

Transgene Delivery of Plasmid DNA to Smooth Muscle Cells and Macrophages from a Biostable Polymer-coated Stent

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Running Title: Stent-mediated gene delivery to vascular cells

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Summary

Metallic stents coated with a polyurethane emulsion containing plasmid DNA were implanted in rabbit iliac arteries to evaluate transgene delivery and expression in the vessel wall. The expression of the plasmid-encoded marker genes, β -galactosidase, luciferase and green fluorescence protein (GFP), were evaluated at 7 days after implantation. In all cases plasmid transfer was confined to the vessel wall at the site of stent implantation, plasmid DNA was not observed in vessel segments immediately proximal or distal to the stent and dissemination of plasmid DNA to lung, liver or spleen was not observed. Expression of transgenes occurred only in vessel segments in contact with the stent and analysis of the GFP expression pattern revealed a high frequency of marker protein-positive cells occurring at or near the luminal surface. The extent of transgene expression was dependent upon the quantity of DNA loaded onto the stent and no signal was detected in vessel segments that received polymer-coated stents lacking plasmid DNA. Of significance, co-localization studies identified transgene expression not only in vascular smooth muscle cells but also in macrophages. Hence polymer coated stents provide a new capability for transgene delivery to immune cells that are believed to contribute to the development of in-stent restenosis.

Keywords: experimental, vasculature, cellular, molecular biology, gene therapy, macrophages, restenosis, smooth muscle, stents

Introduction

Intra-arterial stent implantations have become a common revascularization procedure ¹ since they frequently result in superior angiographic results ^{2,3} and reduce the need for repeated revascularization procedures. However, the stent implant itself can act as a stimulus for intimal hyperplasia, particularly if the stent becomes imbedded in the vessel wall, leading to inflammation. ⁴⁻⁶ Compared with balloon angioplasty-induced restenosis, in-stent restenosis is largely the consequence of intimal hyperplasia. These lesions are highly cellular with some inflammatory cell infiltrate but composed primarily of phenotypically-modulated smooth muscle cells displaying a high frequency of cell cycle protein expression (PCNA, cdk2 and cyclin E). ⁷ In addition to being highly proliferative, the cells within the lesion also display a high frequency of apoptotic cell death, indicating that the tissue within the stent is dynamic with cell turnover occurring at an elevated rate.

Due to the inflammatory-fibroproliferative nature of the lesion, in-stent restenosis may be controlled by the delivery of an antiproliferative agent locally in conjunction with stent implantation. Recently, studies using coated stents that release rapamycin and paclitaxel have reported substantial decreases in the extent of in stent restenosis during animal studies ⁸⁻¹⁰ and the initial phases of clinical trials. ^{11,12} However, coated stents could also be used to deliver genetic material to the cells of the vessel wall by diffusion from the polymer coating. The transformed cells could then provide for the prolonged expression of a therapeutic protein(s) chosen to aid the wound healing process and thereby minimize vessel re-occlusion. The recent report ¹³ that bone marrow cells home to sites of vascular injury and constitute a significant portion of the neointimal

cells in a vascular lesion suggests that extended expression of therapeutic proteins may be required to target these progenitor cells.

Recently, Klugherz et al.¹⁴ demonstrated the expression of green fluorescent protein expression from plasmid DNA delivered to the vessel wall from a stent coated with a biodegradable polymer. Due to limitations posed by the biodegradable polymers, we evaluated the utility of biostable polymer-coated stents for the delivery of genetic material to the vessel wall. In this study, in addition to characterizing the gene delivery properties of the non-degradable polymer coating, we examine the expression of multiple marker genes in the vessel wall and demonstrate a dose-response relationship for transgene expression. We also demonstrate the ability to deliver transgene to inflammatory cells at the site of the stent implantation. This unique capability of polymer coated stent delivery provides a new capability to the vascular gene therapy field to modulate the activity of immune cells that are believed to be a significant contributing factor to in-stent restenosis.¹⁵⁻¹⁸

Results

A number of non-degradable polymer matrix materials are currently being evaluated for coating stents. Of those, polyurethane has been shown to be effective in coating stents to prevent the thrombosis that can occur with uncoated stents and with some of the degradable polymers that have been tested in vivo.¹⁹ In this study we examined the ability of this non-thrombogenic polymer to also function as a slow release agent for plasmid mediated gene transfer to the vessel wall. Stainless steel stents were dip-coated with a resin mixture containing either high or low concentrations of super-coiled plasmid DNA and synthetic polyurethane resulting in a thin layer of polymer coating and spanning the struts. Under standard conditions the procedure resulted in the application of 3.6 ± 1.4 mg of coating corresponding to 0.12 ± 0.04 mg of plasmid DNA to a 3.0 x 12 mm stent.

Samples of the DNA absorbed polyurethane were incubated in buffer at 37°C with constant gentle shaking to examine the elution properties of the plasmid. DNA was released in a time dependent manner (Figure 1A) as detected by absorbance measurements at 260 nm (A_{260}). DNA release from the coated stents was examined in vitro in TSE buffer (pH 8.0) and under these conditions DNA elution was characterized by an early burst of DNA release (See inset), with 50% DNA eluted during the first four hours of incubation followed by a slower sustained release out to at least 7 days, after which only insignificant changes were observed in the DNA concentration. Supercoiled plasmid (Figure 1B) was the predominant form after 16 days of elution indicating that the plasmid survived the polymerization process structurally intact. In addition, agarose gel electrophoresis of eluted DNA samples digested with the restriction endonuclease XhoI indicated no apparent difference between original plasmid and the eluted samples.

The plasmid-absorbed polyurethane-coated stents were then evaluated for vascular gene transfer following implantation in rabbit iliac arteries (Figure 2A). The stents were implanted using oversized balloon angioplasty and retrieved seven days after implantation by excising the artery and adjacent regions. When vessel segments were examined for luciferase activity only extracts from the segment of the artery that had been in direct contact with the stent contained luciferase activity (Figure 2A). No luciferase activity was observed above background in arterial segments proximal or distal to the stent, indicating the utility of the DNA-loaded stents for local delivery of therapeutic genes.

