

***Introduction to Experimental Biophysics Biological
Methods for Physical Scientists 2nd edition Nadeau
Solutions Manual***

SKU: 9781498799591-SOLUTIONS

Solutions for Chapter 2

1. 1 mol of 1 bp weighs 660 g. So a 2 kb plasmid is 1320 $\mu\text{g/nmole}$, or say approximately 1 mg is 1 nmole of plasmid, which is 6×10^{14} molecules. Say 1000 plasmids grow in each cell, so it's 6×10^{11} cells needed, or $\sim 10^{12}$ cells for a 50% yield. For optimal plasmid recovery, cells are grown to just past log phase, or $3\text{-}4 \times 10^9$ cells/mL. Should get 1 mg from 250-300 mL of culture.

2. The conversion factors are:

Average dsDNA MW = $660 \times N$ (where N = number of base pairs)

$$C (\mu\text{M or pmol}/\mu\text{l}) = A_{260} / (0.01 \times N)$$

$$C (\text{ng/ml}) = (A_{260} \times \text{MW}) / (0.01 \times N)$$

For RNA, A₂₆₀ reading of 1.0 is equivalent to about 40 $\mu\text{g/ml}$

3. 1 in 4096 (so 0.5-1 for an average plasmid); 1 in 65536

4. Use some uncut plasmid as a control that is transformed and plated next to your ligation. When plating, make sure to dilute the uncut plasmid enough to be able to count the colonies.

5. The smallest NcoI fragment is barely visible, so we can call this the limit. Assume it's a typical screening gel from a miniprep, so you've cut ~ 200 ng of DNA total. It's about a 250 bp fragment out of a 4 kb fragment, so the amount of DNA in that band is ~ 12.5 ng. This is a reasonable value for a limit.

6. (1) Some uncut plasmid could be left in and transform with very high efficiency. The control with no insert and no ligase controls for this. If anything grows on this one, throw the ligation away and try again. (2) The plasmid could close back in on itself. This can even happen with incompatible sticky ends, especially if they have short overhangs. The no-insert control controls for this.

7. 3:1 is a good compromise to maximize the chance of the insert finding a vector while still making the probability of getting two inserts per vector very small. **(1)** Rarely, there may be more than one insert lined up in the final product. This is hard to control for; gel screening may show it. The frequency should be roughly the frequency of expected successful ligations^(number of inserts), so extremely rare if successful ligations are 1 in 10^6 ! **(2)** The plasmid may recombine to give a result much smaller than expected, without

the insert. Gel screening will reveal a vector that's too short and/or no insert. The likelihood of this depends upon the presence of homologous sequences in the plasmid. Sometimes the frequency of this type of recombination is close to 1, and *E. coli* defective in recombination may need to be used. (3) Part of the insert could be edited out. Gel screening will show an insert that's wrong. The frequency depends upon the properties of the insert; it's more likely with very long or repetitive inserts.

8. Blue/white selection works when an insert is cloned into a polylinker that is deliberately placed inside the *lacZ* coding sequence. If an insert is cloned, the coding sequence is disrupted and no β -galactosidase will be produced. If there is no insert, the β -galactosidase is made. β -galactosidase turns the colorless substrate X-gal from clear to blue. So no insert = blue colonies. Insert = white colonies.

9. (A) Pick a few colonies and grow in 3-5 mL of LB-Amp, then miniprep. (B) Restriction digest screening of minipreps using *XbaI*/*XhoI*. (C) There may be no promoter in the vector, or at least no mammalian promoter. Alternatively, the gene might not express well in mammalian cells and may need to be codon optimized. (D) First lane: the digest just cut out CFP, so you're good to go. Second lane: the digest cut a piece out of the vector. As long as you can separate those last two bands and cut out the one corresponding to CFP, you're good to go. You probably want to run the gel longer than was done in the example to better separate the bands. Third lane: The vector appears to be linearized, but the gene didn't cut out. You're in trouble unless one of your enzymes has gone bad and you can replace it. Fourth lane: *XbaI* and *XhoI* cut in a lot of places, but you should be able to isolate the gene. For all except lane #3, your cloning strategy should be to put the isolated gene into a vector that you understand, which has a mammalian promoter. (E) If the gene doesn't cut out with *XbaI* and *XhoI*, you have no real choice but to PCR it out. The sequence should be in GenBank and you can sequence the ends of the gene to figure out what lies on either side, then develop PCR primers to flank the gene; or you can use the known sequence to develop primers for precisely the gene ends with no flanking sequence.

