Supplementary material

Experimental procedures for CircRNA detection

1. Sample preprocessing

The peripheral blood (about 5 ml) collected from pregnant women using EDTA anticoagulant tubes was immediately stored at 4°C and processed within 2 h. These samples were centrifuged twice at 3000 rpm at 4°C and the supernatant (i.e. plasma) was retained and stored at –80°C until testing.

2. Total RNA

According to the manufacturer's instructions, the total RNAs of these samples were extracted using Trizol reagent (Invitrogen, Gaithersburg, MD, United States), and purified using a NucleoSpin RNA Clean-up kit (Macherey-Nagel, Germany). Then, a NanoDrop 2000 spectrophotometer (NanoDrop, USA) was used to quantify purified total RNA and an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) was used to verify the RNA integrity.

3. RNase R treatment

For each sample, 5 µg of RNA, 2 µl of RNase R 10X Reaction Buffer, and 0.5 µl of RNase R were mixed in the 20 µl reaction system, followed by instantaneous centrifugation. The digestion reaction was performed at 37°C for 30 min. Then, RNA was purified using a NucleoSpin RNA Clean-up kit (Macherey-Nagel, Germany).

4. cDNA synthesis

4.1 First strand cDNA

Total RNA 100-500 ng was added to the 0.2 ml nuclease-free microcentrifuge tube and then the appropriate volume of spike-in controls was added (Agilent Technologies, USA) according to the table below.

Starting amount of RNA		Serial dilutions			Spike-in mix volume to be used in each labeling reaction [µl]
Total RNa [ng]	Maximum volume of RNA [µl]	First	Second	Third	
200	8.3	1 : 20	1 : 25	1 : 10	2
300	7.3	1 : 20	1 : 25	1 : 10	3
400	6.3	1 : 20	1 : 25	1 : 10	4
500	5.3	1 : 20	1 : 25	1 : 10	5

The reverse transcription Master Mix was prepared by gently mixing 4 µl of First Strand Buffer Mix and 1 µl of First Strand Enzyme Mix. It was instantaneously centrifuged and placed in an ice bath. The Master Mix (5 µl) was added to the previously prepared 0.2 ml nuclease-free microcentrifuge tube containing total RNA and spike-in controls.

The solution was gently mixed, instantaneously centrifuged, and then reacted at 42°C for 2 h. After the reaction, it was instantaneously centrifuged and ice-bathed in sequence, and then the subsequent steps were performed immediately.

4.2 Second strand cDNA

The Second Strand Master Mix was prepared by gently mixing 13 µl of Nuclease-free Water, 5 µl of Second Strand Buffer Mix, and 2 µl of Second Strand Enzyme Mix. It was instantaneously centrifuged and placed in an ice bath. Then, 20 µl of Second Strand Master Mix was added to the reacted system generated in step 4.1.

The solution was gently mixed and reacted at 16°C for 1 h, then at 65°C for 10 min.

5. cRNA synthesis

The In vitro Transcription Master Mix was prepared by gently mixing 4 µl of Nuclease-free Water, 20 µl of T7 Buffer Mix, and 6 µl of T7 Enzyme Mix. It was instantaneously centrifuged and added to the reacted system generated in step 4.2.

The solution was gently mixed and reacted at 40°C for 8–14 h. Following this, the cRNA was purified using a NucleoSpin RNA Clean-up kit (Macherey-Nagel, Germany).

6. cRNA reverse transcription

The purified cRNA (5 µg/7.5 µl) and Random Primer (4 µl) were added to a 0.2 ml nuclease-free centrifuge tube, mixed gently, and reacted at 65°C for 5 min, followed by an ice bath for 5 min.

The cRNA reverse transcription Master Mix was prepared by gently mixing 5 µl of 4X Script II Buffer, 2 µl of 0.1 M DTT, and 1.5 µl of CbcScript II. It was instantaneously centrifuged and transferred to the solution generated in the last step.

