

Quick, Consistent and Easy Automated DNA Purification for *Drosophila* Genotyping.



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Rachel Mann¹, Cynthia Staber²

¹Promega Corporation, Madison, WI; ²Brown University, Providence, RI
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1. Abstract

DNA purification from *Drosophila melanogaster* typically requires time-consuming manual homogenization and careful preparation and handling of multiple reagents and buffers. Variability in overall DNA quality due to an individual's technique and expertise can compromise the results of important downstream applications. Automation of this routine laboratory task improves consistency and frees up valuable researcher time and talent to accelerate the discovery process. The Maxwell[®] 16 System combines compact instrumentation, prefilled reagent cartridges and optimized automated methods to perform DNA purification of 1-16 samples in ~45 minutes with minimum handling. Here we report the utility of Maxwell[®] 16 to automate *Drosophila* DNA purification from whole animals and body parts for genotyping by PCR and Southern blot.

2. Introduction

Recently, several manufacturers have introduced modest-scale platforms to bring automation of routine nucleic acid purification within reach of many smaller labs. The Promega Maxwell[®] 16 System was designed to meet the needs of low- to moderate-throughput users without considerable capital investment, training or maintenance (1). The prefilled cartridge design and mechanical plunger action of the Maxwell[®] 16 System make it ideal for simple, rapid purification of a wide variety of sample types.

The Maxwell[®] 16 System uses paramagnetic MagneSil[®] particles that bind and move nucleic acids through a series of wash steps prior to elution. This technology has been adapted to a range of purification technologies for DNA, RNA and protein, making Maxwell[®] 16 a highly versatile, integral tool in the laboratory. The mechanical action of the Maxwell[®] 16 plunger breaks apart many sample types, allowing fully automated extraction without the need for preprocessing – a particular advantage to *Drosophila* researchers.

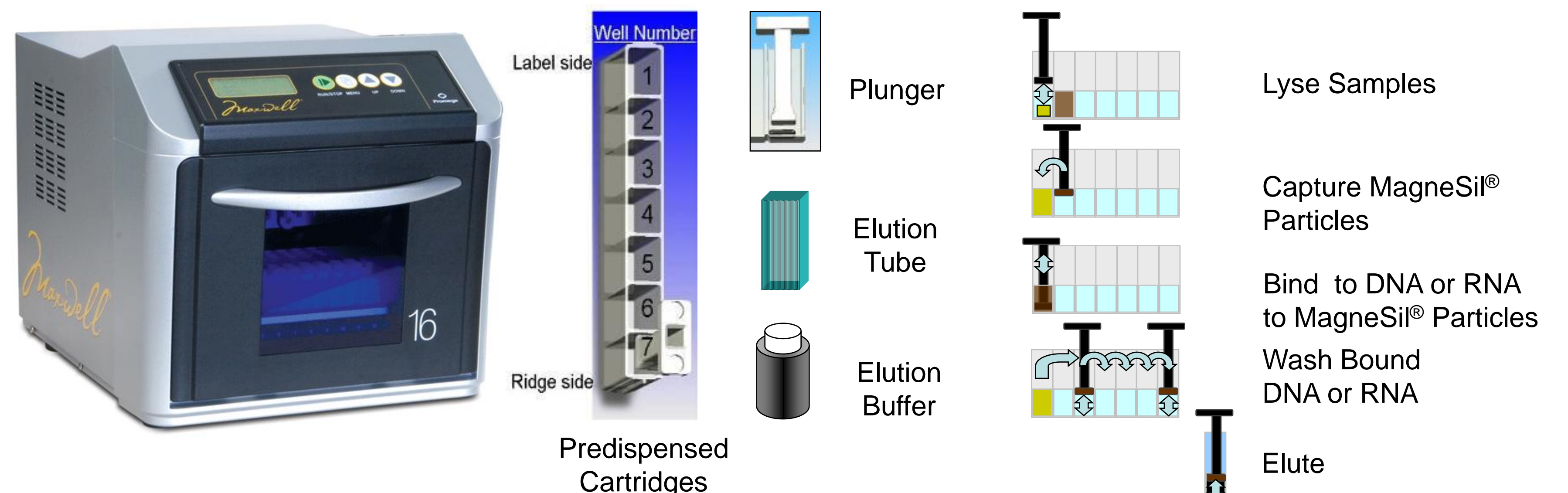
The Maxwell[®] 16 System is available in two configurations. The Standard Elution Volume (SEV) format allows elution in 300µl, which is best for assays requiring higher yield of nucleic acid. The Low Elution Volume (LEV) format allows elution in a minimal volume (30µl), which is ideal for extraction of trace nucleic acids (2) or for extraction of higher concentrations of nucleic acids.

