Rotaphor 6.0 System

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Coventional agarose gel electrophoresis

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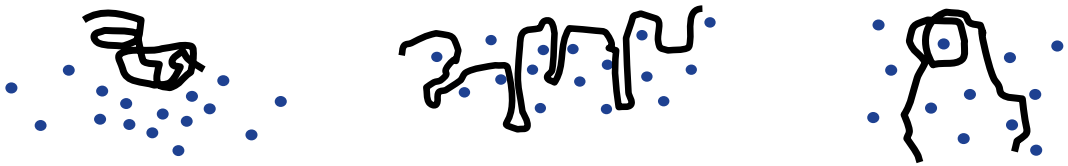
- Upper limit of separation: ~ 25.000 bp (25 kb)
- Problem: trapping of larger molecules



The patented Rotaphor principle

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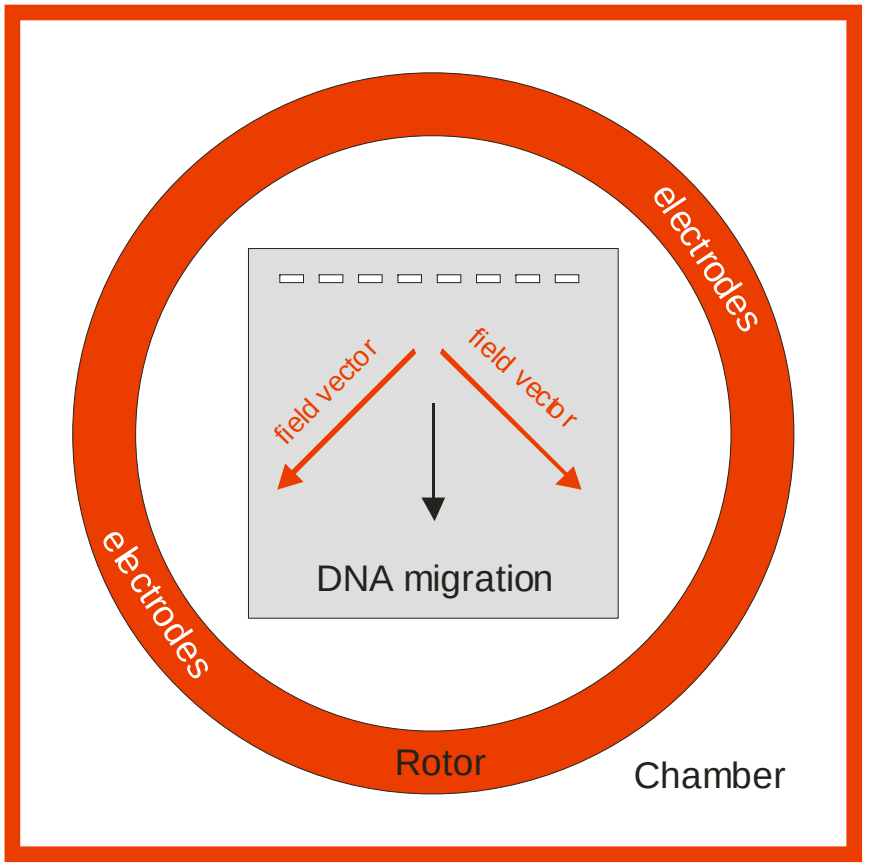
ROFE

ROtating

Field

Elektrophoresis



Rotaphor Version 6.0 - News

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- 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

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- 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

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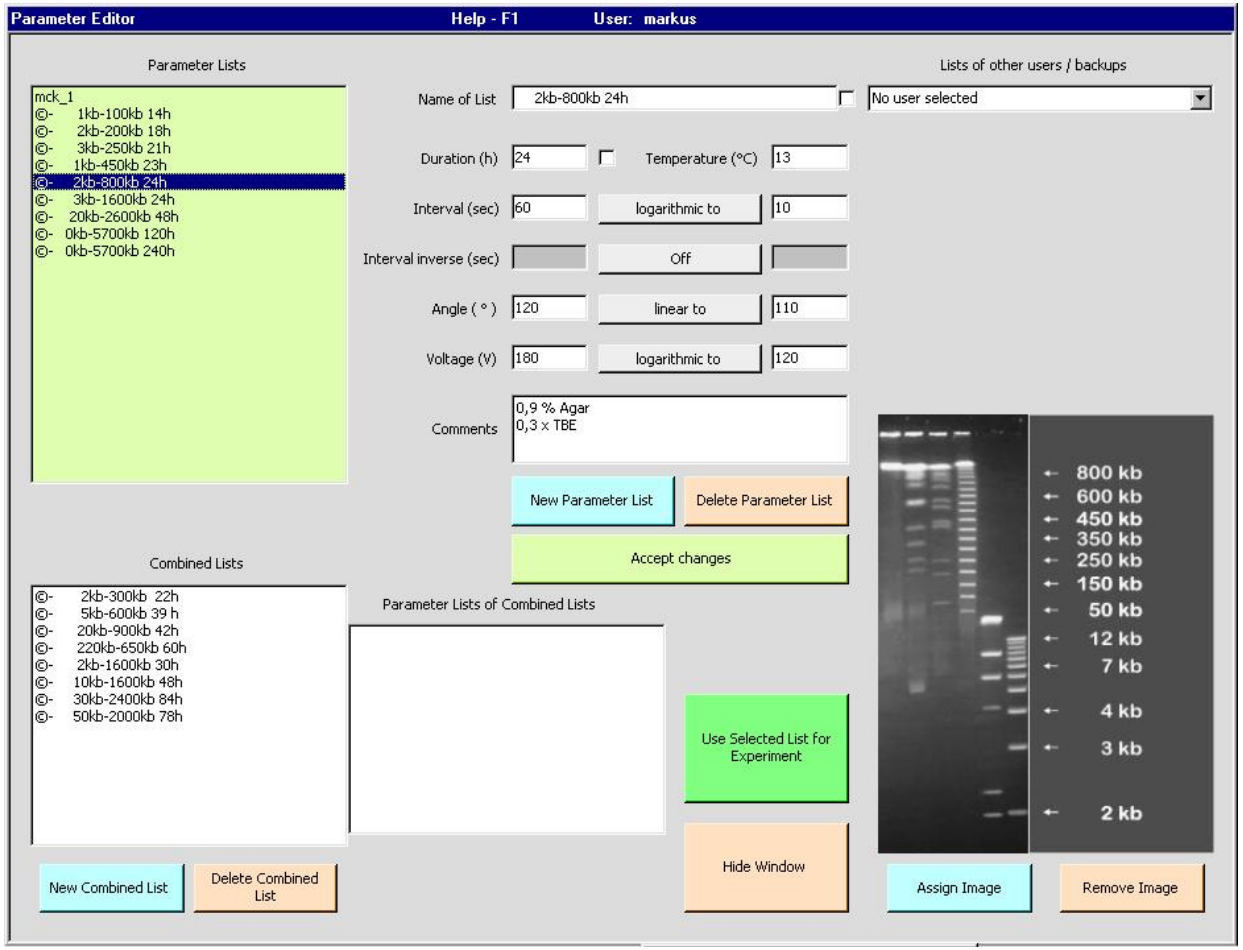

Electrophoresis Control

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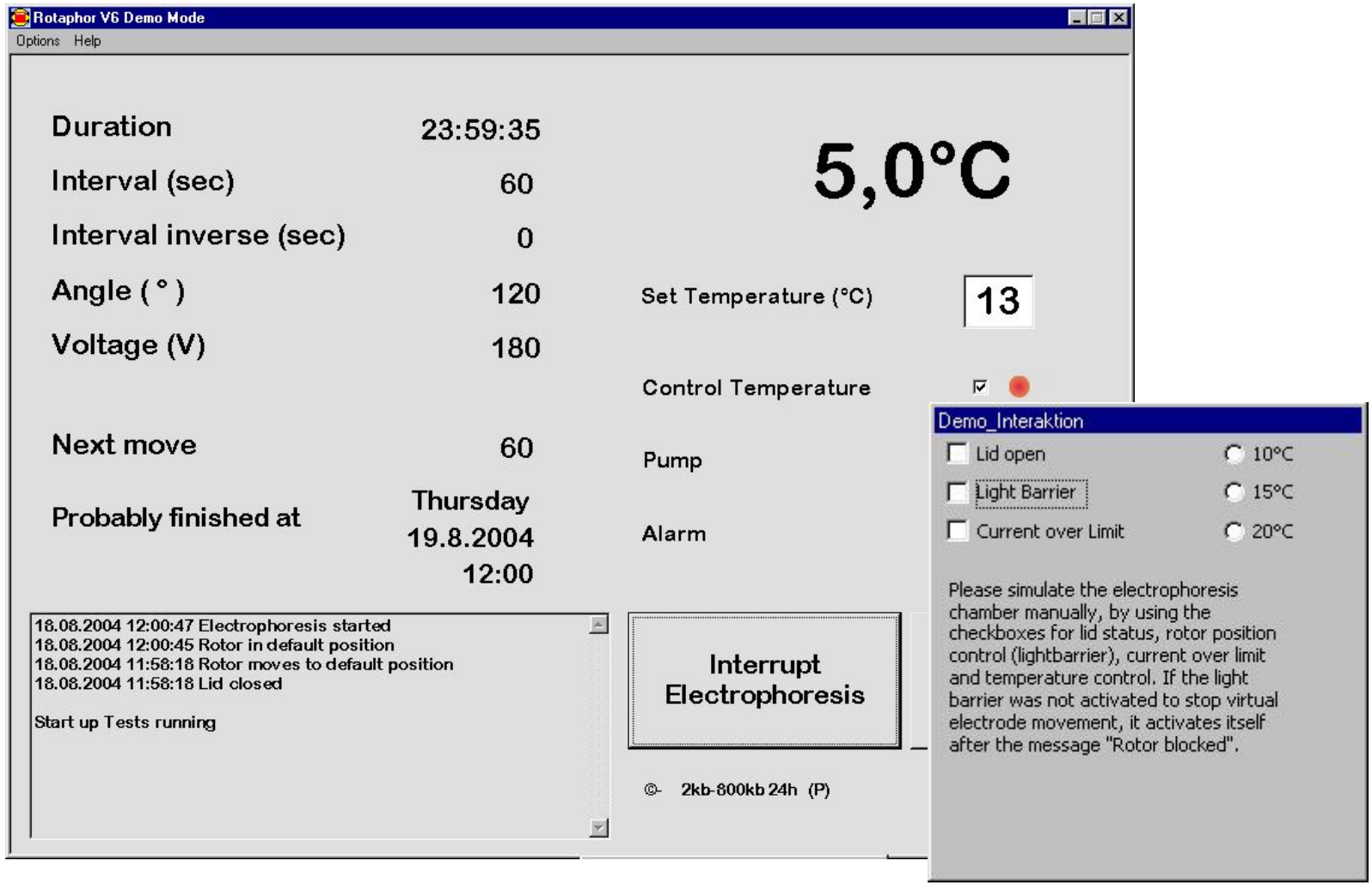

Basics of Pulse Field Gel Electrophoresis (PFGE)

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- 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

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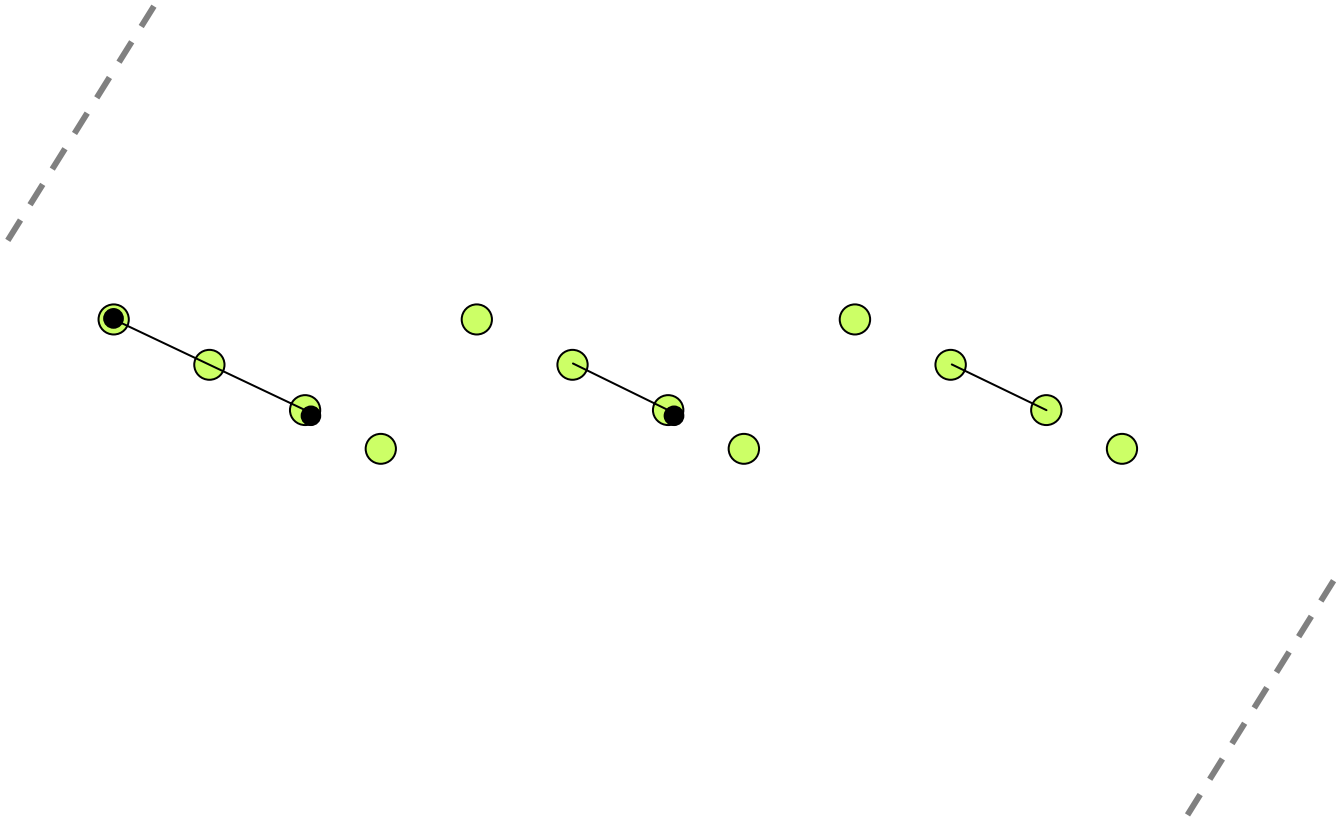

