D. CLONE-FISH

Here we describe a method using clones for adjusting the hybridization conditions. In brief, clones carrying the target sequence of the new probe are grown with chloramphenicol and IPTG. If a vector with an inducible promoter upstream of the multiple cloning site is chosen then this treatment leads to an in vivo transcription of the cloned 16S rDNA and accumulation of 16S rRNA of the uncultured organism inside the E. coli cell. After standard fixation of these clones they could be used as analogs to cultured organisms for determining the melting point of probes.

please cite for this protocol: *Schramm, A., B. M. Fuchs, J. L. Nielsen, M. Tonolla, and D. A. Stahl. 2002. Fluorescence in situ hybridization of 16S rRNA gene clones (Clone-FISH) for probe validation and screening of clone libraries. Environmental Microbiology 4:713-720.*

Theory

Chloramphenicol:

- blocks protein synthesis at the ribosome;
- Rop -protein is not produced any more => leads to uncontrolled (increased) plasmid copy number (for ColE1 origin plasmids like pMB1, pGEMt, pBR322, pUC, pBLUESCRIPT)
- cell elongation

IPTG:

- induces transcription of T7-polymerase => in turn transcribes the insert from T7 promoter on plasmid
- induces transcription of lacZ gene => insert in opposite direction than T7 transcribed
- T7 has higher efficiency than lacZ

Protocol

1. select appropriate vector-host combination [e.g. pGEM-T in JM109(DE3) (for easy A-T-cloning of PCR products) or pET-23(+) in NovaBlue(DE3) (for blunt- or sticky-end cloning)];
2. grow the clones to OD600 of 0.3-0.4 from 1:1000 dilution of an overnight culture;
3. add IPTG (1 mM) and incubate for 1 h;
4. add chloramphenicol (170 mg l^{-1}) and incubate for 4 h;
5. fix with paraformaldehyde and store in PBS:ethanol (1:1) at -20°C

Clones can be used for FISH for more than one year.

Note: only probes that do not target rRNA of the E. coli host cells are suitable for Clone-FISH.