Protocol No. 5

DNA extraction from Next-Gen Prep Gels for next generation sequencing[#]

Suitable for up to 20 µg of genomic DNA.
Next-Gen Prep Gels guarantee a *recovery yield of 50 to 70 %*.

1. Extract genomic DNA with any conventional method. Elute in ddH_2O.

2. Collect up to 20 µg of genomic DNA in 1 Eppendorf tube. Prepare an overnight restriction digest:

    i. Genomic DNA (max. 20 µg in total)
    ii. Restriction enzyme (as suggested by manufacturer)
    iii. Digestion buffer (as suggested by manufacturer)
    iv. H_2O to final volume

    Total volume: 64 µl

3. If necessary, continue digestion the following day for an additional two hours by adding new restriction enzyme (please enquire star activity of the manufacturer).

4. Load **Next-Gen Prep Gels (S-2x4) PN 9026** (max. well volume: 100 µl[*]) with 80 µl of genomic DNA digest: 64 µl of reaction digest + 16 µl of **5 x Elchrom Scientific Sample Loading Buffer PN 3033**).

    [*] *Please note that other gel formats for higher loading volumes are available from Elchrom Scientific. Please enquire.*

5. Load a suitable DNA size marker to precisely define the fragment size range of interest: (e.g.: **M1 Marker (PN 3204)** or **M3 Marker (PN 3203)** from Elchrom Scientific).
    Please note: due to the larger well dimensions, the amount of marker per lane needs to be increased by approximately 10 fold as compared to a standard sized gel pocket (e.g. 3 µl of concentrated M3 Marker [PN 3203] + 17 µl of ddH_2O + 5 µl of 5x Elchrom Scientific Sample Loading Buffer [PN 3033]).

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6. Run the electrophoresis in **ORIGINS by Elchrom™ (PN 2100)** using the following parameters:

    i. 120 Volts, constant,
    ii. 30 mM TAE running buffer (NOTE: follow the instructions in the technical manual of the ORIGINS),
    iii. Pump delay control 4.5 minutes
    iv. Running temperature at 55° C
    v. Running time defined by **ELQuant Software** (please see http://www.elchrom.com/index.php?id=72)
        - Select Spreadex EL 1200
        - XXX = fragment length in bp for the upper selection barrier
        - YYY = fragment length in bp for the lower selection barrier

Select a gel:

Spreadex EL 1200

Select a temperature:

(•) 55 °C
( ) 20 °C

DNA fragment A
(The Longer one) XXX

DNA fragment B
(The Shorter one) YYY

Go

7. After the run, post-stain the gel with any dye of choice (e.g. SYBR, GelRed, EtBr, etc). Destain the gel if necessary.

8. Place the gel up side down on a UV transilluminator: the covalently bound plastic backing will be facing upwards.

9. To prevent UV exposure of the sample DNA, we recommend placing a UV-impermeable plastic foil between the UV screen and the gel. This foil should cover the sample DNA but allow excitation of the marker DNA.

10. Switch on the UV transilluminator and indicate the relevant marker bands on the plastic backing.

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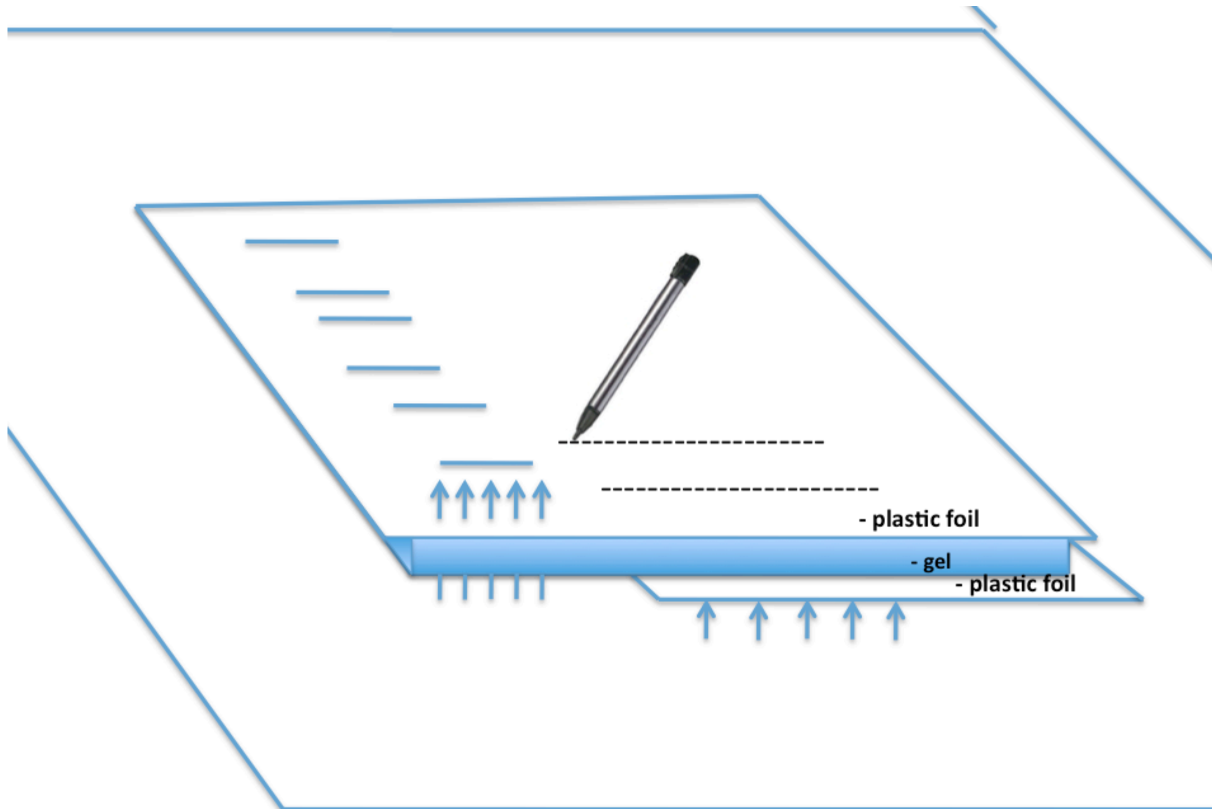

