**Supplementary Information**

**A systematic investigation of key factors of nucleic acid precipitation towards optimized DNA/RNA isolation**

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**Supplementary Figure S1**. Key influence factors and their corresponding conditions of nucleic acid precipitation.

**Supplementary Table S1.** The average recovery rate and its corresponding standard deviation of each type of nucleic acid by adding alcohol and salt independently or both.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Incubation conditions** | **Average recovery** **rate (%)** | **Standard deviation** | **A260/A280** | **A260/A230** |
| **miRNA** | Ethanol + NaAc | 44.92 | 1.56 | 2.00 | 2.08 |
|  | ethanol | 10.64 | 0.95 | 1.98 | 1.68 |
|  | NaAc | 12.35 | 1.24 | 1.93 | 1.79 |
| **Primer** | Ethanol + NaAc | 60.51 | 1.86 | 1.98 | 2.22 |
|  | ethanol | 1.08 | 0.06 | 1.98 | -0.71 |
|  | NaAc | 1.04 | 0.09 | 1.44 | 0.85 |
| **DNA** | Ethanol + NaAc | 19.50 | 0.89 | 1.88 | 2.00 |
|  | ethanol | 3.41 | 0.15 | 1.88 | 2.75 |
|  | NaAc | 1.87 | 0.15 | 1.78 | 1.29 |
| **Plasmid** | Ethanol + NaAc | 73.39 | 3.69 | 1.99 | 2.00 |
|  | ethanol | 0.65 | 0.05 | 1.99 | 0.94 |
|  | NaAc | 2.72 | 0.29 | 1.58 | 1.06 |

**Supplementary Table S2**. The average recovery rate and its corresponding standard deviation and significance level of each type of nucleic acid under each incubation condition\*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of nucleic acid** | **Incubation conditions** | **Average recovery rate** | **Standard deviation** | **A260/A280** | **A260/A230** | **Significance \*\*** |
|  **MiRNA** | -20˚C overnight | 62.52 | 2.78 | 1.98 | 2.12 | a |
| 4˚C overnight | 53.12 | 3.03 | 1.97 | 2.1 | b |
| -20˚C for 2 hrs | 53.5 | 1.42 | 1.97 | 2.1 | b |
| 4˚C for 15 min | 53.55 | 2.83 | 1.98 | 2.1 | b |
| RT for 15 min | 44.84 | 1.54 | 1.98 | 2.2 | c |
| -80˚C for 5 min | 59.62 | 2.54 | 1.98 | 2.2 | ab |
| RT for 0 min | 41.92 | 2.63 | 1.92 | 2.12 | c |
| **Primer** | -20˚C overnight | 69.64 | 3.08 | 1.89 | 2.17 | a |
| 4˚C overnight | 69.88 | 3.51 | 1.89 | 2.04 | a |
| -20˚C for 2 hrs | 43.96 | 2.84 | 1.86 | 2.04 | b |
| 4˚C for 15 min | 60.54 | 2.95 | 1.84 | 2.05 | c |
| RT for 15 min | 62 | 2.01 | 1.83 | 2.05 | c |
| -80˚C for 5 min | 61.9 | 1.87 | 1.88 | 2.04 | c |
| RT for 0 min | 60.51 | 1.86 | 1.86 | 2.05 | c |
| **PCR product** | -20˚C overnight | 34.39 | 1.25 | 1.87 | 2.08 | a |
| 4˚C overnight | 32.75 | 2.96 | 1.87 | 2.2 | a |
| -20˚C for 2 hrs | 26.67 | 3.11 | 1.88 | 2.2 | b |
| 4˚C for 15 min | 24.42 | 0.83 | 1.84 | 2.07 | b |
| RT for 15 min | 23.49 | 1.46 | 1.85 | 2.2 | b |
| -80˚C for 5 min | 15.42 | 0.82 | 1.89 | 21 | c |
| RT for 0 min | 24.17 | 1.96 | 1.85 | 2.2 | b |
| **Plasmid** | -20˚C overnight | 73.79 | 3.08 | 1.9 | 2 | a |
| 4˚C overnight | 62.87 | 3.14 | 1.89 | 2.02 | b |
| -20˚C for 2 hrs | 69 | 2.69 | 1.88 | 2.02 | a |
| 4˚C for 15 min | 66.42 | 2.38 | 1.88 | 2 | ab |
| RT for 15 min | 71.78 | 3.63 | 1.88 | 2.04 | a |
| -80˚C for 5 min | 56.12 | 2.78 | 1.89 | 2.01 | c |
| RT for 0 min | 73.93 | 3.37 | 1.89 | 2.01 | a |

\* The data in this table is the original data representing Figure 2 (the recovery rates of different nucleic acids under different incubation conditions of the paper.

