Genomic DNA extraction from mosquitoes

Fabio Gomes

Abstract

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Before start

Heat dry block at 65o C

Fly Griding Buffer (Adjust pH to 9.2)

	For 100 mL
0.1M NaCl	584 mg
0.2M Sucrose	6.85g
0.1M Tris-HCl pH 9.1	1.21g
0.05M EDTA	1.861
0.5% SDS	5mL 10% SDS

Protocol

Step 1.
Freeze mosquitoes and separate 1 mosquito per tube. Add 25 uL of fly grinding buffer. Grind mosquitoes with a pestle.

Step 2.
Rinse pestle with 25 uL of buffer.

Step 3.
Incubate at 65o C for 30 min

DURATION
00:30:00

Step 4.
Add 7uL 8M KAc

Step 5.
Incubate at ice for 30 min

DURATION
00:30:00

Step 6.
Collect supernatant and add 0.7 vol (28 uL over 40 uL) of room temperature of isopropanol (or 1 vol of ice cold 100% EtOH) and incubate for 5 min at room temperature. Centrifuge at RT for 15 min at max

speed.

DURATION

00:05:00

Step 7.

Centrifuge at max speed for 15 min at RT