BIOL 3020 Fall 2007.
The "Keys of Corn" Project
or "How to clone genes for study in 2007"

DNA Technology – The Awesome Skill

In 1983, the editors of TIME magazine referred to DNA technology as "***….the most awesome and powerful skill acquired by man since the splitting of the atom.***" At that time, this technology included such techniques as gene isolation and purification, gene detection (hybridization techniques – part 2 of this course), gene sequencing, and the ability to copy DNA – known as DNA cloning. Since that time the technique of PCR has been developed (the past two weeks of this course) and the ability to sequence DNA at vastly accelerated rates has spawned the fields of genomics and bioinformatics. DNA technology (often referred to as genetic engineering) has become a part of our lives from forensic examinations to early genetic disease detection and disease treatment (somatic gene therapy).

In this part of the course, you will learn a key component of DNA technology –namely **DNA cloning**. In the lecture and in your manual, you will learn about **traditional cloning** using DNA restriction enzymes, DNA ligase, and plasmid vectors. The humble gut bacterium known as ***E. coli*** is the workhorse of DNA cloning because it is the organism into which we insert and propagate most genetically engineered molecules (yeast is also widely used). Although we will discuss traditional cloning we will perform a new method of DNA cloning called **Gateway Cloning**™ (recombination mediated cloning) that is gathering wide interest because of its ability to simplify and speed up the rate at which cloning is performed.

The "Keys of Corn"

This year we will not clone pieces of lambda phage in order to teach cloning techniques. Instead we are embarking on a new project dubbed "Keys of Corn" that will allow you to learn DNA cloning while generating a new resource that will help researchers to investigate the molecular biology of the largest crop in the world – corn (*Zea mays*). Corn was first domesticated by Native American peoples thousands of years ago and enabled their civilization to flourish in Central and South America. Today, nearly 700 million metric tons of corn are harvested worldwide each year (http://www.ncga.com/WorldOfCorn/main/index.htm). Corn is important not only as a source of food, starch, fiber, oil, but also as renewable biomass for the production of ethanol. Plant scientists aim to understand corn as doctors aim to understand the human body. With this understanding better crop varieties are being developed to help ensure food and fuel for the future. We will study the molecular biology of corn by cloning a number of genes for further study.

We will focus on **Transcription Factor genes (TFs)** because they are key to understanding the regulation of corn growth and metabolism. TFs are like keys because they are the proteins

that turn on and off other genes like the keys of a car. Unlike a car which has one key, the genome of a eukaryote has thousands of TFs. In humans approximately 10% of the genes encode TFs that is about 3000 TFs!. **In corn there may be as many as 5000 or more TFs** ! To understand the amount of research that needs to be done on corn TFs, first imagine that you had a large building with 5000 doors and you are handed 5000 unmarked keys – then you are asked which key opens which door? It is a similar problem with TFs because although the DNA sequence may reveal that a gene encodes a TF similar to other TFs –it is not predictable which genes it turns on or off – just like the handle of a key looks familiar but the shaft is unique for the lock. To make things more complicated one TF may turn on or off one or more genes!

So where do YOU come in? - You will help begin to generate the bunch of keys corresponding to all of the TFs of corn. When these are collected then they will be made available to researchers who will discover how they function in plant growth and metabolism (i.e. which doors are opened – which genes are regulated by each TF).

Right now the maize genome, - which is as large as the human genome, is being sequenced but it will take another few years to be completed. In the meantime scientists have isolated messenger RNAs from many tissues, made **complementary DNA clones (cDNAs)** of many genes and stored them away. They have then sequenced the ends of these at random and these are called **Expressed Sequence Tags (ESTs).** These sequences are then deposited in Genbank - which you have learned to access **(Entrez)** and search **(BLAST)** in the earlier parts of this course. By searching these sequences one may find new TF genes but then in order to study them you need to clone them in the lab. We will identify in the Genbank TFs that are full length or flcDNAs (many cDNAs are not full length because the mRNA is quickly degraded so they are partial – missing the beginning of the Open Reading Frame (ORF). By aligning several ESTs and translating then we can deduce which one corresponds to a flcDNA. We then order that from the cDNA depository (Arizona Genomics Institute) and use the **Polymerase Chain reaction (PCR)** to amplify the entire ORF and YOU will clone it into a useful DNA vector. YOU will then transform this into *E. coli*, grow up the bacteria and isolate the Recombinant DNA clone (Plasmid Preparation –week two). We will send the plasmid for sequencing and when it comes back YOU will verify that there are no mistakes due to PCR or in the cloning. These ENTRY clones will start the collection of corn TFs (keys) that can be used by <u>other</u> researchers (which means that YOU now have become a researcher too!). Some of you may wish to get involved in further research after the class is over.

