FAQ's EZ:TN™ Transposon Tools

Can I sequence my plasmid with this kit?

Can you tell me how the transposon works?

Can I get the transposon to precisely jump back out of my DNA once it has hopped in?

I want to use the EZ::TN in vitro Insertion Kits to sequence a clone that I have, but your standard kits all have antibiotic resistance markers that I can't use. Is there a way that I can still use your system?

The clone I want to sequence is only present in one or two copies in my host cell. My cloning vector already has a kanamycin resistance gene in it, and you say that the Tet-1 transposon isn't really suitable for low-copy clones. What should I do?

My boss won't let me order the EZ::TN Insertion Kit unless he can see some published references for this kit. Are there any papers in the literature about this you can send to me/direct me to?

How much antibiotic should I use with the kit when I'm selecting for my transposition clones?

I'm doing a plasmid rescue using the R6K?/KAN-2 Insertion Kit. Do I HAVE to use your EC 100D cells or can I use DH10B or DH5a as a host for the R6k? ori-containing plasmids? Why can't I use my own E. coli host?
Can I use this kit for mutagenesis of genomic DNA in vitro?

How can I make sure that I only get only 1 transposon per target DNA molecule?

I've got a mixed population of cryptic plasmids I want to transpose with the R6K?/KAN-2 transposon, and the plasmid sizes go from from very small to very large. What's the best way to set up a transposition reaction so that I can get, on average, only 1 transposon per plasmid?

Do you have a list of which organisms have been mutated using Transposomes

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Can I use chemically competent cells with Transposomes?

What are the best conditions to use to electroporate the Transpsome into my bacterium?

Can Transposomes be used with filamentous fungi or yeast?

Can you use transposomes to make knockout mutations in mammalian cells?

How big is the biggest transposon anyone has directly transformed into a bacterial cell?

How many knockout mutants can I expect when I try to mutagenize my particular bacteria?

It takes an awful lot of DNA to transform my particular organism and I'm worried about the cost of the amount of transposon I need to successfully transform my cells, even to get just one mutant. Is there any good way to get around this problem?

The bacterium I want to make a knockout library in is already resistant to the antibiotics you have resistance markers for in your standard transposon offerings. What can I do to make my transposon?

How well do the transposomes work in Gram-positive bacteria?

I'm just getting ready to use the Transposomes for the first time. Are there any particular things I have to watch out for when I do the experiment?

I'm making my own Transposome and I'm concerned about the amount of Transposase I have to use according to your directions. Transposase is expensive and I don't want to use it all in only two custom Transposome preps. Is there any way to scale back on the amount of transposase I need?

I'm doing a gene rescue experiment as you described in an article from the Epicentre Forum. After I isolate the genomic DNA and cut with a restriction enzyme, what kind of ligation conditions should I use?

Making Custom Transposons using pMOD-2 and pMOD-3

What is the best way to purify the transposon away from the pMOD vector?

What is the difference between using the PCR primers supplied with the pMOD vector and using the 19-base ME repeat-based primers

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If I use the PCR primers supplied with the pMOD kit, there will be some short tails at each end of the transposon. Won't that decrease the efficiency of transposition?

How much DNA can I insert between the 19-base MEs?

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Can I sequence my plasmid with this kit?
Yes.

Can you tell me how the transposon works?

The Transposon-plus Transposase forms a “Transposome”. The Transposome binds randomly to some double-stranded DNA, it spans nine base pairs, and Transposase ‘inserts’ the transposon into the DNA.

Can I get the transposon to precisely jump back out of my DNA once it has hopped in?

No. Once it’s in, adding transposase to the DNA will cause a considerable number of random, uncontrollable rearrangements of the DNA.

I want to use the EZ::TN in vitro Insertion Kits to sequence a clone that I have, but your standard kits all have antibiotic resistance markers that I can’t use. Is there a way that I can still use your system?

Yes. You can use either the pMOD-2 or pMOD-3 Transposon Construction Vectors to make your own custom transposon, and Transposome. Call Epicentre’s Technical Services for specific details.

The clone I want to sequence is only present in one or two copies in my host cell. My cloning vector already has a kanamycin resistance gene in it, and you say that the Tet-1 transposon isn’t really suitable for low-copy clones. What should I do?

Use the DHFR-1 Transposon, or create your own transposon using the pMOD-2 or pMOD-3 transposon construction vectors.

My boss won’t let me order the EZ::TN Insertion Kit unless he can see some published references for this kit. Are there any papers in the literature about this you can send to me/direct me to?

Yes. Contact technical support for the current list current list.

How much antibiotic should I use with the kit when I’m selecting for my transposition clones?

