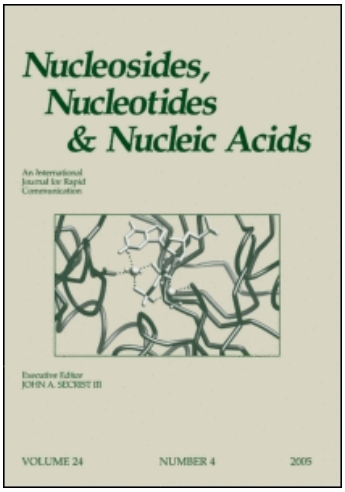


**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# NEW APPROACHES TOWARDS FLUORESCENCE LABELLING OF MESSENGER RNA TRANSCRIPTS

Arlette Garnier; Dieter Hüsken; Jan Weiler

Online publication date: 31 March 2001

**To cite this Article** Garnier, Arlette , Hüsken, Dieter and Weiler, Jan(2001) 'NEW APPROACHES TOWARDS FLUORESCENCE LABELLING OF MESSENGER RNA TRANSCRIPTS', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1181 — 1185
**To link to this Article: DOI:** 10.1081/NCN-100002515
**URL:** http://dx.doi.org/10.1081/NCN-100002515

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1181–1185 (2001)

# NEW APPROACHES TOWARDS FLUORESCENCE LABELLING OF MESSENGER RNA TRANSCRIPTS

**Arlette Garnier, Dieter Hüsken, and Jan Weiler***

Functional Genomics, Novartis Pharma AG,
CH-4002 Basel, Switzerland



## ABSTRACT

Fluorescence labelling of nucleic acids is being used for a wide range of biological applications. The performance of these techniques is dependent on fluorochrome labels with a high sensitivity and high resistance to photobleaching. Indo-cyanine dyes such as Cy3, Cy5 or Cy7 have been found to fulfil these requirements. This study describes several different RNA labelling techniques allowing for a Cy5 based detection of mRNA transcripts.

While the fluorescent labelling of DNAs for routine use as probes in hybridisation assays is well described, few procedures for the effective fluorescent labelling of long RNA fragments have been published, until recently. Hereby, commercially available nucleotides, e.g. fluorescein-, biotin- or digoxygenin modified UTP or CTP are incorporated into RNA by *in vitro* transcription (IVT). Fluorescein itself is not an ideal marker because of its pH sensitivity, its photosensitivity and self-quenching effects. Other compounds such as biotin- or digoxygenin modified UTP or CTP cannot be detected directly but require an additional incubation step with strepdavidin-conjugated dyes. The latter techniques are currently being widely used in e.g. chip-based mRNA expression profiling experiments.

Indo-cyanine dyes such as Cy5, absorbing and emitting in the far-red range, are notable for their increased sensitivity thus making techniques of direct detection

*Corresponding author.

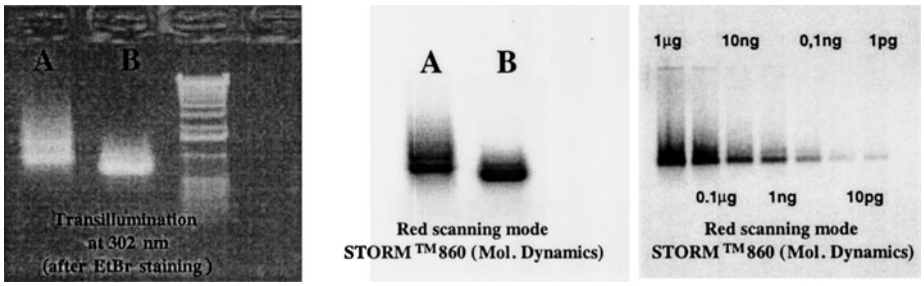


***Figure 1.*** Left and middle: mRNA transcripts (A: 2,2 and B: 1,4 kb) labelled with Cy5-NTPs by IVT following the protocol of the manufacturer. Right: Decreasing concentrations of a chemically Cy5 labelled (*Label* IT Cy5™ kit) mRNA transcript (native gel electrophoresis (0.8% agarose, 1 × TAE buffer).



much more feasible. They operate over a broad range of pH levels, maintaining fluorescence intensity at physiological pH, unlike fluorescein, which dampens below pH 8.0. Furthermore, the use of far-red dyes for of solid-phase hybridisation assays is advantageous due to the lower background of common support materials at higher wavelengths. Recently, Cy5-labelled riboNTP became commercially available allowing for combination of the labelling step with the IVT reaction, i.e. avoiding any post mRNA modifying steps. mRNA transcripts of various lenghts (0.4–2.5 kb) have been obtained with a high incorporation rate of Cy5 labels (Fig. 1, left and middle).

