

16S Universal Bacterial PCR

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Abstract

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Guidelines

Primers:

926F: AAA CTY AAA KGA ATT GAC GG

1492R: TAC GGY TAC CTT GTT ACG ACT T

OR

GM3: AGA GTT TGA TCM TGG C

These primers are for almost full-length 16s (8F/1492R)

GM4: TAC CTT GTT ACG ACT T

Reaction Mix for 926F/1492R:

| | |
|----------------------|---------|
| Water | 16.25µl |
| 10x LA Buffer | 2.5µl |
| dNTP mix, 2.5mM each | 4.0µl |
| Primer 926F, 10µM | 0.5µl |
| Primer 1492R, 10µM | 0.5µl |
| LA Takara HS 2.5U/µl | 0.25µl |
| DNA | 1.0µl |
| Total | 25µl |

Cycling with 926F/1492R:

95°C 3min

95°C 30sec

55°C 45sec 25 cycles

72°C 90sec

72°C 10min

15°C Hold

Reaction Mix for GM3/GM4:

| | |
|----------------------|----------|
| Water | 17.875µl |
| 10x LA Buffer | 2.5µl |
| dNTP mix, 2.5mM each | 2.0µl |
| Primer GM3, 100µM | 0.25µl |
| Primer GM4, 100µM | 0.25µl |
| LA Takara HS 2.5U/µl | 0.125µl |
| DNA | 2.0µl |
| Total | 25µl |

Cycling with GM3/GM4:

95°C 5min

95°C 1min

48°C 2min 30 cycles

72°C 3min

72°C 10min

15°C Hold

Protocol