
# Minicircle DNA Vector Technology
# Cat. #MNXXX Series

***User Manual***

Check Individual PACs for storage conditions

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ver. 4-030812

# Contents

# I. Introduction and Background

## A. The Minicircle Technology

Minicircles (MC) are circular DNA elements that no longer contain antibiotic resistance markers or the bacterial origin of replication. These small vectors can be used *in vivo* or *in vitro* and provide for long-term transient expression of one or more transgenes without the risk of immunogenic responses that can be caused by the bacterial backbone in standard plasmids.

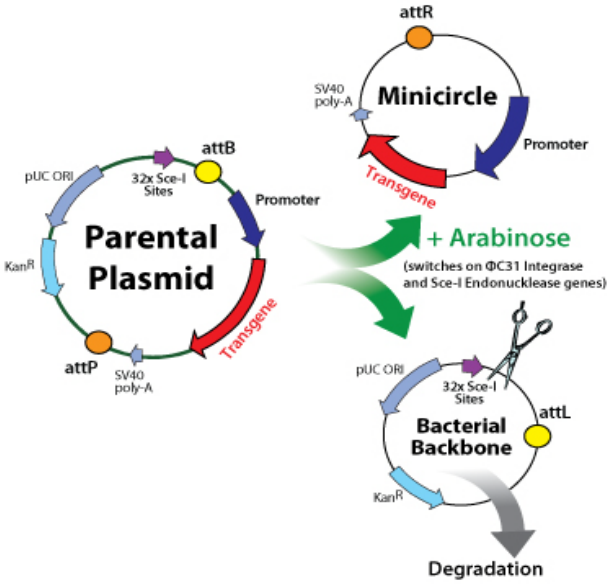
Production of minicircles requires a special parental plasmid and an engineered *E. coli* strain that allows both propagation of the parental plasmid and the production of the minicircles. Minicircles are conditionally generated by an expression of inducible ΦC31 integrase via intramolecular (cis-) recombination.

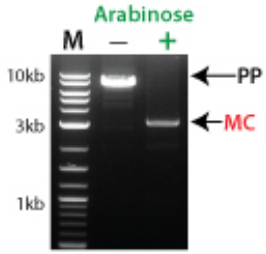
The full-size MC-DNA construct is grown in a special host *E. coli* bacterial strain that harbors an Arabinose-inducible system to express the ΦC31 integrase and the I-SceI endonuclease simultaneously. The ΦC31 integrase produces the MC-DNA molecules and PP-DNA from the full-size MC-DNA upon arabinose induction. The PP-DNA contains a number of engineered I-SceI restriction sites that are subject to the digestion of I-SceI endonuclease and ultimate destruction of the PP-DNA. The MC-DNA is lacking I-SceI restriction site so that it remains intact. The 32 copies of I-SceI sites in the PP-DNA secure the production of superclean MC-DNA without PP-DNA contamination. This bacterial strain produces purified MC-DNA in a time-frame and quantity similar to those of routine plasmid DNA preparation.

Minicirle DNA vectors allow sustained trangene expression in quiescent cells and tissues. These vectors have been demonstrated 10- to 1,000-fold enhancement compared to regular plasmids in long-term transgene expression in quiescent tissues in vivo and in vitro. The mechanism of enhanced transgene expression may result from eliminating heterochromatin formation

induced by the plasmid backbone and methylation and transgene silencing.

The major obstacle to the widespread use of mincircles has been the time-consuming, labor-intensive production. The MC-Easy™ System from SBI enables a simple, reproducible and efficient way to produce high quality Minicircle DNA. The finely tuned growth and induction media produces minicircle DNA that is free of parental and genomic DNA contamination. The Kit also includes an additional, innovative method for degrading any contaminating genomic DNA using an ATP-dependent DNAse reagent that will selectively remove genomic DNA but will ot affect minicircle DNA yield. This method produces clean and effective minicircle every time. http://www.systembio.com/minicircle-dna-vectors/literature





