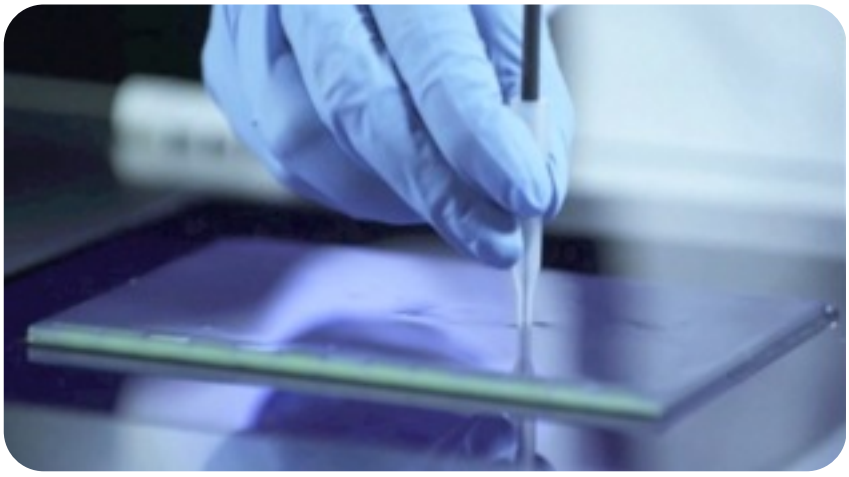
# Technical Manual



## P/N 4000 Pick'n'Go - Direct DNA Recovery Kit - LTP
## P/N 4010 Pick'n'Go - Direct DNA Recovery Kit - HTP

## P/N 4200 DNA Recovery Gel - LTP
## P/N 4210 DNA Recovery Gel - HTP

## P/N 4300 Pick'n'Go Elution Buffer 1.5 ml
## P/N 4310 Pick'n'Go Elution Buffer 20 ml

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# Packaging

## Pick'n'Go - Direct DNA Recovery Kit

**P/N 4000 Pick'n'Go - Direct DNA Recovery Kit - LTP (Low Throughput):**

- ✓ 1 x box of 4 gels, each containing 1 x 13 wells w/EtBr
- ✓ 12 BandPick™ gel cutting device
- ✓ 1 x Catamaran for gel positioning and fixation
- ✓ 1 x Special Forceps
- ✓ 1 ml Sample Loading Buffer
- ✓ 1.5 ml Pick'n'Go Elution Buffer
- ✓ 1 x Nylon String

**P/N 4010 Pick'n'Go - Direct DNA Recovery Kit - HTP (High Throughput):**

- ✓ 1 x box of 4 x 2 gels each containing 1 x 13 wells w/EtBr
- ✓ 24 BandPick™ gel cutting device
- ✓ 1 x Catamaran
- ✓ 1 x Special Forceps
- ✓ 1 ml Sample Loading Buffer
- ✓ 2 x 1.5 ml Pick'n'Go Elution Buffer
- ✓ 1 x Nylon String

**P/N 4200 Direct DNA Recovery Gel - LTP**

- ✓ 1x box of 4 gels, each with 1 x 13 wells w/EtBr

**P/N 4210 Direct DNA Recovery Gel - HTP**

- ✓ 1x box of 4 x 2 gels, each with 1 x 13 wells w/EtBr

**P/N 4300 Pick'n'Go Elution Buffer 1.5 ml**

- ✓ 1 x 1.5 ml Elution Buffer

**P/N 4310 Pick'n'Go Elution Buffer 20 ml**

- ✓ 1 x 20 ml Elution Buffer

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# 1. Important information

*Caution: Ethidium Bromide (EtBr) is a powerful mutagen! Wear appropriate protective clothing, gloves and safety googles. Solutions and gels contaminated with EtBr must be disposed of in compliance with local regulations.*

## 1. 1 Specifications

- Direct DNA Recovery Gels should be stored at 4 °C (40 °F). Do not freeze! Handle gels with care to avoid physical damage.
- Shelf-life of gels: up to 18 months (at least 6) at 4 °C (40 °F).
- All sample wells are multi-channel pipette compatible. Each row follows the SBS standard format and provides one additional well for marker/controls.
- Sequencing reliability after elution (test fragment size 1085 bp, n = 6):
  Reading length from 977 bp to 1002 bp (average 991 bp) with an accuracy of 99.4% or more.

