



# NEPA21 system

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# Workflow

- NEPA21 system on loan
- Optimization protocols for different cell lines
- Follow up with dose-response curves for best electroporation condition
- Benefits:
  - Low cuvette costs
  - No special buffers needed
- Disadvantage:
  - High volume requirement (100  $\mu$ L)

# Jurkat cells - RT

- Jurkat cells
- 4  $\mu\text{M}$  RNP
- HPRT38285
- 4.8  $\mu\text{M}$  EE
- 72 hrs culturing
- T7EI

**Viability (%)**

|     |     |     |   |     |     |    |
|-----|-----|-----|---|-----|-----|----|
| 300 |     |     |   | 36  |     |    |
| 275 |     | 65  |   | 25  | 6   |    |
| 250 |     |     |   |     | 17  |    |
| 225 |     |     |   |     | 27  |    |
| 200 |     |     |   |     | 36  | 14 |
| 175 |     |     |   |     | 51  | 24 |
| 150 |     |     |   |     |     | 33 |
| 0   | 100 |     |   |     |     |    |
|     | 0   | 0.5 | 1 | 1.5 | 2.5 | 5  |

Length (ms)

**T7EI Total Editing Efficiency (%)**

|     |     |      |   |      |      |      |
|-----|-----|------|---|------|------|------|
| 300 |     |      |   | 47.9 |      |      |
| 275 |     | 49.5 |   | 46.9 | 48.0 |      |
| 250 |     |      |   |      | 44.6 |      |
| 225 |     |      |   |      | 46.4 |      |
| 200 |     |      |   |      | 47.5 | 42.9 |
| 175 |     |      |   |      | 48.3 | 45.5 |
| 150 |     |      |   |      |      | 48.6 |
| 0   | 0.0 |      |   |      |      |      |
|     | 0   | 0.5  | 1 | 1.5  | 2.5  | 5    |

Length (ms)

# HEK293 - BT

- HEK293 cells
- HPRT38285
- 4  $\mu$ M RNP
- 4  $\mu$ M EE
- 42 hrs culturing
- T7EI

**Viability (1-4)**

|             |                    |   |     |     |   |
|-------------|--------------------|---|-----|-----|---|
| Voltage (V) | 275                | 3 | 1   |     |   |
|             | 200                |   |     | 1   |   |
|             | 175                |   | 3   | 1.5 |   |
|             | 150                |   | 3   | 2   |   |
|             | 125                |   | 4   | 3   | 2 |
|             | 110                |   |     | 4   |   |
|             |                    | 1 | 2.5 | 5   | 7 |
|             | <b>Length (ms)</b> |   |     |     |   |

**T7EI Total Editing Efficiency (%)**

|             |                    |       |       |       |       |
|-------------|--------------------|-------|-------|-------|-------|
| Voltage (V) | 275                | 33.87 | 32.98 |       |       |
|             | 200                |       |       | 37.28 |       |
|             | 175                |       | 29.93 | 37.62 |       |
|             | 150                |       | 30.89 | 35.44 |       |
|             | 125                |       | 20.48 | 36.41 | 41.49 |
|             | 110                |       |       | 27.23 |       |
|             |                    | 1     | 2.5   | 5     | 7     |
|             | <b>Length (ms)</b> |       |       |       |       |

# K562 - BT

- K562 cells
- HPRT38285
- 4  $\mu$ M RNP
- 4  $\mu$ M EE
- 72 hrs culturing
- T7EI

Viability (1-4)

|     |   |   |     |   |     |
|-----|---|---|-----|---|-----|
| 300 | 3 | 1 |     |   |     |
| 275 | 2 |   | 1.5 |   |     |
| 250 |   | 2 |     |   |     |
| 225 |   | 3 |     |   |     |
| 200 |   |   |     | 4 |     |
| 175 |   | 4 |     |   | 3   |
| 150 |   |   |     |   | 3.5 |
| 125 |   |   |     |   | 4   |
|     | 1 | 2 | 2.5 | 3 | 5   |

Length (ms)

