# Expression

**Transform E. coli BL21(DE3) with pOpenTaq**
Any standard method is fine, such as chemical or electrotransformation.

**Transfer the transformed cell mixture into LB containing 100 mg/L ampicillin**
It is not necessary to select individual clones.

**Grow the culture overnight.**
At 37C under shaking. This is the starter culture.

**Transfer 20 ml of the starter culture into fresh LB/amp for each 1 l of expression culture.**
The precise amounts may vary.

**Grow the expression culture until its OD600 reaches 70% of the starter culture.**
Grow under heavy shaking. Good aeration is important in this step to make the healthiest cells you can have. Measure the OD using any spectrophotometric device. The precise wavelength is unimportant. Any visible wavelength will work, as long as you use the same wavelength on both expression culture and starter culture. The precise induction point has some room for error. Anything 50% to 90% of the starter culture will work almost equally well. It should take 3-5h to reach the 70% value.

**Induce the culture overnight with 1 mM IPTG.**
Time your day so that this overnight step will go for 8-16 hours. We have not found any differences in this time frame, and have not tried other times. If you are short on IPTG, it's OK to use less. We have successfully expressed the polymerase with as little as 0.05 mM IPTG final concentration (This will not generally work on other proteins! pOpenTaq seems to be somehow special in this regard. We don't know why).

**Spin the expression culture down.**
For example, 4,000 rpm for 10 minutes works well in most floor centrifuges. If your tubes are not big enough, you can accumulate the culture in the same tubes with several spins. It is not necessary to refrigerate. Taq polymerase is happy at 72$^{o}$C. Anything you do with it outside of a Sauna will be to Taq pol what cryo-storage is to other proteins.

**Lyse the culture.**
This could be done with a high-pressure lysis device, sonication, or enzymatic lysis kit. If using a DNAse-containing method, it can be advantageous to minimize the amount of DNAse used and extend the treatment time. For example, 1/20$^{th}$ of the amount of DNAse most vendors prescribe for 2 hours time can yield good results with Gene And Cell Technologies enzymatic lysis kit.
Since the intended use of Taq polymerase usually involves DNA in one way or another, it is important that the final Taq Pol preparation contains neither DNA nor DNAse. So you have to strike a somewhat delicate balance during the DNAse treatment step – undertreat, and you will not get rid of all the contaminating E. coli DNA, potentially resulting in false positive amplification of these types of sequences. Overtreat, and you might have a hard time inactivating all the DNAse after it did its job, potentially harming your PCR templates. This step may require some optimization, depending on the preparation of DNAse is used. DNAse from many vendors is a natural product that can vary in quality and concentration.

But this is not meant to be discouraging – optimizing the amount of DNAse can yield a Taq polymerase preparation that is reliably free from both DNA and DNAse and ready to use (the Standard Grade preparation). These steps should be done, even if an additional, redundant ion-exchange purification ("polishing") step is planned (the UltraPure Grade preparation). Learn more about Taq DNA polymerase purity grades.

## Protein Extraction (using the GATC Lysis kit)

**Resuspend cell pellet in 1/10$^{th}$ of culture volume of Lysis Buffer**
E.g. use 100 ml of Lysis Buffer for a cell pellet derived from 1 liter of original culture. Mix the pellet up into homogeneous suspension. Dissociate all clumps. Avoid making bubbles.

**Add 1/1000$^{th}$ of culture volume of Lysozyme**
E.g. use 1 ml of dissolved Lysozyme for 1 liter of original culture. Mix by shaking or gentle vortexing.

**Freeze the cell suspension at -20$^{o}$C**
Make sure the lysate freezes completely. This step damages the cell wall, giving lysozyme access to its molecular targets.

**Thaw the cell suspension in a 37$^{o}$C water bath**
Wait until warmed up. Lysozyme becomes active and digests the cell wall. Clarification and/or viscosity indicate lysis.

**Add 1/1000$^{th}$ of culture volume of $CaCl_2$**
$CaCl_2$ is the required co-factor for the DNAse. It will neutralize and overcome the EDTA present in the lysis buffer, permitting DNAse to become active. Do not forget to add it!