\*\* Letters a, b, c and d represent statistical significance among different incubation conditions of each nucleic acid (*p* < 0.05). The recovery rate with same letter(s) represent there have no significant difference among them. “ab” means that the recovery rate have no significant difference with the recoveries with letter “a”, as well as the recoveries with letter “b”.

**Supplementary Table S3**. The average recovery rate and its corresponding standard deviation and significance level of each type of nucleic acid under each centrifugation force\*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of nucleic acid** | **Centrifugation force** | **Average recovery rate** | **Standard deviation** | **A260/A280** | **A260/A230** | **Significance \*\*** |
| **MiRNA** | 7500 g | 40.31% | 0.01 | 2.00 | 2.12 | a |
| 12000 g | 60.85% | 0.03 | 1.87 | 2.16 | b |
| 18000 g | 60.94% | 0.002 | 1.88 | 2.00 | b |
| 21000 g | 65.88% | 0.05 | 1.87 | 2.05 | c |
| **Primer** | 7500 g | 51.43% | 0.05 | 1.96 | 2.04 | a |
| 12000 g | 60.85% | 0.06 | 1.85 | 2.05 | b |
| 18000 g | 70.91% | 0.03 | 1.84 | 2.05 | c |
| 21000 g | 73.99% | 0.05 | 1.84 | 2.05 | c |
| **PCR product** | 7500 g | 30.94% | 0.04 | 1.88 | 2.04 |  a |
| 12000 g | 34.67% | 0.03 | 1.86 | 2.07 | a |
| 18000 g | 35.29% | 0.03 | 1.86 | 2.05 | a |
| 21000 g | 36.94% | 0.04 | 1.85 | 2.05 | a |
| **Plasmid** | 7500 g | 73.41% | 0.03 | 1.89 | 2.04 | a |
| 12000 g | 75.65% | 0.05 | 1.82 | 2.00 | a |
| 18000 g | 76.04% | 0.03 | 1.80 | 2.00 | a |
| 21000 g | 79.71% | 0.03 | 1.84 | 2.00 | a |

\* The data in this table is the original data representing Figure 3 (the recovery rates of different nucleic acids under different conditions of centrifugation force) of the paper.

\*\* Letters a, b and c represent statistical significance under different centrifugation force of each nucleic acid (*p* < 0.05). The recovery rate with same letter(s) represent there have no significant difference among them.

**Supplementary Table S4**. The average recovery rate and its corresponding standard deviation and significance level of each type of nucleic acid under each centrifugation time \*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of nucleic acid** | **Centrifugation time** | **Average recovery rate** | **Standard deviation** | **A260/A280** | **A260/A230** | **Significance \*\*** |
| **MiRNA** | 10 min | 62.77% | 0.03 | 1.94 | 2.10 | a |
| 20 min | 63.53% | 0.02 | 1.94 | 2.00 | a |
| 30 min | 65.86% | 0.02 | 1.90 | 2.15 | a |
| 1 h | 68.79% | 0.04 | 1.90 | 2.25 | a |
| **Primer** | 10 min | 73.25% | 0.02 | 1.85 | 2.00 | a |
| 20 min | 75.11% | 0.02 | 1.84 | 2.00 | a |
| 30 min | 76.22% | 0.01 | 1.85 | 2.02 | a |
| 1 h | 79.74% | 0.05 | 1.85 | 2.01 | a |
| **PCR product** | 10 min | 36.94% | 0.03 | 1.87 | 2.08 | a |
| 20 min | 37.41% | 0.02 | 1.86 | 2.00 | a |
| 30 min | 42.38% | 0.01 | 1.87 | 2.13 | a |
| 1 h | 44.12% | 0.03 | 1.87 | 2.13 | a |
| **Plasmid** | 10 min | 77.43% | 0.06 | 1.80 | 2.07 | a |
| 20 min | 79.27% | 0.003 | 1.80 | 2.05 | a |
| 30 min | 79.39% | 0.02 | 1.81 | 2.03 | a |
| 1 h | 83.09% | 0.03 | 1.80 | 2.02 | a |

\* The data in this table is the original data representing Figure 4 (the recovery rates of different nucleic acids under different conditions of centrifugation time) of the paper.

\*\* Letter a represent statistical significance under different centrifugation time of each nucleic acid (*p* < 0.05).

