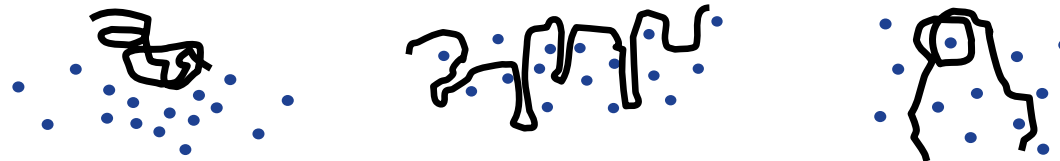


Rotaphor 6.0 System



Conventional agarose gel electrophoresis

- Upper limit of separation: ~ 25.000 bp (25 kb)
- Problem: trapping of larger molecules



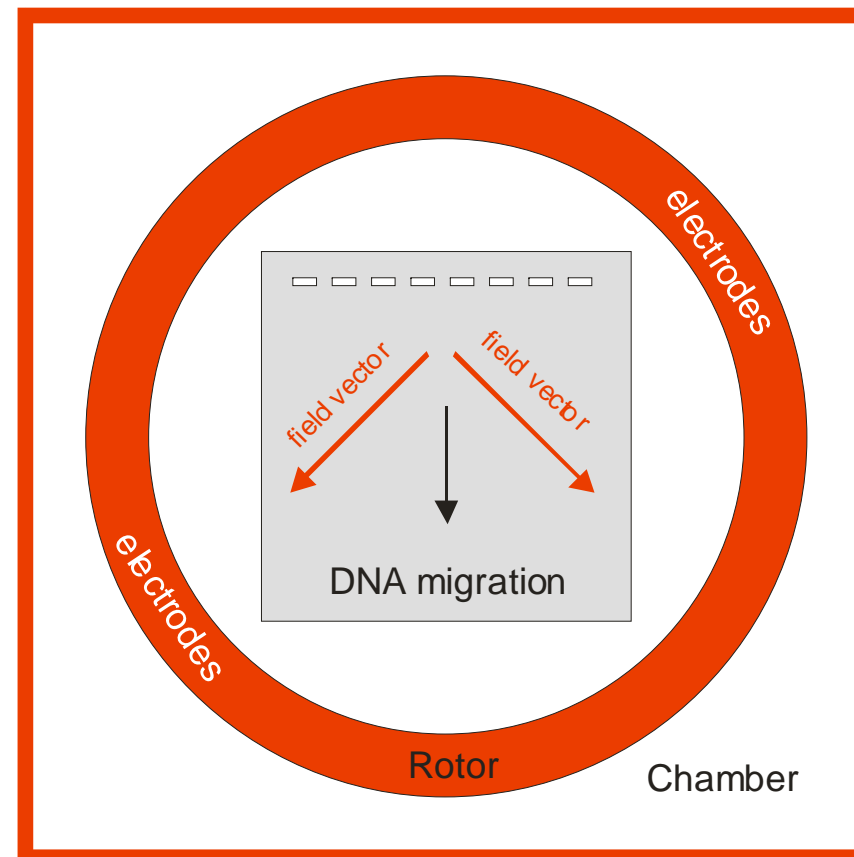
The patented Rotaphor principle

ROFE

ROtating

Field

Elektrophoresis



Rotaphor Version 6.0 - News

- What is required for completing the system?



Rotaphor system

+

Monitor



Refrigerated
Circulator KH-5



043-400
043-490

Rotaphor Version 6.0

– News vs. Version 5.0

- No external controller
- PC software for programming and control of PFGE
- For each program a real gel picture can be displayed
- Comprehensive online help
- User administration
- Program store virtually unlimited
- Launched in January 2005

The Parameter Editor

The screenshot shows the 'Parameter Editor' window with the following components:

- Header:** 'Parameter Editor', 'Help - F1', 'User: markus'
- Parameter Lists (Left):** A list of parameter lists for 'mck_1'. The selected list is '2kb-800kb 24h'. Other lists include '1kb-100kb 14h', '2kb-200kb 18h', '3kb-250kb 21h', '1kb-450kb 23h', '3kb-1600kb 24h', '20kb-2600kb 48h', '0kb-5700kb 120h', and '0kb-5700kb 240h'.
- Configuration (Center):**
 - Name of List: 2kb-800kb 24h
 - Duration (h): 24
 - Temperature (°C): 13
 - Interval (sec): 60, logarithmic to 10
 - Interval inverse (sec): Off
 - Angle (°): 120, linear to 110
 - Voltage (V): 180, logarithmic to 120
 - Comments: 0,9 % Agar, 0,3 x TBE
- Buttons (Center):** 'New Parameter List', 'Delete Parameter List', 'Accept changes'.
- Combined Lists (Bottom Left):** A list of combined lists including '2kb-300kb 22h', '5kb-600kb 39 h', '20kb-900kb 42h', '220kb-650kb 60h', '2kb-1600kb 30h', '10kb-1600kb 48h', '30kb-2400kb 84h', and '50kb-2000kb 78h'. Buttons: 'New Combined List', 'Delete Combined List'.
- Parameter Lists of Combined Lists (Bottom Center):** An empty box.
- Buttons (Bottom Center):** 'Use Selected List for Experiment', 'Hide Window'.
- Lists of other users / backups (Top Right):** A dropdown menu showing 'No user selected'.
- Image (Bottom Right):** A gel electrophoresis image with a ladder on the right. The ladder has markers at 800 kb, 600 kb, 450 kb, 350 kb, 250 kb, 150 kb, 50 kb, 12 kb, 7 kb, 4 kb, 3 kb, and 2 kb. Buttons: 'Assign Image', 'Remove Image'.

Electrophoresis Control

Rotaphor V6 Demo Mode
Options Help

Duration	23:59:35	5,0°C	
Interval (sec)	60		
Interval inverse (sec)	0		
Angle (°)	120		
Voltage (V)	180	Set Temperature (°C)	<input type="text" value="13"/>
Next move	60	Control Temperature	<input checked="" type="checkbox"/> ●
Probably finished at	Thursday 19.8.2004 12:00	Pump	<input type="checkbox"/>
		Alarm	<input type="checkbox"/>

18.08.2004 12:00:47 Electrophoresis started
18.08.2004 12:00:45 Rotor in default position
18.08.2004 11:58:18 Rotor moves to default position
18.08.2004 11:58:18 Lid closed

Start up Tests running

Interrupt Electrophoresis

©- 2kb-800kb 24h (P)

Demo_Interaktion

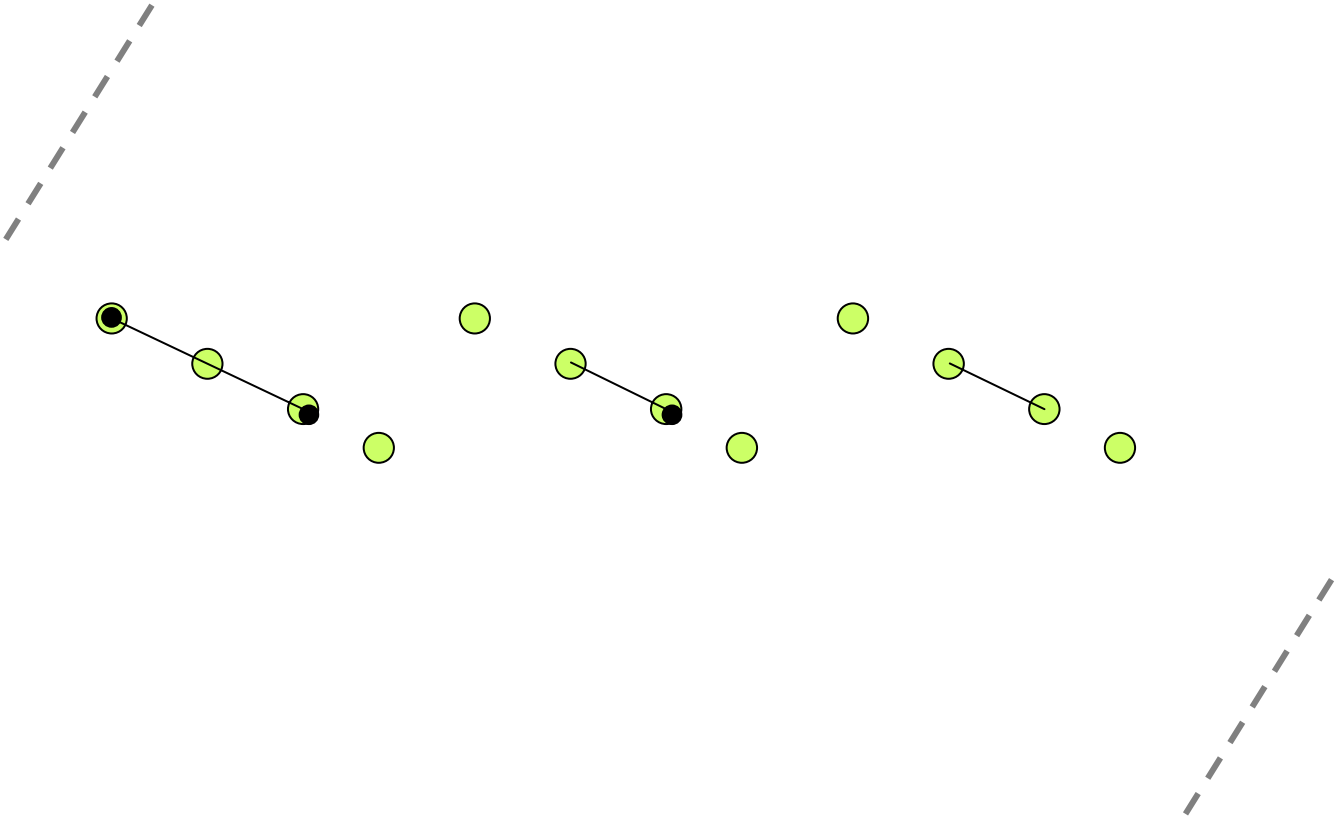
<input type="checkbox"/> Lid open	<input type="radio"/> 10°C
<input type="checkbox"/> Light Barrier	<input type="radio"/> 15°C
<input type="checkbox"/> Current over Limit	<input type="radio"/> 20°C

Please simulate the electrophoresis chamber manually, by using the checkboxes for lid status, rotor position control (lightbarrier), current over limit and temperature control. If the light barrier was not activated to stop virtual electrode movement, it activates itself after the message "Rotor blocked".

Basics of Pulse Field Gel Electrophoresis (PFGE)

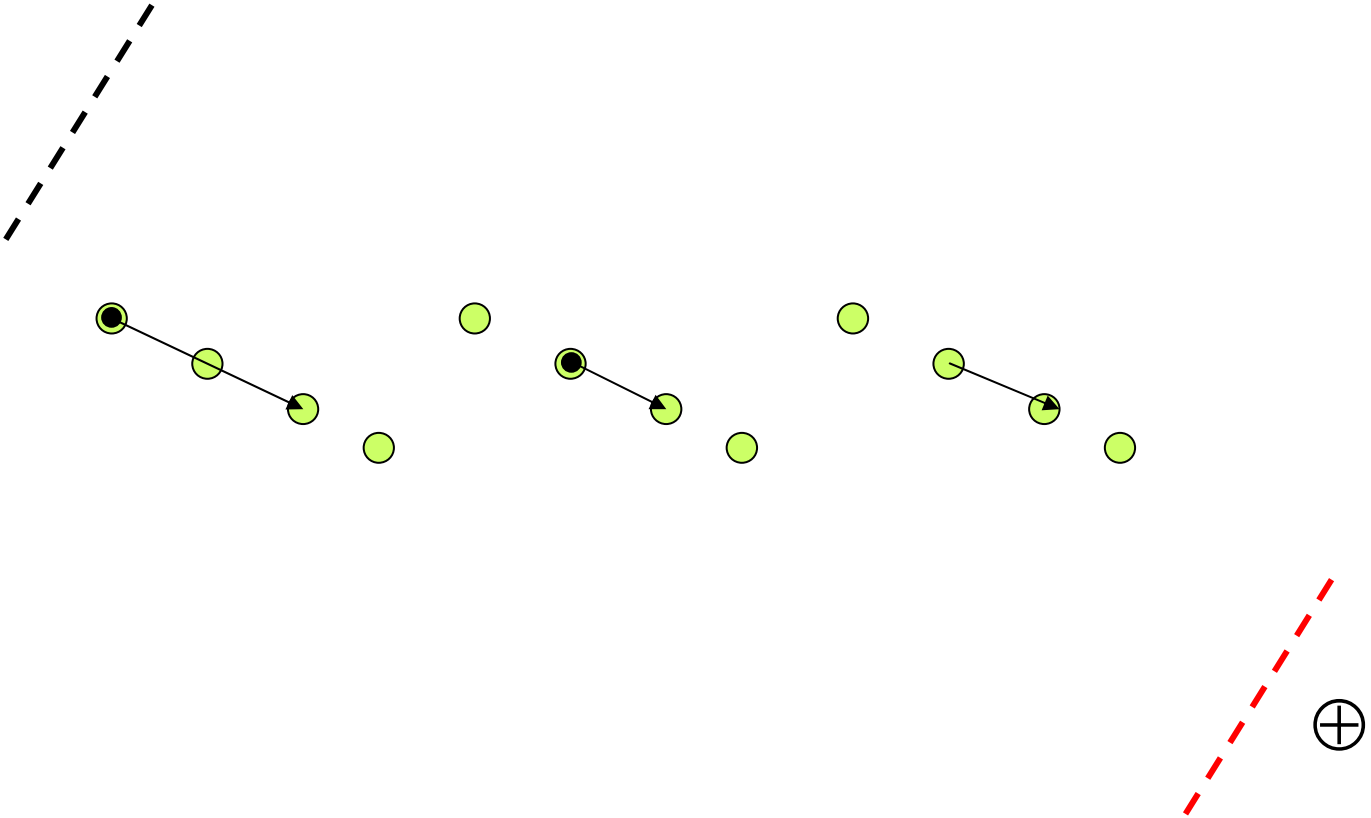
- Separation of large DNA molecules (up to 8.000 kb)
- The electrical field is applied in changing angles
- The large DNA molecules „meander“ through the gel pores
- Run times are much longer than with conventional Agarose electrophoresis (up to 80h), thus the buffer has to be cooled and circulated.

