Bacteria and Yeast Colony PCR

Humberto Pereira[a], Paulo César Silva[a], Björn Johansson[a,*]

[a]*CBMA - Center of Molecular and Environmental Biology Engineering*

* Corresponding author.

E-mail address: bjorn_johansson@bio.uminho.pt (B. Johansson).

Abstract

The bacteria *Escherichia coli* and the yeast *Saccharomyces cerevisiae* are currently the two most important organisms in synthetic biology. *E. coli* is almost always used for fundamental DNA manipulation while yeast is the simplest host system for studying eukaryotic gene expression and performing large-scale DNA assembly. Yeast expression studies may also require altering the chromosomal DNA by homologous recombination. All these studies require the verification of the expected DNA sequence and the fastest method of screening is colony PCR, which is direct PCR of DNA in cells without prior DNA purification. Colony PCR is hampered by the difficulty of releasing DNA into the PCR mix and by the presence of PCR inhibitors. We hereby present one protocol for *E. coli* and two protocols for *S. cerevisiae* differing in efficiency and complexity as well as an overview of past and possible future developments of efficient *S. cerevisiae* colony PCR protocols.

Keywords: PCR, Colony, Yeast, *Saccharomyces cerevisiae*, *Escherichia coli*, Direct lysis

1 Introduction

Colony or whole-cell PCR is the direct PCR amplification of target sequences inside cells without prior isolation or purification of DNA. Colony PCR is possible if enough cells lyse as a consequence of the high temperature in the initial template denaturation step alone or in combination with extra procedures to make DNA more accessible. The material containing the cells or the cells themselves must also not present PCR inhibition to an extent that prevents PCR amplification. The advantage of colony PCR over using purified DNA is savings in time and cost, as the time-consuming DNA extraction step is omitted. Minimizing sample handling by omitting DNA purification can also increase sensitivity if the starting material is limiting as it might be in, for example, forensic applications. Very low amounts of starting material may prohibit DNA purification as all purification procedures are associated with a loss. Less sample handling also lowers the risk of cross-contamination of samples, an important consideration since PCR is a sensitive technique prone to false positive results.

The need for detecting the presence or absence of specific sequences within cells is routinely needed in a wide range of disciplines, such as clinical microbiology, genetic engineering, and forensic sciences. The most common application of colony PCR in genetic engineering is probably the amplification of ligation product sequences within *Escherichia coli* transformants after cut-and-paste cloning. This procedure is straightforward with few associated problems. The first report on *E. coli* colony PCR describes the resuspension of one colony in half a mL of water and subsequent boiling for 5 min [1]. After centrifugation for 2 min at maximum speed in a microcentrifuge (15 - 20 000 g), 5 µL of the supernatant was used as the template for PCR. The authors later succeeded in using *E. coli* directly without prior dilution or boiling. Few variations of this simple protocol have been published, indicating that it is generally applicable with a reasonable rate of success. The current iteration of the protocol simply involves adding a small amount of an *E. coli* colony to a PCR reaction which is thereafter handled as if an amplification from pure DNA.

Another common application of colony PCR is the analysis of transformants of the yeast *Saccharomyces cerevisiae* after genetic engineering or DNA assembly experiments. *S. cerevisiae* can assemble large and complex constructs through homologous recombination in one step [2]. This technique has found many applications in the field of synthetic biology [3–7].

Colony PCR from *S. cerevisiae* is unfortunately non-trivial, which is evident from the myriad of available protocols, both published under peer review and available online (see www.bit.ly/pcr_prot for a compilation). This indicates that there may not be one protocol that is optimal for all use cases. False-negative results are a general problem affecting yeast colony PCR. Factors that seem to affect yeast colony PCR efficiency are the chronological age of the culture, growth phase, growth rate, size of the desired PCR product, the copy number of the target sequence, and media components [8].

Fresh cultures of rapidly growing yeast, where the target amplicon is short and present in multiple copies, seem to present the least problems. Early published yeast colony PCR protocols were essentially *E. coli* protocols adapted for yeast, where yeast cells are simply added to the PCR mixture and the cells are presumably lysed in the initial denaturation step [9].

We have adapted one protocol for *E. coli* colony PCR (Subheading 3.1) and two different protocols for *S. cerevisiae* (Subheadings 3.2 and 3.3) that are routinely used in our laboratory. The protocol in Subheading 3.2 is very simple and rapid, involving only a short preincubation step in a microwave oven, while the protocol in Subheading 3.3 is a version of the LiAc SDS protocol [10], which is more sensitive and robust in our hands, but also more laborious.

The LiAc SDS protocol [10] stands out in another respect. Most publications describing yeast colony PCR protocols have a relatively low number of citations, often in publications describing different yeast colony PCR protocols or publications from the laboratory where the protocol originated. The LiAc SDS has 162 PubMed citations as of January 2023 from a wide range of laboratories, indicating that it is generally applicable.