**Supplementary Table S5**. The average recovery rate and its corresponding standard deviation and significance level of each type of nucleic acid under each volume ratio of ethanol or isopropanol to nucleic acid solution \*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of nucleic acid** | **volume ratio of ethanol/isopropanol** | **Average recovery rate** | **Standard deviation** | **A260/A280** | **A260/A230** | **Significance \*\*** |
| **MiRNA** | Ethanol 2 times | 67.09% | 0.04 | 1.98 | 2.11 | a |
| Ethanol 3 times | 70.53% | 0.04 | 1.95 | 2.00 | ab |
| Ethanol 4 times | 74.46% | 0.06 | 1.93 | 2.00 | b |
| Isopropanol 0.5 time | 52.45% | 0.04 | 1.93 | 2.05 | a |
| Isopropanol 0.75 time | 61.25% | 0.02 | 1.96 | 2.03 | b |
| Isopropanol 1 time | 60.26% | 0.05 | 1.93 | 2.00 | b |
| **Primer** | Ethanol 2 times | 58.99% | 0.06 | 1.85 | 2.02 | a |
| Ethanol 3 times | 80.27% | 0.07 | 1.85 | 2.13 | b |
| Ethanol 4 times | 85.28% | 0.03 | 1.85 | 2.16 | b |
| Isopropanol 0.5 time | 15.96% | 0.01 | 1.85 | 2.00 | a |
| Isopropanol 0.75 time | 41.83% | 0.03 | 1.85 | 2.07 | b |
| Isopropanol 1 time | 60.50% | 0.05 | 1.85 | 2.09 | c |
| **PCR product** | Ethanol 2 times | 47.04% | 0.05 | 1.87 | 2.13 | a |
| Ethanol 3 times | 42.02% | 0.04 | 1.87 | 2.05 | a |
| Ethanol 4 times | 42.63% | 0.04 | 1.86 | 2.06 | a |
| Isopropanol 0.5 time | 24.28% | 0.02 | 1.86 | 2.17 | a |
| Isopropanol 0.75 time | 44.97% | 0.02 | 1.88 | 2.24 | b |
| Isopropanol 1 time | 39.14% | 0.03 | 1.88 | 2.08 | b |
| **Plasmid** | Ethanol 2 times | 73.09% | 0.04 | 1.80 | 2.05 | a |
| Ethanol 3 times | 78.09% | 0.06 | 1.84 | 2.09 | a |
| Ethanol 4 times | 76.09% | 0.07 | 1.81 | 2.04 | a |
| Isopropanol 0.5 time | 46.97% | 0.06 | 1.83 | 2.00 | a |
| Isopropanol 0.75 time | 67.97% | 0.03 | 1.83 | 2.06 | b |
| Isopropanol 1 time | 76.97% | 0.08 | 1.84 | 2.00 | b |

\* The data in this table is the original data representing Figure 5 (The recovery rates of different nucleic acids under different conditions of volume ratio of ethanol/isopropanol to nucleic acid solution) of the paper.

\*\* Letters a, b and c represent statistical significance under different volume ratio of ethanol/isopropanol (*p* < 0.05). The recovery rate with same letter(s) represent there have no significant difference among them. “ab” means that the recovery rate have no significant difference with the recoveries with letter “a”, as well as the recoveries with letter “b”.

