CYCV05-CHANGYU Universal T-Vector Colony PCR Identification Kit

Contents	80-100 reactions (CYCV0501)	400-500 reactions (CYCV0502)
2× Taq PCR MasterMix	1 ml	5 ml
Universal primer F	50 μl	250 μl
Universal primer R	50 μl	250 μl
ddH_2O	1 ml	5 ml

Note: Store at -20 °C.

Description

Colony PCR is the most convenient and rapid way to identify positive clones containing an insert after T-vector cloning and ligation. Colony PCR kits based on T3, T7 or SP6 primers can identify positive clones only from a particular T vector, so working with different T vectors requires several kits. This kit uses universal T-vector primers and can identify positive clones from common T vectors, including the pGEM, pUC and pBluescript series.

With T3, T7 or SP6 primers, the primer sites lie very close to the insertion site. When an insert of about 100 bp or smaller is identified, the primer-dimer band of a negative clone and the amplified band of a positive clone are close in size and cannot be distinguished, which can cause false-positive or false-negative results. With the universal T-vector primers in this kit, the primers are at least 150 bp apart without an insert. With the insert length added, the band amplified from the insert can be clearly distinguished from a primer-dimer band. This helps avoid false-positive or false-negative results and substantially improves identification accuracy. The one-tube PCR MasterMix system requires no separate addition of each component: add the single colony to be screened directly, and the result is obtained after about 1 hour of PCR and electrophoresis.

Expected Amplicon Sizes

T vector	Vector source	Primer distance without insert	Expected amplicon (300 bp insert as example)
pGEM-T Easy	pGEM	281 bp	581 bp
pGEMX-T Easy	pGEM	289 bp	589 bp
pMD18-T	pUC18	158 bp	458 bp
pMD19-T	pUC19	158 bp	458 bp
pSURE-T	pUC19	239 bp	539 bp
pUCm-T	pUC19	239 bp	539 bp
pBLUE-T	pBluescript	300 bp	600 bp

Notes

1. Mark the colonies before picking single colonies for identification, so they can be used after identification.

2. Pick a single colony and do not pick too many cells, to avoid affecting the PCR result.

3. Set the extension time according to insert size. For inserts below 1 kb, 30-45 seconds is sufficient; for longer fragments, increase the extension time proportionally.

❖ Procedure (25 μl reaction as example; a 20 μl reaction may also be used)

1. Prepare the 1× Taq MasterMix reaction solution containing primers as follows:

2× Taq PCR MasterMix	12.5 μl
Universal primer F (10 μM)	0.5 μl
Universal primer R (10 μM)	0.5 μl
ddH_2O	11.5 μl

2. Number the colonies on the overnight plate. Using a sterile pipette tip, pick part of a single colony, add it to the reaction solution, and pipette to disperse the colony fully. A negative control tube without colony may be included to help analyze the result.

3. Place the PCR tube in a thermal cycler and run under the following recommended conditions:

94 °C	5 min
94 °C	30 sec
55 °C	30 sec (30-33 cycles of the three steps 94 °C / 55 °C / 72 °C)
72 °C	0.5-1 min
72 °C	5 min

Note: Adjust the extension time according to insert size.

4. After the reaction, load 5-10 μl directly for electrophoresis.

For laboratory use only. For in vitro use only.