Future developments of yeast colony PCR protocols should separate the effects of DNA release and PCR inhibition, and how these effects vary with variables such as culture medium, age, and growth phase of the cultures, and then systematically apply the relevant conditions based on the results for other direct PCR protocols.

1.1 DNA release

The addition of preincubation with a yeast lytic enzyme such as zymolyase or lyticase can improve efficiency [11,12]. Lyticase usually refers to pure β-1,3-glucanase while Zymolyase is a mixture of lytic enzymes. The factor targeted by this enzyme is the strong yeast cell wall which is weakened or removed. The downside is the enzyme cost and potentially the addition of phosphate in the incubation buffer, which may lead to PCR inhibition by interaction with the magnesium ions in the PCR buffer. Recombinant lyticase from the bacteria *Oerskovia xanthineolytica* is easily produced by the cultivation of cells harbouring a plasmid carrying the glucanase structural gene [13]. The resulting lyticase is cost-effective, but PCR strategies should be designed with care since the resulting enzyme is often contaminated with the expression plasmid and *E. coli* chromosomal DNA. We previously used a protocol based on homemade recombinant lyticase, but while effective, not ultimately considered worth the extra work unless lyticase has some other use in the laboratory.

A brief treatment of cells with sodium hydroxide [14] is a method that has several potential targets. The authors suggest that the modes of action could be increased cell wall permeability, dissociation of DNA from bound proteins, or degradation of RNA. Additionally, sodium hydroxide might neutralize

intercalated PCR inhibitors by denaturing DNA [15]. The addition of the strong anionic detergent sodium dodecyl sulfate (SDS) alone [16] or in combination with ethanol [17] or lithium acetate (LiAc) [10] has also been described as a method for achieving PCR amplification from whole yeast cells. SDS efficiently dissolves membrane lipids but is also a potent PCR inhibitor [18]. The presence of SDS also potentially eliminates DNA-protein interactions as SDS is used to prevent gel shifts in the electrophoresis of DNA. Ethanol would precipitate DNA as soon as it is liberated from the cells and may be a way to selectively wash away inhibitors and concentrate DNA [17]. LiAc is commonly used in yeast transformation [19], where the mode of action may be to turn the cell wall more porous [20], which probably improves cell lysis.

Physical methods such as heating, boiling, grinding with glass beads, or rapid freeze-thaw cycles [21] have also been employed but may be more laborious if the number of samples is large. Glass beads in combination with the metal chelating resin Chelex 100 has been reported to permit PCR from whole yeast cells [22]. The role of the chelator is to remove metal ions necessary for nucleases, thereby protecting DNA. The use of chelating resins has also been reported to allow PCR amplification from forensic samples [23]. Sonication has proved beneficial for colony PCR of Gram-positive bacteria [24] and protein extraction in *S. cerevisiae* [25]. However, its effectiveness for yeast colony PCR is yet to be established.

1.2 Future developments

There is substantial development of techniques for direct amplification of DNA in complex matrixes with as few or no manipulation steps involved. Rapid genetic typing of human blood or tissue and detection of human pathogens, as well as forensic science, are likely the strongest motivation for this development. It is possible that at least some of these new procedures could benefit new methods for direct colony PCR from difficult sources such as *S. cerevisiae*.

One of the most attractive recent developments is thermostable DNA polymerases engineered for higher PCR inhibitor tolerance [26]. Examples of these are the addition of DNA binding domains [27] and polymerases developed through gene shuffling or compartmentalized self-replication. The last approach has yielded DNA polymerases resistant to the potent PCR inhibitor heparin [28] and a broad range of environmentally derived inhibitors [29].

PCR enhancers are another area of development that could potentially aid colony PCR protocols. Common PCR enhancers include N,N,N-trimethylglycine (betaine), bovine serum albumin (BSA), dithiothreitol (DTT), glycerol, and dimethyl sulfoxide (DMSO). DMSO was first reported as improving Sanger DNA sequencing quality [30] of PCR products, possibly by preventing reannealing of the strands. Formamide, glycerol, DMSO, Tween-20, and NP-40 are suggested as remedies for difficulties in the amplification of GC-rich templates [31] as well as betaine at 1 M [32], 1.3 M [33], 2

M [34] or at 1 M in combination with DMSO [35,36]. DMSO disrupts DNA base pairing without affecting fidelity [37], while betaine has been reported to affect the base pair composition dependence of DNA strand composition [38].

Trehalose [39], protein BSA, and gelatin stabilize the DNA polymerase during thermal cycling. Nonionic detergents Tween 20 and NP-40 might have a beneficial effect in this respect as they are added to Taq DNA polymerase purification protocols for this reason [40]. Triton X-100 is thought to have the same effect [41,42]. Tween 20 and NP-40 alone or in combination with DMSO also have been reported to improve specificity and raise the yield of PCR in general [43] and also neutralize the negative effects of sodium dodecyl sulfate (SDS) [44]. Several mono- and disaccharides were recently reported to be effective PCR enhancers, with sucrose surpassing trehalose and DMSO for the conditions tested [45].