PFGE principle

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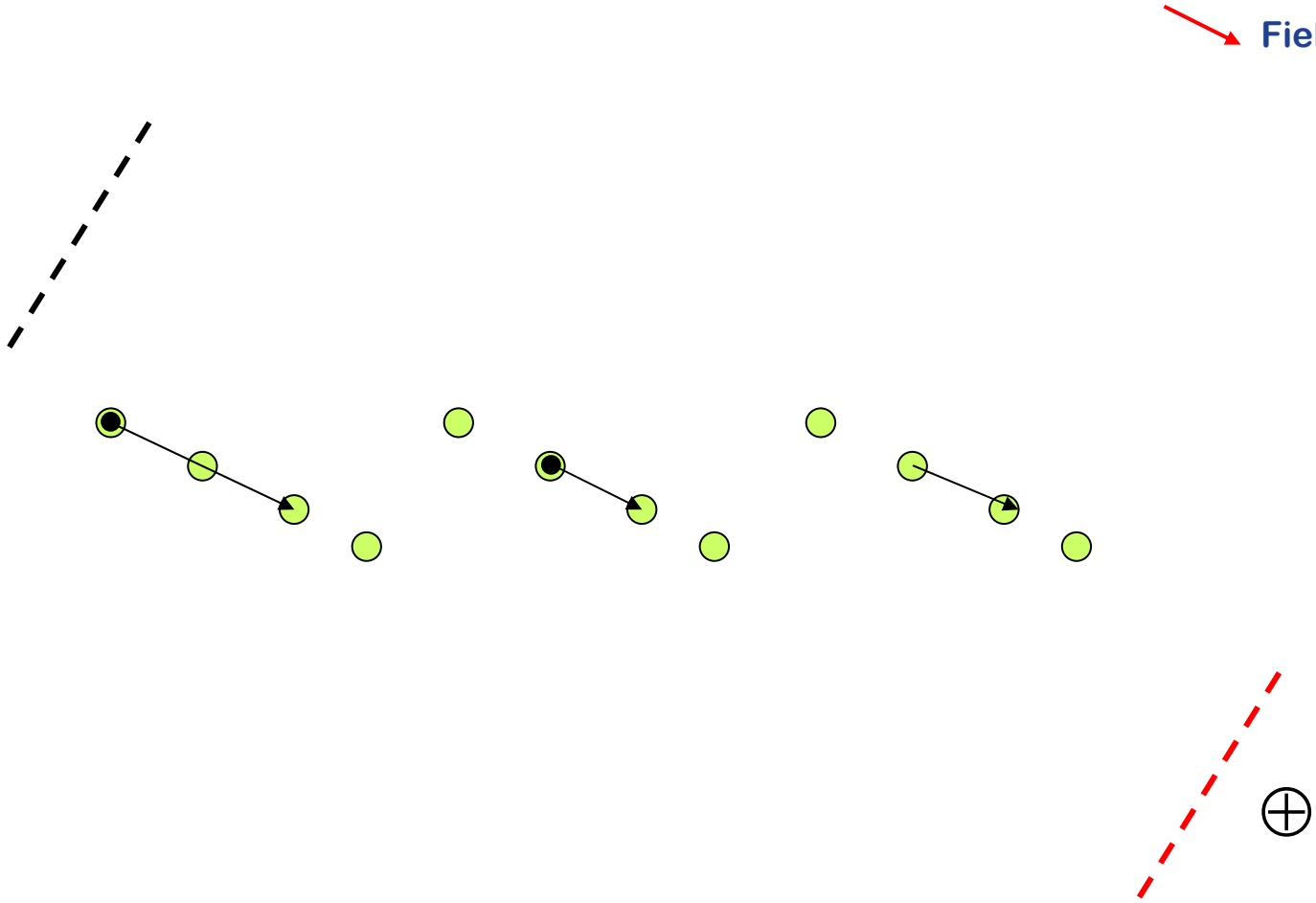

PFGE principle

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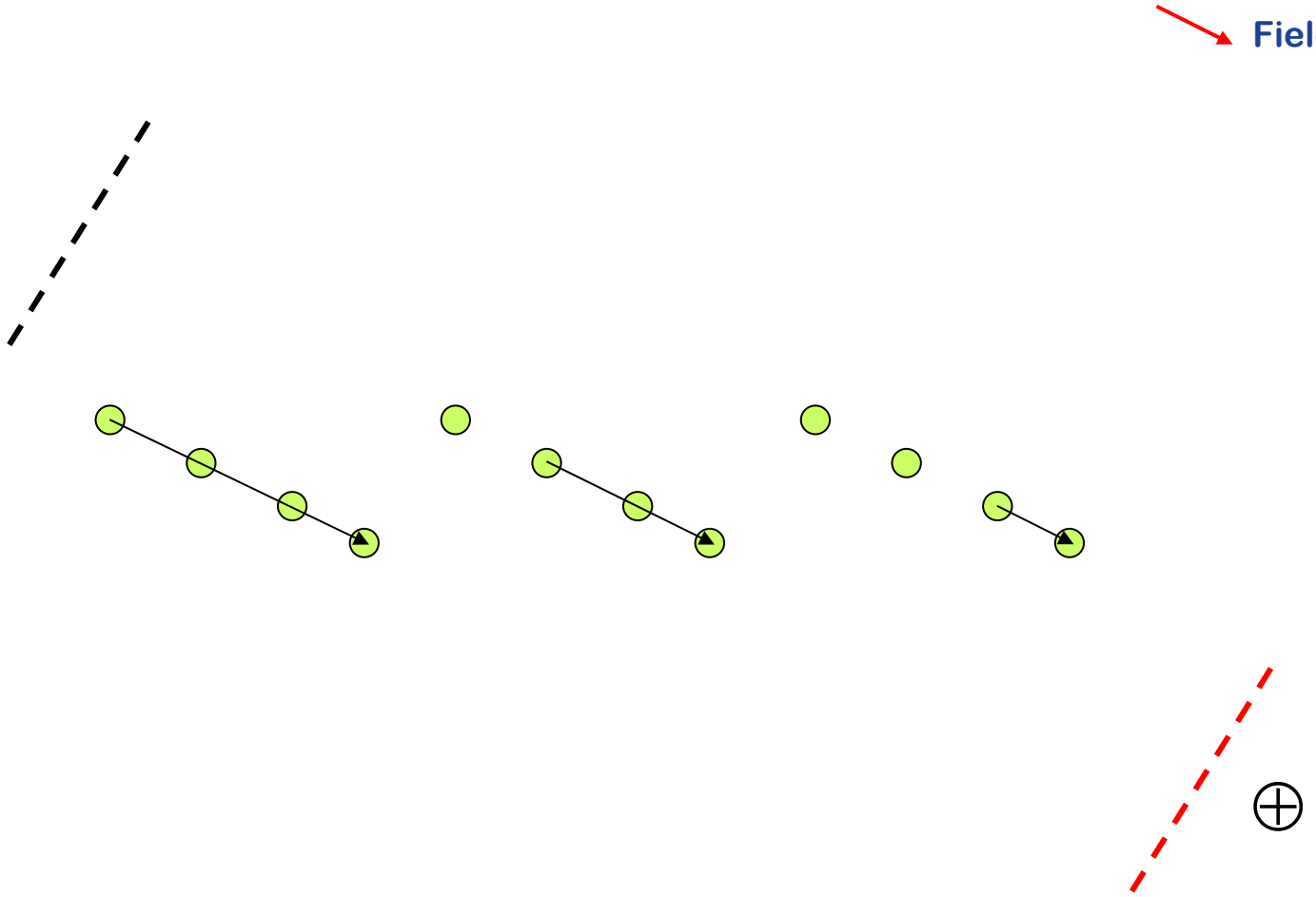
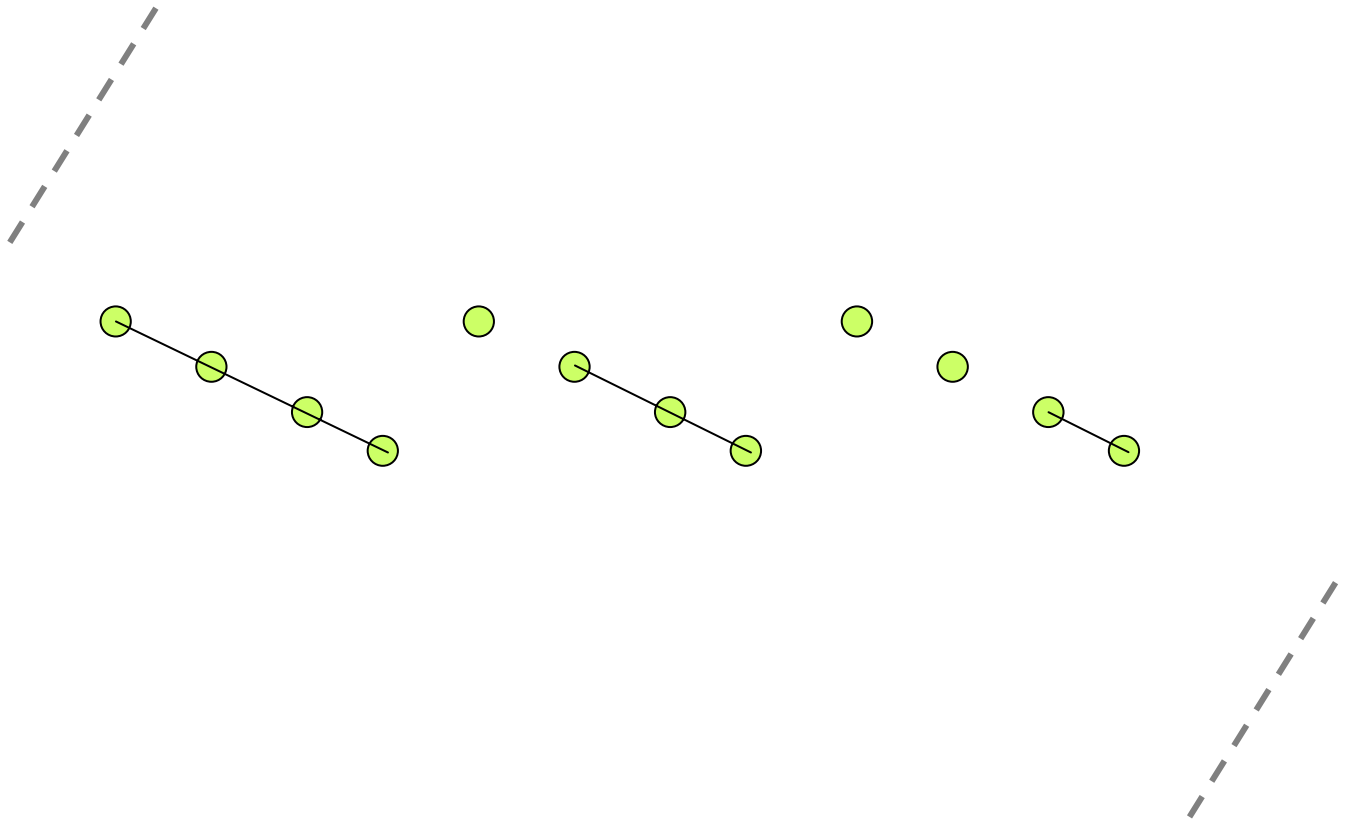
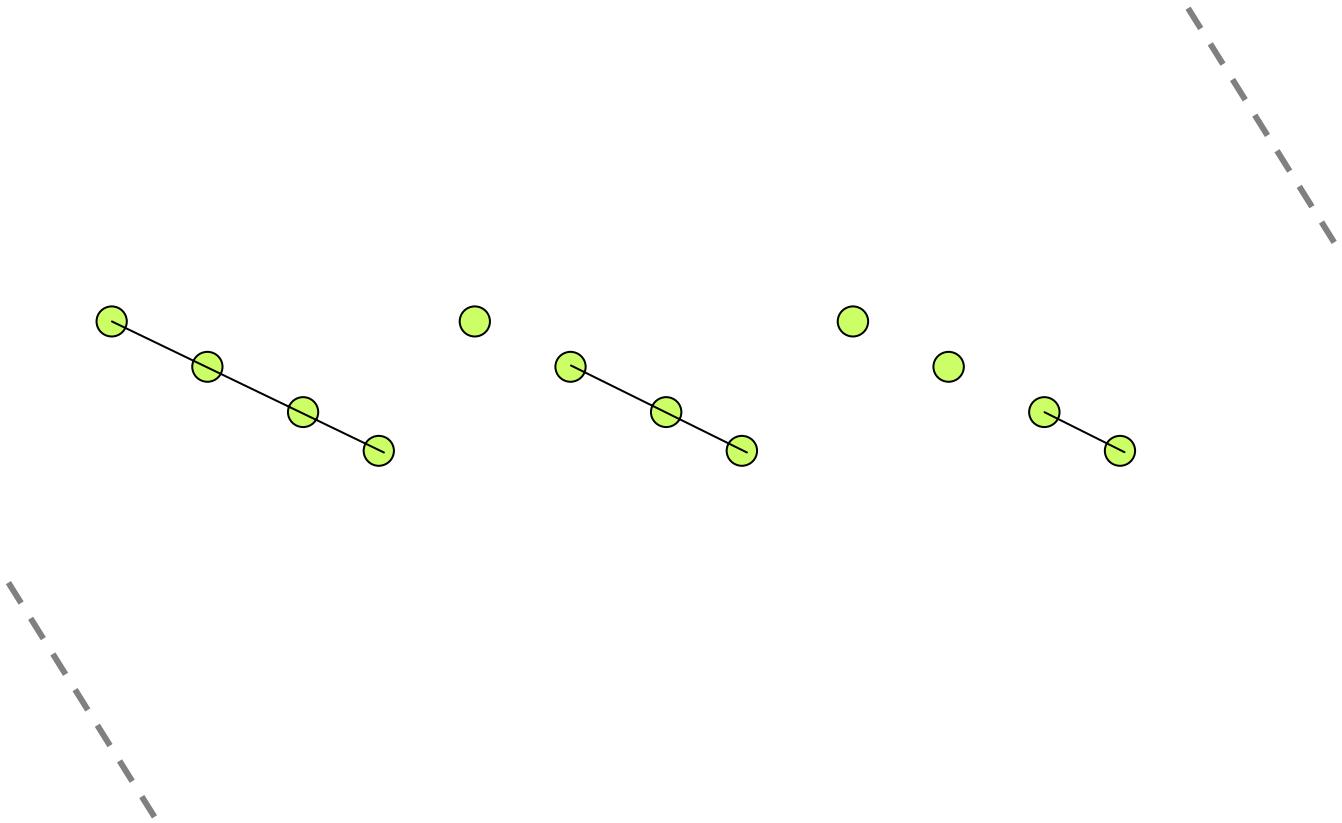