T7EI Total Editing Efficiency (%)

|     |       |       |       |       |       |
|-----|-------|-------|-------|-------|-------|
| 300 | 63.03 | 64.25 |       |       |       |
| 275 | 62.42 |       | 59.01 |       |       |
| 250 |       | 68.43 |       |       |       |
| 225 |       | 62.89 |       |       |       |
| 200 |       |       |       | 62.64 |       |
| 175 |       | 64.77 |       |       | 61.83 |
| 150 |       |       |       |       | 56.07 |
| 125 |       |       |       |       | 65.80 |
|     | 1     | 2     | 2.5   | 3     | 5     |

Length (ms)

Voltage (V)

Voltage (V)

# HeLa - BT

- HeLa cells
- HPRT38285
- 4  $\mu$ M RNP
- 4  $\mu$ M EE
- 48 hrs culturing
- T7E1

**Viability (1-4)**

|             |     |     |   |     |
|-------------|-----|-----|---|-----|
| Voltage (V) | 150 | 4   | 2 |     |
|             | 125 | 4   | 4 | 2   |
|             | 110 | 4   | 4 |     |
|             |     | 2.5 | 5 | 7.5 |

**Length (ms)**

**T7E1 Total Editing Efficiency (%)**

|             |     |       |       |       |
|-------------|-----|-------|-------|-------|
| Voltage (V) | 150 | 34.92 | 62.40 |       |
|             | 125 | 21.39 | 47.69 | 63.47 |
|             | 110 | 9.42  | 30.28 |       |
|             |     | 2.5   | 5     | 7.5   |

**Length (ms)**

# Hepa1-6 - BT

- Hepa1-6 cells (mouse)
- Rosa26-227
- 4  $\mu$ M RNP
- 4  $\mu$ M EE
- 48 hrs culturing
- T7EI

Viability (1-4)

|             |     |             |     |     |
|-------------|-----|-------------|-----|-----|
| Voltage (V) | 225 | 1           | 1   |     |
|             | 200 | 3           | 1   |     |
|             | 175 | 4           | 4   | 2   |
|             | 150 | 4           | 3.5 |     |
|             | 125 | 4           | 4   |     |
|             |     | 2.5         | 5   | 7.5 |
|             |     | Length (ms) |     |     |

T7E1 Total Editing Efficiency (%)

|             |     |             |       |       |
|-------------|-----|-------------|-------|-------|
| Voltage (V) | 225 | 14.05       | 0.00  |       |
|             | 200 | 23.88       | 15.66 |       |
|             | 175 | 27.07       | 13.96 | 17.48 |
|             | 150 | 28.60       | 27.19 |       |
|             | 125 | 28.30       | 29.29 |       |
|             |     | 2.5         | 5     | 7.5   |
|             |     | Length (ms) |       |       |

# RAW - BT

- RAW cells (mouse)
- Rosa26-227
- 4  $\mu$ M RNP
- 4  $\mu$ M EE
- 48 hrs culturing
- T7EI

**Viability (1-4)**

|     |     |   |     |   |   |     |
|-----|-----|---|-----|---|---|-----|
| 275 | 4   | 4 |     | 1 |   |     |
| 225 |     |   | 3   |   |   |     |
| 200 |     |   |     |   | 2 |     |
| 175 |     | 4 |     | 4 | 3 |     |
| 150 |     |   |     | 3 |   |     |
| 125 |     | 4 |     | 4 |   |     |
|     | 0.5 | 1 | 2.5 | 3 | 5 | 7.5 |

**Length (ms)**

**T7E1 Editing Efficiency (%)**

|     |       |       |       |       |       |     |
|-----|-------|-------|-------|-------|-------|-----|
| 275 | 27.38 | 24.64 |       | 15.78 |       |     |
| 225 |       |       | 24.21 |       |       |     |
| 200 |       |       |       |       | 29.17 |     |
| 175 |       | 28.50 |       | 27.29 | 27.65 |     |
| 150 |       |       |       | 28.25 |       |     |
| 125 |       |       | 14.67 |       | 22.93 |     |
|     | 0.5   | 1     | 2.5   | 3     | 5     | 7.5 |

