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Temporal endogenous gene expression profiles in response to polymer-mediated transfection and profile comparison to lipid-mediated transfection

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Abstract

Background Design of efficient nonviral gene delivery systems is limited by the rudimentary understanding of specific molecules that facilitate transfection.

Methods Polyplexes using 25-kDa polyethylenimine (PEI) and plasmid encoding green fluorescent protein (GFP) were delivered to HEK 293T cells. After treating cells with polyplexes, microarrays were used to identify endogenous genes differentially expressed between treated and untreated cells (2 h of exposure) or between flow-separated transfected cells (GFP+) and treated, untransfected cells (GFP–) at 8, 16 and 24 h after lipoplex treatment. Cell priming studies were conducted using pharmacologic agents to alter endogenous levels of the identified differentially expressed genes to determine effect on transfection levels. Differentially expressed genes in polyplex-mediated transfection were compared with those differentially expressed in lipoplex transfection to identify DNA carrier-dependent molecular factors.

Results Differentially expressed genes were *RGS1*, *ARHGAP24*, *PDZD2*, *SNX24*, *GSN* and *IGF2BP1* after 2 h; *RAP1A* and *ACTA1* after 8 h; *RAP1A*, *WDR78* and *ACTA1* after 16 h; and *RAP1A*, *SCG5*, *ATF3*, *IREB2* and *ACTA1* after 24 h. Pharmacologic studies altering endogenous levels for *ARHGAP24*, *GSN*, *IGF2BP1*, *PDZD2* and *RGS1* were able to increase or decrease transgene production. Comparing differentially expressed genes for polyplexes and lipoplexes, no common genes were identified at the 2-h time point, whereas, after the 8-h time point, *RAP1A*, *ATF3* and *HSPA6* were similarly expressed. *SCG5* and *PGAP1* were only upregulated in polyplex-transfected cells.

Conclusions The identified genes and pharmacologic agents provide targets for improving transfection systems, although polyplex or lipoplex dependencies must be considered.

Keywords GFP, HEK 293, LF2000, microarray analysis, nonviral gene delivery, PEI, temporal gene expression profile

Introduction

Nonviral DNA delivery provides a mechanism to directly alter endogenous gene expression and cellular behavior with applications in functional genomics, tissue engineering, medical devices and gene therapy [1]. Because unaided delivery of naked plasmid DNA (pDNA) to cells has low efficacy, nonviral carriers are a common approach to enhance delivery. Nonviral carriers such as cationic polymers (polyplexes) [2,3] and lipids (lipoplexes) [4–9] are used to electrostatically complex DNA to protect it against degradation and facilitate DNA transfer [10–12]. However, current nonviral DNA delivery systems are inefficient for therapeutic use [11,13–15] because our rudimentary understanding of the molecules and processes that facilitate nonviral gene delivery limits the design of efficient delivery systems.

Previous studies have advanced the understanding of nonviral gene delivery, including particle tracking to identify extracellular [16–19] or intracellular [20–28] barriers to DNA transfer, or microarray analysis to understand the toxicogenomic response of cells and tissues to nonviral gene delivery systems [29]. Those particle tracking studies have identified compartments and components of the cell that interact with lipoplexes or polyplexes, at the same time as providing pharmacokinetic information on the movement of the complexes throughout the cell. Those data have been integrated into mathematical models to further our understanding of the gene transfer process [30–33]. Similarly, previous microarray studies have provided global gene expression profiles in cells and tissues exposed to cationic lipoplexes [29] or polyplexes [14] and identified specific genes involved in cytotoxicity and apoptosis [34], which can be dependent on the length of time of exposure to the DNA carrier, cell type, and DNA carrier [14,29,34]. These studies have been instrumental in providing information necessary for targeted physicochemical modification of the DNA carrier [31,35–39] and, although achieving promising *in vitro* and *in vivo* transfection from lipid and polymer DNA carriers [40], transfection still remains below desired levels. Those previous microarray studies were limited in that molecules that facilitate transfection were not identified (i.e. comparisons of gene profiles between treated and transfected cells to treated but untransfected cells were not made), even though it is the treated but untransfected cell population that limits the therapeutic efficacy and use of nonviral DNA delivery systems. A large gap in knowledge remains about molecules that actively facilitate DNA transfer [41], which will have to be exploited in the design of enhanced gene delivery systems.

To address the gap in knowledge concerning mechanisms that facilitate transfection, we previously published studies where, 24 h after delivery of DNA complexes encoding the green fluorescent protein (GFP) transgene, we isolated human embryonic epithelial kidney (HEK 293T) cells that expressed the transgene (GFP+) and compared their gene expression profile to the treated-but- untransfected cells (GFP–). In doing so, we identified specific genes and pathways utilized by transfected cells relative to untransfected cells and confirmed the ability of *RAP1A* and *HSP6A* to enhance lipoplex transfection [42] or *RAP1A*, *SCG5*, *ACRC*, *ATF3*, *ACTA1* and *IREB2* to enhance polyplex transfection [43]. Because those studies were performed at the 24-h time point when transgene expression is fully established, we now extend our approach in the present study for polyplexes by identifying genes at earlier time points because: (i) gene expression profiles have been shown to vary over time [44]; (ii) targeting a single barrier or step in DNA transfer will probably not lead to a therapeutically relevant transfection system [32]; and (iii) other genes and pathways largely remain unidentified that are implicated at earlier stages of transfection (e.g. internalization, endosomal escape, nuclear localization, nuclear entry) [3,19,30,31,39,41,45–52].

As a first objective of the present study, we identified genes and pathways implicated at 2, 8, 16 and 24 h after delivery of GFP transgene to HEK 293T cells using a cationic polymer vector (polyethylenimine; PEI), whereas our other work focuses on the lipid vector (Lipofectamine 2000; LF2000) [53]. PEI is a one of the most widely used polymer gene carriers because it transfects a broad range of cells and can be easily modified [54]. Relative to untreated cells, transcriptome profiling of HEK 293T cells at 2 h post-delivery of polyplexes was used to identify those endogenous genes that may act in response to treatment stress such as toxicity induced by the complex [13] or inherent intracellular defenses against foreign nucleic acids [55]. Relative to GFP– cells, transcriptome profiling was used to identify those endogenous genes used by transfected cells (GFP+) that may aid in overcoming cellular barriers known to occur during DNA transfer [3,19,30,31,39,41,45–52] at 8, 16 and 24 h post-delivery of polyplexes. Events that typically take place 8 h after delivery of complexes to the cells include high rate of endosomal escape [41,45], unpacking of pDNA from vector [45,46], some nuclear localization of complexes or pDNA [19,30,31,45,47], very small amounts of nuclear internalization of complexes or pDNA [30,39,47] and very little production of transgenic protein [30,48]. Events that typically take place 16 h after delivery of complexes to the cells include a high rate of nuclear localization of complexes or pDNA [19,30,47], continued nuclear internalization of complexes or pDNA [3,30,39,47] and continued production of transgenic proteins [30,48]. Events that take place 24 h after delivery of complexes to the cells include the continued nuclear internalization of pDNA [30,31,39,47], the highest synthesis of transgenic proteins [30,31,48] and mitosis (including distribution of transgenic proteins and pDNA to daughter cells) [47,49]. Therefore, the time points chosen for the studies in the present study were selected to capture several key cellular events that occur throughout the gene delivery process. We also ascertained the potential role of several of the identified genes as far as their ability to affect transfection by use of pharmacologic activators or inhibitors of the target endogenous gene. As a second objective of the present study, we compared those genes identified in the present study for polyplexes to those genes identified for lipoplexes [53]. Several different genes were expressed at each time point and may explain some of the mechanistic differences observed in transfection from the two nonviral gene delivery systems: internalization kinetics, mechanism of endosomal escape, and transfection levels [14,29–34,44,56–64]. The identification of molecules and pathways that facilitate transfection as identified in the present study offers new targets for engineering enhanced transfection systems or for cell priming strategies for increased transfection.

Materials and methods

Cell culture and plasmid preparation

HEK 293T cells (ATCC, Manassas, VA, USA) were cultured in T-75 flasks in Dulbecco's modified Eagle's medium containing 4.5 g/l glucose, supplemented with 10% fetal bovine serum, 1mM sodium pyruvate, 100 units/ml of penicillin-streptomycin (all Gibco, Carlsbad, CA, USA) and maintained at 37°C in a humidified 5% CO_2 atmosphere. For seeding, cells were dissociated at confluence with 1mM ethylenediaminetetraacetic acid (EDTA) and viable cells were counted using a hemocytometer and trypan blue dye exclusion assay. Plasmid pEGFP-LUC encodes both the enhanced green fluorescent protein (EGFP) and firefly luciferase protein (LUC) under the direction of a cytomegalovirus promoter (Clontech, Mountain View, CA, USA), and was used for transfection experiments (see below). Plasmid was purified from bacteria culture using Qiagen (Valencia, CA, USA) reagents and stored in Tris–EDTA buffer solution (10mM Tris, 1mM EDTA, pH 7.4) at –20°C.

Transfection and sample isolation for microarray

Cells were seeded at a density of 44.8×10^3 cells/cm^2 into multiple T-75 flasks. After adherence (approximately 18 h after seeding), polyplexes were formed using 25-kDa branched polyethylenimine (PEI; Sigma-Aldrich, St Louis, MO, USA). PEI was diluted in 1X Tris-buffered saline (TBS) and was added dropwise to pEGFPLuc diluted in 1X TBS at an N:P of 20 to deliver 0.25µg/cm^2 pDNA to the cells. After forming at room temperature for 15 min, the polyplexes were delivered to the media above the cells. Those transfection conditions were optimal for high transfection (typically 50–70%) and low cytotoxicity with optimization performed as previously described [43]. The complexes remained in contact with the cells for the duration of each experiment: 2, 8, 16 and 24h. After the incubation period of the polyplexes at each time point, cells were dissociated using 0.05% Trypsin-EDTA (except only EDTA for the 2-h condition) and suspended in 1X PBS at 2–5 million cells/ml and placed on ice. For the 2-h time point, because very little GFP had been produced, untreated samples were obtained identically as were the treated samples except no polyplexes were delivered to the cells. For the 8-, 16- and 24-h time points, after dissociation, the treated cells were separated into transfected (GFP+) and untransfected (GFP–) pure cell populations using fluorescence activated cell sorting (FACS), as described previously [42]. Briefly, at 8h, 16h, or 24h after addition of complexes, cells were dissociated, counted, and concentrated in 1X PBS and placed on ice. For the 8-, 16- and 24-h time points, after dissociation, the treated cells were separated into transfected (GFP+) and untransfected (GFP–) pure cell populations using fluorescence activated cell sorting (FACS), as described previously [45]. Briefly, at 8, 16 or 24 h after the addition of complexes, cells were dissociated, counted, and concentrated in 1X PBS and placed on ice, as described above. Flow cytometric analysis was performed using a B-D FACSVantage SE three-laser, high speed cell sorter (University of Nebraska-Lincoln's Center for Biotechnology Flow Cytometry Core Facility) equipped with a 530/15nm SE laser. A live gate was set on the GFP+ cell population in forward scatter versus side scatter plot to remove cell debris or clumped cells from the sort. A minimum of 2×10^6 cells for each population (GFP+, GFP–) was collected. Cells were collected at each time point on three different days providing the following independent samples (n=3 for each sample): 2 h treated, 2 h untreated, 8 h GFP+, 8 h GFP–, 16 h GFP+, 16 h GFP–, 24 h GFP+ and 24 h GFP– (for an overview of the experimental design, see Figure 1). RNA from each sample was then extracted, purified and hybridized to microarrays (see below).

RNA extraction and microarray hybridization

RNA extraction and quality check was performed as described previously [42]. Briefly, after obtaining each sample (24 in total: three each for 2 h treated, 2 h untreated, 8

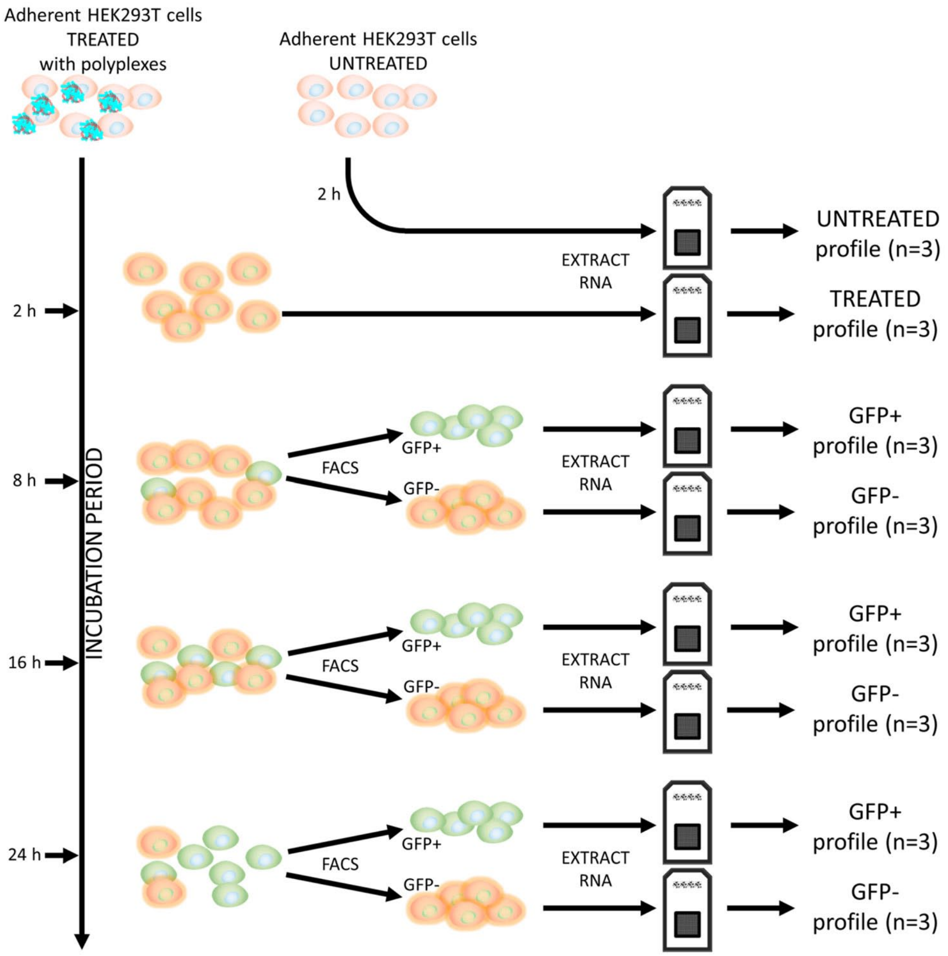

Figure 1. Overview of the experimental design (see Materials and methods). HEK 293T cells were seeded and allowed to become adherent (approximately 18 h). Then, lipoplexes were formed and delivered to adherent cells and allowed to remain in contact for 2, 8, 16 or 24 h. At the 2-h time point, treated cells were harvested and RNA was extracted and hybridized to microarrays. The treated profile was compared with cells that underwent the same treatments, except no lipoplexes were delivered. At the 8-, 16- or 24-h time points, treated cells were FACS separated into GFP positive (GFP+; transfected) and GFP negative (GFP–; untransfected) cell populations and RNA was extracted and hybridized to microarrays. The process was repeated on separate days to achieve *n*=3 for each population at each time point.

h GFP+, 8 h GFP–, 16 h GFP+, 16 h GFP–, 24 GFP+ and 24 h GFP–; see also above and Figure 1), total RNA was TRIzol extracted and further purified using a Qiagen RNeasy column (Qiagen) to achieve a 260/280 ratio greater than 1.8 on Nanodrop 2000 (Thermo Scientific, West Palm Beach, FL, USA). After quality assessment using an RNA 6000 Nano LabChip on an Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA), purified RNA was reverse transcribed to cDNA and hybridized to Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays (Affymetrix, Santa Clara, CA, USA) overnight at 45°C, in accordance with the manufacturer's instructions. After streptavidin-phycoerythrin conjugate staining, expression data were read with the GeneChip Scanner 3000 7G (Affymetrix) to obtain the expression data of over 47 000 transcripts and variants annotated for all known genes of the human genome. Affymetrix GeneChip Operating Software (GCOS, version 1.3) was used for washing, scanning and basic data analysis, including calculation of absolute values and normalization of the data with respect to internal standards.

Microarray and bioinformatics analyses

Each microarray provides 11 independent measures of gene expression (*n*=11) for over 47 000 transcripts and variants annotated for all known genes of the human genome. Microarray expression data were background adjusted and normalized [65] and quality tested using R/Bioconductor with AffyCoreTools library package with all arrays showing good hybridization quality, as described previously [42,43]. The gene expression data has been deposited at the NCBI Gene Expression Omnibus (GEO) accession GSE59665. Differentially expressed genes between treated and untreated (2-h time point) or GFP+ and GFP– (8-, 16- and 24-h time points) were determined using a nonparametric mean variance smoothing method developed for R programming environment [66]. Microarray data for the 24-h time point were previously collected and analyzed using linear models [42] and were downloaded from GEO accession GSE38422. Those microarrays were again analyzed here, using the nonparametric smoothing method, which resulted in a larger gene set differentially expressed between GFP+ and GFP– gene expression profiles than has been previously reported. Those results are included in the present study, along with the pathways and ontologies enriched to the new gene set (see below). Genes differentially expressed greater than two-fold and posterior probability greater than 0.99 were used for the bioinformatics analysis. Enrichr [67], an open source and freely available gene list enrichment analysis tool, was used to identify enriched pathways for Kyoto Encyclopedia of Genes and Genomes (KEGG) [68], WikiPathways (WIKI) [69], BioCarta [70], protein–protein interacting proteins (PPI Hub Proteins) [71], The Comprehensive Resource of Mammalian Protein Complexes (CORUM) [72], Reactome [73] and Gene Ontology Process (GO) [74]. Each list of differentially expressed genes was uploaded to the Enrichr [67] website and pathways or ontologies enriched greater than a combined score (Score) of 5 were reported. The combined score is computed by taking the log of the *p*-value from the Fisher's exact test multiplied by the Z-score of the deviation from the expected rank [67]. Among the differentially expressed genes, the most promising genes that may affect transfection were identified. The genes were selected based on high differential expression, putative gene role and potential to play a role in processes that are known to occur during DNA transfer [3,19,30,31,39,41,45–52]. After identifying target genes, the NextBio [75] Pharmaco Atlas was used to identify pharmacologic agents known to upregulate or downregulate the target gene with a score above 70. Those pharmacologic agents were then used for transfection studies in the presence of pharmacologic agents (see below).

Transfection in the presence of pharmacologic agents

These experiments were performed as previously described to alter the expression of endogenous target genes identified from microarray analysis [42]. The studies acted primarily as a screening tool to confirm whether a particular gene plays an important role in the DNA transfer process. Briefly, transfection studies were performed in the presence and absence of pharmacologic activators or inhibitors of the target genes (selected as described above). HEK 293T cells were seeded in 48-well plates at 44.8×10^3 cells/cm^2 and, 18 h later, pharmacologic agents were delivered to the media on the cells to achieve the final desired drug concentration. The pharmacologic agent was allowed to incubate for 1 h, and then lipoplexes were formed (as described above) and delivered to the media above the cells (still containing the pharmacologic agent). The pharmacologic agent and lipoplexes remained in contact with the cells for the next 24 h and then the cells were lysed and transfection levels were quantified by measuring the luciferase activity (relative light units) using the Luciferase Assay System (Promega, Madison, WI, USA) and luminometer (Turner Designs, Sunnyvale, CA, USA), and luciferase activity was normalized to the total protein amount determined with the BCA protein assay (Pierce, Rockford, IL, USA) and compared with transfection levels in vehicle-only control cells. The results were reported as the fold-change to eliminate variability in absolute measures of transfection, respectively, for *RGS1*, *ARHGAP24*, *PDZD2*, *SNX24*, *GSN* and *IGF2BP1* genes; pharmacologic activators included immunoglobin G [76], 5-episisomicin [77], apramycin [77], 4-hydroxytamoxifen [78], folic acid [79] and tetrachloroethylene [80]; and pharmacologic inhibitors included eicosapentaenoic acid [81], cytoclasin B [82], tolazoline [77], benzoic acid [77], hydrocortisone [83] and propanil [84] (all from Sigma-Aldrich, St Louis, MO, USA); the concentrations of pharmacologic agents selected for the present study were comparative to those tested in the literature showing gene activity [77,82–84] but were optimized for high transfection and minimal toxicity (not shown) (see Supporting information, Figure S1). Statistical analysis was performed using Prism, version 5 (GraphPad, La Jolla, CA, USA) using Student's *t*-test at a 95% confidence level. Data are reported as the mean ±SEM ($n=3$).

Results

PEI polyplexes, carrying a plasmid encoding a GFP reporter gene, were delivered to HEK 293T cells. Then, 2 h after delivery of the polyplexes, we identified genes and pathways regulated in the initial response to treatment with polyplexes by comparing changes in the transcriptome between cells treated by polyplexes with cells not treated by polyplexes (Figure 1). All genes found to be differentially expressed two-fold or greater are listed in the Supporting information (Table S1). Among the greatest differentially expressed genes in cells treated compared to untreated (at the 2-h time point) were: *RGS1*, *ARHGAP24*, *PDZD2*, *SNX24*, *GSN* and *IGF2BP1*, which were

Table 1. Cell shutdown in response to treatment with polyplexes

Gene	Fold Δ	Name
RGS1	−6.9	Regulator of G-protein signaling 1
ARHGAP24	−11.8	Rho GTPase activating protein 24
PDZD2	−14.9	PDZ domain containing 2
MARK1	−35.5	MAP/microtubule affinity-regulating kinase 1: accession AL049302
DENND4A	−37.1	DENN/MADD domain containing 4A: accession T86874
SNX24	−38.3	Sorting nexin 24
ZNF79	−40.2	Zinc finger protein 79
VNN3	−41.7	Vanin 3
C6orf148	−44.2	Chromosome 6 open reading frame 148: accession T97641
GSN	−45.4	Gelsolin
IGF2BP1	−48.8	Insulin-like growth factor 2 mRNA binding protein 1
CPB2	−49.8	Carboxypeptidase B2 (plasma)
IGLV3–25	−54.6	Immunoglobulin lambda variable 3–25
LGSN	−57.0	Lengsin, lens protein with glutamine synthetase domain
LOC400084	−57.6	Uncharacterized LOC400084
EXOC3L4	−58.3	Exocyst complex component 3-like 4
KRT27	−69.7	Keratin 27

Fold change indicates differential expression by comparing microarrays of HEK 293T cells treated with pEGFPLuc/LF2000 (n=3) with microarrays from untreated cells (n=3) with a posterior probability greater than 0.99 at the 2-h time point. Negative numbers indicate downregulation.

downregulated by 6.9-, 11.8-, 38.3-, 45.4- and 48.8-fold, respectively (Table 1). Of all genes differentially expressed between treated and untreated cells at the 2-h time point (see Supporting information, Table S1), only *YWHAE*, *EGR1* and *GSN* were found to be enriched to cellular pathways, including kinase maturation, emerin, EGR-EP300 and prunegelsolin (Table 2). Taken together, treated cells were shown to undergo rapid transcriptional changes in response to treatment with polyplexes in a highly downregulated manner.

Because most cells internalize polyplexes within the first few hours of exposure [3,30,41,45,50–52; unpublished results, S.A.P] but not all cells eventually express the transgene, we next set out to identify those endogenous genes that may facilitate transfection. To do so, polyplexes were delivered to the cells and, after 8, 16 or 24 h, the treated cell population was sorted into GFP+ and GFP– subpopulations (Figure 1). Using microarray analysis, changes in the transcriptome were identified by comparing endogenous gene expression profiles between the GFP+ and GFP– subpopulations. All genes found to be differentially expressed two-fold or greater are listed in the Supporting information (Table S1). At the 8-h time point, the greatest differentially expressed genes between the GFP+ and GFP– populations were *RAP1A*, *ACRC* and *ACTA1*, upregulated by 22.71-, 7.75- or 6.01-fold, respectively (Table 3). At the 16-h time point, the greatest differentially expressed genes were *RAP1A*, *CHORDC1*, *NEB*, *WDR78*, *ACTA1*, *ACRC* and *PGAP1*, upregulated by 13.20-, 9.36-, 7.32-, 6.29-, 5.48-, 5.42- or 5.26-fold, respectively (Table 3). At the 24-h time point, the greatest differentially expressed genes were *RAP1A*, *SCG5*, *ACRC*, *ATF3*, *IREB2* and *ACTA1*, upregulated by 14.59-, 6.15-, 6.14-, 5.83-, 5.71- or 5.04-fold, respectively (Table 3).