PFGE principle



PFGE principle



PFGE principle

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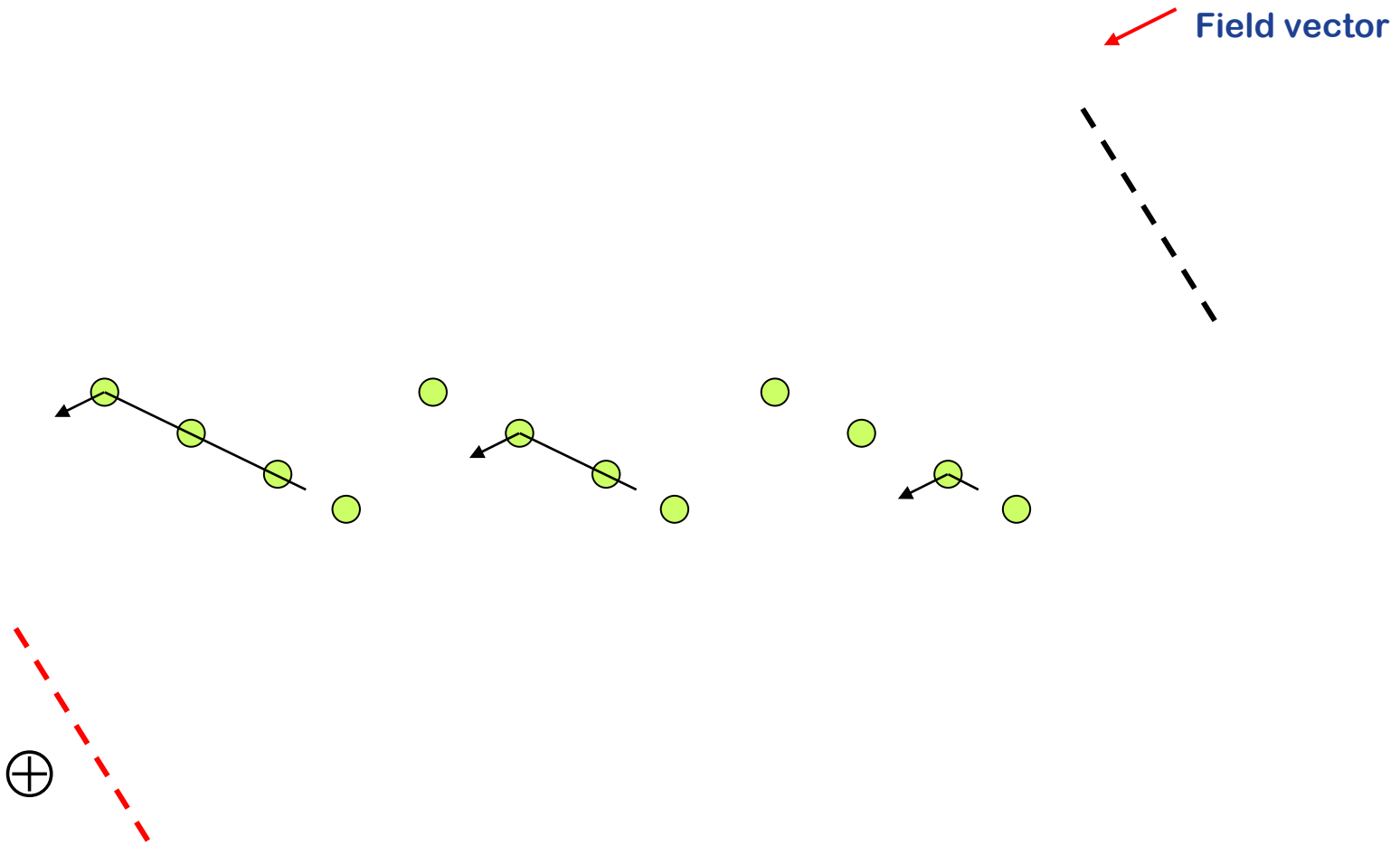

PFGE principle

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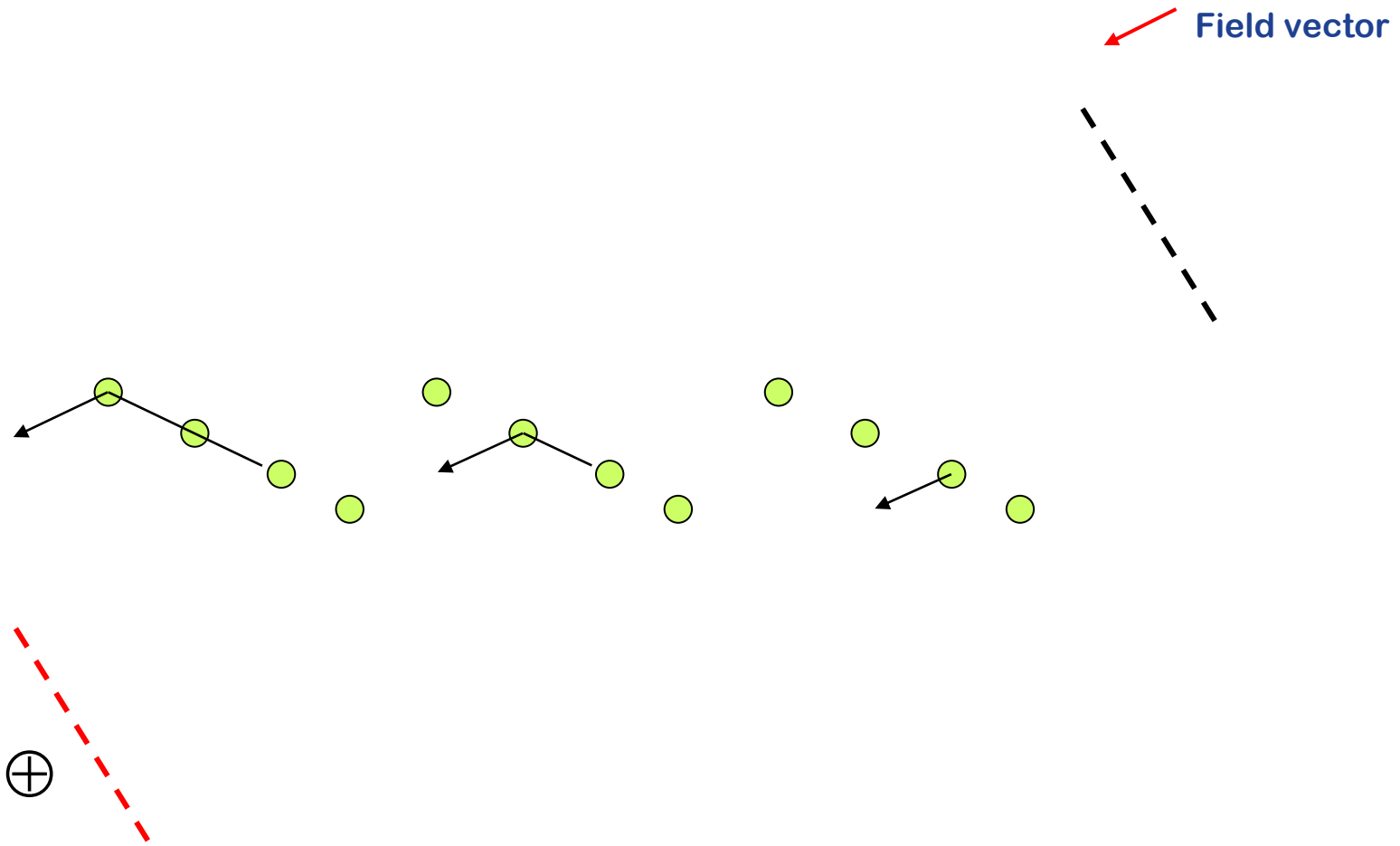

PFGE principle

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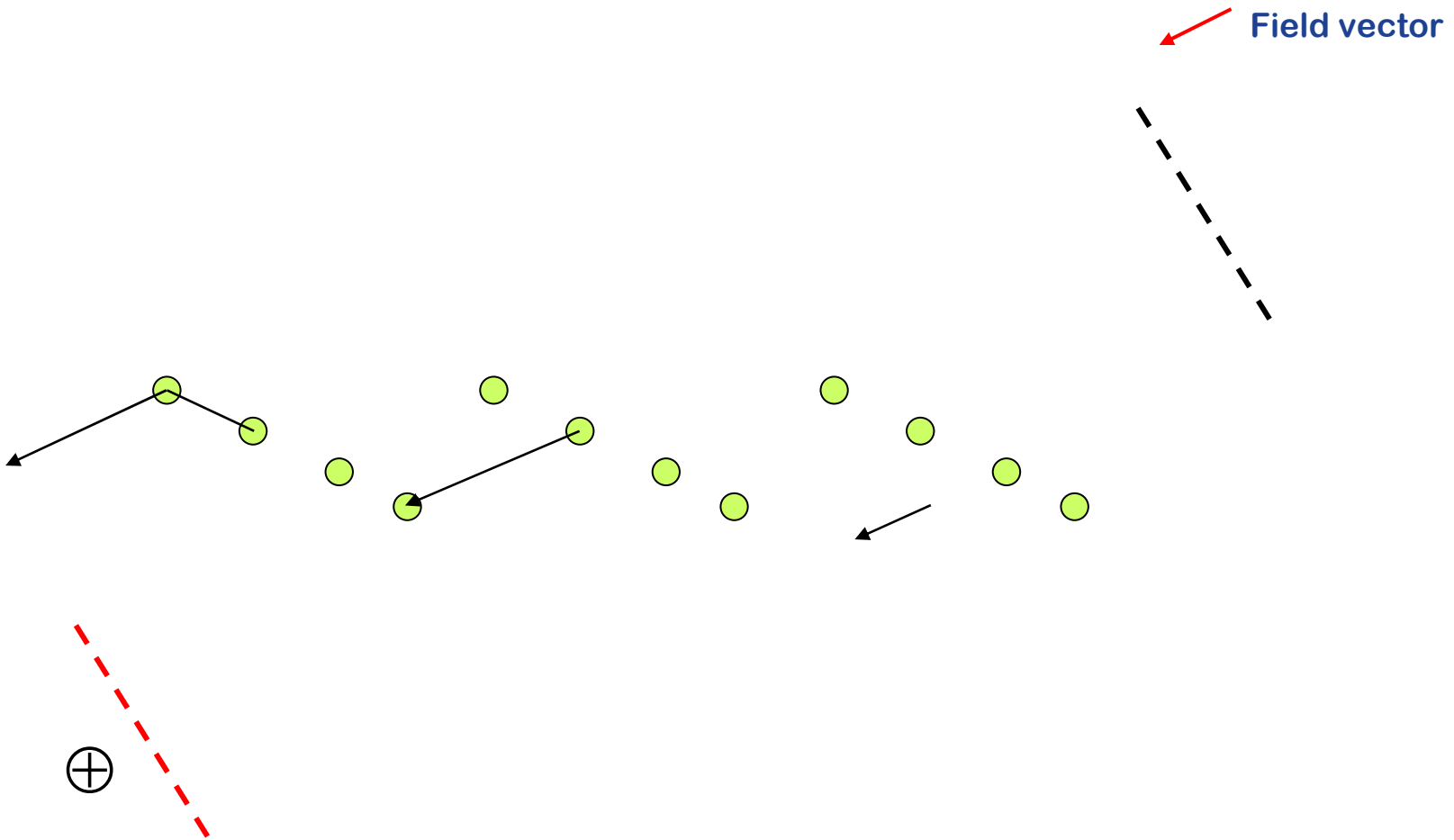