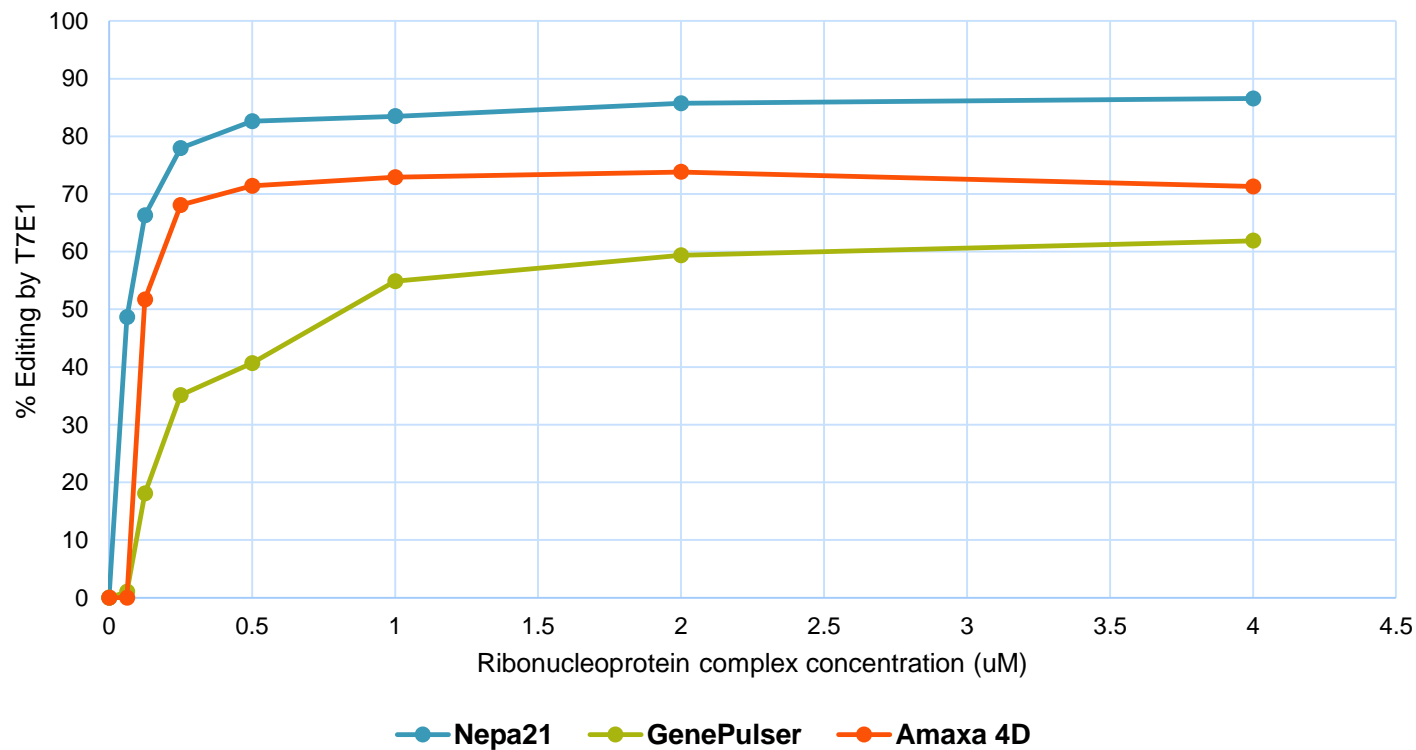
**Length (ms)**



# Final recommendations

- Human cell lines
  - Jurkat : 275 V, 0.5 ms
  - HEK293: 125 V, 5 ms
  - K562: 200 V, 3 ms
  - HeLa: 125 V, 5 ms
- Mouse cell lines
  - Hepa1-6: 125 V, 5 ms
  - RAW: 175 V, 2.5 ms

## Conventional Electroporation Cuvettes Comparing BioRad GenePulser, Amaxa 4D, and Nepa21 systems



- Jurkat cells
- 0-4  $\mu\text{M}$  RNP
- HPRT38087
- 4  $\mu\text{M}$  EE
- 72 hrs culturing
- T7E1



- Jurkat cells
- 0-4  $\mu\text{M}$  RNP
- HPRT38087
- 0 or 4  $\mu\text{M}$  EE
- 72 hrs culturing
- T7EI

### Nepa21 with and without IDT Alt-R Cas9 Electroporation Enhancer