**Add 1/20,000$^{th}$ of culture volume of DNAse**
Mix well. For example, add 50 ul of DNAse per 1 liter of original culture. Incubate at 37$^{o}$C for 2 hours. While you are incubating continue to mix and swirl occasionally, while the viscosity caused by DNA is slowly getting reduced. Your goal is to make sure DNAse penetrates the entire slurry. Because PCR is such an extremely sensitive technique, it is important to make sure no single droplet of E.coli DNA-containing solution gets to hide from the DNAse. Make sure the tube walls and the cap get exposed repeatedly during your mixes.

## Protein Purification

**Heat the slurry at 75$^{o}$C for 1 hour**
The time has come to play the strength of the Taq polymerase, its heat stability. Every other E. coli protein will be denatured and precipitate during this step.

**Cool to room temperature, and spin the aggregates down**
Capture the clarified supernatant containing Taq pol.

**Heat the clarified supernatant at 85$^{o}$C for 1 hour**
This increased, repeated heat step will get rid of any minor contaminant that may have escaped the previous step.

**Cool to room temperature, and spin any remaining aggregates down**

Carefully capture the clarified supernatant containing Taq pol. Avoid the pellet at all costs, even if that means leaving some Taq pol in the tube. The Taq pol containing solution should now be translucent. Residual turbidity that cannot be spun down indicates DNA contamination, and something may have gone wrong during the DNAse step. Turbidity that can be spun down indicates protein contamination. Do spin it down until the supernatant is fully clarified.

**Precipitate Taq pol with 30% Ammonium Sulfate**

Here, we are separating it from many denatured fragments or short peptides that have not aggregated, and other remaining molecules. It's OK to add the Ammonium Sulfate in solid form. For example, if you have 100 ml of supernatant at this stage, add 30 g of Ammonium Sulfate in one swoop (I know it sounds brutal. But your Taq pol is tough. It will be just fine). Mix by inversion until all the ammonium sulfate crystals are dissolved. This may be hard to see because massive amounts of Taq pol will fall out of solution. It should take about 10 minutes of *gentle* shaking. Taq pol is shock sensitive. Do not shake it too hard.

**Spin the Taq pol down and keep the Pellet.**

Taq pol is now a solid Ammonium Sulfate Precipiate. Discard contaminants in the supernatant.

**Resuspend the pellet in storage buffer**

Storage Buffer pH 8.6 1 l

| Component | Want | | M | Use | |
|---|---|---|---|---|---|
| Tris-HCl | 50 | mM | 157.6 | 7.9 | g |
| NaCl | 50 | mM | 58.4 | 2.9 | g |
| EDTA | 0.1 | mM | 292.2 | 29.2 | mg |
| DTT | 1 | mM | 154.3 | 154.3 | mg |
| PMSF | 0.5 | mM | 174.2 | 87.1 | mg |
| Glycerol | 10% | | | 10% | |

# Characterization

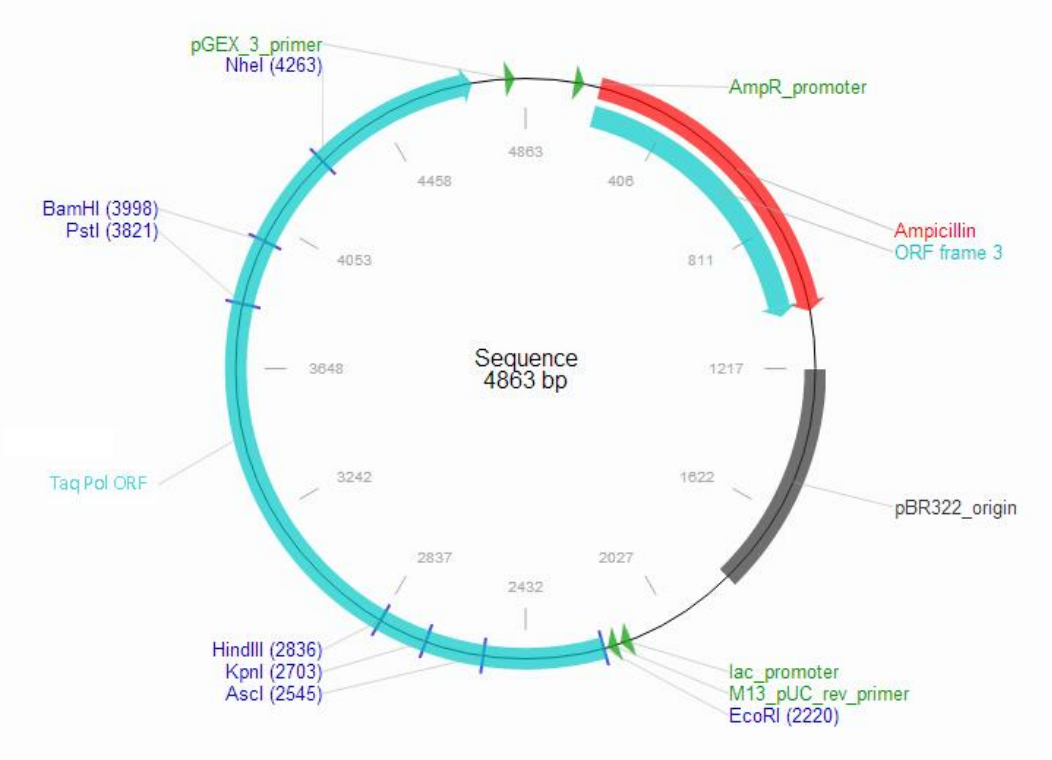
**Taq pol should appear pure by SDS-PAGE.**
The protein runs at 90 kDa. It is possible to get rid of all of them with this purification protocol. However, if some proteinaceous contaminants should still be present, don't worry. This will almost certainly not hurt your PCR reactions in any way.