The level of transgene expression can be modulated by altering the DNA-loading of the stent, resulting in a gene dosage effect as can be observed in the results obtained from stents absorbed with β -galactosidase plasmid DNA (Figure 2B). In this experiment β -galactosidase activity was measured in extracts prepared from the iliac arteries of rabbits that had been implanted with polyurethane-coated stents that had been loaded with 96 μ g or 40 μ g concentrations of the expression plasmid. A statistically significant ($P < 0.01$) increase in the β -galactosidase activity was noted in extracts from arteries that received stents coated with a high dose of plasmid DNA compared to those receiving a low dose and both were significant ($P < 0.01$) compared to background levels. We were unable to demonstrate β -galactosidase activity by histochemical analysis in treated vessels, even at the high dose level (data not shown), indicating that the level of transfection using this method is extremely low.

As has been observed by others,^{19,20} an inflammatory response to the stent was noted (Figure 3). Based upon observation of multiple tissue sections stained with hematoxylin and eosin, inflammation appeared to be predominantly influenced by the

extent of tissue damage by the stent strut, rather than the presence or absence of polymer. The inclusion of DNA within the polymer did not increase inflammation in the vessel wall relative to bare metal or polymer-coated stents without DNA. In addition, no significant vessel stenosis was observed at this early time point in any of the stented arteries.

Green fluorescent protein (GFP) expression plasmid absorbed polyurethane-coated stents were then implanted in rabbit iliac arteries in order to allow determination of the spatial localization of gene transfer. Gene transfer was found to be localized to the areas between the struts and may be related to the fine webbing of polymer bridging the space between the struts. Photomicrographs of vessels from the stented arteries exhibited extensive GFP activity in the medial layers of the arteries, as can be observed in Figure 4. In the absence of DNA only the auto-fluorescence internal and external elastic lamina are seen in Figure 4A. The extent of GFP gene transfer increased as the DNA content of the coating was increased from 40 to 420 μg as can be observed in Figures 4B & 4C. The GFP expression also served to determine the identity of the cells in the vessel wall taking up the plasmid. Immunofluorescent labeling of the cells using primary antibodies directed against either smooth muscle myosin heavy chain (SM1) or against the macrophage antigen RAM11 indicated that both smooth muscle and macrophage express the transgene as can be observed with the double-immunofluorescent labeled cells in Figure 5.

Finally, we examined vector dissemination by PCR analysis of genomic DNA samples from various distal organs for the presence of the GFP gene (Figure 6). A representative analysis is shown of organ extracts from a rabbit that had a GFP-plasmid absorbed stent placed in the iliac artery. In this case, we obtained PCR-mediated

amplification with GFP directed primers only with DNA obtained from artery sections in contact with the stent. The GFP transgene was not detected in samples from adjacent artery sections or distal organs.

Discussion

Recently, there has been considerable interest in the treatment of vascular proliferative diseases using local gene therapy.^{21,22} Adenovirus has been the vector of choice, generally delivered by an infusion catheter device since efficient transfer must be achieved in a clinically relevant delivery time of 2 minutes or less. However, in practice relatively low levels of adenoviral gene expression have been found with infusion devices due to poor entry and transport of the virus within the vessel wall and low transgene expression that has complicated the assessment of the potential benefits of therapeutic genes in the treatment of vascular disorders. Additionally, the immunogenicity of the vector can produce local and systemic adverse reactions.²³⁻²⁵ Therefore, we have examined the use of polymer carriers to facilitate gene delivery using plasmid DNA. The release of genetic material from intravascular stents could represent a new therapeutic modality¹⁴ since, unlike balloon catheters, the stent remains within the vessel, avoiding the limitations associated with the delivery of large quantities of genetic material within a few minutes.²⁶ Instead, the slow release of therapeutic genes from the stent struts embedded in the vessel wall would likely be advantageous in this system.

The development of polymer-coated stents has been considered to be a promising opportunity to target the intractable problem of in-stent restenosis by local delivery of drugs eluting from the polymer coatings. However, the coatings may result in further complications as noted in a study by De Scheerder et al.²⁰ that found 81% luminal stenosis of normal porcine coronary arteries receiving implants of stents coated with a biodegradable poly(organo)phosphazene derivative. By contrast, stents coated with a non-degradable polyurethane polymer resulted in just 32% stenosis, comparable to the 39% rate

obtained with bare metallic stents. In addition, substantial vessel injury was observed in the arteries receiving the poly(organo)phosphazene coated stents, which De Scheerder et al. attribute to a pronounced histolymphocytic response to the coating. In light of the relatively low luminal stenosis associated with a polyurethane polymer we chose to explore the use of this polymer for the delivery of DNA to the vessel wall. A DNA/polymer emulsion was used to coat stainless steel stents and the stents were then delivered to rabbit external iliac arteries and gene transfer was evaluated at 7 days after implantation. At this timepoint we did not observe an inflammatory response that could be attributed to the polymer or DNA. However, others have reported that nonbiodegradable polyurethane polymer-coated stents can evoke extensive inflammatory responses and fibrocellular proliferation at later timepoints in a porcine model ²⁰. With three different plasmid-encoded marker genes, luciferase, β -galactosidase and green fluorescence protein (GFP), we defined the elution and transfection characteristics of this polymer coating.

Our findings extend those previously reported by Klughertz et al. ¹⁴ that used stainless steel stents coated with a biodegradable polylactic-polyglycolic acid polymer impregnated with an expression plasmid encoding GFP to demonstrate gene transfer to porcine coronary arteries. First, the expression of transgene-encoded protein was shown to be dependent upon the dose of DNA loaded onto a biostable polymer-coated stent, and no signal could be detected in vessel segments that received bare metallic stents or polymer-coated stents that lacked plasmid DNA. Consistent with the findings of Klughertz et al., ¹⁴ plasmid transfer occurred only at the site of stent implantation, and dissemination of plasmid DNA to the lung, liver or spleen was not observed, nor was plasmid DNA observed in the vessel segments immediately proximal or distal to the stent. Finally, analysis of GFP expression pattern revealed a high frequency of marker protein-positive cells occurring at or

near the luminal surface. The low level of transfection observed in this study, as indicated by the patchiness of the expression observed in Figure 5 and the low level of luciferase and β -galactosidase activities measured in tissue extracts (Figure 2), suggest that effective clinical application of this method will require the use of therapeutic genes that demonstrate a bystander effect, e.g. thymidine kinase and Fas ligand^{27 23}.