**Supplementary Table S6**. The average recovery rate and its corresponding standard deviation and significance level of each type of nucleic acid under different conditions of cations \*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of nucleic acid** | **volume ratio of ethanol/isopropanol** | **Type of cations** | **Average recovery rate** | **Standard deviation** | **A260/A280** | **A260/A230** | **Significance \*\*** |
| **MiRNA** | Ethanol 4 times | NaAC | 73.88% | 0.03 | 1.95 | 2.04 | ab |
| NaAC+MgCl2 | 66.76% | 0.01 | 1.94 | 2.00 | bc |
| MgCl2 | 80.00% | 0.004 | 1.94 | 2.00 | a |
| NH4AC | 68.01% | 0.04 | 1.95 | 2.01 | bc |
| NH4AC+MgCl2 | 63.73% | 0.02 | 1.94 | 2.01 | c |
| Isopropanol 0.75 time | NaAC | 61.08% | 0.02 | 1.94 | 2.01 | a |
| NaAC+MgCl2 | 42.98% | 0.04 | 1.95 | 2.14 | b |
| MgCl2 | 59.01% | 0.06 | 1.95 | 2.14 | ac |
| NH4AC | 54.22% | 0.01 | 1.85 | 2.19 | c |
| NH4AC+MgCl2 | 46.06% | 0.02 | 1.85 | 2.02 | b |
| **Primer** | Ethanol 4 times | NaAC | 87.94% | 0.06 | 1.86 | 2.01 | a |
| NaAC+MgCl2 | 72.57% | 0.06 | 1.84 | 2.12 | b |
| MgCl2 | 82.94% | 0.08 | 1.84 | 2.10 | a |
| NH4AC | 52.10% | 0.02 | 1.85 | 2.00 | c |
| NH4AC+MgCl2 | 45.55% | 0.03 | 1.87 | 2.03 | c |
| Isopropanol 1 time | NaAC | 67.87% | 0.07 | 1.85 | 2.02 | a |
| NaAC+MgCl2 | 58.43% | 0.03 | 1.84 | 2.06 | b |
| MgCl2 | 69.99% | 0.04 | 1.85 | 2.05 | a |
| NH4AC | 40.13% | 0.04 | 1.85 | 2.06 | c |
| NH4AC+MgCl2 | 50.79% | 0.03 | 1.85 | 2.03 | b |
| **PCR product** | Ethanol 2 times | NaAC | 59.45% | 0.05 | 1.82 | 2.01 | a |
| NaAC+MgCl2 | 56.39% | 0.02 | 1.84 | 2.11 | ab |
| MgCl2 | 62.95% | 0.04 | 1.84 | 2.09 | a |
| NH4AC | 58.07% | 0.05 | 1.84 | 2.10 | a |
| NH4AC+MgCl2 | 50.73% | 0.02 | 1.84 | 2.08 | b |
| Isopropanol 0.75 time | NaAC | 54.98% | 0.02 | 1.80 | 2.05 | a |
| NaAC+MgCl2 | 57.95% | 0.01 | 1.86 | 2.13 | ab |
| MgCl2 | 59.15% | 0.01 | 1.84 | 2.14 | b |
| NH4AC | 51.02% | 0.01 | 1.83 | 2.15 | c |
| NH4AC+MgCl2 | 50.69% | 0.02 | 1.88 | 2.11 | c |
| **Plasmid** | Ethanol 3 times | NaAC | 79.02% | 0.07 | 1.83 | 2.07 | a |
| NaAC+MgCl2 | 68.54% | 0.06 | 1.79 | 2.06 | bc |
| MgCl2 | 72.82% | 0.07 | 1.80 | 2.10 | ab |
| NH4AC | 59.65% | 0.03 | 1.88 | 2.05 | cd |
| NH4AC+MgCl2 | 50.37% | 0.05 | 1.83 | 2.06 | d |
| Isopropanol 1 time | NaAC | 71.90% | 0.01 | 1.82 | 2.10 | a |
| NaAC+MgCl2 | 64.15% | 0.05 | 1.81 | 2.10 | b |
| MgCl2 | 60.33% | 0.05 | 1.80 | 2.20 | b |
| NH4AC | 53.33% | 0.03 | 1.82 | 2.07 | c |
| NH4AC+MgCl2 | 43.91% | 0.03 | 1.82 | 2.07 | d |

\* The data in this table is the original data representing Figure 6 (The recovery rates of different nucleic acids under different conditions of cations) of the paper.

\*\* Letters a, b, c and d represent statistical significance under different cation conditions (*p* < 0.05). The recovery rate with same letter(s) represent there have no significant difference among them. “ab” means that the recovery rate have no significant difference with the recoveries with letter “a”, as well as the recoveries with letter “b”.

**Supplementary Table S7**. The average recovery rate and its corresponding standard deviation of each type of nucleic acid upon addition of tRNA as co-precipitator (carrier).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Type of nucleic acid** | **Volume ratio of ethanol/isopropanol** | **Carrier** | **Average recovery rate** | **Standard deviation** | **A260/A280** | **A260/A230** |
| **MiRNA** | Ethanol 4 times | tRNA | 149.16% | 0.16 | 2.00 | 2.11 |
| Isopropanol 0.75 time | tRNA | 104.98% | 0.11 | 2.00 | 2.09 |
| **Primer** | Ethanol 4 times | tRNA | 129.23% | 0.11 | 1.87 | 2.48 |
| Isopropanol 1 time | tRNA | 91.14% | 0.06 | 1.86 | 2.21 |
| **PCR product** | Ethanol 3 times | tRNA | 93.98% | 0.11 | 1.84 | 2.19 |
| Isopropanol 1 time | tRNA | 90.28% | 0.07 | 1.83 | 2.19 |
| **Plasmid** | Ethanol 2 times | tRNA | 113.69% | 0.04 | 1.84 | 2.22 |
| Isopropanol 0.75 time | tRNA | 80.34% | 0.08 | 1.8 | 2.15 |