PFGE principle



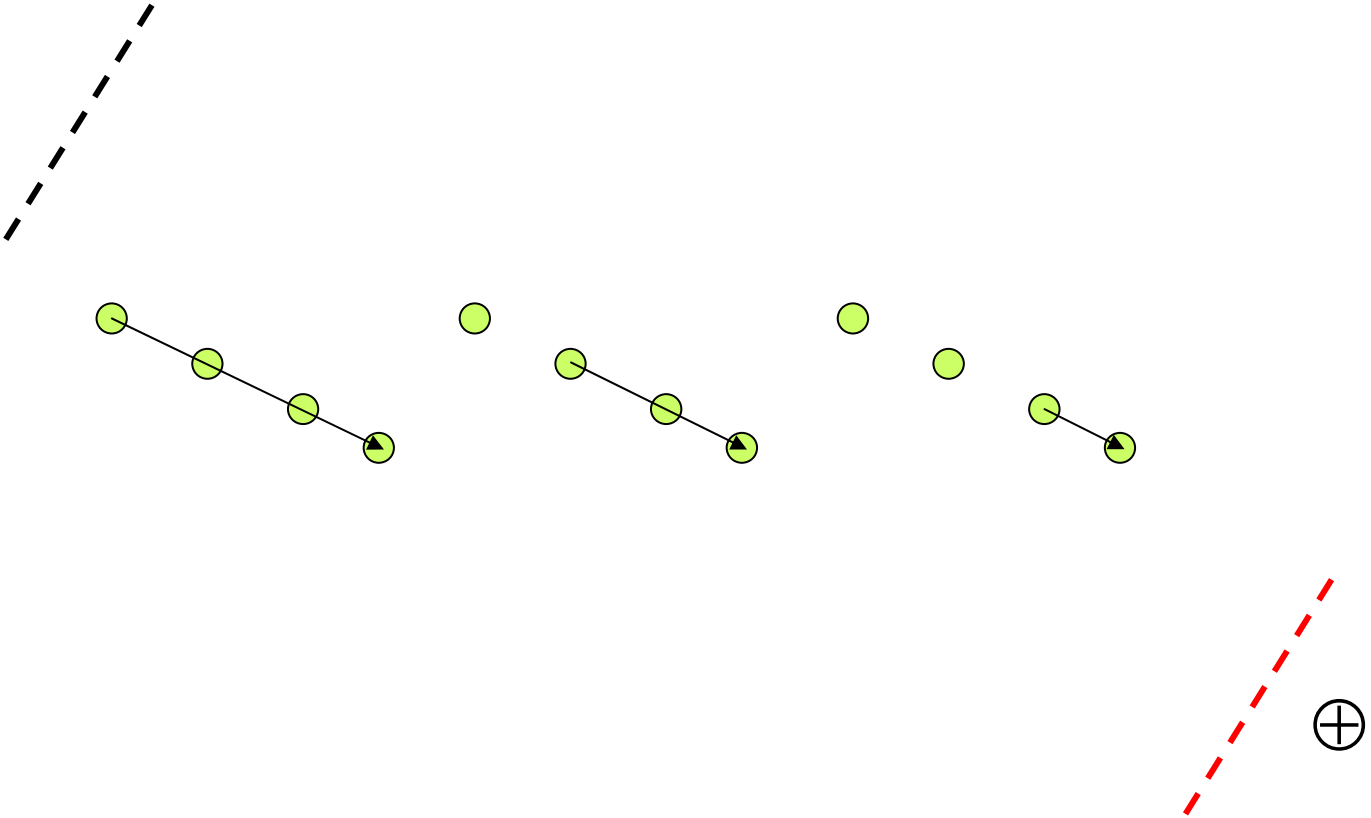
PFGE principle

Field vector

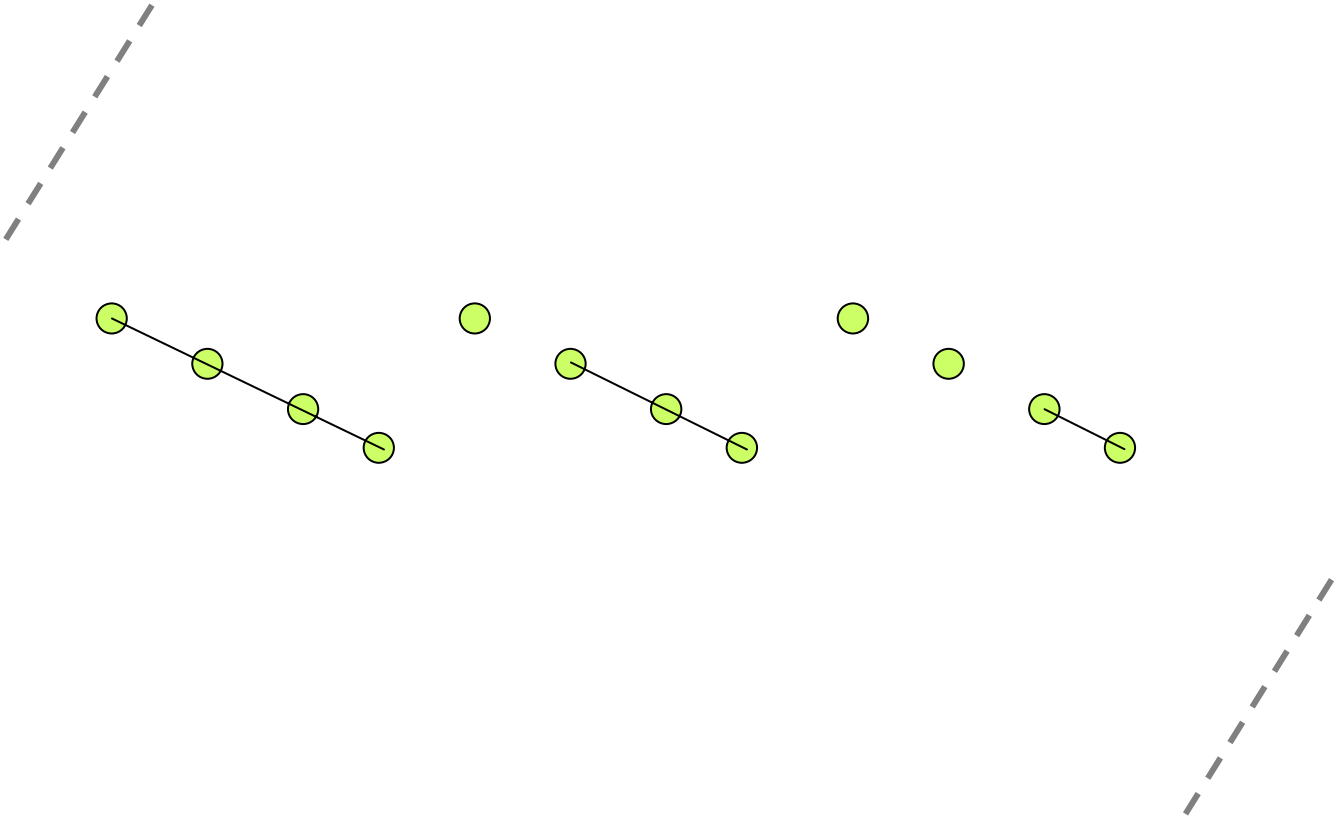


PFGE principle

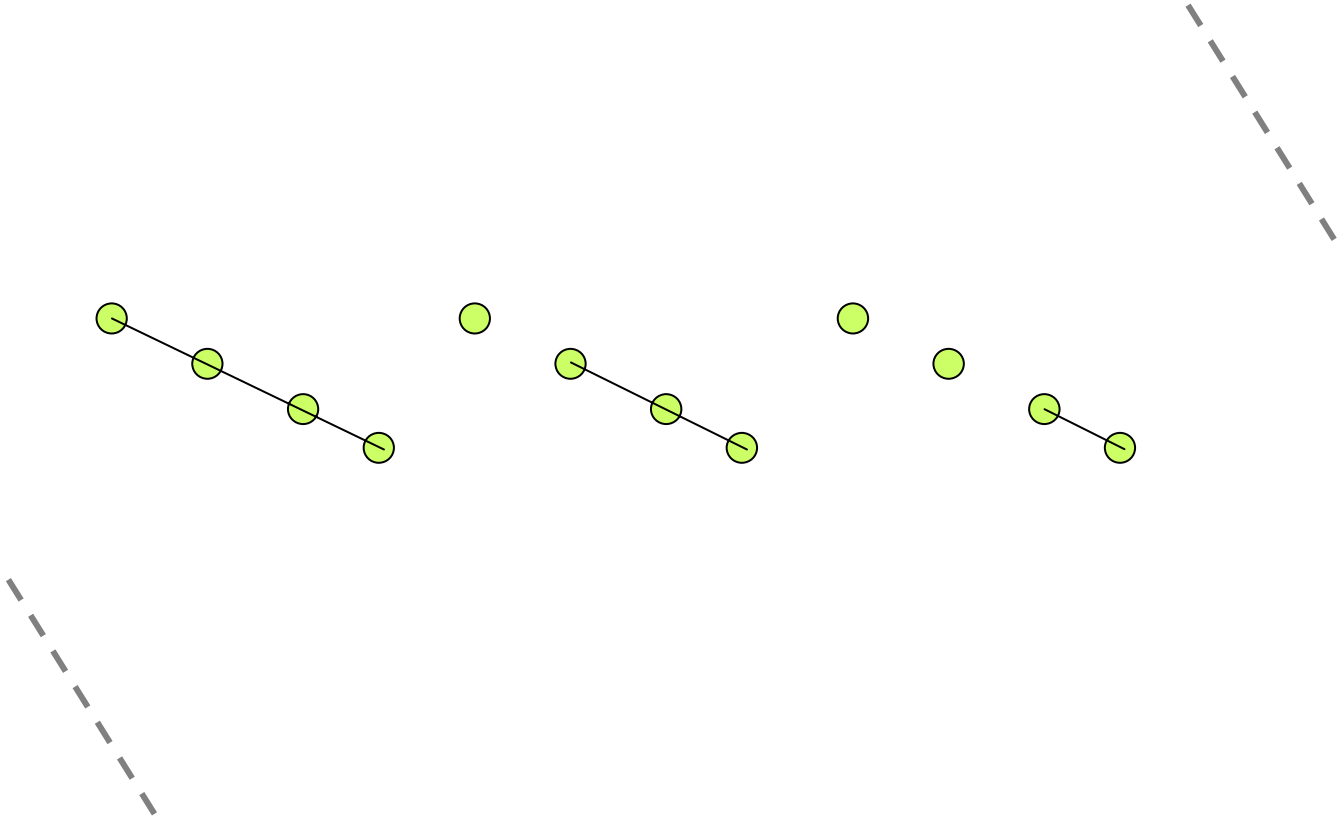
Field vector



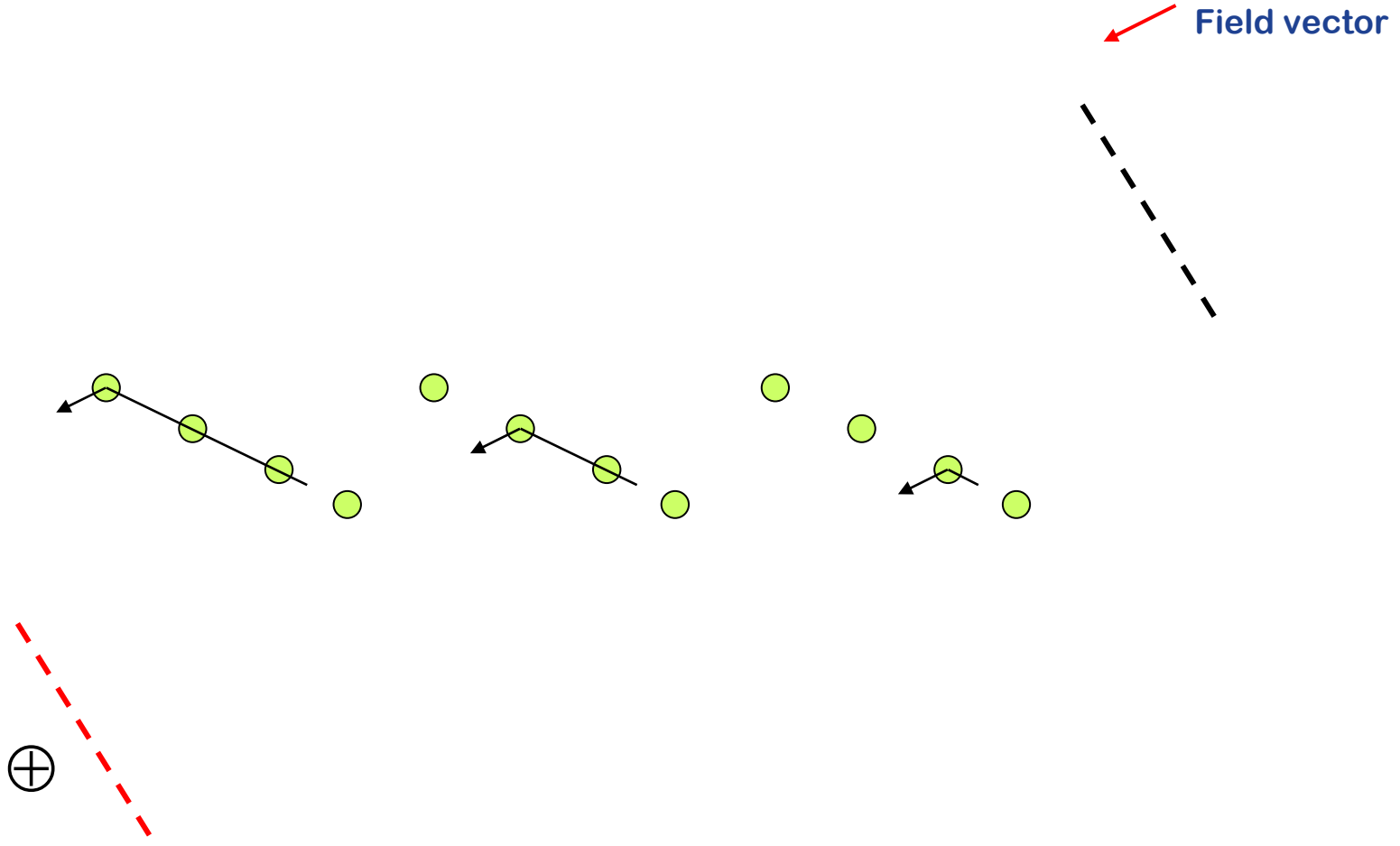
PFGE principle



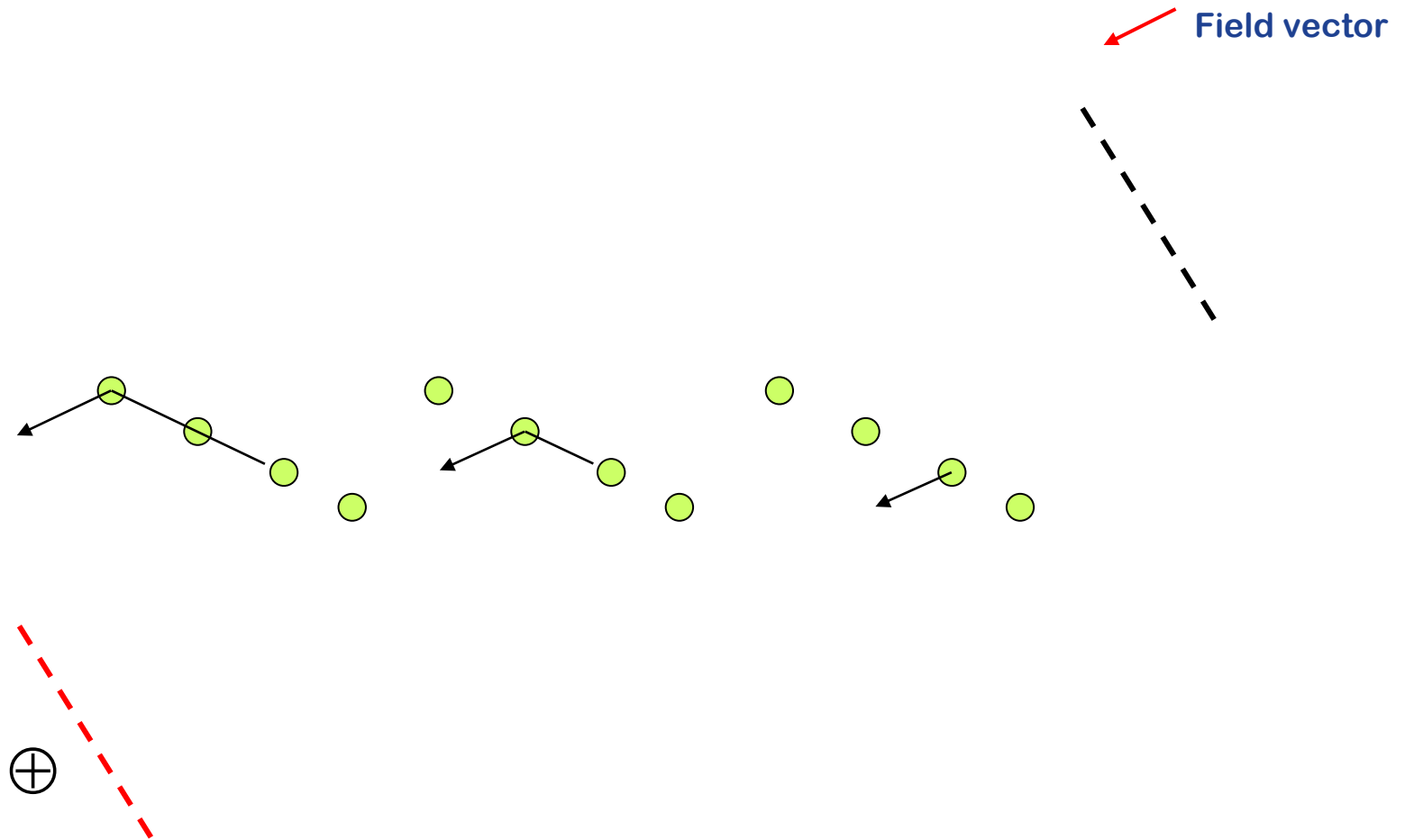
PFGE principle



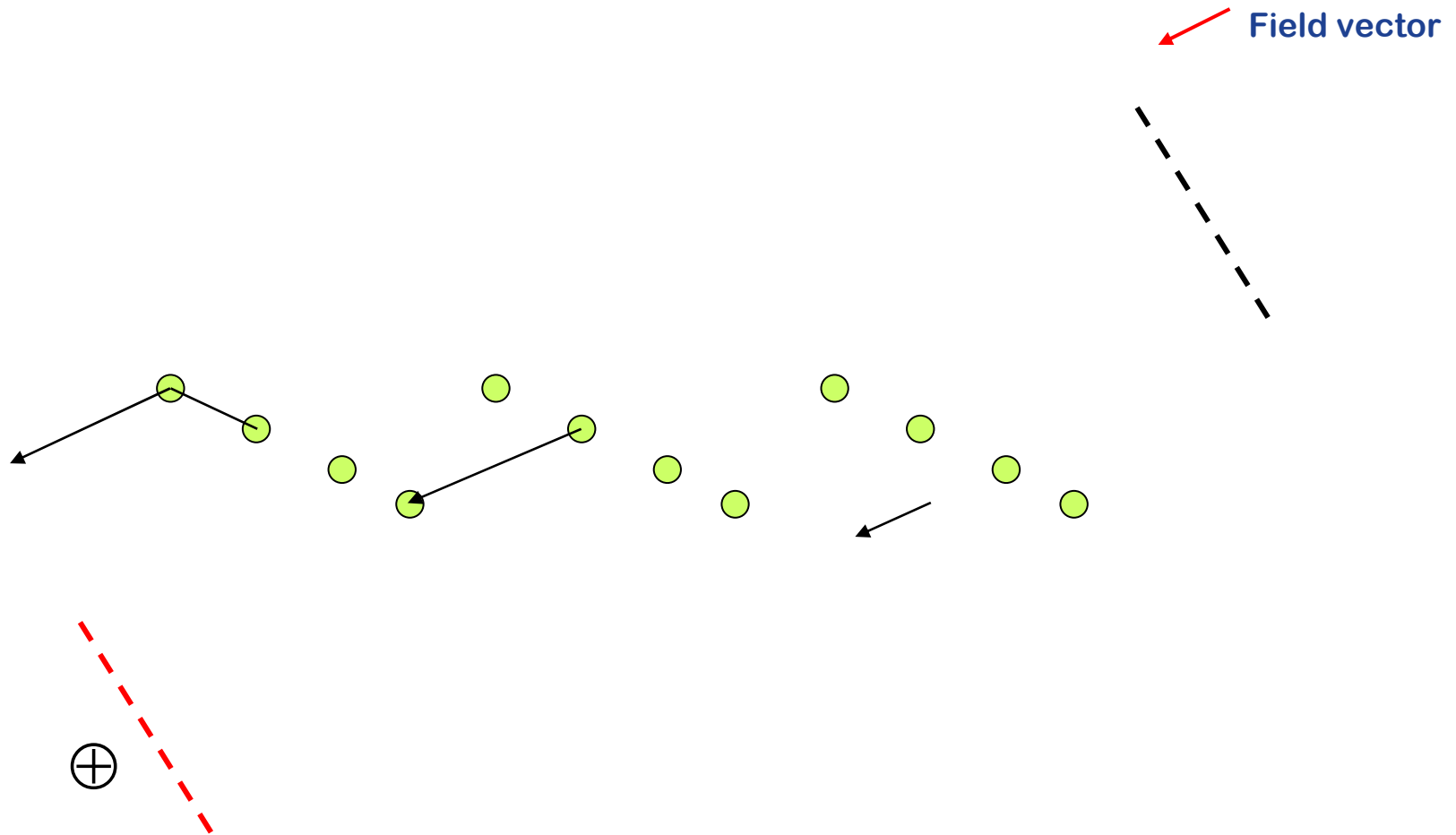
PFGE principle



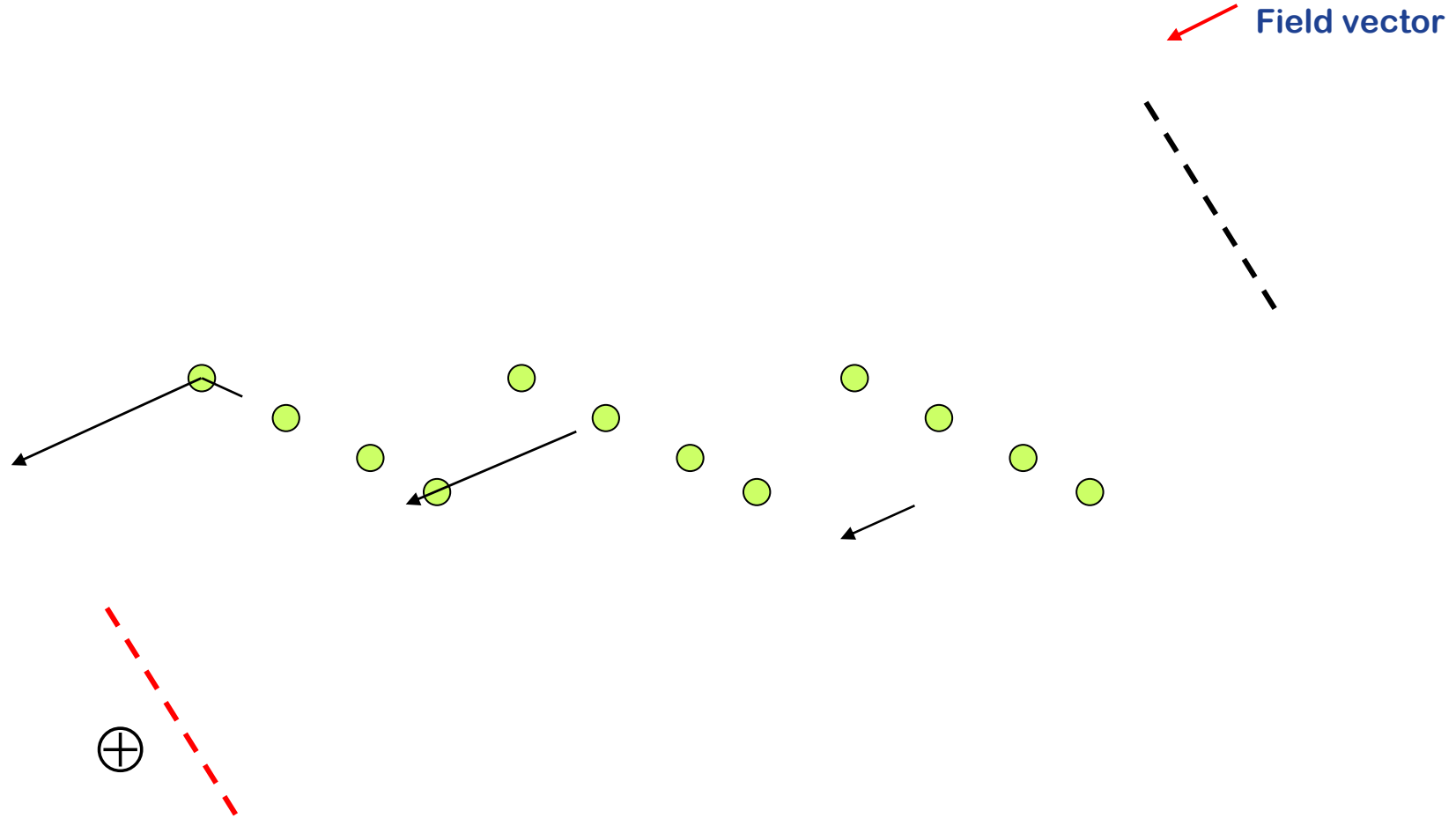
PFGE principle



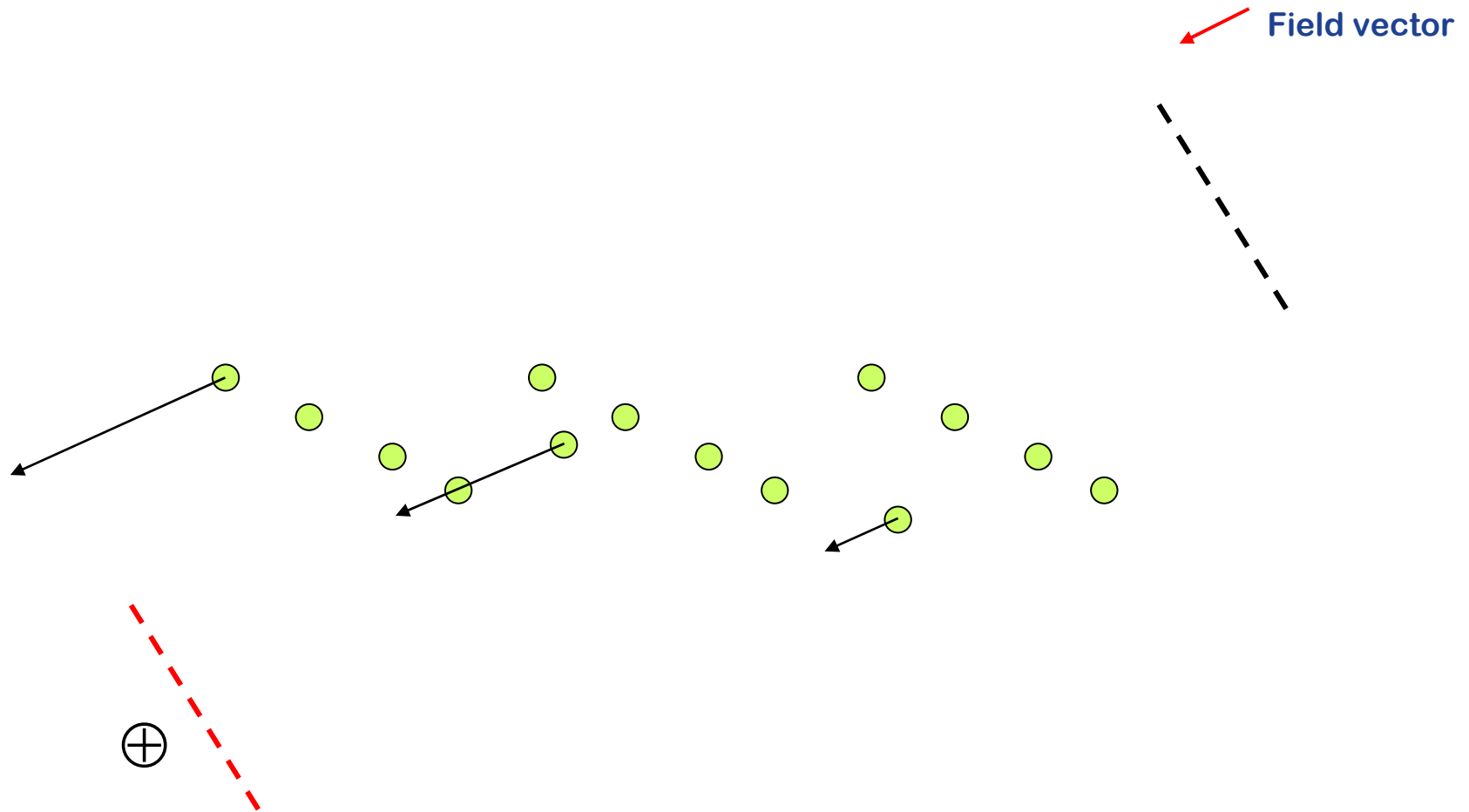
PFGE principle



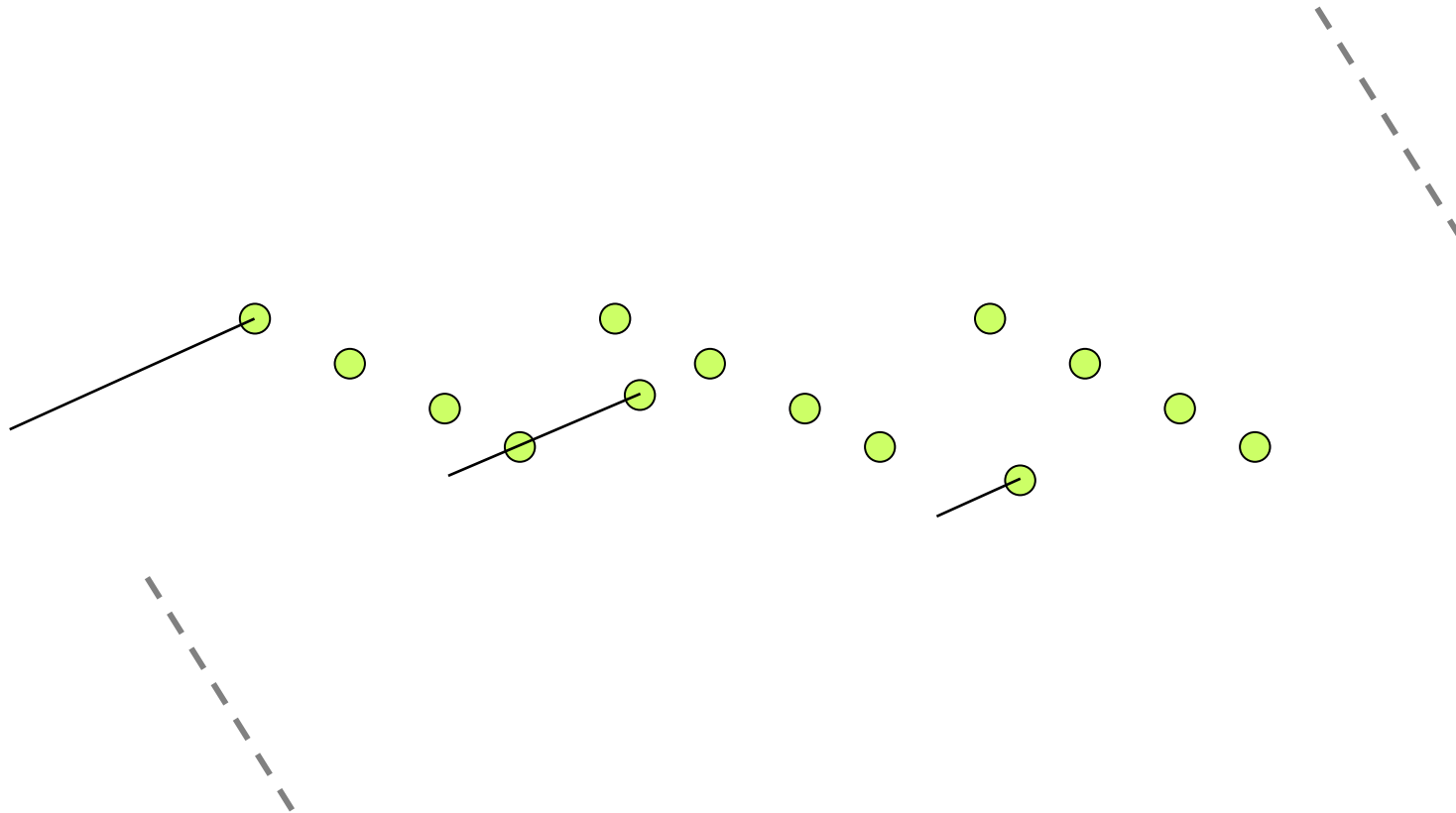
PFGE principle