PFGE principle

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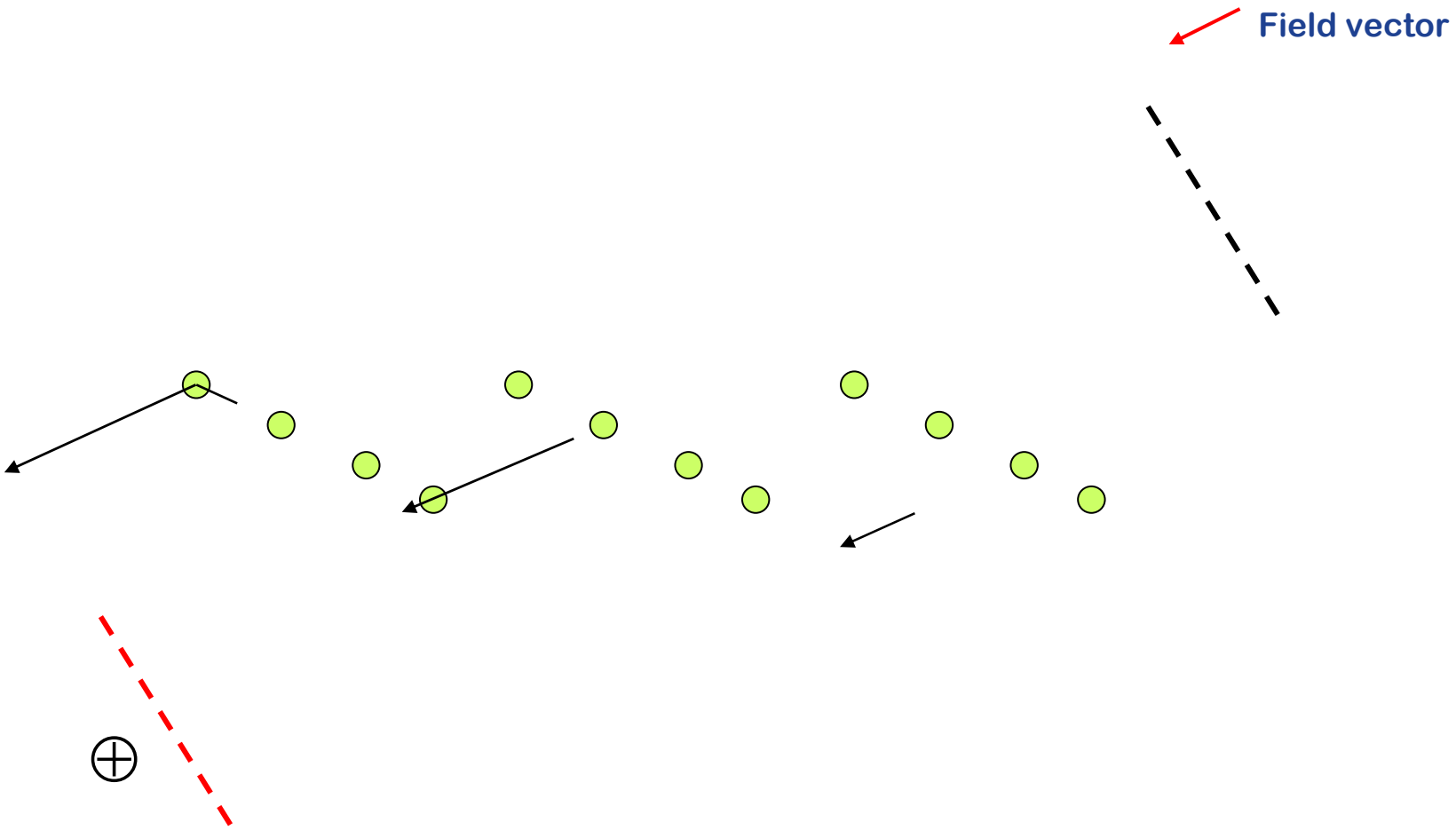

PFGE principle

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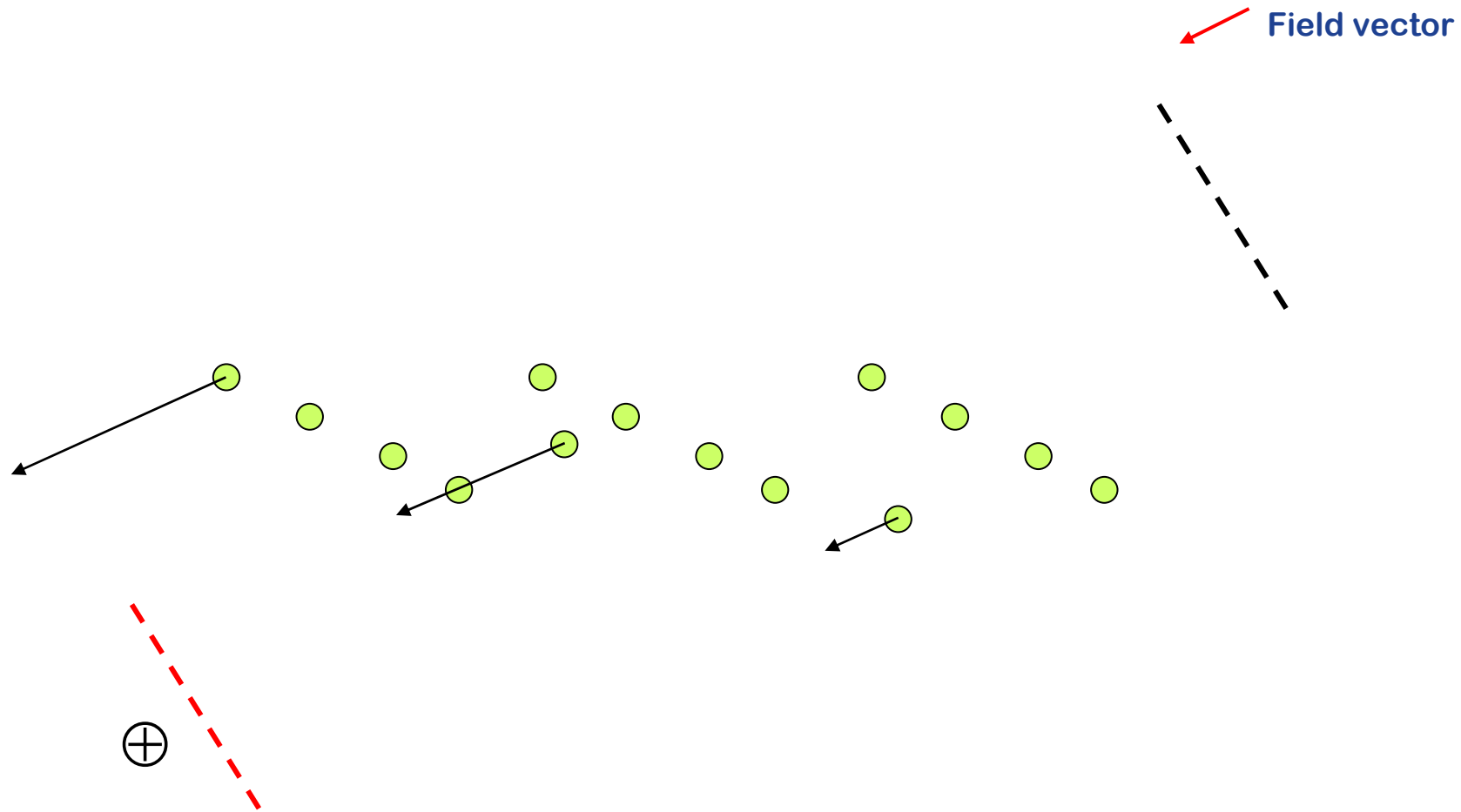
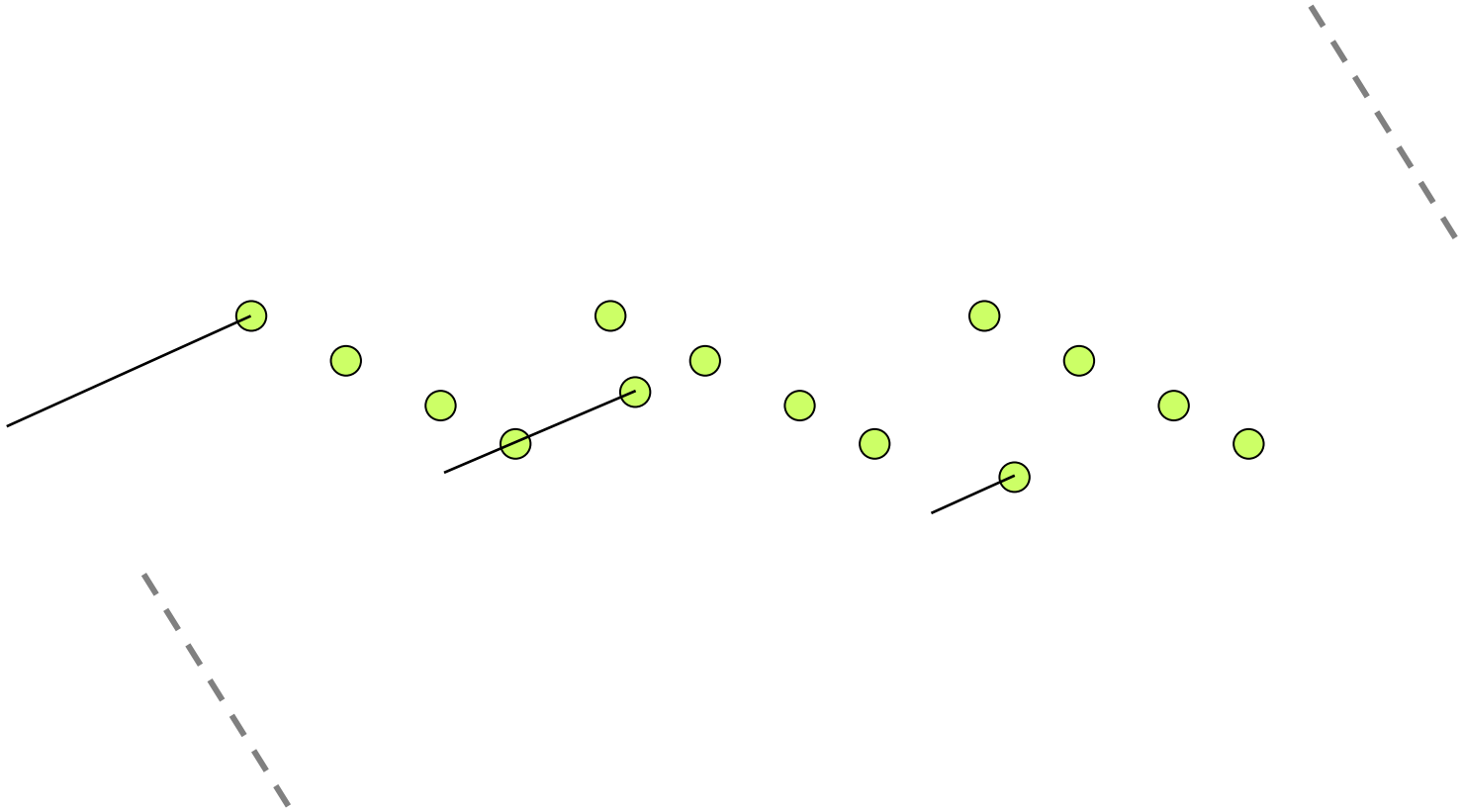
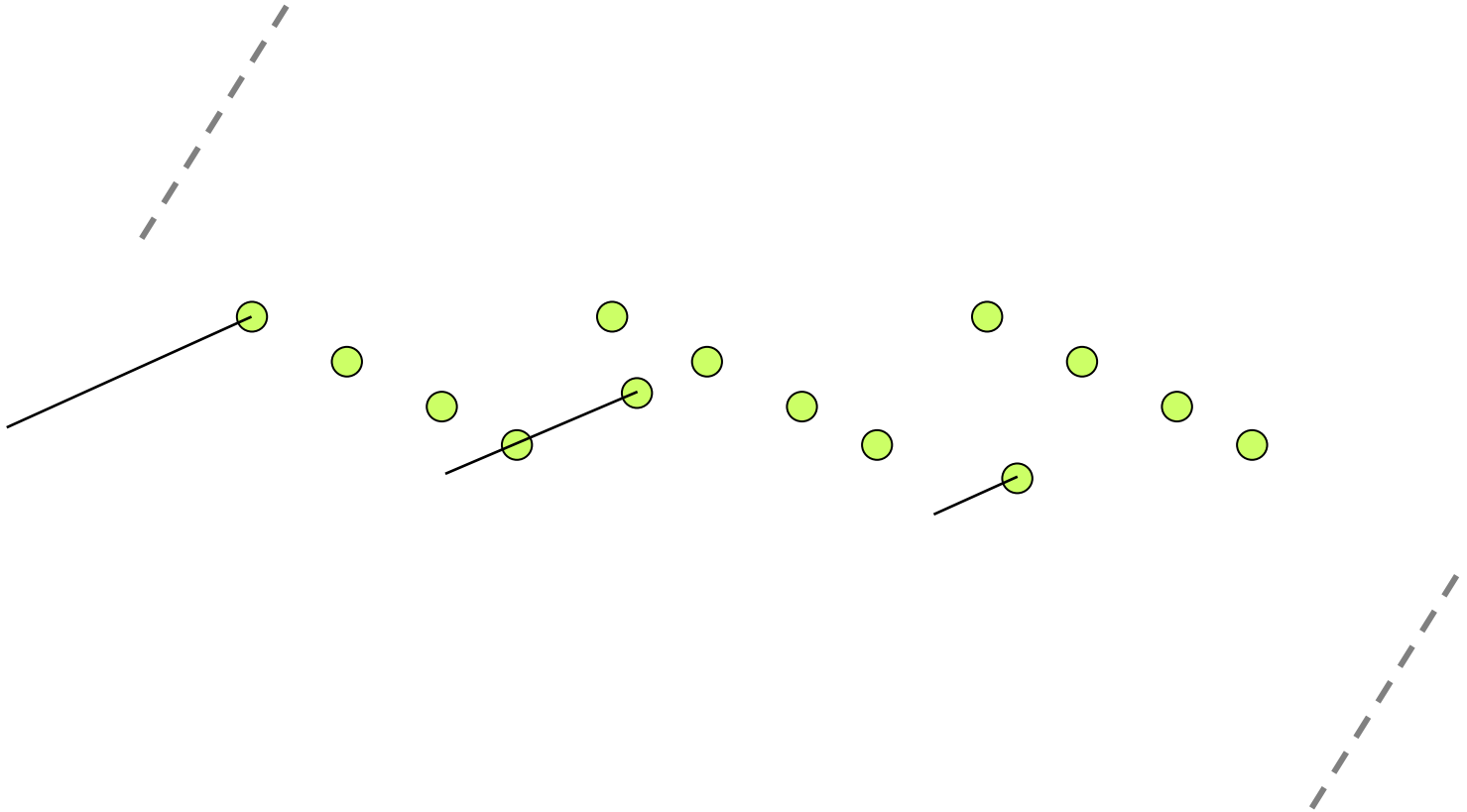