We describe automated protocols for isolation of DNA from whole *Drosophila* animals and *Drosophila* body parts using the Maxwell[®] 16 nucleic acid purification kits. Additional details for sample types purified by the Maxwell[®] 16 System are available in the Maxwell[®] Applications Database at <http://www.promega.com/maxwell16/applications/default.asp>.

3. References

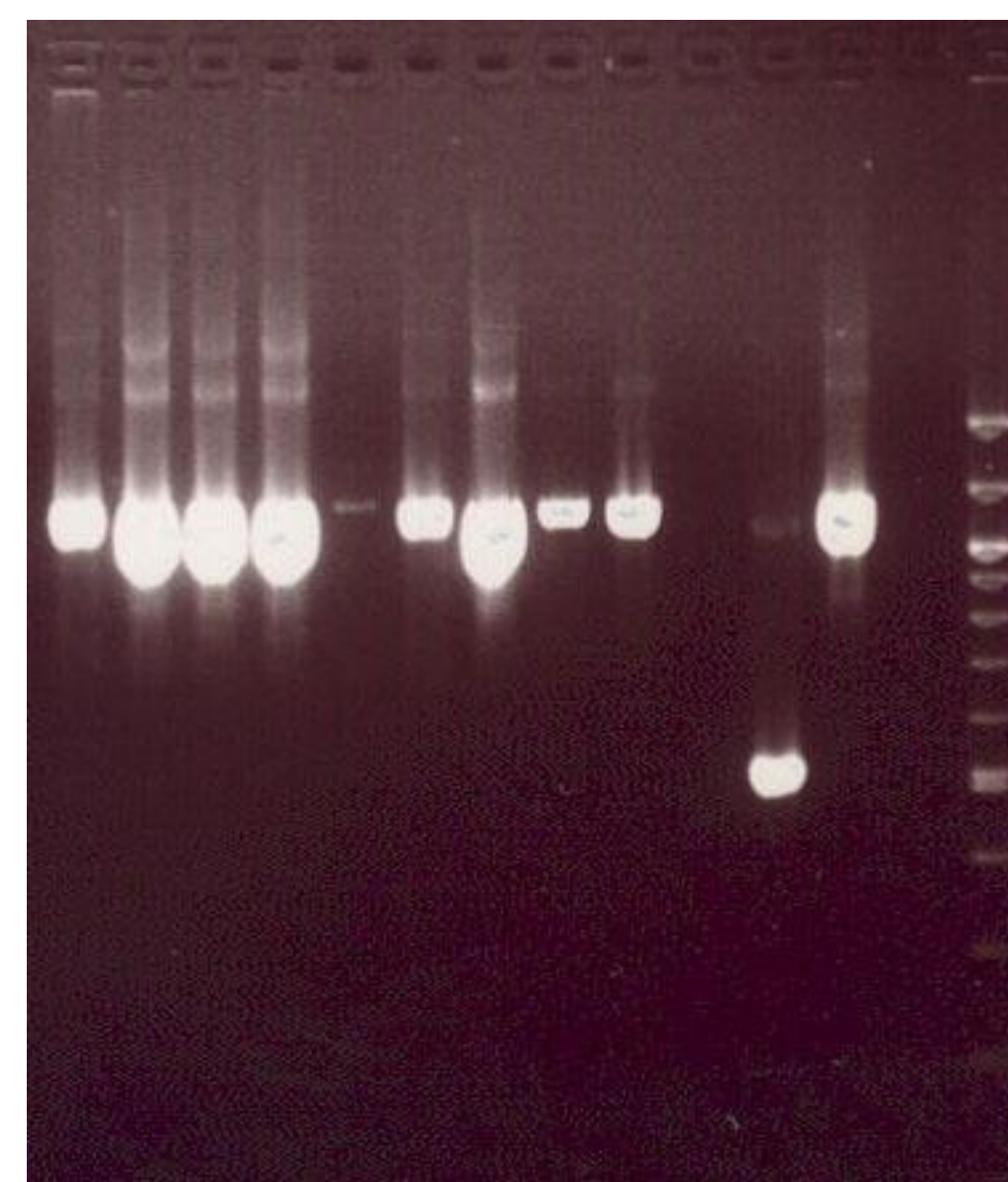
1. Kephart, D. et al. (2006) *Promega Notes* 92, 20–3.
2. Grunst, T. (2007) *Promega Notes* 97, 4–5.