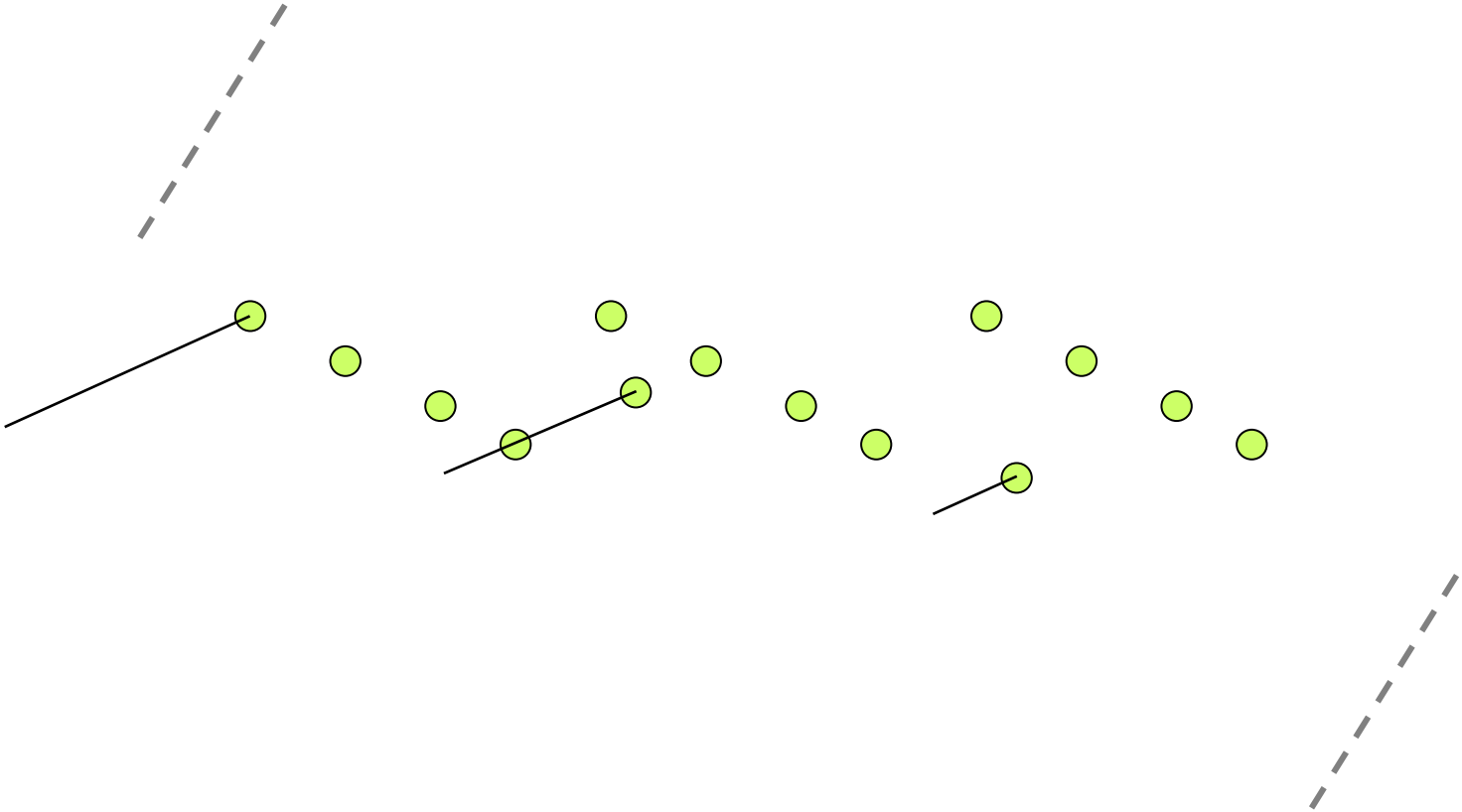
PFGE principle



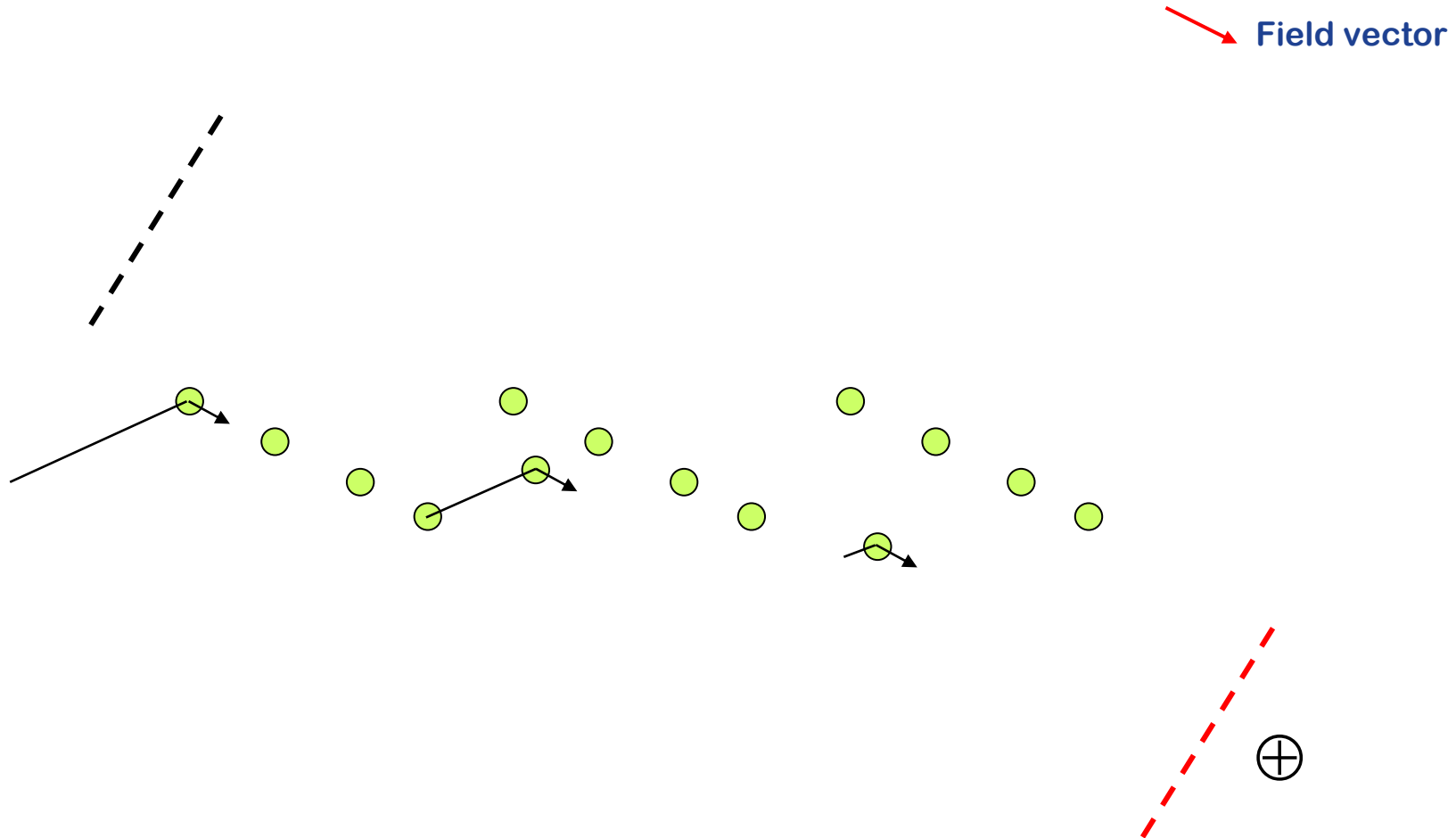
PFGE principle



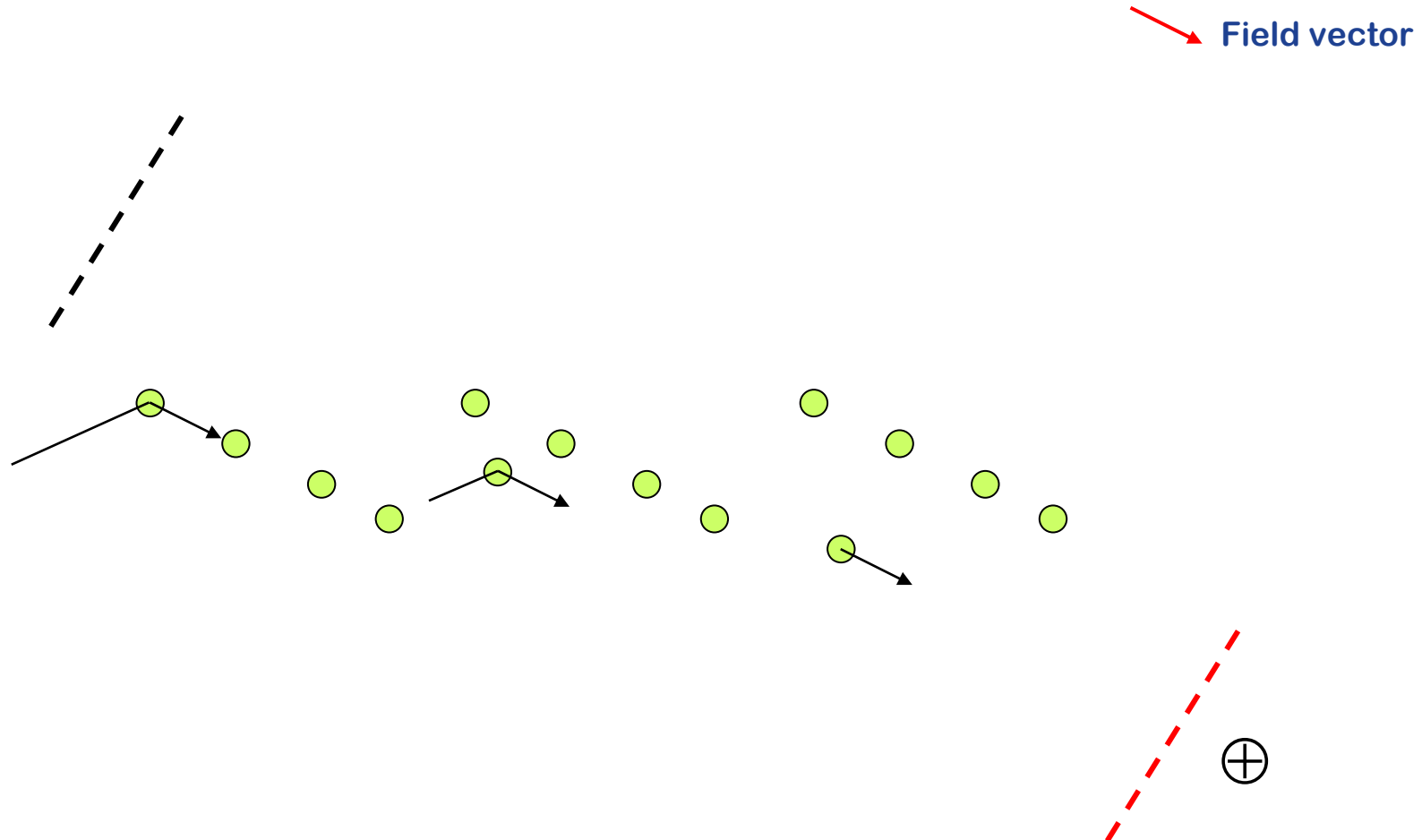
PFGE principle



PFGE principle

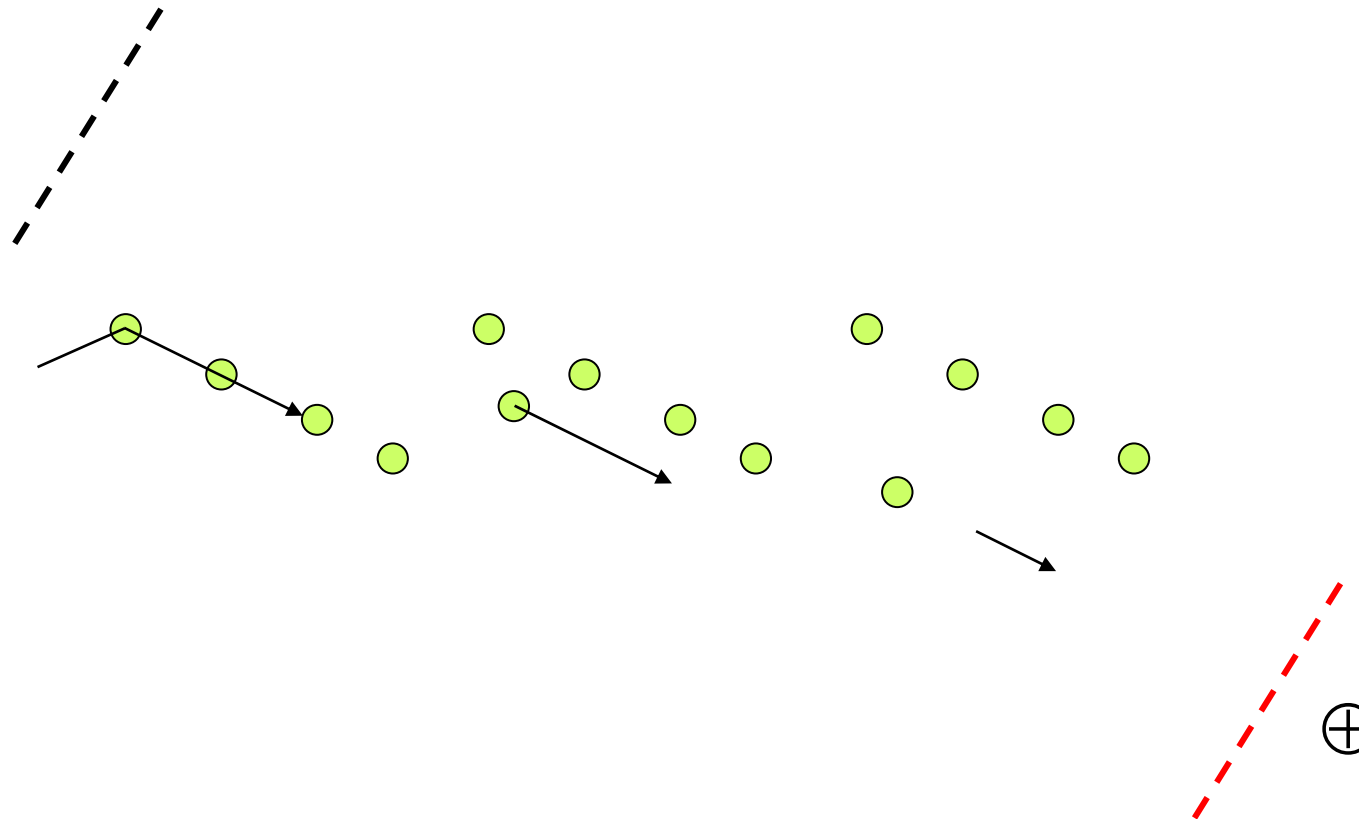


PFGE principle

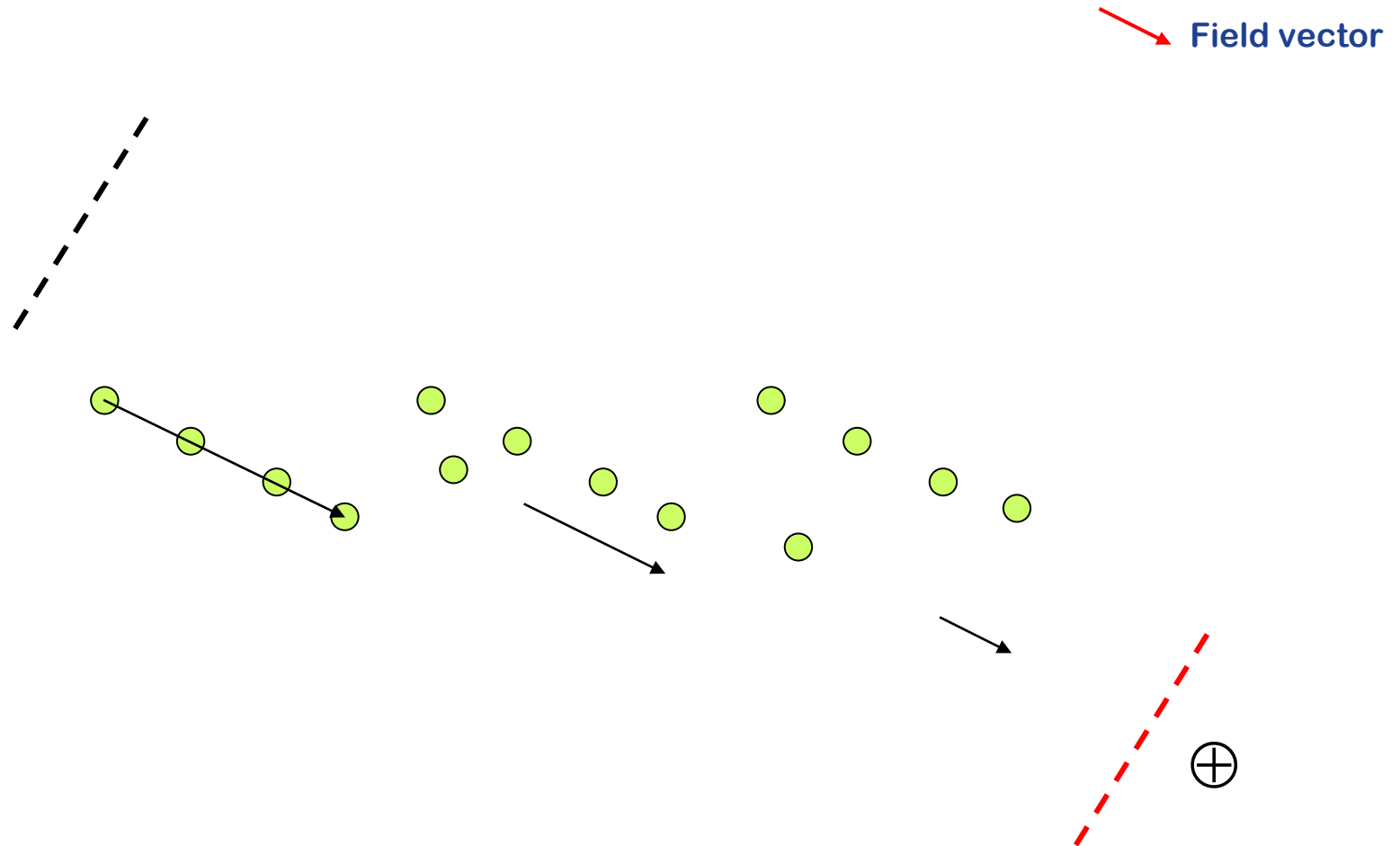


PFGE principle

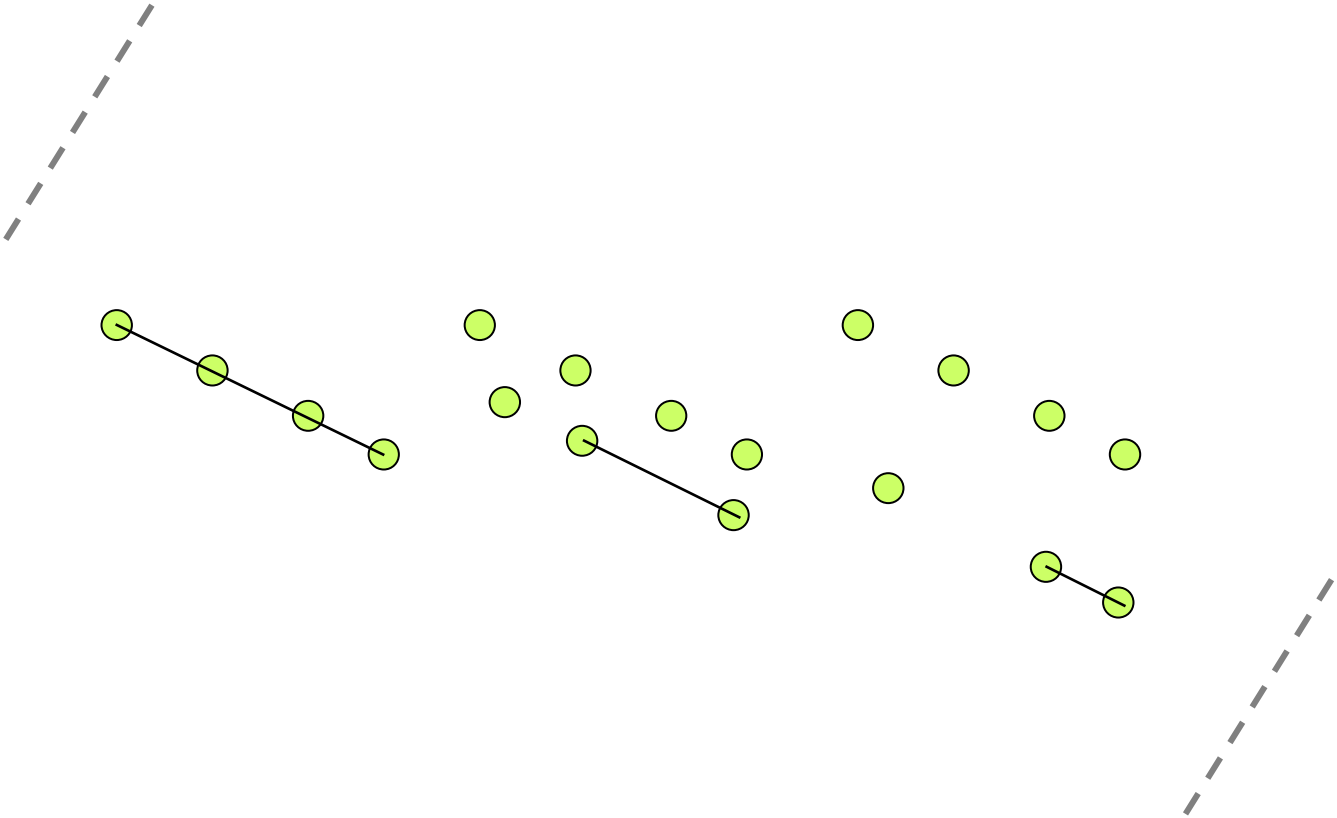
 Field vector



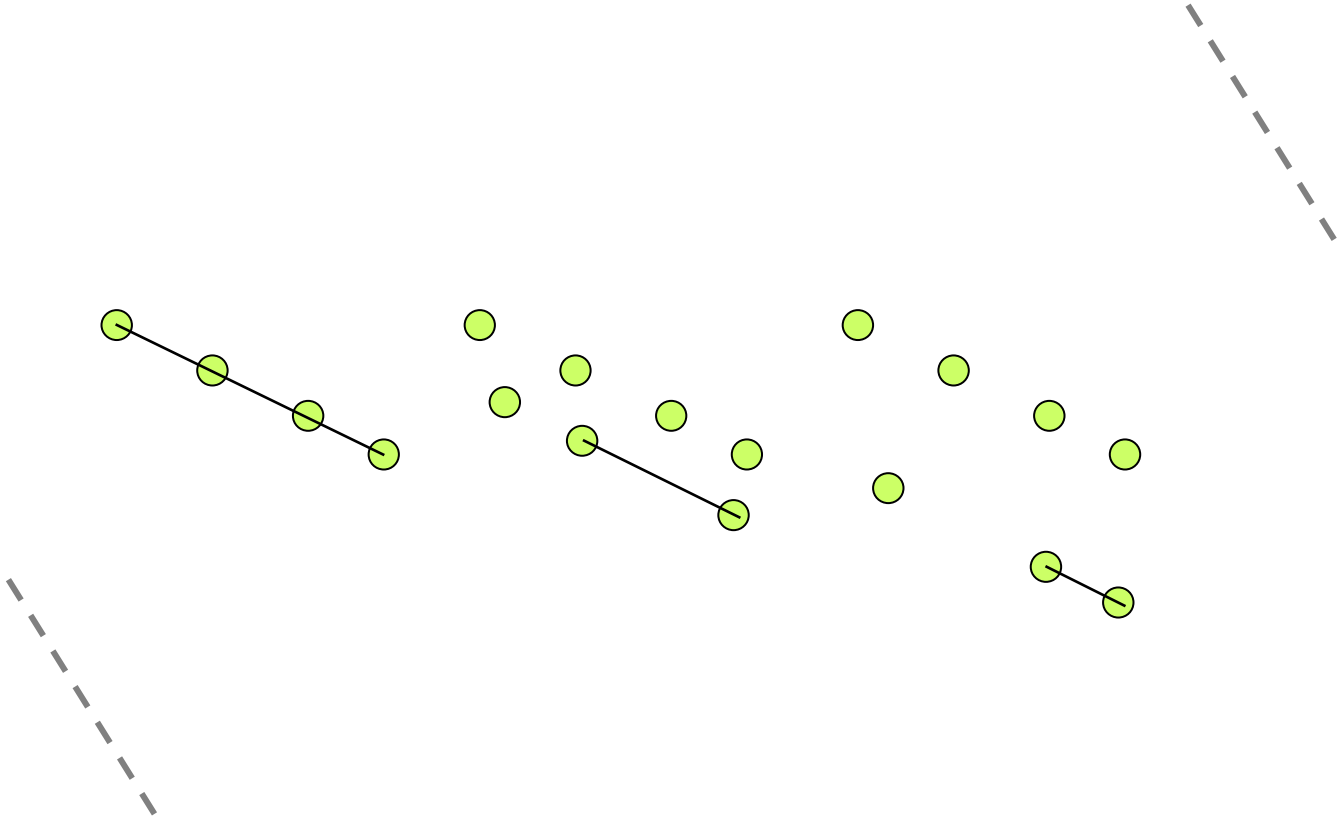
PFGE principle



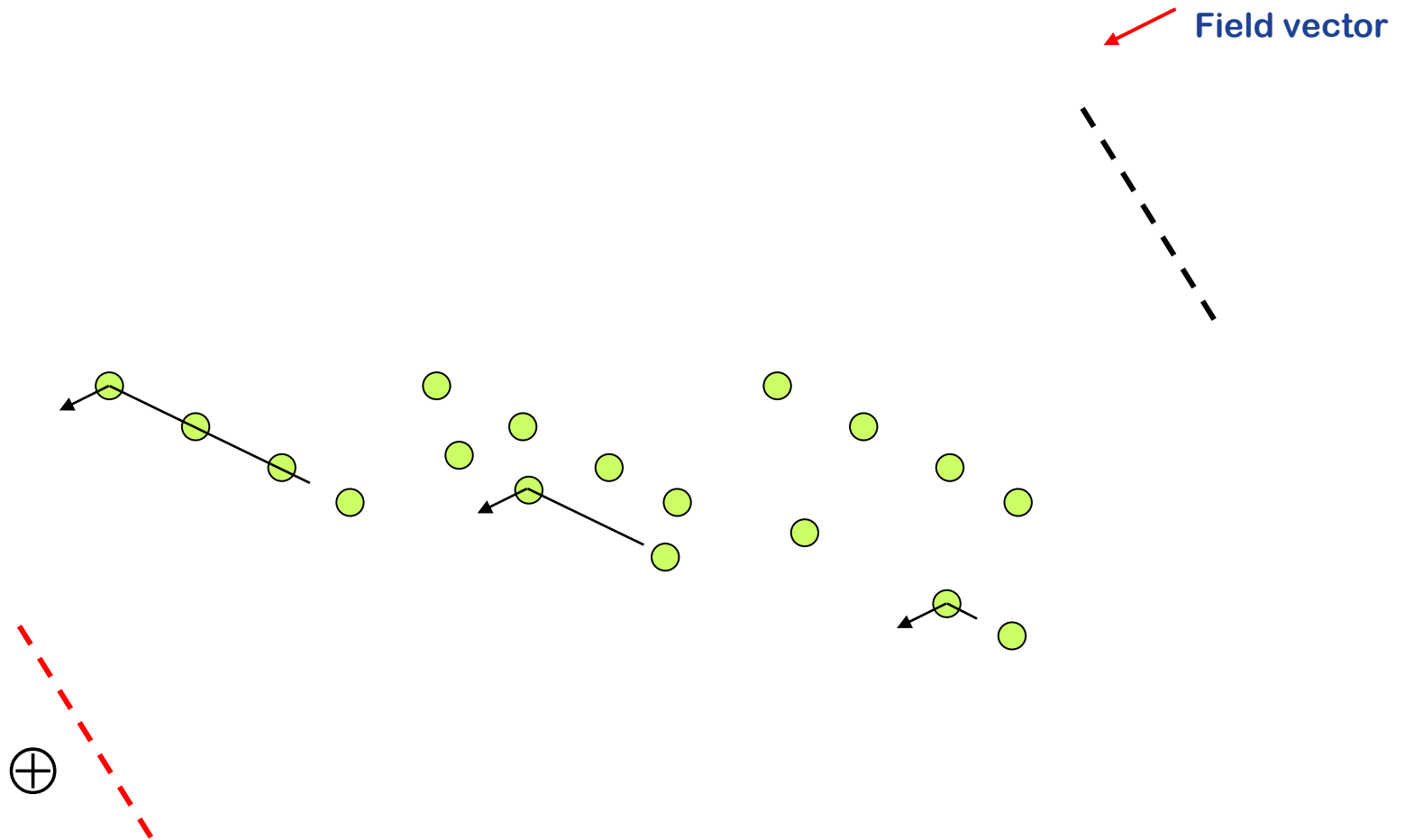
PFGE principle



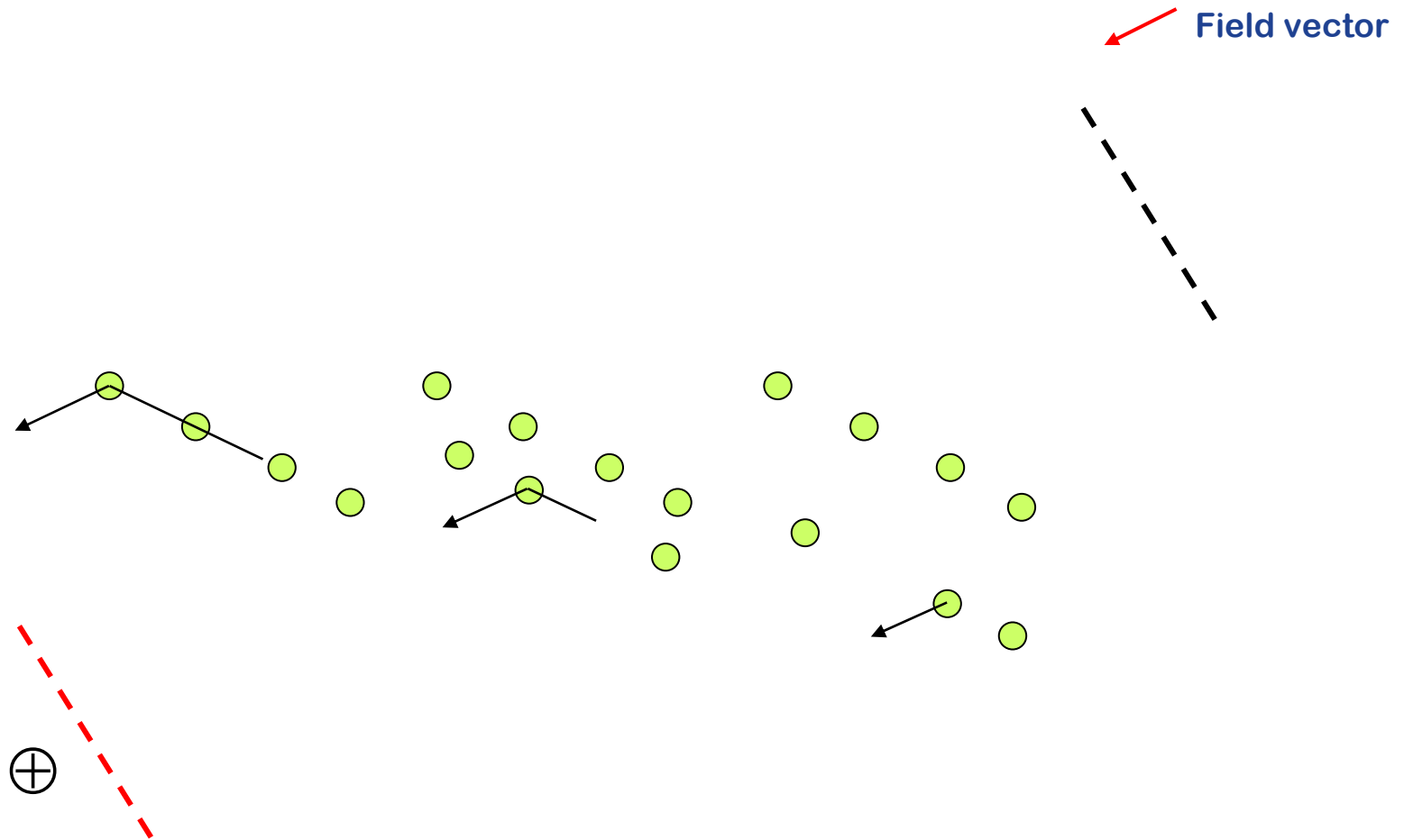
PFGE principle