The mixture was gently mixed, instantaneously centrifuged, and then reacted at 25°C for 10 min and 37°C for 1.5 h.

After the reaction, Terminate Solution (5 µl) was added to the system and gently mixed. The mixture was reacted at 65°C for 10 min and stood at room temperature for 5 min. Neutralize Solution (1 µl) was added to this mixture and gently mixed.

The cDNA was purified using NucleoSpin Extract II (Macherey-Nagel, Germany) following the

manufacturer's instructions. The purified cDNA was quantified using the spectrophotometer (1OD260 = 40 µg/µl).

7. Fluorescence labeling

The volume of the purified cDNA was concentrated to 14 µl. The Random Primer 4 µl was added to it, mixed well, instantaneously centrifuged, denatured at 95°C for 3 min, and ice-bathed for 5 min. Then, 5 µl of 5X Klenow Buffer, 1 µl of Cy5-dCTP (or Cy3-dCTP), and 1.2 µl of Klenow Fragmen were added to it. The mixture was gently mixed, instantaneously centrifuged, and then reacted at 37°C for 1.5 h and 70°C for 5 min.

The labeled production of this step was purified using NucleoSpin Extract II (Macherey-Nagel, Germany) following the manufacturer's instructions and then quantified using the spectrophotometer (1OD260 = 50 µg/µl).

8. Microarray hybridization

The hybridization mixture was prepared according to the following table:

Reagent	Standard volume
2X GEx Hyb Buffer (HI-RPM)	55.0 µl
Formamide	27.5 µl
Sample	27.5 µl
Total volume prepared	110 µl

The hybridization mixture was added to the circRNA microarray (CapitalBio Corporation, Beijing, China), and hybridization was performed in the hybridization oven (Agilent Technologies, USA) at 20 rpm for 16 h.

9. Microarray washing and scanning

After hybridization, the circRNA microarray was washed in wash solution I containing 0.2% SDS and 2×SSC at 42°C for 5 min and wash solution II containing 0.2×SSC at room temperature for 5 min.

The washed microarray was scanned by a G2565CA Microarray Scanner (Agilent Technologies, USA) to obtain hybridization images.

10. Data extraction and analysis

Agilent Feature Extraction (v10.7) software was used to analyze the hybridization images and extract the data, which were then normalized using Agilent GeneSpring software.

All the processes of microarray analysis were conducted by the Bioassay Laboratory of CapitalBio Corporation (Beijing, China).

Supplementary Table SI. Clinical characteristics of pregnant women

Patients ID	Age	BMI	Primi-gravida	Primi-parity	Delivery modes	Delivery [gesta-tional weeks]	Sampling 1 [gestational weeks]	Interval from sam-pling 1 to delivery [h]	Sampling 2 [hours after delivery]
L281	27	28.2	Yes	Yes	Vaginal	38.3	38.1	16	5
L284	28	32.4	No	No	Caesarean	39.9	39.7	26	44
L302	31	33.3	No	No	Vaginal	30.6	30.3	51	15
L317	28	23.7	No	Yes	Vaginal	29.6	29.1	108	30
L325	27	25.2	Yes	Yes	Vaginal	34.9	34.4	69	12
L328	27	29.0	Yes	Yes	Caesarean	34.3	34.1	16	42
L332	38	24.4	No	No	Caesarean	32.4	32.3	29	40

Supplementary Table SII. The expression of Y-circRNA in circulation of male and female neonate