Websites that maybe useful for this section of the course:

Maize Genetic Database (Maize GDB) http://www.maizegdb.org/

Invitrogen™ Gateway Cloning Information

http://www.invitrogen.com/content.cfm?pageid=4072&CID=FL-GATEWAY

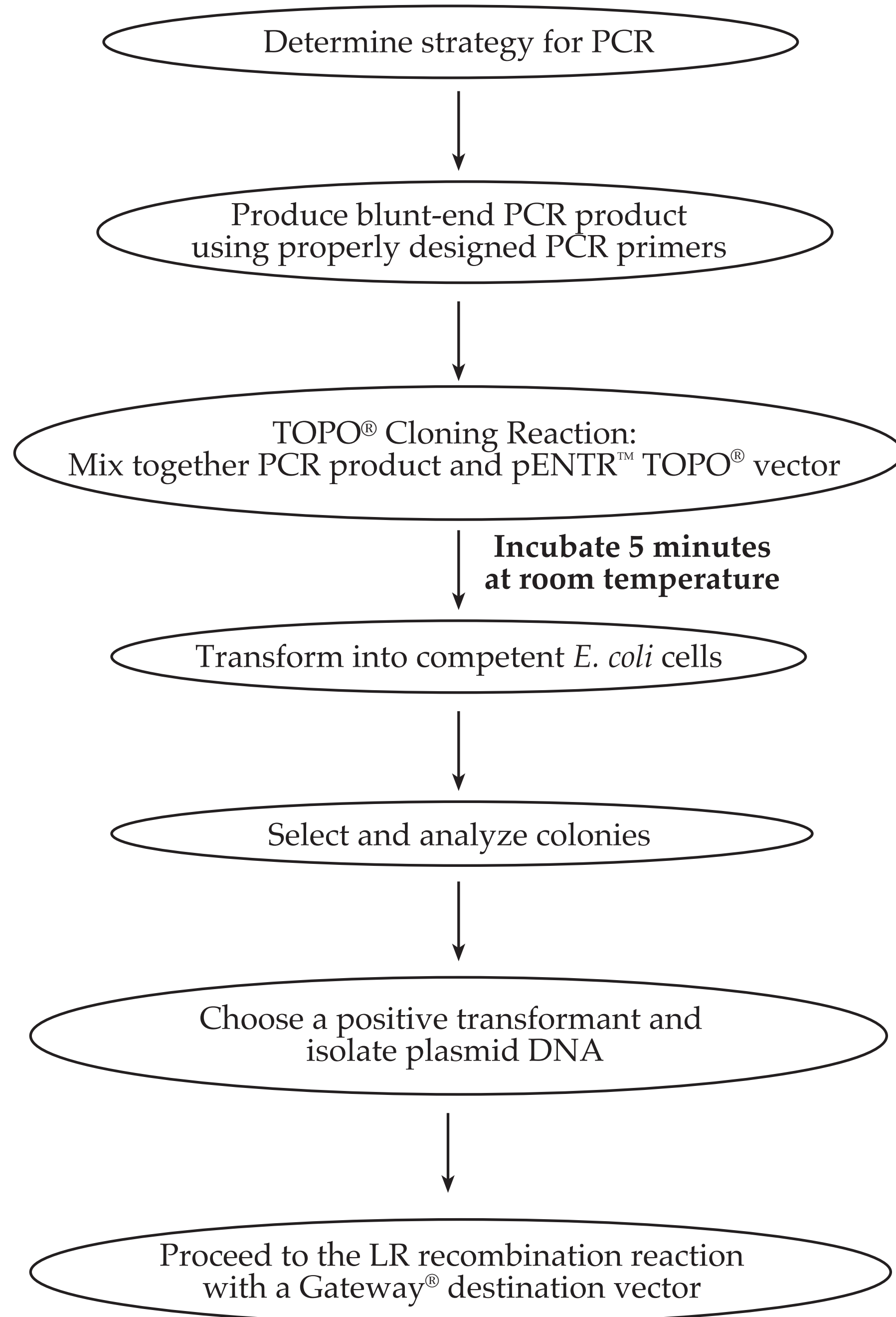
(Click on Online Seminar or OnLine Interview on lower left side of page)

The protocols for Week one are taken from the pENTR Directional TOPO cloning Kit manual and are on the following pages – the entire manual is also placed on WEBCT for your reference.