Table 2. Enriched pathways involved in the cell shutdown response

Source	Term	Score	Genes
CORUM	Kinase maturation complex 1	6.62	*YWHAE*
CORUM	Emerin complex 25 (human)	4.55	*YWHAE*
CORUM	EGR-EP300 complex (human)	4.44	**EGR1**
CORUM	Prune-Gelsolin complex (human)	4.39	***GSN***

Genes found in Table 1 were found to be over-represented to specific terms. The first column indicates the source of pathway database; the second column lists the enriched term, the third column lists the enrichment score; and the last column indicates which of the differentially expressed genes belong to the enriched term. Downregulated genes are shown in italic, upregulated genes are underlined, and genes greater than five-fold differentially expressed are shown in bold. For a description of the source or score, see Materials and methods.

We were next interested in understanding how the gene expression profile changes over time, in terms of number of genes expressed, as well as which genes were found to be differentially expressed over multiple time points. The number of genes differentially expressed (between GFP+ and GFP– cell populations) decreased from 8 h to 16 h from 179 genes to 156 genes, respectively, with 14 genes commonly expressed between the two time points (see Supporting information, Table S2). The number of genes differentially expressed increased from 16 h to 24 h from 156 genes to 457 genes (Figure 2), respectively, with 33 genes commonly being expressed between the two time points (see Supporting information, Table S2), and with *IREB2* notably overexpressed five-fold. The largest number of genes differentially expressed occurred after 24 h of exposure to polyplexes (*n*=457) (Figure 2;

Table 3. Temporal cell response after treatment with polyplexes comparing GFP+ with GFP– gene expression profiles

Time	Gene	Fold Δ	Posterior probability	Accession number	Name
8 h	*RAP1A	22.71	1.000	AB051846	RAP1A, member of RAS oncogene family
	ERVK3-2	8.97	1.000	NM–022155	Endogenous retrovirus group K3, member 2
	WDR78	7.90	1.000	BC032406	WD repeat domain 78
	*ACRC	7.75	1.000	AI091533	Acidic repeat containing
	NEB	6.45	1.000	NM–004543	Nebulin
	*ACTA1	6.01	1.000	NM–001100	Actin, alpha 1, skeletal muscle
	TPH1	5.54	1.000	AI350339	Tryptophan hydroxylase 1
	KIAA1731	5.33	1.000	AL833615	Centrosomal protein KIAA1731
	CKS2	–6.16	0.992	NM–001827	CDC28 protein kinase regulatory subunit 2
16 h	*RAP1A	13.20	1.000	AB051846	RAP1A, member of RAS oncogene family
	CHORDC1	9.36	1.000	AI473843	Cysteine and histidine-rich domain (CHORD) containing 1
	ERVK3-2	7.51	1.000	NM–022155	Endogenous retrovirus group K3, member 2 [NM–022155]
	NEB	7.32	1.000	NM–004543	Nebulin
	WDR78	6.29	1.000	BC032406	WD repeat domain 78
	*ACTA1	5.48	1.000	NM–001100	Actin, alpha 1, skeletal muscle
	*ACRC	5.42	1.000	AI091533	Acidic repeat containing
	PGAP1	5.26	1.000	BF063546	Post-GPI attachment to proteins 1
	KIAA1731	5.24	1.000	AL833615	Centrosomal protein KIAA1731
24 h	*RAP1A	14.59	1.000	AB051846	RAP1A, member of RAS oncogene family
	CHORDC1	10.95	1.000	AI473843	Cysteine and histidine-rich domain (CHORD) containing 1
	WDR78	8.78	1.000	BC032406	WD repeat domain 78
	PGAP1	7.70	1.000	BF063546	Post-GPI attachment to proteins 1
	ERVK3-2	6.65	1.000	NM–022155	Endogenous retrovirus group K3, member 2 [NM–022155]
	NEB	6.59	1.000	NM–004543	Nebulin
	*SCG5	6.15	1.000	NM–003020	Secretogranin V (7B2 protein)
	*ACRC	6.14	1.000	AI091533	Acidic repeat containing
	*ATF3	5.83	1.000	NM–001674	Activating transcription factor 3
	*IREB2	5.71	1.000	BC017880	Iron-responsive element binding protein 2
	AI939588	5.10	1.000	AI939588	NA
	*ACTA1	5.04	1.000	NM–001100	Actin, alpha 1, skeletal muscle
	AW627671	–5.16	0.996	AW627671	NA
	AL530869	–5.34	0.997	AL530869	NA
	NDUFA13	–5.58	0.994	NM–015965	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13
	KIAA1324	–5.66	0.993	AB037745	KIAA1324
	RBBP4	–5.71	0.991	BC003092	Retinoblastoma binding protein 4
	FAF2	–5.72	0.990	AB020694	Fas associated factor family member 2
	HEATR1	–5.78	0.992	NM–018072	HEAT repeat containing 1
	AGBL3	–6.22	0.996	NM–024856	ATP/GTP binding protein-like 3
	MAPK1IP1L	–6.51	0.996	AI671747	Mitogen-activated protein kinase 1 interacting protein 1-like
	DNAJA1	–6.85	0.996	NM–001539	DnaJ (Hsp40) homolog, subfamily A, member 1
	SNHG6	–7.63	0.997	BG169443	Small nucleolar RNA host gene 6 (nonprotein coding)
	EIF4A2	–8.60	0.996	NM–001967	Eukaryotic translation initiation factor 4A2
	TRA2B	–9.44	0.998	NM–004593	Transformer 2 beta homolog (Drosophila)
	CKB	–9.84	0.991	NM–001823	Creatine kinase, brain
	ACTB	–15.16	0.995	NM–001101	Actin, beta
	ND2	–17.83	0.998	NM–173709	MTND2
	AF253979	–18.74	0.997	AF253979	NA

Comparison of GFP+ with GFP– gene expression levels. Differential expression greater than or less than 1 represents upregulation or downregulation, respectively. Data represent genes differentially expressed comparing transfected microarrays (n=3) with treated, untransfected microarrays (n=3) with a posterior probability greater than 0.99 and differential expression greater than five-fold. The Supporting information (Table S1) lists all genes differentially expressed greater than two-fold. Genes indicated with an asterisk (*) represent genes previously identified to alter transfection [43]. NA: not available.

see also Supporting information, Table S2). Additionally, *RAP1A*, *ERVK3-2*, *WDR78*, *ACRC*, *NEB*, *ACTA1*, *CHORDC1*, *SCG5*, *PGAP1*, *ATF3*, *AI939588* and *AL833615* may play a role in multiple aspects that facilitate DNA transfer because they were overexpressed at each time point (Figure 2; see also Supporting information, Tables S1 and S2).

To determine the role of all differentially expressed genes at each time point (8, 16 and 24 h; see Supporting information, Table S1), we performed a pathway and ontology enrichment analysis (Table 4; see Materials and methods). Pathways enriched from genes differentially expressed at the 8-h time point were muscle contraction, cell cycle process, cellular pathways (MPR, G2 and integrin) and protein complexes (HDAC1, RC and ING2) (Table 4). Pathways enriched at the 16-h time point were muscle contraction and the integrin pathway (Table 4). Pathways

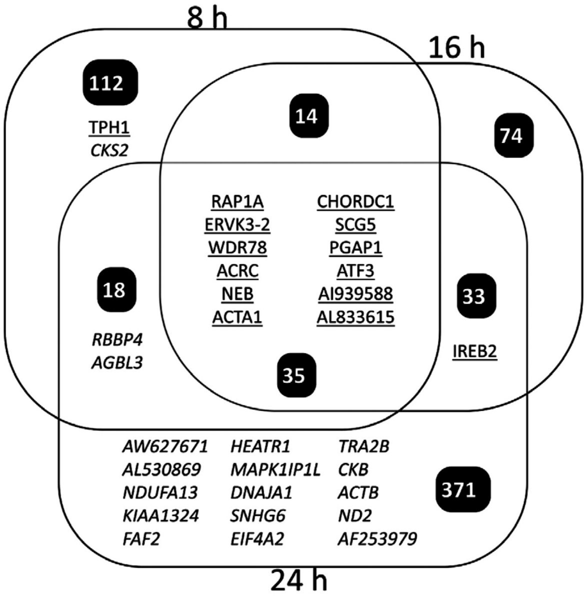

Figure 2. The overlap of genes differentially expressed between the GFP+ and GFP– gene expression profiles is shown for each time point. Numbers in white encircled by a black background represent the total number of genes greater than two-fold differentially expressed comparing transfected microarrays (GFP+; *n*=3) with treated, untransfected microarrays (GFP–; *n*=3) with a posterior probability greater than 0.99. Genes that are greater than five-fold differentially expressed are listed by name. The specific genes associated with the Venn diagram are listed in the Supporting information (Table S2). The level of differential expression for each gene is provided in the Supporting information (Table S1).

enriched at the 24-h time point nucleic acid binding, regulation of transcription, RNA metabolism and processing, response to DNA damage, nuclear factor-kappa B (NF-κB) protein hub and the androgen receptor signaling pathway (Table 4). Because the transfected cells (GFP+) exhibited different genes and enriched pathways at each time point compared to untransfected cells (GFP–), those genes and pathways may play a role in transfection.

As reported in the present study, microarray analysis revealed genes highly differentially expressed in treated cells compared to untreated cells at the 2-h time point and in GFP+ cells compared to GFP– cells at the 8-, 16- and 24-h time points (Tables 1 and 3). To discriminate whether any of the genes identified in the present study have a potential role in transfection as opposed to genes that are a treatment effect, pharmacologic studies were conducted. Those genes highly differentially expressed at 2-, 8-, 16- or 24-h time points (Tables 1 and 3) were narrowed to a smaller subset to further test based on a literature review of each gene's putative function and potential role in the DNA transfer process. Highly differentially expressed genes *RAP1A*, *ACRC*, *ACTA1*, *SCG5*, *ATF3* and *IREB2* (Table 3) were not studied here using pharmacologic agents because we have previously shown that perturbing those genes prior to polyplex delivery can enhance transfection by up 2.5-fold [43], suggesting a role for those genes in transfection. The list of genes was narrowed to *RGS1*, *ARHGAP24*, *PDZD2*, *SNX24*, *GSN* and *IGF2BP1*. Pharmacologic agents known to activate or inhibit each gene were determined using NextBio [75] and from previous studies in the literature [77,82,85–87] and used as a screening tool to confirm whether a particular gene may play an important role in the DNA transfer process. The effects of the pharmacologic agent on transfection levels are summarized in Table 5 with transfection levels provided in Figure 3. In all pharmacologic studies reported here, little-to-no cytotoxicity was observed (see Supporting information, Figure S1) at the concentrations used.

For every target gene investigated, opposing pharmacologic treatments resulted in opposing transfection trends (i.e. if activating a target gene resulted in enhanced transfection, then inhibiting that same target gene resulted in decreased transfection) (Figure 3 and Table 5). There was no trend of whether activation of the target gene always lead to increased transfection or whether inhibition of the target gene lead to decreased transfection (Figure 3 and Table 5). Targets and treatments that resulted in increased transfection relative to untreated controls were: *RGS1* inhibited by 3 μM eicosapentaenoic acid [81] (1.2- fold), *ARHGAP24* inhibited by 1 μM cytoclasin B [82] (1.7-fold), *SNX24* activated by 10 μM 4-hydroxytamoxifen [78] (5.3-fold), *GSN* inhibited by 10 μM hydrocortisone [83] (2.5-fold) and *IGF2BP1* activated by 1 nM tetrachloroethylene [80] (1.2-fold) (Figure 3 and Table 5). Targets and treatments that resulted in decreased transfection relative to untreated controls were: *RGS1* activated by 0.1 μM immunoglobin G [76] (5.7-fold), *ARHGAP24* activated by 0.1 μM 5-episisomicin [77] (5.2-fold), *PDZD2* inhibited by 0.1 μM tolazoline [77] (4.4-fold), *SNX24* inhibited by 1 μM benzoic acid [77] (4.0-fold), *GSN* activated by 1 μM folic acid [79] (5.2-fold) and *IGF2BP1* inhibited by 1 μM propanil [84] (4.8-fold) (Figure 3 and Table 5). When *PDZD2* was activated by 30 μM apramycin [77] prior to delivery of polyplexes, transfection was unchanged. Taken together, altering *RGS1*, *ARHGAP24*, *PDZD2*, *SNX24*, *GSN* and *IGF2BP1* gene activity can affect transfection, showing that microarray analysis can identify genes possibly playing a role in transfection.

We next set out to identify a molecular component to explain the mechanistic differences of transfection observed for polyplexes and lipoplexes [14,29–34,44,58–64]. The number and type of differentially expressed genes for each DNA carrier over time was examined. Relative to control, 2 h after treatment of cells with polyplexes (present study) or lipoplexes [53], a similar number of genes were downregulated in response to treatment with complexes (46 genes for polyplexes; 44 genes for lipoplexes)

Table 4. Top enriched pathways

Source	Term	Score	Genes
8 h			
WIKI	Hs_striated_muscle_contraction_ WP383_21484	7.15	ACTA1, MYH3, *ACTN2*, **NEB**
Reactome	Muscle contraction	6.03	MYH3, *ACTN2*, **NEB**
Reactome	Cell cycle checkpoints	5.13	*ATR, BUB3, CDC25C*
BioCarta	MPR pathway	5.98	**ACTA1,** *CDC25C, CCNB1*
BioCarta	G2 pathway	5.43	*ATR, CDC25C, CCNB1*
BioCarta	Integrin pathway	5.06	ACTA1, *ACTN2*, **RAP1A**
PPI HUB	HDAC1	7.08	SUDS3, ATR, RBBP4, ACTA1, BUB3, REPIN1, MORF4L2, **RAP1A**
CORUM	Cell cycle kinase complex CDC2 (human)	7.33	*CCNB1, CDK1*
CORUM	RC complex during G2/M-phase of cell cycle	5.43	*CDK1, CCNB1*
CORUM	ING2 complex (human)	5.07	*RBBP4, SUDS3*
16 h			
WIKI	Hs_striated_muscle_contraction_ WP383_21484	8.88	ACTA1, MYH3, ACTN2, NEB
Reactome	Muscle contraction	6.86	MYH3, ACTN2, NEB
BioCarta	Integrin pathway	7.52	**ACTA1**, ACTN2, **RAP1A**
24 h			
GO	Nucleic acid binding (GO: 0003676)	8.88	*RAD23A, EIF4A2, RBM15B, NR2C2, TEAD2,* ATF3, *GTF3C2, GRSF1, PRDM2, HNRNPA1, GLI2, RAD52, RC3H2, CARS,* FUBP1, *ZNF45, HEXIM1, KLF10, ZNF217, EIF5, RBM5, SIX2,* DDIT3, *RBPMS,* **IREB2**, *RBMX, POLD3, NFX1, TSC22D3,* CHD2, *RAD51C, POLH, SUB1, SNAPC4, TBX1, MEN1*
GO	Regulation of transcription (GO: 0045449)	14.38	*EPC1, RBM15B, NR2C2, TEAD2,* HDAC8, *ID3, PRDM2, AFAP1L2, GLI2, DEDD2, UBE2N,* MDM2, *TRIB1, CTNNB1, ZNF45, HEXIM1, KLF10,* ***NDUFA13***, GTF2H1, DDIT3, *UIMC1, NFX1, NCOA4, TSC22D3,* CHD2, *KDM3A, SUB1, TBX1, HOXC6, MEN1, SP3*
GO	RNA metabolic process (GO: 0016070)	12.88	*ISG20,* ZNF346, GTF2H1, *QTRT1, SF3B3, RNASET2,* DDIT3, *RBPMS, METTL3, RBMX, GTF3C2, GRSF1, EIF2C3, BOP1, HNRNPA1, POLRMT, DEDD2, SNAPC4, CARS, SMC1A,* FUBP1, ***TRA2B***, *TRA2A*
GO	Response to DNA damage stimulus (GO: 0006974)	6.31	*RAD51C, RAD23A, AEN,* GTF2H1, DDIT3, *UIMC1, POLD3, SMC1A, MEN1, UBE2N, PPP1R15A*
PPI HUB	NFKB1	5.30	*CTNNB1, ISG20,* ***CKB, DNAJA1,*** ATF3, PLK2, *AKT1, PPP1R15A,* ***ACTB***
WIKI	Hs_mRNA_processing_WP411_26223	5.97	*RBM5, PTBP2, METTL3, NXF1, RBMX, HNRNPA1, SMC1A,* ***TRA2B***, *SF3B3*
WIKI	Hs_androgen_receptor_signaling_pathway_ WP138_20973	5.45	*CTNNB1, NR2C2, AKT1, PSMC3IP, SLC25A4,* GTF2H1, *NCOA4,* MDM2

Selected enriched terms are listed for differentially expressed transcripts comparing GFP+ microarrays with GFP– microarrays at each indicated time point after delivery of pEGFPLuc/LF2000 or PEI complexes to HEK 293T cells. The first column indicates the name of pathway database; the second column lists the enriched term; the third column lists the enrichment score (see Materials and methods); and the last column indicates which of the differentially expressed genes belong to the enriched term. Downregulated genes are shown in italic, upregulated genes are underlined, and genes greater than five-fold differentially expressed are shown in bold. CORUM, the comprehensive resource of mammalian protein complexes; GO, Gene Ontology; PPI HUB, protein–protein interacting proteins; WIKI, WikiPathways; Reactome KEGG, Kyoto Encyclopedia.

Table 5. Transfection in the presence of pharmacologic agents

Gene	Putative gene function	Pharmacologic agent[a]	Vehicle (Control+)	Transfection fold change[b]
RGS1	GAP or negative regulator of RhoGTPases and may play an inflammatory and immunological role after bacterial and viral infection [105]	Immunoglobin G ↑ [76]	NaCl	*–57****
		Eicosapentaenoic acid ↓ [81]	MeOH	1.2
ARHGAP24	GAP or negative regulator of RhoGTPases, acting to disrupt actin stress fibers and formation of filopodia [96], promote cell polarity and prevent cell migration [136]	5-episisomicin ↑ [77]	ddH_2O	*–52**
		Cytoclasin B ↓ [82]	DMSO	1.7
PDZD2	Involved in clustering transmembrane receptors, organizing cell junctions, organizing plasma membrane domains (e.g. lamellipodia) [137], interacting with the cortical scaffold [138] and stimulating proliferation [139]; involved in endocytosis via interaction with syndecans and proteoglycans [112]	Apramycin ↑ [77]	ddH_2O	1.0
		Tolazoline ↓ [77]	ddH_2O	*–44**
SNX24	Binds endodomain of receptors on clathrin-coated pits to cause their internalization; involved in membrane trafficking to facilitate exocytosis or trafficking of endosomes to the perinuclear region [113]	4-hydroxytamoxifen ↑ [78]	MeOH	5.3***
		Benzoic acid ↓ [77]	MeOH	*–40**
GSN	Plays a role in ciliogenesis via a calcium-regulated assembly and disassembly of actin filaments, and also to inhibit apoptosis by stabilizing the mitochondria [97]	Folic acid ↑ [79]	NaOH	*–52***
		Hydrocortisone ↓ [83]	MeOH	2.5**
IGF2BP1	Associates with microtubules, microfilaments, and lamellipodia; affects cellular growth and proliferation; increases cell survival during stress [56,140,101]	Tetrachloroethylene ↑ [80]	MeOH	1.2
		Propanil ↓ [84]	MeOH	*–48**

a. Effect of pharmacologic agent on gene or protein activity as reported in the literature: ↑, activation; ↓, inhibition.

b. Transfection fold change comparing cells treated with pharmacologic agent with cells treated with vehicle only (Control+) (Figure 3). Transfection levels were measured 24 h after delivery of complexes to HEK 293T cells. The concentration of the pharmacologic agent was selected for minimal cytotoxicity and maximal effect (see Supporting information, Figure S3).

NaCl = 0.1M sodium chloride; MeOH = methanol; ddH_2O = double distilled water; DMSO = dimethyl sulfoxide; NaOH = 1M sodium hydroxide

Data are reported as the mean (n=3) and significant changes between treated and vehicle-only transfection levels are indicated as: * $p < 0.05$: ** $p < 0.01$; *** $p < 0.001$.

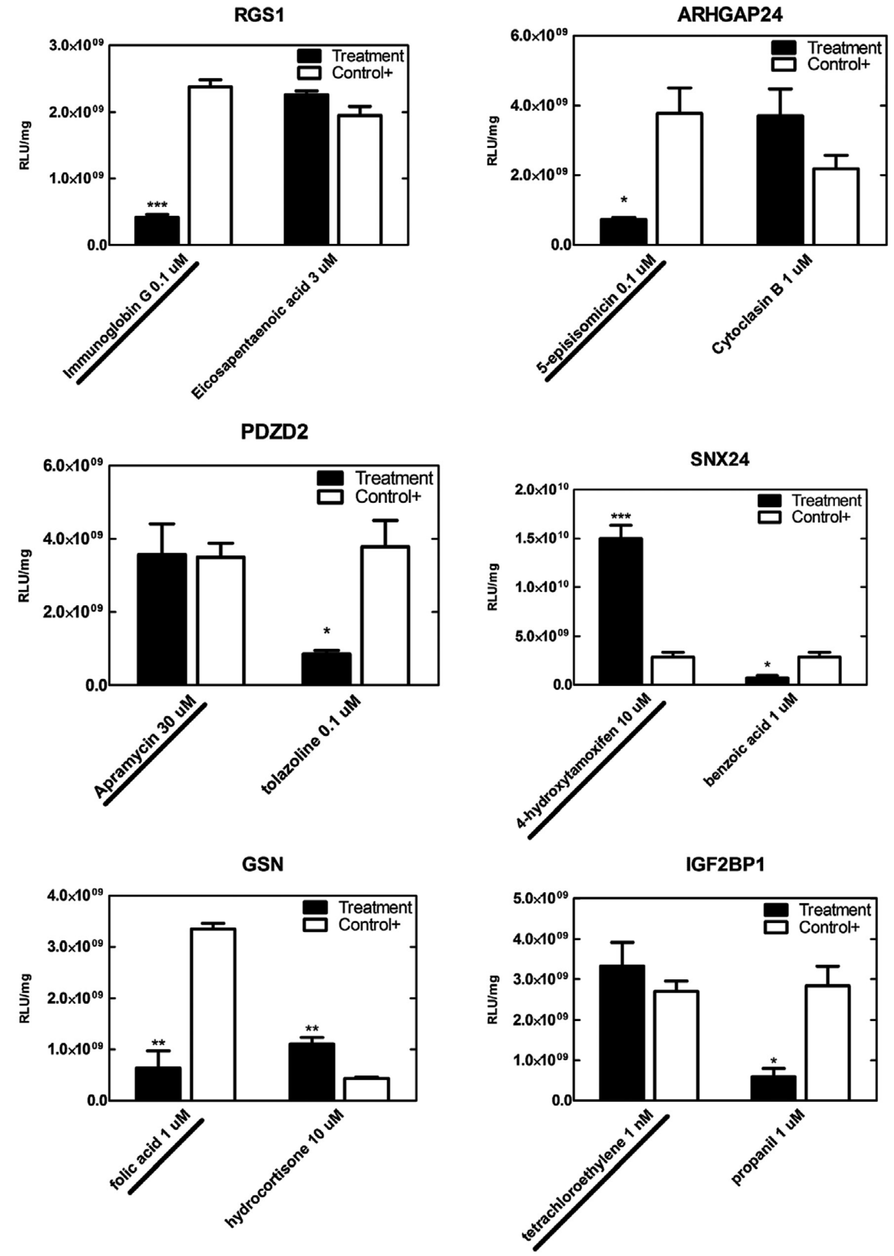

Figure 3. Effect of target gene on transfection for HEK 293T cells treated with indicated pharmacologic agent (black bars) or treated only with vehicle used to deliver the pharmacologic agent (Control+; open bars) (Table 5) to activate (underlined text) or inhibit (plain text) indicated genes. After a 1-h incubation period, lipoplexes were delivered and transfection levels were assayed after 24 h (see Materials and methods). Data are reported as the mean±SEM (*n*=3) and significant changes between treated and vehicle-only transfection levels are indicated as: * $p < 0.05$; ** $p < 0.01$; or *** $p < 0.001$.

