptome, we can determine the location of ribosomes by first generating these fragments (termed "ribosome footprints", or simply "footprints"), and then isolating them and determining their sequence [3–5]. Knowing the transcriptome-wide locations of ribosomes allows the determination of which mRNAs, and which regions of a given mRNA, are engaged with a ribosome, and therefore likely to be undergoing translation. Examining the frequency distribution of ribosome footprints along a given mRNA(s) we can learn about the nature of ribosomal movement and how it might be influenced by mRNA sequence. Furthermore, by counting the number of ribosomes engaged with a given mRNA, we can obtain an estimate of the degree to which the mRNA is translated. After proper consideration of confounding factors (for example, more abundant mRNAs will tend to produce more footprints), these measurements can be compared between different mRNAs, and for a given mRNA across different physiological conditions. When further combined with genetic and chemical manipulation, ribosome profiling gives us unprecedented ability to examine the process of translation and its regulation *in vivo*. Ribosome profiling was initially developed using yeast [4], but has since been applied to cells, tissues, and embryos from diverse

E-mail addresses: mcglincy@berkeley.edu (N.J. M[c]Glincy), ingolia@berkeley.edu (N.T. Ingolia)

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metazoans. For recent perspectives on ribosome profiling and its applications, readers are referred to several excellent reviews: Jackson & Standart [6], Ingolia [7], Brar & Weissman [8], and Andreev *et al.* [9].

This article describes the current state of the ribosome profiling protocol as routinely performed in our laboratory. It has been the basis of ribosome profiling experiments from recent studies associated with our laboratory, including but not limited to: Iwasaki *et al.* [10], Werner *et al.* [11], and Ishikawa *et al.* [12]. In essence, the procedure is similar to that described in Ingolia *et al.* [13]: cells are rapidly harvested and lysed under conditions that are expected to preserve *in vivo* ribosome positioning. This cell lysate is then subjected to nuclease footprinting with RNase I. Ribosomes are pelleted from the digested lysate by ultracentrifugation through a sucrose cushion, and then RNA is extracted from the pellet. To isolate ribosome footprints, the RNA from the ribosomal pellet is resolved by electrophoresis through a denaturing gel, and then fragments of the expected size range extracted from the gel. The 3′ ends of these RNA fragments are treated with T4 PNK to allow ligation of a pre-adenylated DNA linker with T4 Rnl2(tr) K227Q. This RNA-DNA hybrid molecule is used as a template for reverse transcription, and following gel-based purification of full-length products the resulting single-stranded cDNA is then circularized using a CircLigase. A double-stranded DNA library of suitable structure and concentration for Illumina sequencing is then constructed from the single-stranded cDNA circles by means of a PCR reaction.

As the result of our continuing development and optimization, the protocol described in this article has a number of innovations with respect to our previously published protocol (see Fig. 1):

1. We have designed a number of oligonucleotide synthetic controls to guide the remaining gel extraction steps, judge the efficacy of the circularization reaction, and to aid in the planning and execution of the final library construction PCR. These are outlined in Section 2.3.
2. A number of the enzymes employed in the footprinting and library construction have changed: we now obtain RNase I from Epicentre; use Protoscript II for the reverse transcription, and CircLigase II for cDNA circularization.

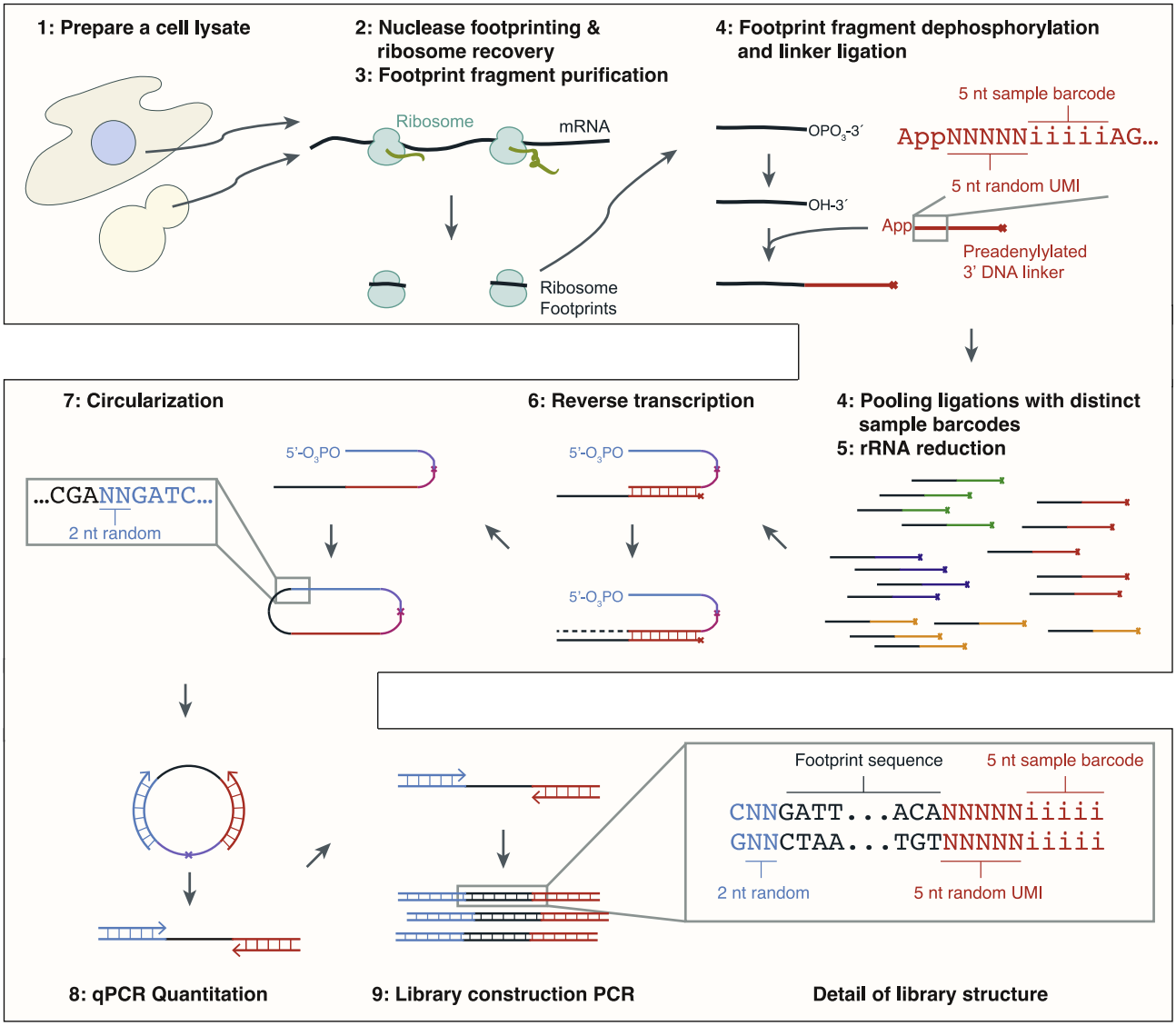

Fig. 1. A schematic of the ribosome profiling protocol. The major steps of the updated ribosome profiling protocol, highlighting several of the most important developments. Each step has the same name and number as the corresponding part of this protocol. UMI – Unique Molecular Index.