Experimental Outline

Flow Chart

The flow chart below describes the general steps required to produce and clone your blunt-end PCR product.



Introduction

Overview

Introduction

The pENTR™ Directional TOPO® Cloning Kits utilize a highly efficient, 5-minute cloning strategy ("TOPO® Cloning") to directionally clone a blunt-end PCR product into a vector for entry into the Gateway® System or the MultiSite Gateway® System available from Invitrogen. Blunt-end PCR products clone directionally at greater than 90% efficiency, with no ligase, post-PCR procedures, or restriction enzymes required.

A choice of pENTR™ Directional TOPO® vectors is available for optimal expression of your PCR product after recombination with the Gateway® destination vector of interest (see table below).

Vector	Benefit
pENTR™/D-TOPO®	For efficient expression of your gene of interest after recombination with a Gateway® destination vector
pENTR™/SD/D-TOPO®	Contains a T7 gene 10 translational enhancer and a ribosome binding site (RBS) for optimal expression of native protein after recombination with a prokaryotic Gateway® destination vector **Note:** Also suitable for efficient expression of your gene of interest in other hosts after recombination with a Gateway® destination vector (*e.g.* mammalian, insect, yeast)
pENTR™/TEV/D-TOPO®	Contains a Tobacco Etch Virus (TEV) recognition site for efficient TEV protease-dependent cleavage of an N-terminal tag from your recombinant protein after recombination and expression from a Gateway® destination vector

The Gateway® Technology

The Gateway® Technology is a universal cloning method that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest using the Gateway® Technology, simply:

1. TOPO® Clone your blunt-end PCR product into one of the pENTR™ TOPO® vectors to generate an entry clone.
2. Generate an expression construct by performing an LR recombination reaction between the entry clone and a Gateway® destination vector of choice.
3. Introduce your expression construct into the appropriate host (*e.g.* bacterial, mammalian, yeast, insect) and express your recombinant protein.

For more information about the Gateway® Technology, refer to the Gateway® Technology with Clonase™ II manual which is available for downloading from www.invitrogen.com or by contacting Technical Service (see page 35).

continued on next page

How Directional TOPO® Cloning Works

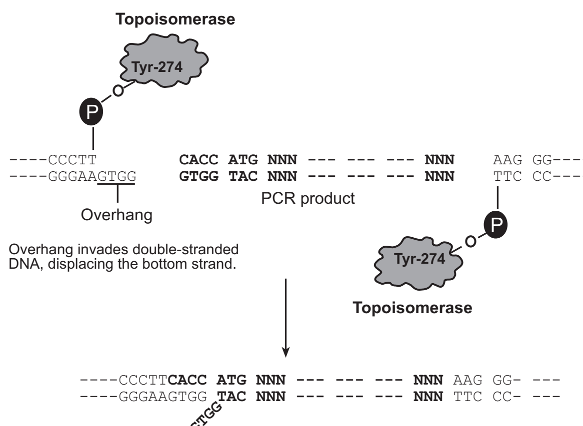
How Topoisomerase I Works

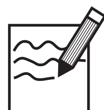
Topoisomerase I from *Vaccinia* virus binds to duplex DNA at specific sites (CCCTT; see **Note** below) and cleaves the phosphodiester backbone in one strand (Shuman, 1991). The energy from the broken phosphodiester backbone is conserved by formation of a covalent bond between the 3′ phosphate of the cleaved strand and a tyrosyl residue (Tyr-274) of topoisomerase I. The phospho-tyrosyl bond between the DNA and enzyme can subsequently be attacked by the 5′ hydroxyl of the original cleaved strand, reversing the reaction and releasing topoisomerase (Shuman, 1994). TOPO® Cloning exploits this reaction to efficiently clone PCR products.

Directional TOPO® Cloning

Directional joining of double-strand DNA using TOPO®-charged oligonucleotides occurs by adding a 3′ single-stranded end (overhang) to the incoming DNA (Cheng and Shuman, 2000). This single-stranded overhang is identical to the 5′ end of the TOPO®-charged DNA fragment. At Invitrogen, this idea has been modified by adding a 4 nucleotide overhang sequence to the TOPO®-charged DNA and adapting it to a 'whole vector' format.