Sample	SRR376	SRR377	SRR378	SRR380	SRR381	SRR395	SRR396	SRR397	SRR398	SRR400	SRR405	SRR406	SRR379	SRR382	SRR383	SRR384	SRR385	SRR386	SRR387	SRR388	SRR389	SRR390	SRR391	SRR392	SRR393	SRR394	SRR399	SRR401	SRR402	SRR403	SRR404
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
chrY:14510475|14518736	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25,19446	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:14652909|14655340	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	86,41327	0
chrY:14802255|14826722	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42,72677	0	0	0	0	0	0	0	0
chrY:14813939|14821476	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9,230132
chrY:14821321|14821476	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10,28787	0	0	0	0	0	0	0	0	0	0
chrY:14821321|14834120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51,15338	0	0	0	21,53697
chrY:14821321|14851563	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	184,5864	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:15021271|15024974	0	0	0	0	0	0	0	0	0	0	0	0	92,07494	0	245,646	0	108,5262	223,884	228,4123	289,6622	164,6059	115,0427	163,7859	134,9833	296,1674	186,9946	276,2283	220,8564	264,6385	489,6752	313,8245
chrY:15024875|15025765	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41,5295	0	0	0	0	0	0	0	0	0	0	0	0
chrY:15024875|15026561	0	0	0	0	0	0	0	0	0	0	0	0	170,9963	0	125,9723	0	0	74,62799	186,8828	50,69088	66,87114	82,17337	135,3014	376,0249	122,33	51,94295	122,7681	0	381,3909	124,8192	233,83
chrY:15362897|15438230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35,60564	0	0	0	0	0	0	0	0
chrY:15372158|15438230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18,89585	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:15390121|15448215	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50,69088	0	0	0	0	0	0	0	0	0	0	0
chrY:15409587|15417992	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42,72677	0	0	0	0	0	0	0	0
chrY:15409587|15438230	0	0	0	0	0	0	0	0	0	0	0	0	65,76781	0	107,0765	290,0643	0	117,2726	0	137,5895	87,44688	65,73869	56,96903	77,13331	0	93,4973	153,4601	0	0	0	70,76434
chrY:15409587|15448215	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25,75369	0	102,3068	0	0	0	0
chrY:15414808|15470401	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	124,8192	0
chrY:15418066|15425648	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36,00754	0	0	0	0	0	0	0	0	0	0
chrY:15418066|15433592	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32,19211	0	0	0	0	0	0
chrY:15435435|15438230	0	0	0	0	0	0	0	0	0	0	0	0	0	0	258,2432	0	0	0	134,9709	79,6571	164,6059	147,9121	192,2705	96,41664	38,63054	114,2745	132,9988	126,2037	0	0	98,45474
chrY:15435435|15447680	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37,79169	0	0	74,62799	31,14713	43,44933	92,59081	0	0	0	0	0	0	0	0	0	0
chrY:15435435|15448215	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39,99724
chrY:15435435|15508852	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12,30684
chrY:15441739|15469849	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	62,33154	0	0	0	0	0
chrY:15447443|15448215	0	0	0	0	0	0	0	0	0	0	0	0	39,46069	0	75,58338	0	0	0	0	0	0	0	42,72677	0	0	0	0	0	0	0	0
chrY:15447443|15472408	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54,48441	0	0
chrY:15466666|15478273	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20,76475	0	0	0	0	0	0	0	0	0	0	0	0
chrY:15466883|15467898	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	62,29426	0	0	0	35,60564	0	0	0	143,2295	0	0	0	0
chrY:15466883|15478273	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37,79169	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:15466883|15481229	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63,10183	0	0	0
chrY:15469757|15526673	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21,32228	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:15470344|15471102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49,8479	0	12,87685	0	0	0	0	0	0
chrY:15481136|15515768	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32,19211	0	0	0	0	0	0
chrY:15505739|15515768	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32,19211	0	0	0	0	0	0
chrY:15511818|15512566	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	57,93244	0	115,0427	0	0	0	0	0	0	0	0	24,61368
chrY:15511818|15515768	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	85,28913	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:15551567|15582109	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	94,14021	0	0	0	0	0	0	0	0	0	0	0
chrY:21094629|21095361	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	66,87114	0	0	0	0	0	0	0	0	0	0
chrY:21094629|21144882	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42,06789	0	0	0
chrY:21205049|21206581	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49,33009	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:21738591|21752658	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	28,48451	0	0	0	0	0	0	0	0
chrY:21749096|21749393	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100,7778	0	0	106,6114	176,5004	115,8649	66,87114	0	113,9381	154,2666	173,8374	114,2745	0	0	0	0	46,15066
chrY:21751407|21761717	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43,44933	0	0	0	0	0	0	0	0	0	0	0
chrY:21753666|21761717	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	57,84998	0	0	0	0	0	0	0
chrY:21901414|21903743	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100,7778	0	0	149,256	83,05901	43,44933	108,0226	0	92,57467	0	135,2069	62,33154	51,15338	94,65275	0	0	95,37803
chrY:22626965|22632841	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51,91188	0	0	0	0	0	0	0	0	0	0	0	0
chrY:22627554|22631867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	62,98615	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chrY:22669238|22683186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	170,5783	0	36,20777	0	65,73869	64,09015	0	57,9458	0	51,15338	0	0	0	18,46026
chrY:22737686|22744579	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20,76475	0	0	0	0	0	0	0	0	0	93,40184	0	0
chrY:22737686|22746410	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	77,13331	0	0	0	0	0	0	0
chrY:22737702|22741577	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36,92053
chrY:22741215|22744079	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18,46026
chrY:22741494|22744579	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44,09031	0	0	0	0	0	0	0	42,72677	0	0	0	0	0	0	115,2177	61,53421
chrY:22741494|22749991	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12,30684
chrY:22741494|22751461	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	43,44933	0	0	0	0	0	0	0	0	0	0	0

