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Supplementary Information for

Non-invasive Preimplantation Genetic Testing for Aneuploidy (niPGT-A) in Spent Medium may be More Reliable than Trophectoderm Biopsy

Lei Huang[a,b], Berhan Bogale[b], Yaqiong Tang[c,d], Sijia Lu[e], Xiaoliang Sunney Xie[a,c,d,1], and Catherine Racowsky[b,1]

[a]Department of Chemistry & Chemical Biology, Harvard University, Cambridge, MA 02138; [b]Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115; [c]Beijing Advanced Innovation Center for Genomics (ICG), Peking University, Beijing 100871, China; [d]Biomedical Pioneering Innovation Center (BIOPIC), Peking University, Beijing 100871, China; and [e]Department of Clinical Research, Yikon Genomics Company, Ltd., Shanghai 201499, China.

[1]To whom correspondence should be addressed. E-mail: sunneyxie@pku.edu.cn (X.S.X.), cracowsky@bwh.harvard.edu (C.R.)

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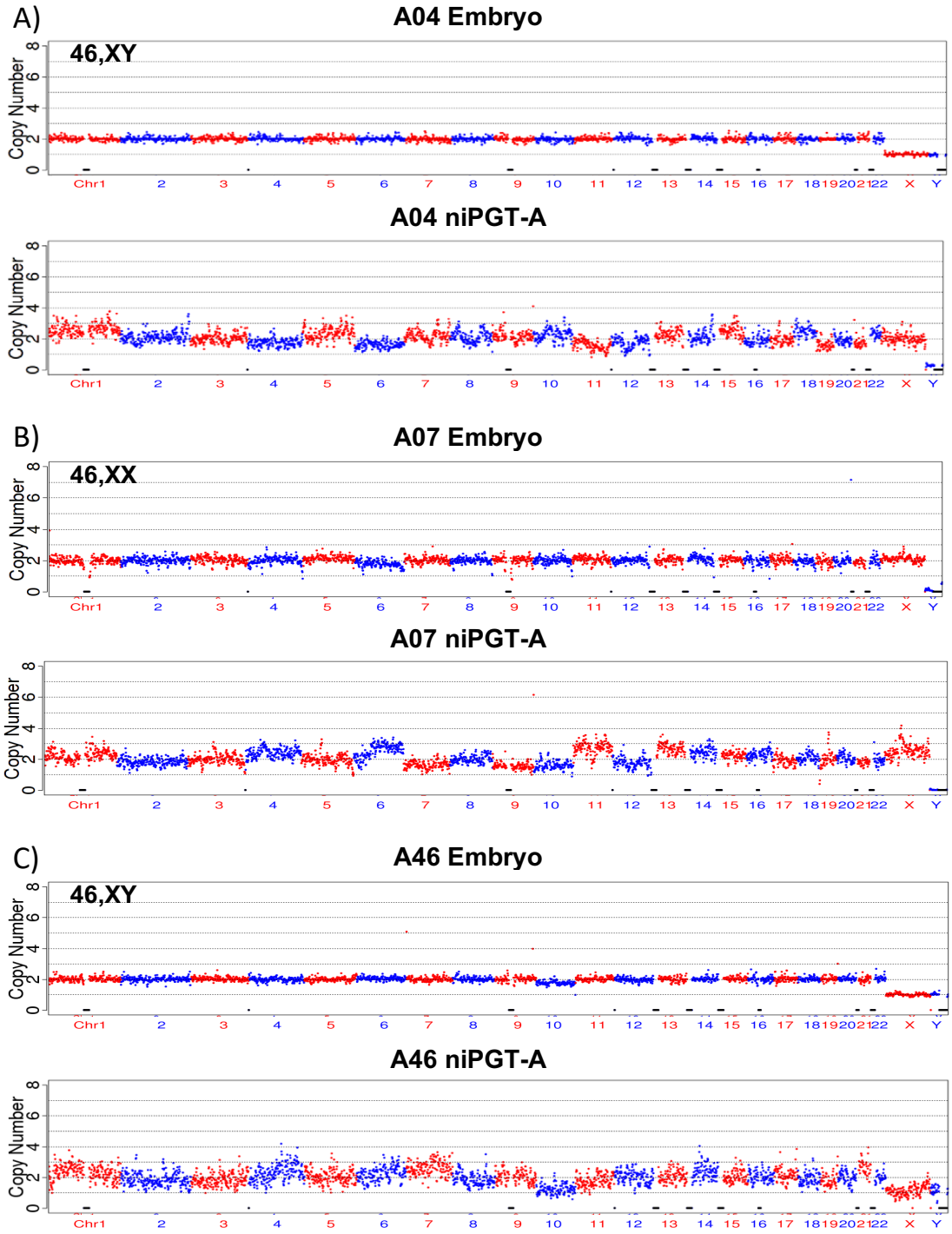