(Figure 4) but no gene was commonly involved in the transcriptional shutdown of each vector. Comparing GFP+ cells with GFP– cells, 8 h after exposure of complexes to cells, 179 genes were differentially expressed for polyplexes and only seven genes for lipoplexes (Figure 4), with *RAP1A* and *ATF3* commonly being differentially expressed for both DNA carrier systems. Comparing GFP+ cells with GFP– cells, 16 h after exposure of complexes to cells, 156 genes were differentially expressed for polyplexes and only 27 genes for lipoplexes (Figure 4), with *ZNF236*, *RAP1A*, *ZRSR1*, *ZNF79* and *ATF3* commonly differentially expressed for both DNA carrier systems. Comparing GFP+ cells with GFP– cells, 24 h after exposure of complexes to cells, 456 genes were differentially expressed for polyplexes and only two for lipoplexes (Figure 4), with *RAP1A* and *HSPA6* commonly being differentially expressed for both DNA carrier systems. Taken together, cells immediately respond to treatment with complexes via a downregulation of transcription with either lipid or polymer gene carriers. At later time points, GFP+ cells

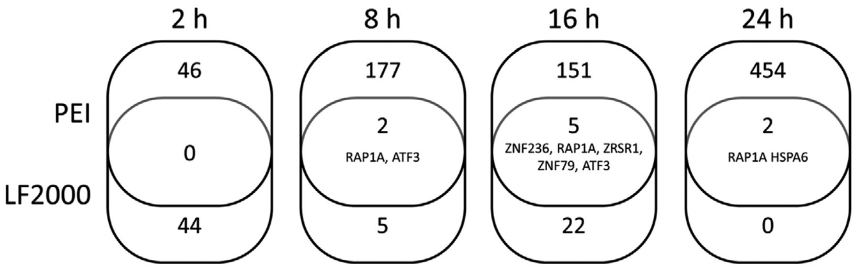

Figure 4. Overlap of the number of differentially expressed genes when comparing those genes identified in polyplex transfection (present study) with lipoplex transfection [53]. The number of genes differentially expressed are shown for each time point (2 h comparing treated with untreated gene expression profiles; 8, 16 and 24 h comparing GFP+ with GFP– gene expression profiles) and DNA carrier (PEI, LF2000). Data represent genes greater than two-fold differentially expressed comparing treated and untreated microarrays (*n*=3) or GFP+ and GFP– microarrays (*n*=3) with a posterior probability greater than 0.99. The specific genes associated with the Venn diagram are listed in the Supporting information (Table S3). The level of differential expression for each gene is provided in the Supporting information (Table S1) for polyplexes or as supplemental information in our other work for lipoplexes [53].

exhibit a different gene profile relative to GFP– cells after exposure to complexes, although polyplexes overexpress a much greater number of genes compared to lipoplexes.

Finally, when comparing GFP+ cells with GFP– cells, the level of differential expression of genes from microarrays found in the present study for polyplexes (*RAP1A*, *SCG5*, *IREB2*, *PGAP1* and *ACTA1*; Table 4) was compared with the level of differential expression of genes found for lipoplexes (*RAP1A*, *HSPA6* and *PACSIN3*) in our complementary study [53] at 2, 8, 16 and 24 h after delivery of complexes to the cells (Figure 5). The differential expression trend and values for *RAP1A*, *HSPA6* and *ATF3* appeared to be similar in both lipoplex- and polyplex-mediated transfection at each time point (2, 8, 16 and 24 h) after delivery of either type of complex, except for the 2-h time point for *RAP1A*, where it was downregulated by 12.5-fold in polyplexes but upregulated by 7.5-fold in lipoplexes (Figure 5). For *PACSIN3*, the differential expression trend and values after treatment of cells by lipoplexes or polyplexes was generally that of downregulation (Figure 5), although a 6.6-fold upregulation of *PACSIN3* was observed at the 8-h time point for lipoplexes. At the 2-h time point, *SCG5*, *IREB2*, *PGAP1* and *ACTA1* were downregulated for both polyplexes and lipoplexes. For lipoplexes, these genes

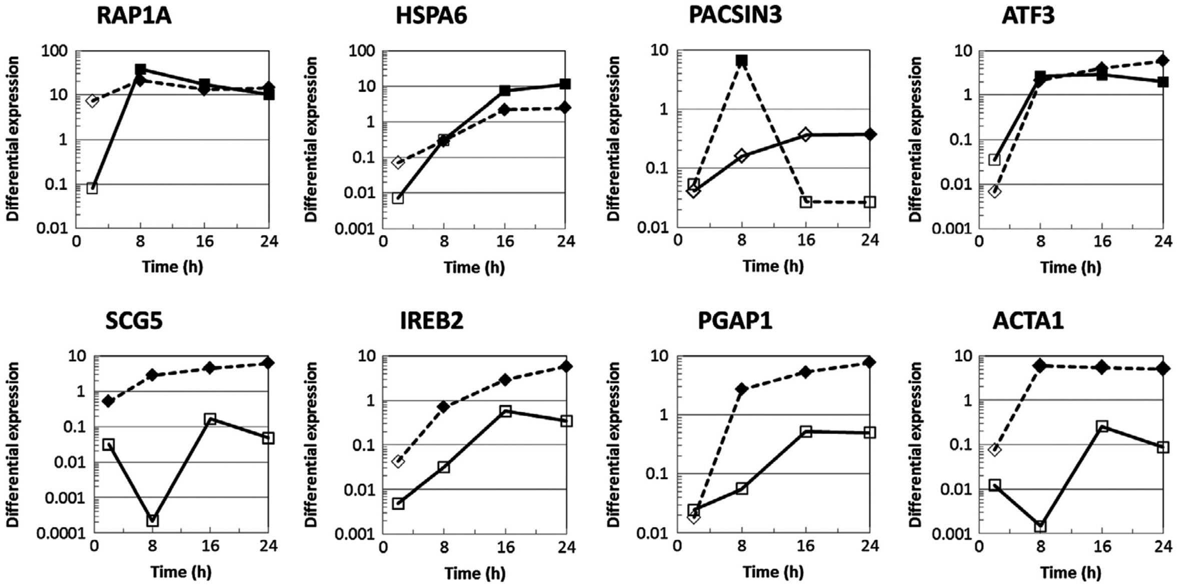

Figure 5. DNA carrier dependency on expression of differentially expressed genes for PEI (dashed line with diamonds) and LF2000 (solid line with squares). Data represent the values obtained from microarray analysis [66] (see Methods and Materials) comparing transfected cell arrays (GFP+; *n*=3) with treated, untransfected cell arrays (GFP–; *n*=3). Filled markers represent posterior probability values ≥ 0.9, whereas unfilled markers represent a posterior probability <0.9. The level of differential expression for each gene is provided in the Supporting information (Table S1) for polyplexes or in our other work for lipoplexes [53].

were then upregulated at the 8-, 16- and 24-h time points but remained downregulated for polyplexes over the same time period (Figure 5). Taken together, relative to GFP cells, GFP+ cells act to overcome the cellular downregulation of transcription observed 2 h after delivery of lipoplexes or polyplexes using some of the same genes (*RAP1A*, *HSPA6* and *ATF3*) (Figure 5), whereas polyplexes exhibit a much larger and sustained expression of genes over time (Figure 4), with a polyplex-specific response to upregulate *SCG5*, *IREB2*, *PGAP1* and *ACTA1* genes (Figure 5).

Discussion

Understanding the intracellular molecules and pathways that may facilitate DNA transfer provides insight into the potential mechanisms required for efficient transfection [88], offering targets for designing enhanced nonviral gene delivery systems. Gene transfer requires molecular mediators to actively transfer the delivered DNA [41] through intracellular barriers, into the nucleus [20–28,45] and for transgene expression [89]. Although the routing kinetics of the polyplexes have been observed as they occur over time and enable the development of transfection models [30–33,64], the molecular mediators used by the cell to route the DNA complex are poorly understood. In the present study, we attempted to fill that gap in knowledge using a transcriptomics approach aimed at identifying molecules and pathways involved in key cellular events known to influence transfection at 2, 8, 16 and 24 h after polyplex delivery. Because mRNA metabolism and catabolism can be condition- and cell-specific and mRNA half-life widely varies from approximately 10 min to 10 h or more, our approach may miss the identification of molecules involved in short-lived signaling pathways. To address this challenge of using microarrays and a few selected time points, we integrated the identified endogenous genes and pathways from the present study with other studies that identified short-lived signaling events into a proposed preliminary model of the biology of transfection of HEK 293T cells by polyplexes (Figure 6). The endogenous genes and pathways are discussed below in the context of the cellular processes and timing of those processes that occur during DNA transfer. Additionally, we compared and contrasted molecular mechanisms of transfection used by polyplexes and lipoplexes since both vectors show promising *in vitro* and *in vivo* transfection [40], although no molecular differences have been identified to account for the mechanistic differences in transfection from the two vectors [14,29–34,44,58–64].

Treatment of cells with polyplexes results in a cellular downregulation of transcription

In the present study, gene expression profiles were obtained for HEK 293T cells treated with polyplexes for 2 h compared to untreated cells (Figure 1). Relative to untreated cells, our results show that HEK 293T cells treated with polyplexes exhibited a marked downregulation of 46 genes (Figure 4; see also Supporting information, Table S1). During this period, polyplexes have been shown to internalize into and escape from the endosomes [13,30,31] at the same time as the cell is actively coping with the cytotoxic stress induced by polyplexes [13] or foreign DNA [90] (Figure 6). Various strategies have been devised to reduce the cytotoxicity by altering delivery techniques, such as complex maturation time, polymer amine to DNA phosphate charge ratio (N:P) or size of polyplexes [90,91], and formulations, such as molecular weight [13], polymer blending (e.g. PEI) [92] or including lipid coatings such as with lysinylated and histidylated cholesterol [93]. Another group used microarrays to identify molecules involved in endosomal escape of a PEGylated polyplex formulation to guide improvements of a new delivery system, which included a GALA fusogenic peptide [59]. That study [59], as well as our previous microarray studies [42,43], demonstrates that the identification of the molecules involved in transfection can be used for targeted improvements to gene delivery systems. Because all of the genes identified in the present study at the 2-h time point were highly downregulated, the cellular response to exposure of polyplexes is suggestive of a type of cellular shutdown of transcription, presumably to prevent further cytotoxic assault [13] and further uptake of the foreign DNA [90]. We also noted that total RNA was reduced in samples treated with polyplexes compared to untreated samples (data not shown). The transcriptional shutdown in response to short-term stress is reported to occur in other eukaryotic systems that act to rapidly destabilize mRNAs and coordinate mRNA turnover within a few minutes to achieve strong activity of stress genes [94]. In the present study, we identified *RGS1*, *ARHGAP24*, *PDZD2*, *SNX24*, *GSN* and *IGF2BP1* genes (Table 1) to be downregulated after a 2-h exposure to polyplexes; those genes are discussed below in terms of potential hallmarks of the cell shutdown. However, whether the shutdown is a positive or negative event for transfection is unclear because sometimes activating the target gene improved transfection, at the same time as inhibiting a different target gene also improved transfection (Figure 3 and Table 5). The results from our studies demonstrate that there are many genes responsible for DNA transfer at different time points and that activating or inhibiting a single gene is unlikely to

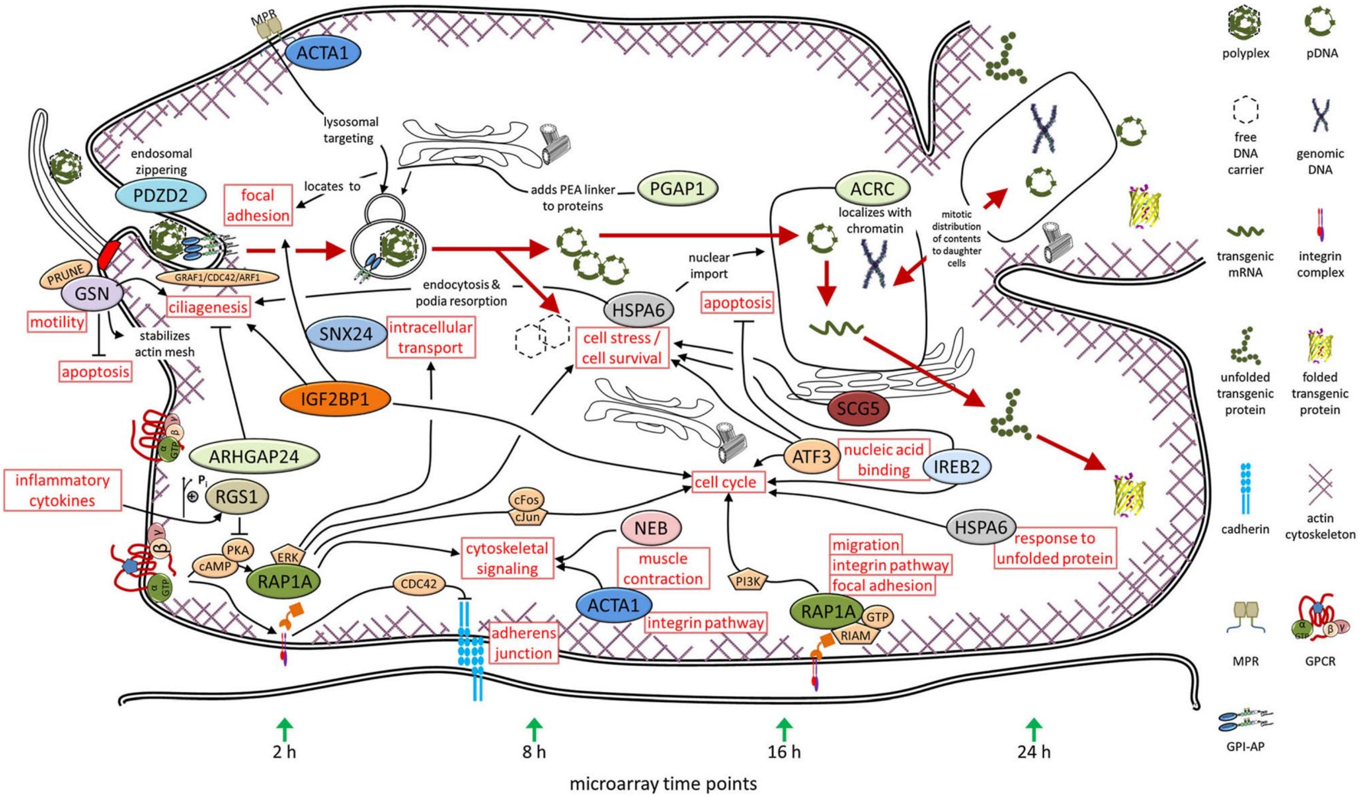

Figure 6. Proposed interaction of genes and pathways during pDNA transfer as identified in the present study. Shown is the outline of a HEK 293T cell with a partial outline of a daughter cell shown in the upper right. The DNA transfer process is shown within the cell, with the initial event of the complex binding to the cell occurring at the far left at time 0 h and subsequent events (large red arrows) occurring over time, from left-to-right. The time points chosen for microarray analysis in the present study are indicated with a green arrow at the bottom and correspond with molecular events shown above the arrow within the cell. Genes, enriched pathways and molecular intermediates as identified or discussed in the present study at each time point appear in the context of the DNA transfer process. Multiple molecular intermediates remain unlisted. PEA, phosphorylethanolamine; GPI-AP, glycosylphosphatidylinositol-anchored proteins; MPR, mannose 6-phosphate receptor.

have a prodigious effect on DNA transfer, agreeing with our pharmacologic studies.

One mechanism that may participate in the cell shutdown event is the interactions of filopodia with polyplexes. Filopodia are slender, actin-rich protrusions that extend outward from the cell body to sense the extracellular environment. Filopodia have recently been reported to bind and process complexes prior to cellular entry via endocytosis [58,95]. Three genes identified in the present study (*ARHGAP24*, *GSN* and *IGF2BP1*) are implicated in the generation of such podia protrusions, which the cell may downregulate to avoid further endocytosis of foreign DNA via cilia (Figure 6). *ARHGAP24*, when overexpressed, disrupts actin stress fibers and the formation of filopodia [96] and, when activated (5-episisomicin, an aminoglycoside antibiotic) in the present study, transfection was decreased by 5.2-fold (Table 5). Similarly, inhibiting the disruption mechanism using cytoclasin B (inhibits actin filament polymerization) resulted in a 1.7-fold increase in transfection. *GSN* also promotes ciliogenesis and inhibits apoptosis in stressed cells by means of a stabilizing actin mesh [97]. Activating GSN by use of folic acid (B vitamin needed for DNA synthesis, repair or methylation) resulted in a 5.2-fold decrease in transfection, even though endocytosis was probably enhanced as a result of promoting the production of filopodia. The loss in transfection levels was probably a result of the polymerized actin mesh acting to impede nuclear localization of the pDNA [98]. Inhibiting GSN by hydrocortisone (steroid hormone that improves metabolism and suppresses immune response) resulted in a 2.5-fold increase in transfection (Table 5), presumably by enhancing nuclear localization of pDNA as reported previously [99]. Finally, cells with *IGF2BP1* downregulated have been previously reported to exhibit reduced focal complexes [100], reduced filopodia [100] and reduced proliferation [101], processes shown to affect transfection. Hence, inhibiting *IGF2BP1* by propanil (herbicide with immunomodulatory activity in eukaryotes) resulted in a 4.8-fold decrease in transfection, at the same time as activating *IGF2BP1* (tetrachloroethylene, a lysosomal membrane disruptor) resulted in a 1.2-fold increase in transfection (Table 5). Taken together, we confirm the role of *ARHGAP24*, *GSN* and *IGF2BP1* genes on transfection and also that those genes may be contributing to low transfection efficiency, potentially by altering cellular processes affecting transfection in cytoskeletal signaling [102], filopodia [58,103,104] and the ability to cope with cellular stress [42,43,100] (Figure 6).

A second mechanism that may participate in cell shutdown is GTPase signaling. *ARHGAP24*, *GSN* and *IGF2BP1* genes (discussed above in the context of filopodia) and *RGS1* are all GTPases that were downregulated at the 2 h time point in treated cells compared to untreated (Figure 6). *RGS1* is as a GTPase activating protein (GAP), which rapidly acts to turn off G-protein-coupled receptor signaling and may play an inflammatory and immunological role after bacterial and viral infection [105]. *RGS1* reportedly exhibits an immediate upregulation after bacterial or viral infection and is subsequently downregulated after 30 min [105]. The altered expression of *RGS1* observed in the present study after polyplex exposure to HEK 293T cells (Table 1) may act to disrupt GTPase signaling and prevent further uptake of the foreign DNA (Figure 6). Therefore, we hypothesized that activating or inhibiting *RGS1* prior to delivery of polyplexes would lead to decreased or enhanced transfection, respectively. In support of our hypotheses, activating (immunoglobin G, an antibody isotype) or inhibiting (eicosapentaenoic acid, involved in immune and inflammatory responses) *RGS1* resulted in a 5.7-fold decrease or a 1.2-fold increase in transfection, respectively (Table 5). One potential explanation of the altered transfection is that a similar mechanism may be at play as in a recent study showing that activation of regulators of G-protein signaling (RGSs) prevent cyclic adenosine monophosphate and protein kinase A activity [106], and protein kinase A has been recently shown to modulate intracellular routing of polyplexes [107]. Another potential mechanism of altered transfection is that *RGS1* is a GAP that would alter cellular cytoskeleton dynamics via RhoGTPase signal transduction and therefore alter internalization and intracellular processing of polyplexes [108]. In either event, altering the expression level of *RGS1* can alter nonviral transfection possibly via G-coupled signal transduction (GTPases Rho, Rac and CDC42) [108] ending in processes known to affect transfection, such as endocytosis [109], cell spreading, migration, stress fibers, focal adhesion [102] and cell motility (Figure 6).

One last mechanism that may also participate in the cell shutdown is endosomal uptake, a critical process involved in transfection [109–111]. In the present study, two genes that play a role in endocytosis, *PDZD2* and *SNX24*, were highly differentially expressed in HEK 293T cells after 2 h of exposure to polyplexes (Table 1). The protein encoded by *PDZD2* contains the PDZ (postsynaptic density 95/discs large/zonus occludens-1) domain that interacts with syndecans and proteoglycans that facilitate zippering of vesicles around polyplexes for endocytosis [112]. Hence, *PDZD2* may be involved in endocytosis of polyplexes because inhibiting the gene (by tolazoline, an adrenergic α-antagonist) resulted in a 4.4-fold decrease in transfection. In addition, the sorting nexin (SNX) family of proteins is involved in various aspects of endocytosis [113]. *SNX24*, identified as differentially expressed, may be involved in endocytic uptake of polyplexes because, in activating (by 4-hydroxytamoxifen, binds to estrogen receptor and alters expression of estrogen-dependent genes) or inhibiting (by benzoic acid, which alters glycine-dependent metabolism) *SNX24*, we saw a 5.3-fold increase or 4.0-fold decrease in transfection, respectively (Table 5). Taken together, given the downregulation of so many genes at the 2-h time point, the cell may act to shutdown certain processes to prevent further uptake of polyplexes such as filopodia [18,50] (*ARHGAP24*, *GSN*, *IGF2BP1*, and *PDZD2*), GTPases [108] (*ARHGAP24*, *GSN*, *IGF2BP1*, *RGS1*) and endocytic uptake or escape [109–111] (*PDZD2* and *SNX24*) (Figure 6). Such a shutdown has been reported after viral infection, where host-cell transcription and nucleocytoplasmic trafficking is halted [114], although the event has not been reported for nonviral transfection. Nonetheless, by modulating the expression of genes identified in the present study, we were able to alter transfection, although how the concerted cellular shutdown of transcription affects transfection remains unclear.

Transfected cells overcome cell shutdown and barriers to transfection

As described above, 2 h after polyplex delivery, all cells exhibited a ‘shutdown’, as indicated by the highly downregulated nature of all differentially expressed genes (see Supporting information, Table S1) and reduction of total RNA (data not shown). However, although not all cells will go on to express the transgene, some cells (GFP+) do overcome the shutdown event and express the transgene. Several cellular processes are known to occur over the course of the next hours (until transgene expression is detected) (Figure 6), as shown in trafficking studies in the literature [3,19,30,31,39,41,45–49]. What remains unknown are the molecules and pathways that participate in those processes. By comparing gene expression profiles for transfected cells (GFP+) with untransfected cells (GFP–) over a time course of transfection, we were able to identify potential genes that may aid in overcoming barriers to transfection.

Relative to GFP– cells, we found several genes to be upregulated after exposure of polyplexes to cells at the 8-h time point (*ACTA1*, *NEB* and *RAP1A*, among others), 16-h time point (*ACTA1* and *RAP1A*) and 24-h time point (*ATF3* and *IREB2*). *ACTA1* encodes the protein that forms microfilaments, a major component of the cytoskeleton, and can affect cytoskeletal organization to enhance transfection [43,115]. *NEB* encodes nebulin that has several domains that may play essential roles in cell migration, motility, cytoskeletal dynamics and focal adhesions [116,117]. *ATF3* encodes an immediate-early gene that acts as a repressor of ATF promoter sites and is inducible through the NF-kB and JNK/SAPK signal transduction pathway [46,118,119], such as by cell stress from nondegradable, free PEI [120] or the presence of foreign DNA [121,122]. *IREB2* (iron-responsive element binding protein 2) is activated in response to oxidative stress and energy metabolism [123,124], such as increased tricarboxylic acid (cycle activity required during the cell cycle [123,125]. The differentially expressed genes identified in the present study (*ACTA1*, *NEB*, *RAP1A*, *ATF3* and *IREB2*) are known to affect processes that would affect transfection such as endocytosis [126,127], cytoskeletal signaling [102], nuclear localization [19,30,47], nuclear entry [3,30,39,47], GFP synthesis [30,48] and the cell cycle [49,109,110] (Figure 6). In our previous work, inhibiting *ACTA1* or activating *RAP1A* prior to delivery of polyplexes resulted in a 1.7-fold increase or 2.0-fold decrease in transfection [43], respectively. Similarly, in our previous work, inhibiting *ATF3* or activating *IREB2* prior to delivery of polyplexes resulted in a 2.3-fold decrease or a 2.2-fold increase in transfection [43], respectively. Taken together, *ACTA1*, *NEB*, *RAP1A*, *ATF3* and *IREB2* genes may help to overcome the cell shutdown and barriers to transfection by upregulating mechanisms involved in binding DNA and trafficking [30,31,39,47], regulating cell stress, regulating cell cycle [47,49] and regulating protein folding [30,31,43,48] (Figure 6).

Molecular and mechanistic differences between polyplex-mediated transfection and lipoplex-mediated transfection

Apparently, lipid- and polymer-based gene delivery systems achieve the same goals in gene transfer in that the pDNA must be internalized and then translocated and imported into the nucleus. Yet, transfection efficiencies from LF2000 lipoplexes are typically better than PEI polyplexes, presumably as a result of the mechanistic differences in transfection, such as: (i) the size and charge of the nanoparticle formed, which influences the uptake pathway and transfection [56,57]; (ii) the nondegradable nature of PEI, which may lead to immediate and sustained cytosolic stress [3,13]; (iii) the endosomal escape mechanism (i.e. PEI causes plasmids to escape the endosome as the endolysomal pressure builds, whereas LF2000 causes plasmids release to be slow and sustained) [58]; and (iv) the reported pharmacogenomics [14,29,34,44,59–63] and pharmacokinetic [30–33,64] differences. In the present study, by comparing gene expression profiles between LF2000- and PEI-mediated transfection, we attempted to identify potential molecular differences that may account for the observed mechanistic differences listed.