**The best way to determine Taq pol's activity is to try varying amounts in PCR reactions directly.**
Add varying amounts of your Taq pol to your reaction. Start at 0.1 ul of taq pol, work your way up to 5 ul or so. Amplify your favorite primer/template pair. You should see a range of ideal Taq pol activity about 4x wide. At lower concentration, the signal should be weaker, and at higher concentration, smearing and false bands will occur. Pick the highest concentration that does not give you any smearing. To be safe, try a couple of other primer/template combinations with the same Taq pol concentration. Be sure to include some variations, such as primer overhangs, genomic templates, "difficult" reactions, to make sure your mastermix is robust. See the manual of the Open Biotechnology mastermix product (link above) for our full product release testing.

**You can convert into standard "units" as a universal activity measure.**
This step is highly optional, but some people like to express the activity of their polymerase in standardized "units". You can follow protocols published by other vendors that involve the incorporation of radiolabeled nucleotides into acid-insoluble material (DNA). Alternatively, you can use a taq pol preparation that has standard units already published as a calibration standard, and determine your units that way.

- By Open Biotechnology, Inc

# pOpenTaq Map & Sequence




>protein
MNSGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGEPVQAVYGFAKSLLKALKEDGDAVIVVFDAKAPSFRHEAYGGYKAGRAPTPEDFPRQL
ALIKELVDLLGLARLEVPGYEADDVLASLAKKAEKEGYEVRILTADKDLYQLLSDRIHALHPEGYLITPAWLWEKYGLRPDQWADYRALTGDESDNL
PGVKGIGEKTARKLLEEWGSLEALLKNLDRLKPAIREKILAHMDDLKLSWDLAKVRTDLPLEVDFAKRREPDRERLRAFLERLEFGSLLHEFGLLES
PKALEEAPWPPPEGAFVGFVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRDLKEARGLLAKDLSVLALREGLGLPPGDDPMLLAYLLDPSNTTP
EGVARRYGGEWTEEAGERAALSERLFANLWGRLEGEERLLWLYREVERPLSAVLAHMEATGVRLDVAYLRALSLEVAEEIARLEAEVFRLAGHPFNL
NSRDQLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKSTYIDPLPDLIHPRTGRLHTRFNQTATATGRLSSSDPNL
QNIPVRTPLGQRIRRAFIAEEGWLLVALDYSQIELRVLAHLSGDENLIRVFQEGRDIHTETASWMFGVPREAVDPLMRRAAKTINFGVLYGMSAHRL
SQELAIPYEEAQAFIERYFQSFPKVRAWIEKTLEEGRRRGYVETLFGRRRYVPDLEARVKSVREAAERMAFNMPVQGTAADLMKLAMVKLFPRLEEM
GARMLLQVHDELVLEAPKERAEAVARLAKEVMEGVYPLAVPLEVEVGIGEDWLSAKE

