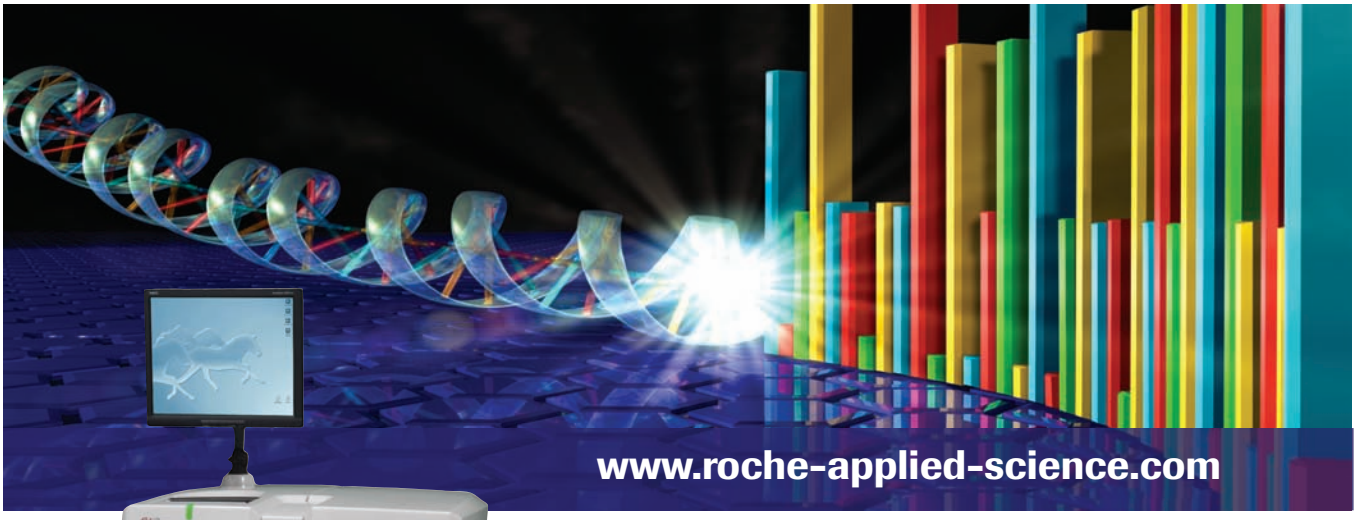


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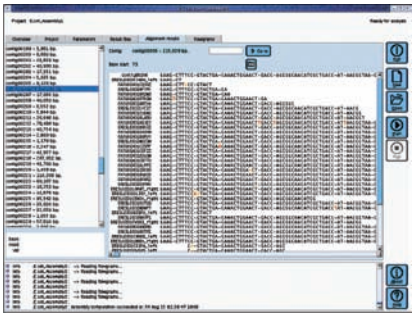
*Whole Genome De Novo Sequencing*



**GS Assembler.** Example of the GS Assembler Alignment Results window showing the result of a successful assembly. The screen displays multiple alignment of reads within contigs with reference to the consensus sequence.

Whole Genome Sequencing with the GS FLX System can be used for *de novo* assembly and assembly against a reference sequence with the GS Assembler or GS Reference Mapper ("Newbler").

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## Eukaryote Whole Genome Sequencing

**Genic Regions of a Large Salamander Genome Contain Long Introns and Novel Genes.** Smith JJ, Putta S, Zhu W, Pao GM, Verma IM, Hunter T, Bryant SV, Gardiner DM, Harkins TT, Voss SR.
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**The complete genome of an individual by massively parallel DNA sequencing** David A. Wheeler, Maithreyan Srinivasan, Michael Egholm, Yufeng Shen, Lei Chen, Amy McGuire, Wen He, Yi-Ju Chen, Vinod Makhijani, G. Thomas Roth, Xavier Gomes, Karrie Tartaro, Faheem Niazi, Cynthia L. Turcotte, Gerard P. Irzyk, James R. Lupski, Craig Chinault, Xing-zhi Song, Yue Liu, Ye Yuan, Lynne Nazareth, Xiang Qin, Donna M. Muzny, Marcel Margulies, George M. Weinstock, Richard A. Gibbs, Jonathan M. Rothberg
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**SNP discovery in swine by reduced representation and high throughput pyrosequencing.** Wiedmann RT, Smith TP, Nonneman DJ.
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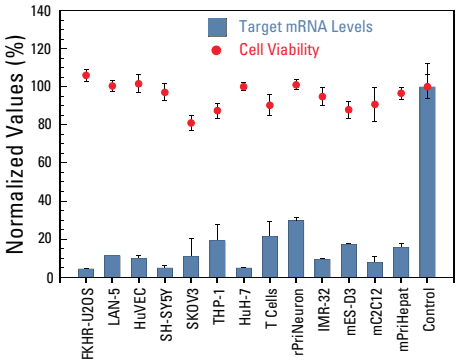