PFGE principle



PFGE principle



PFGE principle

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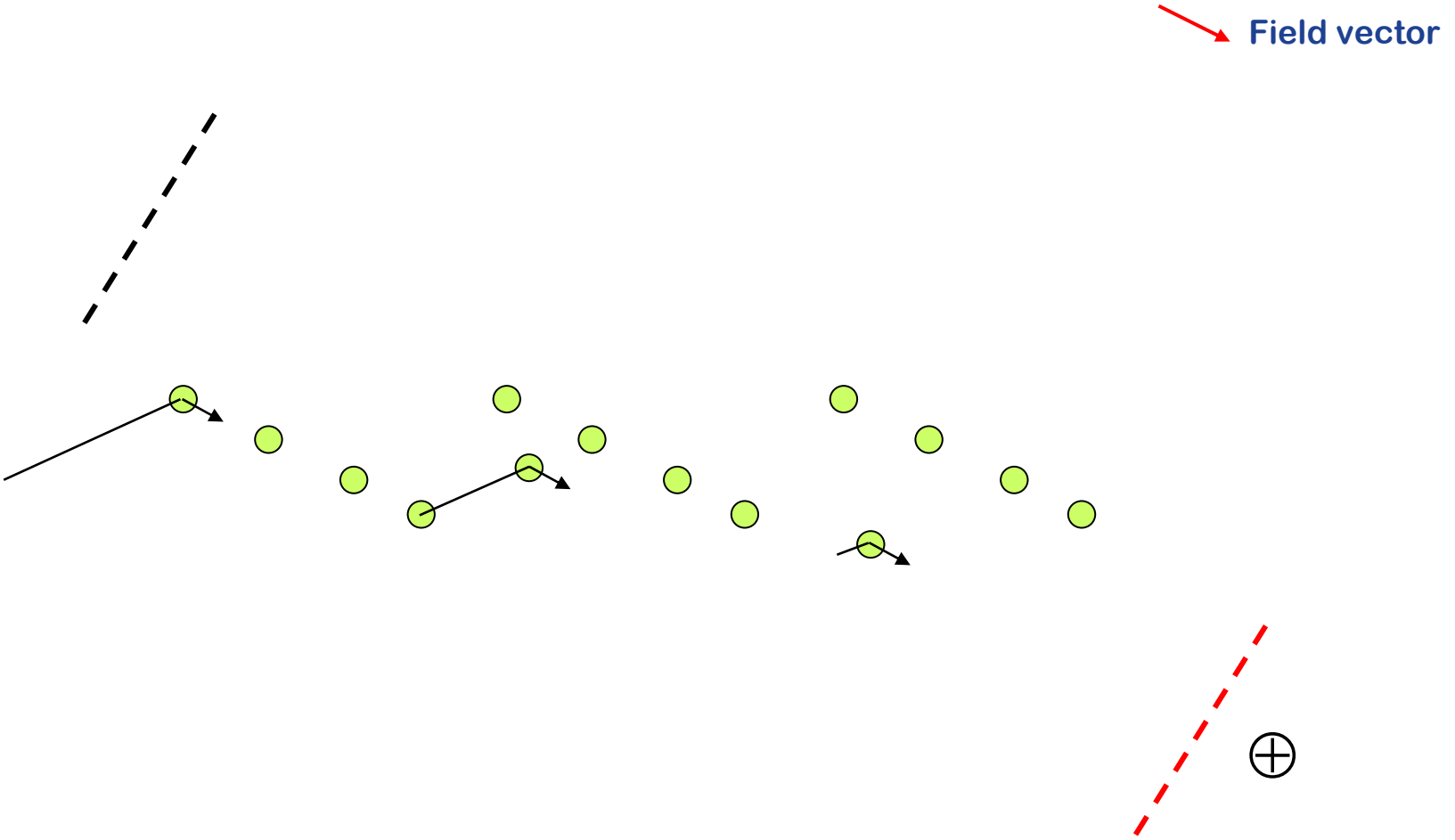

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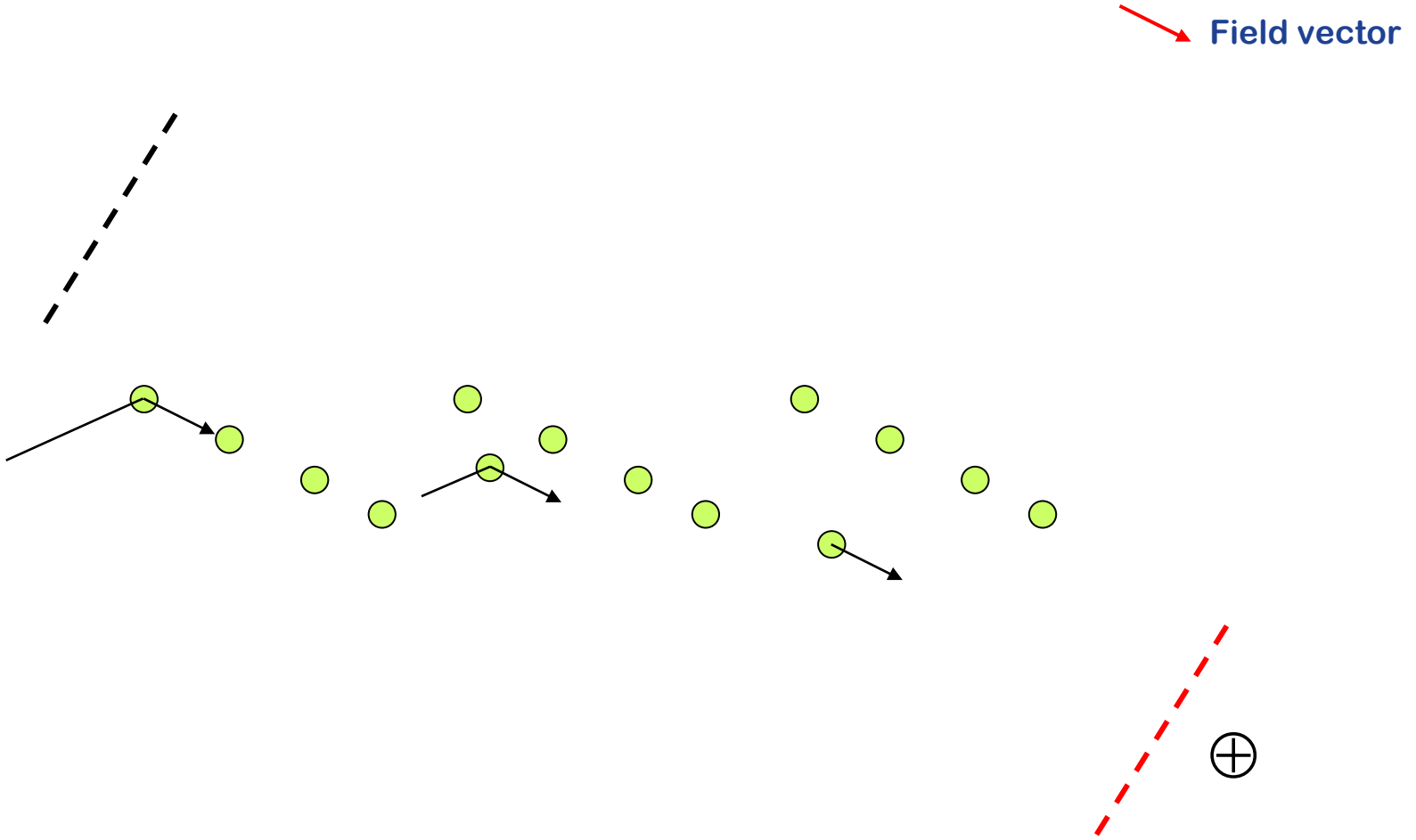

