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Using single-molecule approach to visualize the nucleosome assembly in yeast nucleoplasmic extracts

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1	Using single-molecule approach to visualize the nucleosome assembly in yeast nucleoplasmic
2	extracts
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10	
11	Abstract
12	
13	In eukaryotic cells, the smallest subunit of chromatin is the nucleosome, which consists of a segment of
14	DNA wound on histone protein cores. Despite many years of effort, the process of nucleosome assembly
15	and disassembly is still not very clear. Here, we present a convenient method to investigate the process of
16	nucleosome assembly at the single molecule level. We invented a novel system derived from the yeast
17	nucleoplasmic extracts (YNPE), and demonstrated that the YNPE supports the nucleosome assembly
18	under physiological condition. By combining the total internal reflection fluorescence microscopy with
19	microfluidic flow-cell technique, the dynamic process of nucleosome assembly in YNPE was visualized
20	at single-molecule level. Our system provides a novel in vitro single-molecule tool to investigate the
21	dynamics of nucleosome assembly under physiological conditions.
22	
23	Keywords: Nucleosome assembly, Single-molecule, Yeast nucleoplasmic extracts, Total internal
24	reflection fluorescence microscopy, Microfluidic flow-cell
25	1. Introduction
26	
27	The Eukaryotic genome is packaged into chromatin for fitting in the nucleus. The fundamental unit of
28	chromatin is nucleosome which consists of an octamer of four core histones (H2A, H2B, H3 and H4)
29	wrapped by 146 base pairs of DNA[1]. The repeating nucleosomes are further compacted into higher-

31 assembly is a key to controlling multiple biological processes that require access to nuclear DNA, such as

order structures by the linker histone H1[2]. It is well known that the dynamic regulation of nucleosome

32 transcription, DNA replication, and DNA damage repair [3, 4].

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