No common genes were found to be differentially expressed 2 h after exposure to lipoplexes or polyplexes (Figure 4), yet a similar number of genes was downregulated. It is increasingly recognized that the initial binding and uptake route of the lipoplex or polyplex largely affects its cellular fate, and hence, transfection capacity [56,110]. Genes potentially affecting uptake and found for polyplexes (in the present study; *RGS1*, *ARHGAP24*, *PDZD2*, *SNX24*, *GSN* and *IGF2BP1*) (Table 4) or lipoplexes (*ALMS1*, *ITGB1*, *FCGR3A*, *DOCK10*, *PACSIN3* and *ZDDHC13*; in our complementary work) [53] have a similar function in influencing transfection by filopodia [18,50], GTPase signaling [108] and endocytic uptake or escape [109–111]. Despite different genes being downregulated, the cellular response to both polyplexes and lipoplexes is one exhibiting transcriptional shutdown. However, the different genes reported in the present study may account for the mechanistic differences observed in physiochemical properties (chemistry, size, charge) [128–130], uptake pathway (which is cell type-, size- and vector-dependent) [56,57,96,128–132], endosomal escape [58] and transfection from lipoplexes and polyplexes.

After the cellular shutdown of transcription as a result of treatment of cells with complexes (Table 1), only a portion of the treated cells can overcome the shutdown and barriers to transfection (GFP+). We also identified potential genes and pathways involved in overcoming cell shutdown and overcoming barriers to transfection 8, 16 or 24 h after delivery of polyplexes (*RAP1A*, *SCG5*, *IREB2*, *PGAP1*, *ATF3* and *ACTA1* (Figures 4 and 5 and Table 4) or lipoplexes (*RAP1A*, *HSPA6*, *ATF3* and *PACSIN3*; in our complementary work) [53] to cells. Similar expression level trends during DNA transfer were observed in the GFP+ cells for *RAP1A*, *ATF3* and *HSPA6* after both polyplex- and lipoplex-mediated transfection (Figure 5), highlighting a common role for these genes in transfection. Because the expression levels were sustained over many hours, those genes may play a broad role in cellular processes that occur to overcome barriers to transfection such as escape [41,45], followed by unpacking of plasmid [45,46], nuclear localization [19,30,31,41,45,47], nuclear entry [30,39,47] and GFP synthesis [30,48]. By contrast, relative to GFP– cells, GFP+ cells overexpressed *SCG5*, *IREB2*, *PGAP1* and *ACTA1* after polyplex-mediated transfection but not for lipoplex-mediated transfection at the 8-, 16- and 24-h time points (Figure 5). Those genes may contribute to the mechanistic differences in polyplex and lipoplex transfection such as cytosolic stress [3,13], intracellular accumulation within endolysosome compartments [111,133,134], transgene expression kinetics [30–33,64] and level [135]. As one example, *SCG5* and *PGAP1* are overexpressed after polyplex-mediated transfection (Figure 5) but, in our previous work, activating *SCG5* prior to delivery of polyplexes leads to reduced transfection [43]. However, *SCG5* and *PGAP1* are not overexpressed throughout lipoplex-mediated transfection (Figure 5), and may suggest a molecular mechanism by which lipoplexes transfect better than polyplexes. Taken together, HEK 293T cells that overcome cell shutdown and cellular barriers to transfection may use some similar mechanisms (*RAP1A*, *ATF3*, *HSPA6*) and distinct mechanisms (*SCG5*, *PGAP1*) in response to PEI polyplex transfection or LF2000 lipoplex transfection, respectively.

The number of genes differentially expressed at any time point were always lower for LF2000 compared to PEI (Figure 4). LF2000-mediated transfection typically exhibits lower cytotoxicity compared to PEI-mediated transfection (unpublished results, T.M.M.) and may account for the differences in gene expression responses between the two vectors. Many of the genes elicited for PEI in the present study were indicative of cellular stress, with differentially expressed genes identified in pathways such as DNA damage, cell cycle checkpoints, and regulators of metabolism and transcription (Table 4) (*ATF3*, *IREB2*, *NDUFA13*, *DDIT3*). By contrast, many of the genes elicited for LF2000 were enriched in pathways involved in integrin signaling, spindle formation and focal adhesion (*RAP1A* and *AURKA*; in our complementary work) [53], although evidence of cytotoxicity may exist because the response to unfolded protein pathway was enriched involving *HSPA6*. The nondegradable nature of PEI may lead to immediate and sustained cytosolic stress [3,13], and explain why such a higher number of genes was differentially expressed over time. That stress may be contributing to the reduced transfection observed from polyplexes, relative to lipoplexes, and could represent a major target for improving transfection from new gene delivery systems.

Conclusions

Identifying those genes and pathways that aid in overcoming barriers to transfection provide targets for engineering enhanced delivery systems and enabling their therapeutic use. In the present study, we began such an endeavor using microarray analysis to provide a temporal and high throughput view of the genes and pathways that occur during DNA transfer when using polyplexes, and extended our endeavor to lipoplexes (in our complementary work) [53]. Treating cells with polyplexes or lipoplexes resulted in a cellular shutdown of transcription of distinct genes involved in similar processes, including filopodia (polyplexes: *ARHGAP24*, *PDZD2*, *GSN*, *IGF2BP1*; lipoplexes: *ALMS1*, *FCGR3A*, *DOCK10*), GTPase signaling (polyplexes: *RGS1*, *ARHGAP24*; lipoplexes: *DOCK10*, *ZDDHC13*) and membrane trafficking (polyplexes: *SNX24*; lipoplexes: *PACSIN3*, *ZDDHC13*). The genes appear to be important to DNA delivery because of their ability to modulate transfection. Possibly more important, however, are the genes and pathways utilized by transfected cells to overcome barriers to transfection. We found *RAP1A*, *ATF3* and *HSPA6* genes to facilitate polyplex- and lipoplex-mediated DNA delivery, although polyplex-mediated DNA delivery elicited a much greater transcriptomics response, with distinct genes potentially a result of higher toxicity. Genes such as *ATF3*, *IREB2*, *NDUFA13* and *DDIT3* were only differentially expressed between GFP+ and GFP– cells treated with polyplexes but not lipoplexes. Those genes were found, in the present study, to be involved in DNA damage, cell cycle checkpoints, and regulators of metabolism and transcription. The genes, pathways and pharmacologic agents identified to alter transfection for polyplexes (present study) and lipoplexes (in our complementary work) [53] provide a basis to further explore mechanisms of DNA transfer, prime cells for enhanced transfection from existing DNA delivery strategies and engineer novel nonviral systems that can achieve enhanced transgene expression.

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Supporting information follows.

Supporting information

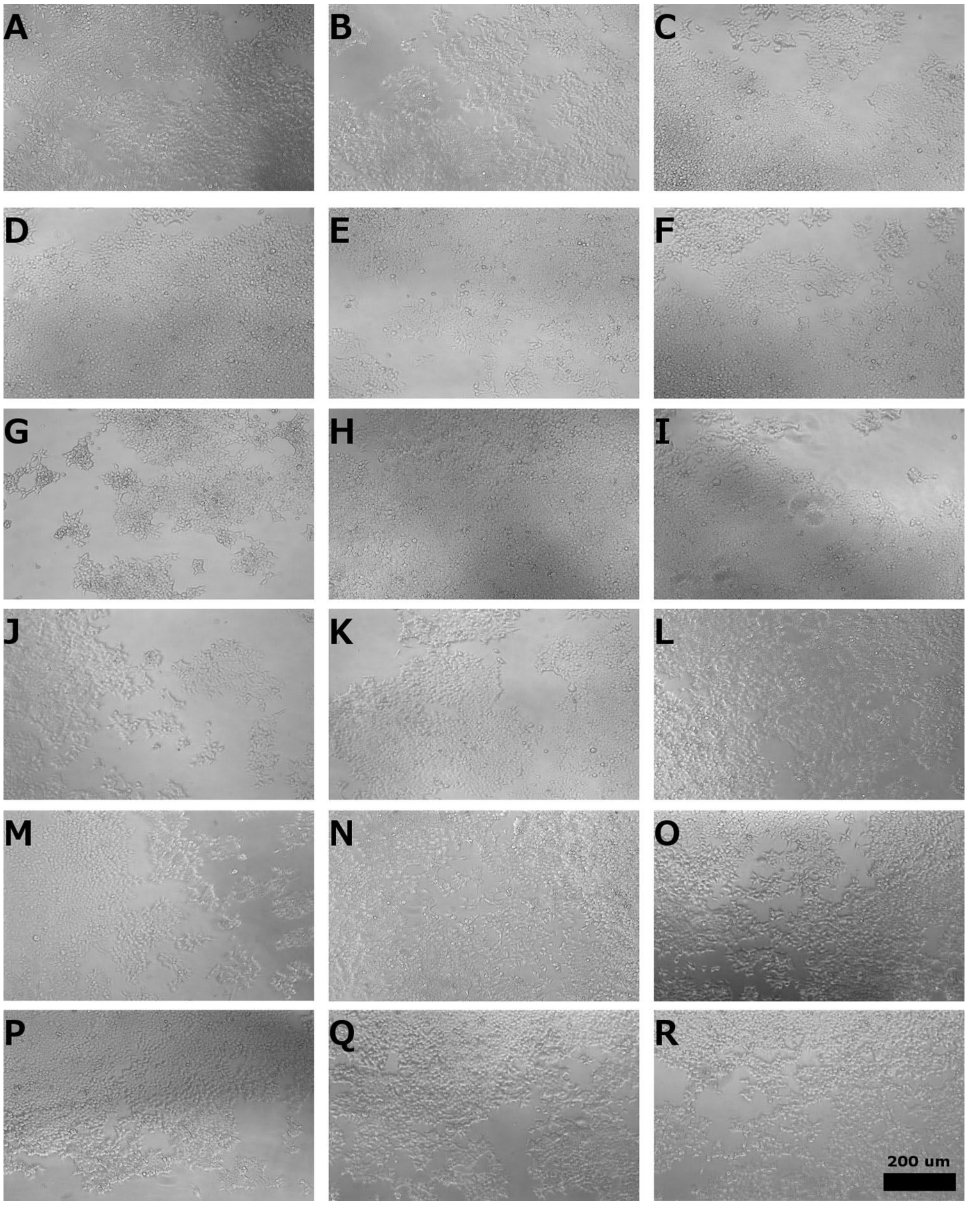

Figure S1. Phase images taken on Leica DMI 3000B (Bannockburn, IL, USA) to assess cell viability and morphology. HEK 293 T cells were seeded, allowed to adhere (approximately 18 h); then pharmacologic agent or vehicle control + was delivered to the media above the cells and after a 1-h incubation, lipoplexes were formed and delivered to the cells. After 24 h, images were taken at ×100 magnification (scale bar is the same for all images and as shown is 200 μM), with representative images shown. (A) Immunoglobin G 0.1 μM, (B) eicosapentaenoic acid 3 μM, (C) 5-episisomicin 0.1 μM, (D) cytoclasin B 1 μM, (E) apramycin 30 μM, (F) tolazoline 0.1 μM, (G) 4-hydroxytamoxifen 10 μM, (H) benzoic acid 1 μM, (I) folic acid 1 μM, (J) hydrocortisone 10 μM, (K) tetrachlorethylene 1 nM, (L) propanil 1 μM, (M) 0.1M NaCl vehicle control+, (N) MeOH vehicle control+, (O) ddH_2O vehicle control+, (P) DMSO vehicle control+, (Q) 1M NaOH vehicle control+ and (R) control–.

Table S1. Genes differentially expressed with a two-fold cut-off limit.

Table S2. Overlap of genes differentially expressed with a two-fold cut-off limit for polyplexes over time.

Table S3. Overlap of genes differentially expressed with a two-fold cut-off limit comparing polyplex gene expression profile with the lipoplex gene expression profile at each time point.

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
2 h	201693_s_at	EGR1	early growth response 1	AV733950	0.999	4.404
	210996_s_at	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	U43430	0.995	0.474
	220047_at	SIRT4	sirtuin 4	NM_012240	0.996	0.449
	242408_at	STYX	serine/threonine/tyrosine interacting protein	AW968935	0.993	0.414
	1560760_s_at	NA	NA	BC042056	1.000	0.345
	1558449_at	NA	NA	BC040475	0.996	0.273
	1554785_at	CCDC82	coiled-coil domain containing 82	BC018663	0.995	0.272
	236613_at	RBM25	RNA binding motif protein 25	BE466195	0.993	0.255
	210148_at	HIPK3	homeodomain interacting protein kinase 3	AF305239	1.000	0.250
	238548_at	NA	NA	AW863675	0.998	0.221
	214942_at	RBM34	RNA binding motif protein 34	D38491	1.000	0.163
	202988_s_at	RGS1	regulator of G-protein signaling 1	NM_002922	0.993	0.145
	1552649_a_at	RFFL	ring finger and FYVE-like domain containing E3 ubiquitin protein ligase	NM_057178	0.994	0.136
	220224_at	HAO1	hydroxyacid oxidase (glycolate oxidase) 1	NM_017545	0.993	0.113
	239110_s_at	W79643	NA	W79643	0.996	0.108
	217547_x_at	ZNF675	zinc finger protein 675	BF308250	0.998	0.103
	238520_at	TRERF1	transcriptional regulating factor 1	BF724270	0.998	0.102
	232451_at	AL110176	NA	AL110176	0.997	0.097

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	221030_s_at	ARHGAP24	Rho GTPase activating protein 24	NM_031305	1.000	0.085
	241790_at	T57946	NA	T57946	0.999	0.075
	1559990_at	LOC283070	uncharacterized LOC283070	BQ636703	0.997	0.073
	1569062_s_at	IQGAP3	IQ motif containing GTPase activating protein 3	BC033549	0.994	0.072
	210611_s_at	DTNA	dystrobrevin, alpha	U26744	0.998	0.072
	1562111_at	BC039439	NA	BC039439	0.999	0.069
	209493_at	PDZD2	PDZ domain containing 2	AF338650	0.997	0.067
	231367_s_at	AW300131	NA	AW300131	1.000	0.060
	1560028_at	C11orf57	chromosome 11 open reading frame 57	AK094203	0.990	0.059
	234050_at	TAGAP	T-cell activation RhoGTPase activating protein	AK025272	0.997	0.059
	236848_s_at	TEX13A	testis expressed 13A	AW291477	0.996	0.051
	231335_at	MS4A6E	membrane-spanning 4-domains, subfamily A, member 6E	AA917066	0.999	0.050
	1556694_a_at	AA716482	NA	AA716482	0.996	0.042
	1561362_at	AI419968	NA	AI419968	0.999	0.038
	204205_at	APOBEC3G	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G	NM_021822	0.995	0.037
	1570290_at	BC033970	NA	BC033970	0.995	0.032
	240488_at	AW444934	NA	AW444934	0.992	0.029
	1564628_at	AL049302	NA	AL049302	0.998	0.028
	230607_at	T86874	NA	T86874	0.997	0.027
	233883_at	SNX24	sorting nexin 24	AK021473	0.997	0.026
	216482_x_at	ZNF79	zinc finger protein 79	X65232	0.997	0.025

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	1553514_a_at	VNN3	vanin 3	NM_078625	0.995	0.024
	243714_at	T97641	NA	T97641	0.994	0.023
	227957_at	GSN	gelsolin	BE962069	0.995	0.022
	241574_s_at	IGF2BP1	insulin-like growth factor 2 mRNA binding protein 1	H93038	0.994	0.021
	206651_s_at	CPB2	carboxypeptidase B2 (plasma)	NM_016413	0.992	0.020
	211655_at	IGLV3-25	immunoglobulin lambda variable 3-25	D01059	0.992	0.018
	220393_at	LGSN	lengsin, lens protein with glutamine synthetase domain	NM_016571	0.991	0.018
	216209_at	LOC400084	uncharacterized LOC400084	AL137270	0.993	0.017
	231209_at	EXOC3L4	exocyst complex component 3-like 4	BF511215	0.990	0.017
	240388_at	KRT27	keratin 27	AI160083	0.992	0.014

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[2]
8 h	1555340_x_at	RAP1A	RAP1A, member of RAS oncogene family	AB051846	1.000	22.712
	221200_at	NM_022155	NA	NM_022155	1.000	8.968
	1554140_at	WDR78	WD repeat domain 78	BC032406	1.000	7.899
	238825_at	ACRC	acidic repeat containing	AI091533	1.000	7.746
	205054_at	NEB	nebulin	NM_004543	1.000	6.446
	203872_at	ACTA1	actin, alpha 1, skeletal muscle	NM_001100	1.000	6.007
	214601_at	TPH1	tryptophan hydroxylase 1	AI350339	1.000	5.538

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	1565830_at	AL833615	NA	AL833615	1.000	5.330
	1562209_at	DCAF4L1	DDB1 and CUL4 associated factor 4-like 1	BU561160	1.000	4.350
	1568807_a_at	RBM26-AS1	RBM26 antisense RNA 1 (non-protein coding)	AI301081	1.000	4.136
	239436_at	CHORDC1	cysteine and histidine-rich domain (CHORD) containing 1	AI473843	1.000	3.986
	217609_at	LRRC23	leucine rich repeat containing 23	BG420747	1.000	3.897
	1557104_at	ZSCAN30	zinc finger and SCAN domain containing 30	AW028277	0.999	3.739
	241156_at	AI939588	NA	AI939588	1.000	3.706
	237058_x_at	SLC6A13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	AI802118	1.000	3.688
	231084_at	WDR96	WD repeat domain 96	AW665251	1.000	3.595
	240089_at	BF508868	NA	BF508868	1.000	3.477
	239203_at	C7orf53	chromosome 7 open reading frame 53	AW014728	1.000	3.431
	229323_at	LOC387723	uncharacterized LOC387723	AI991561	1.000	3.401
	208498_s_at	NM_004038	NA	NM_004038	1.000	3.307
	204894_s_at	AOC3	amine oxidase, copper containing 3 (vascular adhesion protein 1)	NM_003734	1.000	3.235
	214409_at	RFPL3-AS1	RFPL3 antisense RNA 1 (non-protein coding)	AL021937	1.000	3.182
	220703_at	IDI2-AS1	IDI2 antisense RNA 1 (non-protein coding)	NM_018470	1.000	3.058
	238139_at	AI810034	NA	AI810034	0.999	2.993
	232826_at	AU144129	NA	AU144129	1.000	2.970

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	235803_at	AA843122	NA	AA843122	1.000	2.957
	236430_at	TMED6	transmembrane emp24 protein transport domain containing 6	AA708152	1.000	2.902
	222227_at	AK000847	NA	AK000847	1.000	2.851
	205940_at	MYH3	myosin, heavy chain 3, skeletal muscle, embryonic	NM_002470	1.000	2.827
	202450_s_at	CTSK	cathepsin K	NM_000396	1.000	2.813
	244497_at	AI650407	NA	AI650407	0.998	2.788
	203889_at	SCG5	secretogranin V (7B2 protein)	NM_003020	1.000	2.783
	244321_at	PGAP1	post-GPI attachment to proteins 1	BF063546	1.000	2.683
	205786_s_at	ITGAM	integrin, alpha M (complement component 3 receptor 3 subunit)	NM_000632	1.000	2.660
	219947_at	CLEC4A	C-type lectin domain family 4, member A	NM_016184	1.000	2.613
	220623_s_at	TSGA10	testis specific, 10	NM_025244	1.000	2.610
	214094_at	FUBP1	far upstream element (FUSE) binding protein 1	AA156865	1.000	2.607
	227532_at	LRRC39	leucine rich repeat containing 39	BF111992	1.000	2.572
	239845_at	AI885066	NA	AI885066	1.000	2.449
	202887_s_at	DDIT4	DNA-damage-inducible transcript 4	NM_019058	1.000	2.222
	238250_at	AI911318	NA	AI911318	0.999	2.202
	227591_at	LOC100505696	uncharacterized LOC100505696	AI123416	1.000	2.201
	232752_at	LOC100287616	uncharacterized LOC100287616	AK001164	1.000	2.194

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	205871_at	BC005379	NA	BC005379	1.000	2.144
	202672_s_at	ATF3	activating transcription factor 3	NM_001674	1.000	2.140
	221035_s_at	TEX14	testis expressed 14	NM_031272	1.000	2.134
	225442_at	DDR2	discoidin domain receptor tyrosine kinase 2	AI799915	0.999	2.117
	215338_s_at	NKTR	natural killer-tumor recognition sequence	AI688640	0.999	0.497
	223113_at	TMEM138	transmembrane protein 138	AF151030	0.994	0.497
	216169_at	AK025430	NA	AK025430	0.998	0.497
	220235_s_at	LRIF1	ligand dependent nuclear receptor interacting factor 1	NM_018372	0.995	0.496
	1565975_at	AK074226	NA	AK074226	0.999	0.496
	209311_at	BCL2L2	BCL2-like 2	D87461	0.993	0.496
	236323_at	PVRL3-AS1	PVRL3 antisense RNA 1 (non-protein coding)	AI253027	0.991	0.495
	230388_s_at	KANSL1-AS1	KANSL1 antisense RNA 1 (non-protein coding)	AI797017	0.998	0.494
	215022_x_at	ZNF33B	zinc finger protein 33B	BG429214	0.990	0.494
	223420_at	DNAJC14	DnaJ (Hsp40) homolog, subfamily C, member 14	AA156470	0.998	0.494
	224471_s_at	BTRC	beta-transducin repeat containing E3 ubiquitin protein ligase	BC006204	0.995	0.492
	1555007_s_at	WDR66	WD repeat domain 66	BC036233	0.997	0.491
	228729_at	CCNB1	cyclin B1	N90191	0.999	0.489
	223427_s_at	EPB41L4B	erythrocyte membrane protein band 4.1 like 4B	AB032179	0.996	0.489
	223421_at	CYHR1	cysteine/histidine-rich 1	BC005073	0.991	0.482
	219880_at	LOC100507619	uncharacterized LOC100507619	NM_022907	0.994	0.480

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	212153_at	POGZ	pogo transposable element with ZNF domain	AB007930	0.995	0.479
	223256_at	G2E3	G2/M-phase specific E3 ubiquitin protein ligase	AW007694	0.992	0.478
	236502_at	AI808359	NA	AI808359	0.994	0.478
	203863_at	ACTN2	actinin, alpha 2	W63731	0.992	0.477
	238797_at	TRIM11	tripartite motif containing 11	BF059582	0.994	0.477
	202241_at	TRIB1	tribbles homolog 1 (Drosophila)	NM_025195	0.999	0.477
	231437_at	SLC35D2	solute carrier family 35, member D2	AA693722	0.996	0.477
	203245_s_at	LINC00094	long intergenic non-protein coding RNA 94	BC002791	0.993	0.475
	228790_at	FAM110B	family with sequence similarity 110, member B	AW264082	0.990	0.473
	202767_at	ACP2	acid phosphatase 2, lysosomal	NM_001610	0.996	0.472
	225203_at	PPP1R16A	protein phosphatase 1, regulatory subunit 16A	AI742931	0.995	0.472
	206085_s_at	CTH	cystathionase (cystathionine gamma-lyase)	NM_001902	0.998	0.469
	1565786_x_at	FLJ45482	uncharacterized LOC645566	BQ953917	0.997	0.468
	236117_at	AA706701	NA	AA706701	0.990	0.466
	226408_at	TEAD2	TEA domain family member 2	AA905942	0.990	0.465
	209590_at	BMP7	bone morphogenetic protein 7	AL157414	0.997	0.465
	214148_at	LOC100507424	uncharacterized LOC100507424	U90917	0.992	0.462
	236282_at	AA769100	NA	AA769100	0.996	0.461

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	229069_at	BF477573	NA	BF477573	0.993	0.461
	229738_at	DNAH10	dynein, axonemal, heavy chain 10	AI700446	0.992	0.460
	238220_at	KDM6A	lysine (K)-specific demethylase 6A	BE670257	0.991	0.459
	222595_s_at	DIDO1	death inducer-obliterator 1	AL578222	0.991	0.458
	219168_s_at	PRR5	proline rich 5 (renal)	NM_017701	0.993	0.457
	203778_at	MANBA	mannosidase, beta A, lysosomal	NM_005908	0.996	0.457
	232284_at	AI206345	NA	AI206345	0.990	0.454
	230328_at	BF057656	NA	BF057656	0.993	0.454
	230215_at	SEC61A2	Sec61 alpha 2 subunit (S. cerevisiae)	AK022640	0.994	0.452
	202545_at	PRKCD	protein kinase C, delta	NM_006254	0.992	0.452
	217010_s_at	CDC25C	cell division cycle 25 homolog C (S. pombe)	AF277724	0.991	0.450
	243683_at	MORF4L2	mortality factor 4 like 2	H43976	0.993	0.449
	226599_at	FHDC1	FH2 domain containing 1	AA527080	0.991	0.449
	235010_at	LOC729013	uncharacterized LOC729013	AA833832	0.996	0.448
	215786_at	AK022170	NA	AK022170	0.991	0.447
	215942_s_at	GTSE1	G-2 and S-phase expressed 1	BF973178	0.997	0.447
	225300_at	C15orf23	chromosome 15 open reading frame 23	BF792864	0.998	0.446
	227792_at	ITPRIPL2	inositol 1,4,5-trisphosphate receptor interacting protein-like 2	AA490685	0.995	0.446
	221983_at	FAM134A	family with sequence similarity 134, member A	AL040896	0.993	0.446