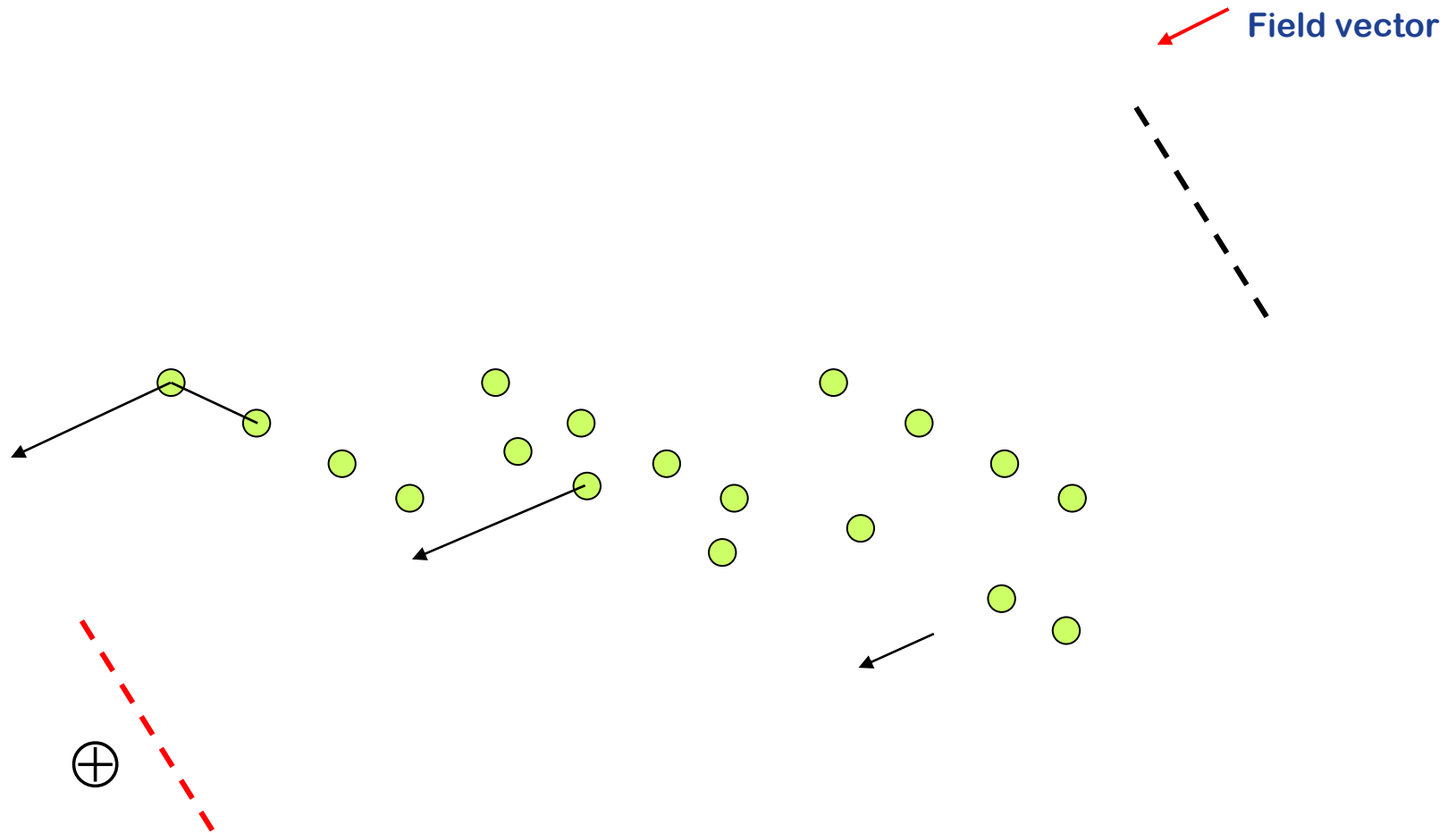
PFGE principle



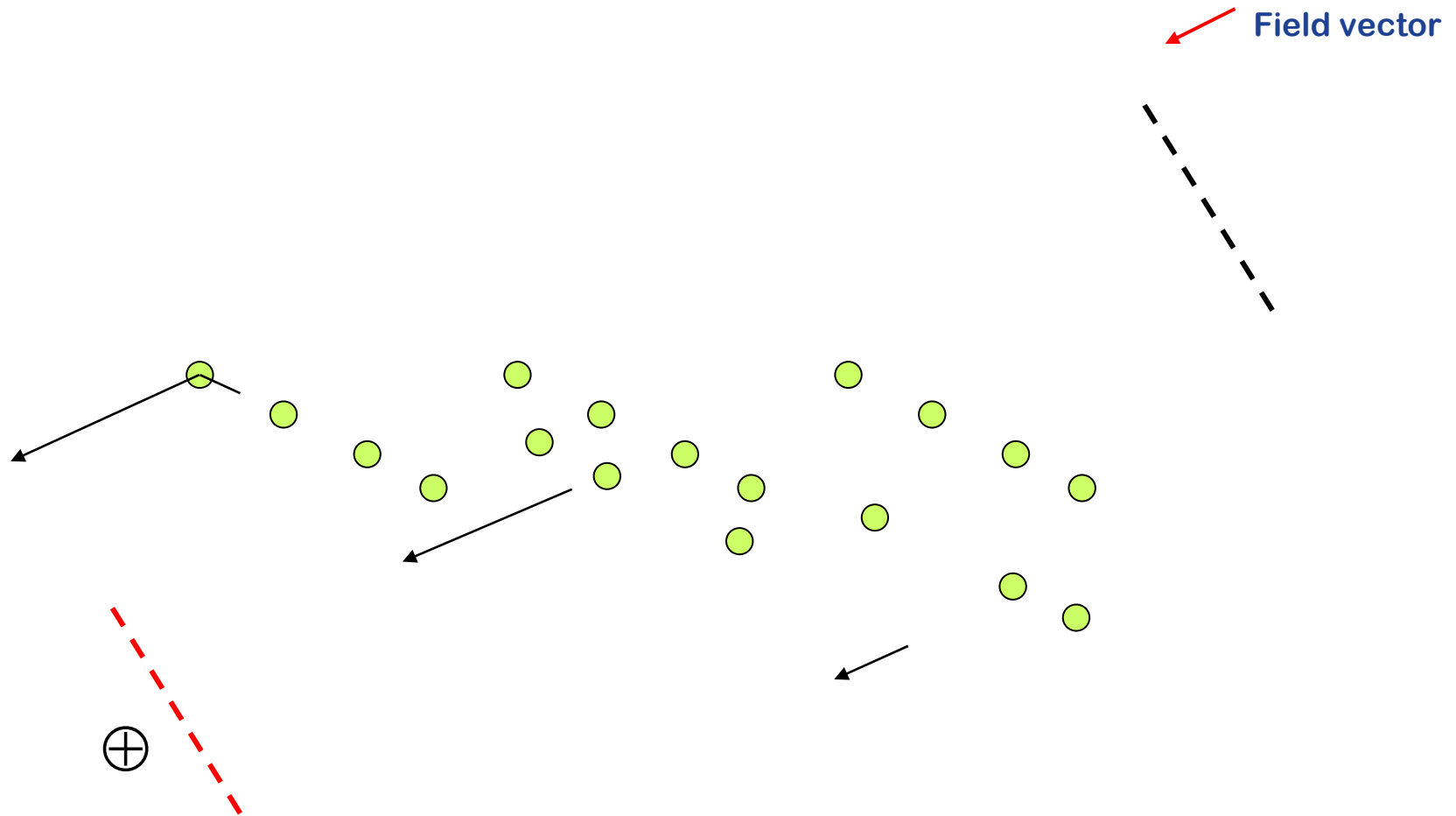
PFGE principle



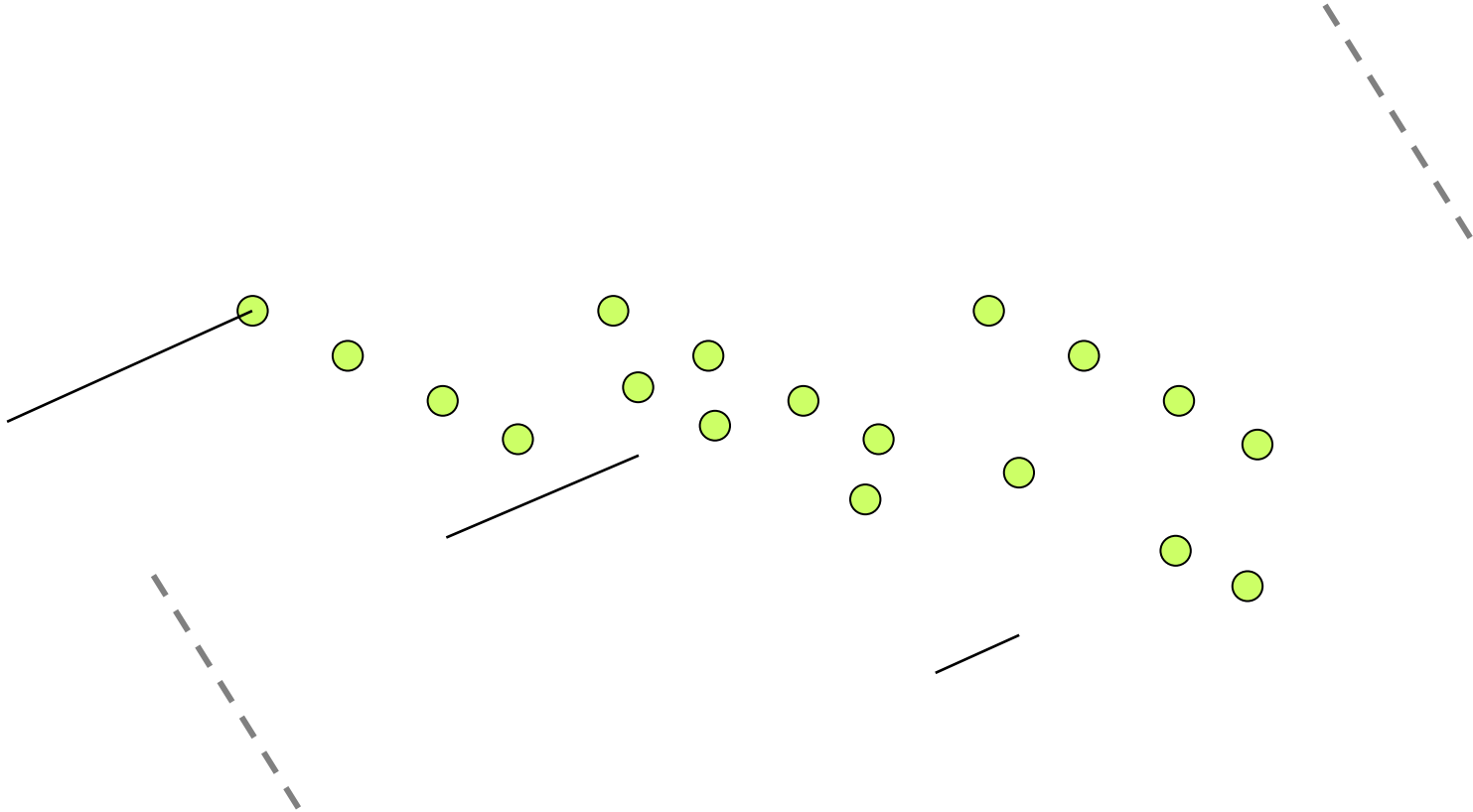
PFGE principle



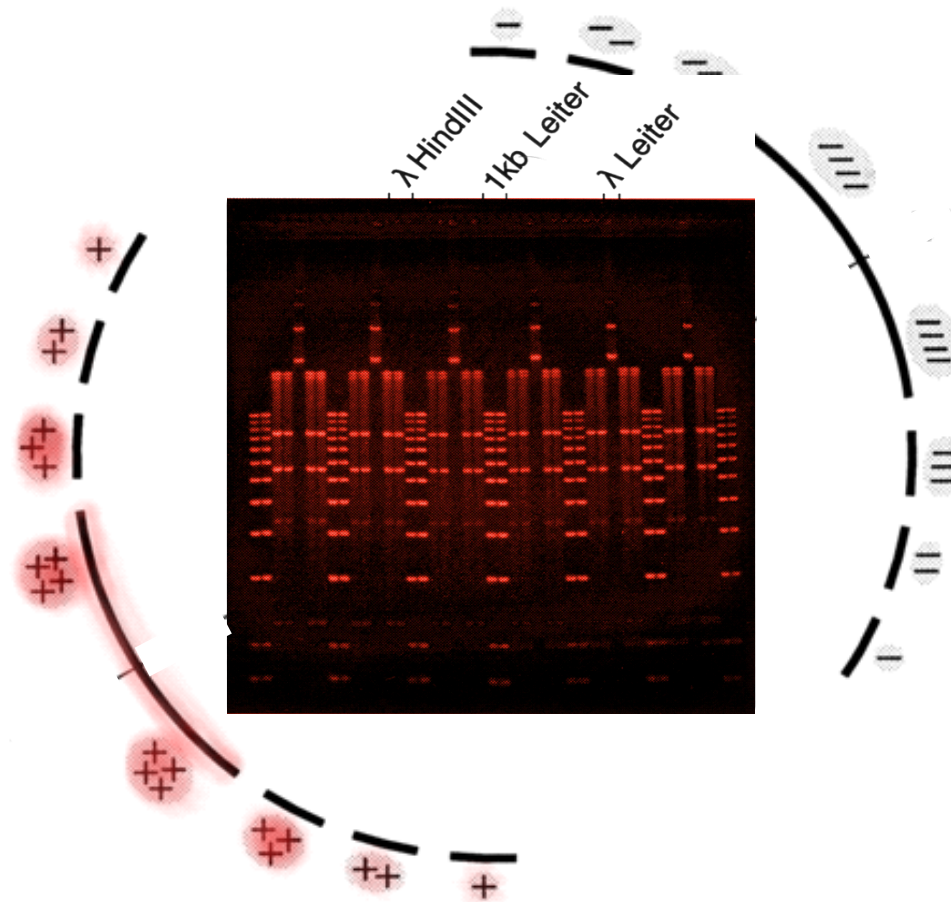
PFGE principle



PFGE principle



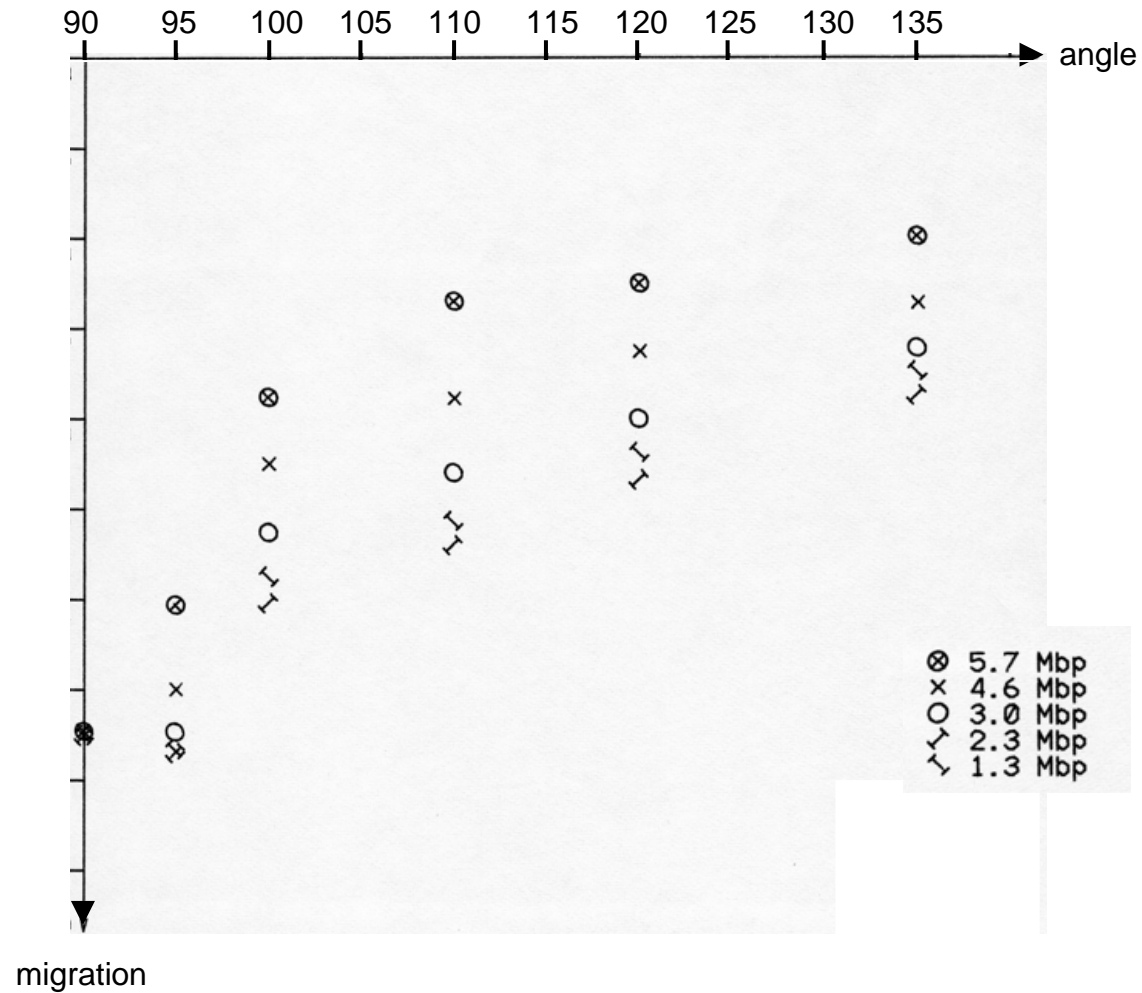
PFGE principle



Important parameters for PFGE

- Voltage (V)
- Field strength (V/cm)
- Pulse length
- Angle / change of angle
- Buffer
- Agarose type
- Agarose concentration (handling limit: 0.5% [w/v])
- Temperature
- Run time

PFGE at different angles