pI 5.97
Mw 93927

>gene
GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCG
CGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGG
AAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAG
TAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACG
TTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTAT
TCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGA
GTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCT
TGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTA
ACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGG
CTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCA
GACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCA
AAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTG
CTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGA
GCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCC
TGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAAC
GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAA
GGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTC
CTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTT
ACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCT
GATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTT
GGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGC
ACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGAATTCGGGGAT
GCTGCCCCTCTTTGAGCCCAAGGGCCGGGTCCTCCTGGTGGACGGCCACCACCTGGCCTACCGCACCTTCCACGCCCTGAAGGGCCTCACCACCAGC
CGGGGGGAGCCGGTGCAGGCGGTCTACGGCTTCGCCAAGAGCCTCCTCAAGGCCCTCAAGGAGGACGGGGACGCGGTGATCGTGGTCTTTGACGCCA
AGGCCCCCTCCTTCCGCCACGAGGCCTACGGGGGGTACAAGGCGGGCCGGGCCCCCACGCCGGAGGACTTTCCCCGGCAACTCGCCCTCATCAAGGA
GCTGGTGGACCTCCTGGGGCTGGCGCGCCTCGAGGTCCCGGGCTACGAGGCGGACGACGTCCTGGCCAGCCTGGCCAAGAAGGCGGAAAAGGAGGGC
TACGAGGTCCGCATCCTCACCGCCGACAAAGACCTTTACCAGCTCCTTTCCGACCGCATCCACGCCCTCCACCCCGAGGGGTACCTCATCACCCCGG
CCTGGCTTTGGGAAAAGTACGGCCTGAGGCCCGACCAGTGGGCCGACTACCGGGCCCTGACCGGGGACGAGTCCGACAACCTTCCCGGGGTCAAGGG
CATCGGGGAGAAGACGGCGAGGAAGCTTCTGGAGGAGTGGGGGAGCCTGGAAGCCCTCCTCAAGAACCTGGACCGGCTGAAGCCCGCCATCCGGGAG
AAGATCCTGGCCCACATGGACGATCTGAAGCTCTCCTGGGACCTGGCCAAGGTGCGCACCGACCTGCCCCTGGAGGTGGACTTCGCCAAAAGGCGGG
AGCCCGACCGGGAGAGGCTTAGGGCCTTTCTGGAGAGGCTTGAGTTTGGCAGCCTCCTCCACGAGTTCGGCCTTCTGGAAAGCCCCAAGGCCCTGGA
GGAGGCCCCCTGGCCCCCGCCGGAAGGGGCCTTCGTGGGCTTTGTGCTTTCCCGCAAGGAGCCCATGTGGGCCGATCTTCTGGCCCTGGCCGCCGCC
AGGGGGGGCCGGGTCCACCGGGCCCCCGAGCCTTATAAAGCCCTCAGGGACCTGAAGGAGGCGCGGGGGCTTCTCGCCAAAGACCTGAGCGTTCTGG
CCCTGAGGGAAGGCCTTGGCCTCCCGCCCGGCGACGACCCCATGCTCCTCGCCTACCTCCTGGACCCTTCCAACACCACCCCCGAGGGGGTGGCCCG
GCGCTACGGCGGGGAGTGGACGGAGGAGGCGGGGGAGCGGGCCGCCCTTTCCGAGAGGCTCTTCGCCAACCTGTGGGGGAGGCTTGAGGGGGAGGAG
AGGCTCCTTTGGCTTTACCGGGAGGTGGAGAGGCCCCTTTCCGCTGTCCTGGCCCACATGGAGGCCACGGGGGTGCGCCTGGACGTGGCCTATCTCA
GGGCCTTGTCCCTGGAGGTGGCCGAGGAGATCGCCCGCCTCGAGGCCGAGGTCTTCCGCCTGGCCGGCCACCCCTTCAACCTCAACTCCCGGGACCA
GCTGGAAAGGGTCCTCTTTGACGAGCTAGGGCTTCCCGCCATCGGCAAGACGGAGAAGACCGGCAAGCGCTCCACCAGCGCCGCCGTCCTGGAGGCC
CTCCGCGAGGCCCACCCCATCGTGGAGAAGATCCTGCAGTACCGGGAGCTCACCAAGCTGAAGAGCACCTACATTGACCCCTTGCCGGACCTCATCC
ACCCCAGGACGGGCCGCCTCCACACCCGCTTCAACCAGACGGCCACGGCCACGGGCAGGCTAAGTAGCTCCGATCCCAACCTCCAGAACATCCCCGT
CCGCACCCCGCTTGGGCAGAGGATCCGCCGGGCCTTCATCGCCGAGGAGGGGTGGCTATTGGTGGCCCTGGACTATAGCCAGATAGAGCTCAGGGTG
CTGGCCCACCTCTCCGGCGACGAGAACCTGATCCGGGTCTTCCAGGAGGGGCGGGACATCCACACGGAGACCGCCAGCTGGATGTTCGGCGTCCCCC
GGGAGGCCGTGGACCCCCTGATGCGCCGGGCGGCCAAGACCATCAACTTCGGGGTCCTCTACGGCATGTCGGCCCACCGCCTCTCCCAGGAGCTAGC
CATCCCTTACGAGGAGGCCCAGGCCTTCATTGAGCGCTACTTTCAGAGCTTCCCCAAGGTGCGGGCCTGGATTGAGAAGACCCTGGAGGAGGGCAGG
AGGCGGGGGTACGTGGAGACCCTCTTCGGCCGCCGCCGCTACGTGCCAGACCTAGAGGCCCGGGTGAAGAGCGTGCGGGAGGCGGCCGAGCGCATGG
CCTTCAACATGCCCGTCCAGGGCACCGCCGCCGACCTCATGAAGCTGGCTATGGTGAAGCTCTTCCCCAGGCTGGAGGAAATGGGGGCCAGGATGCT
CCTTCAGGTCCACGACGAGCTGGTCCTCGAGGCCCCAAAAGAGAGGGCGGAGGCCGTGGCCCGGCTGGCCAAGGAGGTCATGGAGGGGGTGTATCCC
CTGGCCGTGCCCCTGGAGGTGGAGGTGGGGATAGGGGAGGACTGGCTCTCCGCCAAGGAGTGATAAGCCAGCCCCGACACCCGCCAACACCCGCTGA
CGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATCA
CCGAAACGCGCGA

