DNA extraction protocol (Salting out) Modified V.2
Afaq M.M. Niyas¹, Caterina Villari²
¹University of Georgia

ABSTRACT
This protocol describes how to extract DNA with modified salting out method.


DOI
dx.doi.org/10.17504/protocols.io.bpgkmjuw

DOCUMENT CITATION
Afaq M.M. Niyas, Caterina Villari 2020. DNA extraction protocol (Salting out) Modified. protocols.io https://dx.doi.org/10.17504/protocols.io.bpgkmjuw

LICENSE
This is an open access document distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED
Nov 06, 2020

LAST MODIFIED
Nov 06, 2020

DOCUMENT INTEGER ID
44268

ABSTRACT
This protocol describes how to extract DNA with modified salting out method.


Modified Salting Out - mycelium only


Citation: Afaq M.M. Niyas, Caterina Villari (11/06/2020). DNA extraction protocol (Salting out) Modified. https://dx.doi.org/10.17504/protocols.io.bpgkmjuw

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited
1. Preheat the water bath at 55°C
2. Prepare **Lysis Buffer** as following (For 1 Reaction)
   a. Add $140 \mu l$ of the **extraction buffer** (Standard extraction buffer)
   b. Add $17.5 \mu l$ of **SDS 10%**
   c. Add $2 \mu l$ of **proteinase K** (20 mg/ml)
3. Add $\sim 50-100$ mg of mycelium in a 1.5 mL centrifuge tube (Use less amount if it has pigments)
4. Freeze it using liquid $N_2$ and crush it with a sterilized micro pestle
5. Add $159.5 \mu l$ of **Lysis Buffer** as soon as possible and mix it by pipetting
6. Repeat step 2 to all tubes (If you extract more than one tube)
7. Incubate at 55°C overnight

2nd Day
8. Turn on and cool centrifuge to 4°C
9. Preheat incubator or hot plate to 37°C
10. Add 2 $\mu l$ of **RNase A** (10 mg/ml) and leave it act for 10 minutes at 37°C (15 minutes at RT)
11. Add 40 $\mu l$ of a **saturated solution of NaCl** in water (>6M, autoclaved)
12. **Vortex** for 20 min
13. **Centrifuge** at 14000 rpm for 30 min
14. Transfer the **supernatant** to a new tube
15. Add one volume (200 $\mu l$ or more if needed) of **chilled isopropanol**
16. **Mix** it by flipping the tube or vortex 5 seconds
17. Keep in the **freezer or ice** for 10 minutes
18. Precipitate the DNA by **centrifugation** at 14000 rpm at 4°C for 20 min
   a. Be sure to orient tubes so that you know where the pellet will be
   b. Never vortex from this point on
19. **Discard** the supernatant by pouring it out with one single movement, without disturbing the pellet. Always pour from the side of the vial opposite to the pellet. Do not turn over the vial again until dry.
   a. Leave it to dry 10 min
   b. Dry by leaving upside down on paper towel with cap held down, tap out excess liquid
20. **Wash** the pellet with 500 $\mu l$ of 70% EtOH
21. **Centrifuge** at 14000 rpm at 4°C for 10 min
22. **Discard** the supernatant
   a. Be quick in discarding and ensure that the tube is oriented such that the pellet will not dislodge
23. **Dry** the pellet in the vacuum
   a. Start with small increments of time (3 min) and keep going until dry. Over-drying will make re-suspension difficult. Alternatively, dry the pellet in a 37°C drying oven.
24. Add 20 to 40 $\mu l$ of **sterile H$_2$O (PCR water)** to the DNA
25. Let it stay at 4°C (Refrigerator) for 20-30 minutes and mix well by tapping the tubes. Centrifuge briefly before storing in the freezer.

**Buffers and solution ingredients/concentrations**
- Extraction buffer for Standard method: 0.1 M EDTA, 0.05 M, Tris pH 8. Autoclave before use.
- Saturated NaCl: greater than 6M solution with visible salt still not in solution. Autoclave before use.
- Store 70% EtOH and isopropanol in freezer

---

**Citation:** Afq M.M. Niyas, Caterina Villari (11/06/2020). DNA extraction protocol (Salting out) Modified. [https://dx.doi.org/10.17504/protocols.io.bpgkmjuw](https://dx.doi.org/10.17504/protocols.io.bpgkmjuw)

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.