10. The simple answer is to knock in a growth gene using a tooth-specific promoter. More advanced students will provide ideas of how to deliver the gene (e.g. stem-cell recombination, transfection of spermatozoa, etc).

11. Get the gene sequence from the most closely related possible cat (perhaps a lynx or lion). Extract mRNA from the smilodon muscle sample and use the lynx cDNA as probe on either Southern or Northern blot to verify it will bind. If successful, use it to probe a Smilodon cDNA library, pull out ryanodine receptor cDNA and sequence. Verify that you have the right gene by comparing to the sequence from the lynx.

12. The sequence may be found at GenBank: GQ354859.1

```

1  cgtcctcctt attattcaga agagatgttt gaacaagatt ttgaaaaacc caaagaaaaa    61  gatcctccta
aagcagagag tccagccact gaacctcgg gtaattcaac aggtcctgag    121  actaattcta ctcaaccaa
tgcacctgga gggaatcagg gcggaaatga caccagccca    181  acaggaaaca gtggccatgg cccaaacact
gtgagtaatc ctacagctca aaacgggggt    241  attcacccc ctacagttaa tgttcaggc caggagtagc
caagaactca aatctcatgg    301  ggaccaagtc agccaaatat ttatgaaaac tatccaaatc ctaacatccg
aaatttcct    361  gcaggaagac aatggcgtcc cactggtact atcatggggc gtagacagaa tgggcctttt    421
tacagaaatc aacaggtcca aaggggtcct cgttgaact ccttgcttt ggaaggcaaa    481  caagcatttc
gttcaggaaa tccaatttat cgcaaagctt atgcttctac tgcaagaggc    541  aatttccca ctcatgcagg
aaatccagga aatgtcagaa gaaagcctca gggaccaa    601  aaacacccta tgggaaccaa cgttgctcct
ctgggtccca aacatggtac tgtgtccgc    661  aatgaaaaaa tccaaaatcc agcagagaaa ccagtaggtc
caaaagaaag aatagtcatt    721  cctacaagag atcatctgg cccctggaga aactctcaag attatggagt
taataaatca    781  aactataaac tgcctcatcc tgagagtaac atgctagtcc caaattttaa ttctgttgg    841
caacatgaaa actcttatta cccaagagga gagtccaaa gagcccaaaa ttctgatgga    901  caaacccaaa
gccagaattt gcccaaaggg attattttgg agccaagaag aatcccttat    961  gaatcagaaa ctaatgcacc
agaattaaag cacagtacat atcaacgtgt ataccctgag    1021  gaaatccctt ccaatgcaag agaacattt
cctgctggaa gaaatatgtt gaatcatcaa    1081  ggaatctctc cacctattaa ggaagatccc gggatgcagg
aagaacactt acttcatcct    1141  tccatggct caaggggagg tgttactac cctgactata actcttataa tccaggggaa
1201  aactcaccat accttagaag caatacatgg gatgagagag atgattctcc caatactatg    1261  gggcaacca
aaaattcact gtaccccatg aatactccag acctgaaaga aacagtcctt    1321  tataatgaag aggaccaat
tgatgcaact ggagatgaaa ctttccagg acaaagtaga    1381  tggggatgagg aggagccaag cttaaagaa
gggccaacag ttaggcacta tgaaggcgag    1441  caatatacct caaatcaacg gaaagaatac ctccctatt
ccttagataa tccatgaaa    1501  cccagggagg atttcctta tggatgaatt tatccctgga accaagatga gaattttcca
1561  tcatataata cagctcccag tgcaccacca cctgtggaga gcaggggcta ttatgcta    1621  aatgctgttg
gacaggaaga aagcactctg tcccctctt ggaactcctg ggaccatgag    1681  attcaagccc aagggcagaa
agaagaaga ccatatttta acagaaactt ctgggatcga    1741  ccaacaactt tacacaaagc tcccctagt
ccaccacatg agaaagagaa ccagccttat    1801  cctagcaatt cccagctgg gcttcagaaa agtccaacat
ggcatgaaag tgagaatttg    1861  aattatggca tgcacattac taggataaat tcaccagaga gagaacagtt
ggctttccca    1921  gacttaattc ctccaagtta cccatcaggt caaaaagaag cgcagtatt tcatttaagc    1981
cagagaggcc ctgctgtgc tggcagctcc acaggacaca aagaagatcc acttgctcta    2041  caggactaca
ctccatcctt tggcttgca ccaggggaga accaagacac cagtcctccg    2101  tatacagaag atagtcatac