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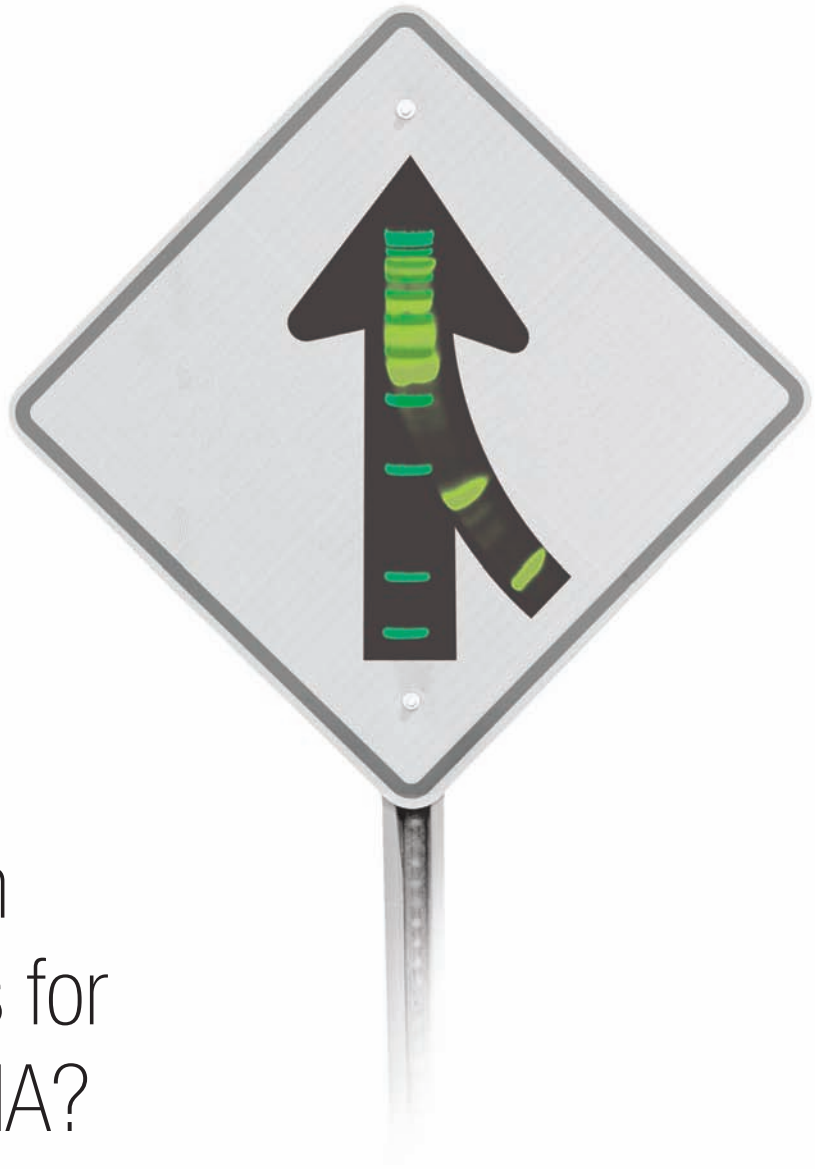