Sample	SRR376	SRR377	SRR378	SRR380	SRR381	SRR395	SRR396	SRR397	SRR398	SRR400	SRR405	SRR406	SRR379	SRR382	SRR383	SRR384	SRR385	SRR386	SRR387	SRR388	SRR389	SRR390	SRR391	SRR392	SRR393	SRR394	SRR399	SRR401	SRR402	SRR403	SRR404
Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
chrY:22744476|22751461	0	0	0	0	0	0	0	0	0	0	0	0	0	0	75,58338	0	0	42,64457	0	0	92,59081	0	156,6648	0	0	0	0	0	202,3707	0	49,22737
chrY:22749910|22751461	0	0	0	0	0	0	0	0	0	0	0	0	0	134,0695	0	0	0	0	0	101,3818	102,8787	41,08668	0	28,92499	0	0	0	0	0	0	86,1479
chrY:22891809|22902966	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12,87685	0	0	0	0	0	0
chrY:23300639|23314859	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20,57574	0	0	0	0	0	0	0	0	0	0
chrY:23310428|23314859	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	64,09015	0	0	0	0	0	0	0	0
chrY:23359026|23360427	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30,8636	0	0	38,56665	0	0	0	0	62,26789	0	0
chrY:23387378|23390345	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20,57574	0	0	0	0	0	0	0	0	0	0
chrY:23412200|23415079	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9,230132
chrY:2661808|2682407	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15,4318	0	0	0	0	0	0	0	0	0	0
chrY:2692622|2713784	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27,6904
chrY:2819679|2822038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44,09031	0	0	0	0	0	56,58327	65,73869	92,57467	0	32,19211	0	30,69203	0	0	0	33,84382
chrY:2821950|2829687	0	0	0	0	0	0	0	0	0	0	0	0	236,7641	150,8282	403,1114	224,1406	138,1242	245,2063	0	173,7973	154,318	205,4334	455,7522	318,1749	309,0443	72,72013	81,84541	126,2037	93,40184	182,428	187,6793
chrY:2821950|2843695	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12,30684
chrY:2829115|2829687	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	85,61835	0	0
chrY:2947325|2970235	0	0	0	0	0	0	0	0	0	0	0	0	78,92138	0	37,79169	0	0	0	0	137,5895	77,15901	0	0	0	57,9458	155,8288	0	0	319,123	0	86,1479
chrY:7209156|7224271	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	93,40184	0	0