```

taaacatgca agacacacca tctccctcc aagcaaccta 2161 cctggccaaa gaaacagctc agagaaaaga
 ctgcctggag aaagtgaaaa cccaagtcct 2221 ttagagatg atgtgtccac cctgaggagg aacacaccat
 gtccatgaa gaatcagctg 2281 agccaaaggg gaattatgcc ctttctgaa gccagttccc ttaatcaaa
 gaatatgcct 2341 tgcctcaaaa gtgatcttg aggagatggg gacaatgtt tagaacaat attgaaggc 2401
 aaccagctca gtgaaagaac tgtgacctt actcctgagc agcttggtat gggtagacct 2461 gaagaagccc
 ctaatccaga aggaatccaa agtgaagtac aaggaaatga gggtagaaagg 2521 cagcaacaaa gaccatcaag
 catcctgcag ttaccatgct ttggctcaa attggcaaag 2581 tatcattcct ccagcactgg aactccatct ggcattggaa
 ggcaaggccc attgatgaa 2641 gatccgatta cgctactga aagtctaac tcattgtcta ggtagctac
 tggggcacag 2701 ttccagagta taaatgtga ccacttaat gcagatgaac acatttcatt tgattccctt 2761
 caaataga

The primers should be similar in T_m and have the appropriate BamHI overhangs.

13. Use either *in vivo* transfection or a viral vector with a strong promoter such as CMV.

Inject into your skin in the desired pattern. Don't inject the GFP protein; it won't be stable.

14. There are two parts to the pET expression system. First, the plasmid contains YFP-DNA cloned downstream of a T7 promoter site. This plasmid also contains amp resistance and origin of replication. The host cell has a modified genome such that the T7 RNA pol gene is inserted into its chromosome and can be activated with the addition of IPTG. When IPTG is added, T7 RNA pol is made, binds to the promoter upstream of YFDNA and transcribes it. The cell then translates it and you have tons of YFP.

15. Get answers from the NEB catalog section on "compatible cohesive ends." Neither Sal nor Xho will recleave the first ligation. Xho will recleave the second one.

16. Restriction enzymes are found in all bacteria, and sequence specificities cover just about every possible 4-6 bp sequence. These enzymes protect bacteria against foreign DNA.

17. May be obtained from manufacturers of plasmid-prep kits.

18. Steps as in Fig. 2.10. Before the discovery of the heat-stable polymerase from *T. aquaticus*, polymerases would be destroyed at each denaturation cycle. This meant that someone trying to run a reaction would have to add fresh polymerase each cycle.

19. Called the Ames test. Bacteria that require histidine are used, and mutations make them revert to a state where they do not require histidine.

20. Find sequence on GenBank. Create mutagenic primers. PCR.

21. Answers will vary.

22. Mechanosensitive channel from *Mycobacterium tuberculosis*. Protects cells from osmotic shock. Restriction sites: BamHI, SspI, FspI.

