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**Submit content:** [adsubmissions@biotechniques.com](mailto:adsubmissions@biotechniques.com)

# SPECIFICATIONS - PRINT

	Width	Height
<b>Full Page</b>		
Trim Area:	8.05"	9.98"
	204.55 mm	269.62 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.175 mm) from trim area	

	Width	Height
<b>1/2 pg Horizontal</b>		
Trim Area:	8.05"	5.08"
	204.55 mm	129.1 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.175 mm) from trim area	

	Width	Height
<b>1/2 pg Vertical</b>		
Trim Area:	3.72"	10.61"
	94.6 mm	269.62 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.175 mm) from trim area	

	Width	Height
<b>1/3 pg Square</b>		
Trim Area:	4.90"	4.90"
	124.61 mm	124.61 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.175 mm) from trim area	

	Width	Height
<b>1/3 pg Vertical</b>		
Trim Area:	2.53"	10.61"
	64.36 mm	269.62 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.175 mm) from trim area	

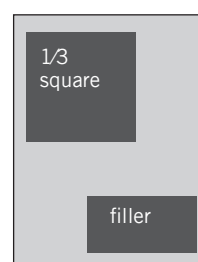
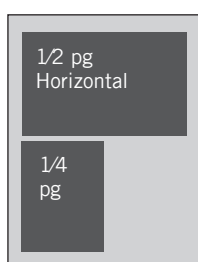
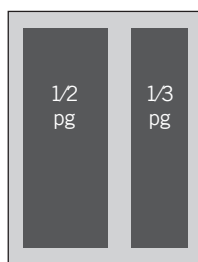
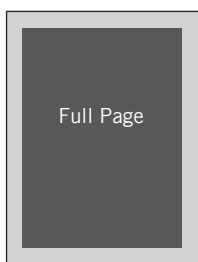
	Width	Height
<b>1/3 pg Horizontal</b>		
Trim Area:	8.05"	3.40"
	204.55 mm	86.56 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.17 mm) from trim area	

	Width	Height
<b>Full Page Internal Insert Tip (Perfect Bound)</b>		
Trim Area:	7.67"	10.61"
	195.025 mm	269.63 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.175 mm) from trim area	

	Width	Height
<b>Full Page Internal Insert Tip (Saddle Stitch)</b>		
Trim Area:	7.99"	10.61"
	202.96 mm	269.63 mm
Live Area:	0.25" (6.35 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.175 mm) from trim area	

	Width	Height
<b>Covertip #1</b>		
Trim Area:	7.80"	4.80"
	198.2 mm	122 mm
Live Area:	0.5" (12.7 mm) inside the trim	
Bleed Area:	Extends 0.125" (3.17 mm) from trim area	

**NOTE:** Text/logos should fit within the live area. Crop marks should be outside the bleed area. Live bleed area for fractional ad along "outer edge" only.



## File Preparation Guidelines

### File Format

- PDF
- Standard high resolution of at least 300 dpi
- Fonts embedded
- Images embedded – resolution at least 300 dpi and in CMYK
- No RGB, Color Management, ICC profiles, or LAB
- Do not use security/password options

### Fonts

- Must be embedded, no multiple master fonts

### Color

- CMYK only
- No Spot/PMS (Pantone) colors
- No RGB, Color Management, ICC profiles, or LAB

### Images

- Resolution of at least 300 dpi
- Color format in CMYK
- No RGB, Color Management, ICC profiles, or LAB

### Submissions

- adsubmissions@biotechniques.com

# SPECIFICATIONS – APPLICATION FORUM

**Sponsored Paper**  
**ApplicationForum**  
**Development and Optimization of a High Titer Recombinant Lentivirus System**  
 Mirus Bio LLC (Madison, Wisconsin USA)

To enable simple and effective high titer recombinant lentivirus production, we examined key parameters for the generation of lentivirus packaging transduction conditions, DNA vector selection, media change, and inoculation time. Achieving or suspension-adapted 293T-derived cells can be used for production hosts. These results define the importance of optimizing transduction processes for high titer recombinant lentivirus production.