Our co-localization studies identified transgene-encoded GFP not only in vascular smooth muscle cells but also in macrophages. This latter finding is significant since macrophages appear to be key participants in the chronic inflammatory response that results following stent implantation. Macrophage accumulation has been observed to be the major inflammatory response in stented arteries both in rabbits and primates.¹⁵⁻¹⁸ In addition, several animal studies have demonstrated an ability to reduce neointima formation in stented arteries using anti-inflammatory strategies including antibody mediated blocking of Mac1 binding, blockade of the MCP-1 receptor CCR2 and immunosuppression by intravenous injection of IL-10.^{15,16,18} Our ability to transduce immune cells with marker gene is also suggestive of sustained gene transfer in vivo, since macrophage accumulation in stented vessel segments is the result of a time-dependent process involving monocyte attachment, extravasation and differentiation. In addition, studies by Van der Giessen et al.¹⁹ found upon comparison that each of the 8 polymers used to coat stents implanted in porcine coronary arteries resulted in neointimal thickening associated with a marked inflammatory response consisting of a chronic inflammatory reaction and a persistent foreign body response. Thus it would appear that the use of polymer coatings for drug or DNA delivery requires the development of strategies to deal with the inflammatory response, and our findings suggest that it is possible to directly target, by gene transfer, the cells that contribute to the immune response.

Polymer coated stents have already entered into clinical trials as delivery devices for the timed release of small molecule drugs such as paclitaxel and rapamycin. It is significant that one of these, rapamycin, which has shown much promise in early reports, ^{11,12} is believed to have significant effects on the immune cells that contribute to in-stent restenosis, ¹⁰ in addition to affecting smooth muscle. ^{28,29} In this paper we have shown that we can also target both smooth muscle cells and immune cells such as macrophages by use of polymer coated stents as a means for the delivery of a transgene. It is conceivable that stent-based gene delivery may represent an advantage over therapies based on the elution of small molecules from polymer coated stents. Whereas a polymer reservoir will be depleted of small molecule drug over time, a genetically modified vascular cell could provide a relatively continuous supply of recombinant protein at the stent site. In fact, an extended period of transgene expression by vascular cells may be possible due to the limited immunogenicity of plasmid-transfected cells. In conclusion, polymer mediated gene transfer may allow for long-term delivery of therapeutic and immuno-regulatory genes designed to aid the wound healing process.

Materials and Methods

Stent preparation. The plasmid vectors used in this study were purified by resin absorption as recommended by the manufacturer (Qiagen, Inc., Valencia, CA). The GFP expression plasmid (pQBI25-fPA) expresses a codon and fluorophore optimized variant of the *Aequorea victoria* green fluorescent protein and was obtained from Q•BIOGENE (Carlsbad, CA). The β -galactosidase expression plasmid (pCMV β) was obtained from Clontech (Palo Alto, CA) and encodes full length *E. coli* β -galactosidase. The luciferase expression plasmid (pCI) was kindly supplied by C.S. Lim and J.L. Swain and encodes the firefly luciferase gene.³⁰ All plasmid vectors express the transgene under the transcriptional control of the CMV immediate early promoter-enhancer.

The 3.0 x 12 mm stainless steel stents (Guidant Corporation) were coated with a polymer prepared and applied by Surface Solutions Labs, Inc. (Carlisle, MA). The stents were partially expanded, fixtured for dip coating, washed in toluene and dried for at least an hour. They were then primed with a 5% silane solution (w/w) and allowed to ambient cure overnight on the fixturing rig. The stents were coated by dipping in a soft, extensible aliphatic polycarbonate\polyurethane coating containing approximately 3-20% plasmid DNA (w/w of resin solids) and allowed to dry. Multiple treatments resulted in a coating weight of approximately 4 mg and 35 microns thick with the struts completely coated and bridged by fine webs of polymer. For one experiment to examine the dose response of gene transfer from the stent, low and high doses of the GFP expression plasmid were obtained by performing either single or multiple dips in a 20% DNA/polymer (w/w) solution that resulted in coated stent containing either a low dose of 40 μ g of DNA or a high dose of 420 μ g. In all cases the struts were completely coated and the coating was stable and resistant to stent expansion, crimping and

general abrasion in handling. Stents were handled aseptically but were not sterilized prior to implantation.

Elution characteristics of plasmid DNA from the polymer matrix. A portion of the polyurethane/polycarbonate polymer containing 1.5 mg of the GFP expression plasmid was incubated in 20 ml of TSE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA, 150 mM NaCl). The sample was gently shaken at 37°C and 15 µl samples were taken at the indicated time points and diluted with 135 µl of TSE before the optical density at 260 nm was measured. The integrity of the plasmid was determined by agarose gel electrophoresis and restriction endonuclease analysis performed according to standard techniques.

Stent implantation. Animal protocols were approved by St. Elizabeth's Medical Center Institutional Animal Care and Use Committee. 13 Male New Zealand white rabbits weighing 3.0 to 3.5 kg were used for this study, each receiving bilateral placement of a stent resulting in a total of 26 stent placements. The rabbits were anesthetized with ketamine (10 mg/kg) and acepromazine (0.2 mg/kg) following premedication with xylazine (2 mg/kg). After left carotid artery cutdown, a 5 French introducer sheath (Terumo, Tokyo, Japan) was positioned in the carotid artery. All catheters were subsequently introduced through this sheath. A balloon catheter (Boston Scientific, Watertown, MA) was advanced over a 0.014-in guide wire (Hi-Torque Floppy II; Advanced Cardiovascular Systems, Temecula, CA) into the external iliac artery after reference angiogram. The coated stents were loaded onto a 2.5 mm diameter, 2 cm channel balloon catheter (Boston Scientific) and advanced to the site in the artery where the balloon was inflated to a pressure reading of 6 atmospheres for 30 seconds to implant the stent. Animals were allowed to recover from the surgery after which

the stented vessel segments were harvested at seven days after implantation. Vessel segments adjacent to both ends of the stent were retained to assess the extent of plasmid dissemination. In some instances liver, lung and spleen specimens were obtained and for PCR analysis of plasmid dissemination.

