

Protocol for amplifying DNA by the PCR from mouse and human libraries

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| DNA Source: | Clones from bacterial cultures |
| | 1. Incyte Genomics mouse GEM1 Library (8,734 clones) |
| | 2. Research Genetics Human UNIGENE Library (31,742 clones) |
| | ** 6ul of bacterial culture used in 100 µl reaction |

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| PCR Kit: | Array Tracker Display Taq™ FL DNA Polymerase (CAT NO: 550-100 or 53-472-UC). Distributed by PGC. | |
| | dNTP Master Mix CAT NO: 62-6113-40 or 61-474-UC. Distributed by PGC. | |
| Mouse Primers | Forward: | 5'-gtg agc gga taa caa ttt cac aca gga aac agc-3' |
| | Reverse: | 5'-ctg caa ggc gat taa gtt ggg taa c-3' |
| Human Primers | Forward: | 5'-gtt ttc cca gtc acg acg ttg-3' |
| | Reverse: | 5'-tga gcg gat aac aat ttc aca cag-3' |

PROTOCOL:

Reaction Setup

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|------------------------|---------|---|
| 10x PCR Buffer | 10.0 µl | MgCl ₂ - 1.5mM (present in 10x PCR Buffer) |
| dNTP Master Mix | 1.6 µl | 0.2mM ea dNTP = total 0.8mM (from PGC) |
| Primer 1 | 2.0 µl | 20pmol ea / 100µl reaction |
| Primer 2 | 2.0 µl | |
| Taq Polymerase | 0.6 µl | 3.0 units / 100µl reaction |
| Add ddH ₂ O | 77.8 µl | |

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|-------------------------------------|--------------------|--|
| Make master mix of the above | 94 μ l | |
| DNA | 6.0 μ l | |
| Total | 100 μ l | |

Cycling Conditions

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| 95°C, 2 min | |
| Cycle 35 times: | 95°C, 30 sec |
| | 52°C, 30 sec |
| | 72°C, 2 min |