**Introduction**  
 Lentivirus is an enveloped single-stranded RNA virus from the Retroviridae family. Its ability to infect dividing and non-dividing cells has led to the widespread utilization of recombinant lentiviruses as a gene delivery vehicle. In addition, lentivirus has an efficient integration mechanism which leads to robust and stable transgene expression in target cells.

Recombinant lentivirus production is frequently accomplished in human embryonic kidney derived cells that stably express the SV40 large T antigen tag, HEK293T cells. These cells are transiently transfected with three or four plasmids that encode the genes of interest (G), the vesicular stomatitis virus envelope protein (Glycoprotein, *gag*, and *pol*), and the essential lentiviral *gag*, *pol*, and *rev*. Once all the genes are expressed at sufficient levels the virus assembles and buds through the plasma membrane to form an enveloped virus encapsulated with VSV-G which confers the ability to transduce a broad range of mammalian cell types. The virus-containing supernatant can be collected and filtered to remove any cells. Target cells are transduced with the recombinant lentivirus in the presence of hexamethylamine (HMT) or TransIT<sup>TM</sup> Transfection Reagent to increase virus aggregation and cellular uptake.

**Cell Confluency**  
 A key parameter for successful transduction is adequate virus production in the host cell. High confluency at the time of transduction. Low confluency can lead to suboptimal and lower transduction efficiency. Performing transductions at high confluency, e.g. 85-90%, will ensure the protein expression levels and subsequent titer.

**Reagent to DNA Ratio**  
 The transfection reagent to DNA ratio is a critical parameter for efficient nucleic acid delivery. The previously optimized transfection reagent must be adjusted to sufficient quantities to effectively transfect and coat the negatively charged plasmid DNA. The same volume of reagent is formulated in the TransIT<sup>TM</sup> Lentiviral Transfection Reagent to perform consistently at a 3:1 reagent-to-DNA ratio (column weight). Deviating from the optimal reagent-to-DNA ratio can adversely affect the virus titer (Figure 1). Under these conditions, the total plasmid concentration of 1 µg/ml of culture resulted in maximal lentivirus titer. Depending on the potency of the vector system, varying the total DNA concentration while maintaining the reagent-to-DNA ratio may increase lentivirus titer.

The order and/or composition and stoichiometry of packaging and transfer plasmids can greatly influence the titer obtained. Depending on the operation of the packaging system, there are four plasmids that need to be present prior to the DNA being added to the transfection reagent to ensure that the transduction complex is not formed preferentially with the plasmid over another. In addition, if using individual packaging systems we recommend a starting ratio of 6:4:1:1 of packaging:pol:G:G vectors. If using a lentivirus packaging plasmid we recommend an initial ratio of 1:1 between the packaging structure and the transfer plasmid.

Figure 1. Optimization of reagent to DNA ratio. Achieving 293T-derived cells were transfected in 6-well plates using polyplex DNA. TransIT<sup>TM</sup> Lentiviral Transfection Reagent was used for transduction. The virus was harvested and titered using a lentivirus titration assay. The virus was then used to transduce HEK293T cells. The reagent to DNA ratio was varied from 1:1 to 8:1. The results are shown. The reagent to DNA ratio was varied from 1:1 to 8:1. The results are shown. The reagent to DNA ratio was varied from 1:1 to 8:1. The results are shown.

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**Figure 4. Single Inoculum and Media Change Required.** Achieving 293T-derived cells were transfected in 6-well plates using polyplex DNA. TransIT<sup>TM</sup> Lentiviral Transfection Reagent was used for transduction. The virus was harvested and titered using a lentivirus titration assay. The virus was then used to transduce HEK293T cells. The reagent to DNA ratio was varied from 1:1 to 8:1. The results are shown.

Figure 4. Single Inoculum and Media Change Required. Achieving 293T-derived cells were transfected in 6-well plates using polyplex DNA. TransIT<sup>TM</sup> Lentiviral Transfection Reagent was used for transduction. The virus was harvested and titered using a lentivirus titration assay. The virus was then used to transduce HEK293T cells. The reagent to DNA ratio was varied from 1:1 to 8:1. The results are shown.