Fig. S1. Noisy copy number plots of three of the four niPGT-A samples and their corresponding embryos (sequencing depth of 0.02×).
Chromosomes are shown in alternating red and blue colors. In all cases, the embryo is euploid and the niPGT-A profiles in A) and B) are similar (CV = 0.20 and 0.19, respectively); in contrast, the niPGT-A profile in C) is much noisier (CV = 0.31).

Table S1. Complete chromosome copy number profiles for niPGT-A, Embryo and TE-biopsy when the percent mosaicism (M) threshold was set at ≥ 30%

ID	Embryo Stage/ Grade	Day of Biopsy/ Frozen	niPGT-A with M ≥ 30% (the minimum threshold level reported)	Embryo	TE-biopsy
A01	5Bb	D5	46,XY,-5(pter→q31.3,~141M,×1),+10(p12.31→qter,~108M,×3)	46,XY,-5(pter→q31.3,~142M,×1),+10(p12.2→qter,~111M,×3)	46,XY,-5(pter→q33,×1),+10(p13→qter,×3)
A02	5Bc	D5	45,XX,-19(×1),+21(×3,mos,~30%)	46,XX,-19q(×1,mos,~70%)	45,XX,-19(×1)
A03	5Bb	D5	45,XY,-3p(pter→p12.2,~84M,×1),-16(×1)	46,XY,-16(×1)	46,XY,-16(×1)
A04	5Bb	D5	Aneuploidy,XXY#,+X(×2),-Y(×0,mos,~30%),+1(p34.3→qter,~210M,×3,mos,~60%),+5q(q22.3→qter,~66M,×3,mos,~50%),-6(×1,mos,~40%),-11q(q11→qter,~79M,×1,mos,~60%),-12q(q12→q21.2,~39M,×1,mos,~30%),+13(×3,mos,~30%),+15(pter→q25.3,~66M,×3,mos,~50%),+18(×3,mos,~50%),-22(×3,mos,~30%)	46,XY	46,XY,-14q(q32.13-qter,×1)
A05	6Bb	D5	45,XX,-4(×1)	45,XX,-4(×1)	45,XX,-4(×1)
A06	6Bc	D5	46,XX,-22(×1, mos,~50%)	46,XX	45,XX,-13(×1)
A07	5Bb	D5	Aneuploidy,XX,+X(×3,mos,~50%),+6q(×3,mos,~70%),-7(×1,mos,~40%),-9(×1,mos,~50%),-10q(×1,mos,~50%),+11p(×3,mos,~70%),+11q(q13.4→q23.3,~43M,×3),+13(×3,mos,~60%),+14(×3,mos,~40%)	46,XX	47,XX,+10(×3)
A08	6Bc	D5	46,XX,-12p(p13.33→p12.1,~25M,×1)	46,XX,-12p(p13.33→p12.1,~25M,×1)	46,XX,+8p(pter-p23,×3),-12p(pter→p11.21,×1)
A09	7Ab	D5	46,XX,-3q(q21.3→q29,~71M,×1,mos,~40%),+12p(p13.33→p12.1,~25M,×3)	46,XX,+12p(pter→p12.1,~24M,×3)	46,XX,+8p(pter-p23,×1),-12p(pter→p11.21,×3)
A10	5Bb	D5	46,XY,+8q(q21.13→qter,~57M,×3, mos,~30%),+12(p12.3→q24.31,~106M,×3)	46,XY,+12(p12.3→q24.31,~108M,×3)	46,XY,+8p(pter-p23,×3),+12(p11.2-qter,×3)
A11	6Ba	D6	46,XX,+12p(pter→p11.21,~33M,×3)	46,XX,+12p(pter→p12.1,~24M,×3)	46,XX,-8p(pter-p23,×1),-12p(pter→p11.21,×3)