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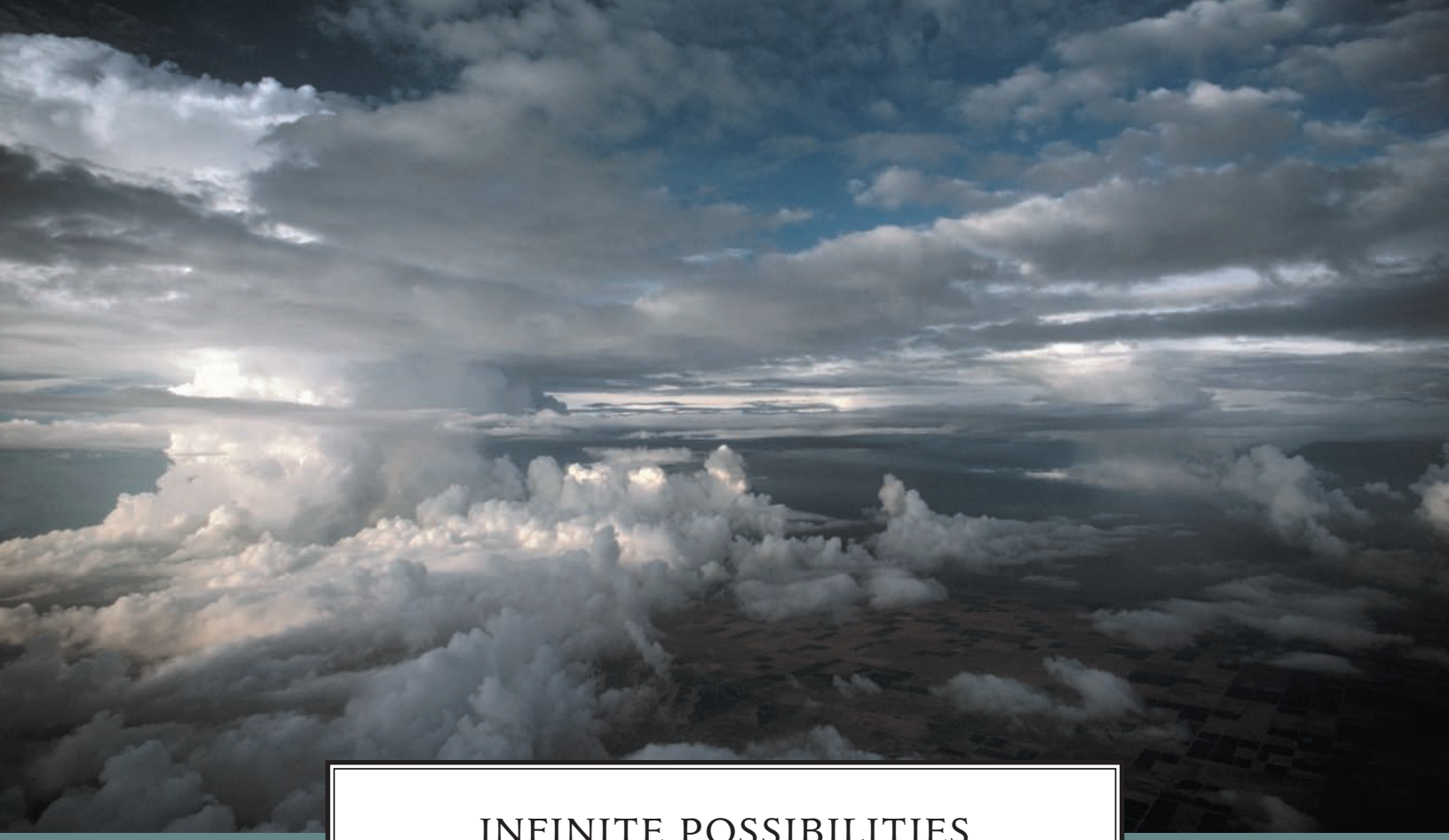