## 1. 2 General remarks

- Before using the kit for the first time, please read the technical manual carefully.
- Direct DNA Recovery Gels are conditioned for a 30 mM TAE running buffer. Elchrom Scientific offers standardised 40 x TAE Stock Solution (P/N 3031).
- All gels are covalently bound to a plastic backing for easy and safe handling. Additional Special Forceps (P/N 2366) can be ordered from Elchrom Scientific.
- Direct DNA Recovery Gels are orientated and positioned properly with a special plastic frame (‘Catamaran’). Additional Catamarans (P/N 2015) are available from Elchrom Scientific.
  Direct DNA Recovery Gels (high throughput version) can be used as individual gels by cutting the plastic backing between the gel matrices.
- Direct DNA Recovery Gels are suitable for any standard electrophoresis tank with the dimensions of: 13.5 cm (width) x 10.8 cm (length). *NOTE: the hight of the Catamaran is 2.7 cm.*
- *Direct DNA Recovery Gels are more sensitive than conventional agarose gels leading to significantly lower DNA detection limits.*
- Elchrom Scientific reserves the right to make changes to the following instructions without notification. For the latest version of the technical manual, please refer to our web page: www.elchrom.com

# 2. Electrophoresis

*Individual wells of DNA Recovery Gels can be used sequentially in multiple runs. However, we recommend to post-stain the gel with 0.5 µg/ml of EtBr in dd$H_2O$ in all subsequent runs to ensure sufficient sensitivity. We recommend to detach the gel from the plastic backing for post-staining to increase staining efficiency.*

## 2. 1 Running buffer

DNA Recovery Gels must be run in 1 x TAE running buffer. Elchrom Scientific offers a standardised 40 x stock solution (P/N 3031; 20 x 50 ml).
For in-house preparation, please follow those instructions:

| Components | Amount for 1 litre (40 x) |
|---|---|
| Tris (hydroxymethyl) aminomethane: | 145.37 g |
| $Na_2EDTA$ x 2 $H_2O$: | 11.16 g |
| Acetic Acid (glacial): | 34.4 ml |

Dissolve Tris and $Na_2EDTA$ in 800 ml of dd$H_2O$. Add acetic acid in a fume hood and adjust to 1 litre with dd$H_2O$.

## 2. 2 Sample preparation

Add 1 µl of Loading Buffer (5 x; provided with kit) to 4 µl of sample. DNA sample may have to be diluted in dd$H_2O$ prior to electrophoresis.
The maximal loading volume per well is 25 µl.

We recommend to run at least 100 ng (optimal 200 ng) of DNA per band of interest.

## 2. 3 Electrophoresis

- Remove the aluminium bag by cutting one short side carefully with scissors.
- Remove the inside plastic bag containing the gel and place it on the work bench.
  *Caution: the gel is immersed in a solution containing EtBr.*
- Cut the plastic bag on three sides and carefully remove the gel by gripping the plastic backing with Special Forceps (included in kit).
- Place the gel in the electrophoresis tank and position it properly with the Catamaran.
- Load the samples.

## 2. 4 Running conditions

Set the voltage or amperage to the required values and start the electrophoresis. Running time depends on the fragment size and the resolution needed. However, we recommend to reduce running time to a minimum for higher sensitivity and easier detection.
*NOTE: high voltage/amperage will substantially increase the running buffer temperature and therefore influence DNA migration.*

# 3. DNA detection and isolation

## 3. 1 Detection and marking of bands

After electrophoresis, remove the DNA Recovery Gel from the tank and rinse the gel once in $ddH_20$[1]. Do not detach the gel from the plastic backing.

For DNA detection, we recommend:

a) UV light of a long wavelength (312 nm) and low intensity.
*Note: the gel backing is not UV-transparent.*
Place the gel with the gel side facing down on the UV table (plastic backing resting on top of the gel).
Mark the bands of interest on the plastic backing with a water-resistant felt pen.
*UV light is highly damaging to DNA! Minimise exposure time!*
Switch off the UV light, turn the gel and cut the signed region with BandPick™ as described in step **3.2**.

b) Blue light visualisation.
Place the gel with the gel side facing down on the blue light transilluminator.
Mark the bands of interest on the plastic backing with a water-resistant felt pen.