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	215008_at	TLL2	tolloid-like 2	AA582404	0.999	0.445
	205802_at	TRPC1	transient receptor potential cation channel, subfamily C, member 1	NM_003304	0.996	0.444
	225675_at	C14orf101	chromosome 14 open reading frame 101	AW976269	0.994	0.444
	227936_at	TMEM68	transmembrane protein 68	AI671172	0.992	0.443
	229790_at	TERF2	telomeric repeat binding factor 2	AW006832	0.994	0.442
	208763_s_at	TSC22D3	TSC22 domain family, member 3	AL110191	0.996	0.442
	207197_at	ZIC3	Zic family member 3	NM_003413	0.994	0.440
	243491_at	AW993257	NA	AW993257	0.993	0.437
	233841_s_at	SUDS3	suppressor of defective silencing 3 homolog (S. cerevisiae)	AK026749	0.994	0.432
	227656_at	C6orf70	chromosome 6 open reading frame 70	AW968493	0.996	0.432
	231574_at	D59630	NA	D59630	0.992	0.431
	206186_at	MPP3	membrane protein, palmitoylated 3 (MAGUK p55 subfamily member 3)	NM_001932	0.993	0.429
	201626_at	INSIG1	insulin induced gene 1	BG292233	0.999	0.425
	220649_at	AGBL3	ATP/GTP binding protein-like 3	NM_024856	0.994	0.425
	242601_at	HEPACAM2	HEPACAM family member 2	AA600175	0.997	0.424
	205139_s_at	UST	uronyl-2-sulfotransferase	NM_005715	0.992	0.423
	230312_at	AI146812	NA	AI146812	0.995	0.421
	242610_x_at	AI569997	NA	AI569997	0.991	0.420
	219477_s_at	NM_018676	NA	NM_01867	0.993	0.419

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
				6		
	211298_s_at	ALB	albumin	AF116645	0.997	0.419
	203556_at	ZHX2	zinc fingers and homeoboxes 2	NM_014943	0.990	0.418
	235681_at	BE894882	NA	BE894882	0.992	0.418
	234408_at	IL17F	interleukin 17F	AL034343	0.992	0.417
	229268_at	FAM105B	family with sequence similarity 105, member B	AA723152	0.992	0.409
	201625_s_at	INSIG1	insulin induced gene 1	BE300521	0.997	0.408
	209431_s_at	PATZ1	POZ (BTB) and AT hook containing zinc finger 1	AF254083	0.990	0.407
	1568706_s_at	AF318328	NA	AF318328	0.996	0.407
	235441_at	BF217471	NA	BF217471	0.991	0.406
	219647_at	POPDC2	popeye domain containing 2	NM_022135	0.994	0.405
	219737_s_at	PCDH9	protocadherin 9	AI524125	0.997	0.405
	225864_at	FAM84B	family with sequence similarity 84, member B	AL039862	0.999	0.403
	202957_at	HCLS1	hematopoietic cell-specific Lyn substrate 1	NM_005335	0.998	0.402
	221257_x_at	FBXO38	F-box protein 38	NM_030793	0.995	0.401
	233402_at	AU159414	NA	AU159414	0.990	0.400
	221971_x_at	BE672818	NA	BE672818	0.990	0.397
	214569_at	IFNA5	interferon, alpha 5	NM_002169	0.997	0.396
	228655_at	BE466077	NA	BE466077	0.995	0.395
	206268_at	LEFTY1	left-right determination factor 1	NM_020997	0.990	0.394
	1560899_at	H50121	NA	H50121	0.998	0.391

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	232803_at	FLJ31958	uncharacterized LOC143153	H09470	0.991	0.391
	209733_at	MID2	midline 2	AL034399	0.990	0.390
	244872_at	RBBP4	retinoblastoma binding protein 4	BE514107	0.996	0.388
	1569021_at	PIK3C2A	phosphoinositide-3-kinase, class 2, alpha polypeptide	BC040952	0.994	0.387
	1570394_at	XRN1	5'-3' exoribonuclease 1	BC039314	0.998	0.385
	224688_at	C7orf42	chromosome 7 open reading frame 42	BE962299	0.994	0.383
	238571_at	BF055200	NA	BF055200	0.991	0.383
	209902_at	ATR	ataxia telangiectasia and Rad3 related	U49844	0.993	0.379
	200632_s_at	NDRG1	N-myc downstream regulated 1	NM_006096	0.995	0.372
	1555411_a_at	CCNL1	cyclin L1	AF367476	0.990	0.372
	207826_s_at	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	NM_002167	0.992	0.371
	229447_x_at	N32025	NA	N32025	1.000	0.369
	212168_at	RBM12	RNA binding motif protein 12	AL514547	0.998	0.369
	217779_s_at	NM_017761	NA	NM_017761	0.996	0.366
	202302_s_at	RSRC2	arginine/serine-rich coiled-coil 2	NM_023012	0.998	0.360
	205085_at	ORC1	origin recognition complex, subunit 1	NM_004153	0.990	0.358
	203378_at	PCF11	PCF11, cleavage and polyadenylation factor subunit, homolog (S. cerevisiae)	AB020631	0.993	0.355
	203178_at	GATM	glycine amidinotransferase (L-arginine:glycine	NM_001482	0.999	0.353

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
			amidinotransferase)			
	239477_at	EFHB	EF-hand domain family, member B	BF941046	0.996	0.347
	222760_at	ZNF703	zinc finger protein 703	BG290193	0.998	0.345
	209464_at	AURKB	aurora kinase B	AB011446	0.993	0.341
	209806_at	HIST1H2BK	histone cluster 1, H2bk	BC000893	0.999	0.339
	226740_x_at	BF740216	NA	BF740216	1.000	0.325
	206976_s_at	HSPH1	heat shock 105kDa/110kDa protein 1	NM_006644	0.993	0.322
	201103_x_at	BE299495	NA	BE299495	0.999	0.308
	214280_x_at	HNRNPA1	heterogeneous nuclear ribonucleoprotein A1	X79536	1.000	0.302
	223081_at	PHF23	PHD finger protein 23	BC002509	0.990	0.300
	203714_s_at	TBCE	tubulin folding cofactor E	NM_003193	0.998	0.299
	222700_at	ATL2	atlastin GTPase 2	AV700003	0.994	0.295
	1560652_at	AL832136	NA	AL832136	0.999	0.295
	222162_s_at	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	AK023795	0.993	0.289
	225786_at	HNRNPU-AS1	HNRNPU antisense RNA 1 (non-protein coding)	AI440495	0.990	0.282
	213612_x_at	AI800419	NA	AI800419	0.999	0.280
	1561741_at	BC042016	NA	BC042016	0.996	0.278
	212854_x_at	AB051480	NA	AB051480	0.997	0.251
	222501_s_at	REPIN1	replication initiator 1	BE674760	0.991	0.250
	223096_at	NOP58	NOP58 ribonucleoprotein homolog (yeast)	AF161469	0.997	0.237
	203213_at	CDK1	cyclin-dependent kinase 1	AL524035	0.990	0.236
	209974_s_at	BUB3	budding uninhibited by benzimidazoles 3 homolog	AF047473	0.997	0.219

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
			(yeast)			
	218009_s_at	PRC1	protein regulator of cytokinesis 1	NM_003981	0.991	0.200
	204170_s_at	CKS2	CDC28 protein kinase regulatory subunit 2	NM_001827	0.992	0.162
Time Point	**Affy Probe ID**	**Gene symbol**	**Gene name**	**Accession number**	**Posterior Probability**	**Differential Expression[2]**
16 h	1555339_at	RAP1A	RAP1A, member of RAS oncogene family	AB051846	1.000	13.198
	239436_at	CHORDC1	cysteine and histidine-rich domain (CHORD) containing 1	AI473843	1.000	9.356
	221200_at	NM_022155	NA	NM_022155	1.000	7.509
	205054_at	NEB	nebulin	NM_004543	1.000	7.321
	1554140_at	WDR78	WD repeat domain 78	BC032406	1.000	6.286
	203872_at	ACTA1	actin, alpha 1, skeletal muscle	NM_001100	1.000	5.475
	238825_at	ACRC	acidic repeat containing	AI091533	1.000	5.421
	244321_at	PGAP1	post-GPI attachment to proteins 1	BF063546	1.000	5.263
	1565830_at	AL833615	NA	AL833615	1.000	5.245
	208498_s_at	NM_004038	NA	NM_004038	1.000	4.453
	203889_at	SCG5	secretogranin V (7B2 protein)	NM_003020	1.000	4.450
	214601_at	TPH1	tryptophan hydroxylase 1	AI350339	1.000	4.181
	231084_at	WDR96	WD repeat domain 96	AW665251	1.000	4.046
	1554980_a_at	ATF3	activating transcription factor 3	AB066566	1.000	3.962

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	236430_at	TMED6	transmembrane emp24 protein transport domain containing 6	AA708152	0.996	3.827
	1568807_a_at	RBM26-AS1	RBM26 antisense RNA 1 (non-protein coding)	AI301081	1.000	3.821
	238139_at	AI810034	NA	AI810034	1.000	3.819
	1561759_at	LOC645513	uncharacterized LOC645513	AF085995	1.000	3.616
	239203_at	C7orf53	chromosome 7 open reading frame 53	AW014728	1.000	3.600
	237058_x_at	SLC6A13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	AI802118	1.000	3.419
	239282_at	CCDC41	coiled-coil domain containing 41	AI651679	1.000	3.405
	214409_at	RFPL3-AS1	RFPL3 antisense RNA 1 (non-protein coding)	AL021937	0.999	3.385
	244497_at	AI650407	NA	AI650407	1.000	3.328
	217609_at	LRRC23	leucine rich repeat containing 23	BG420747	1.000	3.251
	232826_at	AU144129	NA	AU144129	1.000	3.229
	220346_at	MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like	NM_025001	1.000	3.224
	210941_at	PCDH7	protocadherin 7	AB006756	1.000	3.215
	205871_at	BC005379	NA	BC005379	1.000	3.213
	214138_at	ZNF79	zinc finger protein 79	AA284829	0.998	3.203
	241156_at	AI939588	NA	AI939588	1.000	3.164
	205940_at	MYH3	myosin, heavy chain 3, skeletal muscle, embryonic	NM_002470	1.000	3.122
	216250_s_at	LPXN	leupaxin	X77598	0.998	2.991
	221035_s_at	TEX14	testis expressed 14	NM_03127	1.000	2.987

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
				2		
	1555476_at	IREB2	iron-responsive element binding protein 2	BC017880	0.996	2.891
	227301_at	BE644816	NA	BE644816	1.000	2.858
	229323_at	LOC387723	uncharacterized LOC387723	AI991561	1.000	2.807
	219947_at	CLEC4A	C-type lectin domain family 4, member A	NM_016184	0.999	2.797
	235803_at	AA843122	NA	AA843122	1.000	2.787
	206512_at	NM_005083	NA	NM_005083	0.999	2.785
	227532_at	LRRC39	leucine rich repeat containing 39	BF111992	0.999	2.785
	238158_at	MEIG1	meiosis expressed gene 1 homolog (mouse)	BF509612	0.999	2.782
	1553759_at	MCM9	minichromosome maintenance complex component 9	NM_153255	1.000	2.775
	229170_s_at	TTC18	tetratricopeptide repeat domain 18	AW024437	1.000	2.773
	238250_at	AI911318	NA	AI911318	0.996	2.768
	214094_at	FUBP1	far upstream element (FUSE) binding protein 1	AA156865	1.000	2.755
	232641_at	ZNF596	zinc finger protein 596	AC004908	1.000	2.733
	204894_s_at	AOC3	amine oxidase, copper containing 3 (vascular adhesion protein 1)	NM_003734	1.000	2.731
	203455_s_at	SAT1	spermidine/spermine N1-acetyltransferase 1	NM_002970	0.999	2.707
	202450_s_at	CTSK	cathepsin K	NM_000396	1.000	2.707
	236097_at	AA442776	NA	AA442776	1.000	2.705
	220623_s_at	TSGA10	testis specific, 10	NM_02524	0.997	2.704

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
				4		
	1554293_at	TTBK2	tau tubulin kinase 2	BC041876	1.000	2.675
	214748_at	N4BP2L2	NEDD4 binding protein 2-like 2	U50529	0.998	2.651
	243581_at	AW117229	NA	AW117229	0.999	2.620
	240089_at	BF508868	NA	BF508868	1.000	2.614
	214415_at	N58120	NA	N58120	0.997	2.611
	223908_at	HDAC8	histone deacetylase 8	AF212246	1.000	2.596
	205786_s_at	ITGAM	integrin, alpha M (complement component 3 receptor 3 subunit)	NM_000632	0.995	2.594
	225077_at	CHD2	chromodomain helicase DNA binding protein 2	AA890703	1.000	2.586
	211298_s_at	ALB	albumin	AF116645	0.999	2.579
	213570_at	EIF4E2	eukaryotic translation initiation factor 4E family member 2	BF516289	1.000	2.572
	207757_at	ZFP2	zinc finger protein 2 homolog (mouse)	NM_030613	1.000	2.547
	240319_at	AI023795	NA	AI023795	0.999	2.544
	216733_s_at	GATM	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	X86401	1.000	2.538
	209911_x_at	HIST1H2BD	histone cluster 1, H2bd	BC002842	0.998	2.515
	232753_at	ZNF346	zinc finger protein 346	AU147613	1.000	2.481
	1552634_a_at	ZNF101	zinc finger protein 101	NM_033204	0.999	2.450
	240206_at	TARS	threonyl-tRNA synthetase	AA701890	0.996	2.449
	230683_at	W56760	NA	W56760	0.999	2.440
	207201_s_at	SLC22A1	solute carrier family 22 (organic cation transporter), member 1	NM_003057	1.000	2.409

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	230292_at	AA868809	NA	AA868809	0.998	2.397
	231382_at	FGF18	fibroblast growth factor 18	AI798863	0.999	2.377
	1560973_a_at	AF086424	NA	AF086424	0.996	2.374
	220148_at	ALDH8A1	aldehyde dehydrogenase 8 family, member A1	NM_022568	0.996	2.365
	222019_at	PFDN6	prefoldin subunit 6	AW007185	0.996	2.354
	228234_at	AI423165	NA	AI423165	0.999	2.354
	230535_s_at	ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit	BF109387	0.998	2.348
	242656_at	GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	AI377688	0.999	2.347
	1554038_at	LARP1B	La ribonucleoprotein domain family, member 1B	BC030516	1.000	2.341
	202887_s_at	DDIT4	DNA-damage-inducible transcript 4	NM_019058	0.995	2.339
	1569022_a_at	PIK3C2A	phosphoinositide-3-kinase, class 2, alpha polypeptide	BC040952	0.999	2.321
	229130_at	AU145323	NA	AU145323	0.998	2.320
	1552326_a_at	CCDC11	coiled-coil domain containing 11	NM_145020	1.000	2.320
	239845_at	AI885066	NA	AI885066	1.000	2.316
	222227_at	AK000847	NA	AK000847	1.000	2.309
	218371_s_at	PSPC1	paraspeckle component 1	NM_018282	0.992	2.297
	240834_at	FAM105B	family with sequence similarity 105, member B	AI813337	0.999	2.296
	242586_at	FSD1L	fibronectin type III and SPRY domain containing 1-like	H07986	1.000	2.280
	210910_s_at	POMZP3	POM121 and ZP3 fusion	BC000487	0.999	2.266

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	225442_at	DDR2	discoidin domain receptor tyrosine kinase 2	AI799915	0.999	2.262
	210077_s_at	SRSF5	serine/arginine-rich splicing factor 5	U30884	0.997	2.258
	236213_at	AI809760	NA	AI809760	1.000	2.255
	227963_at	BF515913	NA	BF515913	0.999	2.253
	1559397_s_at	PRR14	proline rich 14	BE788667	0.999	2.245
	206110_at	HIST1H3H	histone cluster 1, H3h	NM_003536	0.999	2.244
	207623_at	ABCF2	ATP-binding cassette, sub-family F (GCN20), member 2	NM_005692	0.993	2.241
	229754_at	LOC100507297	uncharacterized LOC100507297	AW104619	0.999	2.234
	229627_at	AI831514	NA	AI831514	1.000	2.230
	1557104_at	ZSCAN30	zinc finger and SCAN domain containing 30	AW028277	0.996	2.229
	232196_at	LCA5L	Leber congenital amaurosis 5-like	AI825840	0.999	2.226
	235540_at	GNRH1	gonadotropin-releasing hormone 1 (luteinizing-releasing hormone)	AW296153	0.994	2.223
	228736_at	HELQ	helicase, POLQ-like	AW084661	0.997	2.219
	224989_at	AI824013	NA	AI824013	0.997	2.218
	239434_at	T78087	NA	T78087	0.999	2.213
	1569157_s_at	ZNF846	zinc finger protein 846	BC037359	0.998	2.210
	1556062_at	RPP30	ribonuclease P/MRP 30kDa subunit	AW182934	0.997	2.205
	1568696_at	CDNF	cerebral dopamine neurotrophic factor	BC037872	0.997	2.194
	220444_at	ZNF557	zinc finger protein 557	NM_024341	0.991	2.190

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	244066_at	DENND4C	DENN/MADD domain containing 4C	N34297	0.999	2.187
	214683_s_at	AI251890	NA	AI251890	0.999	2.183
	235535_x_at	AI369073	NA	AI369073	0.996	2.177
	243802_at	DNAH12	dynein, axonemal, heavy chain 12	AI005163	0.999	2.165
	207064_s_at	AOC2	amine oxidase, copper containing 2 (retina-specific)	NM_009590	1.000	2.163
	203862_s_at	ACTN2	actinin, alpha 2	H16245	0.998	2.159
	243378_at	BE673747	NA	BE673747	0.998	2.159
	227172_at	TMEM116	transmembrane protein 116	BC000282	0.999	2.149
	242754_at	AW132066	NA	AW132066	1.000	2.148
	236892_s_at	HOXB-AS3	HOXB cluster antisense RNA 3 (non-protein coding)	BF590528	0.994	2.148
	208107_s_at	LOC81691	exonuclease NEF-sp	NM_030941	1.000	2.147
	1557417_s_at	AA844689	NA	AA844689	0.999	2.143
	213124_at	ZNF473	zinc finger protein 473	BG538800	0.990	2.143
	242708_at	BF224430	NA	BF224430	0.999	2.142
	229962_at	LRRC37A3	leucine rich repeat containing 37, member A3	W68731	0.993	2.142
	242051_at	AI695695	NA	AI695695	0.999	2.141
	215819_s_at	N53959	NA	N53959	0.997	2.129
	239797_at	AA503877	NA	AA503877	0.995	2.122
	237127_at	AA142959	NA	AA142959	0.997	2.119
	239248_at	LOC100507495	uncharacterized LOC100507495	BE742802	0.999	2.093
	235033_at	NPEPL1	aminopeptidase-like 1	AL577823	0.996	2.087

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	230120_s_at	PLGLB2	plasminogen-like B2	AI088455	0.998	2.085
	217555_at	SMC1A	structural maintenance of chromosomes 1A	AI042030	0.999	2.081
	209292_at	ID4	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	AL022726	1.000	2.081
	203665_at	HMOX1	heme oxygenase (decycling) 1	NM_002133	1.000	2.076
	214950_at	L39064	NA	L39064	0.997	2.072
	206862_at	ZNF254	zinc finger protein 254	NM_004876	0.999	2.071
	235608_at	AI863194	NA	AI863194	0.999	2.069
	230355_at	SEPT7P2	septin 7 pseudogene 2	BF445841	0.996	2.065
	222953_at	GPR83	G protein-coupled receptor 83	BE670361	0.999	2.063
	1570394_at	XRN1	5'-3' exoribonuclease 1	BC039314	0.999	2.062
	231095_at	LOC100507049	uncharacterized LOC100507049	AW193811	0.998	2.060
	220064_at	TTC21B	tetratricopeptide repeat domain 21B	NM_024753	0.999	2.060
	222680_s_at	DTL	denticleless E3 ubiquitin protein ligase homolog (Drosophila)	AK001261	1.000	2.060
	208958_at	ERP44	endoplasmic reticulum protein 44	AI827677	0.999	2.048
	49452_at	ACACB	acetyl-CoA carboxylase beta	AI057637	0.999	2.042
	238528_at	UBR1	ubiquitin protein ligase E3 component n-recognin 1	AI361043	0.992	2.040
	208118_x_at	NM_031211	NA	NM_031211	0.999	2.039
	213557_at	CDK12	cyclin-dependent kinase 12	AW305119	0.998	2.022
	1568787_at	LOC1005060	uncharacterized	R64146	1.000	2.021

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
		83	LOC100506083			
	230657_at	AI423466	NA	AI423466	0.998	2.020
	238094_at	AW292905	NA	AW292905	0.994	2.016
	1559509_at	R17062	NA	R17062	0.994	2.012
	228124_at	ABHD12	abhydrolase domain containing 12	AW303865	0.998	2.010
	223825_at	KIAA1432	KIAA1432	AL136875	1.000	2.010
	229628_s_at	AI831514	NA	AI831514	0.999	2.001
	1569867_at	EME2	essential meiotic endonuclease 1 homolog 2 (S. pombe)	BC041011	0.996	0.499
	229318_at	BF509796	NA	BF509796	0.999	0.491
	242715_at	AA331548	NA	AA331548	0.999	0.444
Time Point	**Affy Probe ID**	**Gene symbol**	**Gene name**	**Accession number**	**Posterior Probability**	**Differential Expression[2]**
24 h	1555339_at	RAP1A	RAP1A, member of RAS oncogene family	AB051846	1.000	14.588
	239436_at	CHORDC1	cysteine and histidine-rich domain (CHORD) containing 1	AI473843	1.000	10.948
	1554140_at	WDR78	WD repeat domain 78	BC032406	1.000	8.779
	244321_at	PGAP1	post-GPI attachment to proteins 1	BF063546	1.000	7.703
	221200_at	NM_022155	NA	NM_022155	1.000	6.654
	205054_at	NEB	nebulin	NM_004543	1.000	6.589
	203889_at	SCG5	secretogranin V (7B2 protein)	NM_003020	1.000	6.151
	238825_at	ACRC	acidic repeat containing	AI091533	1.000	6.144

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	202672_s_at	ATF3	activating transcription factor 3	NM_001674	1.000	5.834
	1555476_at	IREB2	iron-responsive element binding protein 2	BC017880	1.000	5.714
	241156_at	AI939588	NA	AI939588	1.000	5.105
	203872_at	ACTA1	actin, alpha 1, skeletal muscle	NM_001100	1.000	5.041
	221035_s_at	TEX14	testis expressed 14	NM_031272	1.000	4.540
	231084_at	WDR96	WD repeat domain 96	AW665251	1.000	4.468
	1565830_at	AL833615	NA	AL833615	1.000	4.439
	209911_x_at	HIST1H2BD	histone cluster 1, H2bd	BC002842	1.000	4.374
	205940_at	MYH3	myosin, heavy chain 3, skeletal muscle, embryonic	NM_002470	1.000	4.353
	214409_at	RFPL3-AS1	RFPL3 antisense RNA 1 (non-protein coding)	AL021937	1.000	4.300
	232826_at	AU144129	NA	AU144129	1.000	4.171
	236430_at	TMED6	transmembrane emp24 protein transport domain containing 6	AA708152	1.000	4.151
	237058_x_at	SLC6A13	solute carrier family 6 (neurotransmitter transporter, GABA), member 13	AI802118	1.000	4.100
	214138_at	ZNF79	zinc finger protein 79	AA284829	1.000	4.045
	220346_at	MTHFD2L	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2-like	NM_025001	1.000	3.982
	1557104_at	ZSCAN30	zinc finger and SCAN domain containing 30	AW028277	1.000	3.975
	210941_at	PCDH7	protocadherin 7	AB006756	1.000	3.863
	243581_at	AW117229	NA	AW117229	1.000	3.844
	232753_at	ZNF346	zinc finger protein 346	AU147613	1.000	3.809

