

GENE PREP

Application Guide

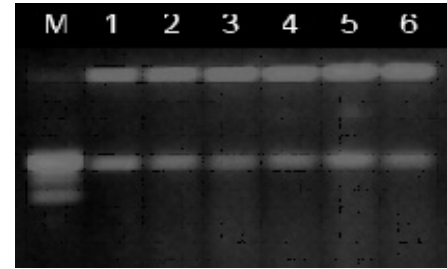
Oragene•DNA Saliva 2



- I. Purpose: To extract genomic DNA from saliva samples collected using the Oragene•DNA Saliva Self-Collection Kit.
- II. Chemical Principal: Proteinase K digestion & non-organic extraction method.
- III. Pretreatment of Saliva:
 - A. Collect saliva by following the Oragene•DNA Saliva Self-Collection Kit User Instructions.
 - B. Incubate at 50C for 2 hours to overnight with shaking in a waterbath or dry incubator.
- IV. Protocol Parameters:
 - A. Sample Volume: 2.0 mL of digest solution plus 2.0 μ l of glycogen (5mg/mL).
 - B. Maximum Number of Samples: 48 samples per run (192 samples with optional stacker)
 - C. Processing Time: ~2 hours for 48 samples (includes 1 hour drying time).
 - D. Yield: 20 - 100 μ g of purified DNA per 2 mL Oragene•DNA saliva solution.
 - E. Quality: Typical OD_{260/280} values are \geq 1.7
The DNA can be used directly in downstream processes such as fluorescence DNA sequencing, PCR, Southern blotting and restriction endonuclease digestions.
- V. Running the Protocol:
 - A. Load Reagents and Samples
 - B. Select a Protocol
 - C. Enter Number of Samples
 - D. Start the Run

VI. Example of genomic DNA obtained from Oragene•DNA saliva samples:

2mL of saliva was pre-treated with 2mL of Oragene•DNA liquid. 2mL of Oragene•DNA saliva solution plus 2µL of glycogen (5mg/mL) were loaded onto the AutoGen system for purification. Approximately 97 µg DNA per 2ml Oragene•DNA saliva solution was obtained with an average purity of OD260/280 = 1.7



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Manual	Digest proteins	Mix 2mL saliva with 2mL Oragene•DNA liquid and 2µL of glycogen (5mg/mL), outside of the instrument.
2. Automated	Precipitate protein	Add Reagent TD-R3, mix and centrifuge. Transfer supernatant to DNA tube.
3. Automated	Precipitate DNA	Add Reagent TD-R4, agitate, centrifuge and discard supernatant.
4. Automated	Wash DNA	Add Reagent TD-R5, mix, centrifuge and discard supernatant. Repeat a few times.
5. Automated	Dry DNA	Transfer DNA tube to agitation rack.
6. Automated	Resuspend DNA	Add Reagent TD-R6, mix and centrifuge.

*Oragene•DNA Saliva Self-Collection Kits are available from DNA Genotek (www.dnagenotek.com)