Volz, 1988
Uni Tübingen

Basic guideline for PFGE parameters

	1 – 100 kb	0.1 – 2.0 Mb	2 – 4 Mb	> 4 Mb
% Agarose	1.0–1.2%	0.8–1.2%	0.6–1%	0.5–0.8%
Buffer	0.5x TBE	0.5x TBE	1.0x TAE	1.0x TAE
Temperature	14 °C	14 °C	14 °C	14 °C
Field strength	6–9 V/cm	4.5–6 V/cm	2–3 V/cm	1.5–2.5 V/cm
Pulse length	0.05–10 sec	10–200 sec	200–1.800 sec	10–60 min
Run time	2–15 hr	15–30 hr	24–72 hr	72–144 hr
Angle	110-130°	110-130°	105-120°	95 to 105°

Examples of PFGE Application (I)

- **Application**

Identification of bacterial strains and/or species

- **Target groups**

Hospitals, Institutes related to health care,
Institute of Microbiology, etc.

- **References**

- Identification of *Azospirillum* species by RFLP and pulsed-field gel electrophoresis.
Microb Releases. 1993;2(1):41-5.
- Clonal diversity among recently emerged strains of *Vibrio parahaemolyticus* O3:K6 associated with pandemic spread.
J Clin Microbiol. 1999 Jul;37(7):2354-7.

Examples of PFGE Application (II)

- **Application**
Investigation of genomic libraries (artificial chromosomes like BAC, PAC, YAC) or large plasmids
- **Target groups**
Diverse research groups, Genome Sequencing Centers, etc.
- **References:**
 - Two-dimensional screening of the Wageningen chicken BAC library. *Mamm Genome. 2000;11(5):360-3.*
 - Plasmid transfer between introduced and indigenous bacteria in leaf litter, soil and vermicompost as affected by soil invertebrates. *Biol. Fertil. Soils 1998; 28(2): 169-76.*

Examples of PFGE Application (III)

- **Application**
Physical gene or genome mapping

- **Target groups**
Diverse research groups

- **References**
 - Nibrin, a Novel DNA Double-Strand Break Repair Protein, Is Mutated in Nijmegen Breakage Syndrome.
Cell 1998; 93: 467-476.

 - Physical-genetic map of the rythromycinproducing organism *Saccharopolyspora erythraea*.
Microbiology. 1998;144 (Pt 8): 2151-9.

Examples of PFGE Application (IV)

- **Further PFGE applications**
 - Evaluation of DNA damages caused by radiation or chemical treatment at cells of cell cultures
 - 2-D PFGE for
 - high resolution physical mapping
 - studies of multigene families
 - Apoptosis studies at intermediate formed larger DNA molecules during the phase of the process