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression^1
	205871_at	BC005379	NA	BC005379	1.000	3.762
	216250_s_at	LPXN	leupaxin	X77598	1.000	3.714
	238139_at	AI810034	NA	AI810034	1.000	3.677
	206512_at	NM_005083	NA	NM_005083	1.000	3.536
	227296_at	MFSD3	major facilitator superfamily domain containing 3	AA523543	1.000	3.406
	219947_at	CLEC4A	C-type lectin domain family 4, member A	NM_016184	1.000	3.366
	225442_at	DDR2	discoidin domain receptor tyrosine kinase 2	AI799915	1.000	3.349
	1569022_a_at	PIK3C2A	phosphoinositide-3-kinase, class 2, alpha polypeptide	BC040952	1.000	3.231
	227301_at	BE644816	NA	BE644816	1.000	3.222
	1568807_a_at	RBM26-AS1	RBM26 antisense RNA 1 (non-protein coding)	AI301081	1.000	3.154
	235803_at	AA843122	NA	AA843122	1.000	3.121
	207623_at	ABCF2	ATP-binding cassette, sub-family F (GCN20), member 2	NM_005692	1.000	3.103
	1562209_at	DCAF4L1	DDB1 and CUL4 associated factor 4-like 1	BU561160	1.000	3.070
	1561759_at	LOC645513	uncharacterized LOC645513	AF085995	1.000	3.003
	222851_at	ZNF654	zinc finger protein 654	AL120354	1.000	3.002
	216009_at	SLC39A9	solute carrier family 39 (zinc transporter), member 9	U92027	1.000	2.931
	229170_s_at	TTC18	tetratricopeptide repeat domain 18	AW024437	1.000	2.926
	214094_at	FUBP1	far upstream element (FUSE) binding protein 1	AA156865	1.000	2.910

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	239944_at	AA431379	NA	AA431379	1.000	2.905
	1559509_at	R17062	NA	R17062	1.000	2.903
	217373_x_at	MDM2	Mdm2, p53 E3 ubiquitin protein ligase homolog (mouse)	AJ276888	1.000	2.902
	214748_at	N4BP2L2	NEDD4 binding protein 2-like 2	U50529	1.000	2.874
	239203_at	C7orf53	chromosome 7 open reading frame 53	AW014728	1.000	2.849
	235033_at	NPEPL1	aminopeptidase-like 1	AL577823	1.000	2.832
	239794_at	AI356405	NA	AI356405	1.000	2.797
	209383_at	DDIT3	DNA-damage-inducible transcript 3	BC003637	1.000	2.780
	242656_at	GTF2H1	general transcription factor IIH, polypeptide 1, 62kDa	AI377688	1.000	2.757
	208498_s_at	NM_004038	NA	NM_004038	0.996	2.739
	209315_at	HBS1L	HBS1-like (S. cerevisiae)	AW297143	1.000	2.639
	201939_at	PLK2	polo-like kinase 2	NM_006622	1.000	2.602
	236613_at	RBM25	RNA binding motif protein 25	BE466195	0.999	2.582
	220148_at	ALDH8A1	aldehyde dehydrogenase 8 family, member A1	NM_022568	1.000	2.572
	238528_at	UBR1	ubiquitin protein ligase E3 component n-recognin 1	AI361043	1.000	2.559
	213418_at	HSPA6	heat shock 70kDa protein 6 (HSP70B')	NM_002155	1.000	2.500
	225077_at	CHD2	chromodomain helicase DNA binding protein 2	AA890703	1.000	2.495
	228532_at	C1orf162	chromosome 1 open reading frame 162	AW662189	1.000	2.409
	217609_at	LRRC23	leucine rich repeat	BG420747	1.000	2.394

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
			containing 23			
	227532_at	LRRC39	leucine rich repeat containing 39	BF111992	1.000	2.372
	242838_at	MAP6D1	MAP6 domain containing 1	AA283642	1.000	2.366
	210077_s_at	SRSF5	serine/arginine-rich splicing factor 5	U30884	1.000	2.341
	229072_at	BF968097	NA	BF968097	1.000	2.314
	223908_at	HDAC8	histone deacetylase 8	AF212246	1.000	2.252
	218371_s_at	PSPC1	paraspeckle component 1	NM_018282	1.000	2.243
	244497_at	AI650407	NA	AI650407	1.000	2.227
	214601_at	TPH1	tryptophan hydroxylase 1	AI350339	1.000	2.208
	235608_at	AI863194	NA	AI863194	1.000	2.203
	218504_at	FAHD2A	fumarylacetoacetate hydrolase domain containing 2A	NM_016044	1.000	2.198
	1557078_at	SLFN5	schlafen family member 5	AK054668	1.000	2.198
	241668_s_at	AI033967	NA	AI033967	1.000	2.191
	239797_at	AA503877	NA	AA503877	1.000	2.149
	214792_x_at	VAMP2	vesicle-associated membrane protein 2 (synaptobrevin 2)	AI955119	1.000	2.143
	1569157_s_at	ZNF846	zinc finger protein 846	BC037359	1.000	2.127
	237020_at	CATSPERD	catsper channel auxiliary subunit delta	AI203106	1.000	2.125
	215228_at	NHLH2	nescient helix loop helix 2	AA166895	1.000	2.123
	240910_at	BF431313	NA	BF431313	1.000	2.119
	228234_at	AI423165	NA	AI423165	1.000	2.116
	1552634_a_at	ZNF101	zinc finger protein 101	NM_033204	1.000	2.094

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	1556090_at	BM970306	NA	BM970306	1.000	2.083
	202450_s_at	CTSK	cathepsin K	NM_000396	1.000	2.082
	207183_at	GPR19	G protein-coupled receptor 19	NM_006143	1.000	2.063
	1553759_at	MCM9	minichromosome maintenance complex component 9	NM_153255	0.997	2.041
	1557196_a_at	AL831886	NA	AL831886	1.000	2.033
	231487_at	COX8C	cytochrome c oxidase subunit VIIIC	AW269746	1.000	2.004
	204711_at	KIAA0753	KIAA0753	NM_014804	0.999	0.500
	37028_at	PPP1R15A	protein phosphatase 1, regulatory subunit 15A	U83981	0.994	0.500
	227212_s_at	PHF19	PHD finger protein 19	BE544837	0.999	0.499
	202146_at	IFRD1	interferon-related developmental regulator 1	AA747426	0.997	0.498
	222044_at	PCIF1	PDX1 C-terminal inhibiting factor 1	AI199589	0.994	0.497
	1554116_s_at	PARP11	poly (ADP-ribose) polymerase family, member 11	BC031073	0.993	0.497
	229153_at	SLC7A6OS	solute carrier family 7, member 6 opposite strand	BF432635	0.999	0.496
	221096_s_at	TMCO6	transmembrane and coiled-coil domains 6	NM_018502	0.996	0.495
	239816_at	POLD3	polymerase (DNA-directed), delta 3, accessory subunit	AW337142	0.999	0.494
	242720_at	ITIH4	inter-alpha-trypsin inhibitor heavy chain family, member 4	AI004137	0.995	0.493
	205244_s_at	SLC13A3	solute carrier family 13 (sodium-dependent dicarboxylate transporter),	NM_022829	0.993	0.493

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			member 3			
	227689_at	ZNF227	zinc finger protein 227	BF739795	0.994	0.492
	210396_s_at	AF271775	NA	AF271775	0.999	0.492
	1569307_s_at	BC038430	NA	BC038430	0.999	0.492
	221270_s_at	QTRT1	queuine tRNA-ribosyltransferase 1	NM_031209	0.996	0.492
	218743_at	CHMP6	charged multivesicular body protein 6	NM_024591	0.999	0.491
	217555_at	SMC1A	structural maintenance of chromosomes 1A	AI042030	0.998	0.491
	209488_s_at	RBPMS	RNA binding protein with multiple splicing	D84109	0.997	0.490
	32062_at	LRRC14	leucine rich repeat containing 14	D25216	0.996	0.490
	212338_at	MYO1D	myosin ID	AA621962	0.992	0.490
	51200_at	C19orf60	chromosome 19 open reading frame 60	AI744084	0.998	0.490
	225417_at	EPC1	enhancer of polycomb homolog 1 (Drosophila)	AA766646	0.999	0.489
	1555902_at	ARMCX5	armadillo repeat containing, X-linked 5	BC022066	0.993	0.488
	218280_x_at	NM_003516	NA	NM_003516	0.998	0.488
	207163_s_at	AKT1	v-akt murine thymoma viral oncogene homolog 1	NM_005163	0.993	0.487
	230522_s_at	C9orf100	chromosome 9 open reading frame 100	BG028209	0.997	0.487
	221267_s_at	FAM108A1	family with sequence similarity 108, member A1	NM_031213	0.996	0.487
	222563_s_at	TNKS2	tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2	AF329696	0.995	0.486
	212563_at	BOP1	block of proliferation 1	BG491842	0.998	0.486

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	225779_at	SLC27A4	solute carrier family 27 (fatty acid transporter), member 4	AK000722	0.998	0.485
	204019_s_at	SH3YL1	SH3 domain containing, Ysc84-like 1 (S. cerevisiae)	NM_015677	0.993	0.485
	218023_s_at	FAM53C	family with sequence similarity 53, member C	NM_016605	0.999	0.485
	239737_at	AW028687	NA	AW028687	0.997	0.484
	235581_at	AA478537	NA	AA478537	0.998	0.484
	222028_at	ZNF45	zinc finger protein 45	AI967981	0.999	0.484
	218875_s_at	FBXO5	F-box protein 5	NM_012177	0.997	0.484
	237923_at	AI733801	NA	AI733801	0.999	0.483
	236926_at	TBX1	T-box 1	AW074836	0.994	0.482
	205647_at	RAD52	RAD52 homolog (S. cerevisiae)	NM_002879	0.995	0.482
	238999_at	AI610347	NA	AI610347	0.996	0.482
	210273_at	PCDH7	protocadherin 7	AB006757	0.999	0.482
	214395_x_at	EEF1D	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	AI335509	0.991	0.481
	203163_at	KATNB1	katanin p80 (WD repeat containing) subunit B 1	NM_005886	0.999	0.481
	229833_at	LOC100506713	uncharacterized LOC100506713	BF507533	0.996	0.480
	228642_at	BF593636	NA	BF593636	1.000	0.479
	209568_s_at	RGL1	ral guanine nucleotide dissociation stimulator-like 1	AF186779	0.998	0.479
	236999_at	AA767131	NA	AA767131	0.995	0.479
	1558750_a_at	LOC100288637	OTU domain containing 7A pseudogene	BG109249	0.996	0.478

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	230591_at	LOC729887	uncharacterized LOC729887	AI792242	0.997	0.478
	238333_s_at	SPRN	shadow of prion protein homolog (zebrafish)	AI355435	0.992	0.478
	219958_at	TMEM74B	transmembrane protein 74B	NM_018354	0.996	0.478
	222998_at	MAF1	MAF1 homolog (S. cerevisiae)	AL136937	0.998	0.477
	227864_s_at	FAM125A	family with sequence similarity 125, member A	AI091713	0.999	0.476
	208763_s_at	TSC22D3	TSC22 domain family, member 3	AL110191	1.000	0.476
	225616_at	SPRYD4	SPRY domain containing 4	AI570493	0.997	0.476
	217734_s_at	WDR6	WD repeat domain 6	NM_018031	0.999	0.475
	202814_s_at	HEXIM1	hexamethylene bis-acetamide inducible 1	AW193511	1.000	0.475
	217551_at	OR7E14P	olfactory receptor, family 7, subfamily E, member 14 pseudogene	AA719797	0.997	0.474
	209553_at	VPS8	vacuolar protein sorting 8 homolog (S. cerevisiae)	BC001001	0.999	0.473
	211721_s_at	ZNF551	zinc finger protein 551	BC005868	0.995	0.473
	202393_s_at	KLF10	Kruppel-like factor 10	NM_005655	0.996	0.472
	219722_s_at	GDPD3	glycerophosphodiester phosphodiesterase domain containing 3	NM_024307	0.992	0.472
	223500_at	CPLX1	complexin 1	BC002471	0.995	0.470
	222408_s_at	YPEL5	yippee-like 5 (Drosophila)	BC000836	0.997	0.469
	225951_s_at	AV756026	NA	AV756026	1.000	0.467
	1556645_s_at	BC043400	NA	BC043400	0.995	0.465
	220746_s_at	UIMC1	ubiquitin interaction motif	NM_01629	0.999	0.465

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
			containing 1	0		
	218479_s_at	XPO4	exportin 4	NM_022459	0.999	0.464
	201834_at	PRKAB1	protein kinase, AMP-activated, beta 1 non-catalytic subunit	BC001007	0.993	0.464
	225675_at	C14orf101	chromosome 14 open reading frame 101	AW976269	0.994	0.462
	200974_at	ACTA2	actin, alpha 2, smooth muscle, aorta	NM_001613	0.990	0.462
	225860_at	BG469257	NA	BG469257	0.999	0.461
	224763_at	RPL37	ribosomal protein L37	BF724210	0.999	0.461
	49679_s_at	MMP24	matrix metallopeptidase 24 (membrane-inserted)	AA243774	0.997	0.460
	1569108_a_at	ZNF589	zinc finger protein 589	BC028160	0.997	0.460
	229574_at	TRA2A	transformer 2 alpha homolog (Drosophila)	AI268231	0.993	0.460
	222138_s_at	WDR13	WD repeat domain 13	AF158978	0.997	0.460
	229954_at	CHDH	choline dehydrogenase	AI025415	0.996	0.459
	210869_s_at	MCAM	melanoma cell adhesion molecule	M29277	0.995	0.459
	239834_at	AW874669	NA	AW874669	0.995	0.459
	227859_at	DNAJC27	DnaJ (Hsp40) homolog, subfamily C, member 27	AV706343	0.998	0.459
	1556339_a_at	BM353142	NA	BM353142	0.997	0.458
	230835_at	KRTDAP	keratinocyte differentiation-associated protein	W69083	0.994	0.458
	204862_s_at	NME3	non-metastatic cells 3, protein expressed in	NM_002513	0.999	0.458
	41512_at	BRAP	BRCA1 associated protein	AL042733	0.998	0.457
	238035_at	SP3	Sp3 transcription factor	N66313	0.992	0.456

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	226576_at	ARHGAP26	Rho GTPase activating protein 26	AI768563	0.994	0.456
	225605_at	TP53I13	tumor protein p53 inducible protein 13	AL540867	0.998	0.455
	218526_s_at	RANGRF	RAN guanine nucleotide release factor	NM_014185	1.000	0.455
	224798_s_at	C15orf17	chromosome 15 open reading frame 17	AI079857	0.998	0.454
	228001_at	TMEM50B	transmembrane protein 50B	N51405	0.991	0.454
	238491_at	LOC100506161	uncharacterized LOC100506161	BF241692	0.998	0.453
	204793_at	GPRASP1	G protein-coupled receptor associated sorting protein 1	NM_014710	0.992	0.452
	1557224_at	BM682057	NA	BM682057	0.995	0.452
	1553564_at	MACROD2	MACRO domain containing 2	NM_080676	0.998	0.451
	230154_at	WAC	WW domain containing adaptor with coiled-coil	AW003119	0.993	0.451
	229711_s_at	MDM2	Mdm2, p53 E3 ubiquitin protein ligase homolog (mouse)	AA902480	0.996	0.451
	1557410_at	LOC100506606	uncharacterized LOC100506606	AK090590	0.997	0.450
	225477_s_at	NR2C2	nuclear receptor subfamily 2, group C, member 2	AL138444	0.999	0.449
	215519_x_at	SGSM3	small G protein signaling modulator 3	AI081779	0.997	0.449
	219230_at	TMEM100	transmembrane protein 100	NM_018286	0.995	0.449
	228033_at	E2F7	E2F transcription factor 7	AI341146	1.000	0.448
	226408_at	TEAD2	TEA domain family member 2	AA905942	0.994	0.448
	219361_s_at	AEN	apoptosis enhancing nuclease	NM_022767	0.996	0.448

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	228652_at	ZNF776	zinc finger protein 776	AI279532	0.999	0.448
	227561_at	DDR2	discoidin domain receptor tyrosine kinase 2	W73819	0.996	0.447
	204538_x_at	NPIP	nuclear pore complex interacting protein	NM_006985	1.000	0.447
	209889_at	SEC31B	SEC31 homolog B (S. cerevisiae)	AF274863	0.998	0.446
	242316_at	AI810103	NA	AI810103	0.995	0.445
	214857_at	LOC100505761	uncharacterized LOC100505761	AL050035	0.993	0.445
	228595_at	HSD17B1	hydroxysteroid (17-beta) dehydrogenase 1	AA894611	0.998	0.445
	212528_at	PPPDE2	PPPDE peptidase domain containing 2	AI348009	0.998	0.444
	233010_at	AU158573	NA	AU158573	0.995	0.444
	209751_s_at	AF291676	NA	AF291676	0.999	0.444
	1553103_at	NFX1	nuclear transcription factor, X-box binding 1	NM_147134	0.998	0.443
	214035_x_at	LOC399491	GPS, PLAT and transmembrane domain-containing protein	AA308853	1.000	0.443
	228816_at	ATP6AP1L	ATPase, H+ transporting, lysosomal accessory protein 1-like	AK022625	0.995	0.443
	244618_at	W58255	NA	W58255	0.995	0.442
	231115_at	POLH	polymerase (DNA directed), eta	AI890529	0.996	0.441
	1556613_s_at	DPY19L4	dpy-19-like 4 (C. elegans)	BC038568	0.994	0.441
	230312_at	AI146812	NA	AI146812	0.999	0.441
	214706_at	ZNF200	zinc finger protein 200	AU149447	0.999	0.441
	37590_g_at	AL109698	NA	AL109698	0.993	0.440
	227921_at	AI797678	NA	AI797678	1.000	0.440

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	235702_at	AA507442	NA	AA507442	0.999	0.439
	226647_at	TMEM25	transmembrane protein 25	AL562445	0.995	0.438
	214121_x_at	PDLIM7	PDZ and LIM domain 7 (enigma)	AA086229	0.994	0.437
	211029_x_at	FGF18	fibroblast growth factor 18	BC006245	0.995	0.437
	237083_at	H46176	NA	H46176	0.996	0.437
	209852_x_at	PSME3	proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki)	BC001423	0.992	0.437
	221042_s_at	CLMN	calmin (calponin-like, transmembrane)	NM_024734	0.996	0.437
	202378_s_at	LEPROT	leptin receptor overlapping transcript	NM_017526	0.997	0.436
	228852_at	ENSA	endosulfine alpha	AV726322	0.996	0.436
	204839_at	POP5	processing of precursor 5, ribonuclease P/MRP subunit (S. cerevisiae)	NM_015918	0.999	0.435
	205658_s_at	SNAPC4	small nuclear RNA activating complex, polypeptide 4, 190kDa	NM_003086	0.997	0.435
	220755_s_at	C6orf48	chromosome 6 open reading frame 48	NM_016947	1.000	0.433
	209509_s_at	DPAGT1	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)	BC000325	0.997	0.433
	203164_at	SLC33A1	solute carrier family 33 (acetyl-CoA transporter), member 1	BE464756	0.992	0.433
	212751_at	UBE2N	ubiquitin-conjugating enzyme E2N	BG290646	0.997	0.432
	235181_at	TYW5	tRNA-yW synthesizing protein 5	H12075	0.993	0.432

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	226552_at	IER5L	immediate early response 5-like	BF110608	0.994	0.431
	225698_at	EPB41L4A-AS1	EPB41L4A antisense RNA 1 (non-protein coding)	BF314746	1.000	0.431
	221727_at	SUB1	SUB1 homolog (S. cerevisiae)	AA456973	0.991	0.431
	233455_at	AL110133	NA	AL110133	1.000	0.431
	204135_at	FILIP1L	filamin A interacting protein 1-like	NM_014890	0.997	0.430
	212708_at	MSL1	male-specific lethal 1 homolog (Drosophila)	AV721987	0.993	0.429
	232274_at	CCNL2	cyclin L2	AK000685	0.991	0.429
	40472_at	LPCAT4	lysophosphatidylcholine acyltransferase 4	AF007155	0.992	0.427
	223692_at	NMNAT1	nicotinamide nucleotide adenylyltransferase 1	AF312734	0.991	0.426
	33304_at	ISG20	interferon stimulated exonuclease gene 20kDa	U88964	0.996	0.425
	223421_at	CYHR1	cysteine/histidine-rich 1	BC005073	0.996	0.425
	1569973_at	SEPT7P2	septin 7 pseudogene 2	BC015774	0.992	0.425
	219426_at	EIF2C3	eukaryotic translation initiation factor 2C, 3	NM_024852	0.997	0.424
	201703_s_at	PPP1R10	protein phosphatase 1, regulatory subunit 10	NM_002714	0.997	0.423
	207877_s_at	NVL	nuclear VCP-like	NM_002533	0.999	0.423
	230759_at	SNX14	sorting nexin 14	AI476227	0.998	0.422
	212791_at	C1orf216	chromosome 1 open reading frame 216	AL042729	0.995	0.422
	202802_at	DHPS	deoxyhypusine synthase	NM_001930	0.996	0.422
	208922_s_at	NXF1	nuclear RNA export factor 1	BC004904	1.000	0.421

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	203992_s_at	KDM6A	lysine (K)-specific demethylase 6A	AF000992	0.997	0.421
	231375_at	LOC202181	chromosome 5 open reading frame 25 pseudogene	AI027731	0.991	0.418
	229913_at	C7orf61	chromosome 7 open reading frame 61	BF940911	0.992	0.417
	224774_s_at	NAV1	neuron navigator 1	AK022622	0.994	0.416
	230588_s_at	LOC285074	anaphase promoting complex subunit 1 pseudogene	AA906142	0.996	0.416
	229351_at	AA634138	NA	AA634138	0.995	0.415
	217630_at	ANGEL2	angel homolog 2 (Drosophila)	AI188346	0.997	0.414
	223095_at	MARVELD1	MARVEL domain containing 1	BC004995	0.998	0.413
	223284_at	NAT14	N-acetyltransferase 14 (GCN5-related, putative)	AB038651	0.995	0.413
	214870_x_at	AC002045	NA	AC002045	1.000	0.413
	228171_s_at	PLEKHG4	pleckstrin homology domain containing, family G (with RhoGef domain) member 4	AI056683	0.993	0.412
	1558094_s_at	C3orf19	chromosome 3 open reading frame 19	AL526467	0.997	0.412
	207034_s_at	GLI2	GLI family zinc finger 2	NM_030379	0.991	0.412
	237299_at	T71642	NA	T71642	0.995	0.411
	226993_at	AA973551	NA	AA973551	0.990	0.411
	228983_at	AI692591	NA	AI692591	0.994	0.410
	44111_at	VPS33B	vacuolar protein sorting 33 homolog B (yeast)	AI672363	0.997	0.409
	242568_s_at	BF995452	NA	BF995452	0.999	0.409

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	1554411_at	CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	AB062292	0.996	0.409
	219481_at	TTC13	tetratricopeptide repeat domain 13	NM_024525	0.998	0.408
	230626_at	TSPAN12	tetraspanin 12	AI056699	0.991	0.408
	211950_at	UBR4	ubiquitin protein ligase E3 component n-recognin 4	AB007931	1.000	0.408
	204028_s_at	RABGAP1	RAB GTPase activating protein 1	NM_012197	0.998	0.407
	205339_at	STIL	SCL/TAL1 interrupting locus	NM_003035	0.998	0.407
	214100_x_at	NSUN5P1	NOP2/Sun domain family, member 5 pseudogene 1	AI284845	0.993	0.407
	241453_at	PTK2	PTK2 protein tyrosine kinase 2	AA912743	0.996	0.406
	218410_s_at	PGP	phosphoglycolate phosphatase	NM_024118	0.996	0.406
	213206_at	GOSR2	golgi SNAP receptor complex member 2	AW149492	0.992	0.404
	223742_at	MRPL4	mitochondrial ribosomal protein L4	BC000756	0.992	0.404
	210069_at	U62733	NA	U62733	0.995	0.403
	225508_at	KIAA1468	KIAA1468	BF055274	0.992	0.403
	227755_at	AA042983	NA	AA042983	0.991	0.402
	219249_s_at	FKBP10	FK506 binding protein 10, 65 kDa	NM_021939	0.996	0.402
	1555964_at	ARL17A	ADP-ribosylation factor-like 17A	BU623906	0.995	0.402
	222752_s_at	TMEM206	transmembrane protein 206	AK024066	0.997	0.402
	228661_s_at	AI768374	NA	AI768374	0.992	0.401
	202458_at	PRSS23	protease, serine, 23	NM_007173	0.997	0.401