Assessment of gene expression in vivo. Arterial samples harvested at 7 days after stent implantation and vessel segments were opened with a longitudinal incision in order to remove the stent. When luciferase or β -galactosidase expression were to be determined specimens were snap frozen in liquid nitrogen and stored at -70°C . The frozen samples were then pulverized using a mortar and pestle and suspended in either reporter lysis buffer (Promega) or lysis buffer (Tropix, Bedford, MA). Cleared lysates were assayed for luciferase activity using luciferase assay system (Promega) or for β -galactosidase activity using Galacto-Star assay system (Tropix). Protein concentration was determined using BCA Protein Assay Reagent from Pierce (Rockford, IL) and reporter activities were normalized in relation to protein concentration. When GFP expression was being assessed, harvested tissue samples were embedded in OCT and stored at -70°C prior to preparation of 8 μm cryosections. Upon thawing, cryosections were fixed with 4% paraformaldehyde, counterstained with DAPI and GFP expression was examined by fluorescent microscopy. Other sections were stained with hematoxylin and eosin.

PCR analysis of plasmid dissemination. Frozen tissue samples were pulverized using a mortar and pestle and the resulting powder was suspended in DNA extraction buffer (150 mM NaCl, 10 mM Tris-HCl pH 8.0, 10 mM EDTA, 0.1% SDS and 100 $\mu\text{g}/\text{ml}$ Proteinase K) and incubated at 55°C for 1 hour. Tissue debris was removed by brief centrifugation and

the supernatant was extracted with phenol/chloroform, after which the DNA was precipitated from the aqueous layer by ethanol precipitation, all procedure performed using standard techniques. PCR amplification was performed using GFP-specific oligonucleotide primers (5'-CTACTCTGTGCTATGGTGTTCATG-3' and 5'-TGTTGATAATGGTCTGCTAGTTGAA-3') on a MJ Research thermal cycler and afterwards the amplified samples were analyzed by agarose gel electrophoresis.

Cellular Localization. Immunohistological analysis was performed on GFP-expressing arterial sections to determine which cell types within the arterial wall were expressing the transgene. Frozen sections (8 μm) were fixed with 4% paraformaldehyde for 10 minutes at room temperature and following washes in PBS the sections were incubated for 1 hour in antibody dilution buffer (Tris-buffered saline with 0.1% Tween 20 and 1% BSA) with a mouse monoclonal antibody specific for smooth muscle myosin heavy chain (anti-SM1; Yamasa Soy Sauce, Chiba, Japan) or an antibody specific for rabbit macrophage (anti-RAM11, Dako, Carpinteria, CA). After washing in PBS, bound antibody was detected by incubating the sections for 1 hour at 4°C with rhodamine-conjugated anti-mouse antibody (Dako, Carpinteria, CA).

Statistical Analysis. Values are expressed as the mean \pm the standard error of the mean. Data was examined by a one-way analysis of variance (ANOVA) and once the difference among group means was considered significant, pairs of groups were compared using Fisher's PLSD. A p value of less than 0.05 was considered indicative of a significant difference.

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Titles and Legends to Figures

Figure 1. The kinetics of DNA release in vitro from a polyurethane matrix containing the GFP reporter plasmid DNA. **(A)** Time course of DNA release from the polymer. Examples shown illustrate cumulative release from a polyurethane matrix. DNA was quantified by measuring absorbance at 260 nm (A_{260}). The release of DNA from the polymer is characterized by an initial rapid burst that is better illustrated by the inset graph. Results shown are the means of three separate determinations \pm the standard error of the mean. **(B)** Agarose gel electrophoresis demonstrates the release of intact plasmid DNA from the polymer. Original plasmid (lane 2); a sample obtained after 16 days of elution (lane 3). The plasmid released from the stent was structurally intact as demonstrated by restriction analysis. An *Xho*I digestion of the original plasmid (lane 4) appears identical to the *Xho*I digestion of a sample obtained after 16 days of elution; (lane 5); DNA molecular weight standards (lanes 1 and 6).

Figure 2. In vivo gene transfer of luciferase or β -galactosidase reporter plasmids from DNA/polymer coated stents to the vessel wall of rabbit iliac arteries. To assess luciferase and β -galactosidase gene transfer, stented artery segments were harvested at seven days after stent implantation. Lysates of the tissue were assayed for **(A)** luciferase or **(B)** β -galactosidase activity. **(A)** Luciferase activity was observed only in the region of the artery in contact with the stent but not in adjacent arterial segments (proximal or distal) or at the site of the abdominal aorta. The results of two independent experiments are shown. **(B)** In this experiment the stents were coated with polymer containing no DNA (Control), low DNA (40 μ g) or high DNA (96 μ g). The extent of β -galactosidase expression found in the extracts was dependent on the DNA content of the polymer coating. An asterisk (*) designates