In this system, PCR products are directionally cloned by adding four bases to the forward primer (CACC). The overhang in the cloning vector (GTGG) invades the 5′ end of the PCR product, anneals to the added bases, and stabilizes the PCR product in the correct orientation. Inserts can be cloned in the correct orientation with efficiencies equal to or greater than 90%.





Note

The 5′ TOPO® recognition site in pENTR™/TEV/D-TOPO® is encoded by the sequence TCCTT rather than CCCTT. This is because the 5′ TOPO® recognition site directly follows the TEV recognition site, and studies have shown that TEV protease does not cleave efficiently if the first amino acid following the TEV recognition sequence is proline (Kapust *et al.*, 2002) as would be the case if the 5′ TOPO® recognition site was encoded by **CCC**TT. By changing the sequence of the 5′ TOPO® recognition site to **TCC**TT, the first amino acid following the TEV recognition site is now serine. **This change does not affect TOPO® Cloning efficiency and allows efficient TEV cleavage.**

Setting Up the TOPO® Cloning Reaction

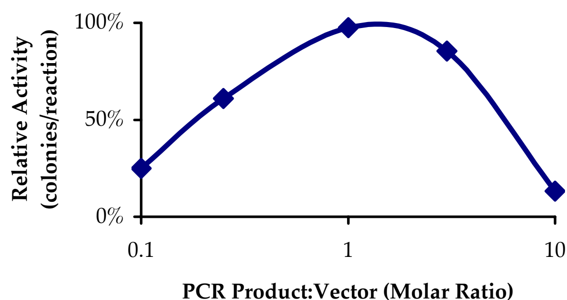
Introduction

Once you have produced the desired blunt-end PCR product, you are ready to TOPO® Clone it into the pENTR™ TOPO® vector and transform the recombinant vector into One Shot® competent *E. coli*. You should have everything you need set up and ready to use to ensure that you obtain the best possible results. We suggest that you read this section and the section entitled **Transforming One Shot® Competent *E. coli*** (pages 13-14) before beginning. If this is the first time you have TOPO® Cloned, perform the control reactions on pages 23-25 in parallel with your samples.

Amount of PCR Product to Use in the TOPO® Cloning Reaction

When performing directional TOPO® Cloning, we have found that the molar ratio of PCR product:TOPO® vector used in the reaction is critical to its success. **To obtain the highest TOPO® Cloning efficiency, use a 0.5:1 to 2:1 molar ratio of PCR product:TOPO® vector (see figure below).** Note that the TOPO® Cloning efficiency decreases significantly if the ratio of PCR product: TOPO® vector is <0.1:1 or >5:1 (see figure below). These results are generally obtained if too little PCR product is used (*i.e.* PCR product is too dilute) or if too much PCR product is used in the TOPO® Cloning reaction. If you have quantitated the yield of your PCR product, you may need to adjust the concentration of your PCR product before proceeding to TOPO® Cloning.

Tip: For pENTR™ TOPO® vectors, using 1-5 ng of a 1 kb PCR product or 5-10 ng of a 2 kb PCR product in a TOPO® Cloning reaction generally results in a suitable number of colonies.



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Setting Up the TOPO® Cloning Reaction, continued

Using Salt Solution in the TOPO® Cloning Reaction

You will perform TOPO® Cloning in a reaction buffer containing salt (*i.e.* using the stock salt solution provided in the kit). **Note that the amount of salt added to the TOPO® Cloning reaction varies depending on whether you plan to transform chemically competent cells (provided) or electrocompetent cells (see page x for ordering information).**

- If you are transforming chemically competent *E. coli*, use the stock Salt Solution as supplied and set up the TOPO® Cloning reaction as directed below.
- If you are transforming electrocompetent *E. coli*, the amount of salt in the TOPO® Cloning reaction **must be reduced** to 50 mM NaCl, 2.5 mM $MgCl_2$ to prevent arcing during electroporation. Dilute the stock Salt Solution 4-fold with water to prepare a 300 mM NaCl, 15 mM $MgCl_2$ Dilute Salt Solution. Use the Dilute Salt Solution to set up the TOPO® Cloning reaction as directed below.

Performing the TOPO® Cloning Reaction

Use the procedure below to perform the TOPO® Cloning reaction. Set up the TOPO® Cloning reaction depending on whether you plan to transform chemically competent *E. coli* or electrocompetent *E. coli.* **Reminder:** For optimal results, be sure to use a 0.5:1 to 2:1 molar ratio of PCR product:TOPO® vector in your TOPO® Cloning reaction.