Relatively new PCR enhancers are nanoparticles from gold (AuNPs) [46,47], titanium dioxide (TiO2) [48], and graphene oxide (GO) or reduced graphene oxide (rGO) [49]. The mode of action of nanoparticles has not been elucidated in detail. Finally, attempts have been made to combine several enhancers in an attempt to find synergistic positive effects [50–52].

The PCR reagents can be altered in order to enhance PCR specificity. This was observed when locked nucleic acid (LNA)- modified primers were used instead of unmodified oligonucleotides [53]. Replacing the canonical dNTPs with 2'-Deoxynucleoside 5'-(alpha-P-seleno)-triphosphates (dNTPαSe) [54] was capable of increasing PCR specificity by over 240-fold [55].

2 Materials

2.1 *E. coli* colony PCR

1. Water. PCR components and other solutions should be prepared using the best available water. We routinely use double-deionized water with a specific conductance of 18.2 MΩ/cm at 25 °C.
2. 2x PCR master mix with DMSO (Table 1). We have found it practical to prepare a two times concentrated PCR master mix containing all components except PCR primers and template DNA, as this minimizes pipetting errors and improves consistency across PCR experiments. The PCR master mix can be stored at -20 °C without a noticeable loss of efficiency. We routinely include 1% DMSO in the final PCR mixture.
3. 5x PCR-compatible loading buffer (Table 2). A PCR-compatible loading buffer can be added directly into the PCR mix, saving post-PCR pipetting steps that might potentially contaminate

the laboratory. We have adopted such a loading buffer made in-house to lower PCR costs. Tartrazine food coloring is a commercial food coloring sold in grocery stores.

4. Thermocycler.
5. Electrophoresis running buffer. We use 1X Tris-acetate-EDTA (TAE) made from a 50X TAE (2 M Tris-base, 5.71% (v/v) glacial acetic acid, 50 mM EDTA) stock solution. This 50X TAE is prepared by dissolving 242g Tris, 18.61g EDTA and 57.1 mL glacial acetic acid in 500 mL of water and adding water up to 1 L.
6. Agarose gel 1%. Add 100 mL of the chosen running buffer for each gram of agarose and heat it until the agarose melts completely. After adding a pre-staining DNA dye, pour the gel into an appropriate mold and let it solidify.
7. Electrophoresis equipment, including power supply and electrophoresis tank.

2.2 *S. cerevisiae* colony PCR using a microwave oven.

All the items listed on section 2.1 plus:

8. Microwave oven.

2.3 PCR using *S. cerevisiae* LiAc permeabilized cells

All the items listed on section 2.1 plus:

8. 1 M Lithium Acetate Stock Solution. The lithium acetate solution is prepared as a 1 M stock in water. Add 10.2 g lithium acetate dihydrate ($LiOAc*2H_2O$, Mw 102.02 g/mol) in 80 mL water and dissolve. Add water to 100 mL and autoclave.
9. SDS Stock Solution 20% (w/v). Add 10 g SDS to 40 mL H_2O. Heat to 60 °C to dissolve the SDS. Adjust pH to 7-8 using sodium hydroxide. Adjust volume to 50 mL with water. Do not autoclave as SDS will precipitate.
10. LiOAc-SDS Solution. Mix 75 µL water, 20 µL 1 M LiOAc, and 5 µL 20% (w/v) SDS for each DNA extraction. Aliquot 100 µL in 1.5 mL microcentrifuge tubes.
11. TE buffer. Add 10 mL 1 M Tris-HCl pH 8.0 and 2 mL 0.5 M EDTA pH 8.0 to 988 mL water. The resulting solution will be 10 mM Tris-HCl and 1 mM EDTA. Autoclave to sterilize. This buffer is used to resuspend DNA in the last step. Other recipes of TE buffer can probably also be used.

3 Methods

Carry out all procedures at room temperature unless otherwise specified. PCR master mixes should be kept on ice at all times, but PCR tubes can be handled at room temperature during the preparation of the mix.

3.1 *E. coli* colony PCR

This protocol can be used to amplify new constructs in *E. coli* transformants. We have found it efficient to use a three-primer strategy using two vector-specific primers flanking the insertion location of the insert and one gene-specific primer, usually one of the primers used to amplify the insert (Fig. 1). The two vector-specific primers should differ in the distance to the insertion site by 200-400 bp. Using this strategy, an empty clone will produce a short PCR product corresponding to the distance between the vector-specific primers while one of two longer bands will arise from a successful clone, depending on the orientation of a cloned insert (*see* **Note 1**).

1. Prepare a 1x PCR master mix containing all PCR components except template DNA. We use a homemade 2x PCR master mix containing DNA polymerase, buffer, Mg^{2+}, dNTPs, and DMSO to which PCR primers are added to a final concentration of 1µM and water. We prepare 110% of the theoretical required volume, which is calculated as the total volume of each PCR reaction times the number of clones including two negative controls (no cells and cells with the empty vector, i.e., vector without the insert) and a positive control if available. The cells are assumed to take up no volume in the calculation.