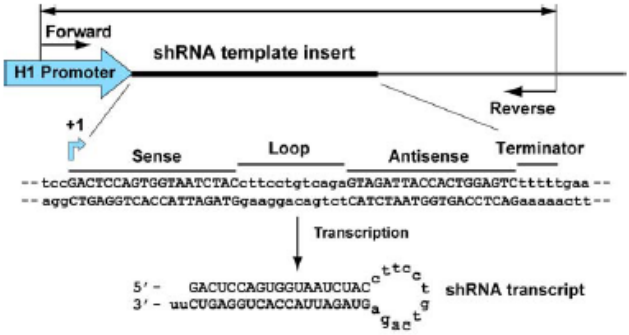

## B. ZYCY10P3S2T *E.coli*

ZYCY10P3S2T *E.coli* Minicircle producer competent cells have been prepared and tested by a modification of the procedure of Kay (Kay MA, He CY, Chen ZY. 2010, Nature Biotechnology). These cells are suitable for the cloning of minicircle-based plasmids. ZYCY10P3S2T *E.coli* minicircle producer cells are derived from a BW27783 bacterial strain that stably expresses a set of inducible minicricle-assembly enzymes, ΦC31 integrase and I-SceI homing endonuclease. This bacterial strain produces purified minicircles in a time frame and quantity similar to those of routine plasmid DNA preparation, making it feasible to use minincircles in place of plasmids in mammalian transgene expression studies.

**Note: Other competent *E. coli* strains cannot be substituted for minicircle production as they do not have the appropriate genome modifications to produce minicircles.**

## C. Minicircle shRNA Expression Vectors

The Minicircle shRNA parental plasmids contain either a CMV or EF1 promoter that drive expression of GFP or GFP and Puromycin resistance, followed by an H1 promoter that drives expression of the shRNA construct.




Typically, 3-5 target sequences in the gene of interest need to be selected and tested to identify functional shRNAs with at least 70% silencing efficiency of the target mRNA. Although there is no standard rule for selecting the target mRNA binding sites for shRNA sequences, we have found the following criteria useful:

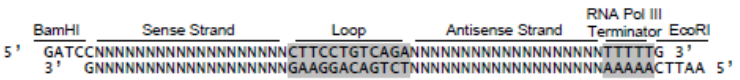
- 19-29 nt in length, usually longer oligos (25-27 nt) are more robust and give better silencing efficiencies although 19 nt oligos could be also used.
- Unique sequences with less than 70% homology with other mRNA sequences in a RefSeq database. Especially avoid homology to other non-target mRNA sequences in the central portion of shRNA. Flanking sequences usually tolerate mismatches without reduction in silencing efficiency.
- 40-55% GC content
- No more than 4 consecutive A's or T's.
- No more than 5 consecutive G's or C's
- No thermodynamically stable secondary structure (0 Kcal/mol)
- A 5' terminus (3-5 flanking nucleotides) on the antisense strand should be more AT-rich than the 3' terminus.

The template sequences coding for the shRNA targeted to each selected target site must contain both the sense and anti-sense strand, and be designed to form a stem-loop structure when transcribed. In addition, both the top and bottom strands of the entire shRNA sequence (sense-loop-antisense-terminator) must be synthesized and annealed to make a double-stranded DNA

sequence that can be cloned into the vector. The features of the oligonucleotides coding for the shRNA template sequence should include the following:

- The 19-29 nucleotide sense and antisense mRNA sequences. Usually longer siRNAs (25-27 nt) have better silencing efficiencies although 19-nt oligos are more commonly used.
- A hairpin loop sequence between sense and antisense portion. The 9-nt loop sequence (5'-TTCAAGAGA-3') is most commonly used in RNA silencing experiments, but we have used a 12-nt sequence (5'-CTTCCTGTCAGA-3'), which generates similar results. Loop sequences of 3 to 15 nucleotides have been used successfully by different investigators.
- A TTTTT terminator sequence for RNA polymerase III.
- A BamHI and EcoRI restriction site overhang sequences for directional cloning of annealed shRNA template oligonucleotides into the vector.
- Using of initiation G nucleotide in the first position of sense portion of SHRNA is not necessary, as RNA polymerase III could initiate transcription from any +1 nucleotide of the H1 promoter.