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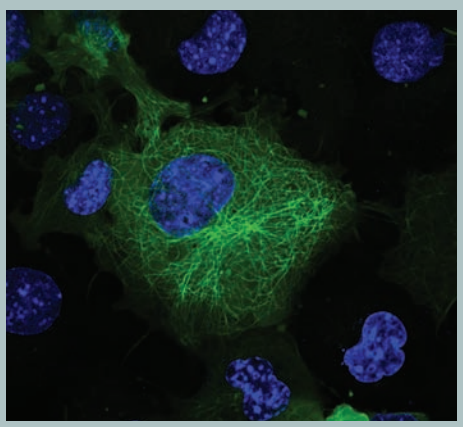
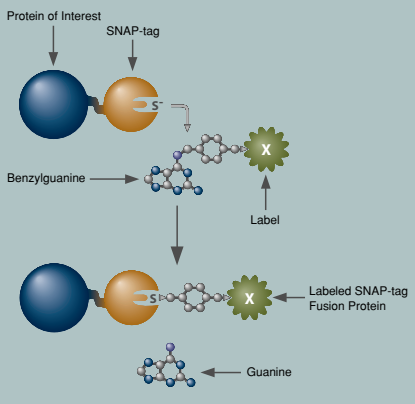


*Live COS-7 cells transiently transfected with pSNAPm-Tubulinβ. Cells were labeled with SNAP-Cell TMR-Star (green pseudocolor) for 30 minutes and counterstained with Hoechst 33342 (blue) for nuclei.*

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## Methods Overview

A general overview of the ExactSTART transcript tagging and amplification process is shown (Fig. 1) and summarized as follows:

1. Treat a total RNA or size-selected RNA sample with a discrete set of RNA modifying enzymes (Table 1) to generate substrates for tagging.
2. Ligate a tagging sequence specific for the downstream application to the exact 5′ nucleotide of the desired RNA.
3. Reverse-transcribe the tagged RNA into cDNA that is now tagged at both ends.

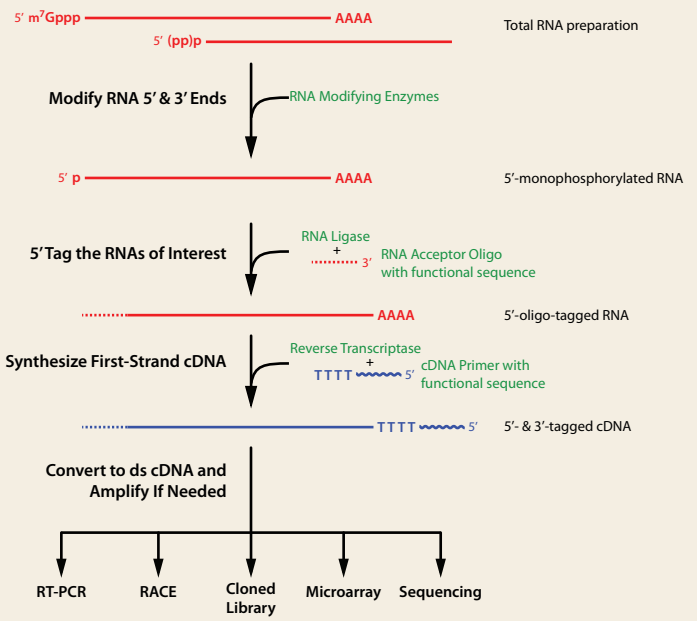


**Figure 1. Overview of the ExactSTART™ transcript discovery and analysis process.** The actual RNA modifying enzymes used (Table 1) and their order of use is dictated by the RNA species that you wish to tag. The tagging sequence included in the RNA Acceptor Oligo and the cDNA Primer is dependent on the intended downstream application.

**Table 1. The ExactSTART™ Platform of transcriptome discovery and profiling tools uses a select group of RNA modifying enzymes with strict enzymatic specificity.** Shaded enzymes are unique to EPICENTRE.