**Conclusions**  
 We systematically optimized the experimental conditions surrounding recombinant lentivirus production including cell confluency, transfection reagent to DNA ratio, reagent formulation, DNA vectors, media change, and inoculation time. The highest titer was achieved when 293T17 cells are transfected at 90% confluency using the TransIT<sup>TM</sup> Lentiviral Transfection Reagent at a 3:1 reagent to DNA ratio (column weight). Using these conditions, a media change is not required post-transduction and a single harvest at 48 hours post-transduction offers convenience and less handling of the cells. In addition, the transfer and packaging plasmid also play a critical role in the titer achieved. The combination of transfection reagent, transfer vector and packaging plasmid can lead to a 100-fold difference in titer in head to head comparisons. The TransIT<sup>TM</sup> Lentiviral Transfection System consistently allows recombinant lentivirus production in suspension 293-derived cell types. The TransIT<sup>TM</sup> Lentiviral Transfection System containing Mirus' novel lentivirus formulation and Full Lentiviral Packaging Mix (ProVirus) MESSON<sup>TM</sup> Genomics provides exceptional lentivirus titer, increased stability and reduced workload.

**Cell Type and Growth Characteristics**  
 Achieving 293T cells are commonly used for recombinant lentivirus production. However, when large quantities of virus are required or when a chemically defined system that lacks animal-derived components is needed, it is advantageous to employ suspension adapted 293 cell lines. Transient transfection efficiency can vary widely in different serum-free media formulations possibly due to charged components interacting with the electrostatic complex formation between the transfection reagent and the plasmid DNA. For a suspension cell platform, we have found that Thermo Fisher Scientific's FreeStyle<sup>TM</sup> 293-F cells grown in FreeStyle<sup>TM</sup> F1 Expression Medium, supplemented with 0.2% polybrene 969 is amenable to single cell growth and high titer lentivirus production using a standard transfection protocol. In head-to-head comparisons with commonly used transfection technologies the TransIT<sup>TM</sup> Lentiviral Transfection Reagent yields the highest titers (Figure 5).

Figure 5. Suspension 293 cells support lentivirus production. Suspension adapted 293 cells grown in FreeStyle<sup>TM</sup> 293-F medium were transfected with the Full Lentiviral Packaging Mix provided by MESSON<sup>TM</sup> Genomics (1:1 ratio, 1 µg/ml) and used for the transgene expression. FreeStyle<sup>TM</sup> 293-F cells were transfected with the Full Lentiviral Packaging Mix provided by MESSON<sup>TM</sup> Genomics (1:1 ratio, 1 µg/ml) and used for the transgene expression. FreeStyle<sup>TM</sup> 293-F cells were transfected with the Full Lentiviral Packaging Mix provided by MESSON<sup>TM</sup> Genomics (1:1 ratio, 1 µg/ml) and used for the transgene expression.

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## 2-page app note example

## Content Guidelines

Word count	In copy images	Additional content
<p><b>First page of App Note:</b></p> <ul style="list-style-type: none"> <li>Total word count: 630</li> </ul>	<p>In general, in-copy images will replace about 90 words. The total word count will be reduced accordingly, depending on the number of images you include.</p>	<p><b>Web Link</b></p> <ul style="list-style-type: none"> <li>Please provide web page to link your app note and logo to</li> </ul>
<p><b>All pages after first page:</b></p> <ul style="list-style-type: none"> <li>Total word count: 840</li> </ul>		<p><b>Logo</b></p> <ul style="list-style-type: none"> <li>Please provide company logo for branding of app note</li> </ul>

\* If no web link is provided, your content and logo will automatically be linked to your homepage

## File preparation

- All text files must be MS Word documents (.doc).
- Images must be provided separately, with a resolution of at least 300 dpi.
- Acceptable formats for images are EPS, TIFF and PDF.