2. Prepare the appropriate number of tubes containing 1x PCR master mix. We keep the tubes open before adding the *E. coli* cells as we have found that the proximity to a Bunsen burner provides a sufficiently clean environment to avoid contamination.
3. Add a part of the *E. coli* colony to the inside of the tube, by swirling the toothpick against the wall of the tube (*see* **Notes 3-5**).
4. Transfer the remaining cells on the toothpick to fresh liquid or solid medium for preserving the clone and possibly preparing plasmid DNA.
5. Vortex and place the PCR tubes in a preheated thermal cycler as soon as possible.
6. Run the PCR program; time periods and temperatures depend on the polymerase used, size of the expected PCR product, and the melting temperature of the primers. We have found that 5 min initial denaturation (94-98 °C), 35 cycles of the main program, and 5 min of post-extension at 72 °C is sufficient.
7. Analyze 5-10 µL of the PCR amplification by gel electrophoresis. We add dyes to the loading buffer, in which case we can omit the addition of a loading buffer to the PCR products.

3.2 *S. cerevisiae* colony PCR using a microwave oven.

This protocol usually represents the best compromise between cost, work, and success rate and should probably be the first protocol tested for a laboratory wishing to implement *S. cerevisiae* colony PCR. We have found it to be efficient for PCR products up to 2 kb, with occasional success for products up to 3 kb in size.

1. Prepare 1x PCR master mix according to the same principles as for the *E. coli* protocol (Subheading 3.1).
2. Pick a small, well-isolated colony with a sterile toothpick or a sterile 200 µL pipette tip (*see* **Notes 4 and 6**).
3. Transfer part of the colony to the side of a PCR tube. The most common mistake is to transfer too much cell material to the tube. We usually swirl the toothpick on the inside of the tube.
4. Transfer the remaining cells on the toothpick to fresh solid or liquid medium.
5. Incubate the tubes for 1-2 min at full power (800-1000W) using a stock domestic microwave oven.
6. Cool the tubes by placing them on ice or by a 3-5 min incubation at -20 °C in a freezer.
7. Add the PCR master mix, we use a total PCR volume of 20 µL to save on reagents. A larger scale such as 50 µL will be less sensitive to excess biomass in the PCR reaction which might be useful for optimization.
8. Run the PCR program (*see* **Note 7**) and analyze 5-10 µL of the PCR product by gel electrophoresis.

3.3 PCR using *S. cerevisiae* LiAc permeabilized cells

This protocol may not qualify as colony PCR, as DNA is effectively purified from the cells. However, this protocol is considerably less laborious than methods relying on any combination of glass beads, phenol, and chloroform. In our hands, this protocol has succeeded where the microwave oven protocol (Subheading 3.2) failed. This protocol has given more stable results, especially in the hands of less experienced workers. This protocol was first described by Lõoke et al. [10].

1. Prepare one tube of 100 µL LiOAc-SDS mix for each colony.
2. Transfer a small colony from a plate using a sterile toothpick (*see* **Note 6**). The toothpick can also be used to inoculate liquid or solid medium to preserve the clone.
3. Vortex the tubes briefly and incubate at >70 °C for 10 min (*see* **Note 8**).
4. Add 300 µL of 96% ethanol and vortex briefly to precipitate DNA.
5. Spin tubes at least 15 000 g in a microcentrifuge for 3-5 min to precipitate DNA. The cells and cell debris will co-precipitate with the DNA at this point.

6. Remove liquid by inverting the tubes.
7. Add 500 µL 70% ethanol to each tube.
8. Spin tubes like in step 5.
9. Remove liquid by inverting the tubes. Try to remove as much of the liquid as possible in this step (*see* **Note 8**).
10. Resuspend the DNA in 100 µL TE buffer (*see* **Note 10**).
11. Spin down the cell debris for 1 min at top speed in a microcentrifuge.
12. Use 1 µL of the supernatant for 20 µL of total PCR volume.
13. Transfer about half of the supernatant to a fresh tube and store the DNA at -20 °C.
14. Run the PCR program and analyze 5-10 µL of the PCR product by gel electrophoresis.