| | RNA Modifying Enzyme | RNA Substrate(s) End-Product | Comments |
|---|---|---|---|
| 5′ End | RNA 5′ Polyphosphatase* | 5′ pppN—— 3′ → 5′ pN—— 3′ | Removes γ, β phosphates from RNA with 5′-triphosphorylated end. |
| 5′ End | Tobacco Acid Pyrophosphatase (TAP) | 5′ m7GpppN—— 3′ → 5′ pN—— 3′<br>5′ pppN—— 3′ → 5′ pN—— 3′ | Removes the 5′ cap structure from 5′-capped RNA. Also removes γ, β phosphates from RNA with a 5′-triphosphorylated end. |
| 5′ End | Terminator™ 5′-Phosphate*-Dependent Exonuclease | 5′ pN—— 3′ → NMPs | Degrades RNA with a 5′-mono-phophorylated end. |
| 5′ End | APex™ Heat-Labile Alkaline Phosphatase | 5′ pN—— 3′ → 5′ HO—— 3′<br>5′ pppN—— 3′ → 5′ HO—— 3′ | Removes terminal phosphates from RNA. |
| 5′ End | Polynucleotide Kinase (PNK) | 5′ HO—— 3′ → 5′ pN—— 3′<br>5′ ——Np 3′ → 5′ ——OH 3′ | Adds a phosphate to the 5′-hydroxyl end of RNA. Also removes 3′ phosphate. |
| 3′ End | Poly(A) Polymerase | 5′ ——OH 3′ → 5′ ——AAAA 3′ | Adds a poly(A) tail to the 3′-hydroxyl end of RNA. |
| | RNA Ligase | 5′——OH 3′ (acceptor)<br>+<br>5′ pN—— 3′ (donor)<br>↓<br>5′ ——— 3′ | Joins RNA with 5′ monophosphate to RNA with a 3′ hydroxyl. |

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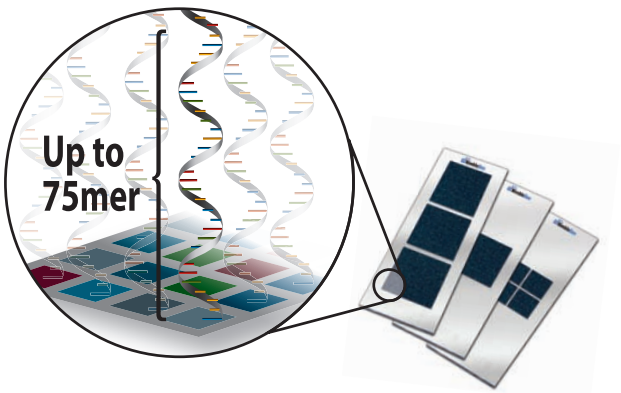


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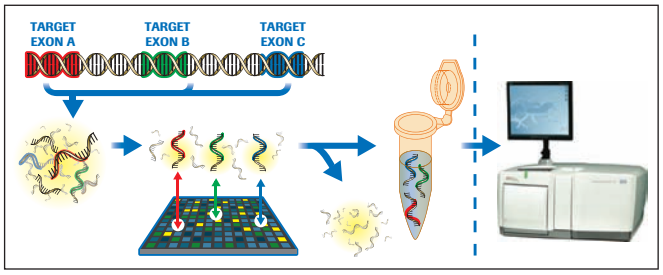


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# EVOLUTION


By **Nicholas H. Barton**, *University of Edinburgh*, **Derek E.G. Briggs**, *Yale University*, **Jonathan A. Eisen**, *University of California, Davis*, **David Goldstein**, *Duke University Medical Center*, and **Nipam H. Patel**, *University of California, Berkeley*