*Alternative method:*
Use the nylon string to peel the gel off the plastic backing.
Cut the bands with BandPick™ directly on the UV table as described in step **3.2**. *Careful: minimise exposure of DNA to UV light!*

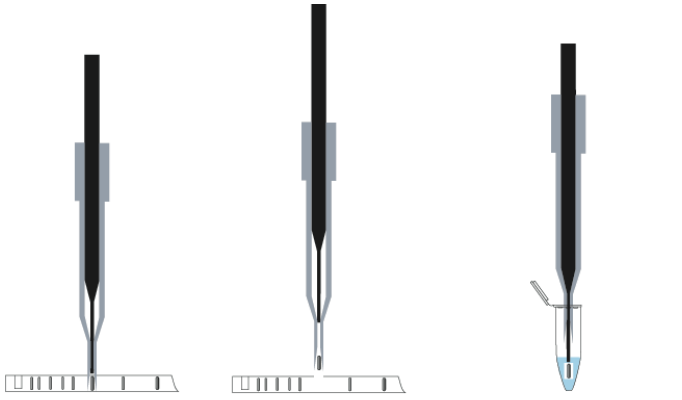
## 3. 2 Cutting bands with BandPick™

- BandPick™: insert the black piston into the white tube till its end resides approximately 2 mm above the outer cutting edge of the tube.
- Press BandPick™ into the band of interest and push it all the way to the bottom of the gel. Turn the BandPick™ clock- and counter-clockwise to cut the gel properly. Also rub the cutting device against the plastic backing to ensure the gel properly detaches.
- Without lifting the whole BandPick™, pull up the piston slowly by about 1 mm; hold in position carefully.
  *CAREFUL: if the piston is lifted too far, the vacuum created in the tube will be lost and the gel piece can not be removed.*

[1] For DNA that is difficult to sequence or very low amounts of DNA, it may be necessary to include a washing step of 3 x 5 min in $ddH_20$ to remove all remaining TAE buffer.

- Lift the whole BandPick™ quickly to excise the cut gel piece.
- Eject the gel piece into a test tube by pushing the piston. The volume of the excised gel piece is precisely 5 µl to allow adjustment of subsequent reaction buffers.

Optional: Check on the UV transilluminator whether the gel pieces were cut out properly.




For a more detailed description of the procedure, please follow the instructions provided in the tutorial movie under http://www.elchrom.com

# 4. Elution

## 4.1 Standard protocol

To perform sequencing and ligation, the DNA needs to be eluted from the gel piece:

- Place the gel piece into a small PCR tube.
- Add 5 µl of Pick'n'Go Elution Buffer. Make sure that the gel piece is fully covered with buffer.
- Incubate for 15 min at 75 °C.
- Spin down briefly at low rpm.
- Transfer the supernatant to a new tube.

## 4.2 High yield DNA recovery

Some downstream protocols may require higher amounts of eluted DNA:

- Extracting DNA fragments of more than 1000 bp.
- Required end volume of more that 5 µl for sequencing.
- Subsequent electrophoresis to determine elution efficiency.

In these cases, we recommend to cut 3 gel pieces from the band of interest and pool them into one tube. Cover the pieces with 10 µl of Pick'n'Go Elution Buffer and incubate for 15 min at 75 °C.

*Note: the amount of DNA eluted from 1 to 3 gel pieces is sufficient to achieve highly-reliable sequencing results (≥ 99.4%) and efficient ligations even though it may not be quantifiable by standard photo-spectrometry.*

To check the elution efficiency, we recommend gel electrophoresis. For best sensitivity, either use the DNA Recovery Gel or, alternatively, a ultra-thin agarose gel.
To visualize, load one complete elution (5 µl) on the gel.

# 5. Downstream applications

## 5.1 Performing PCR directly on the gel piece

Add the PCR reaction mix directly to the tube containing the gel piece. Make sure that the piece is covered with reaction mix. Perform the PCR with your standard conditions. Alternatively, you can elute the DNA as described in step **4**. prior to PCR analysis.

## 5.2 Sequencing

For sequencing, follow you standard protocol or use your local sequencing provider. Use the eluted DNA as template.
We recommend to use a protocol optimised for low DNA-content to increase reading length.

## 5.3 Ligation

For ligation, elute the fragments of interest (backbone and/or insert) and follow the instructions of your ligase kit manual.