Recently, new methods for *post*-attachment of various marker molecules to long nucleic acids have become available. The "Mirus *Label* IT" kits (Panvera) offer a one-step covalent labelling of nucleic acids with a single reagent assumed to involve covalent modification of the C8-position of the guanosines. Similarly to the Cy5 NTP IVT RNA labelling described above, also the Mirus *Label* IT kit provided a high Cy5 incorporation rate and consequently a high sensitivity for the labelled RNA transcripts (Fig. 1, right).

Both labelling methods described above allowed for efficient and fast access to Cy5 labelled mRNA transcripts. However, in both approaches the labelling process of the RNA occurs in an uncontrolled and non site-specific manner, which might be desirable for certain applications (e.g. 2). Therefore, two alternative techniques were investigated that would allow the site-specific incorporation of a Cy5 dye into mRNA transcripts.

Following a modified protocol from literature (1–3) the 2′,3′-diol-function of the 3′-terminus of an mRNA transcript was oxidatively cleaved to the dialdehyde by sodium periodate. After coupling with an ethylenediamine derivative, the product was stabilised by aldimine reduction. The primary amino group introduced at these sites was used for attachment to a commercially available Cy5 N-hydroxysuccinimidyl active ester. Based on this chemical 3′-labelling procedure (Fig. 2), various Cy5-labelled RNA transcripts of different lengths (0.4 to 2.5 kb)



MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

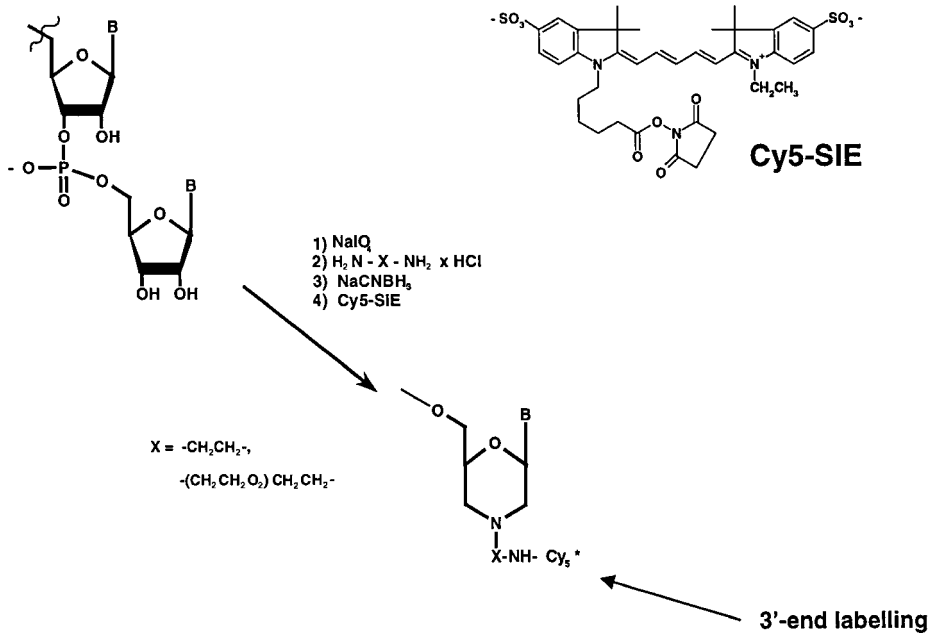


***Figure 2.*** Chemical labelling at the 3′-terminus of RNA with Cy5.

were obtained in amounts up to 1 mg. The labelling reaction was nearly quantitative and no significant degradation of RNA was observed during any of chemical steps (Fig. 3). To confirm that conjugation of the label occurs exclusively at the primary alkyl-amino function, and not at the exocyclic amino groups of the bases, mRNA on which no prior chemical modification step had been performed, was treated with

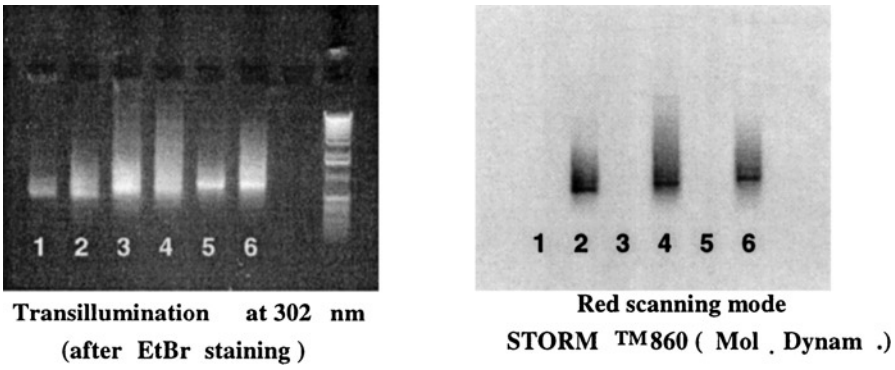


***Figure 3.*** 3′-Cy5 labelled mRNA transcripts (lanes 2, 4, 6; lanes 1, 3, 5 represent the corresponding unlabelled transcripts) of different lengths (600, 800 and 1400 bp) applied to native gel electrophoresis (0.8% agarose, 1 × TAE buffer).