A12	5Ba	D5	47,XX,-X(×1,mos,~60%),-1p(p31.1→p12,~43M,×1),-1q(×1),-5(×1),+7(×3),-9q(q21.31→q34.3,~59M,×1,mos,~40%),+11(×3,mos,~50%),-12q(×1,mos,~40%),+13(×3,mos,~40%),+17q(q12→q24.3,~35M,×3,mos,~40%),+21(×3)	46,XX,-1(p31.1→qter,~179M,×1), +7(p21.2→qter,~143M,×3)	46,XX,+1(p31.2→qter,×1), +7(p15.2→qter,×3)
A13	6Ba	D5	46,XY,+22(×3,mos,~60%)	47,XY,+22(×3)	47,XY,+22(×3)
A14	5Ba	D5	46,XY,-1(×1),+7(×3)	46,XY,-1(×1),+7(×3)	46,XY,-1(×1),+7(×3)
A15	6Bc	D5	46,XY,-1p(pter→p31.1,~74M,×1),7p(pter→p15.3 p15.3,~23M,×3)	46,XY,-1p(pter→p31.1,~74M,×1), 7p(pter→p15.3,~23M,×3)	46,XY,-1p(pter→p31.1,×1), 7p(pter→p15.3,×3)
A16	5Bb	D5	45,XX,-22(×1)	45,XX,-22(×1)	45,XX, -1(×1)
A17	6Bb	D5	46,XY,-1(p31.1→qter,~173M,×1),+7(p15.3→qter,~137M,×3)	46,XY,-1(p31.2→qter,~180M,×1), +7(p21.2→qter,~143M,×3)	46,XY,-1(p31.2→qter,×1), +7(p21.2→qter,×3)
A18	5Bb	D5	45,XY,+Y(×2,mos,~40%),-5(×1),+6(pter→q25.3,~161M,×3),+19(×3),+22(×3,mos,~60%)	47,XY,-5(×1,mos,~70%),+6(×3),+19(×3,mos,~50%),+22(×3,mos,~60%)	48,XY,-5(×1),+6(×3),+19(×3),+22(×3)
A19	5Bb	D6	46,XX,+X(×3,mos,~40%),+11(×3),-16(×1)	47,XX,+11(×3),-16q(×1)	47,XX,+11(×3),-16(×1)
A20	5Bb	D5	45,XX,-2(×1)	45,XX,-2(×1)	45,XX,-2(×1)
A21	6Bb	D5	46,XY,+X(×2,mos,~30%),-Y(×0,mos,~50%)	46,XY	46,XY
A22	6Bb	D5	45,XX,-19(×1)	46,XX	46,XX
A23	6Ab	D5	46,XX	46,XX	46,XX
A24	6Aa	D5	46,XY	46,XY	46,XY
A25	5Bb	D6	45,XX,-21(×1)	45,XX,-21(×1)	45,XX,-21(×1)
A26	5Bc	D5	45,XY,+19(×3,mos,~70%),-22(×1)	46,XY,+19(×3),-22(×1)	46,XY,+19(×3),-22(×1)
A27	5Bc	D5	47,XX,-1(×1,mos,~50%),+16(×3)	48,XX,+16(×4)	48,XX,+16(×3)
A28	5Bb	D5	45,XX,-8(×1),+22(×3,mos,~70%)	45,XX,-8(×1)	46,XX,-8(×1),+22(×3)
A29	5Bc	D5	46,XX,-10(×1),+11(×3),-12(×1),-16p(×1,mos,~30%),+21(×3)	46,XX,-10(×1),+11(×3),-12(×1),+21(×3)	45,XX,-10(×1),+11(×3),-12(×1)
A30	5Bc	D5	47,XX,+4(×3),+8(×3,mos,~70%)	46,XX,+4(×3,mos,~70%),+8(×3,mos,~70%)	48,XX,+4(×3),+8(×3)
A31	5Bb	D5	44,X,-X(×1),-16(×1)	44,X,-X(×1),-16(×1)	Degraded DNA
A32	5Bc	D6	46,XY,+13(×3,mos,~40%),+15(×3,mos,~40%),+17q(×3,mos,~40%),-22(×1,mos,~60%)	46,XY,+13(×3,mos,~30%),+17q(×3,mos,~40%),-22(×1,mos,~60%)	45,XY,-22(×1)
A33	5Cb	D6	46,XY	46,XY	46,XY,-1(pter-p32.3,×1)
A34	5Bb	D6	45,XY,+Y(×2,mos,~40%),-16(×1,mos,~60%),-18(×1)	45,XY,-16q(×1,mos,~50%),-18(×1)	45,XY,-18(×1)
A35	6Bb	D6	47,XX,+16(×3),-18(×1), +22(×3)	46,XX,+16(×3),-18(×1),+22(×3, mos,~60%)	47,XX,+16(×3),-18(×1),+22(×3)