4 Notes

1. The choice of PCR primers can be important. PCR primers should be specific for both vector and insert, as false positive detection may arise by using PCR primers that are specific only for the insert or vector. The explanation for this surprising phenomenon is that DNA from the ligation mixture may adsorb onto the surface of the cells and serve as a PCR template masking the absence of the correct DNA construct inside the cells [56].
2. We provide a web service (http://pydna.pythonanywhere.com) where PCR can be simulated prior to PCR to ensure that PCR primers bind to the template DNA.
3. Each colony must be transferred to both culture medium and PCR reaction. Since many clones are usually screened, keeping track of PCR tubes and clones may be a logistical issue. We have found that stabbing a gridded LB plate with the tip and leaving it there is a good way of keeping track of the picked clones.
4. Many published protocols rely on the use of sterile toothpicks for transferring clones to the PCR tubes. It should be noted that toothpicks have been associated with PCR inhibitors [57]. If this is a concern, sterile pipet tips can be used instead.
5. We have found that toothpicks may absorb some of the PCR mix if cells are added into the master mix. Pipette tips used in the same way may also remove some PCR mix by capillary action. We deposit the cells above the surface of the PCR mix in the tube and vortex the tubes prior to PCR. This has the added benefit of not allowing interaction between PCR mix and template prior to PCR.
6. We usually keep an open petri dish with a suitable solid selective yeast medium nearby to preserve the clones. The petri dish is gridded with 8x8 to 10x10 squares using a marker pen or by placing it on a printable petri dish grid [58]. The toothpicks can be left standing in the agar as a help to keep track of processed clones.
7. This protocol is sensitive to the amount of yeast cells in the PCR tube. During the set-up of this protocol, it is useful to use a PCR test case. We use primers 19_D-DFR1 (5’ GAC TCA GAC AGG TTG AAA AGA AGA C 3’) and 18_A-DFR1 (5’ CAA AGG TTT GGT TTT CAG TTA AGA A 3’) to amplify a 1288 bp PCR product from the DFR1 locus in *S. cerevisiae* using a program consisting of initial denaturation for 4 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 5 min. This PCR reaction is very robust and any yeast colony PCR protocol should do it with success.
8. The original protocol also states that a 10 min incubation at room temperature can be performed instead of the incubation at 70 °C, but the high temperature should inactivate nucleases that can potentially degrade DNA. This might be an issue since DNA and cell debris are present together until the last step.

9. It is important to remove as much as possible of the ethanol in step 9 of Subheading 3.2, as ethanol could be a PCR inhibitor. The tubes can be incubated in a 37 ºC heat block for 3-5 min in order to evaporate traces of ethanol.
10. Unlysed cells and cell debris will also be resuspended in this step.

Acknowledgement

This work was supported by the Fundação para a Ciência e Tecnologia Portugal (FCT) through Project FatVal PTDC/EAM-AMB/032506/2017 funded by national funds through the FCT I.P. and by the ERDF through the COMPETE2020 – Programa Operacional Competitividade e Internacionalizacão (POCI). CBMA was supported by the strategic program UIDB/04050/2020 funded by national funds through the FCT I.P. Humberto Pereira acknowledges FCT for the Ph.D. scholarship, SFRH/BD/148722/2019.

References

[1] D. Güssow, T. Clackson, Direct clone characterization from plaques and colonies by the polymerase chain reaction, Nucleic Acids Res. 17 (1989) 4000.

[2] D.G. Gibson, G.A. Benders, K.C. Axelrod, J. Zaveri, M.A. Algire, M. Moodie, M.G. Montague, J.C. Venter, H.O. Smith, C.A. Hutchison, One-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic Mycoplasma genitalium genome, Proceedings of the National Academy of Sciences. 105 (2008) 20404–20409.

[3] F. Pereira, F. Azevedo, N.S. Parachin, B. Hahn-Hägerdal, M.F. Gorwa-Grauslund, B. Johansson, Yeast Pathway Kit: A Method for Metabolic Pathway Assembly with Automatically Simulated Executable Documentation, ACS Synth. Biol. (2016) 386-394.

[4] V.F. Cataldo, V. Salgado, P.A. Saa, E. Agosin, Genomic integration of unclonable gene expression cassettes in *Saccharomyces cerevisiae* using rapid cloning-free workflows, Microbiologyopen. 9 (2020) e978.

[5] Y. Shi, D. Wang, R. Li, L. Huang, Z. Dai, X. Zhang, Engineering yeast subcellular compartments for increased production of the lipophilic natural products ginsenosides, Metab. Eng. 67 (2021) 104–111.

[6] S. Wang, X. Xu, X. Lv, Y. Liu, J. Li, G. Du, L. Liu, Construction and Optimization of the de novo Biosynthesis Pathway of Mogrol in *Saccharomyces Cerevisiae*, Front Bioeng Biotechnol.

10 (2022) 919526.

[7] H. Pereira, F. Azevedo, L. Domingues, B. Johansson, Expression of *Yarrowia lipolytica* acetyl-CoA carboxylase in *Saccharomyces cerevisiae* and its effect on *in-vivo* accumulation of Malonyl-CoA, Comput. Struct. Biotechnol. J. 20 (2022) 779–787.

[8] C. Bonnet, C. Rigaud, E. Chanteclaire, C. Blandais, E. Tassy-Freches, C. Arico, C. Javaud, PCR on yeast colonies: an improved method for glyco-engineered *Saccharomyces cerevisiae*, BMC Res. Notes. 6 (2013) 201.

[9] G.M. Sathe, S. O'Brien, M.M. McLaughlin, F. Watson, G.P. Livi, Use of polymerase chain reaction for rapid detection of gene insertions in whole yeast cells, Nucleic Acids Res. 19 (1991) 4775.