Note: The blue color of the TOPO® vector solution is normal and is used to visualize the solution.

Reagents*	Chemically Competent *E. coli*	Electrocompetent *E. coli*
Fresh PCR product	0.5 to 4 μl	0.5 to 4 μl
Salt Solution	1 μl	--
Dilute Salt Solution (1:4)	--	1 μl
Sterile Water	add to a final volume of 5 μl	add to a final volume of 5 μl
TOPO® vector	1 μl	1 μl
Final volume	6 μl	6 μl

*Store all reagents at –20°C when finished. Salt solution and water can be stored at room temperature or +4°C.

1. Mix reaction gently and incubate for 5 minutes at room temperature (22-23°C).

   Note: For most applications, 5 minutes will yield a sufficient number of colonies for analysis. Depending on your needs, the length of the TOPO® Cloning reaction can be varied from 30 seconds to 30 minutes. For routine subcloning of PCR products, 30 seconds may be sufficient. For large PCR products (> 1 kb) or if you are TOPO® Cloning a pool of PCR products, increasing the reaction time may yield more colonies.
2. Place the reaction on ice and proceed to **Transforming One Shot® Competent *E. coli***, next page.

   Note: You may store the TOPO® Cloning reaction at –20°C overnight.

Transforming One Shot® Competent *E. coli*

Introduction

Once you have performed the TOPO® Cloning reaction, you will transform your pENTR™ TOPO® construct into competent *E. coli*. One Shot® TOP10 or Mach1™-T1R Chemically Competent *E. coli* (Box 2) are included with the kit to facilitate transformation, however, you may also transform electrocompetent cells (see page x for ordering information). Protocols to transform chemically competent or electrocompetent *E. coli* are provided in this section.

Materials Needed

In addition to general microbiological supplies (*i.e.* plates, spreaders), you will need the following reagents and equipment:

- TOPO® Cloning reaction (from Step 2, previous page)
- One Shot® TOP10 or Mach1™-T1R chemically competent *E. coli* (supplied with the kit, Box 2)
- S. O.C. Medium (supplied with the kit, Box 2)
- pUC19 positive control (to verify transformation efficiency, if desired, Box 2)
- 42°C water bath (or electroporator with cuvettes, optional)
- 15 ml sterile, snap-cap plastic culture tubes (for electroporation only)
- LB plates containing 50 μg/ml kanamycin (two for each transformation)
- LB plates containing 100 μg/ml ampicillin (if transforming pUC19 control)
- 37°C shaking and non-shaking incubator

Note

There is no blue-white screening for the presence of inserts. Most transformants will contain recombinant plasmids with the PCR product of interest cloned in the correct orientation. Sequencing primers are included in the kit to sequence across an insert in the multiple cloning site to confirm orientation and reading frame.

Preparing for Transformation

For each transformation, you will need one vial of One Shot® competent cells and two selective plates.

- Equilibrate a water bath to 42°C (for chemical transformation) or set up your electroporator if you are using electrocompetent *E. coli.*
- Warm the vial of S.O.C. Medium from Box 2 to room temperature.
- Warm selective plates at 37°C for 30 minutes.
- Thaw **on ice** one vial of One Shot® cells from Box 2 for each transformation.

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Transforming One Shot® Competent *E. coli*, continued

One Shot® Chemical Transformation Protocol

Use the following protocol to transform One Shot® TOP10 or Mach1™-T1ᴿ chemically competent *E. coli*.

1. Add 2 µl of the TOPO® Cloning reaction from **Performing the TOPO® Cloning Reaction**, Step 2, page 12 into a vial of One Shot® Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down**.

   Note: If you are transforming the pUC19 control plasmid, use 10 pg (1 µl).
2. Incubate on ice for 5 to 30 minutes.

   Note: Longer incubations on ice seem to have a minimal effect on transformation efficiency. The length of the incubation is at the user's discretion.
3. Heat-shock the cells for 30 seconds at 42°C without shaking.
4. Immediately transfer the tubes to ice.
5. Add 250 µl of room temperature S.O.C. Medium.
6. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
7. Spread 50-200 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
8. An efficient TOPO® Cloning reaction may produce several hundred colonies. Pick 5-10 colonies for analysis (see **Analyzing Transformants**, page 16).