A36	6Bb	D6	45,XY,-22(×1)	45,XY,-22(×1)	45,XY,-22(×1)
A37	5Bb	D5	47,XY,-16p(×1,mos,~30%),+21(×3)	47,XY,+21(×3)	47,XY,+21(×3)
A38	6Ab	D6	45,XX,-22(×1)	45,XX,-22(×1)	45,XX,-22(×1)
A39	6Cc	D6	46,XY,-18q(×1,mos,~30%)	46,XY	No Result
A40	5Bc	D5	46,XY,-16q(×1)	46,XY	47,XY,Tri/polysomy10,Del/Dup 16
A41	5Bb	D5	46,XY,+3(×3,mos,~70%),-16p(×1,mos,~30%)	47,XY,+3(×3)	47,XY,+3(×3)
A42	5Bc	D5	46,XY,-Y(×0,mos,~30%)	46,XY	46,XY
A43	6Bb	D6	46,XY,-Y(×0,mos,~30%)	46,XY	47,XY,+20(×3)
A44	6Bc	D5	46,XX,+19q(×3,mos,~70%)	46,XX,+19(×3,mos,~70%)	47,XX,+19(×3)
A45	6Cb	D5	46,XX	46,XX	47,XX,+3(×3),+10(×3),-18(×1)
A46	6Bb	D5	Aneuploidy,XY,+1p(p36.13→p11.2,~105M,×3,mos,~60%),+4(×3,mos ,~30%),+7(p14.3→qter,~126M,×3,mos ,~60%),-10(×1),+14(pter→q24.3,~54M,×3,mos,~40%),+21(×3,mos ,~60%)	46,XY	47,XY,+10(×3)
A47	7Bb	D5	46,XX,-21(×1,mos,~60%)	46,XX	47,XX,+22(×3)
A48	5Bb	D5	46,XX,-3p(×1,mos,~40%),-16q(×1,mos,~30%)	46,XX	46,XX
A49	6Bb	D5	46,XY,-5(×1,mos,~50%)	46,XY	46,XY
A50	6Bb	D5	Aneuploidy,XY,-X(×0,mos,~50%),-Y(×0,mos,~40%),+4q(×3,mos,~40%),-8q(×1,mos,~50%),-9(×1,mos,~50%),+13(×3,mos,~40%),-14(×1,mos,~60%),-15(×1,mos,~60%)	46,XY	46,XY
A51	5Ab	D5	46,XY	46,XY	46,XY
A52	6Bb	D5	45,XY,-22(×1)	45,XY,-22(×1)	45,XY,-22(×1)

Table S2. Coefficients of variation for embryos identified as male or female by niPGT-A

Sex		Mean CV	Autosome CV	Sex Chr X CV	Sex Chr Y CV
Male (n=23)	Mean	0.15	0.19	0.21	0.27
	SD	0.03	0.03	0.04	0.11
	Lower limit	0.10	0.13	0.14	0.12
	Upper limit	0.21	0.25	0.32	0.66

Sex		Mean CV	Autosome CV	Sex Chr X CV
Female (n=25)	Mean	0.17	0.20	0.19
	SD	0.06	0.06	0.06
	Lower limit	0.10	0.12	0.12
	Upper limit	0.29	0.31	0.35

Table S3. Performance of niPGT-A when varying the number of non-informative copy number profiles excluded

	niPGT-A			TE-biopsy
Non-informative samples excluded	0	2	4	2
False positives	7	5	3	9
False positive rate	36.8% (7/19)	29.4% (5/17)	20.0% (3/15)	50.0% (9/18)
PPV	82.5% (33/40)	86.8% (33/38)	91.7% (33/36)	78.0% (32/41)
% Concordance of embryo ploidy	86.5% (45/52)	90.0% (45/50)	93.8% (45/48)	82.0% (41/50)
% Concordance of chromosome copy numbers	76.9% (40/52)	80.0% (40/50)	83.3% (40/48)	62.0% (31/50)