[10] M. Lõoke, K. Kristjuhan, A. Kristjuhan, Extraction of genomic DNA from yeasts for PCR-based applications, Biotechniques. 50 (2011) 325–328.

[11] M. Ling, F. Merante, B.H. Robinson, A rapid and reliable DNA preparation method for screening a large number of yeast clones by polymerase chain reaction, Nucleic Acids Res. 23 (1995) 4924–4925.

[12] T.J. Kwiatkowski Jr, H.Y. Zoghbi, S.A. Ledbetter, K.A. Ellison, A.C. Chinault, Rapid identification of yeast artificial chromosome clones by matrix pooling and crude lysate PCR, Nucleic Acids Res. 18 (1990) 7191–7192.

[13] S.H. Shen, P. Chrétien, L. Bastien, S.N. Slilaty, Primary sequence of the glucanase gene from *Oerskovia xanthineolytica*. Expression and purification of the enzyme from Escherichia coli, J. Biol. Chem. 266 (1991) 1058–1063.

[14] H. Wang, S.E. Kohalmi, A.J. Cutler, An improved method for polymerase chain reaction using whole yeast cells, Anal. Biochem. 237 (1996) 145–146.

[15] M.T. Bourke, C.A. Scherczinger, C. Ladd, H.C. Lee, NaOH treatment to neutralize inhibitors of Taq polymerase, J. Forensic Sci. 44 (1999) 1046–1050.

[16] R. Akada, T. Murakane, Y. Nishizawa, DNA extraction method for screening yeast clones by PCR, Biotechniques. 28 (2000) 668–70, 672, 674.

[17] B. Linke, K. Schröder, J. Arter, T. Gasperazzo, H. Woehlecke, R. Ehwald, Extraction of nucleic acids from yeast cells and plant tissues using ethanol as medium for sample preservation and

cell disruption, Biotechniques. 49 (2010) 655–657.

[18] L. Rossen, P. Nørskov, K. Holmstrøm, O.F. Rasmussen, Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions, Int. J. Food Microbiol. 17 (1992) 37–45.

[19] R.D. Gietz, R.H. Schiestl, High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method, Nat. Protoc. 2 (2007) 31–34.

[20] T.A. Pham, S. Kawai, K. Murata, Visualization of the synergistic effect of lithium acetate and single-stranded carrier DNA on *Saccharomyces cerevisiae* transformation, Curr. Genet. 57 (2011) 233–239.

[21] S. Harju, H. Fedosyuk, K.R. Peterson, Rapid isolation of yeast genomic DNA: Bust n' Grab, BMC Biotechnol. 4 (2004) 8.

[22] B.A. Blount, M.R.M. Driessen, T. Ellis, GC Preps: Fast and Easy Extraction of Stable Yeast Genomic DNA, Sci. Rep. 6 (2016) 26863.

[23] P.S. Walsh, D.A. Metzger, R. Higuchi, Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, Biotechniques. 10 (1991) 506–513.

[24] M.A.H.M. Jamal, S.P. Sharma, H.-J. Chung, H.-J. Kim, S.-T. Hong, S. Lee, Ultra-High Efficient Colony PCR for High Throughput Screening of Bacterial Genes, Indian J. Microbiol. 57 (2017) 365–369.

[25] D. Liu, X.-A. Zeng, D.-W. Sun, Z. Han, Disruption and protein release by ultrasonication of yeast cells, Innov. Food Sci. Emerg. Technol. 18 (2013) 132–137.

[26] M.B. Kermekchiev, L.I. Kirilova, E.E. Vail, W.M. Barnes, Mutants of Taq DNA polymerase resistant to PCR inhibitors allow DNA amplification from whole blood and crude soil samples, Nucleic Acids Res. 37 (2009) e40.

[27] Y. Wang, D.E. Prosen, L. Mei, J.C. Sullivan, M. Finney, P.B. Vander Horn, A novel strategy to engineer DNA polymerases for enhanced processivity and improved performance *in vitro*, Nucleic Acids Res. 32 (2004) 1197–1207.

[28] F.J. Ghadessy, J.L. Ong, P. Holliger, Directed evolution of polymerase function by compartmentalized self-replication, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4552–4557.

[29] C. Baar, M. d'Abbadie, A. Vaisman, M.E. Arana, M. Hofreiter, R. Woodgate, T.A. Kunkel, P.

Holliger, Molecular breeding of polymerases for resistance to environmental inhibitors, Nucleic Acids Res. 39 (2011) e51.

[30] P.R. Winship, An improved method for directly sequencing PCR amplified material using dimethyl sulphoxide, Nucleic Acids Res. 17 (1989) 1266.

[31] K. Varadaraj, D.M. Skinner, Denaturants or cosolvents improve the specificity of PCR amplification of a G + C-rich DNA using genetically engineered DNA polymerases, Gene. 140 (1994) 1–5.