Transformation by Electroporation

Use ONLY electrocompetent cells for electroporation to avoid arcing. Do not use the One Shot® TOP10 or Mach1™-T1ᴿ chemically competent cells for electroporation.

1. Add 2 µl of the TOPO® Cloning reaction from **Performing the TOPO® Cloning Reaction**, Step 2, page 12 into a sterile microcentrifuge tube containing 50 µl of electrocompetent *E. coli* and mix gently. **Do not mix by pipetting up and down. Avoid formation of bubbles.** Transfer the cells to a 0.1 cm cuvette.
2. Electroporate your samples using your own protocol and your electroporator.

   Note: If you have problems with arcing, see the next page.
3. Immediately add 250 µl of room temperature S.O.C. Medium.
4. Transfer the solution to a 15 ml snap-cap tube (*i.e.* Falcon) and shake for at least 1 hour at 37°C to allow expression of the kanamycin resistance gene.
5. Spread 20-100 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µl of S.O.C. Medium. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies.
6. An efficient TOPO® Cloning reaction may produce several hundred colonies. Pick 5-10 colonies for analysis (see **Analyzing Transformants**, page 16).

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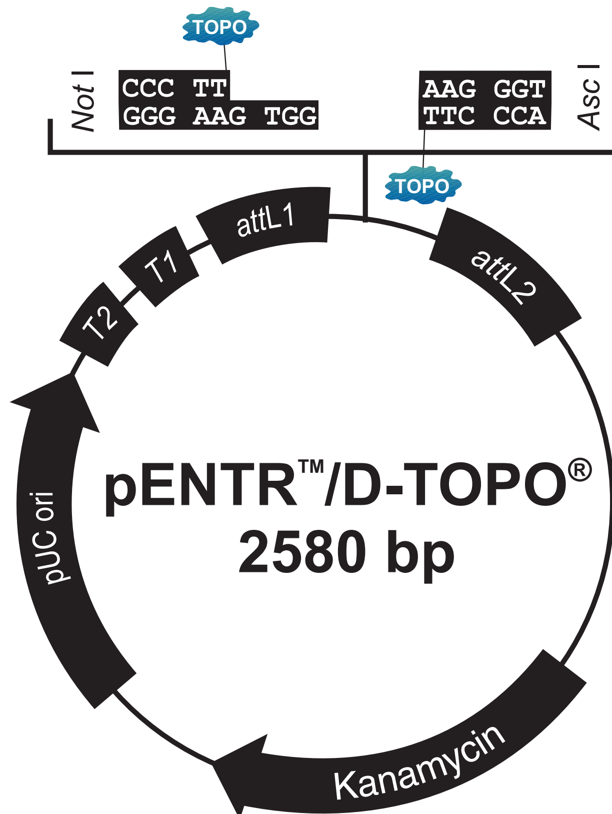
Map and Features of pENTR™/D-TOPO®

pENTR™/D-TOPO® Map

The figure below shows the features of pENTR™/D-TOPO® vector. **The complete sequence of pENTR™/D-TOPO® is available for downloading from www.invitrogen.com)or by contacting Technical Service (see page 35).**



Comments for pENTR™/D-TOPO®
2580 nucleotides

*rrn*B T2 transcription termination sequence: bases 268-295
*rrn*B T1 transcription termination sequence: bases 427-470
M13 forward (-20) priming site: bases 537-552
*att*L1: bases 569-668 (c)
TOPO® recognition site 1: bases 680-684
Overhang: bases 685-688
TOPO® recognition site 2: bases 689-693
*att*L2: bases 705-804
T7 Promoter/priming site: bases 821-840 (c)
M13 reverse priming site: bases 845-861
Kanamycin resistance gene: bases 974-1783
pUC origin: bases 1904-2577