See the product literature for the specific kit you are using.

I'm doing a plasmid rescue using the R6K?/KAN-2 Insertion Kit. Do I HAVE to use your EC 100D cells or can I use DH10B or DH5a as a host for the R6k? ori-containing plasmids? Why can't I use my own E. coli host?

You must use the EC100D pir+ or EC100D pir116 cells for the plasmid rescue function. These cells have the pi (p) gene, which is necessary for replication of a plasmid with the R6k? origin of replication.

Can I use this kit for mutagenesis of genomic DNA in vitro?

Yes. The same "rules" will apply for the insertion reactions as far as molar equivalents of DNA required for balancing the amount of transposon and target genomic DNA.

How can I make sure that I only get only 1 transposon per target DNA molecule?

Follow the calculation in the product literature. Make sure the target DNA is accurately quantitated by fluorimetry (NOT OD260)

I've got a mixed population of cryptic plasmids I want to transpose with the R6K?/KAN-2 transposon, and the plasmid sizes go from from very small to very large. What's the best way to set up a transposition reaction so that I can get, on average, only 1 transposon per plasmid?

While this can be tricky, one approach would be to try to make a "weighted average" of the sizes of the DNA in bases, and try to get a rough proportion of the concentrations of the plasmids. Once the "weighted average" is determined, you can estimate how much transposon you will need to try to achieve a 1:1 balance of transposon to target.

Do you have a list of which organisms have been mutated using Transposomes.

The list presented below is what we know has been done, but you can be sure that there are lots of other bacteria and yeast being tried that we don't know about yet!!!
Many labs are making their own Transposomes for creating gene knockouts in vivo, using the pMOD-2 or pMOD-3 Transposon Construction vectors. Some of these Transposomes are given in the list .

"*Transposome Hit List"*

group	Species	Transposon/Antibiotic Res
Gram negative	"Gasoline isolates"*	???
Gram negative	Acinetobacter baumanii	Kan
Gram negative	Actinobacillus pleuropneumonii	Kan
Gram negative	Aeromonas hydrophila	Kan
Gram negative	Agrobacterium tumefaciens	Kan
Gram negative	Bartonella licheniformis	Kan
Gram negative	Campylobacter jejuni	???
Gram negative	Campylobacter jejuni	Kan
Gram negative	Desulfovibrio spp. (anaerobe)	Kan
Gram negative	E.coli	Kan, Chl, Tmp
Gram negative	E.coli (Enterotoxigenic)	R6Kgamma/KAN-2
Gram negative	Haemophilus influenzae	KAN, Chlor
Gram negative	Helicobacter pylori	Kan?
Gram negative	Neisseria Gonorrhoeae	R6Kgamma/KAN-2
Gram negative	Proteus mirabilis	R6Kgamma/KAN-2
Gram negative	Proteus vulgaris	Kan
Gram negative	Pseudomonas aeruginosa	??
Gram negative	Pseudomonas butanovora	Kan
Gram negative	Pseudomonas mendocina*	Kan
Gram negative	Pseudomonas sp. (MMSS5)	Kan
Gram negative	Pseudomonas syringae	lon::Nal, Spect, Kan???
Gram negative	Pseudomonas syringae	R6Kgamma/KAN-2
Gram negative	Roseobacter spp.	
Gram negative	Rubrivivax gelatinosus*	Kan
Gram negative	Salmonella typhimurium	Kan
Gram negative	Serratia marsescens	
Gram negative	Shigella spp.*	Kan
Gram negative	Sphingomonas spp. *	Kan
Gram negative	Xanthomonas campestris	Kan
Gram negative	Xylella fastidiosa (plant path.)	Kan
Gram positive	Clavibacter michiganensis	Kan
Gram positive	Corynebacterium diptheriae	Kan
Gram positive	Mycobacterium bovis BCG	Kan
Gram positive	Mycobacterium smegmatis	Kan
Gram positive	Mycobacterium tuberculosis	Kan
Gram positive	Mycobacterium ulcerans	Kan
Gram positive	Rhodococcus equi	Kan
Gram positive	Rhodococcus erythropolis	Kan
Gram positive	Rhodococcus opacis	Kan
Gram positive	Rhodococcus rhodochrous	Kan
Gram positive	Staphylococcus aureus*	?
Gram positive	Streptococcus pyogenes	Spectinomycin
Gram positive	Synechococcus spp.	Kan

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Can I use chemically competent cells with Transposomes?
No.

What are the best conditions to use to electroporate the Transpsome into my bacterium?

These conditions must be determined experimentally for each microorganism. Contact the supplier of the electroporator for recommendations. There is some evidence that suggests that lipid-based transfection reagents can be used to get Transposomes into cells but we are not sure how well it works. Testing is underway.