The top and bottom strands of the shRNA template oligonucleotides should be designed to look like the following diagram after annealing:



# II. Minicircle Cloning Protocols

## A. Cloning into Minicircle Parental Plasmids

1) Clone your insert-of-interest into the parental plasmid of choice either by using the multiple cloning site, or by using SBI's Cold Fusion cloning kit (Cat #MC010, MC100, or MC101).
2) Sequences of the multiple cloning sites for the different parental plasmid vectors can be obtained from SBI.
3) Your insert should contain a Kozak sequence, ATG start site, and stop codon.
4) Propagation of the parental plasmid during cloning and screening can be accomplished with any strain of competent *E. coli* or you can use the ZYCY10P3S2Tcells directly.

## B. Cloning into Minicircle shRNA Parental Plasmids

1) Linearize the MNSI vector with EcoRI/ BamHI. Digest the plasmid overnight at 37°C. Then purify the plasmid DNA.

2) Anneal the shRNA template oligonucleotides

    a. Use regular non-phosphorylated oligos. Dissolve the shRNA template oligonucleotides in an appropriate amount of deionized water to a final concentration of 20 µM.

    b. Set annealing reactions for each experimental shRNA template

        1 µl Top strand shRNA template oligo
        1 µl Bottom strand shRNA template oligo
        18 µl 10 mM Tris-HCl, pH 8.5
        20 µl Total Volume

    c. Heat the reaction mix to 95°C for 2 min in a thermocycler.

d. Turn off the thermocycler and let it cool to room temperature over 20 minutes.

3) Ligate the shRNA template into the linearized minicircle shRNA parental plasmid.

a. Set up a ligation reaction for each shRNA template

1 µl linearized vector
1 µl annealed double-stranded shRNA template
1 µl 10x T4 DNA ligase buffer
6 µl deionized water
1 µl T4 DNA ligase (40 U/ µl)
10 µl Total Volume

b. Incubate the ligation reaction at 16°C overnight.

4) Propagation of the parental plasmid during cloning and screening can be accomplished with any strain of competent *E. coli*.

# III. Minicircle Production Protocol

The MC-Easy™ Minicircle DNA Production kit provides a simple, efficient way to produce high quality minicircle DNA.

The most common problems for producing Minicircle DNA are parental and genomic DNA contamination. This kit offers an easy and reliable solution to eliminate genomic and parental DNA contamination. The system comes complete with a special minicircle DNA transfection reagent, MC-Fection™ that works well with most cell lines. Choose from the minicircle production kit that includes the engineered *E. coli* producer strain ZYCY10P3S2T (cat# MN920A-1) or select the minicircle production and isolation kit alone (MN910A-1).

## A. Contents

The MC-Easy Minicircle DNA Production kit provides enough reagents for 5 minicircle production preparations.

| | |
|---|---|
| 5X Minicircle Growth medium | 200 ml |
| 10X Induction Medium | 200 ml |
| Endotoxin-free water | 5 ml |
| Minicircle-safe DNase | 125 µl |
| ATP (25mM) | 125 µl |
| 10X DNase buffer | 250 µl |
| Precipitation Buffer | 0.5 ml |
| 2 ml DNA Centrifugal Filter Columns | 5 filters |
| MC-Fection (transfection reagent for minicircle DNA) | 100 µl |
| ZYCY10P3S2T E.coli Minicircle Producer Strain (***included in Cat# MN920A-1***) | 5 vials |

**Other Reagents Needed (but not included in the kit)**

- Sterile deionized water
- 100% Ethanol
- LB agar + Kanamycin plates

## B. Transforming ZYCY10P3S2T *E. coli* Minicircle producer strain (MN900A-1):

1) Thaw competent cells on ice.

2) Add DNA from ligation reaction, using 1-5 µl of the reaction to one vial of ZYCY10P3S2T cells moving the pipette through the cells while dispensing. Gently tap tubes to mix.

3) **Alternatively, for parental plasmid transformation add 20-100 ng DNA to the vial.**

4) Incubate cells on ice for 30 minutes.

5) Heat-shock cells for 30 seconds in a 42°C water bath. Do not shake.

6) Place cells on ice for 2 minutes.

## C. Recovery

**This procedure is very important for Kanamycin resistant constructs.**

1) Add 0.2 ml of room temperature S.O.C. Medium to the vial. Transfer to a bacterial culture tube.

2) For tubes containing ligation reactions, shake at 250 rpm (30°C or 37°C) for 90 minutes.

3) For tubes containing parental plasmid DNA: shake at 250 rpm (30°C or 37°C) for 60 minutes.