11. Switch off the UV transilluminator, flip the gel and excise the genomic sample DNA between the previously indicated size markers.

12. Electroelution of DNA fragments:
    Prepare dialysis tubes (Carl Roth, Visking), approximately 3 cm longer than the gel slice and seal one end with a plastic clip. *Note: the length of the dialysis tube my vary depending on the type of plastic clip!*
    Gently push the excised gel slice into the tube and position it all the way down next to the closing plastic clip.
    Add 300 - 500 μl of 30 mM TAE buffer. The gel slice should be completely immersed. *Avoid air bubbles!*
    Use a second plastic clip to close the dialysis tube. *Note: orientate both clips the same way for correct positioning in the electrophoresis box.*

13. Position the dialysis bag in the ORIGINS so that the gel slices are orientated in parallel to the electrodes.
    Fill the ORIGINS with 1 x TAE running buffer until the bag is fully submerged. Subsequently lower the buffer level again until the clips sit stably on the bottom of the electrophoresis chamber.
    Electroelute at 120 Volts.

14. Calculation of the required time for complete DNA extraction:

    i. Go to http://www.elchrom.com/index.php?id=72
    ii. Open the ElQuant software.
    iii. Select gel type: Spreadex EL 1200

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iv. Enter the longest fragment of interest in the „*DNA fragment B*“ field: e.g.: 550 bp (see blue arrow below).
v. In the field „*DNA fragment A*“,, enter any fragment length longer than the fragment of interest: e.g. 551 bp.

Select a gel:

Spreadex EL 1200

Select a temperature:

◉ 55 °C
○ 20 °C

DNA fragment A
(The Longer one) 551

DNA fragment B
(The Shorter one) 550

Go

Elquant Software

Elquant > Results

Gel Format	Gel Length	Running distance of the shorter fragment	Distance between the two bands	Running Time
Wide Mini S-2x200 2x104	8 mm	7.2 mm	0 mm	38 min
Wide Mini S-2x104L	17 mm	15.3 mm	0.1 mm	81 min
Wide Mini- S4x13 Wide Mini S-4x25	40 mm	36 mm	0.2 mm	192 min
Mini (12 sample wells)	57 mm	51.3 mm	0.2 mm	out of range min
Mini (8 sample wells) Wide Mini S-2x13 Wide Mini S-2x25	87 mm	78.3 mm	0.4 mm	out of range min

ElQuant parameters:

- Temperature: 55 °C
- Electric field strength: 10 V/cm
- Gel type:Spreadex EL1200
- Fragment A size:551
- Fragment B size:550

vi. Calculate the mobility of the fragment of interest:
running distance of the shorter fragment / running time
⇒ e.g. 15.3 mm / 81 min = 0.188 mm/min

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   vii. The running time for complete elution is calculated from the thickness of the excised gel slice (e.g. 0.8 cm):
   gel length / mobility = 8 mm / 0.188 mm/min = ~ 42 min

15. Collection of eluted DNA:
    DNA shows some tendency to stick to dialysis bags during electroelution. To ensure maximal recovery, we recommend:
    i. Invert the positioning of the dialysis bag inside the ORIGNS: the lower end of the bag initially facing the anode, should now be facing the cathode and vice versa.
    ii. Apply 200 V for approximately 1 to 2 minutes.
    iii. Open the bag and carefully remove as much of the buffer as possible.

16. Use QIAquick PCR Purification Kit or QIAEX II Kit to concentrate your DNA.

17. Calculate the yield by using a spectrophotometer.

18. Barcode your DNA fragments with kit of choice.

19. Proceed to emulsion and sequencing step.

#Please note that this protocol has been developed and tested using Elchrom Scientific precast gels together with ORIGINS by Elchrom™. For regular electrophoresis units, contact service@elchrom.com.

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