(c) = complementary sequence

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Group	Code		GenBank ID	Plasmid ID	Plate	Row	Col	Rice homolog	TF Family	Primer	Sequence	%GC	length	In Tm	IDT Tm	PCR prod
1 Glenn & Heather	B	33	DR785526	ZM_BFb0002B11.r	2	B	11	LOC_Os02g09480	MYB	B33up	caccatgggagtggagtcggagtgcg	64	22	70.05	66.3	915
										B33dn	cggccgtgtgacgacgcc	78	18	72	64.8	
2 Eric & Lisa	B	21	DR814848	ZM_BFb0044L07.r	44	L	7	LOC_Os04g43680	MYB	B21up	caccatggggagggctccgtgct	68	19	68.64	67.8	873
										B21dn	gccagggaactgaaaattctcg	50	22	64.27	56.3	
3 Rick & Sandy	B	19	DR807759	ZM_BFb0034D02.r	34	D	2	LOC_Os01g24070	C2C2-GATA	B19up	caccatgggctccgccgatcgc	72	18	71.2	67.4	378
										B19dn	tccatgaagcaacgtggatgtg	50	22	66.43	57.9	
4 Jenna & Caitlyn	B	34	DR785591	ZM_BFb0002C21.r	2	C	21	LOC_Os06g09370	bHLH	B34up	caccatgccaccgtcaacctattt	45	20	59.32	59.8	~1203
										B34dn	ccgttcaggagggactgc	67	18	61.82	58.2	
5 Ashley & Ryann	B	35	DR785666	ZM_BFb0002E13.r	2	E	13	LOC_Os09g36250	MYB	B35up	caccatgggaggaaggcgaccg	67	18	65.76	64.5	909
										B35dn	gctagctagaactttgctccgttt	46	24	61.62	57.3	
6 Joe & Brian	B	21	DR814848	ZM_BFb0044L07.r	44	L	7	LOC_Os04g43680	MYB	B21up	caccatggggagggctccgtgct	68	19	68.64	67.8	873
										B21dn	gccagggaactgaaaattctcg	50	22	64.27	56.3	
7 Kevin & Gladys	B	35	DR785666	ZM_BFb0002E13.r	2	E	13	LOC_Os09g36250	MYB	B35up	caccatgggaggaaggcgaccg	67	18	65.76	64.5	909
										B35dn	gctagctagaactttgctccgttt	46	24	61.62	57.3	
8 Mitch & Nicholas	B	24	DV507461	ZM_BFb0185K03.r	185	K	3	LOC_Os04g10260	bZIP	B24up	caccatggactcgacccctccc	67	18	62.29	63.7	1107
										B24dn	gggttcgctcttggcagc	67	18	64.39	59.6	
10 Dionne & Casey	B	25	DT643534	ZM_BFb0102J01.r	102	J	1	LOC_Os03g22770	C2C2-CO-like	B25up	caccatgggtgagggtgacgac	61	18	59.29	62.1	801
										B25dn	tggtttgtcctctgttccaa	45	20	59.11	54.1	
11 Claire & Thomas	B	26	EB820373	ZM_BFb0379H09.r	379	H	9	LOC_Os01g01770	C2H2	B26up	caccatggacagcaggagggagt	58	18	59.65	62.9	1008
										B26dn	gtaatccaggcaccagcagt	55	20	60.14	57.4	
12 Vieh	B	5	DV540768	ZM_BFb0235D23.r	235	D	23	LOC_Os05g48690	Trihelix	B5up	caccatggaggttagggagatggc	55	20	60.29	61.2	1029
										B5dn	gaggaaggtggaggtggag	63	19	59.62	57	
13 Kelly & Shequella	B	9	DR821424	ZM_BFb0060L02.r	60	L	2	LOC_Os08g37810	Trihelix	B9up	caccatggccgacgagcctccg	72	18	69.27	66.8	876
										B9dn	cgagtctccatggtctctcctgg	61	23	66.74	60.5	
14 Amanda	B	24	DV507461	ZM_BFb0185K03.r	185	K	3	LOC_Os04g10260	bZIP	B24up	caccatggactcgacccctccc	67	18	62.29	63.7	1107
										B24dn	gggttcgctcttggcagc	67	18	64.39	59.6	
15 Lucas & Kathryn	B	29	DR804210	ZM_BFb0029D03.r	29	D	3	LOC_Os01g19694	HB	B29up	caccatggaggatctgtacagcatcc	50	20	60.36	60.7	885
										B29dn	gggtccaattgtgcctgtat	50	20	59.68	55.2	
16 Adam & Andrea	B	10	EB166014	ZM_BFb0345D13.r	345	D	13	LOC_Os01g55580	CPP	B10up	caccatggagggcaatgaacagg	53	19	60.88	60.5	~1800
										B10dn	gtttttgtccaaaaggccag	45	20	59.59	52.7	
17 Shuyi and Sarah	B	12	EB166629	ZM_BFb0348O23.r	348	O	23	LOC_Os01g33350	PLATZ	B12up	caccatgaaaagggagacaatgcct	43	21	59.95	59.5	642
										B12dn	aagaatggcgcacgttg	56	18	61.4	53.9	
18 Justin & Bradley	B	31	DV543172	ZM_BFb0238O07.r	238	O	7	LOC_Os02g03030	PHD	B31up	caccatggtcgacgtcatgcgc	61	18	64.4	63.1	702
										B31dn	gcgaggaaaggtcatatcctt	48	21	59.57	54.4	
19 Stephanie & Quinn	B	14	DR810487	ZM_BFb0038D06.r	38	D	6	LOC_Os04g50120	PLATZ	B14up	caccatggcaatcgacgacgaa	50	18	61.22	60	759
										B14dn	gaactcctccaccatgaggc	60	20	62	57.7	
													Avg	63.276	60.347	
													Lowest	59.11	52.7	