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	238692_at	BTBD11	BTB (POZ) domain containing 11	AL040935	0.992	0.400
	221920_s_at	SLC25A37	solute carrier family 25, member 37	BE677761	0.995	0.399
	236750_at	AL134451	NA	AL134451	0.992	0.399
	205559_s_at	PCSK5	proprotein convertase subtilisin/kexin type 5	NM_006200	0.996	0.399
	224369_s_at	FBXO38	F-box protein 38	AF251055	0.993	0.398
	231638_at	PRSS30P	protease, serine, 30 homolog (mouse), pseudogene	AA400057	0.991	0.396
	1556429_a_at	WDR67	WD repeat domain 67	BC026969	0.995	0.395
	209020_at	C20orf111	chromosome 20 open reading frame 111	AF217514	0.994	0.394
	213951_s_at	PSMC3IP	PSMC3 interacting protein	BE964655	0.997	0.394
	218348_s_at	ZC3H7A	zinc finger CCCH-type containing 7A	NM_014153	0.994	0.394
	225434_at	DEDD2	death effector domain containing 2	AW245401	0.997	0.393
	225864_at	FAM84B	family with sequence similarity 84, member B	AL039862	0.999	0.393
	234929_s_at	SPATA7	spermatogenesis associated 7	AF144488	0.991	0.393
	214821_at	SLC25A4	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	AF052119	0.993	0.392
	223290_at	BC000320	NA	BC000320	0.994	0.392
	201009_s_at	TXNIP	thioredoxin interacting protein	AI439556	0.994	0.391
	221723_s_at	SLC4A5	solute carrier family 4, sodium bicarbonate cotransporter, member 5	AF243499	0.994	0.390
	203782_s_at	POLRMT	polymerase (RNA) mitochondrial (DNA	NM_00503	0.994	0.390

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
			directed)	5		
	205277_at	PRDM2	PR domain containing 2, with ZNF domain	NM_012231	0.995	0.390
	203739_at	ZNF217	zinc finger protein 217	NM_006526	0.990	0.390
	236080_at	LOC100507670	uncharacterized LOC100507670	BE276063	0.992	0.389
	236330_at	AW292996	NA	AW292996	0.990	0.387
	218083_at	PTGES2	prostaglandin E synthase 2	NM_025072	0.996	0.386
	223706_at	C22orf23	chromosome 22 open reading frame 23	AF324466	0.991	0.385
	216264_s_at	LAMB2	laminin, beta 2 (laminin S)	X79683	0.996	0.383
	212858_at	PAQR4	progestin and adipoQ receptor family member IV	AL520675	0.994	0.383
	238653_at	LRIG2	leucine-rich repeats and immunoglobulin-like domains 2	BF967997	0.994	0.382
	219914_at	ECEL1	endothelin converting enzyme-like 1	NM_004826	0.994	0.382
	208132_x_at	PRRC2A	proline-rich coiled-coil 2A	NM_004638	0.990	0.382
	225070_at	NUS1	nuclear undecaprenyl pyrophosphate synthase 1 homolog (S. cerevisiae)	BF112132	0.992	0.381
	225819_at	TBRG1	transforming growth factor beta regulator 1	AA576946	0.994	0.381
	1555062_s_at	GTPBP3	GTP binding protein 3 (mitochondrial)	AY078987	0.995	0.380
	218858_at	DEPTOR	DEP domain containing MTOR-interacting protein	NM_022783	0.993	0.380
	202645_s_at	MEN1	multiple endocrine neoplasia I	NM_000244	0.994	0.380
	203916_at	NDST2	N-deacetylase/N-sulfotransferase (heparan	NM_00363	0.992	0.379

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
			glucosaminyl) 2	5		
	205106_at	NM_014221	NA	NM_014221	0.996	0.378
	227412_at	PPP1R3E	protein phosphatase 1, regulatory subunit 3E	AK024489	0.993	0.378
	226954_at	UBE2R2	ubiquitin-conjugating enzyme E2R 2	BE221883	0.997	0.378
	224489_at	KANSL1	KAT8 regulatory NSL complex subunit 1	BC006271	0.991	0.377
	214280_x_at	HNRNPA1	heterogeneous nuclear ribonucleoprotein A1	X79536	1.000	0.377
	206510_at	SIX2	SIX homeobox 2	AF332197	0.995	0.376
	201395_at	RBM5	RNA binding motif protein 5	NM_005778	1.000	0.376
	232230_at	LINC00263	long intergenic non-protein coding RNA 263	AU151788	0.992	0.376
	223450_s_at	COG3	component of oligomeric golgi complex 3	AF332595	0.993	0.374
	65630_at	TMEM80	transmembrane protein 80	AI742455	0.992	0.374
	217983_s_at	RNASET2	ribonuclease T2	NM_003730	0.999	0.372
	200617_at	MLEC	malectin	NM_014730	0.996	0.372
	215469_at	AF339807	NA	AF339807	0.992	0.372
	230130_at	AI692523	NA	AI692523	0.996	0.372
	202241_at	TRIB1	tribbles homolog 1 (Drosophila)	NM_025195	0.992	0.372
	238797_at	TRIM11	tripartite motif containing 11	BF059582	0.993	0.371
	202131_s_at	RIOK3	RIO kinase 3 (yeast)	NM_003831	0.992	0.371
	222029_x_at	PFDN6	prefoldin subunit 6	NM_014260	0.997	0.370

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	227922_x_at	LOC441124	uncharacterized LOC441124	AA156779	0.992	0.369
	225360_at	TRABD	TraB domain containing	AL449244	0.993	0.368
	1556646_at	AA714835	NA	AA714835	0.996	0.366
	218243_at	RUFY1	RUN and FYVE domain containing 1	NM_025158	0.998	0.366
	224915_x_at	ZNFX1-AS1	ZNFX1 antisense RNA 1 (non-protein coding)	AV756131	0.997	0.366
	1560577_at	BC031948	NA	BC031948	0.993	0.365
	201037_at	PFKP	phosphofructokinase, platelet	NM_002627	0.992	0.363
	209556_at	NCDN	neurochondrin	AB011179	0.993	0.363
	221261_x_at	NM_030801	NA	NM_030801	0.991	0.362
	228559_at	CENPN	centromere protein N	BF111626	0.990	0.362
	218897_at	TMEM177	transmembrane protein 177	NM_030577	0.998	0.362
	227718_at	PURB	purine-rich element binding protein B	BF337790	0.996	0.362
	216297_at	AL049314	NA	AL049314	0.996	0.362
	226080_at	SSH2	slingshot homolog 2 (Drosophila)	BE676214	0.995	0.360
	217508_s_at	C18orf25	chromosome 18 open reading frame 25	BE783279	0.997	0.360
	238155_at	AI638235	NA	AI638235	0.993	0.359
	228245_s_at	OVOS2	ovostatin 2	AW594320	0.996	0.359
	220201_at	RC3H2	ring finger and CCCH-type domains 2	NM_018835	0.995	0.358
	242222_at	LOC440894	uncharacterized LOC440894	AA069120	0.990	0.357
	218073_s_at	TMEM48	transmembrane protein 48	NM_018087	0.994	0.357

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression^1
	221436_s_at	CDCA3	cell division cycle associated 3	NM_031299	0.994	0.356
	218683_at	PTBP2	polypyrimidine tract binding protein 2	NM_021190	0.993	0.356
	213053_at	HAUS5	HAUS augmin-like complex, subunit 5	AW189966	0.992	0.355
	230063_at	ZNF264	zinc finger protein 264	BF063192	0.990	0.355
	203500_at	GCDH	glutaryl-CoA dehydrogenase	NM_000159	0.993	0.355
	209301_at	CA2	carbonic anhydrase II	M36532	0.999	0.354
	218378_s_at	PRKRIP1	PRKR interacting protein 1 (IL11 inducible)	NM_024653	0.992	0.353
	225264_at	RARS2	arginyl-tRNA synthetase 2, mitochondrial	AK023550	0.997	0.352
	212194_s_at	TM9SF4	transmembrane 9 superfamily protein member 4	AI418892	0.996	0.352
	226987_at	RBM15B	RNA binding motif protein 15B	W68720	0.990	0.352
	1557293_at	LOC440993	uncharacterized LOC440993	CA418406	0.993	0.350
	201828_x_at	FAM127A	family with sequence similarity 127, member A	NM_003928	0.995	0.348
	209480_at	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	M16276	0.991	0.346
	229430_at	C8orf46	chromosome 8 open reading frame 46	AI421311	0.998	0.345
	224986_s_at	PDPK1	3-phosphoinositide dependent protein kinase-1	AL040394	0.993	0.345
	239477_at	EFHB	EF-hand domain family, member B	BF941046	0.999	0.345
	203384_s_at	GOLGA1	golgin A1	NM_002077	0.990	0.345

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Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	243092_at	PAN3-AS1	PAN3 antisense RNA 1 (non-protein coding)	AI140189	0.995	0.344
	52005_at	WIZ	widely interspaced zinc finger motifs	AA422049	0.993	0.343
	1555897_at	KDM1A	lysine (K)-specific demethylase 1A	CA306222	0.998	0.343
	230641_at	AI792670	NA	AI792670	0.993	0.343
	39817_s_at	C6orf108	chromosome 6 open reading frame 108	AF040105	0.996	0.342
	244360_at	FBXL17	F-box and leucine-rich repeat protein 17	AW002273	0.991	0.342
	202402_s_at	CARS	cysteinyl-tRNA synthetase	NM_001751	0.995	0.341
	204992_s_at	PFN2	profilin 2	NM_002628	1.000	0.340
	1558693_s_at	C1orf85	chromosome 1 open reading frame 85	AW090182	0.994	0.339
	224447_s_at	MIEN1	migration and invasion enhancer 1	BC006006	0.997	0.339
	225462_at	TMEM128	transmembrane protein 128	AV705805	0.995	0.337
	202109_at	ARFIP2	ADP-ribosylation factor interacting protein 2	NM_012402	0.993	0.336
	200688_at	SF3B3	splicing factor 3b, subunit 3, 130kDa	D13642	0.991	0.334
	229231_at	H04996	NA	H04996	0.992	0.334
	218257_s_at	UGGT1	UDP-glucose glycoprotein glucosyltransferase 1	NM_020120	0.992	0.334
	214984_at	AC003007	NA	AC003007	0.990	0.333
	223613_at	UQCR11	ubiquinol-cytochrome c reductase, complex III subunit XI	BC000462	0.994	0.332
	200800_s_at	NM_005345	NA	NM_005345	1.000	0.332

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	213365_at	ERI2	ERI1 exoribonuclease family member 2	N64622	0.992	0.331
	224627_at	GBA2	glucosidase, beta (bile acid) 2	AB046825	0.995	0.331
	219647_at	POPDC2	popeye domain containing 2	NM_022135	0.997	0.330
	204366_s_at	GTF3C2	general transcription factor IIIC, polypeptide 2, beta 110kDa	NM_001521	0.997	0.329
	225458_at	PP7080	uncharacterized LOC25845	BF528646	0.991	0.327
	223773_s_at	SNHG12	small nucleolar RNA host gene 12 (non-protein coding)	AF277181	0.994	0.325
	204454_at	LDOC1	leucine zipper, down-regulated in cancer 1	NM_012317	0.993	0.324
	218059_at	ZNF706	zinc finger protein 706	NM_016096	0.995	0.322
	233413_at	AU156421	NA	AU156421	0.997	0.317
	230483_at	AA868500	NA	AA868500	0.991	0.315
	211038_s_at	CROCCP2	ciliary rootlet coiled-coil, rootletin pseudogene 2	BC006312	0.997	0.312
	235099_at	CMTM8	CKLF-like MARVEL transmembrane domain containing 8	AW080832	0.991	0.312
	212429_s_at	GTF3C2	general transcription factor IIIC, polypeptide 2, beta 110kDa	AW194657	0.995	0.312
	220587_s_at	MLST8	MTOR associated protein, LST8 homolog (S. cerevisiae)	NM_022372	0.993	0.310
	225310_at	RBMX	RNA binding motif protein, X-linked	AI928344	1.000	0.310
	1553329_at	C7orf45	chromosome 7 open reading frame 45	BC017587	0.997	0.308
	229980_s_at	SNX5	sorting nexin 5	AA131508	0.999	0.305

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	228357_at	UNK	unkempt homolog (Drosophila)	BE966979	0.996	0.303
	231142_at	BF221525	NA	BF221525	0.991	0.302
	212510_at	GPD1L	glycerol-3-phosphate dehydrogenase 1-like	AA135522	0.995	0.300
	212689_s_at	KDM3A	lysine (K)-specific demethylase 3A	AA524505	0.995	0.300
	213842_x_at	NSUN5P2	NOP2/Sun domain family, member 5 pseudogene 2	AK021688	0.996	0.299
	219639_x_at	PARP6	poly (ADP-ribose) polymerase family, member 6	NM_020213	0.994	0.299
	1559186_at	PRKXP1	protein kinase, X-linked, pseudogene 1	AL833178	0.993	0.297
	225890_at	C20orf72	chromosome 20 open reading frame 72	AI678096	0.994	0.294
	226829_at	AFAP1L2	actin filament associated protein 1-like 2	AW138743	0.994	0.292
	206858_s_at	HOXC6	homeobox C6	NM_004503	0.997	0.290
	224841_x_at	GAS5	growth arrest-specific 5 (non-protein coding)	BF316352	1.000	0.286
	228882_at	TUB	tubby homolog (mouse)	AL042088	0.995	0.286
	216058_s_at	CYP2C19	cytochrome P450, family 2, subfamily C, polypeptide 19	X65962	0.994	0.278
	207826_s_at	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	NM_002167	0.998	0.275
	201520_s_at	GRSF1	G-rich RNA sequence binding factor 1	BF034561	0.995	0.270
	209265_s_at	METTL3	methyltransferase like 3	BC001650	0.998	0.269
	208705_s_at	EIF5	eukaryotic translation initiation factor 5	BG481972	1.000	0.268

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	1560652_at	AL832136	NA	AL832136	1.000	0.262
	213302_at	PFAS	phosphoribosylformylglycinamidine synthase	AL044326	0.996	0.262
	1553494_at	TDH	L-threonine dehydrogenase	NM_152566	0.997	0.261
	221730_at	COL5A2	collagen, type V, alpha 2	NM_000393	0.997	0.260
	216199_s_at	MAP3K4	mitogen-activated protein kinase kinase kinase 4	AL109942	0.991	0.256
	209218_at	SQLE	squalene epoxidase	AF098865	0.998	0.249
	201046_s_at	RAD23A	RAD23 homolog A (S. cerevisiae)	NM_005053	0.995	0.243
	218300_at	C16orf53	chromosome 16 open reading frame 53	NM_024516	0.990	0.243
	209849_s_at	RAD51C	RAD51 homolog C (S. cerevisiae)	AF029669	0.995	0.234
	235547_at	N4BP2L2	NEDD4 binding protein 2-like 2	BG548427	0.993	0.234
	201959_s_at	MYCBP2	MYC binding protein 2, E3 ubiquitin protein ligase	AA488899	0.995	0.232
	1570296_at	BC029614	NA	BC029614	0.996	0.230
	211996_s_at	BG256504	NA	BG256504	0.993	0.230
	220356_at	CORIN	corin, serine peptidase	NM_006587	0.995	0.225
	210774_s_at	NCOA4	nuclear receptor coactivator 4	AL162047	0.993	0.220
	212455_at	YTHDC1	YTH domain containing 1	N36997	0.994	0.211
	243605_at	AW627671	NA	AW627671	0.996	0.194
	224610_at	AL530869	NA	AL530869	0.997	0.187
	220864_s_at	NDUFA13	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13	NM_015965	0.994	0.179

Table S1. Genes differentially expressed greater than two-fold at 2, 8, 16 and 24 h after delivery of polyplexes.

Time Point	Affy Probe ID	Gene symbol	Gene name	Accession number	Posterior Probability	Differential Expression[1]
	221874_at	KIAA1324	KIAA1324	AB037745	0.993	0.176
	210371_s_at	RBBP4	retinoblastoma binding protein 4	BC003092	0.991	0.175
	212108_at	FAF2	Fas associated factor family member 2	AB020694	0.990	0.175
	218594_at	HEATR1	HEAT repeat containing 1	NM_018072	0.992	0.173
	220649_at	AGBL3	ATP/GTP binding protein-like 3	NM_024856	0.996	0.161
	212643_at	MAPK1IP1L	mitogen-activated protein kinase 1 interacting protein 1-like	AI671747	0.996	0.154
	200881_s_at	DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	NM_001539	0.996	0.146
	225547_at	SNHG6	small nucleolar RNA host gene 6 (non-protein coding)	BG169443	0.997	0.131
	200912_s_at	EIF4A2	eukaryotic translation initiation factor 4A2	NM_001967	0.996	0.116
	200893_at	TRA2B	transformer 2 beta homolog (Drosophila)	NM_004593	0.998	0.106
	200884_at	CKB	creatine kinase, brain	NM_001823	0.991	0.102
	AFFX-HSAC07/X00351_3_at	ACTB	actin, beta	AFFX-HSAC07/X00351_3	0.995	0.066
	1553551_s_at	ND2	MTND2	NM_173709	0.998	0.056
	224373_s_at	AF253979	NA	AF253979	0.997	0.053

[1]Differential expression represents comparison of microarray gene expression from cells treated with complexes (n = 3) to microarray gene expression from cells left untreated (n = 3). [2]Differential expression represents comparison of microarray gene expression from cells treated and transfection (GFP+; n = 3) to microarray gene expression from cells treated and untransfected (GFP-; n = 3. Differential expression greater than or less than 1 represents upregulation or downregulation, respectively.

Table S2. Overlapping genes between time points (from Figure 2).

8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes)[1]	16h U 24h (32 genes)[1]	8h U 16h U 24h (35 genes)[1]
BUB3	N53959	VAMP2	ITGAM	ID3	UBR1	RAP1A
AK022170	HMOX1	ZNF45	BF508868	AGBL3	MTHFD2L	CHORDC1
AF318328	ZNF473	PFKP	DDIT4	DCAF4L1	BE644816	AU144129
DNAJC14	HIST1H3H	TMCO6	FAM105B	EFHB	GTF2H1	ZSCAN30
AI569997	NM_031211	C1orf85	AI885066	TRIM11	AA503877	ATF3
EPB41L4B	AF086424	HSD17B1	TSGA10	TSC22D3	CHD2	LRRC23
BF055200	GPR83	AEN	XRN1	KDM6A	MCM9	PGAP1
RBM12	CCDC11	CMTM8	AK000847	TEAD2	N4BP2L2	ACRC
LRIF1	MEIG1	C7orf45	AOC3	CYHR1	NPEPL1	AI810034
CCNB1	ID4	AI033967	LOC387723	AI146812	ALDH8A1	BC005379
G2E3	AI831514	PGP	ALB	RBBP4	PCDH7	RBM26-AS1
TMEM68	CDK12	MRPL4	AI911318	AL832136	PFDN6	AI939588
LOC100507619	PLGLB2	GAS5	GATM	FBXO38	ZNF346	FUBP1
NM_017761	AA142959	AV756026	ACTN2	TRIB1	HDAC8	PIK3C2A
AI800419	CCDC41	MSL1		HNRNPA1	FGF18	WDR96
BF057656	ZNF596	UQCR11		POPDC2	ABCF2	MYH3
BF217471	AI251890	PPP1R15A		C14orf101	R17062	C7orf53
CCNL1	LOC100507495	BG256504		FAM84B	SMC1A	AI650407
TBCE	DNAH12	AL530869			LPXN	TPH1
FLJ45482	ABHD12	NM_014221			TTC18	CLEC4A
ATL2	N58120	AW292996			IREB2	SLC6A13
ADAMTS1	LRRC37A3	UGGT1			AI423165	ACTA1
PCF11	L39064	MEN1			HIST1H2BD	RFPL3-AS1
LOC100507424	LOC100507297	PFAS			ZNF101	CTSK
NDRG1	TMEM116	ND2			PSPC1	TMED6
FAM134A	AA844689	SLC33A1			SRSF5	WDR78
C6orf70	AI369073	SLC25A37			NM_005083	DDR2
PRC1	LARP1B	TRABD			AI863194	TEX14
IDI2-AS1	TTBK2	AA714835			AW117229	SCG5
CDK1	AI809760	BRAP			SEPT7P2	LRRC39
BTRC	POMZP3	SSH2			ZNF846	NEB
IL17F	ZFP2	TSPAN12			LOC645513	NM_004038
ZHX2	BE673747	MAF1				AL833615
BE894882	ERP44	PLK2				NM_022155
NM_018676	LCA5L	NUS1				AA843122
ORC1	TTC21B	AA634138				
SLC35D2	AI023795	C20orf111				
N32025	TARS	NMNAT1				

Table S2. Overlapping genes between time points (from Figure 2).

8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes) [1]	16h U 24h (32 genes) [1]	8h U 16h U 24h (35 genes) [1]
BF740216	BF509796	EPB41L4A-AS1				
LOC729013	EIF4E2	RAD23A				
POGZ	SLC22A1	AFAP1L2				
BE299495	AA442776	CDCA3				
D59630	LOC100506083	PDLIM7				
BC042016	DTL	CYP2C19				
ATR	BF515913	AL049314				
LOC100505696	LOC100507049	PAN3-AS1				
PATZ1	AI423466	UBE2R2				
C7orf42	GNRH1	BC043400				
CDC25C	ZNF254	PRDM2				
ACP2	AI824013	BC000320				
BE672818	DENND4C	OR7E14P				
BCL2L2	T78087	AI692523				
TMEM138	ACACB	AW627671				
DIDO1	AI695695	EIF5				
PRKCD	CDNF	SH3YL1				
H50121	BF224430	C6orf108				
ITPRIPL2	EME2	C8orf46				
NKTR	W56760	MDM2				
PRR5	AU145323	KATNB1				
NOP58	AW132066	BF431313				
DNAH10	AW292905	ZC3H7A				
HIST1H2BK	ATP5E	LOC729887				
HSPH1	KIAA1432	TRA2B				
LEFTY1	ZNF557	STIL				
AK025430	FSD1L	SLC25A4				
AURKB	PRR14	ARMCX5				
PCDH9	RPP30	DHPS				
FHDC1	LOC81691	SNX5				
MORF4L2	AOC2	ZNF706				
MPP3	SAT1	EEF1D				
TLL2	AA331548	NDST2				
BF477573	HELQ	AA868500				
AA706701	HOXB-AS3	PLEKHG4				
TERF2	AA868809	AI638235				
AB051480		TYW5				
AI206345		BOP1				

Table S2. Overlapping genes between time points (from Figure 2).

8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes)[1]	16h U 24h (32 genes)[1]	8h U 16h U 24h (35 genes)[1]
SUDS3		SPRN				
RSRC2		DDIT3				
AI808359		C1orf216				
BMP7		GDPD3				
MANBA		PTK2				
PPP1R16A		LOC100506606				
UST		MYCBP2				
CTH		WIZ				
HEPACAM2		NM_003516				
AW993257		SPRYD4				
TRPC1		U62733				
IFNA5		FAHD2A				
LOC100287616		CKB				
AK074226		ENSA				
C15orf23		KIAA1324				
SEC61A2		BF593636				
ZNF703		W58255				
BE466077		CPLX1				
AU159414		AI610347				
AA769100		QTRT1				
ZIC3		SEC31B				
FAM110B		TMEM50B				
GTSE1		SLC27A4				
MID2		NCDN				
PVRL3-AS1		C7orf61				
FLJ31958		ZNF589				
INSIG1		LOC202181				
REPIN1		AF339807				
LINC00094		TRA2A				
WDR66		PP7080				
HNRNPU-AS1		LPCAT4				
CKS2		PPP1R3E				
PHF23		GPR19				
HCLS1		AA507442				
KANSL1-AS1		NAV1				
ZNF33B		DNAJC27				
		RGL1				
		PSMC3IP				

Table S2. Overlapping genes between time points (from Figure 2).						
8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes)[1]	16h U 24h (32 genes)[1]	8h U 16h U 24h (35 genes)[1]
		AU158573				
		CORIN				
		BTBD11				
		CA2				
		PRSS23				
		GTPBP3				
		ZNFX1-AS1				
		MLST8				
		ISG20				
		TMEM177				
		RBM5				
		WDR6				
		ECEL1				
		PRKRIP1				
		NM_030801				
		BF995452				
		ZNF227				
		TMEM74B				
		COG3				
		BC029614				
		AL134451				
		FBXL17				
		BG469257				
		AW874669				
		CARS				
		NR2C2				
		HLA-DQB1				
		AA767131				
		FAM108A1				
		MAP3K4				
		GRSF1				
		AA431379				
		KIAA1468				
		RBPMS				
		PRRC2A				
		AI810103				
		NPIP				
		CROCCP2				

Table S2. Overlapping genes between time points (from Figure 2).						
8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes) [1]	16h U 24h (32 genes) [1]	8h U 16h U 24h (35 genes) [1]
		GOLGA1				
		UBE2N				
		C3orf19				
		H04996				
		HEXIM1				
		GPRASP1				
		AF291676				
		PARP11				
		WDR67				
		PAQR4				
		POLH				
		RC3H2				
		BC031948				
		PTGES2				
		FILIP1L				
		ACTB				
		PFN2				
		BF968097				
		H46176				
		UIMC1				
		TMEM25				
		COX8C				
		GOSR2				
		SP3				
		HAUS5				
		DEPTOR				
		C18orf25				
		KDM1A				
		CHMP6				
		NDUFA13				
		POLRMT				
		TP53I13				
		NVL				
		LEPROT				
		CCNL2				
		TTC13				
		AF253979				
		GLI2				

Table S2. Overlapping genes between time points (from Figure 2).						
8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes) [1]	16h U 24h (32 genes) [1]	8h U 16h U 24h (35 genes) [1]
		LOC100506161				
		TMEM128				
		SNAPC4				
		ATP6AP1L				
		SLC4A5				
		ANGEL2				
		RBMX				
		TNKS2				
		KRTDAP				
		MARVELD1				
		MLEC				
		PURB				
		SUB1				
		AI733801				
		LAMB2				
		NFX1				
		NHLH2				
		LOC100505761				
		YPEL5				
		AI797678				
		AA478537				
		FKBP10				
		HBS1L				
		RANGRF				
		PTBP2				
		COL5A2				
		SIX2				
		RNASET2				
		C19orf60				
		RARS2				
		UNK				
		C9orf100				
		AU156421				
		RAD52				
		SNHG12				
		TBRG1				
		LOC285074				
		AKT1				

Table S2. Overlapping genes between time points (from Figure 2).