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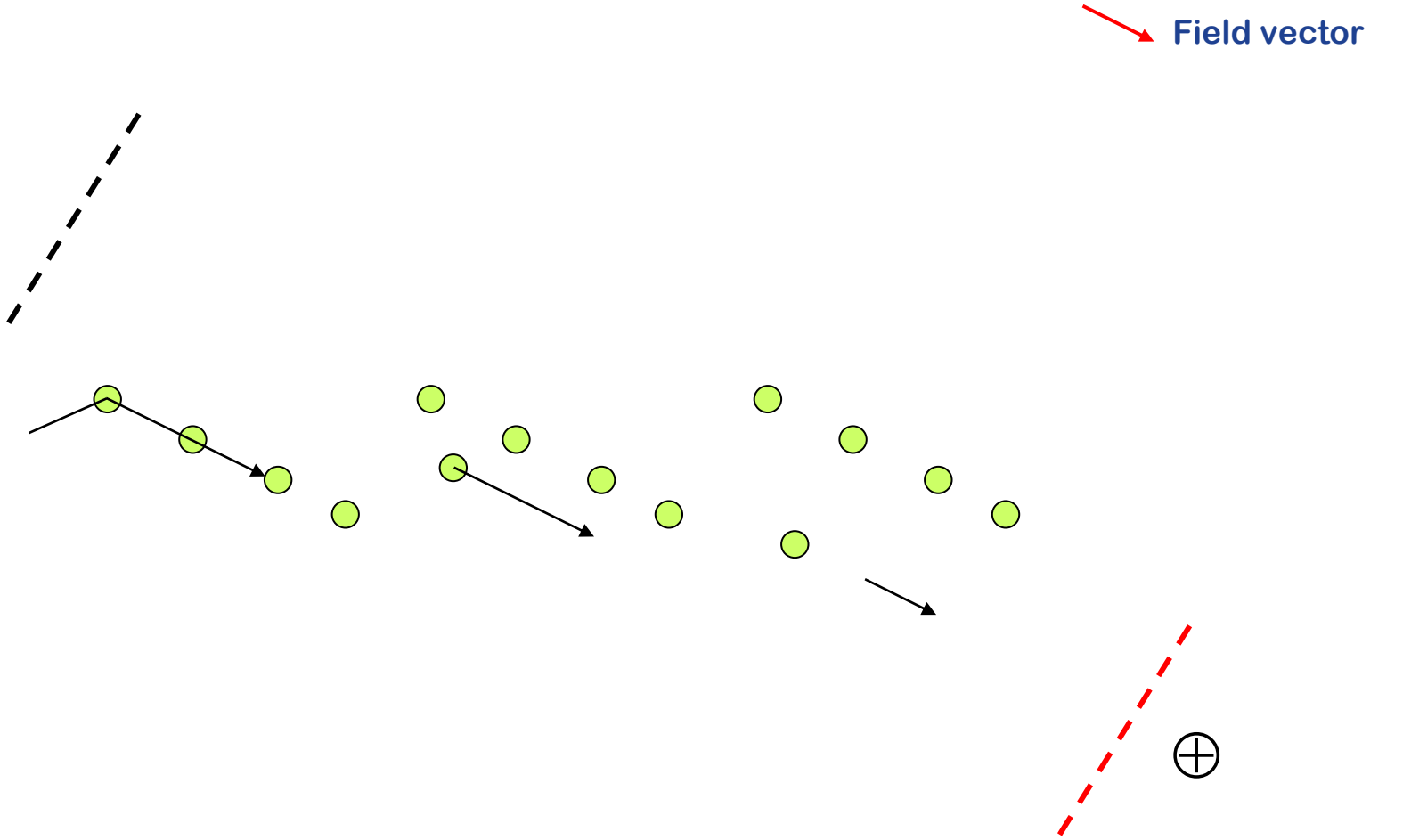

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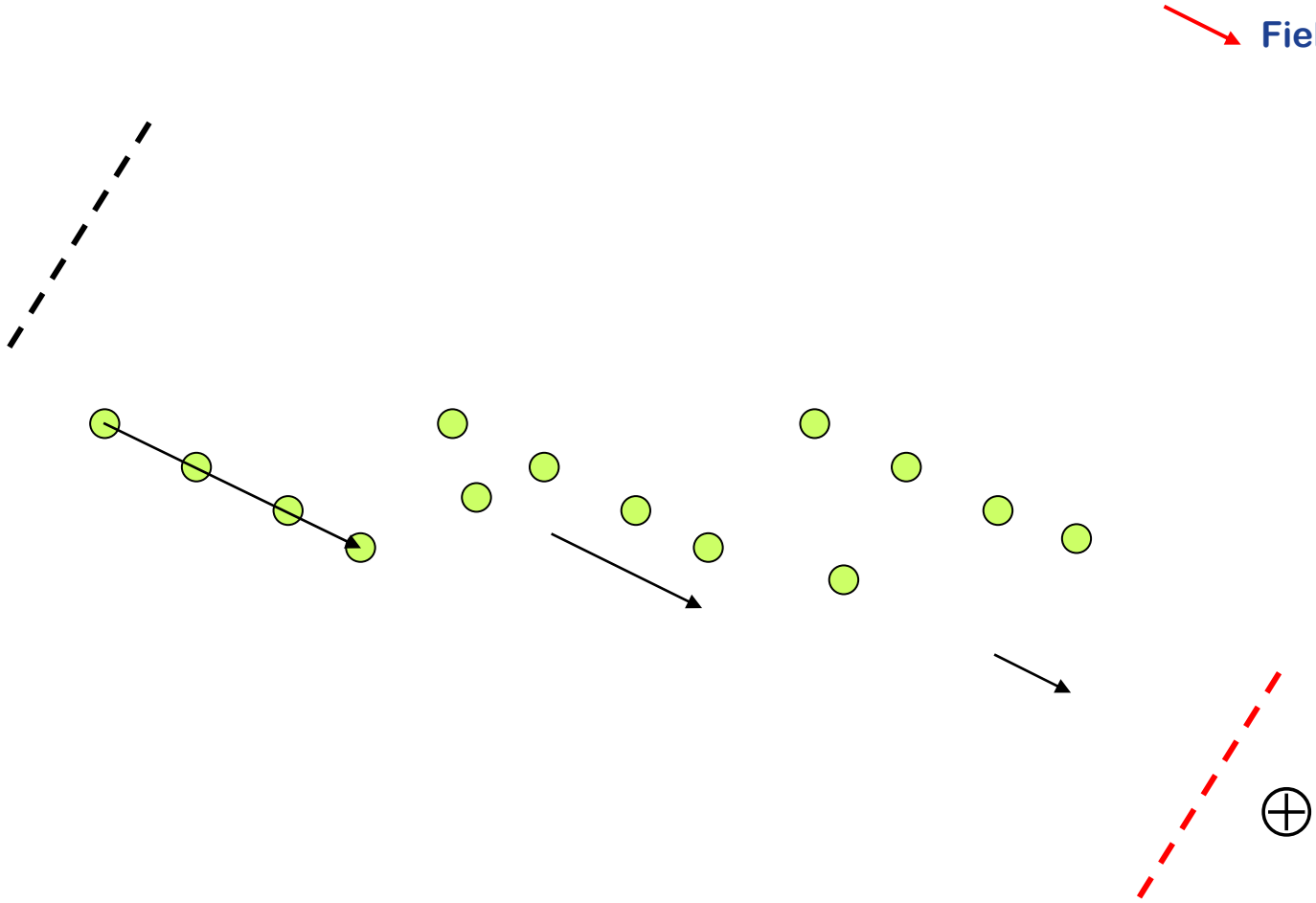
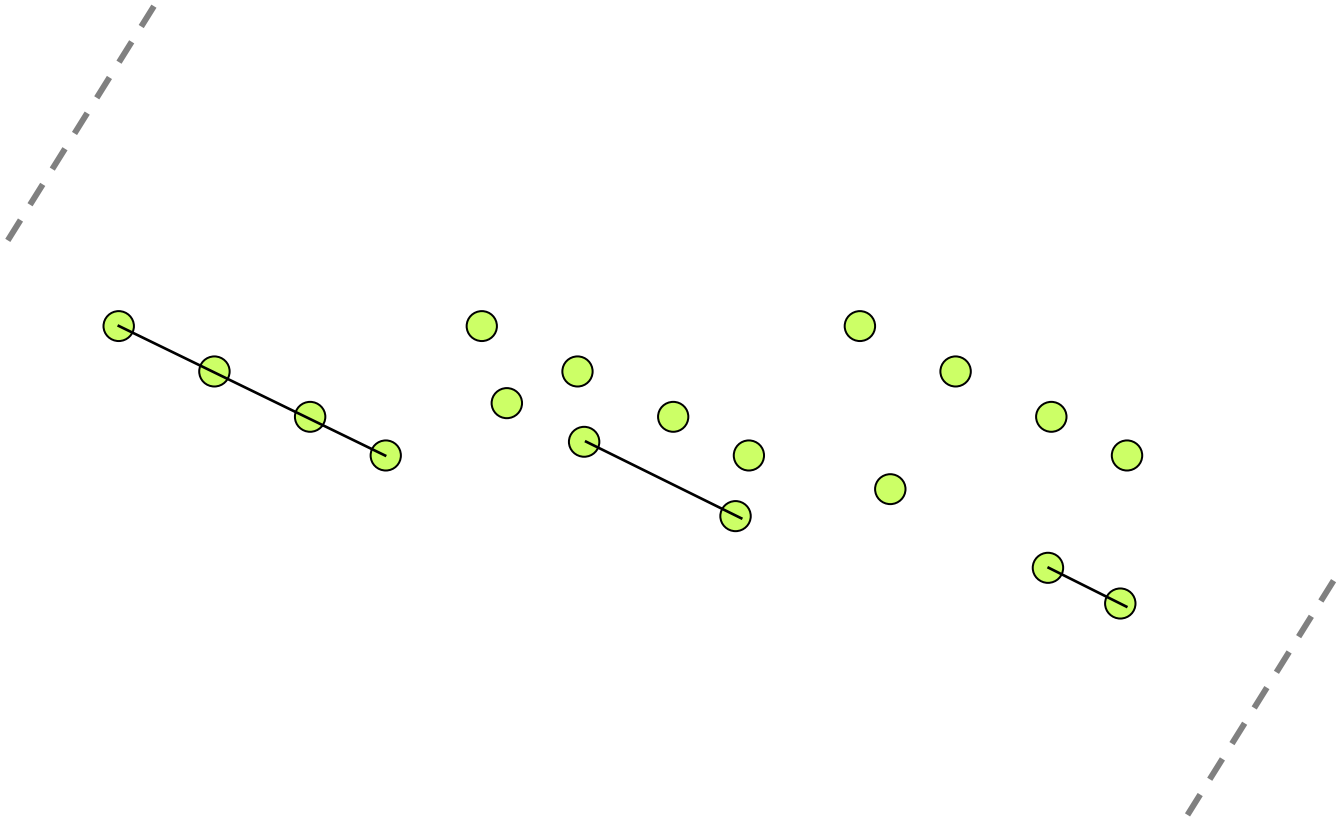
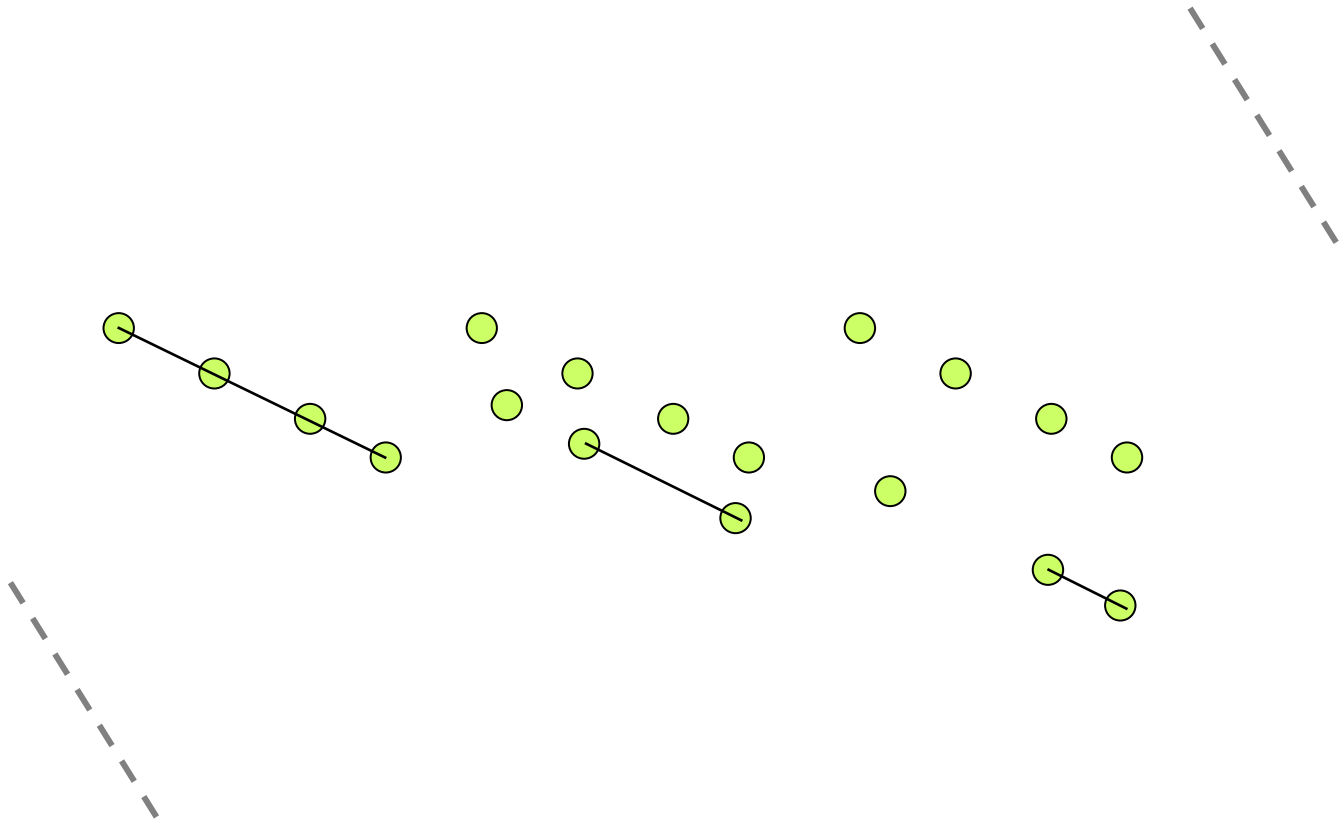