## D. Plating and Mini-Preps

1) Pre-warm culture plates to 37°C. Chilled plates will decrease ZYCY10P3S2T *E coli* transformation efficiency.

2) Spread 50-200 µl of bacterial solution on an LB plate containing 50 µg/ml kanamycin. Incubate overnight at 37°C.

3) Keep the remaining solution at room temperature, and spread onto a new plate the next day in case no colonies are seen.

4) Pick 3-5 colonies to grow in 2 ml of LB containing 50 µg/ml kanamycin. Grow overnight and then extract the plasmid by miniprep.

5) Check the minicircle parental plasmid by restriction digest analysis and sequencing.

6) If the parental plasmid looks correct, make glycerol stocks from the miniprep bacterial culture and store them at -80°C. You are now ready to produce minicircle DNA from the parental plasmid DNA.

## E. Growth and Induction of the Minicircle Producer Strain

The following steps describe production of minicircle DNA from the parental minicircle plasmid which was produced in the previous steps.

**Day 1: Inoculation**

1) On day 1 at about 5:00 PM, inoculate 2 ml of LB containing 50 µg/m kanamycin with a single colony or an inoculation loop of glycerol stock bacteria. Incubate at **30°C** shaking at 250 rpm for 1-2 hours.

**Note: The bacteria must be fresh. LB agar + kanamycin plates stored at 4°C are only fresh for 1 week. Glycerol stocks are good for up to one year.**

2) After 1-2 hrs measure the $OD_{600}$ to calculate the inoculate volume. Use the following formula to calculate inoculate volume:

$$Inoculate\ volume\ (ml) = \frac{2 \times 10^{-4}}{OD_{600}}$$

For example, if the $OD_{600}$ reading is 0.01, the inoculate amount is:

$$Inoculate\ volume\ (ml) = \frac{2 \times 10^{-4}}{0.01}$$

$$Inoculate\ volume\ (ml) = 0.02\ ml = 20\ \mu l$$

3) Dilute 5X Growth medium to 1X:

160 ml of sterile deionized water
+40 ml of 5X Growth Medium
**200 ml Total volume 1X Growth Medium**

4) Inoculate 200 ml of 1X Growth Medium with inoculate volume calculated in step 2.

5) Grow bacteria overnight at 30°C, shaking at 250 rpm in a 1 liter sterile flask*. The overnight culture should not go beyond 16 hrs.

***If using a different sized flask for the overnight culture, keep the ratio of flask size to O/N culture volume at 5:1 (vol:vol).**

**Day 2: Induction**

1) On Day 2 after about 16 hrs of overnight growth, measure the pH and $OD_{600}$ of the culture medium. The pH should be around 7 and the $OD_{600}$ should be between 4-6. **Record the $OD_{600}$ and the pH in your laboratory notebook in case you need to troubleshoot later.**

    a. If the $OD_{600}$ > 8 and the pH < 6.5, you **must** restart the protocol from the beginning. Check the temperature and ventilation of the incubator.
    b. If the $OD_{600}$ is between 6 and 8, it is okay to proceed to the step 3.

2) If the $OD_{600}$ was between 4-6, combine 200 ml of the overnight culture with 200 ml of 1X Induction medium.

    a. Add 20 ml of 10X Induction medium and 180 ml of sterile deionized water (room temperature).

    b. The pH should now be between 6.9 and 7.4. If the pH is <6.9, add NaOH to adjust the pH to 7.

3) If the $OD_{600}$ was between 6-8, combine 200 ml of the overnight culture with 400 ml of 1X Induction medium.

    a. Add 40 ml of 10X Induction medium and 360 ml of sterile deionized water (room temperature).

    b. Divide the culture (600 ml total) into two 1-liter sterile culture flasks (300 ml each).

    c. The pH should now be between 6.9 and 7.4. If the pH is <6.9, add NaOH to adjust the pH to 7.

4) Incubate the mixture again at 30°C, shaking at 250 rpm for 5- 5.5 hours. Longer induction times may increase bacterial death and cause genomic DNA contamination.

5) Take 1ml of the bacterial culture and do a miniprep followed by restriction digest analysis to check the quality of minicircle plasmid.

6) Pellet bacterial at 4°C and store the pellet at -20°C overnight.