**Supplementary Table S8**. The average recovery rate and its corresponding standard deviation and significance level of each type of nucleic acid under different conditions of carriers \*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of nucleic acid** | **volume ratio of ethanol/isopropanol** | **Type of carriers** | **Average recovery rate** | **Standard deviation** | **A260/A280** | **A260/A230** | **Significance \*\*** |
| **MiRNA** | Ethanol 4 times | Without carriers | 77.83% | 0.02 | 2.00 | 2.02 | a |
| Glycogen | 88.76% | 0.03 | 2.00 | 2.02 | b |
| Line polyacrylamide | 85.56% | 0.03 | 1.98 | 2.01 | b |
| Isopropanol 0.75 time | Without carriers | 65.30% | 0.04 | 1.98 | 2.15 | a |
| Glycogen | 70.93% | 0.03 | 1.93 | 2.12 | b |
| Line polyacrylamide | 73.00% | 0.04 | 1.97 | 2.14 | b |
| **Primer** | Ethanol 4 times | Without carriers | 81.28% | 0.04 | 1.88 | 2.05 | a |
| Glycogen | 90.31% | 0.03 | 1.81 | 2.24 | b |
| Line polyacrylamide | 84.18% | 0.03 | 1.90 | 2.24 | a |
| Isopropanol 1 time | Without carriers | 69.06% | 0.03 | 1.87 | 2.05 | a |
| Glycogen | 75.84% | 0.02 | 1.88 | 2.03 | b |
| Line polyacrylamide | 73.27% | 0.03 | 1.93 | 2.05 | ab |
| **PCR product** | Ethanol 3 times | Without carriers | 62.50% | 0.02 | 1.90 | 2.08 | a |
| Glycogen | 71.72% | 0.03 | 1.92 | 2.25 | b |
| Line polyacrylamide | 72.13% | 0.04 | 1.91 | 2.06 | b |
| Isopropanol 1 time | Without carriers | 54.42% | 0.03 | 1.89 | 2.03 | a |
| Glycogen | 65.08% | 0.04 | 1.85 | 2.01 | b |
| Line polyacrylamide | 66.68% | 0.04 | 1.85 | 2.13 | b |
| **Plasmid** | Ethanol 2 times | Without carriers | 81.58% | 0.02 | 1.97 | 2.04 | a |
| Glycogen | 87.67% | 0.01 | 1.94 | 2.09 | b |
| Line polyacrylamide | 93.32% | 0.04 | 1.96 | 2.10 | c |
| Isopropanol 0.75 time | Without carriers | 67.29% | 0.03 | 1.92 | 2.10 | a |
| Glycogen | 75.51% | 0.01 | 1.94 | 2.01 | b |
| Line polyacrylamide | 79.60% | 0.01 | 1.91 | 2.03 | c |

\* The data in this table is the original data representing Figure 7 (The recovery rates of different nucleic acids under different conditions of carriers) of the paper.

\*\* Letters a, b and c represent statistical significance under different conditions of carriers of ethanol/isopropanol (*p* < 0.05). The recovery rate with same letter(s) represent there have no significant difference among them. “ab” means that the recovery rate have no significant difference with the recoveries with letter “a”, as well as the recoveries with letter “b”.

**Supplementary Table S9.** The measuring results of carries in aqueous solutions by NanoDrop 2000

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Working Concentration (μg/mL )** | **Sample Type** | **Nucleic Acid Conc.** | **A260** | **A260/A280** | **A260/A230** |
| **Glycogen** | 50 | DNA | 4.35  | 0.10  | 1.52  | 0.47  |
|  |  | RNA | 2.85  | 0.06  | 1.68  | 0.47  |
|  |  | ssDNA | 2.20  | 0.06  | 1.68  | 0.51  |
| **LPA** | 20 | DNA | 0.60  | 0.01  | 1.05  | 0.47  |
|  |  | RNA | 0.60  | 0.02  | 1.48  | 0.56  |
|  |  | ssDNA | 2.90  | 0.03  | 1.55  | 0.56  |
| **tRNA**  | 20 | DNA | 12.45  | 0.48  | 1.99  | 2.00  |
|  |  | RNA | 21.45  | 0.48  | 2.00  | 1.91  |