4. Maxwell[®] 16 System



5. Single Animal and Population PCR Genotyping

1 2 3 4 5 6 7 8 9 10 11 12 13 M

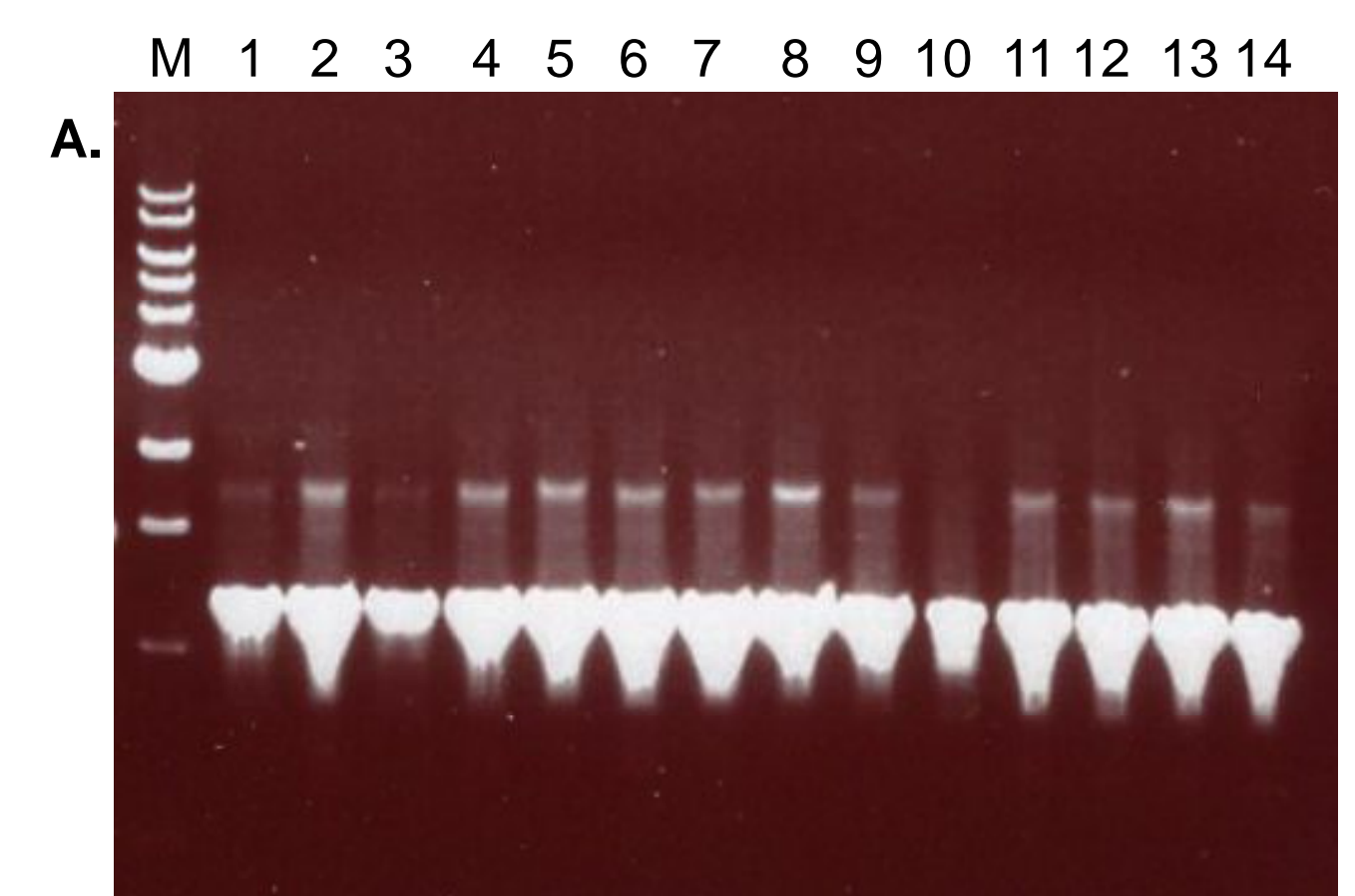


As a proof-of-concept experiment, DNA was isolated from various sample types in the same population using the Maxwell[®] 16 Tissue DNA Purification Kit (Promega), and eluted in 300 µl of water. Either 1 µl (lanes 1–4, 8–9) or 5 µl of a 1:100 dilution (lanes 5–7) of eluate were used as template for PCR. Shown here on a 0.8% agarose gel are ~1,100bp fragments amplified from DNA from the following:

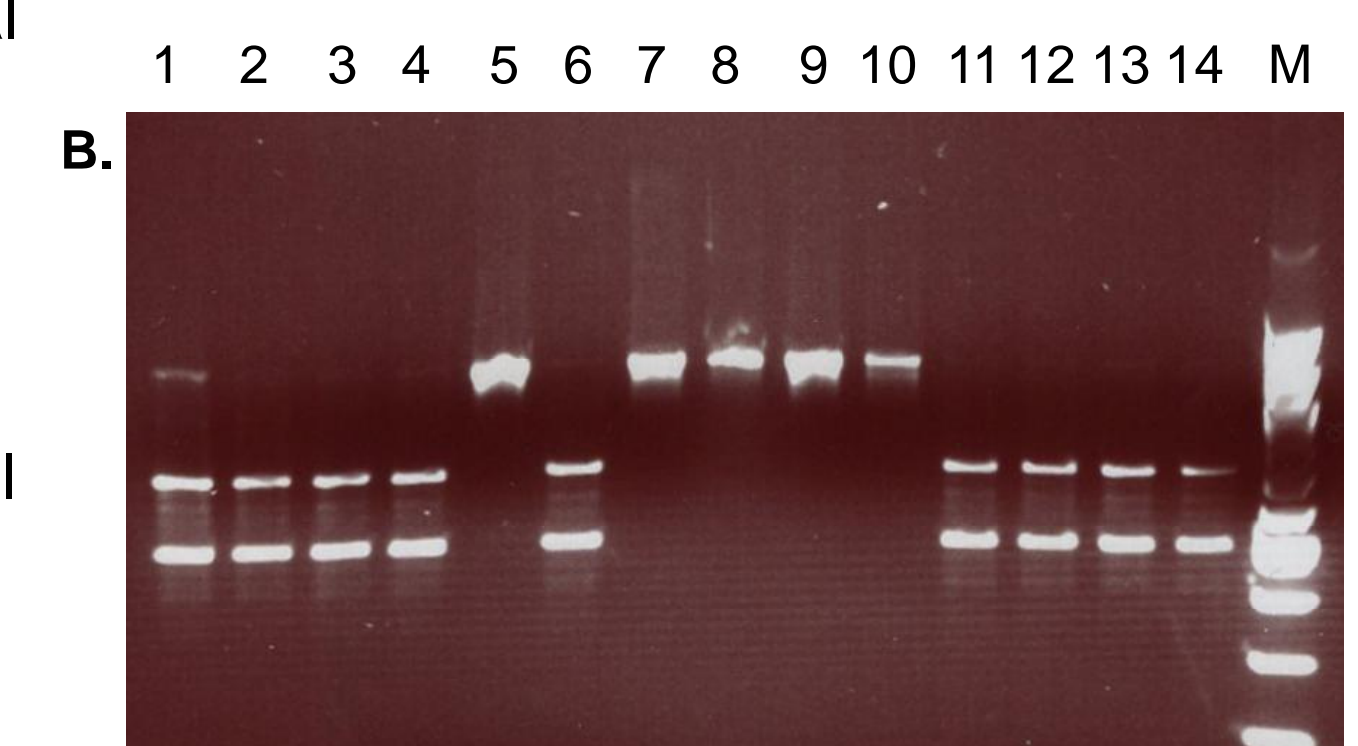
Lane 1	1 whole fly	Lane 9	12 pairs of fly wings (frozen at –80° C. for 5 minutes prior to processing)
Lane 2	12 fly heads	Lane 10	BLANK
Lane 3	1 fly head	Lane 11	Positive control (~550bp)
Lane 4	1 whole fly	Lane 12	Positive control (~1,100bp)
Lane 5	8 pair fly testis	Lane 13	BLANK
Lane 6	~60mg 3 rd instar larvae	Lane M	100bp ladder (NEB)
Lane 7	~60mg pupae		
Lane 8	12 pairs of fly wings		