**Day 3: Extraction of Minicircle plasmid**

1) Follow protocol of Invitrogen's PureLink HiPure Plasmid Purification Kit but use 2-3 Maxi columns for each minicircle construct produced.

2) Alternatively, use the QIAGEN MegaPrep Kit protocol with one column, but use double the volume of P1, P2 and P3 Buffers.

3) Digest 0.5 µg minicircle plasmid with a restriction enzyme and run an agarose gel to check the quality again.

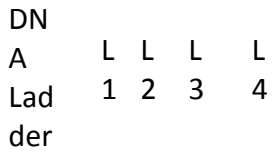
4) If the quality is good, go to the transfection procedure offered in Section I to check the function.

5) If the minicircle preparation contains genomic DNA or parental DNA or both, continue with the next steps (Sections F-H) of this protocol.

    a. If the parental DNA contamination is more than 10% higher than the minicircle DNA yield, you must start again. Please refer to the troubleshooting section for more details.

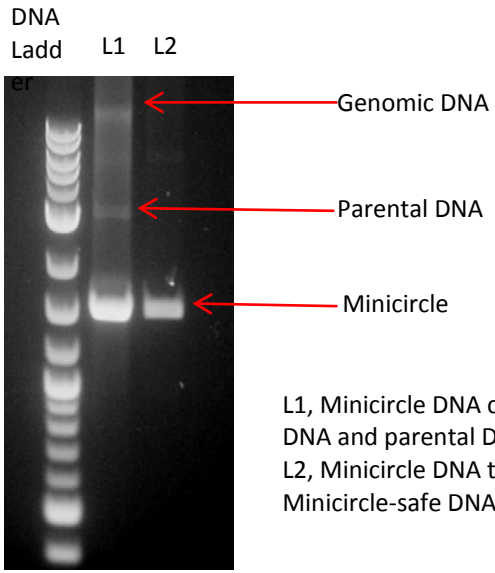
b. It is okay to proceed with excess genomic DNA contamination (in the absence of parental DNA contamination).

This is an example of a purified minicircle plasmid that does not have any genomic or parental plasmid contamination. If your preparataion looks like this, you can proceed by transfecting the minicircles into your cells-of-interest.




L1, Parental DNA of SRM100PA-1 cut by Mfe1
L2, L3,L4, Minicircle DNA of SRM100PA-1 cut by Mfe1

This is an example of a minicircle plasmid that has both genomic DNA and parental plasmid contamination. If your minicircle preparation looks like this, you will need to perform the Removal of Genomic and Parental Plasmid DNA contamination steps (Section F.) before transfecting it into your cells-of-interest.




L1, Minicircle DNA contaminated with genomic DNA and parental DNA.
L2, Minicircle DNA treated with Nde1 and Minicircle-safe DNAse.

## F. Removal of Genomic and Parental Plasmid DNA contamination

Parental DNA and genomic DNA contamination are common problems in minicircle DNA production. Follow this protocol to remove parental plasmid and genomic DNA contamination.

1) Linearize parental DNA with 1 or 2 restriction enzymes that cut the bacterial backbone without cutting inside the minicircle DNA. You should use a plasmid editor software to help you choose the appropriate restriction enzymes.

2) Then use Minicircle-safe DNase to digest the linearized DNA, according to the protocol below. (Minicircle-safe DNase is included in the kit.)

~400 µl plasmid (use the whole amount you obtained in the last step)
20 µl 25 mM ATP
50 µl 10X Reaction Buffer
20 µl DNase
~10 µl water
500 µl Total Reaction Volume

Incubate at 37°C for 2-16 hours

3) Inactivate DNase by incubation at 70°C for 30 minutes.

4) Check Result: Take 1-2 µl from DNase-treated and untreated samples. Digest them with same restriction enzyme and run agarose gel to check the result. If the reaction was successful, you should see minicircle DNA present, but no parental plasmid or genomic DNA contamination in the sample.

## G. Removal of in-activated DNase and Restriction Enzymes

1) Add 50 µl of Precipitation Buffer (1:10 the volume of the reaction in the previous step) to the DNase-treated solution and mix by pipetting.

2) Add 1.1 ml of 100% ethanol and mix by inverting.

3) Put on ice for 30 min.

4) Centrifuge at 15000x *g* for 15 minutes. Carefully discard supernatant. Do not disturb DNA pellet.

