HMW DNA Isolation and Tagging for HD-Mapping

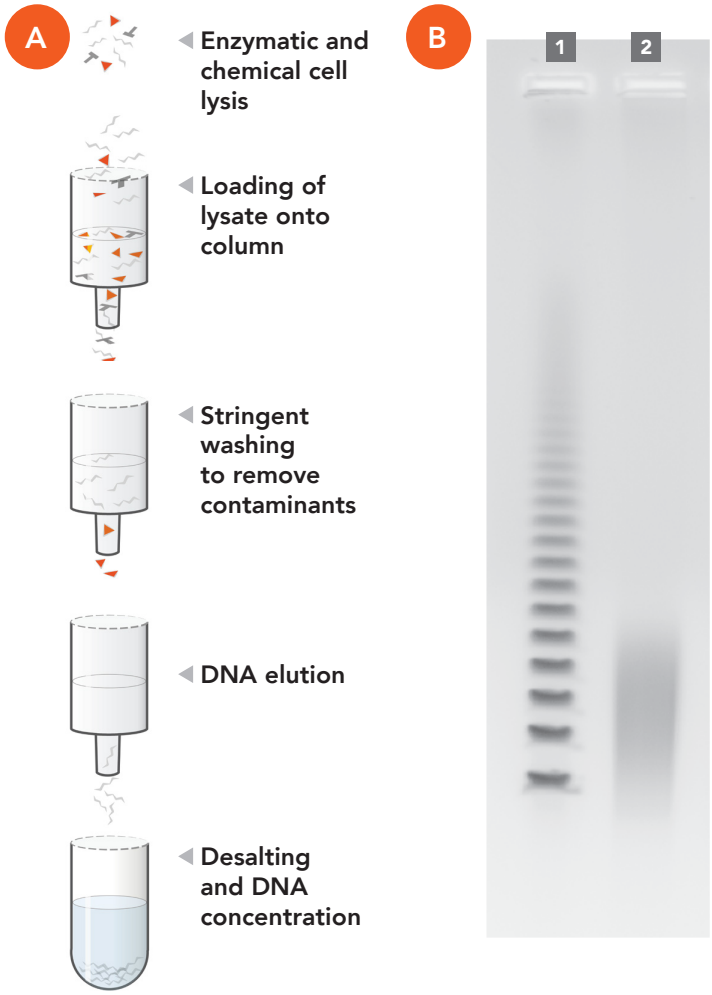
Nabsys

Overview

Whole genome electronic maps enable a number of applications ranging from sequence assembly scaffolding to microbial strain identification. The isolation and sequence-specific tagging of pure, high molecular weight (HMW) DNA is critical for generating high-resolution maps. Due to the resolution of Nabsys HD-Mapping, HMW DNA of sufficient length and purity is relatively easy to obtain using commercially available kits with minor modifications. Here we describe a general approach for HMW DNA isolation followed by sequence-specific tagging in preparation for single molecule electronic detection.

High-Definition Mapping System

DNA Isolation Procedure



Sample Input

The general protocol described here is appropriate for DNA isolation from a variety of source materials including mammalian cells and tissues, yeast, and bacterial cultures. Depending on the source material, an up-front processing step may be required (i.e. tissue dissociation, cell spheroplasting, or nuclei isolation).

DNA Isolation Workflow

For many different sample types, HMW DNA of sufficient length and purity can be isolated using relatively rapid and easy kit-based approaches. While there are a number of HMW DNA kits available from different manufacturers and employing different chemistries, we recommend the NucleoBond® AXG kit from Macherey-Nagel (**FIGURE 1A, TABLE 2**). This approach begins with a combination of enzymatic and chemical lysis followed by ion exchange chromatography for purification. Following a series of wash steps, DNA is eluted from the column and concentrated / desalted by precipitation. The use of wide bore pipet tips and limiting the duration of the vortexing steps minimizes DNA fragmentation without significantly impacting purity or yield. This procedure allows for isolation of 10-20 µg of DNA in the size range of 50-300 kb in ~5 hours (**FIGURE 1B, TABLE 1**).