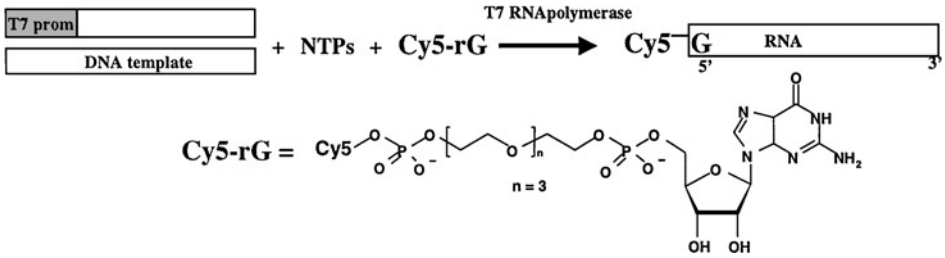


***Figure 4.*** Site-specific (5′-terminus) enzyme-mediated incorporation of Cy5 initiator guanosin monophosphate into RNA transcripts.

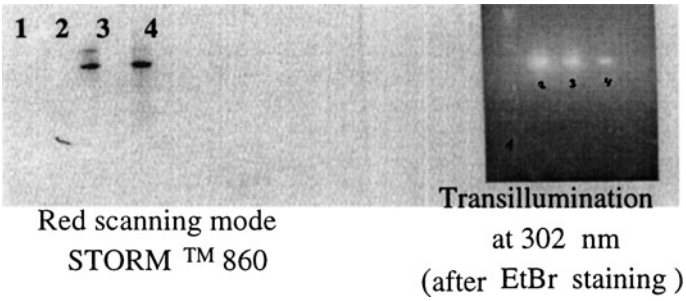


***Figure 5.*** Lane 1: DNA marker; Lane 2: Unlabelled 1,3 kb mRNA transcript; Lane 3 and 4: 5′-Cy5 labelled mRNA transcripts (ratio: Cy5-rG:GTP:ATP,UTP,CTP 1:12:16 and 1:1:8, respectively). Native gel electrophoresis: 0.8% agarose, 1 × TAE buffer.

the Cy5 N-hydroxysuccinimidyl active ester. As expected, no label incorporation was observed. This chemical RNA 3′-end labelling procedure offers an efficient way to specifically incorporate a single label per RNA molecule.

Seelig and Jäschke have described a new type of IVT initiator molecule comprising a guanosine monophosphate attached to an anthracene ring system by a long flexible polyethyleneglycol spacer (4). This initiator nucleotide has been site-specifically incorporated into RNA transcripts yielding RNA conjugates with an anthracene moiety at the 5′-terminus. In an analogous approach, a Cy5 labelled initiator guanosine monophosphate (available from Interactiva, Ulm, Germany) was successfully incorporated at the 5′-terminus of RNA transcripts. Also this approach allowed for the incorporation of a single Cy5 label at a defined position within an RNA sequence (Fig. 4).

Compared to the *post*-chemical 3′-labelling, the enzyme-mediated 5′-labelling process is considerably faster, however, the labelling efficiency is dependent on the relative ratio of dye-labelled vs. unlabelled NTPs: the maximum rate of Cy5 incorporation was found to be app. 50% (Fig. 5).

## ACKNOWLEDGMENTS

We thank Damiano Marinelli for excellent technical assistance.

## REFERENCES

1. Drobyshev, A.; Mologina, N.; Shik, V.; Pobedimskaya, D.; Yershov G.; Mirzabekov, A. *Gene*, **1997**, *188*, 45–52.
2. Wu, T.; Ruan, K.; Liu, W. *Nucleic Acids Res*., **1996**, *24*, 3472–3473.
3. Prudnikov, D.; Mirzabekov, A. *Nucleic Acids Res*., **1996**, *24*, 4535–4542.
4. Seelig B.; Jäschke A. *Tetrahedron Lett*., **1997**, *38*, 7729–7732.





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

# Request Permission or Order Reprints Instantly!