Can Transposomes be used with filamentous fungi or yeast?

No – not with sufficient efficiency to make the experiments worthwhile.

Can you use transposomes to make knockout mutations in mammalian cells?

No. The main problem is getting the Transpsome through both the cell membrane and the nuclear membrane and normal transfection reagents are not effective at doing this. Some of our customers are trying microinjection directly into the nucleus but there is no data available the indicates that this really works. Some people also try using electroporation or transfection reagents... but these techniques only get the Transposome into the cytoplasm where only transient expression of the resistance marker occurs...and the expression will fade away within 20-24 hours.

How big is the biggest transposon anyone has directly transformed into a bacterial cell?

Just under 11,000 bp.

I've got the knockout mutant I'm looking for and I want to sequence the knockout mutant directly. How do I do this?

See the EPICENTRE Forum, Volume 6, Number 3, Use of EZ::TN Transposomes for Genetic Analysis and Direct Sequencing of Bacterial Genomic DNA. We can also provide a couple of literature references in pdf form by e-mail.

How many knockout mutants can I expect when I try to mutagenize my particular bacteria?

The number of mutants you'll get is dependent upon the transformation efficiency, the expression of the antibiotic resistance gene in the cell and the presence/absence of Type I or Type II resitriction modification systems.

It takes an awful lot of DNA to transform my particular organism and I'm worried about the cost of the amount of transposon I need to successfully transform my cells, even to get just one mutant. Is there any good way to get around this problem?

Scale down the transposome synthesis reaction and/or reduce the amount of transposome you are using in the electroporation.
EZ::TN Transposome Kits (continued)

The bacterium I want to make a knockout library in is already resistant to the antibiotics you have resistance markers for in your standard transposon offerings. What can I do to make my transposon?

You can make your own transposon quite easily using the pMOD-2 and pMOD-3 transposon construction vectors

How well do the transposomes work in Gram-positive bacteria?

They can work quite well. For examples, see the "transposome hit list" on a previous page.

I'm just getting ready to use the Transposomes for the first time. Are there any particular things I have to watch out for when I do the experiment?

See above. Make sure your electroporator is set to the proper conditions and use only enough Transposome to get the results (1 µl or less). Any more may cause arc-ing in the transformation process. And, make sure you are using the concentration of antibiotic in the selective medium that's appropriate for your organism.

I'm making my own Transposome and I'm concerned about the amount of Transposase I have to use according to your directions. Transposase is expensive and I don't want to use it all in only two custom Transposome preps. Is there any way to scale back on the amount of transposase I need?

See above about scaling down the transposome formation reaction, and using less transposome in the electroporation.

I'm doing a gene rescue experiment as you described in an article from the Epicentre Forum. After I isolate the genomic DNA and cut with a restriction enzyme, what kind of ligation conditions should I use?

You will need to look at ligation conditions that favor re-circularization rather than "concatenation", and this will depend on the average fragment size and

concentration of the digested chromosomal DNA. See the article from the Epicentre Forum (8:3): “Rapidly Rescue Clone Transposed Genomic DNA”.

Making Custom Transposons using pMOD-2 and pMOD-3

What is the best way to purify the transposon away from the pMOD vector?

It will depend on the nature of the transposon, but restriction digestion with PvuII/PshAI or PCR are the two best methods
.

What is the difference between using the PCR primers supplied with the pMOD vector and using the 19-base ME repeat-based primers?

PCR using the supplied PCR primers will leave short DNA tails outside the Mosaic ends, while PCR with the 19-base ME primers will leave a blunted end right at each end of the transposon. Now, that said, PCR using the 19-base ME-primers will make a good transposon, but this type is best used in vitro. The reason is that the Taq (or whatever PCR enzyme you are using) will “nibble” at the ends of the MEs a bit, This degradation is not fatal, but can render the MEs unsuitable by dropping efficiencies quite significantly! A remedy for this would be to use the End-IT™ End-Repair Kit Epicentre sells to repair the damaged ends. For Transposome applications, we suggest PCR using the forwrd and reverse PCR primers along with the pMOD-2 and pMOD-3 kits to make the transposon product and then polish the ends with PvuII or PshAI.

If I use the PCR primers supplied with the pMOD kit, there will be some short tails at each end of the transposon. Won’t that decrease the efficiency of transposition?

The difference in transposition efficiencies using either method will differ a little but not significantly.

How much DNA can I insert between the 19-base MEs?

We have heard of people inserting as much as 11 kb between the MEs but it must be remembered that the larger the transposon, the lower the transformation and transposition efficiencies.