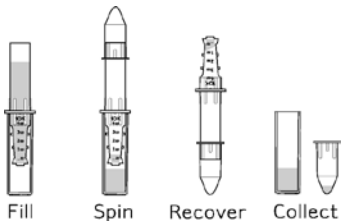
5) Dissolve plasmid with 1-2 ml of TE buffer.

6) Optional: Continue to next step using 2ml DNA Centrifugal column to remove dNTPs and salts.

7) Alternatively, if a the ATP and dNTPs do not influence your downstream experiment, you can wash pellet with 1 ml of 70% ethanol. Centrifuge at 15000x *g* for 5 minutes. Discard supernatant. When pellet is dry, resuspend in endotoxin-free water (supplied) or endotoxin-free TE buffer.

## H. Optional: Removal of ATP and dNTPs with 2ml DNA Centrifugal columns

2ml DNA Centrifugal columns can be used to remove dNTPs, primers, other macromolecular components, salts and buffers.



1) Insert the 2ml DNA Centrifugal filter into a filtrate collection tube.

2) Add up to 2 ml of sample to the device and cover with concentrate collection tube.

3) Place filter device into the centrifuge rotor and counterbalance with a similar device.

    a. For a swinging bucket rotor, spin at 1,000x *g* for 30 min or 3000x *g* for 10 min.

    b. For a fixed angle rotor, spin at 7,500 × *g* for 10-30 min.

4) Wash step: add 2 ml of the buffer you want and repeat spin procedure.

5) Remove the assembled device from the centrifuge and separate the 2ml DNA Centrifugal column from the filtrate collection tube.

6) To recover the concentrated solute, invert the filter column to concentrate collection tube. Spin for 2 minutes at 300-1,000 × *g* to transfer the concentrated sample from the column to the tube. NOTE: For optimal recovery, perform the reverse spin immediately.

7) Measure plasmid concentration and check A260/A280 ratio. The A260/A280 ratio should be 1.8-1.9.

## I. Transfect Minicircle DNA with MC-Fection™ to validate the construct

1) Seed 1.5X10$^5$ target cells/ ml per well in a 12-well plate. SBI uses 293T cells, but you can use any target cells that you prefer.

2) Incubate overnight at 37°C. Cells should be 50%-70% confluent the next day.

3) In a sterile Eppendorf tube add the following (per well):
   100 µl DMEM (without FBS)
   1 µg Minicircle DNA
   2.5 µl MC-Fection™

4) Mix by vortexing vigorously for a few seconds. Incubate at room temperature for 15 min.

5) Add the mixture to one well of a 12-well plate and incubate at 37°C, 5% $CO_2$ overnight.

6) Check for fluorescent reporter protein expression (e.g. GFP or RFP), or other marker gene, or protein-of-interest expression.

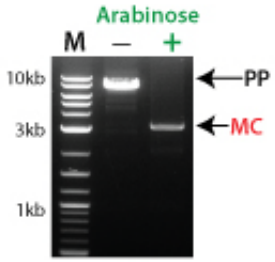
## J. Tail Vein injection of Minicircles for *in vivo* expression of transgenes

Minicircle DNA can be injected into mice by tail vein injection for *in vivo* expression of transgenes. We recommend following the protocols set up by your institution's animal handling facility.

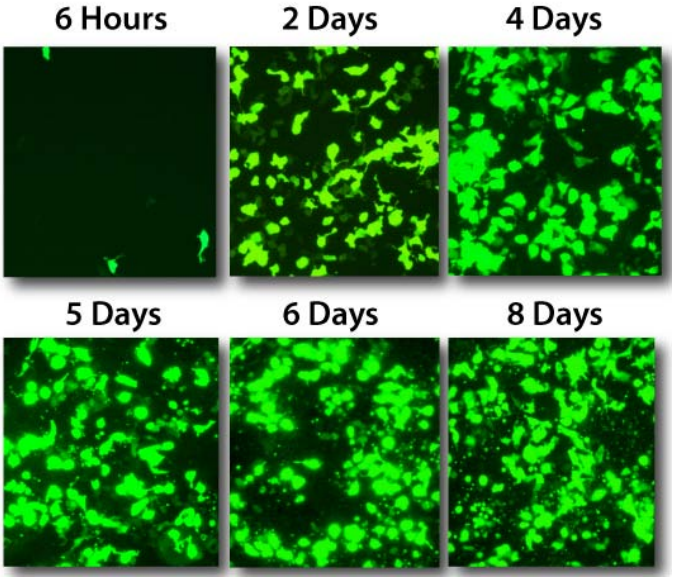

Tail vein
injection

# IV. Example Data

The gel image below shows uninduced (minus arabinose) and induced (plus arabinose) ZYCY10P3S2T *E. coli*. The resulting minicircles (~3kb) are produced.