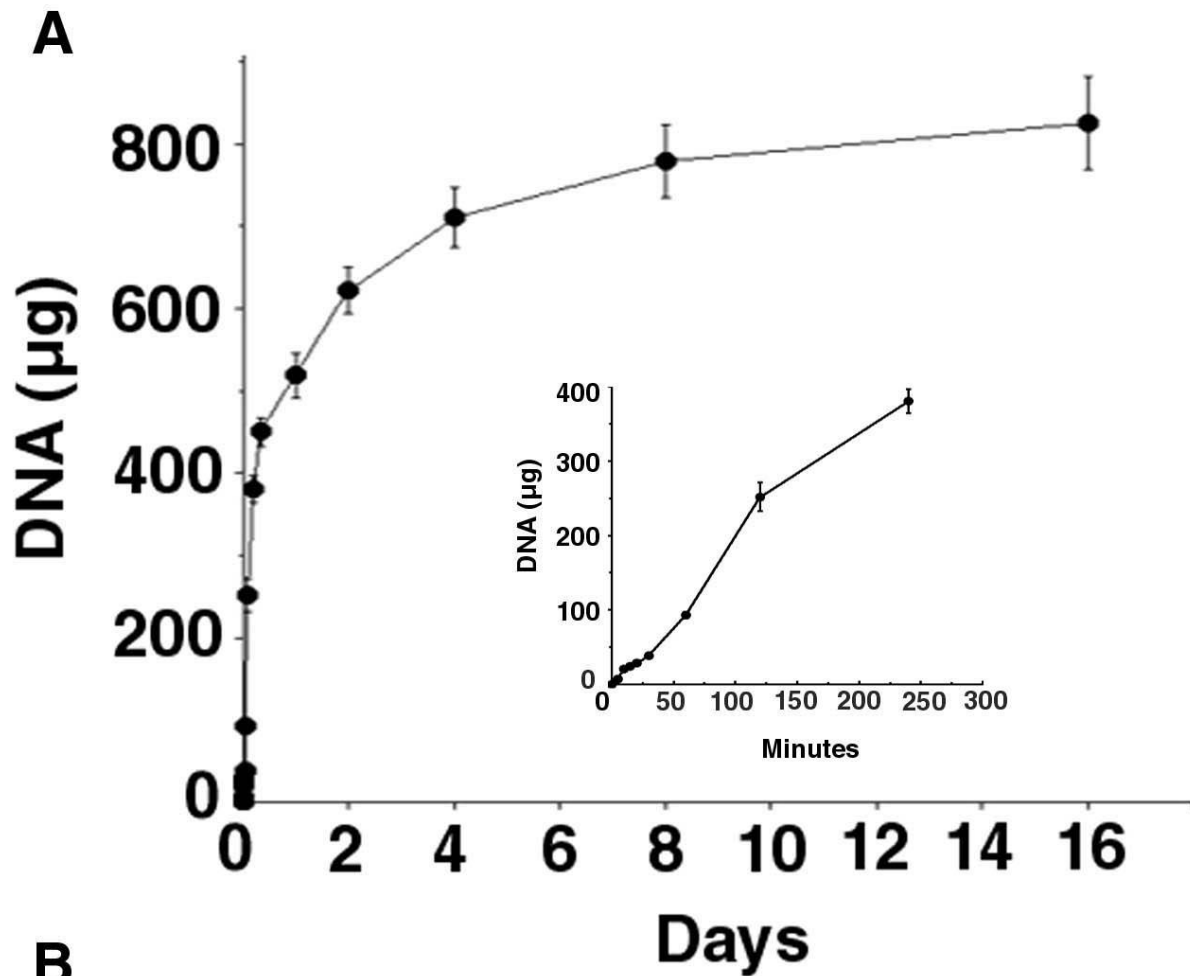
statistical significance in comparison to extracts from control artery that received a polymer-coated stent without DNA. The results shown are the mean \pm the standard error of the mean for 4 deliveries under each experimental condition.

Figure 3. Histological sections of stented and non-stented vessels. Cross-sections of rabbit iliac arteries stented with oversized balloons and harvested at seven days following gene transfer were stained with hematoxylin and eosin. Shown are 40x magnifications of sections from an unstented artery (**A**) or arteries that received either a bare metal stent (**B & C**) or a stent coated with polyurethane polymer alone (**D & E**) or a stent coated with polyurethane polymer containing the GFP plasmid (**F & G**). Representative examples are shown. The degree of inflammatory infiltrate observed was greater in areas where the strut caused damage to the underlying media (B, D & F) compared to areas where the media was intact (C, E & G).

Figure 4. GFP-expressing cells were located in the medial layer of the stented regions of rabbit iliac artery and the number of GFP-expressing cells increased in a dose-dependent manner. GFP expression was assessed at 7 days following GFP-DNA polymer stent implantation. The arteries shown have received a polyurethane coated stent without DNA (**A**); with low (40 μ g) GFP plasmid content (**B**); or high (420 μ g) GFP plasmid content (**C**). Results are representative of 4 individual deliveries under each condition. GFP-expressing cells were located in the medial layer and the number of GFP-expressing cells increased in a dose-dependent manner.

Figure 5. GFP gene transfer was detected in both medial smooth muscle cells and macrophages. To determine the cell types expressed the transgene, GFP-expressing sections of the rabbit iliac arteries stented with an oversized balloon (3 mm diameter) were subjected to immunohistochemical analysis using either a mouse monoclonal antibody specific for smooth muscle myosin heavy chain (anti-SM1) or an antibody specific for rabbit macrophage (anti-RAM11). Primary antibodies were detected by incubating the sections with rhodamine-conjugated anti-mouse antibody (Dako, Carpinteria, CA) to give a red fluorescence image. Results were obtained from 2 individual deliveries. Approximately 4 histological sections were analyzed in each vessel segment and multiple microscopic fields were observed for each histological section. Sections displaying high smooth muscle cell or macrophage frequencies were selected to demonstrate transgene expression. Control sections are from arteries that received a polymer coated stent without plasmid. Co-localization of the cell-type specific labeling with GFP results in a yellow image. Transgene expression was observed in both smooth muscle cells and macrophages.

Figure 6. PCR analysis of tissues for GFP distribution after iliac artery deployment of GFP-coated stents in vivo. Tissues were harvested and DNA extraction was performed using standard techniques. PCR amplification was performed using GFP-specific primers and the amplified DNA samples were subjected to agarose gel electrophoresis. GFP-DNA was detected only in the stent-deployed artery. No dissemination was observed to distal or proximal sections of the artery or to downstream organs. The results from the analysis of a single artery are shown. This experiment was repeated on 2 animals with identical results.



