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# Isolation of genomic DNA from cell culture (CRISPR)

**Required reagents and materials:**

1. Lysis buffer

| | 100 mls | 1 L |
|---|---|---|
| 10mM Tris-HCl pH7.5 | 0.5 ml of 2M stock | 5 ml of 2M stock |
| 10mM EDTA | 2 ml of 0.5M stock | 20 ml of 0.5M stock |
| 10mM NaCl | 0.2 ml of 5M stock | 2 ml of 5M stock |
| 0.5 % sarcosyl (N-lauryl sarcosine) | w/v: 0.5 g | w/v: 5g |

   10 ug/ml Proteinase K (Make stock and aliquot and store in -20C. Add 10ul of stock per 1 ml of lysis buffer fresh each time)
   20ug/mL glycogen (add 4uL of a 5mg/mL stock per sample to lysis buffer fresh each time)

2. Precipitation Buffer – this solution will remain a slurry

| | 100mls |
|---|---|
| 150mM NaCl | 3 ml of 5M stock |
| 100% EtOH | 97 mls |

3. Wash buffer – 70% EtOH
4. Resuspension buffer – TE

**Procedure:**

**For a microfuge tube from 96 well plate:**

1. Spin down collected trypsinized cells at 6k rpm for 5 minutes.
2. Remove all media and add 300uL of Lysis Buffer (with proteinase K and glycogen) to cell pellet (may not be visible). Vortex well. [can freeze overnight at this point at -20C]
3. Heat samples to 60C for 2 hours.
4. Add 900ul (3x lysis buffer) of precipitation buffer to each tube and mix. Incubate at RT 30 min. [place at -20C for longer/overnight]
5. Spin at max speed 4C for 15 min.
6. Wash the precipitated DNA with 1ml 70% EtOH. Spin at max speed 5 min 4C
7. Remove wash buffer and let pellets dry at RT.
8. Resuspend DNA with 25ul of TE.
9. The DNA should be ready to test by PCR. Use 2ul of DNA in PCR reaction.

**For a 24 well plate:**

1. Wash cells 2x with PBS.
2. Add 300ul of lysis buffer to each well of cells. You can lyse the cells 1 of 3 ways: place the plate at -80C for 1 hour, place the plate at -20C for overnight or place the plate at 60C for 2-3 hrs. If you place the plate at 60C dampen some paper towels and place in the bottom of a Tupperware container. Place the plate on the paper towel and seal the lid of the Tupperware. This will prevent the plate from drying out.
3. If you froze the plate, remove from freezer and place in 60C oven to thaw lysis buffer.
4. Remove the lysis buffer from each well and place in an Eppendorf tube.
5. Add 900ul (3x lysis buffer) of precipitation buffer to each tube and mix. Incubate at RT 30 min.
6. Spin at max speed 4C for 15 min.
7. Wash the precipitated DNA with 1ml 70% EtOH. Spin at max speed 5 min 4C
8. Remove wash buffer and let pellets dry at RT.
9. Resuspend DNA with 50-100ul of TE.
10. The DNA should be ready to test by PCR. Use 1ul of DNA in PCR reaction.

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**For a 6 well plate:**

1. Wash cells 2x with PBS.
2. Add 500ul of lysis buffer to each well of cellsand place the plate at 60C for 2-3 hrs. If you place the plate at 60C dampen some paper towels and place in the bottom of a Tupperware container. Place the plate on the paper towel and seal the lid of the Tupperware. This will prevent the plate from drying out.
3. Remove the lysis buffer from each well and place in an Eppendorf tube.
4. Add 1500ul (3x lysis buffer) of precipitation buffer to each tube and mix. Incubate at RT 30 min.
5. Spin at max speed 4C for 15 min.
6. Wash the precipitated DNA with 1ml 70% EtOH. Spin at max speed 5 min 4C
7. Remove wash buffer and let pellets dry at RT.
8. Resuspend DNA with 200ul of TE.
9. The DNA should be ready to test by PCR. Use 1ul of DNA in PCR reaction.

**For a 96 well plate:**

1. Remove media by flipping the plate aka “dumping.” Wash cells once with 1x PBS. Remove PBS.
2. Add 50ul of lysis buffer to each well of cells. Lyse the cells by placing the plate at 60C for 2-3 hrs. Dampen some paper towels and place in the bottom of a Tupperware container. Place the plate on the paper towel and seal the lid of the Tupperware. This will prevent the plate from drying out.
3. Using a multichannel pipet, add 150ul (3x lysis buffer) of precipitation buffer to each well of plate and mix.
4. Incubate at RT 30 min.
5. Spin the plate at 3750 for 15min.
6. Decant the supernatant manually with the multichannel pipet.
7. Wash the precipitated DNA 3x with 150ul 70% EtOH. Removing each wash manually with the multichannel pipet.
8. Let the plate/wells dry with the plate open at RT. This step may take ~15min.
9. Resuspend DNA with 30ul of TE.
10. The DNA should be ready to test by PCR. Use 5ul of DNA in PCR reaction.