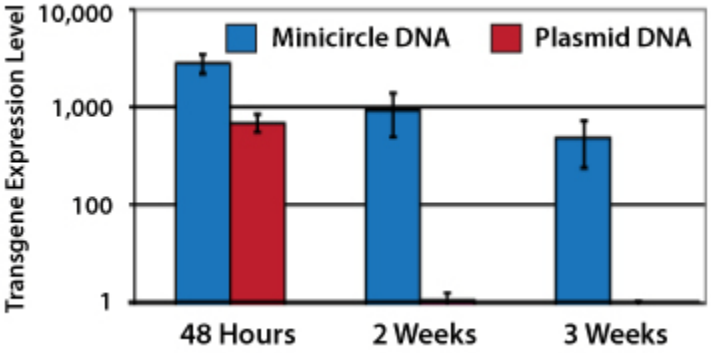



Transfection of 1 µg minicircle DNA (pMC.CMV-MCS0EF1-GFP-SV40PolyA) into HEK293 cells. Expression of GFP persists for at least 8 days.




Example data of *in vivo* transgene expression in a mouse injected with minicircle DNA through tail-vein injection. Plasmid DNA is

degraded after 2 weeks of expression, while minicircle DNA expression remains strong for at least 3 weeks.



# V. Troubleshooting

The most common problems in minicircle production are:

1. Genomic DNA contamination
2. Parental DNA contamination
3. Low yield

| Problems | Possible causes | Solutions |
|---|---|---|
| **Genomic DNA contamination:** | Bacteria over growth. | Shorten culture time and reduce the amount of inoculum. Check ventilation and shake speed. |
| | Bacteria is not fresh or was stored at $4^0$C for > 1 week. | Always use fresh bacteria. |
| | Ventilation is not good. | Keep the ratio of flask size to culture volume at 5:1 (vol:vol). |
| | Culture medium pH is too low or incubation time is too long. | Keep the culture pH >6.5. |
| | Mechanical damage to bacteria, such as vigorously vortexing after freezing. | Resuspend bacteria pellet carefully and completely after freezing by gently vortexing and pipetting up and down. |
| | Lysis time > 5min. | Invert bottles gently after adding lysis buffer and keep at RT <5min. |

| | | |
|---|---|---|
| | Insufficient quantity of SDS in lysis buffer to permit complete binding to the cellular debris. When the buffer is stored at temperatures below 20°C, which causes the SDS to precipitate out of solution. | Warm Lysis buffer at $50^0$C a few minutes to dissolve the SDS completely. |
| | Solution was not mixed soon enough after the addition of buffer P3. This could result in the formation of clusters of PDS (potassium dodecyl sulfate) together with cellular debris including genomic DNA. These clusters have a tendency to float. If these floating pieces are added to a column then genomic DNA contamination almost definitely will occur. | Mix immediately after adding Buffer P3 (or N3, or S3) by inverting vigorously 4-6 times. Remove floating pieces by filter.<br><br>Do not overload the column beyond its capacity. |
| **Parental DNA contamination** | Bacteria is stored at $4^0$C for more than one week. | Always use fresh bacteria. |

| | | |
|---|---|---|
| | pH <6.5 before induction. | Check pH and correct to >6.5 before induction. |
| | Cold induction medium or temperature during induction is too low. | Use room-temperature induction medium. |
| | Induction time is not enough or too long. | Adjust induction time. |
| | >10% parental plasmid contamination | Start the protocol from the beginning. |
| **Low yield** | The plasmid failed to precipitate, which is especially common when the size of minicircle DNA <3.5 kb. | Use high speed centrifuge 15000G for 30-60min to precipitate small size minicircle DNA. |
| | Bacteria was not resuspended or lysed completely. | Use double amount of resuspension buffer, lysis buffer and neutralization buffer.<br><br>Consider adding LyseBlue reagent to resuspension buffer. Solution should turn evenly blue after adding lysis buffer and inverting 4-5 times. |