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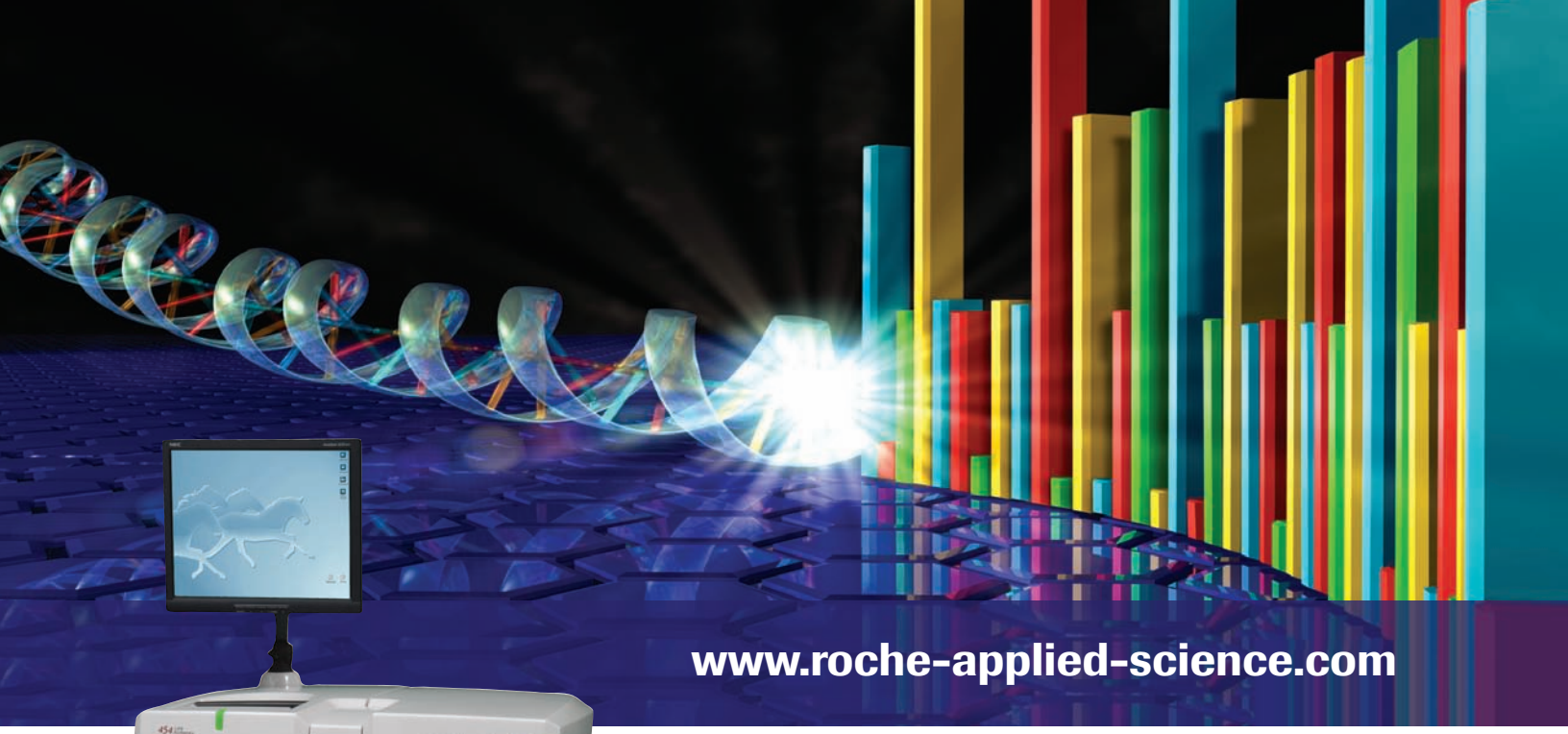


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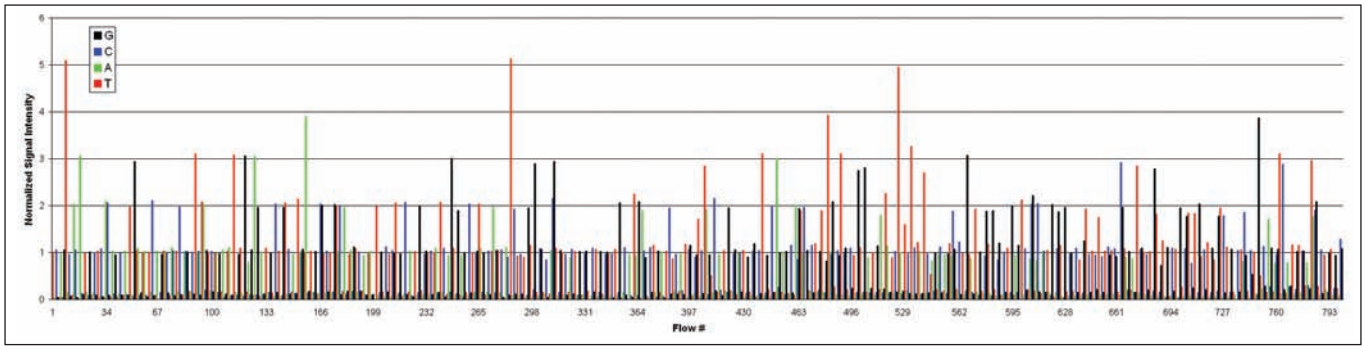