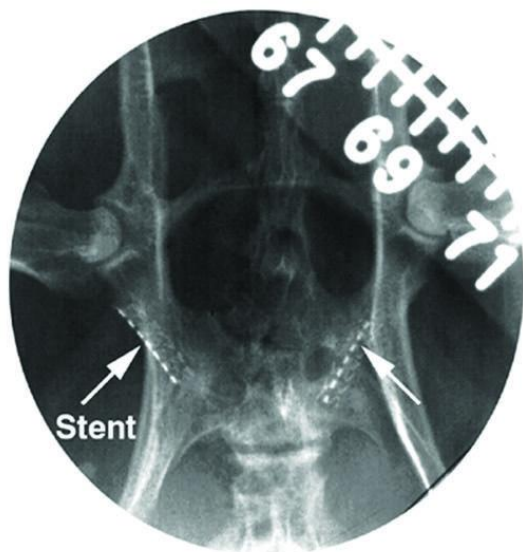
B

1 2 3 4 5 6

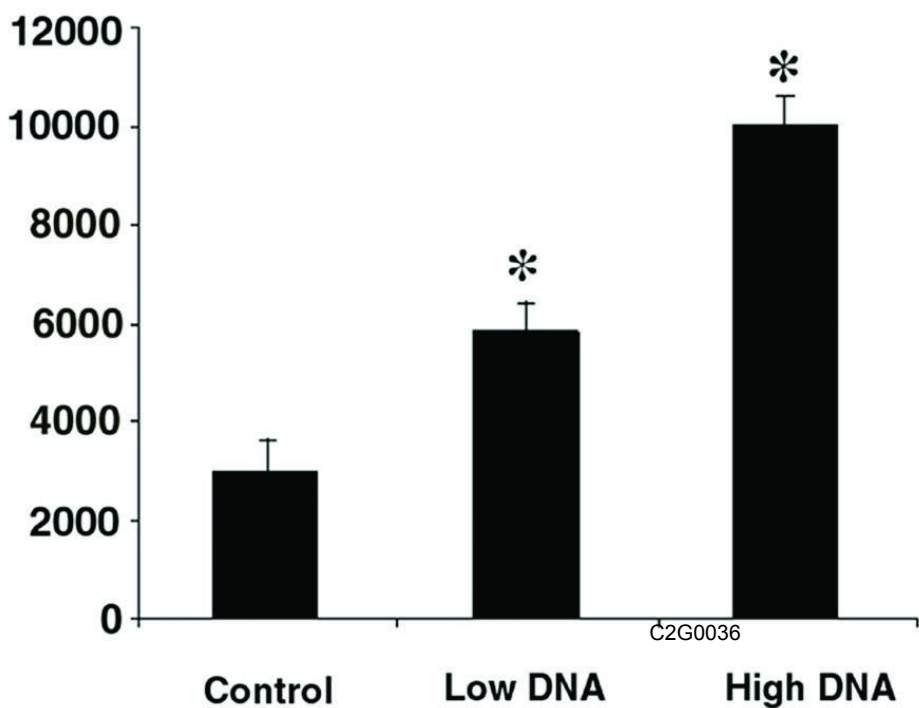


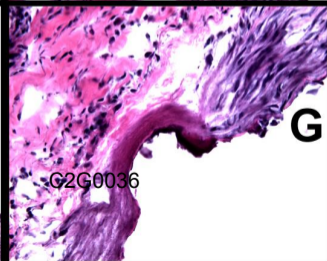
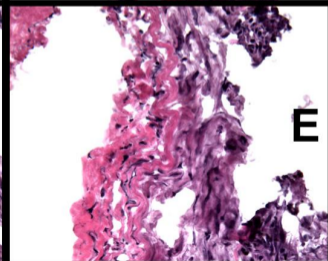
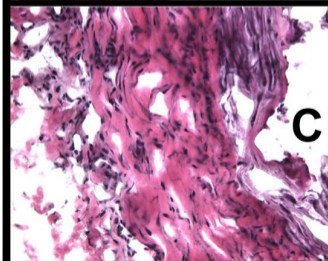
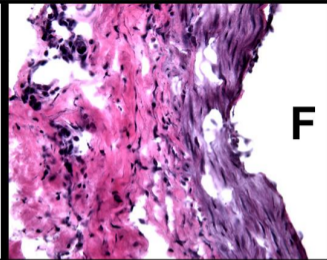
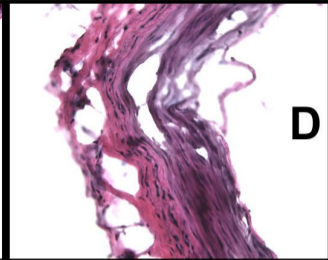
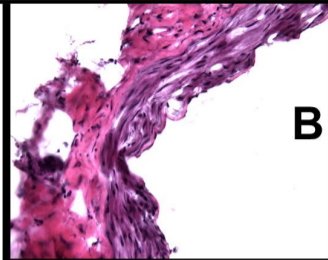
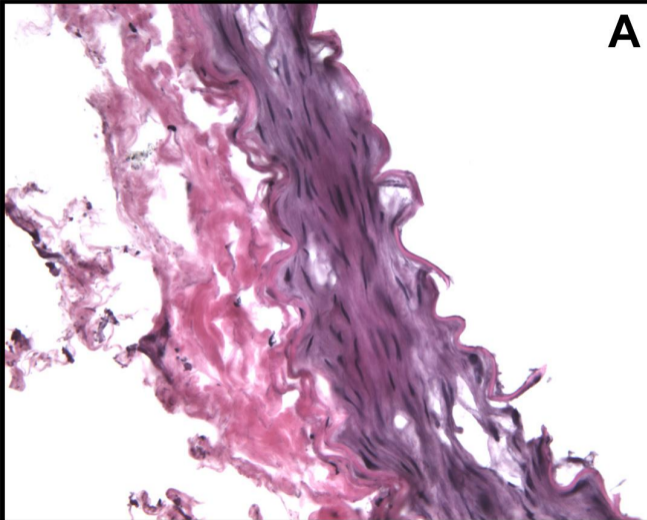
1, 6; DNA ladder
 2; Original plasmid
 3; Day 16 Sample
 4; Original plasmid + XhoI
 5; Day 16 Sample + XhoI