6. Detection of a Transgenic HR Construct by PCR and Restriction Enzyme Digestion

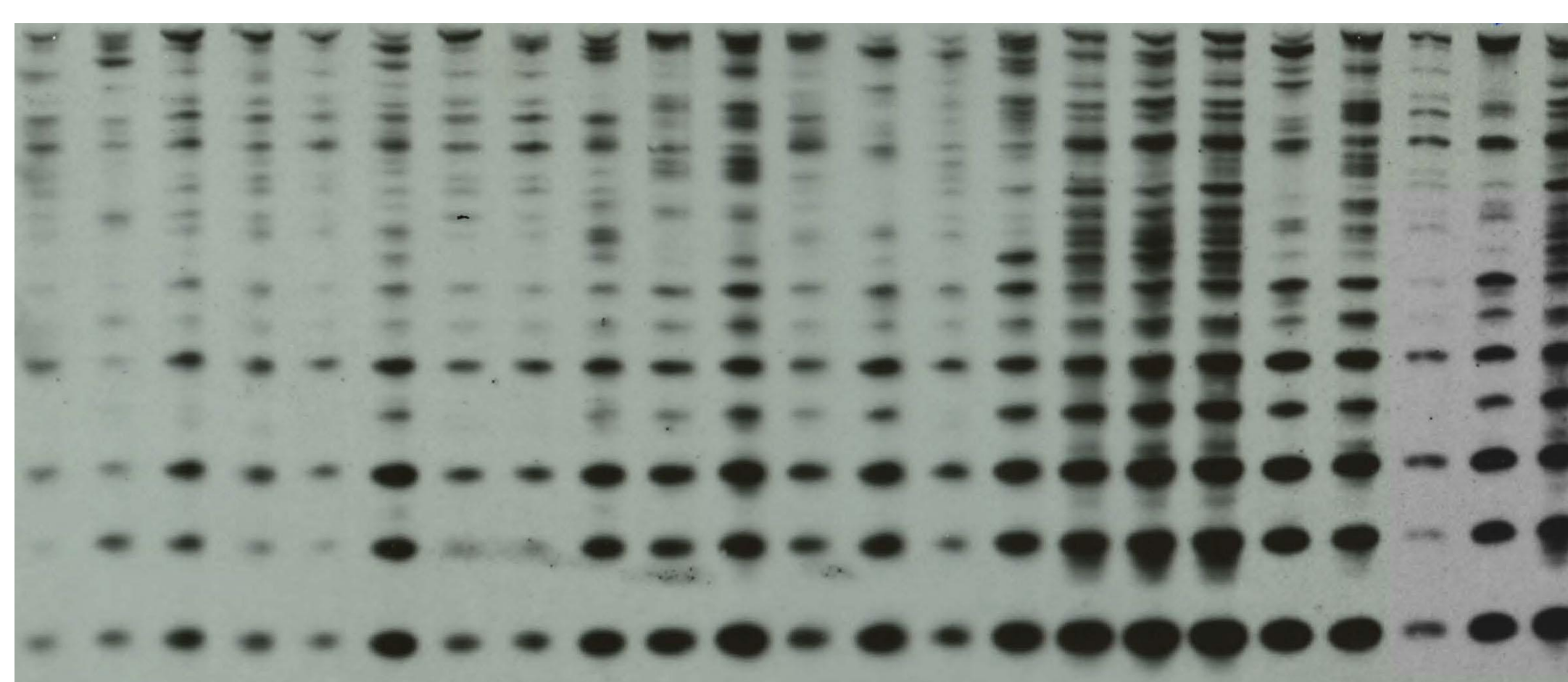
Panel A. DNA was isolated from 14 individual male flies using the Maxwell[®] 16 Tissue DNA Purification Kit (Promega) and eluted in 300 µl of water. Five microliters of eluate was amplified 40 cycles in a 50 µl reaction using 0.25 µl GoTaq DNA Polymerase (Promega). Primers were complementary to the arms of a construct designed for a homologous recombination (HR) experiment, and the endogenous sequence. Ten microliters of PCR product was run on a 0.8% agarose gel with a 1kb ladder (NEB) as a marker (Lane M). The expected 1,197bp sequence was successfully amplified in all samples.



Panel B. Successful integration of homology arms and subsequent Cre removal of the mini-white gene results in 75 bases of vector sequence replacing endogenous sequence between arms 1 and 2. This unique sequence retains two restriction sites that can be used in a diagnostic digest to differentiate recombinant and endogenous loci. Following clean-up using the Wizard[®] SV Gel and PCR Clean-Up System (Promega), 1 µl of eluate from each of the reactions described above was digested by Acc65I in a 10 µl reaction. The 10 µl reaction was run on a 1.5% agarose gel with a 100bp ladder (NEB), (Lane M). The presence of the two smaller bands at ~400bp and ~800bp (lanes 1–4, 6, and 11–14) confirm the presence of the HR construct.



7. Screening Wild Populations for a Repetitive Element via Southern Blot



A collection of wild-caught *Drosophila* populations from diverse geographic locations was analyzed for presence and quantity of a repetitive element (Rsp). Three hundred nanograms of each DNA sample, purified using the Maxwell[®] 16 System, were digested with XbaI and run on a 1.5% agarose gel with known populations as "standards". The gel was blotted and probed with the Rsp element. Probe DNA was directly labeled with HRP (GE Healthcare) and detected using Pierce Bioscience detection reagents.