# SPECIFICATIONS – eNEWSLETTER

## General Guidelines:

- Materials due 1 week prior to launch
- Animated Gifs are not supported by Outlook 2007, 2010 or Windows Mobile 7
- Animated ads can be accepted but advertisers should be aware of the limitations of showing animated ads in the Outlook email program
- Text portion of ads can include basic formatting and unlimited links

## Newsletter Ad Design and Specifications:

- Newsletters alternates advertising content with editorial content
- Most ad spots now offer a combination of images and text, ensuring your ads will be seen by those not downloading images or viewing text-only newsletter versions
- Newsletters feature a 640×80 px top leaderboard image-only ad and also contain a specific number of additional ad spots based on the type of newsletter
- Advertisers can choose any combination of the ad options below to equal the number of ad placements for the sponsored newsletter
- Label ad materials in the order you would like them to appear in the newsletter starting with the top leaderboard image ad of 640×80 px as #1
- Remember to include the links you would like your images or text to link to

## Newsletter Schedule

**Daily Newsletters:** Published Weekdays (*Monday through Friday*)

**Tech Newsletters:** Published Mondays and Wednesdays

**Weekly Newsletters:** Published every Saturday

**E-Table of Contents Alerts:** Published once a month

**Custom Topic Tech Newsletters:** Published on Mondays as sponsored

# SPECIFICATIONS – eNEWSLETTER

## Newsletter Ad Options

### Included in the Top Leaderboard Section of All Newsletters:

- 640 x 80 Image-only Ad

### Included in the Top Mobile Section of All Newsletters:

- 300 x 50 Image-only Ad

### Then, choose any combination from options #1 through #4 below

**Daily:** 2 additional spots

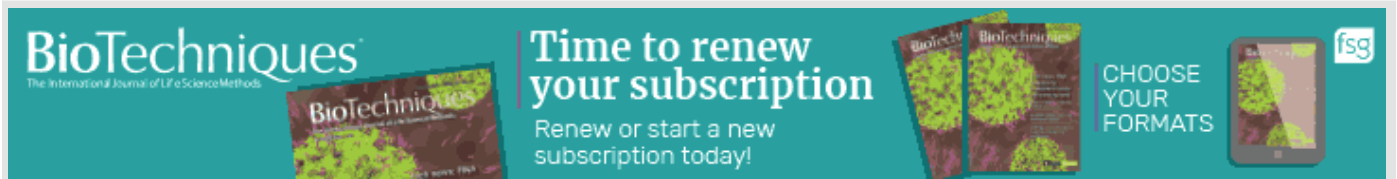
**eToC:** 3 additional spots

**Weekly:** 3 additional spots

**Tech:** 4 additional spots

#### Option #1

640 x 80 image-only



#### Option #2

420 x 80 image/logo, plus 15 words of text



*BioTechniques* offers a variety of newsletters, webinars, discussion forums and more. Don't miss out, **renew** or **subscribe** today!

#### Option #3

300 x 50 image / logo, plus 25 words of text



Stay up to date on industry developments with a free subscription to *BioTechniques*. Don't miss out, **renew** or **subscribe** today!

#### Option #4

200 x 90 image/logo, plus 40 words of text



Are you interested in the latest news on peer-reviewed life science methods? Stay up to date on industry developments with a free subscription to *BioTechniques* and gain access to a variety of newsletters, webinars, discussion forums and more. Don't miss out, **renew** or **subscribe** today!

# SPECIFICATIONS – THIRD PARTY EMAILS

## Required Materials:

- HTML file
- Plain text version (suggested but not required)
- Subject line
- List Selects

## HTML Guidelines:

- Compress images to as small a size as possible
- Main file must be .HTML or .HTM, **not** a pdf, Word Doc, or Word created HTML
- All image files should be in .gif or .jpg format
- All image files should be stored on a publicly accessible Web server. We can host images; if necessary, send the .gif or .jpg files to us along with the HTML
- **Do not use** JavaScript for essential elements, some browsers do not support it and others view it as SPAM
- Comply with Web safe colors (216) for text, backgrounds and solid color areas of images (i.e. logos)
- When specifying a background page color (BGCOLOR), specify within a table rather than the body element
- Use relative (font size="-2") rather than absolute (font size="2") font sizes
- **Do not use** custom backgrounds (BACKGROUND tag within body element)
- **Do not use** page anchors (A NAME="xyz")
- Use absolute (http://www.yourcompany.com/page) rather than relative (/page) links
- **Avoid using** the <BASE> tag (e.g. <BASE HREF="xyz">)
- **Do not use** Style Sheets or Cascading Style sheets if possible; we can try to use it but most often CSS emails do not render correctly in Outlook 07
- You may send your own plain text file if you wish. If you do not, one will be automatically generated and sent to all recipients that may prefer plain text emails
- We will host the HTML and offer a link at the top of the email for recipients to view the HTML online