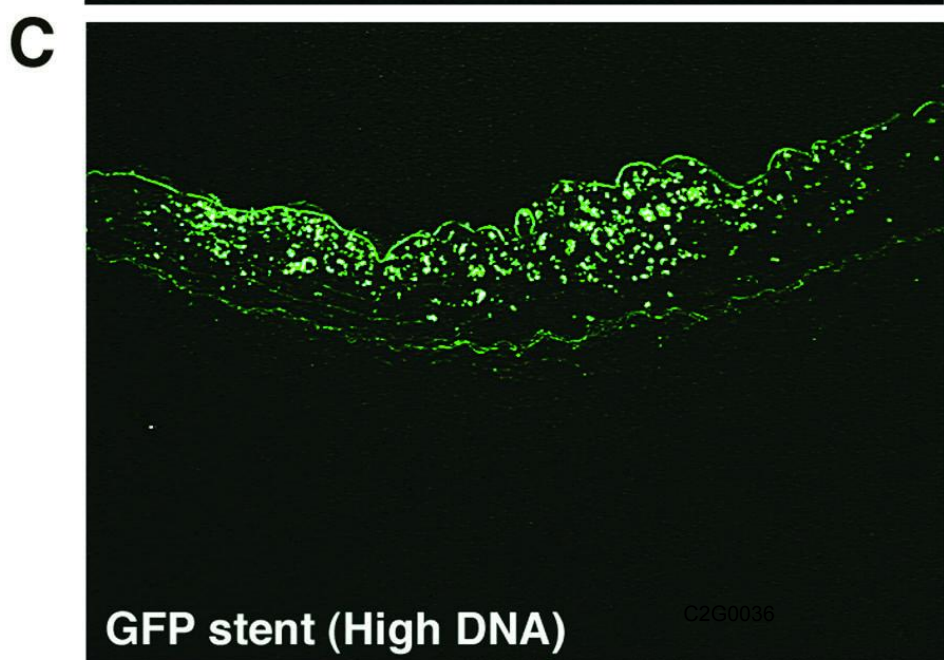
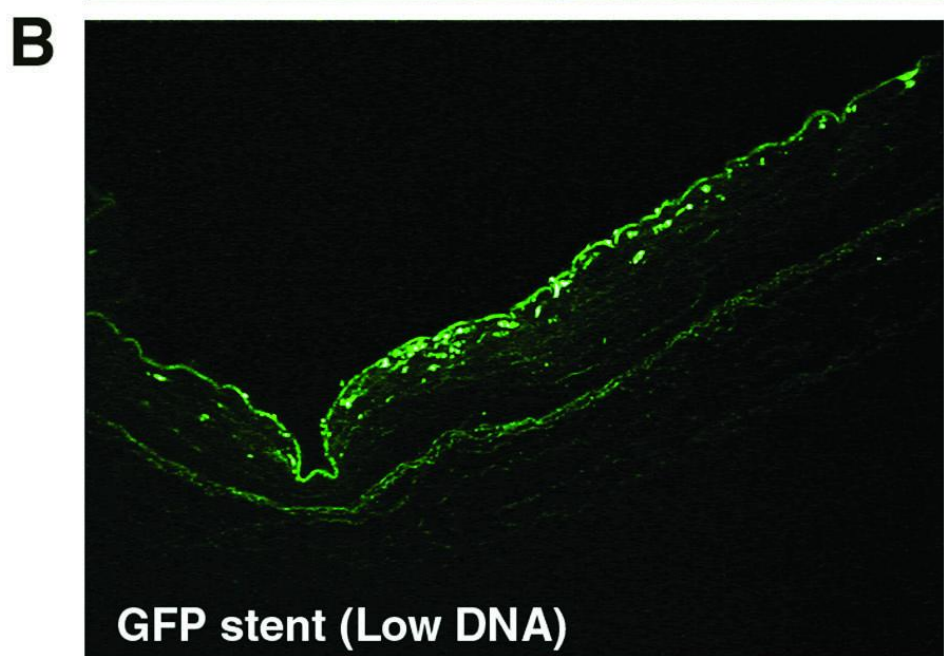
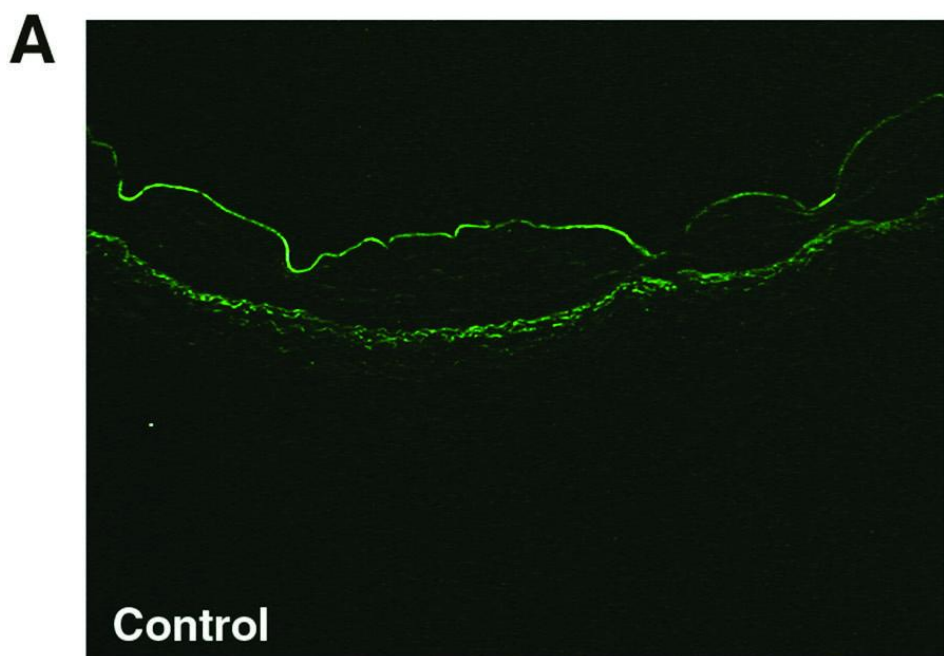
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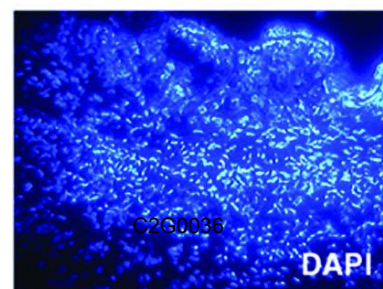
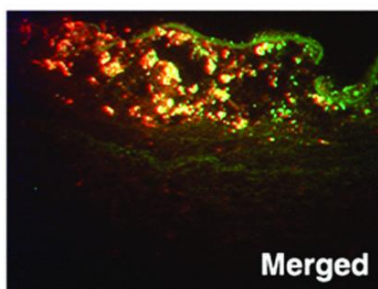
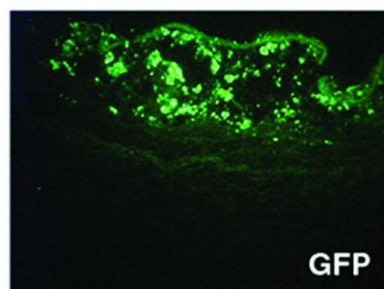
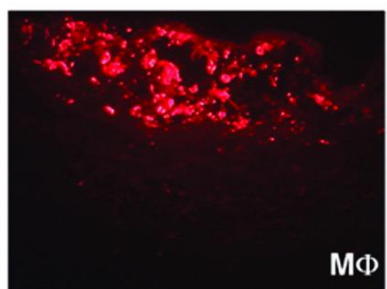
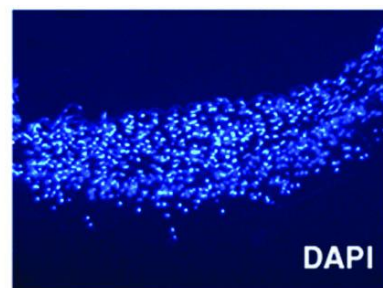
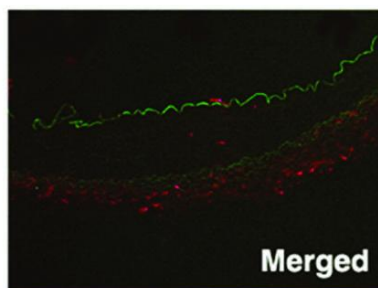
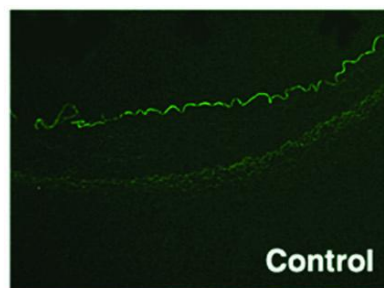
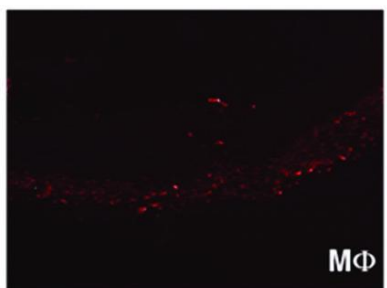
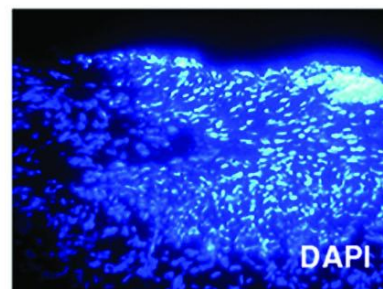