8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes)[1]	16h U 24h (32 genes)[1]	8h U 16h U 24h (35 genes)[1]
		PHF19				
		SLC39A9				
		TM9SF4				
		HSPA6				
		ZNF264				
		AI768374				
		PDPK1				
		EIF4A2				
		SNHG6				
		TDH				
		LOC399491				
		XPO4				
		PCSK5				
		DPY19L4				
		DNAJA1				
		PRSS30P				
		MAP6D1				
		PRKAB1				
		SQLE				
		RAD51C				
		PARP6				
		CTNNB1				
		RABGAP1				
		KIAA0753				
		FBXO5				
		IER5L				
		ZNF654				
		SLFN5				
		POP5				
		LOC100507670				
		C6orf48				
		ZNF217				
		OVOS2				
		KANSL1				
		FAM53C				
		AL109698				
		VPS33B				
		TBX1				

Table S2. Overlapping genes between time points (from Figure 2).

8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes) [1]	16h U 24h (32 genes) [1]	8h U 16h U 24h (35 genes) [1]
		SLC7A6OS				
		AL110133				
		RBM15B				
		EIF2C3				
		AC002045				
		CATSPERD				
		LOC100288637				
		CHDH				
		TUB				
		DEDD2				
		AI692591				
		MCAM				
		ACTA2				
		C20orf72				
		LINC00263				
		NM_005345				
		BF221525				
		CLMN				
		AL831886				
		BM353142				
		POLD3				
		PPP1R10				
		HOXC6				
		ZNF551				
		ARHGAP26				
		TMEM48				
		AF271775				
		SLC13A3				
		CENPN				
		KLF10				
		WDR13				
		PSME3				
		ERI2				
		NXF1				
		NME3				
		RIOK3				
		NSUN5P1				
		ARL17A				

Table S2. Overlapping genes between time points (from Figure 2).						
8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes)[1]	16h U 24h (32 genes)[1]	8h U 16h U 24h (35 genes)[1]
		LOC441124				
		MACROD2				
		DPAGT1				
		FAM127A				
		PPPDE2				
		METTL3				
		EPC1				
		TMEM80				
		RUFY1				
		YTHDC1				
		SGSM3				
		PRKXP1				
		LDOC1				
		KDM3A				
		AI356405				
		C22orf23				
		MYO1D				
		MIEN1				
		SNX14				
		ARFIP2				
		BM970306				
		E2F7				
		GTF3C2				
		C15orf17				
		WAC				
		AW028687				
		GPD1L				
		RPL37				
		IFRD1				
		NCOA4				
		BM682057				
		LRRC14				
		UBR4				
		ITIH4				
		MAPK1IP1L				
		TXNIP				
		ZNF776				
		SF3B3				

Table S2. Overlapping genes between time points (from Figure 2).

8h (112 genes)	16 h (74 genes)	24h (369 genes)	8h U 16h (14 genes)[1]	8h U 24h (18 genes)[1]	16h U 24h (32 genes)[1]	8h U 16h U 24h (35 genes)[1]
		FAM125A				
		FAF2				
		SPATA7				
		T71642				
		NSUN5P2				
		LRIG2				
		C1orf162				
		AA042983				
		TMEM206				
		AC003007				
		HEATR1				
		VPS8				
		GCDH				
		MMP24				
		C16orf53				
		LOC440894				
		TMEM100				
		LOC440993				
		ZNF200				
		BC038430				
		GBA2				
		MFSD3				
		AA973551				
		AI792670				
		PCIF1				
		LOC100506713				
		NAT14				

[1] U stands for the union of the time points, e.g. a gene that was found at multiple time points.

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).

2 h time point			8 h time point			16 h time point			24 h time point		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes)[1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes)[1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes)[1]
C20orf96	T97641		NDUFA10	AK025430	RAP1A	ZNF548	ZNF596	AK000847 (ZNF236)		ACTB	RAP1A
INSL3	TAGAP		PACSIN3	CDK1	ATF3	KRTAP19-1	TARS	RAP1A		PFN2	HSPA6
LOC100130938	ZNF675		ARMC8	AURKB		AA457019	AI023795	NM_005083 (ZRSR1)		BF968097	
UGCG	AW444934		DNAJB11	AK000847		PDP2	MCM9	ZNF79		H46176	
BM849515	ARHGAP24		IFT27	DDR2		GNAL	N4BP2L2	ATF3		VAMP2	
TPTE	IQGAP3			ITGAM		RDH10	DDR2			UIMC1	
NM_024914	TEX13A			BTRC		NIP7	BF509796			AI423165	
PTPN14	DTNA			IFNA5		CCNB1	ITGAM			TMEM25	
AV649908	LOC283070			PCDH9		LINC00032	AI423165			COX8C	
AA416756	CCDC82			AI810034		TTF2	AI810034			GOSR2	
AW195867	LOC400084			IL17F		FOXN3	PSPC1			SP3	
DOCK10	APOBEC3G			ALB		HSPA6	ALB			HAUS5	
FBXO4	ZNF79			ZHX2		BC017896	FAM105B			ZNF45	
DNM3	AI419968			FAM105B		ARMC8	AI911318			PFKP	
ANXA6	BC039439			AI911318		PRR11	AU145323			DEPTOR	
LRRC59	HAO1			BE894882		AURKA	BC005379			TMCO6	
ATP1A3	AA716482			BC005379		TOB1	NPEPL1			C1orf85	
BC040701	PDZD2			LOC100287616		AL117451	ALDH8A1			C18orf25	
ITGAL	T57946			AK074226		AI819238	N53959			HSD17B1	
RHOC	AL049302			C15orf23		TRIM13	UBR1			KDM1A	
AI912723	LGSN			BUB3		SAMD14	AI251890			AEN	
BF829112	STYX			SEC61A2		W80619	PCDH7			CHMP6	
LIMS1	HIPK3			AK022170			MTHFD2L			CMTM8	
SLC24A2	MS4A6E			ZNF703			SLC22A1			NDUFA13	

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).

2 h time point			8 h time point			16 h time point			24 h time point		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
NM_000570	AW300131			NM_018676			EIF4E2			POLRMT	
BC040304	BC033970			TRIB1			AA442776			NVL	
AI809906	W79643			AOC3			AOC3			TP53I13	
SLC3A1	TRERF1			WDR96			BF508868			UBR1	
AW274257	EGR1			BF508868			WDR96			LEPROT	
CYP2C9	RBM34			ORC1			LOC100506083			MTHFD2L	
AL137539	RBM25			BE466077			DTL			C7orf45	
AK021679	GSN			AF318328			HMOX1			AI033967	
AI806127	IGF2BP1			AU159414			BF515913			PGP	
GTF2I	EXOC3L4			SLC35D2			PFDN6			MRPL4	
ITGB1	RGS1			DNAJC14			SRSF5			GAS5	
AF113689	AL110176			N32025			TEX14			CCNL2	
AL117426	IGLV3-25			AI569997			BE644816			TTC13	
FMO1	CPB2			FHDC1			CHORDC1			WDR96	
FBN1	SNX24			TEAD2			LOC100507495			AF253979	
CCDC30	T86874			EPB41L4B			ZNF473			GLI2	
BCL2L10	C11orf57			BF055200			AW132066			LOC100506161	
R51853	KRT27			TEX14			AI885066			AV756026	
ZFR	RFFL			MORF4L2			RBM26-AS1			TMEM128	
AF394782	YWHAE			TRIM11			ZNF346			SNAPC4	
	SIRT4			BF740216			AW292905			MSL1	
	VNN3			ID3			DNAH12			UQCR11	
				RBM12			LOC100507049			ATP6AP1L	

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				LRIF1			AI423466			TEAD2	
				MPP3			HIST1H3H			PPP1R15A	
				TLL2			AU144129			BG256504	
				CHORDC1			GNRH1			AL530869	
				CYHR1			HDAC8			SLC4A5	
				CCNB1			ATP5E			NM_014221	
				AI146812			AI939588			ID3	
				AA769100			MYH3			AW292996	
				RBM26-AS1			NM_031211			ANGEL2	
				AI885066			FGF18			RBMX	
				LOC729013			ZNF254			BE644816	
				G2E3			AI824013			UGGT1	
				ZIC3			DENND4C			KRTDAP	
				TMEM68			KIAA1432			TNKS2	
				POGZ			ZNF557			CHORDC1	
				AU144129			FSD1L			CYHR1	
				BE299495			AF086424			MARVELD1	
				LOC100507619			DDIT4			MEN1	
				AI939588			GTF2H1			PFAS	
				MYH3			GATM			MLEC	
				D59630			GPR83			ND2	
				BC042016			T78087			PURB	
				AGBL3			ABHD12			SUB1	

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				ATR			TSGA10			SLC33A1	
				BF477573			N58120			AI146812	
				NM_017761			SCG5			AI733801	
				LOC100505696			AA503877			SLC25A37	
				PATZ1			ZSCAN30			TRABD	
				AI800419			ABCF2			AA714835	
				FAM110B			R17062			LAMB2	
				AA706701			LRRC37A3			NFX1	
				TSC22D3			L39064			BRAP	
				C7orf42			ACACB			LOC100505761	
				GTSE1			AI863194			NHLH2	
				CDC25C			CCDC11			AU144129	
				DDIT4			C7orf53			MYH3	
				BF057656			PRR14			YPEL5	
				GATM			LOC100507297			AGBL3	
				BF217471			AW117229			SSH2	
				KDM6A			SEPT7P2			AI797678	
				ACP2			AI650407			AA478537	
				RBBP4			TMEM116			TSPAN12	
				CCNL1			SMC1A			FKBP10	
				TSGA10			ACTN2			MAF1	
				SCG5			AA844689			RANGRF	
				BE672818			MEIG1			HBS1L	

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2 h time point			8 h time point			16 h time point			24 h time point		
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				ZSCAN30			TPH1			PTBP2	
				BCL2L2			LPXN			COL5A2	
				MID2			AI695695			SIX2	
				PVRL3-AS1			LRRC39			RNASET2	
				TMEM138			CDNF			C19orf60	
				HNRNPA1			ID4			PLK2	
				TERF2			AI369073			NUS1	
				TBCE			NEB			GTF2H1	
				AB051480			LARP1B			AA634138	
				C7orf53			TTC18			C20orf111	
				AL832136			BF224430			RARS2	
				AI650407			AI831514			NMNAT1	
				FLJ45482			TTBK2			EPB41L4A-AS1	
				AI206345			NM_004038			RBBP4	
				ACTN2			AI809760			RAD23A	
				DIDO1			CDK12			UNK	
				PRKCD			POMZP3			C9orf100	
				H50121			RPP30			AFAP1L2	
				POPDC2			ZFP2			AU156421	
		RGL1		TPH1			LOC81691			ZSCAN30	
		PSMC3IP		ATL2			BE673747			AA503877	
		AU158573		LRRC39			CLEC4A			RAD52	
		CORIN		ADAMTS1			AOC2			SNHG12	
		BTBD11		FLJ31958			EME2			CDCA3	

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LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
		CA2		SUDS3			LRRC23			TBRG1	
		PRSS23		INSIG1			AL833615			LOC285074	
		GTPBP3		NEB			SAT1			AKT1	
		ZNFX1-AS1		ITPRIPL2			FUBP1			PHF19	
		MLST8		RSRC2			AA331548			PDLIM7	
		ISG20		AI808359			ERP44			SLC39A9	
		TMEM177		PCF11			IREB2			CYP2C19	
		RBM5		BMP7			ZNF846			TM9SF4	
		WDR6		REPIN1			SLC6A13			ZNF264	
		ECEL1		LINC00094			LOC387723			AI768374	
		PRKRIP1		NM_004038			XRN1			AL049314	
		NM_030801		NKTR			ACTA1			PDPK1	
		BF995452		NOP58			PLGLB2			EIF4A2	
		ZNF227		PRR5			LOC645513			PAN3-AS1	
		TMEM74B		CLEC4A			AA142959			C7orf53	
		COG3		DCAF4L1			RFPL3-AS1			UBE2R2	
		BC029614		WDR66			LCA5L			AL832136	
		AL134451		LOC100507424			HIST1H2BD			SNHG6	
		FBXL17		DNAH10			PGAP1			AI650407	
		BG469257		LRRC23			ZNF101			TDH	
		AW874669		AL833615			CTSK			LOC399491	
		CARS		HNRNPU-AS1			TTC21B			XPO4	
		NR2C2		FBXO38			W56760			TPH1	

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2 h time point			**8 h time point**			**16 h time point**			**24 h time point**		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
		HLA-DQB1		FUBP1			CHD2			PCSK5	
		AA767131		MANBA			HELQ			DPY19L4	
		FAM108A1		HIST1H2BK			NM_022155			BC043400	
		MAP3K4		NDRG1			CCDC41			DNAJA1	
		GRSF1		CKS2			HOXB-AS3			PRSS30P	
		AA431379		SLC6A13			PIK3C2A			MAP6D1	
		KIAA1468		LOC387723			ACRC			PRDM2	
		RBPMS		XRN1			AA868809			BC000320	
		PRRC2A		ACTA1			TMED6			OR7E14P	
		AI810103		PHF23			AA843122			ATF3	
		NPIP		C14orf101			WDR78			AI692523	
		CROCCP2		RFPL3-AS1						AW627671	
		GOLGA1		FAM84B						EIF5	
		UBE2N		PGAP1						PRKAB1	
		C3orf19		PPP1R16A						SQLE	
		H04996		CTSK						SH3YL1	
		HEXIM1		HCLS1						C6orf108	
		GPRASP1		UST						C8orf46	
		AF291676		KANSL1-AS1						MDM2	
		PARP11		EFHB						RAD51C	
		WDR67		ZNF33B						KATNB1	
		PAQR4		CTH						DCAF4L1	
		POLH		NM_022155						CLEC4A	
		RC3H2		FAM134A						PARP6	

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		BC031948		HEPACAM2						BF431313	
		PTGES2		PIK3C2A						ZC3H7A	
		FILIP1L		ACRC						LOC729887	
		ACTB		TMED6						CTNNB1	
		PFN2		AW993257						LRRC23	
		BF968097		C6orf70						RABGAP1	
		H46176		PRC1						FBXO38	
		UIMC1		TRPC1						IER5L	
		TMEM25		HSPH1						KIAA0753	
		COX8C		IDI2-AS1						FBXO5	
		GOSR2		AA843122						ZNF654	
		SP3		LEFTY1						TRA2B	
		HAUS5		WDR78						STIL	
		DEPTOR								SLC25A4	
		C18orf25								SLFN5	
		KDM1A								POP5	
		CHMP6								LOC100507670	
		NDUFA13								C6orf48	
		POLRMT								ARMCX5	
		TP53I13								ZNF217	
		NVL								DHPS	
		LEPROT								OVOS2	
		CCNL2								SLC6A13	
		TTC13								KANSL1	

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		AF253979								FAM53C	
		GLI2								AL109698	
		LOC100506161								ACTA1	
		TMEM128								SNX5	
		SNAPC4								ZNF706	
		ATP6AP1L								EEF1D	
		SLC4A5								VPS33B	
		ANGEL2								TBX1	
		RBMX								RFPL3-AS1	
		TNKS2								NDST2	
		KRTDAP								HIST1H2BD	
		MARVELD1								PGAP1	
		MLEC								SLC7A6OS	
		PURB								AA868500	
		SUB1								ZNF101	
		AI733801								CTSK	
		LAMB2								AL110133	
		NFX1								EFHB	
		NHLH2								CHD2	
		LOC100505761								RBM15B	
		YPEL5								AC002045	
		AI797678								EIF2C3	
		AA478537								ACRC	

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		FKBP10								CHDH	
		HBS1L								LOC100288637	
		RANGRF								TMED6	
		PTBP2								CATSPERD	
		COL5A2								TUB	
		SIX2								DEDD2	
		RNASET2								PLEKHG4	
		C19orf60								AI638235	
		RARS2								WDR78	
		UNK								TYW5	
		C9orf100								BOP1	
		AU156421								DDIT3	
		RAD52								SPRN	
		SNHG12								AI692591	
		TBRG1								MCM9	
		LOC285074								C1orf216	
		AKT1								N4BP2L2	
		PHF19								DDR2	
		SLC39A9								MCAM	
		TM9SF4								GDPD3	
		HSPA6								PTK2	
		ZNF264								LOC100506606	
		AI768374								MYCBP2	
		PDPK1								AI810034	

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		EIF4A2								WIZ	
		SNHG6								ACTA2	
		TDH								PSPC1	
		LOC399491								C20orf72	
		XPO4								LINC00263	
		PCSK5								NM_005345	
		DPY19L4								NM_003516	
		DNAJA1								SPRYD4	
		PRSS30P								BC005379	
		MAP6D1								NPEPL1	
		PRKAB1								ALDH8A1	
		SQLE								U62733	
		RAD51C								BF221525	
		PARP6								CLMN	
		CTNNB1								FAHD2A	
		RABGAP1								CKB	
		KIAA0753								ENSA	
		FBXO5								AL831886	
		IER5L								BM353142	
		ZNF654								PCDH7	
		SLFN5								POLD3	
		POP5								PPP1R10	
		LOC100507670								HOXC6	
		C6orf48								TRIB1	

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		ZNF217								ZNF551	
		OVOS2								ARHGAP26	
		KANSL1								TMEM48	
		FAM53C								AF271775	
		AL109698								SLC13A3	
		VPS33B								KIAA1324	
		TBX1								BF593636	
		SLC7A6OS								W58255	
		AL110133								CENPN	
		RBM15B								CPLX1	
		EIF2C3								KLF10	
		AC002045								PFDN6	
		CATSPERD								AI610347	
		LOC100288637								SRSF5	
		CHDH								WDR13	
		TUB								TEX14	
		DEDD2								QTRT1	
		AI692591								TRIM11	
		MCAM								PSME3	
		ACTA2								SEC31B	
		C20orf72								TMEM50B	
		LINC00263								ERI2	
		NM_005345								NXF1	
		BF221525								NME3	

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LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes)[1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes)[1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes)[1]
		CLMN								RIOK3	
		AL831886								NSUN5P1	
		BM353142								RBM26-AS1	
		POLD3								ZNF346	
		PPP1R10								ARL17A	
		HOXC6								RBM25	
		ZNF551								NM_005083	
		ARHGAP26								LOC441124	
		TMEM48								SLC27A4	
		AF271775								MACROD2	
		SLC13A3								DPAGT1	
		CENPN								FAM127A	
		KLF10								PPPDE2	
		WDR13								METTL3	
		PSME3								HDAC8	
		ERI2								EPC1	
		NXF1								NCDN	
		NME3								TMEM80	
		RIOK3								C7orf61	
		NSUN5P1								ZNF589	
		ARL17A								AI939588	
		LOC441124								RUFY1	
		MACROD2								LOC202181	
		DPAGT1								AF339807	

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).

2 h time point			8 h time point			16 h time point			24 h time point		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
		FAM127A								TRA2A	
		PPPDE2								PP7080	
		METTL3								LPCAT4	
		EPC1								YTHDC1	
		TMEM80								SGSM3	
		RUFY1								PPP1R3E	
		YTHDC1								PRKXP1	
		SGSM3								LDOC1	
		PRKXP1								GPR19	
		LDOC1								FGF18	
		KDM3A								KDM3A	
		AI356405								AA507442	
		C22orf23								NAV1	
		MYO1D								AI356405	
		MIEN1								C22orf23	
		SNX14								RGL1	
		ARFIP2								DNAJC27	
		BM970306								TSC22D3	
		E2F7								MYO1D	
		GTF3C2								MIEN1	
		C15orf17								SNX14	
		WAC								PSMC3IP	
		AW028687								ARFIP2	
		GPD1L								AU158573	

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2 h time point			8 h time point			16 h time point			24 h time point		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
		RPL37								CORIN	
		IFRD1								BTBD11	
		NCOA4								KDM6A	
		BM682057								CA2	
		LRRC14								BM970306	
		UBR4								E2F7	
		ITIH4								PRSS23	
		MAPK1IP1L								GTPBP3	
		TXNIP								ZNFX1-AS1	
		ZNF776								GTF3C2	
		SF3B3								SCG5	
		FAM125A								MLST8	
		FAF2								C15orf17	
		SPATA7								ISG20	
		T71642								ABCF2	
		NSUN5P2								TMEM177	
		LRIG2								RBM5	
		C1orf162								R17062	
		AA042983								WAC	
		TMEM206								AI863194	
		AC003007								WDR6	
		HEATR1								AW028687	
		VPS8								GPD1L	
		GCDH								HNRNPA1	

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).

2 h time point			8 h time point			16 h time point			24 h time point		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
		MMP24								RPL37	
		C16orf53								IFRD1	
		LOC440894								NCOA4	
		TMEM100								BM682057	
		LOC440993								ECEL1	
		ZNF200								LRRC14	
		BC038430								UBR4	
		GBA2								PRKRIP1	
		MFSD3								ZNF79	
		AA973551								NM_030801	
		AI792670								AW117229	
		PCIF1								SEPT7P2	
		LOC100506713								BF995452	
		NAT14								ITIH4	
										MAPK1IP1L	
										TXNIP	
										SMC1A	
										ZNF776	
										SF3B3	
										ZNF227	
										FAM125A	
										TMEM74B	
										FAF2	

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).

2 h time point			8 h time point			16 h time point			24 h time point		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes)[1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes)[1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes)[1]
										POPDC2	
										COG3	
										LPXN	
										LRRC39	
										BC029614	
										AL134451	
										FBXL17	
										SPATA7	
										BG469257	
										NEB	
										TTC18	
										T71642	
										NSUN5P2	
										LRIG2	
										C1orf162	
										AW874669	
										CARS	
										NR2C2	
										NM_004038	
										AA042983	
										HLA-DQB1	
										TMEM206	
										AC003007	
										HEATR1	

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).											
2 h time point			**8 h time point**			**16 h time point**			**24 h time point**		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes)[1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes)[1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes)[1]
										AL833615	
										AA767131	
										VPS8	
										FUBP1	
										FAM108A1	
										MAP3K4	
										GCDH	
										MMP24	
										ZNF846	
										IREB2	
										C16orf53	
										GRSF1	
										AA431379	
										LOC440894	
										TMEM100	
										KIAA1468	
										C14orf101	
										LOC645513	
										LOC440993	
										FAM84B	
										RBPMS	
										ZNF200	
										PRRC2A	

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).

2 h time point			8 h time point			16 h time point			24 h time point		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
										AI810103	
										NPIP	
										CROCCP2	
										GOLGA1	
										BC038430	
										UBE2N	
										GBA2	
										C3orf19	
										NM_022155	
										MFSD3	
										H04996	
										AA973551	
										HEXIM1	
										PIK3C2A	
										GPRASP1	
										AI792670	
										PCIF1	
										AF291676	
										PARP11	
										LOC100506713	
										WDR67	
										PAQR4	
										POLH	
										RC3H2	
										AA84312	

Table S3. Genes that overlap at each timepoint between two DNA carriers (PEI and LF2000) (from Figure 4).											
2 h time point			**8 h time point**			**16 h time point**			**24 h time point**		
LF2000 (44 genes)	PEI (46 genes)	LF2000 U PEI (0 genes)[1]	LF2000 (5 genes)	PEI (177 genes)	LF2000 U PEI (2 genes) [1]	LF2000 (22 genes)	PEI (151 genes)	LF2000 U PEI (5 genes) [1]	LF2000 (0 genes)	PEI (454)	LF2000 U PEI (2 genes) [1]
										2	
										BC031948	
										PTGES2	
										NAT14	
										FILIP1L	

[1] U stands for the union of the time points, e.g. a gene that was found at multiple time points.