[32] W. Henke, K. Herdel, K. Jung, D. Schnorr, S.A. Loening, Betaine improves the PCR amplification of GC-rich DNA sequences, Nucleic Acids Res. 25 (1997) 3957–3958.

[33] P.N. Hengen, Optimizing multiplex and LA-PCR with betaine, Trends Biochem. Sci. 22 (1997) 225–226.

[34] D.S. Mytelka, M.J. Chamberlin, Analysis and suppression of DNA polymerase pauses associated with a trinucleotide consensus, Nucleic Acids Res. 24 (1996) 2774–2781.

[35] S. Frackman, G. Kobs, D. Simpson, D. Storts, Others, Betaine and DMSO: enhancing agents for PCR, Promega Notes. 65 (1998) 27–29.

[36] J. Kang, M.S. Lee, D.G. Gorenstein, The enhancement of PCR amplification of a random sequence DNA library by DMSO and betaine: application to in vitro combinatorial selection of aptamers, J. Biochem. Biophys. Methods. 64 (2005) 147–151.

[37] A. Hardjasa, M. Ling, K. Ma, H. Yu, Investigating the effects of DMSO on PCR fidelity using a restriction digest-based method, Journal of Experimental Microbiology and Immunology (JEMI). 14 (2010) 161–164.

[38] W.A. Rees, T.D. Yager, J. Korte, P.H. von Hippel, Betaine can eliminate the base pair composition dependence of DNA melting, Biochemistry. 32 (1993) 137–144.

[39] A.-N. Spiess, N. Mueller, R. Ivell, Trehalose is a potent PCR enhancer: lowering of DNA melting temperature and thermal stabilization of taq polymerase by the disaccharide trehalose, Clin. Chem. 50 (2004) 1256–1259.

[40] U.J. Desai, P.K. Pfaffle, Single-step purification of a thermostable DNA polymerase expressed in Escherichia coli, Biotechniques. 19 (1995) 780–2, 784.

[41] A. Adlimoghadam, M.H. Hedayati, S.D. Siadat, H.A.M. Nejati, J. Vandyousefi, D. Norouzion,

Optimization of PCR conditions for detection of human brucellosis from human serum samples, Res. J. Microbiol. 3 (2008) 352–358.

[42] T.C. Lorenz, Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies, J. Vis. Exp. (2012) e3998.

[43] B. Bachmann, W. Lüke, G. Hunsmann, Improvement of PCR amplified DNA sequencing with the aid of detergents, Nucleic Acids Res. 18 (1990) 1309.

[44] I.G. Wilson, Inhibition and facilitation of nucleic acid amplification, Appl. Environ. Microbiol. 63 (1997) 3741–3751.

[45] A.R. Sakhabutdinova, A.V. Chemeris, R.R. Garafutdinov, Enhancement of PCR efficiency using mono- and disaccharides, Anal. Biochem. 606 (2020) 113858.

[46] H. Li, J. Huang, J. Lv, H. An, X. Zhang, Z. Zhang, C. Fan, J. Hu, Nanoparticle PCR: Nanogold-Assisted PCR with Enhanced Specificity, Angew. Chem. Int. Ed. 44 (2005) 5100–5103.

[47] W. Yang, X. Li, J. Sun, Z. Shao, Enhanced PCR amplification of GC-rich DNA templates by gold nanoparticles, ACS Appl. Mater. Interfaces. 5 (2013) 11520–11524.

[48] A. Khaliq R, P.J. Sonawane, B.K. Sasi, B.S. Sahu, T. Pradeep, S.K. Das, N.R. Mahapatra, Enhancement in the efficiency of polymerase chain reaction by TiO 2 nanoparticles: crucial role of enhanced thermal conductivity, Nanotechnology. 21 (2010) 255704.

[49] J. Jia, L. Sun, N. Hu, G. Huang, J. Weng, Graphene enhances the specificity of the polymerase chain reaction, Small. 8 (2012) 2011–2015.

[50] M. Musso, R. Bocciardi, S. Parodi, R. Ravazzolo, I. Ceccherini, Betaine, dimethyl sulfoxide, and 7-deaza-dGTP, a powerful mixture for amplification of GC-rich DNA sequences, J. Mol. Diagn. 8 (2006) 544–550.

[51] M. Ralser, R. Querfurth, H.-J. Warnatz, H. Lehrach, M.-L. Yaspo, S. Krobitsch, An efficient and economic enhancer mix for PCR, Biochem. Biophys. Res. Commun. 347 (2006) 747–751.

[52] Z. Zhang, M.B. Kermekchiev, W.M. Barnes, Direct DNA amplification from crude clinical samples using a PCR enhancer cocktail and novel mutants of Taq, J. Mol. Diagn. 12 (2010) 152–161.

[53] J. Pan, C. Zhang, Y. Teng, S. Zeng, S. Chen, D. Liang, Z. Li, L. Wu, Detection of Spinal Muscular Atrophy Using a Duplexed Real-Time PCR Approach With Locked Nucleic Acid-

Modified Primers, Ann. Lab. Med. 41 (2021) 101–107.