Ori Amp LacP M13 Taq EcoRI BamHI

The size of pOpenTaq is 4863 bp. One good way to confirm for pOpenTaq's identity is a double restriction digest with EcoRI and BamHI. This will give characteristic fragments of 1778 and 3085 bp.

## Reconstituting DNA from filter paper

All plasmids from Gene And Cell Technologies ship dried on a small piece of filter paper in a regular postal envelope. It may be necessary to let your administrative staff know that the plasmid will arrive in this form, and not in a package. We don't want these to end up in a filing cabinet or being discarded.

DNA on filter paper is very stable. It can be preserved for at least several years at room temperature or in the freezer in this state.

**Retrieve the filter from its zip-loc bag with forceps.**
*Optional: If unsure, you can cut the filter in two, and preserve half of it for later as a backup.*

**Place the filter in a standard microcentrifuge tube and add 50 µl of Tris: 10 mM, pH 8.**
*The Tris buffer is user-supplied. Water will work as well. Tris just stabilizes the DNA in case it needs to be stored in the wet state for prolonged periods. Use a pipet tip to push the filter paper into the liquid and make sure it's completely submerged.*

**Soak for 5 minutes and spin in a table top centrifuge at top speed for 5 minutes.**
*This will physically squeeze the trapped plasmid out of the filter mesh. This step is generally necessary to get sufficient plasmid yield.*

**Transform 2 µ into competent E. coli (e.g. 5α, 10β strains) and plate ~5% of the mixture.**
*Most manufacturers call for recovering the E. coli in 1 ml SOC medium after transformation. Plating 50 µl of that should give you a manageable number of colonies on a single 10 cm plate. If your cells aren't quite elite, or you cut some corners during the protocol, it is possible to spin the mixture down and plate all the cells, for increased colony yield.*

**Incubate overnight. Pick a strong-looking colony, and grow it up in liquid culture.**
*Perpare plasmid for digestion. Prepare stocks in 10% glycerol for long-term storage at -80$^{o}$C.*