PFGE principle



PFGE principle



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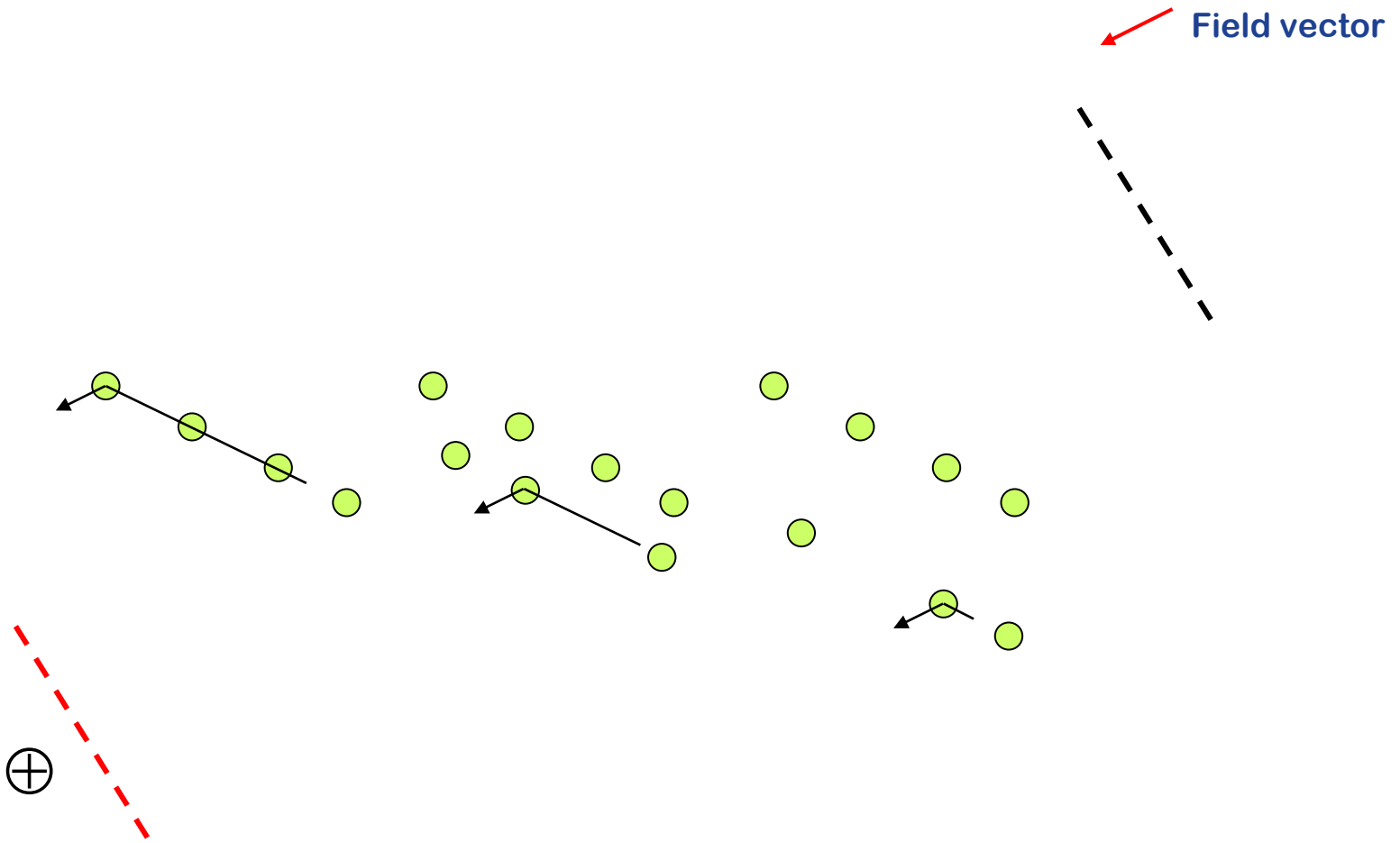

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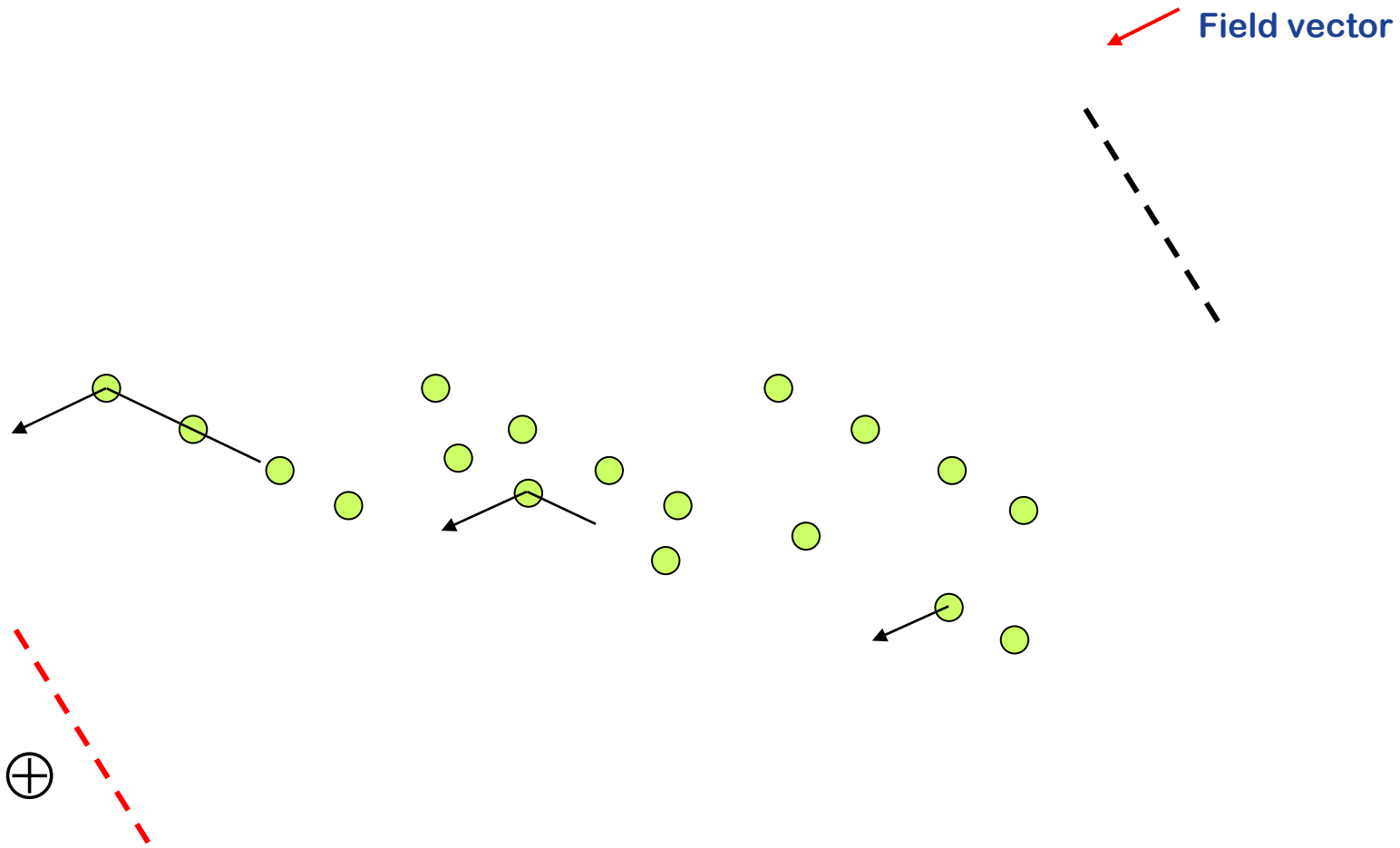

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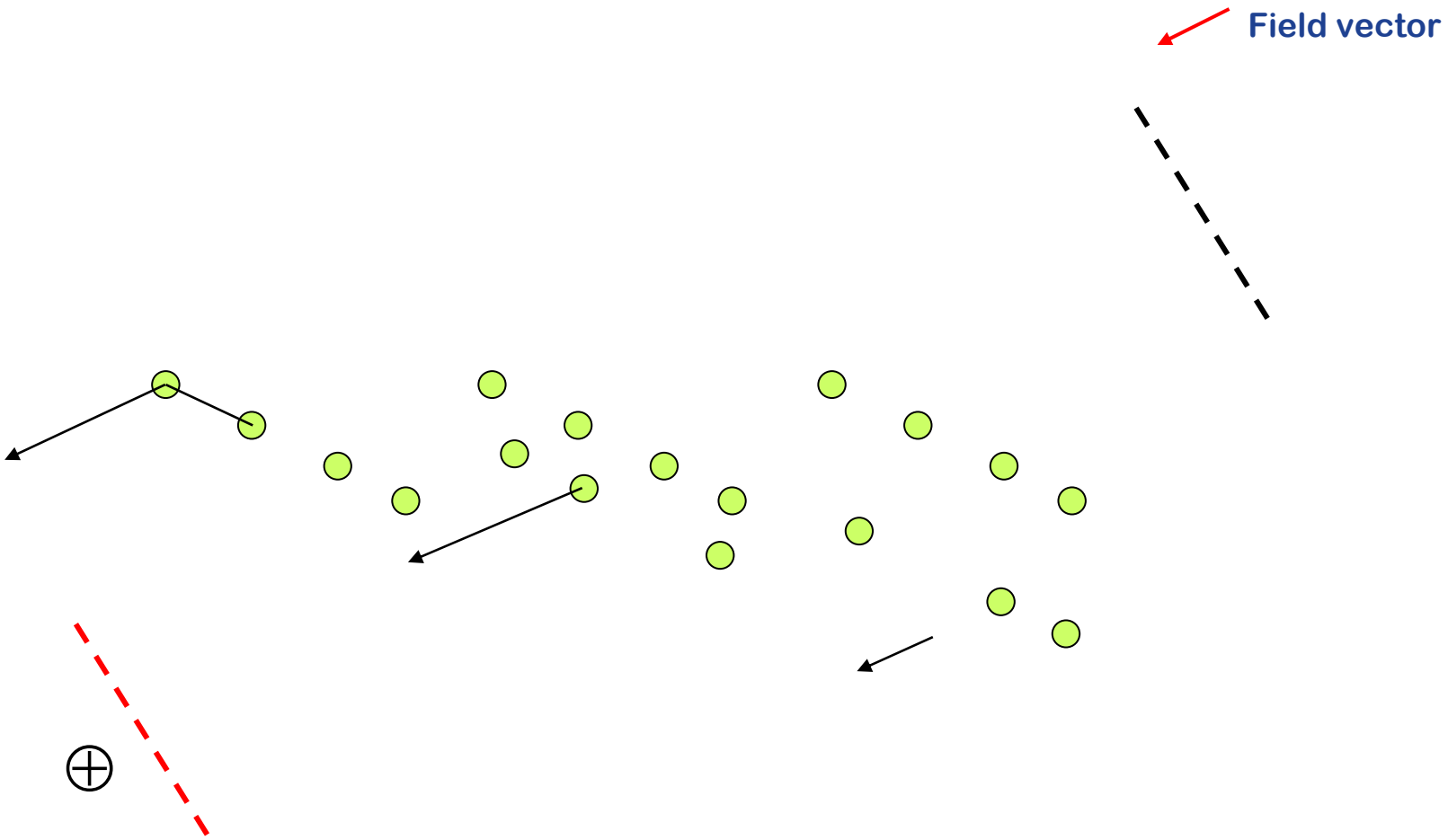