[54] B. Hu, Y. Wang, S. Sun, W. Yan, C. Zhang, D. Luo, H. Deng, L.R. Hu, Z. Huang, Synthesis of Selenium-Triphosphates (dNTPαSe) for More Specific DNA Polymerization, Angew. Chem. Int. Ed Engl. 58 (2019) 7835–7839.

[55] B. Hu, Y. Wang, N. Li, S. Zhang, G. Luo, Z. Huang, Highly convenient and highly specific-and-sensitive PCR using Se-atom modified dNTPs, Chem. Commun. . 57 (2021) 57–60.

[56] Q. Dallas-Yang, G. Jiang, F.M. Sladek, Avoiding false positives in colony PCR, Biotechniques. 24 (1998) 580–582.

[57] A.B. Lee, T.A. Cooper, Improved direct PCR screen for bacterial colonies: wooden toothpicks inhibit PCR amplification, Biotechniques. 18 (1995) 225–226.

[58] T. Lehti, B. Westerlund-Wikström, Colony immunoblotting assay for detection of bacterial cell-surface or extracellular proteins, Bio Protoc. 3 (2013).

Table 1

Recipe for 1 mL twice concentrated PCR master mix containing 2% DMSO suitable for colony PCR.

Component	Volume (µL)
Water	650
Taq buffer with NH_4SO_4 (x10)	200
$MgCl_2$ (50 mM)	80
dNTPs (10 mM each)	40
DMSO (100%)	20
Taq DNA polymerase (5 U/µL)	10

Table 2

Recipe for 5x PCR compatible loading buffer

Component	Volume
25% ficoll	10 mL
Tartrazine food coloring	1 mL
Xylene Cyanol 125 mg/mL	10 µL

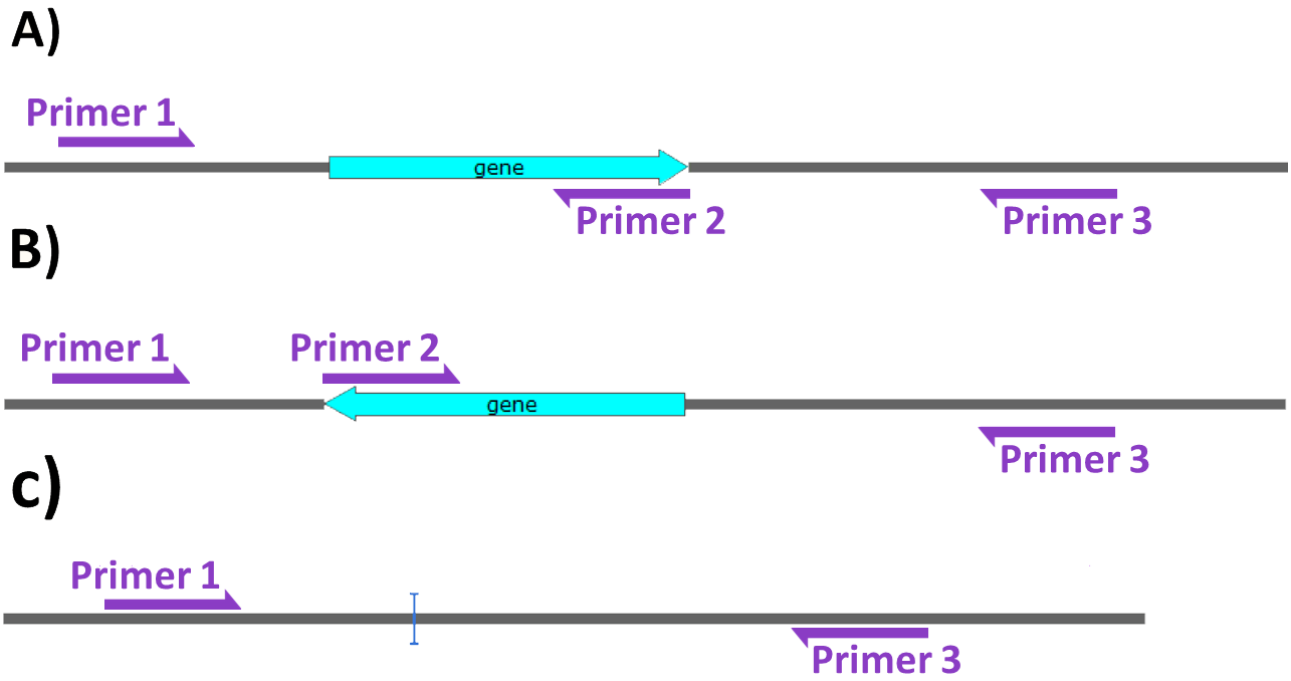

Fig. 1 Illustration of three-primer strategy for confirming cloning results. The annealing primer locations (represented in purple) will be different depending on the outcome: A) plasmid containing an insert with the desired orientation; B) plasmid containing an insert with the inverse orientation; C) plasmid without insert.