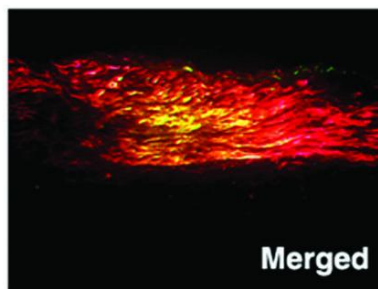
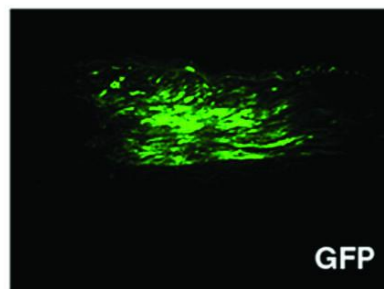
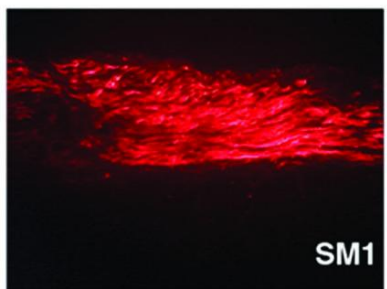
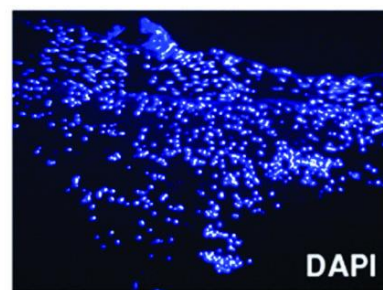
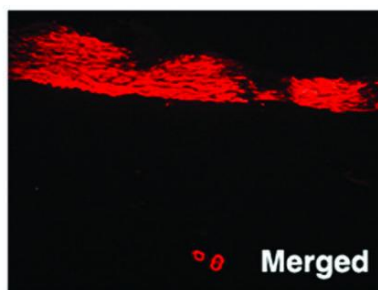
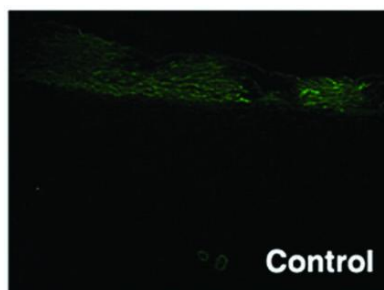
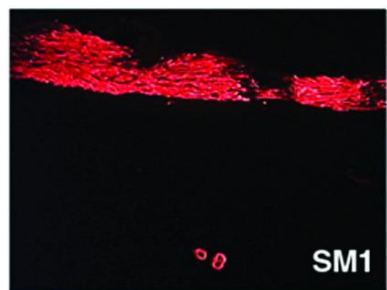
A**Relative Luciferase Expression**

P; Proximal
S; Stent
D; Distal
A; Aorta

B **β -galactosidase Activity**







1 2 3 4 5 6 7



1: Original plasmid
2: Stent site
3: Proximal
4: Distal
5: Lung
6: Liver
7: Spleen

C2G0036