FIGURE 1: Overview of DNA isolation procedure using ion exchange chromatography. (A) The general isolation procedure from cell lysis through DNA elution and precipitation (adapted from www.mn-net.com). (B) A typical pulsed-field gel of *E. coli* MG1655 DNA isolated using the NucleoBond procedure run next to a lambda ladder.

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Sample Type	260/280	260/230
E. coli	1.88	2.31
B. subtilis	1.87	2.54
C. albicans	1.85	2.48
H. sapiens- cultured cells	1.85	2.14

TABLE 1: Observed UV/vis absorbance ratios for HMW DNA isolated from different cell types.

Sequence-Specific DNA Tagging

The general process for sequence-specific HMW DNA tagging for Nabsys HD-Mapping is shown in Figure 3. The sequence specificity is determined by the choice of nicking enzyme(s). The most appropriate nicking approach for a given genome can be determined using a supplied software tool or determined empirically. Nick translation is then used to provide a tag attachment site. Tagged DNA molecules are coated with RecA protein and injected into the Nabsys instrument. A wide range of nicking enzymes is available from commercial suppliers and all enzymes and buffers required for nick translation and coating are provided in the HD-Mapping Sample Preparation Kit (**FIGURE 2, TABLE 2**).

FIGURE 2: HD-Mapping Sample Preparation Kit for sequence-specific tagging. Includes buffers and enzymes required for labeling, tagging, and coating of HMW DNA.

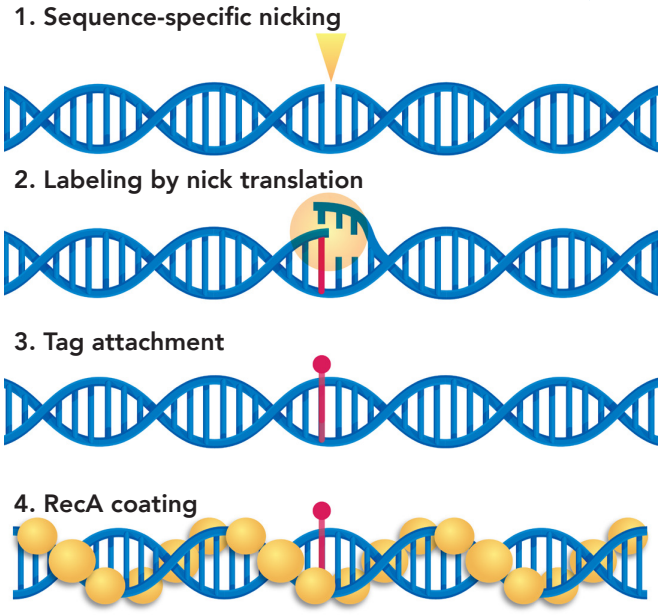

FIGURE 3: Sequence-specific DNA tagging workflow for Nabsys HD-Mapping. (1) HMW DNA is nicked at specific recognition sites. (2) Label is introduced using nick translation. (3) Tags are attached at labeled sites. (4) DNA is coated with RecA prior to injection into instrument.

Conclusions

Isolation of pure HMW DNA appropriate for Nabsys HD-Mapping is rapid and straightforward using a commercially available kit with minor modifications to limit mechanical shearing. The result is high quality HMW DNA suitable for sequence-specific tagging and single molecule electronic detection for a wide range of HD-Mapping applications. For more information about Nabsys HD-Mapping please visit: **www.nabsys.com.**

MFR.	Description	Part #
Nabsys	HD-Mapping Sample Analysis Kit	900-00006
	HD-Mapping Sample Preparation Kit	900-00007
Macherey -Nagel	NucleoBond AXG20 columns	740544
	NucleoBond Buffer Set III	740603

TABLE 2: List of materials for DNA isolation and sequence-specific tagging in preparation for Nabsys HD-Mapping.

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