Use an online codon optimizer to optimize for mammalian expression. Human optimization:

```
ATG CTG AAA GGC TTC AAA GAA TTT CTG GCC CGA GGA AAT ATT GTG GAC CTG GCG
GTA GCC GTT GTA ATA GGA ACC GCG TTT ACT GCG CTG GTA ACA AAA TTC ACG GAT
AGC ATC ATC ACC CCT TTG ATA AAT AGG ATA GGA GTG AAC GCG CAA AGC GAT GTG
GGG ATC CTC CGC ATA GGC ATA GGA GGT GGT CAG ACT ATT GAC CTT AAC GTA CTT
TTG TCA GCG GCG ATC AAT TTC TTC CTC ATA GCC TTT GCC GTT TAT TTT TTG GTA GTT
CTC CCC TAT AAC ACC TTG CGC AAG AAA GGA GAG GTC GAG CAG CCG GGA GAC ACA
CAA GTT GTC TTG TTG ACT GAG ATC AGA GAT TTG CTG GCA CAA ACG AAT GGG GAT
TCA CCA GGG AGG CAT GGA GGA CGA GGA ACA CCG TCA CCT ACA GAC GGA CCC AGA
GCT AGT ACA GAG AGC CAA TAG
```

23. TOPO cloning is a ligase-free method of cloning that takes advantage of the property of Taq polymerase that it adds a single deoxyadenosine (A) to the 3'-end of the PCR products. TOPO vectors are special plasmid vectors that are designed to carry the sequence 5'-(C/T)CCTT-3' at the two linear ends. The linear vector DNA already has the topoisomerase enzyme covalently attached to both 3' ends. This is then mixed with PCR products. When the free 5' ends of the PCR product strands attach to the topoisomerase/3' end of each vector strand, the strands are covalently linked by the topoisomerase.

24. N terminal fusions are usually the safest in terms of protein expression. If protein is desired, then the fusion partner with secretion signals must be on the N terminus. N terminal fusions avoid putting foreign sequences next to translation signals, which can inhibit translation. C terminal fusions can work fine in many cases as well. Trying to put the GFP protein on an extracellular part of an ion channel will almost certainly not work because it will have to pass through the membrane. I know of only one group that claims to have got this to work (Verkman, UCSF). Most N-and C-terminal fusions can be done with simple restriction enzyme cloning, but make sure that the two parts are in frame. Getting them out of frame leads to truncation or just bizarre results. Trying to put the GFP anywhere but the N or C terminus will take PCR cloning. Even if the sequence is perfect, things that can go wrong include the protein getting hung up in the endoplasmic

reticulum or forming inclusion bodies, or simply failing to fluoresce. This is usually obvious from looking at the cells.

25. Either increase or decrease annealing temperature. Increase can prevent formation of nonspecific products; decrease can reduce issues such as primer dimer.

26. Disadvantages: Short product lengths (< 1 kb). Low product amounts. Errors.
Advantages: Simple, fast, and robust.

27. The main disadvantage is that it requires the extraterrestrial life to have some universal sequence, such as ribosomal RNA. For a close by planet such as Mars, that is probably a safe assumption. Life cannot have been extinct for more than thousands of years in order to get a signal. The possibility of contamination must be very carefully controlled for, and the experiment must have the ability to reject known Earth sequences. Within these limits, the experiment can be low-cost, robust, and very sensitive.

28. If not inactivated, the enzyme will cut the ligation product, thereby undoing the ligation (unless of course the resulting product doesn't cut with the same enzyme, in which case it could be good to have the enzyme still in there!) Enzymes may be removed by running the ligation on an agarose gel or using a clean-up kit. A list of temperatures is given here: <https://www.neb.com/tools-and-resources/usage-guidelines/heat-inactivation>

29. The accelerating force on the molecule is ZeE . The retarding force is given by the Stokes formula and is related to the medium viscosity and particle shape; it can be expressed as $f v$ where f is the frictional coefficient and v is the speed. Setting the two forces to balance gives Eq. A1.

30. “Dummy” nucleotides are used to stop synthesis at a specific type of base pair (ACGT). Then the fragments are separated by size, using very long polyacrylamide gels to obtain single base pair resolution.