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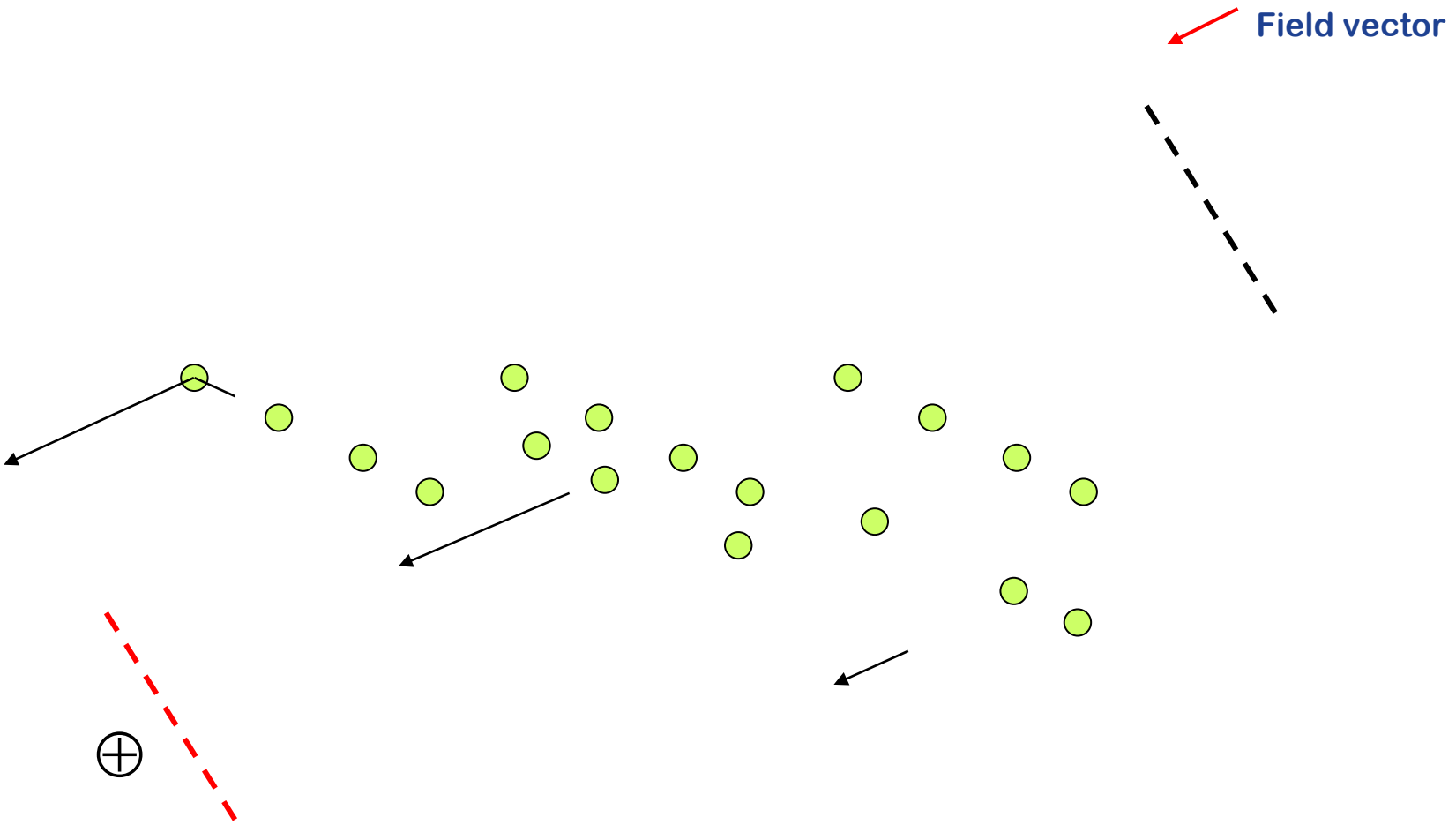

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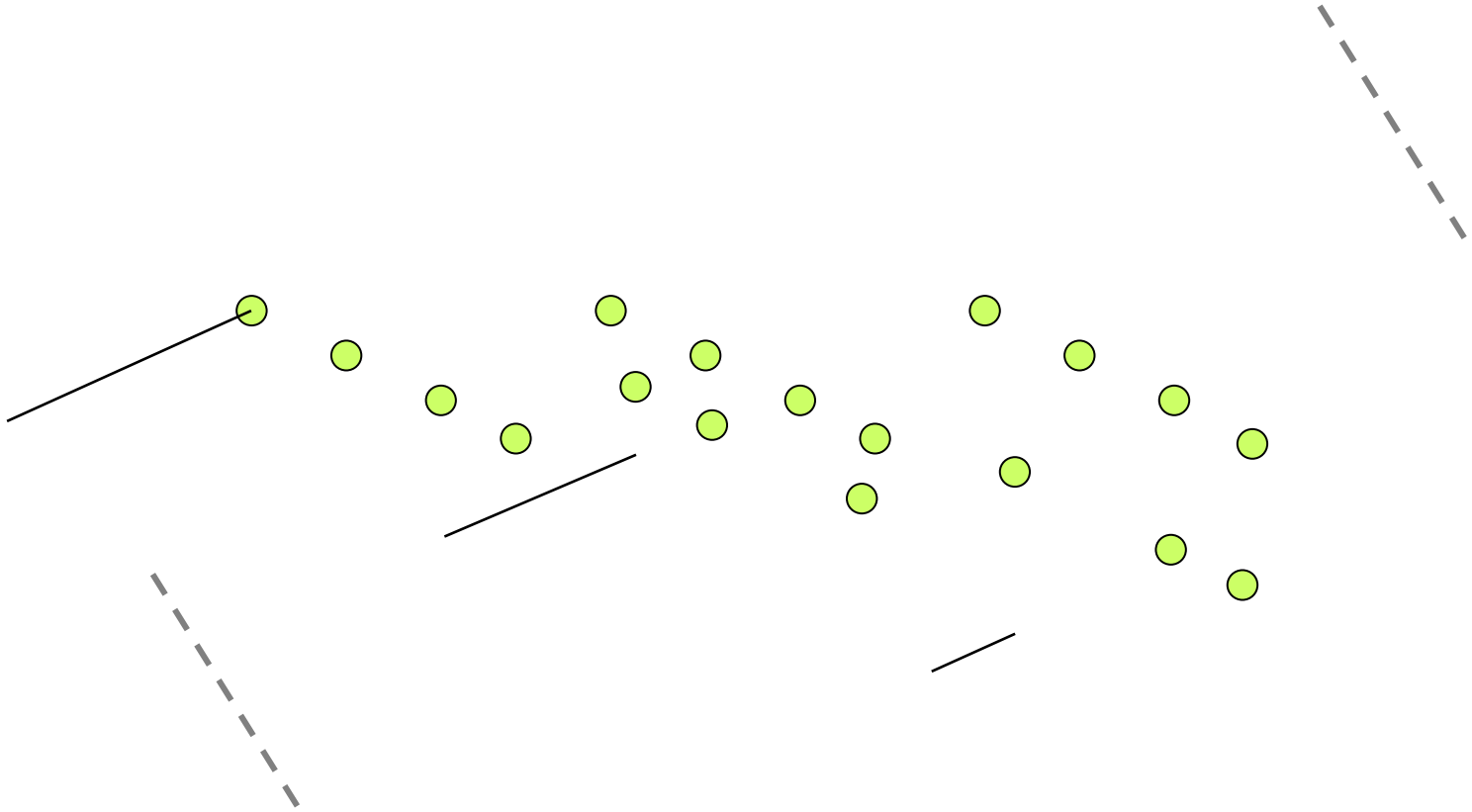
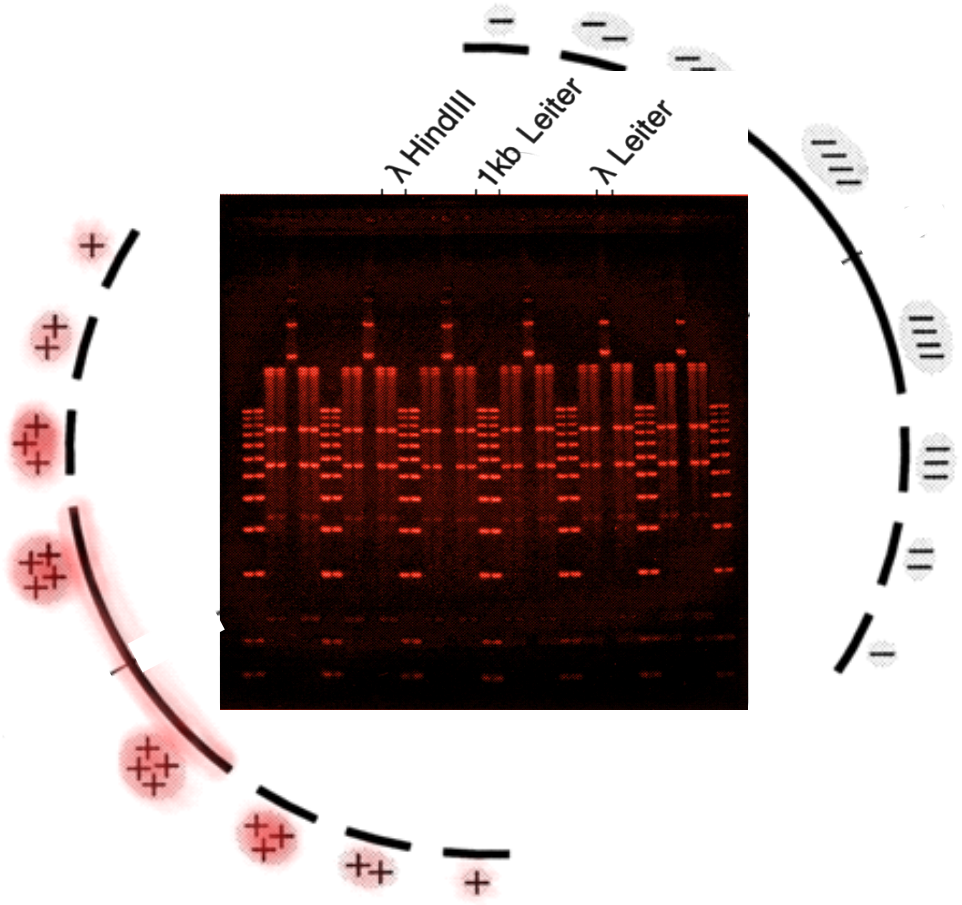


PFGE principle



Important parameters for PFGE

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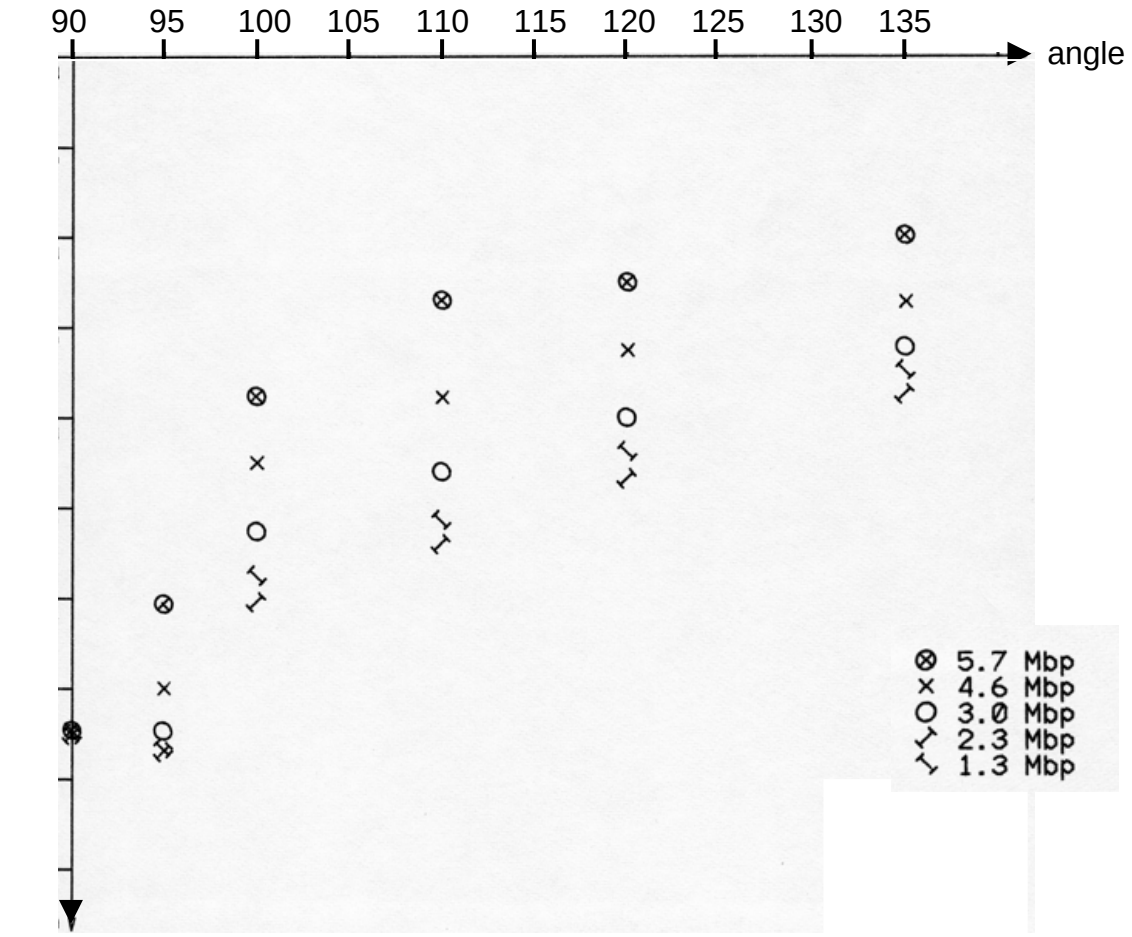
- Voltage (V)
- Field strength (V/cm)
- Pulse length
- Angle / change of angle
- Buffer
- Agarose type
- Agarose concentration (handling limit: 0.5% [w/v])
- Temperature
- Run time

PFGE at different angles



Volz, 1988
Uni Tübingen

Basic guideline for PFGE parameters

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	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)

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- **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)

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- **Application**

  Investigation of genomic libraries (artificial chromosomes like BAC, PAC, YAC) or large plasmids

- **Target groups**

  Diverse research groups, Genome Sequening 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)

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- **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)

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- **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

  – Aptoptosis studies at intermediate formed larger DNA molecules during the phase of the process