Recalcitrant PCRs

Group	Code		GenBank ID	Plasmid ID	Plate	Row	Col	Rice homolog	TF Family	Primer	Sequence	%GC	length	In Tm	IDT Tm	PCR prod
2 Eric & Lisa	B	18	DR804559	ZM_BFb0029K24.r	29	K	24	LOC_Os01g09720	C2C2-Dof	B18up	caccatggttcgctcttccgct	56	18	60.91	62	693
										B18dn	gatggacaggtccggcca	67	18	65.6	60	
7 Kevin & Gladys	B	23	DV505052	ZM_BFb0182D11.r	182	D	11	LOC_Os02g06330	AP2-EREBP	B23up	caccatgcgccgggcgaagccg	78	18	75.92	70.3	591
										B23dn	gaggtggagtagcgtgtcgtagcggg	65	26	73.14	65.7	
8 Mitch & Nicholas	C	14	EE033875	ZM_BFc0095G09.r	95	G	9	LOC_Os01g13520	ARF	C14up	caccatggcgcaaggagcagga	61	18	65.41	64.5	
										C14dn	atagtccctcttcccatcctgc	55	22	62.86	58.2	
14 Kirk & Amanda	B	28	DT645220	ZM_BFb0105C03.r	105	C	3	LOC_Os01g45860	GRAS	B28up	caccatgggcggcgcgttcccctt	70	20	75.47	70.3	1323
										B28dn	gagcggccgcccgtgccag	84	19	77.62	69.9	

Group		Code	DNA Conc ng/ul	A260/ 280	A260/2 30	ORF size (bp)	X ng for ligation	fold dilution for X ng/4ul	Gel picture ul for 200 ng
Section 1									
1 Glenn & Heather	B	33-4	125	1.94	2.05	915	4.6	109	1.6
2 Eric & Lisa*	B	21-3	87	1.96	1.89	873	4.4	80	2.3
3 Rick & Sandy	B	19-2	124	1.92	1.89	378	1.9	262	1.6
4 Jenna & Caitlyn	B	34-3	126	1.93	2.04	1200	6.0	84	1.6
5 Ashley & Ryann	B	35-2	137	1.92	2.01	909	4.5	121	1.5
6 Joe & Brian	B	21-4	127	1.97	1.97	873	4.4	116	1.6
7 Kevin & Gladys*	B	35-4	199	1.97	2.11	909	4.5	175	1.0
8 Mitch & Nicholas*	B	24-4	173	1.94	1.93	1107	5.5	125	1.2
Section 2									
10 Dionne & Casey	B	25-4	124	1.94	1.97	801	4.0	124	1.6
11 Claire & Thomas	B	26-4	186	1.94	1.98	1008	5.0	148	1.1
12 Vieh	B	5-4	109	1.94	2.05	1029	5.1	85	1.8
13 Kelly & Shequella	B	9-4	193	1.95	2.04	876	4.4	176	1.0
14 Kirk & Amanda*	B	24-4	173	1.94	1.93	1107	5.5	125	1.2
15 Lucas & Kathryn	B	29-4	102	1.93	1.98	885	4.4	92	2.0
16 Adam & Andrea	B	10-4	156	1.92	2.05	1800	9.0	69	1.3
17 Shuyi and Sarah	B	12-4	174	1.94	2.15	642	3.2	217	1.1
18 Justin & Bradley	B	31-4	122	1.92	2.01	702	3.5	139	1.6
19 Stephanie & Quinn	B	14-3	136	1.95	1.83	759	3.8	143	1.5

* = different from original assignment

For Cloning reaction each group will use

4ul of diluted PCR product
1ul of salt solution
1 ul of TOPO vector

6ul total volume