# VI. References

Kay et al. A robust system for production of minicircle DNA vectors. Nature Biotechnology. 2010

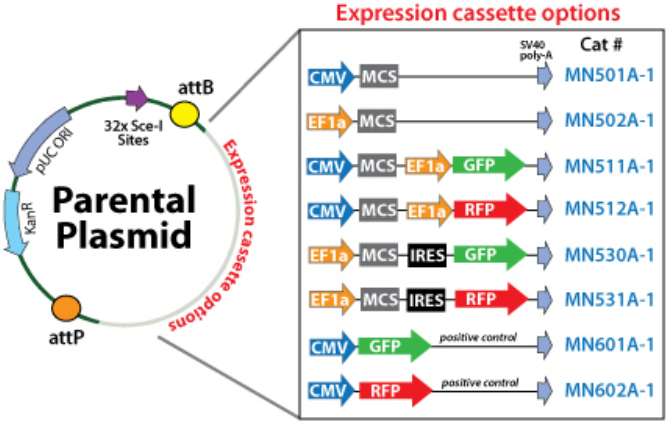
Fangjun Jia et al. A nonviral minicircle vector for deriving human iPS cells. Nature Methods 2010 Mar;7(3):197-9.

Zhi-Ying Chen et al. Improved production and purification of minicircle DNA vector free of plasmid bacterial sequences and capable of persistent transgene expression in vivo. Human Gene Therapy 16 (1) 126-131 (January 2005).

Zhi-Ying Chen et al. Minicircle DNA Vectors Devoid of Bacterial DNA Result in Persistent and High-Level Transgene Expression in Vivo. Molecular Therapy 8 (3) 495-500 (September 2003).

# VII. Vector Maps

This is a partial list of available minicircle Parental Plasmids. A complete list can be found on SBI's website. Full sequences of the parental plasmids can be obtained by contacting SBI.




| Catalog# | Description | Multiple Cloning Sites (MCS) |
|---|---|---|
| **MN100A-1** | pMC.BESPX-MCS1 (empty vector) | **SpeI+EcoR1+BglII+EcoRV+XbaI+ SalI +ApaI** |
| **MN100B-1** | pMC.BESPX-MCS2 (empty vector) | **SpeI+EcoR1+BglII+EcoRV+XbaI+ NheI+ BstBI +SwaI +BamHI +PstI + SacI + SalI +ApaI** |
| **MN501A-1** | pMC.CMV-MCS-SV40polyA | **XbaI + SalI + BamHI + SwaI + BstBI + EcoRI +NheI** |
| **MN502A-1** | pMC.EF1-MCS-SV40polyA | **XbaI + NheI + ApoI + EcoRI + BstBI + SwaI + BamHI + SalI** |
| **MN511A-1** | pMC.CMV-MCS-EF1-GFP-SV40PolyA | **XbaI + NheI + EcoRI + BstBI + SwaI + BamHI** |
| **MN512A-1** | pMC.CMV-MCS-EF1-RFP-SV40PolyA | **XbaI + NheI + EcoRI + BstBI + SwaI + BamHI** |
| **MN530A-1** | pMC.EF1-MCS-IRES-GFP-SV40PolyA | **XbaI + NheI + EcoRI + BstBI + SwaI + BamHI** |
| **MN531A-1** | pMC.EF1-MCS-IRES-RFP-SV40PolyA | **XbaI + NheI + EcoRI + BstBI + SwaI + BamHI** |

# V.Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

**http://www.systembio.com**

For additional information or technical assistance, please call or email us at:

**Phone:** (650) 968-2200
(888) 266-5066 (Toll Free)
**Fax:** (650) 968-2277
**E-mail:**
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

System Biosciences (SBI)
265 North Whisman Rd.
Mountain View, CA 94043

# VIII. Licensing and Warranty

Use of the Minicircle Technology (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

**** This Product shall be used by the purchaser for internal research purposes only and distribution is strictly prohibited without written permission by System Biosciences.**

### Limited Warranty

SBI warrants that the Product meets the specifications described in the accompanying Product Analysis Certificate. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.