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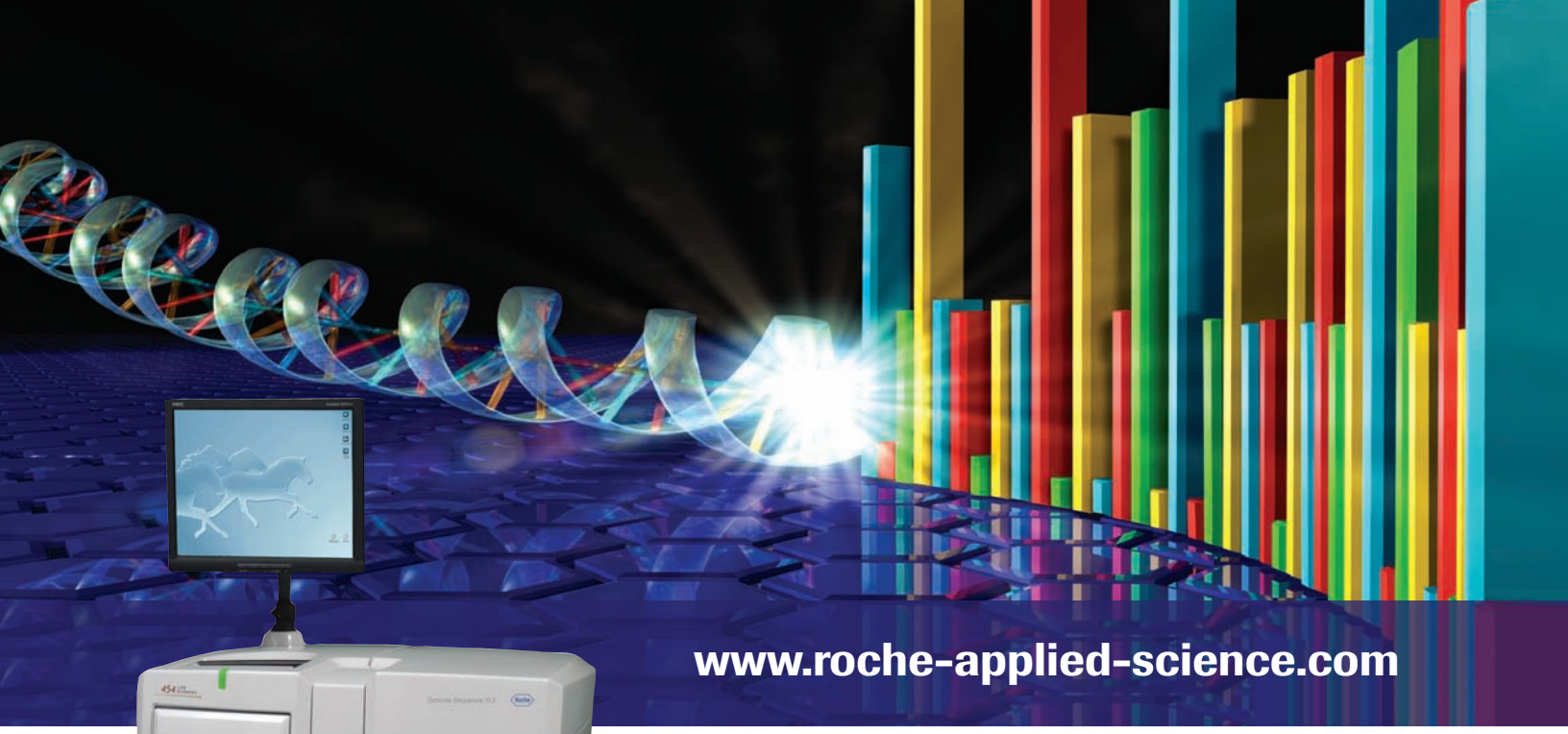

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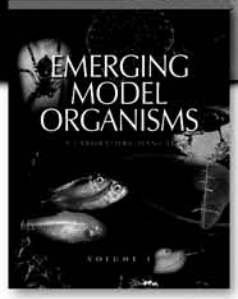


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