**Please note these are only guidelines. If you need help, please contact us and we can test your file and provide assistance.**

# SPECIFICATIONS – BANNER ADVERTS

## General Guidelines

- All creatives must click through to another page and open in a new window
- All creatives must have a border
- Leaderboards need to be supplied in both desktop (728 x 90px) and mobile (300 x 50px) sizes
- **No** expandable banners
- **No** extensive blinking or flashing elements
- **No** ad can spawn or pop from another ad unit
- **No** geotargeting or frequency capping through creatives
- **No** automatic downloads or executable files
- *BioTechniques* cannot guarantee we can provide click reports for Javascript ads for third-party servers. Clients must refer to external reports for click-through information

## Online Banner Ad Sizes

Style	Size
Button (B)	120 x 90px
Top Leaderboard (TLB)	728 x 90px
Mobile Leaderboard (MLB)	300 x 50px
Medium Rectangle Unit (MPU)	300 x 250px
SkyScraper 1 (SKY-1)	120 x 600px
SkyScraper 2 (SKY-2)	120 x 600px
Bottom Leaderboard (BLB)	728 x 90px

## Acceptable Formats/Sizes

- .gif or .jpg: max file size: 40 KB
- Initial Flash File Size: 40 KB
- Looping Limit/Maximum Total Animation Length: 3 loops/15 Seconds Total

# SPECIFICATIONS – OTHER DIGITAL PRODUCTS

## Application Forum and White Papers

- Color mode: RGB
- Resolution: 300 DPI or higher
- Format: PDF, TIFF or EPS
- Fonts must be embedded or converted to outlines
- Size: 8.5" x 11"

### Also required:

- Company name (under which item should be published under)
- 175-word abstract for website entry submitted in MS Word or other text program
- 75-word condensed abstract for newsletter entry submitted in MS Word or other text program

## Webinars

- High-resolution client logo (300 dpi) – .eps file preferred
- Your sales rep and a *BioTechniques* editor will work with you to determine other requirements as we develop the content and promotional portions of the webinar

## Third Party Surveys

- List selects
- Your sales rep will provide you with demographic and list targeting options
- General guidelines for submitting survey questions
- Materials due 10-days prior to launch date
- Up to 10 questions
- Indicate if more than one answer choice is allowed
- Distribution date
- Seed addresses
- Name, email address and phone number of contact authorized to approve distribution



# SPECIFICATIONS – OTHER DIGITAL PRODUCTS

## Video

In addition to the audio/video file, please provide up to an 8-word title, a 20-word homepage description, a 75-word newsletter description, and the company name under which the video should be published.

Audio Formats	Extensions	Details
MP3	.mp3	MPEG-1 or MPEG-2 format required

Video Formats	Extension	Details
MPG	.mpg	MPEG-1 or MPEG-2 format required
MP4	.mp4	Acceptable video format
Apple QuickTime	.mov	Acceptable video format
Microsoft Audio/Video Interlaced	.avi	Acceptable video format

### For Video Files

If submitting video, the following specifications are a guideline for authors/contributors:

- Frame rate: 24 frames per second minimum
- NTSC (4:3) size and frame rate, de-interlaced
- Video Codec: MPEG-2 or MPEG-4 (MPEG-4 preferred)
- Video Bit rate: at least 5mbps
- Resolution: 1280×720 recommended
- Time: no more than 5 minutes

If the software used for the creation of your video cannot deliver one of the above formats, then please save them in one of the accepted formats. Any alternative format supplied may be subject to conversion (if technically possible) prior to online publication.

### For Product Listings

If submitting product listings, the following specifications are a guideline for authors/contributors:

#### BioTechniques.com

- Image: 1920x1020px recommended
- Press release
- Call to action

#### eNewsletter

- Article image: 200x300px (Maximum)
- Article title
- Article copy: 50 words
- Desired links: call to action, image, title

#### Journal

- Article image
- Article title
- Article copy: 150 words (Maximum)
- Call to action