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AutoGen FLEX STAR

Application Guide

Buccal Swabs (Tissue Protocol)

I. **Purpose:** To extract genomic DNA from buccal swabs.

II. **Chemical Principal:** Proteinase K/organic extraction method.

III. **Preparation of Reagents:**

A. **Add proteinase K to reagent AGF-M1 (Tissue Digestion Solution A) to a final concentration of 0.4mg/ml. This should be prepared fresh before each run.**
B. **If desired, RNase A may be added to reagent AGF-M2 (Tissue Digestion Solution B) in any desired concentration immediately before use. Do not store AGF-M2 with RNase A. The RNase A will degrade during storage.**

IV. **Preparation of Buccal Swabs:**

A. **Place Buccal Swab in a 15ml Falcon tube (or equivalent). Cut the brush handle short enough so that it does not interfere with the closing of the tube cap.**
B. **Add 250µl of AGF-M1 containing Proteinase K and 250µl of AGF-M2 to the sample, and incubate at 50-55C (optimal temperature for proK activity) in a shaking incubator at 500rpm for 2-3 hours.**

V. **Protocol Parameters:**

A. **Sample Volume:** 0.5 ml of digest solution.

B. **Maximum Number of Samples:** 30 samples per run .

C. **Processing Time:** 3.4 hours for 30 samples (includes 1 hour drying time).

D. **Yield:** 2-4 µg of purified DNA per buccal swab.

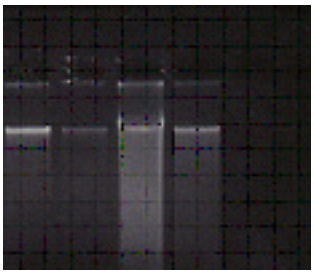
E. **Quality:** Typical OD260/280 values are 1.8-2.0. The DNA can be used directly in downstream processes such as fluorescence DNA sequencing, PCR, Southern blotting and restriction endonuclease digestions.

VI. Running the Protocol:

A. **Transfer the supernatant from Proteinase K digest to the AutoGen sample tubes. Be sure to remove the buccal swab. Presence of the buccal swab in the AutoGen tube unit will interfere with the extraction process.**
B. **Select "Tissue DNA" Protocol on the AutoGenFlex STAR.**
C. **Start the run.**

VII. Example of genomic DNA obtained from Buccal Swabs:

Samples were reconstituted with 20ul of TE buffer and 10ul was run on a gel. The samples were measured for OD and yielded 2-4ug of DNA with a 260/280 purity of 2.0.



VIII. Extraction and Purification Process

Process Site	Purpose	System Process
1. Manual	Digest Proteins	Mix Proteinase K with Reagents M1 and M2, and then add to sample.
2. Automated	Precipitate Protein	Add Reagent R7, mix and centrifuge. Transfer supernatant to DNA tube.
3. Automated	Precipitate DNA	Add Reagent R4, agitate, centrifuge, discard supernatant.
4. Automated	Wash DNA	Add Reagent R5, mix, centrifuge, discard supernatant. Repeat several times.
5. Automated	Evaporate Alcohol	Transfer DNA tube to incubation rack.
6. Automated	Resuspend DNA	Add Reagent R3, mix and centrifuge.

* All reagents and consumables are sold as a complete kit for Tissue DNA extraction on the FLEX STAR Catalog #AGKT-FXTD.

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