## 6. Ordering information:

| P/N | Product | Wells per Gel | Run. Dist. (cm) | Gel Size W x H x D (cm) | Quantity |
|---|---|---|---|---|---|
| 4000 | Pick'n'Go - DNA Recovery Kit LTP | 13 | 8.7 | 13.05 x 0.3 x 9.6 | 4 Gels, 1 x 13 wells w/EtBr<br>12 x BandPick™<br>1 x Catamaran<br>1 x Special Forceps<br>1 ml Sample Loading Buffer<br>1.5 ml Pick'n'Go Elution Buffer<br>1 x Nylon String |
| 4010 | Pick'n'Go - DNA Recovery Kit HTP | 2 x 13 | 4 | 13.05 x 0.3 x 4.8 | 4 x 2 Gels, 1 x 13 wells w/EtBr<br>24 x BandPick™<br>1 x Catamaran<br>1 x Special Forceps<br>1 ml Sample Loading Buffer<br>2 x 1.5 ml Pick'n'Go Elution Buffer<br>1 x Nylon String |
| 4200 | Direct DNA Recovery Gel-LTP | 13 | 8.7 | 13.05 x 0.3 x 9.6 | 4 Gels |
| 4210 | Direct DNA Recovery Gel-HTP | 2 x 13 | 4 | 13.05 x 0.3 x 4.8 | 4 x 2 Gels |
| 4300 | Pick'n'Go Elution Buffer 1.5 ml | | | | 1 x 1.5 ml |
| 4310 | Pick'n'Go Elution Buffer 20 ml | | | | 1 x 20 ml |

# 7. Troubleshooting

| Trouble | Shoot |
|---|---|
| Sample wells are not visible. | - Ensure proper illumination.<br>- Put dark paper under electrophoresis chamber to enhance contrast.<br>- Rinse each well with 1 x loading buffer and remove loading buffer directly with running buffer. The dye in the loading buffer will mark the well for better visibility. |
| DNA is not entering the gel. | - Use TAE buffer instead of TBE buffer (Borate prevents DNA migration) |
| DNA not visible using blue light. | - Try to load at least 200 ng DNA.<br>- Use a UV transilluminator. |
| DNA not visible with UV light, not even the marker. | - Check orientation of the gel: the plastic backing is not UV-transparent |
| No fragments can be detected on the control gel after DNA elution. | - Run more DNA/band.<br>- Cut 3 pieces and elute in 10 µl of elution buffer to increase total amount of eluted DNA. |
| DNA 'smears' on the control gel after elution. | - DNA cross-linked!<br>- Use blue light instead of UV light.<br>- If no blue light transilluminator available, use UV light of long wavelength (312 nm) and low intensity.<br>- Minimise the exposure time. |
| Sequencing reading length is too short Low quality reads. | - Cut 3 pieces and elute in 10 µl elution buffer to increase total amount of eluted DNA. |
| Gel piece is not lifted by the BandPick™. | - Do not lift the piston more than 1 mm otherwise the vacuum is lost.<br>- Turn and scratch the BandPick™ thoroughly to completely detach the gel piece from the plastic backing before lifting the piston. |

## 8. Related Products

| Product Number | Product | Quantity |
|---|---|---|
| 2310 | BandPick™, cutting cylinder plus black piston | 1 box of 10 |
| 2315 | BandPick™, cutting cylinder plus black piston | 1 box of 50 |
| 2366 | Special Forceps | 1 |
| 2355 | Peel-IT™ | 1 |
| 3204 | M1 Marker | 50 loadings |
| 3204EB | Ready-to-use M1 Marker for EtBr staining | 50 µl |
| 3031 | 40 x Running Buffer Stock Solution, 1.2 M TAE buffer | 1 l (20 x 50 ml) |
| 3033 | Sample Loading Buffer 10 ml | 10 x 1 ml |
| 3034 | Sample Loading Buffer 50 ml | 50 x 1 ml |
| 2015 | Catamaran S-13/50, to secure one Wide Mini gel | 1 box of 2 |

For further questions, please visit our web page or contact us.

# 9. Quick protocol

### Electrophoresis

1. Remove the Direct DNA Recovery Gel from the aluminium and plastic bag.
2. Position the gel in the electrophoresis tank containing 1 x TAE running buffer using the catamaran and load the samples.
3. Set the voltage or amperage to the required values and start the electrophoresis.
4. After electrophoresis, remove the gel from the tank and rinse it once in dd$H_20$.

### DNA detection and cutting bands with BandPick™

1. Place the gel with the gel side facing down on the detection table (UV- or blue light).
2. Mark the bands of interest on the plastic backing with a water-resistant felt pen.
3. Switch off the UV light and turn the gel to excise DNA using BandPick™.

### Elution

1. Place the gel piece in a small tube (PCR tube).
2. Add 5 µl of Elution Buffer. Make sure that the gel piece is fully covered with buffer.
   *High yield DNA recovery: use 3 gel pieces with 10 µl of Elution Buffer*.
3. Incubate for 15 min at 75 °C.
4. Spin down briefly at low speed.
5. Transfer the supernatant to a new tube.

The eluted DNA can be used for PCR, sequencing or ligation as described in step **5**.